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CONTENT

FEED TO FOOD

Jovanka Lević, Slavica Sredanović

University of Novi Sad, Institute of Food Technology, 21000 Novi Sad, Bulevar cara Lazara 1

The global feed industry is under increasing pressure to supply sustainable, safe and healthy feed. With the expanding global population, which is forecast to exceed 9 billion people by 2050, comes an associated higher demand for animal protein and, therefore, feed. Globally, industrial feed production was close to an estimated 870 million tons in 2011. IFIF (International Feed Industry Federation) anticipates feed industry will grow by at least 3% a year and this challenge must be met with better technology. Feed directly contribute to the quality of meat, milk and eggs in possitive and negative direction. Throught feed diet content it is possible to manipulate the animal products quality and it is possible to achieve different nutritional, sensoric, physical and chemical characteristics. Also, the different contaminants may be transmit to animal products through feed. This indicates the necessity of research related to determining the impact of feed on animal products quality and following the quality of animal products depending on the composition of diets consumed by animals. Technological processes used in Feed industry have unavoidable impact in food chain and their permanent development and improvement is necessary. They must be optimized in order to ensure that all ingredients of the formulated mixture maintain their prescribed concentrations and activities. Proper physical form, consistency, stability and other characteristics of feed must be also achieved by using adequate processing technologies and equipments.

Like globally, the feed industry is of great importance in the Republic of Serbia and it is an important part of the food chain, which plays a crucial role in the meaning of sustainability and careful use of resources. In order to ensure market surpluses of healthy and high quality food products of animal origin (meat, milk, eggs etc.), it is necessary to expand animal feed production. In Republic of Serbia there are all essential prerequisites for achieving this: agricultural land areas, production of cereals and proteinated vegetable feed, adequate quantities of agricultural and food industry by-products, and developed feed manufacturing facilities. Continued innovation is required to raise efficiency of feed and livestock production.

EU legislation is designed to ensure that feeds are of high quality and are safe for both livestock and consumers. Full harmonization of Serbian legislative with the rules in EU is not finished yet, but activities about it are in progress.

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COST ACTION FEED FOR HEALTH 2008-2012 MAIN ACHIEVEMENTS

*Luciano Pinotti**

Department of Health, Animal Science and Food Safety, Veterinary Faculty, Università degli Studi di Milano, Milan, Italy- *Feed for health Action Chair

Food of animal origin contributes significantly to the total nutrients in the current EU diet. The latest review of livestock production and trade indicates that more than 195 million tonnes of meat, milk and eggs were produced in the EU in 2011. To sustain this scale of livestock production, about 500 million tonnes of feedstuffs are required each year within the EU-27. Clearly, ensuring such high outputs of these traded products conform to adequate quality standards is a major undertaking and it is fair to say that the EU has made significant progress in defining standards and promoting legislation in this area. As a consequence the explicit and detailed formulation of the concepts of food/feed safety and food/feed quality, has given rise, within the EU, to legislation on the traceability, control and labelling of both feed and food. However nowadays both feeds/and foods must be considered not only in terms of their nutritional properties but also in terms of their ability to promote health and protect against disease. As a consequence, the role of animal nutrition in designing foods closer to the optimum composition for long-term human health are becoming increasingly important. In light of these topics a scientific network has been set up: the COST Action Feed for Health is a network supported by the European Cooperation in Science and Technology which involves 28 countries, mainly from EU. The main aim of the COST Action is to develop an integrated and collaborative network of research groups that focuses on the roles of feed and animal nutrition in improving animal health and also the quality, safety and wholesomeness of human foods of animal origin. A further topic of the action is to examine the perception of consumers as regards the effects of animal nutrition on animal health and on the quality and safety of the resulting food products. The Feed for Health project works mainly through four Working Groups (WG): Feed and food for health (WG1), Feed Safety (WG2), Feed Supply (WG3), and Consumer concerns and perceptions (WG4). During its life span the action evidenced that wholesome and balanced feed is essential not only for promoting animal growth, production and health, but also for producing high quality food products. This is particularly likely to be true in large scale animal production, where nutrition-based interventions for health can offer a practical and efficient solution to maintaining animal health. From the consumer point of view, it is generally accepted that FEED is perceived as particularly vulnerable in the livestock chain, and they prefer animal products from livestock systems that used high quality animal feed, safe for consumers, friendly to the environment and the animals.

VALIDATION OF IMMUNOASSAYS FOR THE DETECTION OF PROCESSED RUMINANT PROTEINS IN NON-RUMINANT PROTEINS

Leo W.D. van Raamsdonk[1], Rob J.C.F. Margry[2], Robert G.C. Van Kaathoven[2], Monique G.E.G. Bremer[1]

[1]RIKILT Wageningen UR (University & Research centre), P.O. Box 230, 6700 AE Wageningen, The Netherlands
[2]CCL Nutricontrol, Feed to Food Quality Services, P.O. Box 107, 5460 AC Veghel, The Netherlands

The consumption of food products of animal origin is an inevitable part of our daily diet. As a result of the production of meat, milk and egg products approximately 17 Million Ton of waste animal by-products are produced in the European Union each year. These by-products could be a highly valuable source of nutrients, especially proteins, except for the situation that the consumption by farmed animals is generally prohibited for avoiding mad cow disease

Due to a growing aquaculture industry the demand for high quality proteins for aquafeeds is increasing. Non-ruminant processed animal proteins (PAPs) have shown great potential for this purpose. A 2% tolerance limit for the presence of ruminant PAP in non-ruminant PAP is shown to have negligible impact on the risk of additional BSE cases. Therefore, for a safe re-introduction of non-ruminant PAPs in aqua feed methods are needed that are able to discriminate between ruminant and non-ruminant PAPs at this tolerance level.

The performance of MELISA-TEK™ Ruminant in combination with the MELISA-TEK high Sensitivity Sample Extraction kit was evaluated in an intralaboratory study. The results showed an overall specificity of 99%, which indicates no cross-reaction with non-ruminant PAPs. The sensitivity was sufficient from a contamination level of 0.5% up, although depending on the processing temperature. These results were sufficient to start a large interlaboratory validation study, in which Melisa-Tek was compared with Reveal.

The study comprised a training phase, an entrance test and the final validation experiment. Samples were spiked at 0.5%, 1.0% and 2.0%. Fourteen participants passed the test and investigated the samples. For both Melisa-Tek and Reveal specificity and sensitivity were at 97% or higher. Concordance and accordance were also at good levels. The study complied with AOAC guidelines as far as possible for a qualitative study.

• Immunoassays for the detection of ruminant PAP (Melisa-Tek and Reveal) are validated at 0.5% and higher with non-ruminant PAP as matrix.
• The design of the study can be used as guideline for future studies with qualitative results.

***Keywords**: feed ingredients, former food products, bakery products, packaging materials, quantification*

REINTRODUCTION OF PROCESSED ANIMAL PROTEINS IN FEED: FILLED GAPS AND GAPS TO BE FILLED

Pascal Veys, Marie-Caroline Lecrenier, Vincent Baeten

Walloon Agricultural Research Centre CRA-W, Food and feed quality unit, Valorisation of Agricultural Products Department, Henseval building, Chaussée de Namur, 24. 5030 Gembloux, Belgium

In 2010, the second TSE Roadmap set down the conditions to be met for a partial reintroduction of processed animal proteins (PAPs) in feed in the European Union. Progresses in detection methods have allowed meeting the prescribed conditions allowing a partial lift of the total feed ban. Major improvements in the light microscopic method and the PCR were achieved. Nevertheless none of the method is able on its own to fit all requirements for the accurate identification of prohibited ingredients from animal origin method lead to propose a combinatory approach on which official controls can rely for a better detection and identification of animal constituents in feed. The combination models proposed varies according to the final destination of the feed or feed ingredients. Nonetheless the likely reintroduction of PAPs will be a source of new challenges in accurate identification of certain feed ingredients possibly interfering with light microscopy and PCR. The emergence of new concerns supports the necessity of developing complementary techniques for disclosing contaminations. The lecture will present the recent advances in method combination as regards PAPs detection and shed light on possible interferences with authorized products from animal origins. Some study cases will be presented and discussed in terms of gaps to be filled.

Keywords: *Processed animal proteins, feed ban, combination of methods, future challenges*

IMPLEMENTATION OF THE REAL-TIME PCR AS OFFICIAL METHOD OF DETECTION OF PROCESSED ANIMAL PROTEINS IN THE EUROPEAN UNION REFERENCE LABORATORY NETWORK

Olivier Fumière, Aline Marien, Pascal Veys, Vincent Baeten, Gilbert Berben

Walloon Agricultural Research Centre CRA-W, Valorisation of Agricultural Products Department, Henseval building, Chaussée de Namur, 24. 5030 Gembloux, Belgium

In the TSE Road map 2 describing the strategy on Transmissible Spongiform Encephalopathies for the period 2010-2015, the European Commission envisaged a possible gradual lifting of the feed ban but keeping the level of consumer protection unchanged. To achieve this goal, strict control rules are maintained: 1. Processed Animal Proteins (PAPs) coming from ruminant remain forbidden; 2. Ruminant cannot have PAPs in their diet unless few exceptions like fishmeals as milk replacers for young animals; 3. The intra-species recycling of PAPs is banned. In addition, PAPs will not be allowed in the feeds for herbivores (rabbit, horse,…) whatever their origin.
The implementation of this new legislation requires additional analytical methods for its enforcement. Besides a continual improvement of the light microscopy protocol, real-time PCR tests were or are assessed and validated for the purpose of the detection of PAPs and the determination of their species origin. Different PCR methods already proved their potential through previous inter-laboratory studies but always in the hands of their developers[1]. In 2009, a big step forward in the implementation of PCR methods in a network of labs was the development and the validation of a transfer protocol using plasmid calibrants through an international interlaboratory study conducted by the EURL-AP gathering 18 participants from Europe, Japan and Australia[2]. In March 2012, the ruminant PCR assays developed by TNO Triskelion used in combination with the CRA-W transfer protocol was officially declared as fit for the detection of PAPs in feed based on the results of an interlaboratory study involving 12 European participants[3]. The work to validate a pig and a poultry target is also in progress.

[1] Detection of Ruminant Meat and Bone Meals in Animal Feed by Real-Time Polymerase Chain Reaction: Result of an Interlaboratory Study. M. Prado, G. Berben, O. Fumière, G. Van Duijn, J. Mensinga-Kruize, S. Reaney, A. Boix, Ch. von Holst. *J. Agric. Food Chem.* 2007, 55, 7495-7501.

[2] Determination of the cut-off value of a PCR assay on a specific PCR platform can be essential for the transferability of a qualitative real-time PCR method. O. Fumière, V. Planchon, A. Marien, R. Oger, G. Berben. *In: Proceedings of Rapid Methods Europe 2010, January 25-27, 2010, Noordwijkerhout, The Netherlands.* Bilthoven, The Netherlands: Rapid Methods Europe.

[3] Validation study of a real-time PCR method developed by TNO Triskelion bv for the detection of ruminant DNA in feedingstuffs. O. Fumière, A. Marien, G. Berben. Preliminary report. 9th of March 2012. http://eurl.craw.eu/img/agenda/20120309617d721b.pdf

As the PCR is an indirect method targeting the detection of DNA, the presence of ingredients of animal origin authorised by the legislation (e.g. milk or egg powder, fats and blood powder) could interfere with the results. To solve this problem, different strategies are investigated (e.g. the PCR analysis of the sediment fraction or the combination of a laser microdissection and catapulting system with the real-time PCR).
Another aspect of the implementation of the PCR was the launching in December 2010 of a training program intended in a first step to beginners. Within 4 sessions, people from 19 NRLs attended courses alternating theoretical and practical aspects. Moreover, the EURL-AP produced a DVD explaining through video sequences the good laboratory practices to provide reliable PCR results.A final evaluation of the successful implementation of the PCR in the EURL-AP network is under progress with the interlaboratory study aiming to evaluate the ability of the NRLs to detect the presence of ruminant in DNA samples as well as in feed samples.
This communication is under the responsibility of the authors and does not reflect the view of the European Union Commission.
Keywords: *Real Time PCR, calibrant, plasmid, cut-off, PAP, processed animal protein*

PRODUCTION OF FERMENTED SAUSAGES WITH OLIVE OIL, PROBIOTICS AND DIETARY FIBERS

Taxiarhoula Magra, Nikolaos Soultos, Elias Papapanagiotou, Ioannis Amvrosiadis

Department of Hygiene and Technology of Foods of Animal Origin, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, Greece

Fat is a source of vitamins, essential fatty acids and energy for the consumer. It contributes to better structure and flavour of the sausage as well. However, high consumption of animal fat is related to serious diseases. Trends in meat industry are focalizing in the production of sausages with partial replacement of pork fat with olive oil.

The aim of this study was to produce 'healthier' fermented sausages. Pork fat was totally replaced with extra virgin olive oil; probiotics and dietary fibers were used to improve functional value, sensory properties, and structure of the product.

Sausages were produced according to the traditional technologies with pork and bovine meat. Pork fat was totally replaced with extra virgin olive oil emulsified with turkey meat. Sausages were fermented for 21 days, during which the weight loss was up to 30%.

Samples were subjected to microbiological (counts of lactic acid bacteria, Micrococci, coagulase-positive staphylococci, yeasts/moulds, Enterobacteriaceae) and physicochemical tests (weight loss, pH, aw, % moisture, protein, fat and ash content, oxidation of fatty acids). Sensory evaluation (appearance, colour, consistency, hardness, odor, general acceptance) was performed to final products.

Post fermentation, sausages were pathogens free and obtained high scores in all sensory properties. The emulsion of olive oil, forming a mosaic pattern, was visible in the sliced product in the same way that fat is visible in the conventional fermented sausages. Fermented sausages with olive oil are more stable to oxidation in comparison to sausages with pork fat. Thus, they can be preserved at 5oC, in vacuum or modified atmosphere package, for 12 months.

Fermented sausages with olive oil, probiotics, and dietary fibers, belong to new eco-innovative products that are not only technologically complete, but also safe and healthy for the consumer.

***Keywords**: fermented sausages, olive oil, probiotics, dietary fibers*

DIETARY SPECIES-SPECIFIC PROBIOTIC CAN CONTRAST MULTIRESISTANT E. COLI ISOLATES IN THE GUT OF VEAL CALVES

Alessandro Agazzi, Simone Stella, Erica Tirloni, Serena Maroccolo, Barbara Ripamonti, Carla Bersani, Giovanni Savoini

Università degli Studi di Milano, Dipartimento di Scienze Veterinarie per la Salute, la Produzione Animale e la Sicurezza Alimentare, Via Celoria 10, I-20133 Milan, Italy

Cattle are a reservoir of E. coli, that acquires resistant genes from other microorganisms causing antibiotic resistance: antimicrobial activity of probiotics could contrast this pathogen. The aim of the trial was to investigate the inhibitory effects of a species-specific multistrain probiotic (SMP) on multiresistant E. coli isolates from veal calves. Two hundred fifty four E. coli were randomly isolated from monthly-pooled fecal samples on 24 subjects. Animals were bred in 4 boxes of 6 animals each for 6 months. Isolates E. coli were evaluated for antimicrobial susceptibility using disk diffusion methods. CLSI disk diffusion test was performed on each isolate, with eight classes of antimicrobial agents: penicillins (penicillin, ampicillin), sulphonamide, cephalosporins (cephalothin), tetracyclines (tetracycline), aminoglycosides (neomycin, apramycin), macrolides (spyramicin), lincosamides (lincomycin-spectinomycin), quinolones (nalidixic acid, enrofloxacin). Inhibition test of SMP on multiresistant E. coli was then performed. The requisite for E. coli selection was the resistance to penicillins, sulphonamides, tetracyclines, macrolides and to two of the other antimicrobial classes tested. The first step of the experiment evidenced an extremely high resistance prevalence (> 70%) of isolates E. coli towards penicillin, sulphonamide, tetracycline, ampicillin and spyramicin; 4% of tested strains were resistant to all the considered antimicrobials, and sixty E. coli isolates resulted as multiresistant (23.62%). In the second step, the inhibitory effect of SMP against multiresistant E. coli showed very large inhibition halos toward all the isolates: 76.7% with halo > 20mm, 20.0% with halo between 10 and 20mm and 3.3% with halo <10mm. Obtained results evidenced the positive effect of SMP on multiresistant E. coli inhibition: this gives a new perspective on breeding practices to contrast the prevalence of severe infectious by E. coli strains that usually involve veal calves especially during the first weeks of life.

***Keywords**: calf, species-specific multistrain probiotic, antiobiotic-resistant E.coli*

NEWBORN CALF FED SPECIES-SPECIFIC PROBIOTIC: EFFECTS ON GROWTH PERFORMANCE, HEALTH STATUS, MICROBIOLOGICAL AND HEMATOLOGICAL PARAMETERS AND CELL MEDIATE IMMUNE RESPONSE

Alessandro Agazzi, Serena Maroccolo, Erica Tirloni, Simone Stella, Barbara Ripamonti, Carla Bersani, Vittorio dell'Orto, Giovanni Savoini

Dipartimento di Scienze Veterinarie per la Salute, la Produzione Animale e la Sicurezza Alimentare, Via Celoria 10, 20133 Milan, Italy

Poor performance of young calves is often related to low digestion and absorption of nutrients due to gut colonization of E.coli: species-specific multistrain probiotic (SMP) could improve gut health increasing the digestion efficiency with consequent improved performance. The aim of the study was to evaluate the effects of dietary SMP in calves during the first month of life on performance, microbiological and health status, blood cells count and cell-mediated immune response. Twenty-two Friesian calves, divided in 2 homogenous groups, were fed a milk replacer with (T) or without (C) 1g/d SMP (Lactobacillus animalis-Lactobacillus paracasei-Bacillus coagulans, 30:35:35%, 1.8x1010 CFU/g) plus a concentrate mixture. On 2, 8, 14, 21 and 28d of life growth performance and blood cells count were determined, while fecal Lactobacilli and E. coli enumeration were performed. Daily fecal score and general health score (GHS) were determinated. Skin thickness at 24h post phytohaemoagglutinin (PHA) injection was evaluated on 8 and 28d. Data were analysed by a mixed procedure of SAS. No differences were found on ADG, concentrate intake was higher in T group (14.77kg vs 12.56kg/DM basis; $P<0.05$), but no effect was observed on FCR. E. coli tended to be lower in T animals (3.76 vs 5.01Log CFU/g; $P=0.07$), increasing Lactobacilli/E. coli ratio (2.02 vs 3.73; $P<0.05$). Fecal score increased in T calves during the last weeks on trial with no differences on GHS. Higher basophils content at the end of the trial (0.21% vs 0.16%; $P<0.05$) and a lower eosinophil percentage (0.05% vs 0.22%, $P<0.01$) on 8d of life were found in T group: skin test was not different on 8 and 28d of life. The administration of SMP in calves can benefits the microbial balance in such a stressful time as during the first month of life.

***Keywords**: calf, species-specific multistrain probiotic, E.coli, gut health*

THERMAL ANALYSIS OF REHYDRATED PORK MEAT

Sanja Ostojić[1], Darko Micić[1], Snežana Zlatanović[1], Lato Pezo[1], Danijela Šuput[2], Branislav Simonović[1], Ljubinko Lević[2]

[1]University of Belgrade, Institute of General and Physical Chemistry, Studentski trg 12, 11000 Belgrade, Serbia
[2]University of Novi Sad, Faculty of Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

Rehydration properties have been related to structural changes. In order to improve the drying process it is of interest to know the capability of meat to regain water.
The aim of this work was to follow the influence of rehydratation process on osmotically dehydrated meat by methods of thermal analysis: differential scanning calorimetry (DSC) and thertmogravimetric analysis (TGA).
Water loss, obtained from thertmogravimetric analysis (TGA) and protein stability, obtained from differential scanning calorimetry (DSC), expressed as temperature (Tm) and enthalpy (ΔH) of protein denaturation, during the rehydration process at different temperatures (20 ºC, 30 ºC, 40 ºC) in time period of 15, 30, 40 and 60 min, respectively, was followed. DSC and TGA of fresh, osmotically dried and rehydrated pork meat have been performed on TA Instruments DSC Q 1000, and TGA measurements on TA Instruments TGA Q 500, under N2 purge flow of 50ml/min and 60ml/min respectively. DSC scans were conducted in temperature range from 3 ºC to 150 ºC, and from -80 ºC to 180 ºC with heating rate Hr=5 ºC/min, and TGA scans in temperature range from 25 ºC to 900 ºC, heating rate Hr=5 ºC/min. Obtained thermal characteristics of rehydrated pork meat have been compared to fresh and osmotically dehydrated (in sugar beet molasses) pork meat. It was shown that process of rehydration affects thermal stability (DSC results), and water loss (TGA results) of rehydrated pork meat compared to fresh and dehydrated pork meat. Changes have been induced concerning protein thermal stability: increased enthalpy (ΔH) and temperature of denturation (Tm), suggesting to greater protein stability and higher water contempt of rehydrated comparing to dehydrated meat. Best results consernig protein stability, have been achieved by rehydration at 20 ºC: from ΔH=528 J/g, Tm= 62 ºC for osmotically dehydrated meat to ΔH=750 J/g, Tm= 65 ºC for rehydrated meat at 20 ºC in time of 45 min.

ROBUSTNESS OF NIRS CALIBRATIONS FOR TRANSFERENCE TO PORTABLE INSTRUMENTS

Sagrario Modroño, Ana Soldado, Adela Martínez-Fernández, Begoña de la Roza-Delgado

Department of Nutrition, Grasslands and Forages. Regional Institute for Research and Agro-Food Development. SERIDA, 33300, Villaviciosa. Spain

Near Infrared Spectroscopy (NIRS) has been widely tested for the quantitative prediction of nutritive parameters in feedstuffs. NIR laboratory instruments working in controlled environments are widely accepted for quality and control. However for the establishment of feed controls at farm and industrial level it is necessary to develop robust calibrations able to be transferred from at-lab to on-site/on-line instruments. Taking into account these considerations, the purpose of this work has been to develop a robust calibration evaluating different alternatives to apply the model on the on-site instrument available, and to establish adequate recommendations to users.

Three instruments were involved in this research work: at-lab level a NIR instrument (FossNIRSystem 6500, master) for the development of robust calibrations, and two on-site instruments, a diode array NIR instrument (Corona 45 visNIR 1.7, host-1) and a Phazir handheld MEMS (micro-electro-mechanical system, host-2) from Polycromix. All instruments work in reflectance mode, however their mechanical and optical characteristics (optics, window, etc.) are extremely different and can limit their use. 123 intact feed samples were scanned with the master instrument to develop the calibration model; 20 were scanned with all NIR instruments (master, host-1 and host-2), ten samples were used to evaluate the standardization procedure and ten to validate externally the proposed methodology. The nutritive parameters evaluated have been: Dry matter, crude protein and crude fiber.

The best results for the development of the calibration model were obtained when applying second derivative as math treatment and Standard Normal Variate and Detrending for scatter correction, for all evaluated parameters the coefficient of determination of cross validation was up 0.9. In relation with the transferability, we have obtained satisfactory results for host-1, however, for host-2 instrument we have observed that can be useful when samples are small pellets. The use of host-2 instrument with intact big pellets was not possible.

The results of this transference have shown the importance of the data optical system in the applicability of instrumentation for quality control of intact samples at farm and industrial level. The main reason for the disadvantage of host-2 instrument is the small window size (low sensibility) to extract relevant information and to model data and in order to get a satisfactory prediction of nutritive parameters. However has been proved to be useful when working with fine samples or small pellets.

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INFLUENCE OF Se SUPPLEMENTATION OVER TRACE ELEMENTS IN COW MILK

Amelia González-Arrojo[1], Ana Soldado[1], Fernando Vicente[1], Mª Luisa Fernández Sánchez[2], Alfredo Sanz-Medel[2], Begoña de la Roza-Delgado[1]

[1]Department of Nutrition, Grasslands and Forages. Regional Institute for Research and Agro-Food Development. SERIDA, 33300, Villaviciosa. Spain
[2]Department of Physical and Analytical chemistry, Faculty of Chemistry, University of Oviedo. C/ Julián Clavería, 8, 33006, Oviedo. Spain

Nowadays, many strategies to improve the functional nutrient content in food have the objective of enhancing its nutritive value. In this sense cow milk has got a great interest due to its special place in the human diet, from childhood to elders. The benefits of some trace elements in milk play an important role in our nutrition; in particular Se is an essential trace element that occurs in selenoproteins. Se can modulate the activities of certain transcription factors and kinases. This fact focuses the importance of a natural enrichment of cow milk with Se. A lot of studies have evaluated different strategies to increase Se content in cow milk. However, the influence of natural Se supplementation over total content of other trace elements such as Cu and Zn in cow milk has not been studied. In this work we explore the impact of Se supplementation in dairy cows ration on the Zn and Cu content in milk.

The experiment was carried supplementing cows with 6, 18 and 27 mg of Se on organic form as Se-enriched yeasts. Cows were milked at 07:30 and 19:30 each day and individual milk yields were recorded at each milking. Whole milk samples mineralization was carried out with a microwave digestion unit Ethos One (Millestone). An acid microwave digestion was selected for Se, Cu and Zn quantification using HNO3 65% and H2O2 30%. An ICP-MS (Agilent 7500c Octopole Reaction System) instrument was used for trace element measurements (Se, Cu and Zn).

Milk Se concentrations responded quickly, reaching the steady-state level, on average between from 7 to 14 day of the experiment. In relation with Zn and Cu, results obtained have showed that total Zn content in milk was not affected by Se supplementation, whereas Cu contents decrease with Se supplementation. Cu content for milk coming from cows without supplementation was up 100ng/g milk and those supplemented was lower than 60 ng/g milk.

In our society we widely focus our efforts in the development of functional products; however, it is necessary to evaluate the influence of this enrichment in other critical parameters. In our study we have observed that increasing Se do not affect to Zn, but it reduces Cu content. More studies are necessary to evaluate the influence on other trace metals.

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AROMABIOTIC POULTRY FOR AN IMPROVED FEED EFFICIENCY AND BREAST MEAT YIELD IN BROILERS

Inne Gantois[1], Jan Vervloesem[1], Rejhana Semic[1], Andras Muzsek[2], László Pál[3], Károly Dublecz[3]

[1]Nuscience, Ghent, Belgium
[2]Nuscience Hungary, Szekesfehervar, Hungary
[3]Department of Animal Science, Georgikon faculty, University of Pannonia, Keszthely, Hungary

With the increasing cost prices of broiler feed, the industry is looking for opportunities to reduce the feed cost and to improve performance in order to improve or maintain feed cost/kg broiler meat. In this trial the effect of Aromabiotic Poultry, a carefully balanced mixture of medium chain fatty acids, on zootechnical performances was quantified. One day old broiler chicks (Ross 308, as hatched) were housed in 12 pens, following a randomized block design with 2 dietary treatments with 6 replicates of 32 birds each. The control treatment consisted of a low protein corn/wheat/soy diet. For the second treatment 0.13%, 0.08%, 0.06% and 0.03% of Aromabiotic Poultry was added to the prestarter, starter, grower and finisher diet, respectively. Evaluation of the overall zootechnical performances showed a slightly improved (not significant) average weight (2836.4 vs. 2808.0 g/bird) and daily weight gain (66.7 vs. 65.96 g/bird/day). These growth performances were obtained with a lower feed intake compared to the control (116.6 vs. 117.7 g/bird/day) resulting in a better FCR (1.75 vs. 1.79). Up till the end of the grower phase the improvement even was significant (P= 0.023). There was no significant effect on mortality (4.7% vs. 4.2%). At the end of the trial 10 animals with comparable body weights were selected per treatment to evaluate slaughter results. Carcass yield (63.3 vs. 63.0%) as well as breast meat yield (19.0 vs. 18.7%) was improved. In conclusion, Aromabiotic Poultry proved to be an economical interesting functional feed ingredient to be used in broiler feed.

SALBIOTIC, THE ULTIMATE HURDLE AGAINST SALMONELLA

Inne Gantois, Jan Vervloesem, Rejhana Semic

Nuscience, Ghent, Belgium

Consumption of food contaminated with zoonotic agents or their toxins is a serious threat for public health. Salmonella is one of the key causes of foodborn illnesses of bacterial origin. Poultry meat and eggs are an important source of Salmonella. A sustainable strategy to reduce the Salmonella positive foodstuffs is to tackle this pathogen early in the food chain i.e. the animal. Literature showed that medium chain fatty acids (MCFA) are able to reduce colonization and invasion after infection of chickens with Salmonella. Thanks to this scientific knowledge and years of expertise with MCFA, Nuscience has developed the functional feed ingredient Salbiotic.

Salbiotic was added to the feed at four farms suffering from Salmonella (java and typhimurium) for several cycles. Three of the four farmers did maximal efforts to clean and disinfect whereas the fourth one did no supplementary efforts. Salmonella was determined using the overshoe method and by bacterial counts of caecum content. In addition technical data were collected and compared with the technical performance of 2 previous cycles on the same farm, with a growth promoter added to the feed.

The technical performances clearly demonstrated that Salbiotic has a positive effect on daily weight gain (53.4 vs. 51.1) and feed conversion ratio (1.459 vs. 1524) and has a positive effect on mortality (4.00% vs. 6.50%). The use of Salbiotic during 2 cycles resulted in a complete eradication of Salmonella in 3 farms. In the farm without extra hygiene measures, 33% and 66% of the houses was Salmonella java free after addition of Salbiotic during respectively 2 and 3 cycles.

In conclusion, Salbiotic offers poultry farmers an opportunity to build an extra hurdle against Salmonella.

THE USE OF LACUNAE AREA/FRAGMENT AREA RATIO AS A MARKER IN DISTINGUISH BETWEEN LAND ANIMAL VS. SEA MAMMALS IDENTIFICATION

L Pinotti[1], V. Baeten[2], P. Veys[2], T. Fearn[3], M. Ottoboni[1], F. Cheli[1], V. Dell'Orto[1]

[1]Department of Health, Animal Science and Food Safety, Università degli Studi di Milano, Milan, Italy,
[2]Quality Department of Agro-food Products, Walloon Agricultural Research Centre (CRA-W), Chaussée de Namur, 24, 5030 Gembloux, Belgium/ EURL-AP European Union Reference Laboratory for animal proteins in feedingstuffs,
[3]Department of Statistical Science, University College London, UK

The aim of this study was to evaluate the potential of image analysis measurements in combination with the official analytical method for the detection of constituents of animal origin in feedstuffs [i.e. the microscopic examination technique as described in Regulation EC/2009/152] in distinguishing between land mammals vs. sea mammals. For this purpose, pure samples containing poultry (AV) terrestrial mammalian (TMAM) and Sea mammals (SMAM) material (Sources: Walloon Agricultural Research Centre, Belgium,; VSA, University of Milan and SAFEED-PAP Project) were analysed. Sediment fractions of each sample were observed with a compound microscope (Olympus BX41, Germany) at several magnifications. Through a digital camera and an image analysis software (Image-for Plus 4.5.1), we obtained 772 bone fragment lacunae images at X40. On each lacuna 30 geometric variables plus the lacunae area/fragment area ratio were obtained. Data were analysed by ANOVA (GLM procedure) and by LDA procedure of SAS statistic software. Results obtained in the present study indicated that even though most of variables measured were significantly (<.001) different between TMAM and SMAM vs. poultry in term of mean, no differences between TMAM and SMAM have been detected. However when lacunae area/fragment area ratio was considered some differences have been observed. SMAM material have shown the lowest the ratio lacunae area/fragment area (13.47) compared to the TMAM and AV (23.03 and 26,58, respectively). In both measured variables and lacunae area/fragment area ratio porpoise was the main source of misclassification. In conclusion, data here presented have confirmed that some lacunae variables/descriptors measured by image analysis have some potential in distinguish poultry material from mammal's material, but not in distinguish between AV and TMAM from SMAM. By contrast lacunae area/fragment area ratio appears promising for distinguishing between terrestrial animal vs. sea mammals. (This work has been done in the frame Cost Action FA0802).

MANGANESE IN CHICKEN AND SWINE FEED: A REGIONAL MONITORING IN ITALY

Daniela Marchis, Paola Brizio, Matteo Pellegrino, Stefania Gavinelli, Renata Tarasco, Giuseppina Amato, Stefania Squadrone, Maria Cesarina Abete

National Reference Centre for Surveillance and Monitoring on feed Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta – Turin, Italy

Manganese is a very common element in the earth crust; it can be mainly found as pyrolusite (MnO2), but also as rhodochrosite (MnCO3). The richest fields of Manganese are in South Africa, Russia, Ukraine, Georgia, Gabon and Australia. It is widely used in steel and chemical industries, as well as soil fertilizer, oxidant and disinfectant. As a result of the massive use of this issue in industry and agriculture, surface and ground water are polluted. Manganese takes part in many enzymatic processes, as it is an essential trace element; however, it shows to have harmful effects at higher doses. Its toxic dose depends on its bioavailability. Adverse effect could occur if assumption exceeds the maximum tolerance level. According to Regulation EC 1831/2003, this element is authorized as feed additive in all species. C.Re.A.A. - National Reference Centre for the Surveillance and Monitoring of Animal Feed- completed the official method described in Regulation 152/2009 and organized with Piedmont Region a monitoring of its levels in feedingstuffs and premixtures, in order to check possible exceeding of legal limits in feed. Quantification of Manganese was performed through atomic absorption with air-acetylene flame atomization (FAAS), at 279.6 nm wavelength, after sample mineralization with concentrated nitric acid, hydrofluoric acid and hydrochloric acid, and hydrogen peroxide 30% v/v. This method was validated according to Regulation 882/EC/2204; the following parameters were evaluated: accuracy; applicability (matrix and concentration range); limit of detection; limit of quantification; precision; repeatability; reproducibility; recovery; selectivity; sensitivity; linearity; measurement uncertainty. C.Re.A.A. laboratory analysed 57 samples (45 chicken feed, and 12 swine feed). All samples were negative. As mentioned before, currently manganese levels are checked by Piedmont Region, but its entry into the international market as additive and its adverse effects should suggest a national monitoring.

WHEAT MILLING AND MYCOTOXIN FRACTIONATION IN BY-PRODUCTS: A SYSTEMATIC REVIEW

Federica Cheli, Luciano Pinotti, Luciana Rossi, Vittorio Dell'Orto

Department of Health, Animal Science and Food Safety, Università degli Studi di Milano, Via Celoria 10, 20133, Milano, Italia

Cereals and cereal by-products constitute a major part of human and animal diet. It has been estimated that up to 25% of the world's crops may be contaminated with mycotoxins. The relevance of mycotoxin on human and animal health prompted the European Community to introduce maximum permissible limits in food and feed. Considering the levels indicated by the European legislation, results from literature indicate that sometimes the limits proposed for cereal-derived products may be not warranted by the limit for unprocessed cereals. Therefore, the understanding of how mycotoxin distribution and concentration change during milling process are a worldwide topic of interest due to the high economic and sanitary impact on human/animal health.

This paper reviews recent findings on the effects of milling process on mycotoxin distribution in wheat milling products and by-products. Published data confirm that milling can minimize mycotoxin concentration in fraction used for human consumption, but concentrate mycotoxins into fractions commonly used as animal feed. Other physical processes carried out before milling (such as sorting, cleaning, debranning) are also interesting methods to reduce the mycotoxin content. These processes may be very efficient to reduce the grain mycotoxin content before milling. The concentration of mycotoxins in wheat by-products may be up to three-fold compared to original grain. Published data show a high variability in mycotoxin repartitioning and sometimes appear conflicting but this may be mainly due to the type of mycotoxins, the level and extent of fungal contamination, and a failure to understand the complexity of milling technology and/or to the omission of key processing information. A precise knowledge of such data is vital as they may provide a sound technical basis to mill manager and support risk management and regulatory bodies in order to reduce human and animal exposure to dangerous amounts of mycotoxins and revise legislative limits.

ANTIOXIDANT CAPACITY OF SELECTED GRAINS AND FEEDS

Vida Rezar[1], Alenka Levart[1], Darja Kocjan Ačko[2], Janez Salobir[1]

[1]University of Ljubljana, Biotechnical faculty, Department of animal science, Chair for nutrition, Groblje 3, 1230 Domzale
[2]University of Ljubljana, Biotechnical faculty, Department of agronomy, Jamnikarjeva 101, 1000 Ljubljana

Antioxidants protect living organisms against free radical attacks and prolong shelf life of food and feeds during storage. Antioxidative capacity of plant food and feeds is derived from different water and lipid soluble antioxidants, like vitamin C and E, polyphenols, carotenoids, terpenoids and trace minerals.
The aim of our preliminary research was to determine antioxidant capacity of fat-soluble (ACL) and water-soluble (ACW) antioxidants in selected grains and feeds by measuring the inhibition of the photo induced chemiluminiscent autoxidation of luminol using Photochem (Analytik Jena, Germany).
Samples of grains (wheat, barley, corn, oats, spelt and buckwheat) grown in different geographic locations and under different crop production systems (ecological, integrated or conventional), and samples of feeds commonly used in feedstuffs in Slovenia, were collected. Fat-soluble antioxidants were extracted from grain and feeds samples using hexane, water-soluble antioxidants were extracted using 2 % aqueous solution of m-phosphoric acid (MPA). Antioxidant activities of hexane and MPA extracts of grains and feeds were determined according to manufacturer's instructions. ACL is expressed in nmol of trolox (TR) equivalents per g of sample and ACW is expressed in nmol equivalents of ascorbic acid (AA) per g of sample.
Water-soluble antioxidant activity contributed more than 80 % of total (ACL + ACW) antioxidative capacity of grains and feeds. Variability in ACL among different grains was lower (CV = 19%) in comparison to ACW (CV = 39%). Among grains, spelt had the highest (305 nmol trolox eq/g) and buckwheat had the lowest (135 nmol TR eq/g) antioxidative capacity of fat-soluble antioxidants (ACL), while buckwheat had the highest (2235 nmol AA eq/g) and spelt had the lowest (818 ± 293 nmol AA eq/g) water-soluble antioxidant capacity (ACW). Results of our preliminary study show that antioxidative capacity of grains is not influenced by crop production system, but further investigations are needed.

EFFECT OF LONG-TERM FEEDING OF CRUDE GLYCERINE ON PERFORMANCE, CARCASS TRAITS, MEAT QUALITY AND METABOLIC PARAMETERS OF FINISHING BULLS

Milan Marounek, Luděk Bartoň, Petr Homolka

Institute of Animal Science, Prague, Czech Republic

Previous reports evaluated effects of glycerine fed in different concentrations usually less than for 100 days. The objective of the present study was to follow effects of glycerine feeding in finishing bulls for 266 days.
Forty-eight Fleckvieh bulls (222 kg live weight at the start of trial) were allotted to four dietary treatments: control diet with 0% of glycerine (C), or with 5% (G5), or 10% of glycerine (G10) on the DM basis for the whole experimental period, or with 0% of glycerine for 118 d and then 10% of glycerine for 148 d (CG10). Glycerine substituted barley meal. Bulls were weighed every 2 weeks. Blood was sampled three times and the rumen fluid was collected immediately after the slaughter.
Bulls in groups C, G5, G10 and CG10 consumed 7.59, 7.71, 7.75 and 8.02 kg DM/d, respectively (P = 0.429). Neither weight gain nor feed conversion in control and treated bulls were significantly different (1.33, 1.36, 1.38, 1.43 kg/d and 5.65, 5.56, 5.58, 5.53 kg DM/kg in groups C, G5, G10, CG10, respectively). No treatment effect on carcass yield, chemical composition of m.longissimus lumborum, serum glucose, NEFA, plasma 3-HBA and activities of aminotransferases was observed. Glycerine added at 5% significantly increased concentrations of serum triglycerides in the 2nd and the 3rd sampling. The highest concentration of volatile fatty acids (VFA) was found in the rumen fluid of control bulls. In bulls fed glycerine rumen VFA non-significantly decreased in a dose-dependent manner.
It can be concluded that long-term feeding glycerine in diets of bulls had no influence on performance and meat quality, and little influence on metabolic and rumen parameters. Glycerin at 5% or 10% of the DM diet is a suitable alternative for barley meal in diets of finishing bulls.

STUDY OF TRADITIONAL PONTIAKOS CAVOURMAS MADE FROM WILD BOAR MEAT

Timotheos Zougkouridis[1], Nikolaos Soultos[2], Taxiarhoula Magra[2], Ekaterini Papavergou[2], Ioannis Amvrosiadis[2]

[1]Veterinary Department of Administrative District of Pieria, GR 60100, Katerini, Greece
[2]Department of Food Hygiene and Food Technology of Animal Origin, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, GR 54124 Thessaloniki, Greece

Pontiakos cavourmas is a traditional thermally processed meat product manufactured locally in Katerini area (Northern Greece). It is made by the addition of gelatine, salt, nitrate salt, ascorbic acid and a variation of spices and seasonings to cooked wild boar meat. The mixture is stuffed to artificial casings, put in metallic moulds and cooled. Then the product is sliced, packaged under vacuum and stored under refrigeration. The objective of the present study was to evaluate the effect of re-heating on the possibility of extending the shelf life and improving the safety of the product. Three series of samples were prepared. Half of the samples of each series were prepared using the usual manufacturing process (control). The other half, after being stuffed to casings, were repasteurized by immerging them in a water bath of 85 0C for 2 hours. All samples were vacuum packaged and stored at 4 0C for 60 days. The shelf-life and safety of the samples during storage were assessed by using both microbiological (counts of aerobic mesopholic, psychrotrophic, lactic acid bacteria, Br. thermosphacta, Enterobacteriaceae, yeasts and L. monocytogenes) and physicochemical (pH, aw and biogenic amines) analyses. Re-heating of the product at 85 0C for 2 hours resulted in significantly lower populations for all the above groups of microorganisms, as compared to the control samples. Moreover, repasteurization inhibited the growth of L. monocytogenes which was detected only after 60 days of refrigerated storage (<100 cfu/g) whereas in control samples it reached populations as high as 4.021 log cfu/g within the same period. Repasteurization also resulted in lower levels of the biogenic amines tyramine, putrescine and cadaverine as compared to the control samples. In conclusion, repasteurization extended the shelf life and improved the safety of vacuum packaged pontiakos cavourmas.

***Keywords**: traditional food, gel, cavourmas, re-heating*

PHYTANIC ACID IN ORGANIC MILK – A MATTER OF FEEDING

Mette Krogh Larsen[1], Brita Ngum Che[1], Troels Kristensen[2], Trine Kastrup Dalsgaard[1], Lars I. Hellgren[3], Jette Feveile Young[1]

[1]Department of Food Science, Aarhus University, AU Foulum, DK-8830 Tjele
[2]Department of Agroecology, Aarhus University, AU Foulum, DK-8830 Tjele
[3]Department of System Biology, Technical University of Denmark, DK-2800 Kgs. Lyngby

Phytanic acid (PA) is a branched saturated fatty acid which is produced in the rumen from the phytol side chain of chlorophyll, and PA has been suggested to have health improving properties and a protective effect on metabolic syndrome. Due to the higher amount of grass based feeding a higher PA content has been reported for organic milk and PA has been suggested as a marker of organic milk products. PA occurs naturally as two isomers (SRR and RRR) and the relative amount of the RRR isomer is reported higher in organic milk.
The present study was conducted at five Danish commercial organic herds during the grazing season. Bulk milk was analyzed for the content of PA as well as the distribution of the two isomers and PA results were related to feed composition.
The concentration of total PA in milk fat was around 100 mg/100 g which is half of a value of 200 mg/100 g suggested by a German science team as a marker for organic milk. This difference may be related to differences in management where Danish organic milk production is intensive and uses significant amounts of concentrates. The average concentration of total PA was 26% higher in late summer than spring and differences between farms were small.
Distribution between isomers varied and the share of RRR isomers varied from 24% to 49% of total PA. The share of RRR isomers was positively related to the amount of grazed legumes ($r = 0.85$). This effect may be due to legumes affecting the rumen microorganisms, and the more intense use of legumes in organic farming could explain the higher share of the RRR isomer in organic milk.

CONSUMER PERCEPTION OF ANIMAL FEED IN RELATION TO FOOD SAFETY

Nataša Vukelić[1], Jasmina Živković[2], Djordje Okanović[2], Nikola Puvača[1]

[1]University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia
[2]University of Novi Sad, Institute of Food Technology, Bul. cara Lazara 1, 21000 Novi Sad, Serbia

The texture, taste and nutritional value of meat are directly influenced by the nutrition of the animal concerned. Over the last decades, as in human nutrition, concepts in animal nutrition are also changing. Today, the role of animal feed in the production of safe food is recognized worldwide, and recent health crises have emphasized its influence on human health, feed and food safety.
In the last years, consumers are more and more aware and sensitive of food safety and their linkage to animal production. Among the major concerns are health issues threatening not only animal production, but also the people using the products derived from these animals. Microbiological risks, such as salmonella-related food poisoning, pesticide residues from feed production, and resistance problems following the use of antibiotics in animal production have become the focus of their attention. As a result of that, the aim of this paper is to investigate consumers' opinions of animal feed which improve animal health, animal nutrition which is designing functional food for humans and whether they are willing to buy and pay higher price for such meat.
The research was carried out via individual personal interviews using prepared questionnaire. The sample consist 400 meat consumers from Novi Sad, Subotica, Zrenjanin, Vršac, Ruma and Indjija (Vojvodina, Serbia).
***Keywords**: Consumers' perception, animal feed, food safety, Vojvodina*

TRANSFER OF VOLATILES FROM OREGANO OR CARAWAY ESSENTIAL OILS INTO COW'S MILK

Johan Lejonklev[1], Mette Marie Løkke[1], Mette Krogh Larsen[1], Grith Mortensen[1], Mikael Agerlin Petersen[2], Martin Riis Weisbjerg[3]

[1]Aarhus University, Department of Food Science
[2]University of Copenhagen, Department of Food Science
[3]Aarhus University, Department of Animal Science

The objective of this experiment was to study the transfer of volatile compounds from oregano and caraway essential oils into cow's milk. During normal feeding the animals lungs as well as intestines are exposed to volatile compounds present in the feed. In order to study the differences between respiratory and gastrointestinal exposure Holstein cows equipped with a duodenum cannula were used in two setups with two animals recieving each treatment. In the first experiment animals were placed in a controlled environment to inhale vapours of the essential oils for 9 hours. In the second experiment essential oils diluted in deodorised sesame oil was injected through the cannula over a period of 9 hours, with two different levels being tested. Milk was collected prior to and immediately after treatment, as well as the following morning. Commercially available essential oils from Origanum vulgare plants and Carum carvi seeds were used. The aroma profile of essential oils and milk was analysed using purge-and-trap coupled with GC/MS.

The results show that milk contains a number of terpenes naturally at very low levels. When the animal is exposed to essential oils several terpenes, present in the essential oils, increase or appear in the milk, suggesting that the aroma compounds are absorbed through the lungs as well as the intestine. It also indicates that the absorption, and subsequent transfer from blood into milk, is very fast. In addition to the terpenes two esters were identified that increased in the milk after exposure, despite the essential oils contained no or insignificant amounts of them. This indicates that these esters are more readily absorbed or synthesised within the animal following exposure to essential oils. Little or none of the increased amounts of aroma compounds, terpenes and esters, could be found in the milk one day after exposure.

THE IMPACT OF MYCOTOXINS ON THE INTESTINE

Isabelle Oswald

INRA, UMR 1331, ToxAlim Research Centre in Food Toxicology,
180 chemin de Tournefeuille, BP93173, 31027 Toulouse cedex 03, France

The intestinal epithelium is a single layer of cells lining the gut lumen that acts as a selective filter, allowing the translocation of essential dietary nutrients, electrolytes, and water from the intestinal lumen into the circulation. It constitutes also the largest and most important barrier to prevent the passage of harmful intraluminal entities, including foreign antigens, microorganisms, and their toxins from the external environment. The intestine is also an immune privileged site where immunoregulatory mechanisms simultaneously defend against pathogens but also preserve tissues homeostasis to avoid immune-mediated pathology in response to environmental challenges.
Following ingestion of contaminated food or feed, intestinal epithelial cells could be exposed to a high concentration of toxicants, potentially affecting intestinal functions. Among natural food contaminants, mycotoxins are regarded as an important risk factor for human and animal health. Mycotoxins are structurally diverse fungal metabolites that can contaminate a variety of dietary components consumed by animals and humans. It is considered that 25% of the world crop production is contaminated by mycotoxins during pre-harvest, transport, processing or storage. The major mycotoxin-producing fungal genera are Aspergillus, Fusarium and Penicillium mainly producing aflatoxins, zearalenone, trichothecenes, fumonisins, ochratoxins. The effects of mycotoxins on the intestinal morphology, intestinal barrier function will be discussed together with their impact on the local immune response (antibody production, antimicrobial peptide generation, inflammatory cytokine release…).

EFFECTS OF PROCESSING METHOD ON INTAKE, MILK YIELD AND MILK FATTY ACID PATTERN WHEN FEEDING COWS LINSEED-ENRICHED CONCENTRATE FEEDS

Anjo Elgersma[1], Jeroen Brok[2], Seerp Tamminga[2]

[1]Independent scientist, PO Box 323, 6700 AH Wageningen, The Netherlands
[2]Animal Sciences Group, Wageningen University, The Netherlands

The fatty acid pattern of cows' milk fluctuates throughout the year in systems where fresh forage is fed. Dairy companies aim for delivery of milk with a more stable fatty acid profile. Linseed contains a high content of alpha-linolenic acid and could be a substitute for fresh grass in periods when this is not available. Linseed in dairy cow rations can increase the concentration of unsaturated fatty acid in milk, e.g. conjugated linoleic acid (CLA), but the processing method of linseed into concentrate feed may affect milk composition. Feed processing could also influence palatability and hence intake and milk production. The aims were (i) to test the effects of linseed-based concentrate versus standard concentrate feed as control (C) in dairy cow rations on intake, milk production, milk composition and milk fatty acid profile, and (ii) to compare two feed processing methods: extrusion (Extr) and BOA compactor (BOA).
Fifteen dairy cows were individually housed and received grass silage, maize silage, 1 kg of standard concentrate plus 4 kg of either C, Extr or BOA concentrate. Cows were fed and milked twice daily. Three groups of 5 cows received the 3 concentrates in varying order during 14 days each, in a Latin Square design. Intake was recorded and pooled milk samples were analysed.
Milk production and yields of milk fat and protein were not affected by treatments. Forage intake declined when linseed-enriched concentrate was fed. Concentrate intake and total intake were lowest with Extr, due to unpalatability. Linseed addition increased the unsaturated fatty acid level in milk; the CLA content was highest when feeding Extr concentrate feeds.
***Keywords**: linseed, extrusion, concentrate, intake, cow milk composition, fatty acids*

EFFECT OF FORAGE DRY MATTER CONTENT AND BALE SIZE ON FERMENTATION CHARACTERISTICS AND AEROBIC STABILITY OF ALFALFA ENSILED IN ROUND BALES

Sema Yaman[1], Engin Ünay[1], Vedat Karakaş[1], Murat Yıldırım[2], Şinasi Aşkar[2], Ali Bilgen[1], Mustafa Avcı[3], Tugay Ayaşan[3]

[1]Livestock Central Research Institute, 06852 Lalahan Ankara, Türkiye
[2]Veterinarian Faculty of Kırıkkale University, Kırıkkale, Türkiye
[3]Eastern-Mediterenian Agricultural Research Institute, Adana, Türkiye

The objective of this study was to evaluate effect of forage dry matter and bale size on chemical and fermentation characteristics and aerobic stability of alfalfa conserved as round baled silage. Alfalfa was harvested before beginning of budding stage in autumn and preserved as round bale silage ensiled as 40% and 50% dry matter content in 50 and 500 Kg weights and stored outdoor. Alfalfa round bale silages (ARBS) was opened after two months from baling. For each analysis four samples from each forage dry matter content and bale size were taken.

When dry matter content of alfalfa bales increased, content of cell wall components (neutral detergent fibre, acid detergent fibre and acid detergent lignin) are also significantly increased. Cell wall components and crude fibre content of small round bales are found significantly higher than those of large round bales. Dry matter content of alfalfa bales had no effect on silage fermentation characteristics. In small round alfalfa silages, lactic acid content is higher and acetic acid content and silage acid level (pH) is lower than those of large round alfalfa bales as there was no significant difference between small and large round alfalfa bales considering microbial counts, butyric and propionic acid contents.

Bale dry matter content was not effective on fermentation quality and total bacteria, yeast and mould counts in bale silages exposed to aerobic conditions. In small round bale alfalfa silages exposed to aerobic conditions, lactic acid content was higher, acetic acid content and pH were lower than those of large round bale silages while butyric acid content and microbial counts were not changed by bale size in small and large round bales exposed to aerobic conditions. In alfalfa round bales there was no interaction between dry matter content and bale size and number of day exposed to aerobic conditions.

***Keywords**: alfalfa, round bale silage, dry matter, bale size, fermentation acids*

QUALITY CONTROL OF FEED IN REPUBLIC OF SRPSKA

Tanja Malčić, Tatjana Docić-Kojadinović, Duška Jokić

Agricultural Institute Banja Luka, Knjaza Milosa 17, Republic of Srpska

Department of Agrochemistry and Agroecology, Agriculture Institute Republic of Srpska since 1995. year have been involved in chemical analysis of nutritive value of animal feed. Continuous training of personnel, laboratory equipment, the adoption of new methods and market demands has led to a steady increase the number of analyzed samples so that in 1995. year carried out quality control in 150, and in 2011. year in 670 samples of animal feed.
Samples for analysis come from feed mixers, farms in the Republic of Srpska, as well as border crossings. Depending on the type of sample (components, premixes, forage mixtures) are determined the following quality parameters: moisture, ash, crude protein (Kjeldahl method), crude fiber (method by Homeberg Stohman), crude fat (method by Soxlet) phosphorus (spectrophotometric method), Na, Ca, Mg (flame photometry), Zn, Cu, Mn and Fe (atomic absorption spectrophotometry, AAS). The mid of 2012. year was adopted and the method of determination of selenium (AAS - hydride technique). In order to reduce possible errors in determining the content of macro and micro elements, special attention is given to the destruction (incineration) of the samples and obtaining a common solution. Incineration is done depending on the type of sample. Preparing a common solution in the analysis of mineral nutrients and premixes is done in microwave oven under the pressure, and the finished mixtures with wet burning mixture of acids (HNO3, H2SO4, HClO4).
In the work will be given practical examples of possible errors in analysis and detailed analysis of the results for the period 2009 - 2011 when analyzed 2000 samples, of which 1743 forage mixtures (87.15%), 208 premixes (10.40%) and 49 mineral nutrients (2.45%).

GARLIC IMPAIRS ACTINOBACILLUS PLEUROPNEUMONIAE IN VITRO AND ALLEVIATES PLEUROPNEUMONIA IN A PIG MODEL

Petra Becker[1], Piet van Wikselaar[1], Monique Mul[1], Arjan Pol[2], Bas Engel[3], Jan Wijdenes[4], Carola van der Peet-Schwering[1], Henk Wisselink[4], Norbert Stockhofe-Zurwieden[4]

[1]Wageningen UR Livestock Research, Lelystad, NL;
[2]Department of Microbiology, Faculty of Science, Radboud University Nijmegen, Nijmegen, NL;
[3]Biometris, Plant Sciences Group of Wageningen UR Wageningen, NL;
[4]Central Veterinary Institute, Wageningen UR, Lelystad, NL

A huge number of divers sulfurous compounds have been identified in garlic preparations, and many of them are associated with health-supporting properties. Digestion products of sulfurous compounds from garlic, the most stable volatile one being allyl methyl sulfide (AMS), are to a certain extent excreted via the lungs and could therefore have an effect on the course of pneumonia in pigs. Hence, the objectives of this study were (i) to test the susceptibility of the pig pathogen Actinobacillus pleuropneumoniae to AMS in in vitro experiments, and (ii) to assess the impact of garlic on systemic blood AMS levels and on clinical and pathological symptoms in the lungs of pigs experimentally infected with A. pleuropneumoniae.
In in vitro experiments, the effect of AMS on the growth of A. pleuropneumoniae serotype 9 was examined in closed bottles equipped with a photometer tube. The bottles were incubated at 37°C and the growth of A. pleuropneumoniae was monitored as optical density at 600 nm. In an in vivo challenge trial, 15 seven-week-old pigs, which received a diet with 5% of a commercial garlic feed component, and a control group of 15 pigs, which received a diet without garlic, were infected with A. pleuropneumoniae serotype 2 by exposure to an aerosol, and subsequently followed for 4 days.
In the in vitro experiments, AMS was shown to exhibit an antibacterial effect against A. pleuropneumoniae serotype 9. At 1.1 mM, AMS impaired the growth rate of A. pleuropneumoniae by 8% compared to unimpeded growth. Although causing a delay in the growth of A. pleuropneumoniae when compared to unaffected growth in medium, AMS did not lower the stationary phase yield of A. pleuropneumoniae. In the in vivo challenge trial, blood AMS in the garlic-fed group amounted to 0.32 ± 0.13 µM at the day of the challenge, whereas in the control group no AMS was detected. At the end of the experiment, the occurrence of characteristic pleuropneumonia lesions in 47% of the lungs of the control group and in 27% of the lungs of the garlic-fed group, in combination with a near to significant ($p = 0.06$) lower relative lung weight in the garlic-fed group, indicated a beneficial, alleviating effect of garlic on the course and severity of the A. pleuropneumoniae infection.

FOOD AND FEED-RELATED PATHOGEN AND TOXIN BINDERS FOR AN IMPROVED GUT HEALTH

Petra Becker, Piet van Wikselaar

Wageningen UR Livestock Research, Edelhertweg 15, 8219 PH Lelystad, The Netherlands

Activated carbon is commonly used in the treatment of diarrhea and for detoxification purposes, because of its high absorption capacity. However, activated carbon does not discriminate between beneficial and harmful compounds and cells. Hence, one of the objectives of this study was to find dietary fiber-related, specific binders for enteropathogens and toxins to promote gut health by their egestion. To study the adhesive capacity of different food and feed ingredients, miniaturized adhesion tests were developed for bacterial cells and AB5 toxins, such as the diarrhea-causing E. coli heat-labile toxin LT and cholera toxin.

The binding capacity of natural substances for bacterial cells was tested by allowing bacteria to adhere to different fibrous materials supplied as well coatings in microtitration plates. The amount of bacteria retained on the materials was determined in an automated way as growth after addition of liquid medium. The test principle was based on an inverse relationship between initial cell densities and the appearance of growth: The higher adhering cell numbers are, the shorter are the detection times of growth. The interfering efficiency of natural substances with binding of the diarrhea-causing LT toxin and cholera toxin to the host receptor gangliosid GM1 was tested using an adapted GM1-coated microtiter-well ELISA.

With growth as measurand for bacterial adhesion, a simple, high-throughput method was developed for the screening of huge numbers of different binding matrices and bacterial species. The adhesion screening of different food and feed components for bacteria resulted in highly discriminating product rankings. Konjac gum, for example, was a good binding matrix for Salmonella strains, the pig pathogen E. coli K88ac adhered well to yeast cell wall material, and the calf pathogen E. coli K99 to coffee grounds. Host receptor binding of LT and cholera toxin was most efficiently counteracted by skim milk powder and ground fenugreek seed. Employing the adhesion tests, we were also able to show that pea hulls bind E. coli K88ac and bean hulls bind the ETEC's toxin LT, after a small-intestinal segment perfusion experiment with ETEC K88ac-challenged piglets had indicated that both pea and bean hulls have the potential for successful application in diarrhea prophylaxis and treatment.

POTENTIAL OF MINOR OILSEED MEALS AS FEEDSTUFF FOR ORGANIC & LOW INPUT DAIRY SYSTEMS

Catalin Dragomir[1], Marketta Rinne[2], David Yanez Ruiz[3]

[1]National Institute for Research-Development in Animal Nutrition and Biology, Calea Bucuresti nr. 1, Balotesti, Ilfov, 077015, Romania
[2]MTT Agrifood Research Finland, Jokioinen, Finland
[3]Estacion Experimental del Zaidin, Profesor Albareda 1, 18008, Granada, Spain

The bibliographic data on chemical composition, nutritive value, effects on the rumen environment and productive performances of ruminants are reviewed for three minor oilseed meals: Camelina, Crambe and Safflower. The cold-extracted meals of these low-input cultures have the potential to be used as protein supplements in organic & low input dairy systems, especially in the frame of commercial arrangements with oilseeds processing factories. The wide variability of the processing techniques & conditions leads to large range of the nutritive values of the corresponding meals (determined by the content of protein, residual oil, fibrous fractions of the seeds, etc.), which requires systematic chemical analyses of these meals. On the other hand, data on rumen environment and animal performances are scarce and inconsistent, preventing appropriate valorisation of these feedstuffs in ruminant nutrition. As the areas cultivated with these crops are likely to grow, specific research in this field is currently needed.
***Keywords**: organic, low-input, dairy, oilseeds, feedstuff*

ASSESSMENT OF SOME HYGIENIC PARAMETERS OF ANIMAL FEEDS IN SERBIA

Ana Varga, Jovanka Lević, Ivana Čabarkapa, Bojana Kokić, Dragana Plavšić, Ljubiša Šarić

Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, Novi Sad, Serbia

The role of animal feed in the production of safe food is recognized worldwide. Assessment of microbiological status is an important element in quality assurance system during animal feedstuffs production, commerce and feeding. The main interest of feed industry is the production of safe and hygienic correct animal feed. Animal feed can be contaminated with wide range of microbial contaminants such as bacteria, yeasts, moulds and their toxic metabolites. Such contaminants have negative impact on animal performance and can compromise the safety of animal products.

For the effective prevention of animal and public health, feed safety must be provided at all stages, including primary production. To this effect, feed safety and wholesomeness are controlled and monitored for years all over the world. Considering that feed hygiene quality vary among regions and countries because of different environmental and other conditions during animal feed production and processing, objective of this study is to show microbiological safety of animal feed manufactured in Serbia.

Study results show microbiological safety of 80 samples of animal feed analyzed during one year (2011/2012). Out of a total of 80 analyzed feed samples, 25 samples were feedstuffs (such as maize, maize feed flour, wheat, wheat feed flour, sunflower, sunflower meal, soybean and soybean meal) and 55 samples were complete feed. Procedures of sample examination were based on International Standards. Microbiological safety of analyzed products was evaluated in accordance with the regulations of Serbia („Službeni glasnik RS", broj 41/09). Of a total of 25 analyzed samples of feedstuffs 40% did not comply with microbiological criteria specified in the Regulation because of the increased total number of moulds. Of a total of 55 analyzed samples of complete feed 8.69% of samples also had increased total number of moulds and therefore did not comply with microbiological criteria specified in the Regulation. Total number of moulds in analyzed samples ranged from 100-110.000 cfu/g. The highest number of contaminated samples was sunflower meal samples and complete feed for young animals. Sulphite reducing Clostridia were detected in two samples and Salmonella was detected in one sample. Coagulase positive staphylococci were not detected in analyzed samples.

Application of good agricultural and good hygienic practices in addition to the adequate storage practices resulted in the absence of pathogenic microorganisms, with the exception of sulphite reducing Clostridia, the presence of which may be explained by errors and omissions in the processing technology or by supplemental contamination.

With continuous collection and evaluation of microbial safety-related data during processing, distribution and use of animal feed, and application of adequate agricultural and management practices, microbial feed safety hazards may be considerably reduced and adequate feed quality ensured. Feed quality and safety are important prerequisites for sustainable development of livestock production.
***Keywords**: feed, microbiological safety*

TECHNOLOGICAL RESEARCH ON THE PRODUCTION OF FUNCTIONAL COOKED SAUSAGE FOR CHILDREN

Toni Doncheva[1], Djordje Okanović[2]

[1]Rettenmaier Austria GMBH & Co.KG, Gesslgasse 7/1, 1230 Wien, Austria
[2]Institute of Food Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

Proper nutrition plays a crucial role in the normal growth and development of the child organism due to the relatively high nutrition and energy needs associated with the development of new tissues and the high intensity of the metabolic processes of a child's organism.
The development of specialized products for functional nutrition is of great social importance. They are an integral therapy element in the treatment of various diseases. Properly organized nutrition helps increase the body's defense mechanisms, activates metabolism and leads to health recovery.
Another crucial factor defining the need for balanced and functional nutrition during childhood is the escalating number of obesity cases, making it one of the most common medical conditions among children. Obesity is associated with increased metabolic and cardiovascular risk in childhood and adolescence, and increased morbidity and mortality rate in adults, which determines its social and health importance. The condition is easily recognized but very difficult to treat.
The first practical conclusion that can be drawn is the scientific necessity to fill the deficit of dietary fiber in the peoples' diet. According to some studies, daily consumption of dietary fiber by residents of Western European countries does not exceed 4 – 5 g per day, which represents 20% of the recommended standards. It is considered that the physiological need of the body of dietary fiber varies between 32 and 38%.
The second practical conclusion is one of the mandatory conditions for prophylactic and therapeutic nutrition, diseases that are associated with insufficient dietary fiber are result the low content in products of easily digestible fats and carbohydrates. Low fat meat in itself is a low-calorific, as it contains proteins used in anabolic processes. Increased relative share of connective tissue in meat products at the expense of fat and their enrichment with dietary fiber in even greater degree decreases energy value and retain the previous level of proteins.
The third practical conclusion is based on the "Relatedness" in functional characteristics of meat protein (as water and fat retaining, emulsification and gel formation ability) and dietary fiber. Such interdependence allows maximum approximation of structural, mechanical, organoleptic, etc., quality indicators of functional meat products in comparison to traditional ones.
Keywords: *dietary fiber, cooked sausage, children*

GROWTH PARAMETERS OF CARP FED MIXTURES CONTAINING DIFFERENT LEVELS AND ORIGIN OF PROTEINS

Marko Stanković[1], Vesna Poleksić[1], Nada Lakić[1], Zorka Dulić[1], Ivana Živić[2], Zoran Marković[1]

[1]University of Belgrade, Faculty of Agriculture, 11000 Belgrade, Serbia,
[2]University of Belgrade, Faculty of Biology, 11000 Belgrade, Serbia

ABSTRACT

The aim of this research was to study growth performances of carp yearlings fed mixtures with different shares of total proteins, and different protein origin.
90 days experiment was carried out in the Laboratory for Fish Nutrition, at the University of Belgrade, Faculty of Agriculture. Feed mixtures with total protein content of 38.1% (diet A), 38.5% (diet B), 41.5% (diet C), and 43.7% (diet D), originating from different sources: fishmeal, full fat soybean meal, and yeast were applied. Statistical analysis of data obtained through control measurements on 30 day intervals, showed differences in growth parameters between treatments. Fish fed a diet dominated by fishmeal and the total protein content of 43.7%, achieved the greatest weight gain and feed utilization. Fish fed diets with 41.5% protein and an equal proportion of fishmeal and full fat soybean meal in the mixture, had better feed utilization and greater weight gain than fish fed mixtures with the highest amount of soybean meal and lower content of total proteins.
The obtained results indicate that in addition to protein levels, their origin and the share in mixtures significantly affect the utilization of feed and growth of cultured fish. Full fat soybean meal, that has similar protein content, amino acid composition and approximate digestibility as fishmeal, if present in higher amount in mixtures with lower share of total proteins, result in poorer feed efficiency and lower growth of cultured fish.
***Keywords**: carp yearlings, proteins, feed utilization, growth rate*

INTRODUCTION

Proteins are essential for an organism as the main body structural elements and also for a number of physiological processes. For the utilization of feed and growth of cultured fish protein origin and quantity in feed mixtures is of crucial importance. Fishmeal (FM) is the most important component of feed that provides proteins for carnivorous fish species, but also for omnivorous fish in intensive production. Due to its deficit on the world market and the increasing price [12], uncertain supply and microbiological soundness [3], FM and other feed of animal origin are combined or replaced with alternative sources of plant proteins. There is a constant tendency toward decreasing the share of FM in feed, while optimizing the amount of proteins in the meal [11, 6, 2, 1].

The aim of this research was to study growth performances of carp yearlings fed mixtures with different protein content, and different protein origin.

MATERIAL AND METODS

The experiment was carried out in the Laboratory for Fish Nutrition of the Faculty of Agriculture, University of Belgrade. Feed mixtures with total protein content of 38.1% (diet A), 38.5% (diet B), 41.5% (diet C), and 43.7% (diet D), originating from different sources: FM, full fat soybean meal and yeast were applied (Table 1). In total 12 independent tanks, with 120 L of usable water volume and flow rate of 0.34 $Lmin^{1}$ were used. Water quality parameters (temperature, electroconductivity, dissolved oxygen, and pH) were monitored in each tank every day using MULTI 340i/SET (WTW, Weilheim, Germany). Fish were acclimated to laboratory conditions during the period of 2 weeks. Each tank was stocked with 24 yearlings, average weight 95.6 g. Experiment duration was 90 days.

Fish were fed with same percentage of feed depending on the total quantity of fish in each tank, i.e. 3.5% of the ichthyomass, using semiautomatic feeders with pendulum.

Table 1. Ingredients and chemical composition of experimental diets

Ingredients of experimental diets (%)				
Ingredient:	Diet A	Diet B	Diet C	Diet D
Fish meal	26	28	30	32
Soybean meal	29	30	30	31
Yeast	2	4	6	8
Wheat gluten	5	5	5	5
Wheat	11,5	11,5	11,5	11,5
Corn	24	19	15	10
DCP	1,2	1,2	1,2	1,2
Calcium	0,3	0,3	0,3	0,3
Min. Vit.premix	1	1	1	1
Chemical composition of the experimental diets				
DM gkg^{-1}	937	933	937	892
In DM (g)				
Protein	381,0	384,8	415,2	437,2
Lipid	85,4	89,0	90,7	96,4
Ash	95,0	95,4	96,1	107,6
Fibre	20,3	20,4	24,5	20,2
[1]NFE	41,8	41,0	37,4	33,9
[2]Gross energy	19,6	19,7	19,8	20,0
[3]P/E	19,4	19,5	20,1	21,9

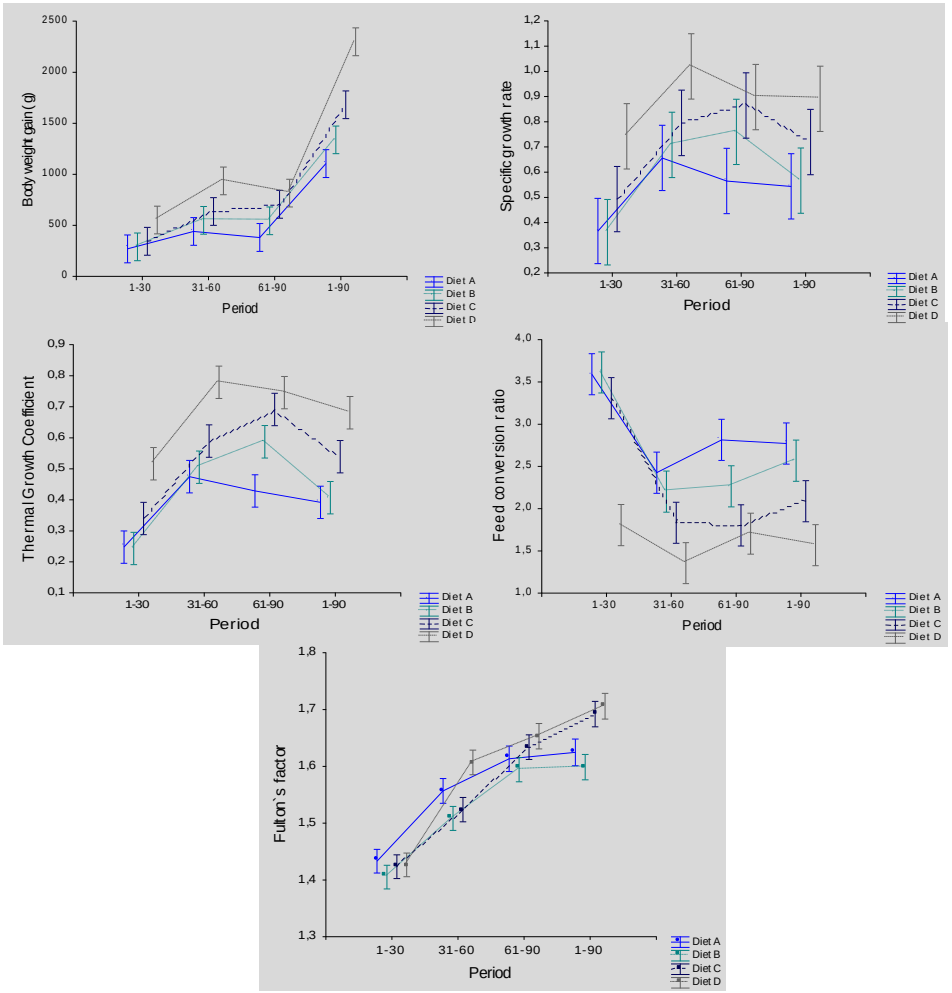
[1]NFE – Nitrogen free extract = 100 – protein (g) – lipid (g) – ash (g) – fibre (g)
[2]Gross energy (MJ/kg) = protein (g) * 23,6 + lipid (g) * 39,5 + NFE (g) *17,3
[3]P/E = Relationship protein and energy (g protein kJ^{-1} gross energy)

For every day measurements of feed quantity, as well as for control measurements each 30 days, a digital balance CASBEE, model MW 120; Casbee, Samsungm Korea, accuracy 0,01 g was used, while an ichthyometer was employed for length and height measurements.
Parameters for growth performances were calculated using following equations:
BWG (body weight gain) = final body weight (g) – initial weight (g);
SGR (specific growth rate) = (ln final weight – ln initial weights) x (days of trial-1) x 100;
FCR (Feed conversion ratio) = (feed intake, kg) x (wet weight gain, kg)$^{-1}$;
TGC (Thermal Growth Coefficient) = [(final weight)$^{1/3}$ – (initial weights)$^{1/3}$] x (days x C^{o})$^{-1}$ x 1000;
CF (Condition factor) = body weight (g) x (fork length, cm)$^{-3}$ x 100;
Statistical analysis was done using two-factorial analysis of variance with factors type of feed and period. Individual comparison of average values was carried out using Tukey test.

RESULTS AND DISCUSSION

During the experiment water temperature varied from 21°C to 24.4°C, with minimal differences in measured values between tanks during the day. Electroconductivity ranged from 487 to 562 µS/cm; dissolved oxygen was on average 6.4mg/l; and pH value was from 6.8 to 8.0. According to Marković [10], Flajšhans and Hulata [4], Hover [7], all the values monitored were within the optimal range for carp growth.
From measurement data, growth parameters: BWG, SGR, FCR, TGC, CF were calculated. The results obtained are shown on Graph 1.
Statistical analysis of data obtained through control measurements carried out in 30 day intervals, showed differences in growth performance of fish fed different diets / treatments: BWG of fish fed feed D and C was significantly different compared to fish fed feed A and B ($p<0.001$). SGR of fish was statistically different between D and A treatment ($p<0.001$), B ($p<0.001$) and C ($p=0.004$), as well as between A and C ($p=0.001$). FCR was significantly different between fish fed D and A diet ($p<0.001$), B ($p<0.001$) and C diet ($p=0.004$), and fed A and C ($p=0.004$). TGC was significantly different between fish fed B and C diet ($p=0.039$), A and D diet ($p<0.001$), diet B ($p<0.001$) and diet C ($p=0.003$). Average values of TGC were statistically different between A and C diet ($p=0.001$). CF was significantly different between fish fed A and D ($p=0.043$), diet B and C ($p=0.041$), and between B and D ($p<0.001$).
Carp fed a diet dominated by FM and the highest total protein content of 43.7% achieved the greatest weight gain and feed utilization. Fish fed diets with 41.5% protein and an equal proportion of FM and full fat soybean meal in the mixture, had better feed utilization and greater weight gain than fish fed mixtures with the highest amount of soybean meal and lower content of total proteins. According to Gatlin [5], omnivorous freshwater fish are less dependent from FM in the supplemented feed. Similar results were obtained by Kumar et al. [9]: FM can be replaced up to 75% with highly nutritive *Jatropha curcas* kernel meal in the diet

for common carp fry. However, Brinker and Reiter [2] are pointing out that in mixtures with lower level of proteins originated from FM better production results can be obtained compared to mixtures containing more proteins originated from FM and/or plant proteins. Accordingly, growth of fish is influenced by both level and origin, as well as feed utilization. FM is often used in fish feed since it offers a balanced amount of amino acids, essential fatty acids, vitamins, minerals, and giving usually better palatability [8].



Graph 1. Mean ± standard error for the tested parameters of fish per treatment and periods

CONCLUSIONS

The obtained results indicate that in addition to protein levels, their origin and the share in mixtures significantly affect the utilization of feed and growth of cultured fish. Full fat soybean meal, that has similar protein content, amino acid composition and approximate digestibility as in FM, if present in higher amount in mixtures with lower share of total proteins, result in poorer feed efficiency and lower growth of cultured fish.

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RED DEER (*CERVUS ELAPHUS*) – NEW PERSPECTIVE ON THE FARMS IN SLOVAKIA EFFECT OF SILAGE QUALITY ON BARK BROWSING AND NUTRIENTS DIGESTIBILITY

Matúš Rajský[1], Miroslav Vodnansky[2], Mária Chrenková[1], Rudolf Kropil[3], Dušan Rajský[3]

[1]Animal Production Research Centre Nitra, Hlohovecká 2, 951 41 Lužianky, Slovakia
[2]Middle European Institute of Wildlife Ecology, Erzherzog Karls-Strasse 33/2/82, A-1220 Vienna, Austria
[3]Technical University, T.G.Masaryka 24, 960 53 Zvolen, Slovakia

ABSTRACT

Red deer belongs among important free living hunting ruminant species in Slovakia. During the last ten years the value of red deer as a new farming species has been increased. One of the main reasons for increase in farming of red deer is the reduced number of cattle; grazing areas are available to red deer farming.
There was observed the effect of the quality of maize and grass silage on bark browsing and on apparent digestibility of nutrients in winter period by red deer. A total of 15 red deer females, aged 5-8 years, placed in pens, were used in the experiment. The animals were divided into five groups; three animals in each group. The highest intensity ($P < 0.001$) of bark browsing per animal was 227 g/d in group fed maize silage of worse quality and meadow hay. The lowest intensity of bark browsing per animal was 2 g/d in group maize silage of good quality and meadow hay.
Four animals (in every treatment) in balance boxes were used to determine the apparent digestibility of nutrients of maize and grass silages were used. There was found significant difference ($P < 0.001$) in digestibility of organic matter between maize silage of average quality (69.8 %) vs. grass silage of average quality (59.9 %). Decreased quality of grass silage significantly affected ($P < 0.001$) the apparent digestibility of nutrients in comparison to silage of average quality: organic matter (40.6 vs. 59.9 %). The results show that quality of feed significantly influences the nutrients digestibility as well as damage on forests caused by red deer.
Keywords: *Red deer, Silage quality, Nutrients digestibility, Bark browsing*

INTRODUCTION

Red deer in Slovakia belongs among important free living hunting ruminant species. Questions of nutrition and impact on the environment are interesting in connection with farming of new species. Slovakia is a country with a high density

of free living deer, especially red deer, and during the last ten years gradually increased also interest in farming these animals. One of the main reasons for increased farming value of red deer is the reduced number of cattle and thus creating the amount of grazing areas to farming red deer. There were established 175 deer farms. Damages caused by deer in forest and agriculture in Slovakia are intensive during the winter period. One of the possibilities used to reduce bark browsing is supplementary feeding. It also significantly influences the animal's condition. Silage of good quality is important for winter feeding of red deer as a ruminant.

MATERIAL AND METHODS

There was observed the effect of quality of maize and grass silage on bark browsing and on apparent digestibility of nutrients in red deer during the winter period. Experiments were performed on International Workplace for Game Nutrition and Ecology in Nitra, which is a common experimental base of the Animal Production Research Centre Nitra and Middle European Institute of Wildlife Ecology Vienna, Brno, Nitra.

Figure 1. Red deer in balance box

A total of 15 red deer females aged 5-8 years, placed in pens, were used in the experiment to examine the intensity of bark browsing. The animals were divided into five groups, three animals each group. In all groups, meadow hay was used as the base of feed ration. The treatments were as follows: MS1-maize silage of good quality, meadow hay; MS2-maize silage of average quality, meadow hay;

MS3-maize silage of worse quality, meadow hay; GS1-grass silage of average quality, meadow hay; GS2- grass silage of worse quality, meadow hay. In addition to feeds the animals received daily fresh spruce stems 6-19 cm thick, placed vertically. The amount of browsed bark was estimated by measuring the browsed surface and converting it to weight in the proportion 100 cm^2 = 26 g. All feeds including bark were available ad libitum.

To determine the apparent digestibility of nutrients of maize and grass silages four animals (in every treatment) in balance boxes were used (*Fig. 1*). There were examined following feeds: maize silage of average quality, grass silage of average quality and grass silage of worse quality.

The nutrient contents of feeds and spruce bark used in our experiments are given in Table 1. The experimental data were subjected to ANOVA using the GLM procedure of SAS (SAS/STAT 2002, v. 9.1, SAS Institute Inc., Cary, NC, USA).

Table 1. Nutrient content of feeds and bark (g.kg^{-1} dry matter)

Nutrient (g.kg^{-1})	Maize silage of average quality	Maize silage of worse quality	Maize silage of good quality	Grass silage of worse quality	Grass silage of average quality	Meadow hay	Spruce bark
Dry matter (%)	39.21	48.56	45.20	31.61	68.13	85.44	44.34
Crude protein	96.01	98.98	91.51	97.81	127.33	79.37	38.3
Trude fibre	174.82	203.98	209.26	407.76	294.95	305.78	298.05
Fat	31.26	24.17	28.05	29.68	28.52	15.41	47.59
Ash	41.67	50.95	41.00	87.36	83.93	66.49	32.35
Nitrogen-free extrakt	656.25	621.92	630.18	377.39	465.26	532.95	583.7
Organic matter	958.33	949.05	959.00	912.64	916.07	933.51	967.65
Ca	2.77	5.99	3.67	1.71	4.59	1.91	11.39
P	2.41	2.54	1.51	5.25	2.9	2.04	0.68
Mg	1.6	2.12	1.9	1.08	1.62	1.24	0.82
Na	0.2	0.14	0.06	0.06	0.09	0.59	0.07
K	8.64	9.34	11.3	29.9	20.3	14.51	2.53
Acetic acid	16.53	*	4.40	4.06	3.36	–-	–-
Propionic acid	0.51	*	0.11	0.58	0.5	–-	–-
Butyric acid+izo	3.93	*	0.24	5.7	0.85	–-	–-
Valeric acid+izo	0.18	*	0.18	0.61	0.32	–-	–-
Caproic acid	0.05	*	0.02	0.09	0.15	–-	–-
Volatile fattid acids total	21.19	*	4.96	11.04	5.18	–-	–-
Lactic acid	31.09	*	27.21	42.8	2.67	–-	–-
Fattid acids total	52.28	*	32.16	53.84	7.85	–-	–-

*Notes: * due to extreme pH (7.4) the sample was not analyzed for volatile fatty acid content (possible damage to instrumentation)*

RESULTS AND DISCUSSION

The results show that quality of silages affects the taste attractiveness and significantly influences damage on forests (bark browsing). Quality of silages also significantly influences digestibility of nutrients in red deer.

Effect of silage quality on bark browsing

The highest intensity ($P < 0.001$) of bark browsing per animal was 227 g/d (4.9 % of total food intake, in dry matter) in group fed maize silage of worse quality and meadow hay. The lowest intensity of bark browsing per animal was 2 g/d (0.3 % of total food intake, in dry matter) in group fed maize silage of good quality and meadow hay. In group fed maize silage of average quality, meadow hay, represented bark browsing per animal 19 g/d. The second highest value was estimated with feeding grass silage of worse quality (with a very high content of crude fiber), meadow hay - average for individual and day was 57 g. In group fed grass silage of average quality with lower content of fiber was estimated 22 g/d bark browsing per animal.

The proportion of silage dry matter intake of the total food intake ranged from 97.6 % in experimental group fed maize silage of good quality and meadow hay. And in group fed maize silage of worse quality and meadow hay we estimated dry matter intake from silage 25.5 % ($P < 0.001$). It is evident that deer prefer consumption of good quality silage over hay and bark consumption.

Also Pheiffer and Hartfiel (1984) demonstrated that proper supplementary feeding markedly decreased the rate of bark consumption. Putman and Staines (2004) came to the same conclusion. Rajský et al. (2008) show the significant effect of wild ruminants feeding on decrease of bark browsing.

Effect of silage quality on nutrients digestibility

There was found significant difference ($P < 0.001$) in digestibility of organic matter between animals fed maize silage of average quality (69.8 %) vs. animals fed grass silage of average quality (59.9 %).

Vodnansky et. al. (2012) described digestibility of organic matter of beet pulp silage in red deer in winter 84.73 % and the same silage in summer 83.55 %. The data indicated that the changes in wild ruminant digestion depend primarily on nutrients intake and not on the season.

We estimated significantly lower ($P < 0.001$) digestibility of nutrients in grass silage of worse quality compared to digestibility of nutrients in grass silage of average quality: organic matter (40.6 vs. 59.9 %), crude protein (25.6 vs. 49.9 %), crude fibre (28.0 vs. 57.0 %) and ash (23.4 vs. 30.9 %).

Supplementary feeding is widely used in the management of ungulate populations in Europe. It is mainly focused on the improvement of animal condition and trophy quality and the reduction of winter mortality. Rajský et. al. (2008) describes body weight increase in red deer fed maize silage with the addition of oats during the winter period by 5,3 %, while red deer fed meadow hay only in the same period showed decrease in body weight of 9,9 %.

As a result, the population density of game may increase. However, supplementary feeding not necessarily leads to improved population status since it may cause overabundance (Cote et al., 2004).

CONCLUSIONS

Reduced number of cattle creates grazing areas for free living hunting ruminant species and also for red deer farming.
The objective of the present study was to explore the effect of various silages on spruce bark browsing and nutrients digestibility in red deer during winter under controlled conditions.
The results show that the quality of feed significantly influences the nutrients digestibility and also the damage on forests caused by red deer.

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CHARACTERISTICS OF BEVERAGE OBTAINED FROM MILK PERMEATE

Dragana Ilić-Udovičić[1], Spasenija Milanović[2], Mirela Iličić[2]

[1]Higher Technological School of Professional Studies in Šabac, Hajduk Veljkova 10, Šabac, Serbia
[2]University of Novi Sad, Faculty of Technology, Bulevar Cara Lazara 1, Novi Sad, Serbia

ABSTRACT

The study presents the possibility of producing a beverage by utilizing milk permeate obtained during UF process of feta cheese and cottage cheese making. Beside permeate, the obtained varieties of the beverage also contain the following ingredients: orange base, sucrose, water and stabilizer (pectin).
The characteristics of the milk permeate-base beverages analyzed in this study are as follows: pH value, titrable acidity, chemical composition and energy value after production and during 30-day storage.
The obtained results revealed that there are no significant differences in the chemical composition of the beverages during 30-day storage, as well as that there is a high correlation coefficient of the content of the tested permeate-base beverages components. The obtained beverages utilizing milk permeate have high nutritive and low energy value and excellent sensory properties (colour typical of an aromatic additive ingredient, distinctive aroma, pleasant and fresh flavour).
***Key words**: milk permeate, beverage, characteristics*

INTRODUCTION

Utilization of by-products of dairy technology is of great significance from ecological, technological and economic aspect. Introduction of modern membrane concentration and fractionation processes, and especially ultrafiltration, after the treatment of milk and whey, resulted in permeate as a by-product [4]. Permeate represents a basic by-product in dairy industry and precious raw material for production of numerous products [2]. The chemical composition of permeate depends on raw material and its factor of concentration [14]. Due to its composition, permeate can pose a serious source of pollution if disposed in environment as waste water [6, 16].
Numerous processes for permeate utilization into food and feed, or as fuel in industry, have been developed worldwide with the purpose to protect the environment. Permeate processing by using selective methods and techniques such as: evaporation, ultrafiltration, demineralization, drying, hydrolysis and fermentation, or their combination, give different products. Principal groups of possible products are: concentrated and dried products (powdered permeate, demineralized permeate, lactose, beverages), hydrolised lactose (glucose-

galactose syrup), as well as fermented products (single cell proteins, ethanol, methane, lactic acid/ammonium lactate) [4, 18, 19]. Taking into consideration the high content of mineral substances, permeate is used directly only in limited quantities for fertilization or in animal feed [10].
Among many possibilities of permeate processing, permeate-base beverages take an important place. Such beverages are clear transparent liquids, similar to traditional juices. They have distinctive fresh flavour and are often saturated with carbonic acid [13] because of permeate components [11, 12]. That products remind of electrolyte beverages used as sports drinks that could be [7] and belong to a group of high nutritive and functional value products [17]. There are different formulations of permeate-base beverages [3]. Nowdays, the industry uses starter cultures in permeate-base fruit beverages production [1]. Orange aroma is most often used in the production [9], and the addition of aromatic concentrates or sugar syrup in permeates, followed by carbonization, give various non-alcoholic beverages [8]. Consumption of this type of beverages is in constant growth, and valorisation of permeate and its further processing is becoming more important [20].
The aim of this study is to investigate variability of the chemical composition of orange permeate-base beverages and to establish inter-relations of specific components in the formulation.

MATERIALS AND METHODS

Permeate is obtained after ultrafiltration of milk in feta cheese and cottage cheese production, with the usage of UF plant with capacity of 5000 L of milk/h (producer DDS Denmark, with polysulfone membrane, plate module).
Besides permeate, the following components were used for the production of milk permeate-base beverage in laboratory conditions: orange base (Dohler, Germany), sucrose, water and chosen stabilizer (pectin). Six beverages were prepared from six permeates according to the same formulation. Each beverage was prepared in accordance with the following normative: 50% permeate, 10% orange base, 5% sugar (sucrose), 35% water and 0,5% stabilizer (pectin). A method of preparation was the same for all six beverages: the necessary quantity of water was measured; sugar was mixed with stabilizer and diluted in the water. Afterwards, the mixture was heated on a water bath at the temperature of 60°C upon which the orange base, previously diluted in permeate, was added to the mixture. This mixture was pasteurized on the water bath at the temperature of 93-95°C, for 5 minutes. The mixture was cooled to 20°C and pH value was measured. The pH was adjusted by adding 10% solution of citric acid (E330).
The following physicochemical analyses: pH value, using a pH-meter (Consort C830, Belgium); titrable acidity, by the Soxhlet-Henkel method; content of milk fat (MF), using the Gerber method; dry matter (DM), using a drying process; total proteins (TP), using the Kjeldahl method and ash content by incineration procedure were determined in beverage obtained from milk permeate [5]. On the basis of the content of total sugar (TS) obtained by calculation (TS=DM-

(TP+MF+Ash)) the energy value of the product was determined in the following way: EV=(4.4x%TP+9.3x%MF+4.1x%TS)x4.186 (kJ/100g).
After 30 days of storage of the beverage at the temperature of +4 ºC the pH value was measured using a pH-meter (Consort C830, Belgium).
All the received results were processed in Microsoft Office Excel 2007.

RESULTS AND DISCUSSION

The quality parameters of beverage obtained from milk permeate are shown in the Table 1. The average value of the dry matter of the beverage is 14.07%, with the variation coefficient of 2.77.

Table 1. Chemical characterization of permeate beverages (n=6)

Beverages	Dry matter (%)	Ash (%)	Total proteins (%)	Milk fat (%)	Total sugar (%)	Energy value (kJ/100g)
Mean	14,07	0,65	0,37	0	13,05	231
Standard deviation	0,39	0,16	0,03	0	0,37	6,35
Coefficient of variation (%)	2,77	24,61	8,11	0	2,84	2,75

n-number of samples

Figure 1 shows the pH values of the beverage after the production and 30 days of storage.
The citric acid was used for adjustment of the acidity, i.e. pH value of the beverages [12]. Certain beverages have pH value that ranges from 3 to 5, or between 4 and 5, which is similar to the pH value of sweet whey [9]. The analysis of beverage pH value suggests that the prepared beverages pH value (I, II, III, IV. V and VI) ranges in the interval from pH 3.76 to pH 3.78 (Figure 1). The average value is 3.77, standard deviation is 0.009, and variation coefficient is 0.24%. Titrable acidity ranges in the interval 40-41°SH, with the average value of 40.5°SH. pH value of the produced beverages is within the limits cited in the literature. During the 30-day period of storage at +4°C pH values of all beverages increased, which is positive from the duration and quality of products aspect (Figure 1).

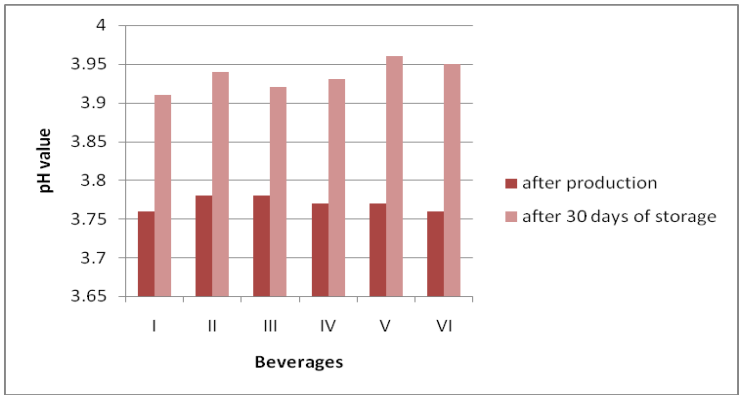

Figure 1. pH value of beverages after production and after 30 days of storage

The interdependence of dry matter and pH value can be shown in the equation (Figure 2):

$$y = 0.1111x^2 - 3.1954x + 26.714 \quad (1)$$

with the determination coefficient $R^2 = 0,8964$. It is valid for dry matter of the beverage in the interval from 13.80 to 14.85%.

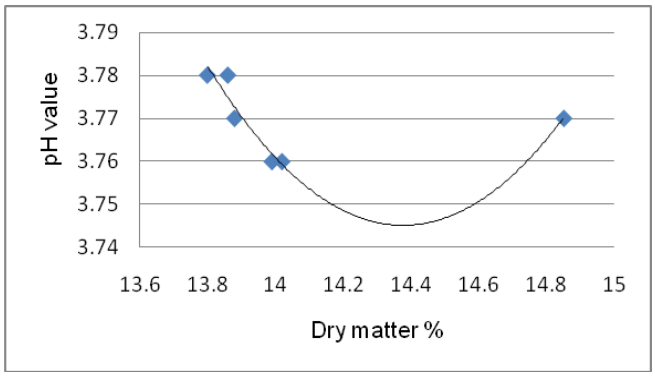

Figure 2. Interrelationship of dry matter and pH value of beverages

The content of total proteins in the beverages varies insignificantly, in the interval from 0.33% to 0.40%. The average value of proteins is 0.37% and the standard deviation amounts to 0,03.
The interdependence of total proteins and pH value is shown in Figure 3 and can be expressed in the equation:

$$y = 785.71x^3 - 867.14x^2 + 318.09x - 35.015 \quad (2)$$

with the determination coefficient R^2 = 0,75. It is valid for the content of total proteins of the beverage in the interval from 0.33% to 0.40%.

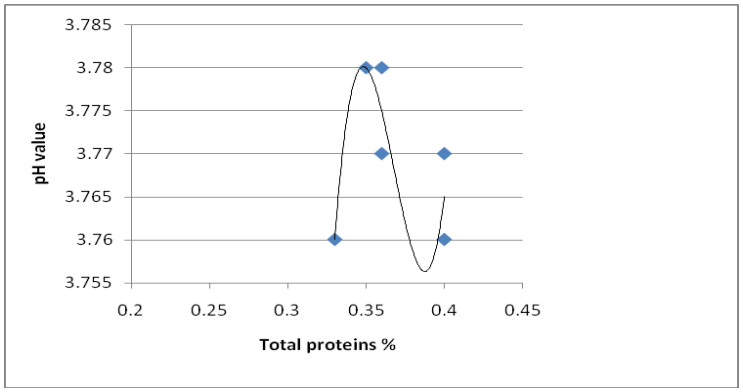

Figure 3. Interrelationship of total proteins and pH value of beverages

The presented formulae show that if one value of quality parameter is known, the other can be calculated provided that there is a certain dependence between them, as well as that with the change of one parameter it could be anticipated change in the quality of final beverage.
The average of energy value is 231 kJ/100g, which is the reason why the produced permeate-base beverages are considered to be low energy diet products aimed at different categories of consumers. The obtained beverages are fat-free, with fresh pleasant flavour so that they are convenient for all those on the weight reduction diets.

CONCLUSION

The experimental results show that there exist interrelationship of certain milk permeate-base beverage components. The correlation between quality parameters can be shown by mathematical formulae with specific determination coefficient. Permeate-base beverages belong to the group of fat-free products. The beverages can be produced with the existing equipment in dairy industry, so that the production of these beverages is possible from technological, economic and ecological aspect.

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EFFECTIVENESS OF *FAGOPYRI HERBA* FEED SUPPLEMENTATION IN NORMAL AND HIGH-FAT FED RATS

Aleksandra Mišan[1], Maja Đurendić-Brenesel[2], Ivan Milovanović[1], Miljan Milić[3], Vladimir Pilija[2], Đuro Vukmirović[1], Radmilo Čolović[1], Anamarija Mandić[1], Jovanka Lević[1]

[1]Institute of Food Technology - FINS, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia
[2]Institute of Forensic Medicine, Clinical Center Vojvodina, Hajduk Veljkova 7-9, 21000 Novi Sad, Serbia
[3]Center of Pathology and Histology, Clinical Center Vojvodina, Hajduk Veljkova 1-3, 21000 Novi Sad, Serbia

ABSTRACT

Fagopyri herba has been used in the treatment of vascular diseases and reported to possess hypolipidemic effects. As a higher food intake is connected with obesity and associated cardiometabolic risk factors, the present work was undertaken to investigate the effects of *Fagopyri herba* consumption on body weight gain, feed intake, feed efficiency ratio and net caloric intake in male Wistar rats fed a standard and a hyperlipidemic diet for 8 weeks. For this purpose, 25 male Wistar rats were randomly divided into five groups: a standard diet group (I), a *Fagopyri herba* supplemented diet group (II), a high-fat diet group (III), a high-fat *Fagopyri herba* supplemented diet group (IV), a group eating the same diet as III group for 7 weeks and the same as group IV for 1 week (V). After 8 weeks, the feed intake was significantly lower in the three high-fat fed groups (III, IV and V) (P<0.05) than in the I and II group. The IV group had a lower body weight gain (85.80 ± 14.89 g *vs.* 131.60 ± 37.73 g; P<0.05) than the III group, which can be connected with its lower net caloric intake in comparison with the III group (259 kJ/100 g *vs.* 410 kJ/100 g). Faecal fat excretion of the V group was significantly higher than of the III group (11.800 ± 0.200% *vs.* 10.06 ± 0.400; P<0.05). This result suggests that the beneficial effect of *Fagopyri herba* in high-fat fed rats is partly mediated by higher excretion of faecal lipids. The results of this experiment suggest that *Fagopyri herba* consumption would be beneficial for regulation of lipid metabolism or prevention of hyperlipidemia in experimental animal models.

***Keywords**: Fagopyri herba, Wistar rats, hyperlipidemic diet, net caloric intake, feed efficiency ratio*

INTRODUCTION

A higher food and dietary fat intake is connected with obesity and associated cardiometabolic risk factors [7]. On the other hand, plant polyphenols have been reported to exert cardiovascular benefits by altering concentrations of blood lipid

components and a high intake of polyphenols (flavonoids) can significantly reduce the risk of mortality from cardiovascular diseases [7].
Fagopyri herba is an herbal drug derived from common buckwheat (*Fagopyrum esculentum* Moench) and has been used in the treatment of vascular diseases [3]. Rutin (quercetin-3-*O*-rutinoside), the dominant flavonol glycoside in *Fagopyri herba*, was reported to possess antioxidant activity [4], antagonize the increase of capillary fragility associated with haemorrhagic disease, reduce high blood pressure [1,10], decrease the permeability of the blood vessels, has an anti-oedema effect and reduces the risk of atherosclerosis [5].
The present work was undertaken to investigate the effects of *Fagopyri herba* consumption on body weight gain, feed intake, feed efficiency ratio and net caloric intake in male Wistar rats fed a standard and a hyperlipidemic diet for 8 weeks.

MATERIAL AND METHODS

Plant and feed material

Fagopyri herba in a powder form was obtained from the Institute for Medicinal Plants Research "Dr Josif Pančić", Belgrade, Serbia where a herbarium voucher specimen (No. 31210911) was deposited. A commercial complete mixture for laboratory rats containing 20% proteins in a powder form was obtained from the Veterinary Institute JSC, Subotica, Serbia. The mixture was processed at FINS pilot plant. First, it was steam conditioned at 80 ºC in a batch type steam conditioner (Muyang SLHSJ0.2A, China). Afterwards, steam conditioned mixture was extruded in a single screw extruder (OEE 8, Amandus Kahl, Germany) at 103 ºC to obtain granules with diameter of 11.5 mm.

Experimental animals and diets

Experiments were carried out on twenty five male Wistar rats, aged four months, body weight 310-440 g, obtained from the vivarium of the pharmaceutical company Galenika a.d., Belgrade, Serbia. All the experiments and protocols employed in the study were reviewed and approved by the Institutional Animal Care and Use Committee [No. III-2011-01].
Experimental animals were housed in groups of two or three per standard cage, in a room with a 12 h light-dark cycle and an ambient temperature of 24 °C. All rats were fed normal chow for 2 weeks after arrival. Normal chow was obtained by extrusion of commercially obtained complete mixture.
They were then randomly divided into five groups of five rats each. The animals of the group I (control) were fed normal chow. The rats of the group II (buckwheat) were fed normal chow supplemented with 5% powdered *Fagopyri herba*. High-fat groups III and IV were fed a lipogenic diet [2] consisting of 2.5% cholesterol, 20% sunflower oil and 0.5% sodium cholate added to normal chow without (group III) or with 5% powdered *Fagopyri herba* (group IV). This regime was maintained for 8 weeks. The animals of the group V were maintained in the same food regime as the animals in group III. After 7 weeks, the animals showed symptoms of hyperlipidemia (data not shown) and they were maintained

in the same food regime as the animals in the group IV for 1 week. The animals were given food and tap water *ad libitum* during the experimental period. Food consumption and weight gain were measured daily and weekly, respectively. Faeces was collected during the last week of the feeding period and kept frozen until the analysis at -20 °C.

Chemical analyses
Standard methods of analysis (AOAC, 1984) were used to determine crude protein, fat, reducing sugar, crude cellulose, starch and water contents. In the protein determination, a nitrogen-to-protein conversion factor of 6.25 was used. Gross energy contents of feed and faeces were measured by oxygen calorimetric bomb (model AC 500, Leco, USA) calibrated by benzoic acid.

Net caloric intake and apparent fat absorption
Net caloric intake was calculated as a difference between gross energy contents of feed and faeces. Apparent fat absorption is calculated as follows: Apparent absorption of fat (%) = (fat consumed – fat in feces)/fat consumed x 100%

RESULTS AND DISCUSSION

General composition of diets
Experimental diets were made by control diet supplementation with *Fagopyri herba* or lipids and their combination, which influenced the proximate composition of the diets. An efficiency of *Fagopyri herba* in the treatment of vascular diseases has been attributed to its rutin content [7]. In our previous experiments, the content of rutin in applied *Fagopyri herba* was shown to be 4.99% [8]. *Fagopyri herba* addition at 5% to the control (BLF-5%) changed the proximate composition of the diet (Table 1.). Protein, starch and fat contents slightly decreased due to increase in cellulose and reducing sugar contents. Crude protein contents as well as the contents of moisture, ash, reducing sugars and starch of high fat diet (HF) and 5% buckwheat supplemented high fat diet (HFBLF-5%) were significantly lower, while their fat content was much higher in comparison with the control and BLF-5%. HFBLF-5% had significantly higher content of cellulose than HF, the diet which was characterized with the highest calculated and gross energy value.

Feed intakes, weight gains and feed efficiency ratio
After 8 weeks of the experiment, body weight gains were significantly higher in the high-fat groups III and V compared to other groups of animals (Table 2.). *Fagopyri herba* addition to the high fat diet caused a reduction in body weight gain of the rats of the IV group. The average weight gain of the IV group was not significantly different from that of the I and II group. Total food intake at the end of the experiment was significantly lower in the high-fat groups III, IV and V compared to I and II group, probably due to the higher energy density of the diets. However, feed efficiency of the III, IV and V group was significantly higher than that of the I and II group. In comparison with I, II, IV and V group, food

efficiency of the III group of animals was higher, but not statistically different from the IV and V group. Interestingly, although the feed efficiency ratio of the IV group is not statistically different from the III group, supplementation of the powdered *Fagopyri herba* significantly lowered the weight gain in rats fed a high-fat diet without significantly suppressing the feed intake.

Table 1. Proximate composition of experimental diets: C-control, BLF-5% Fagopyri herba supplemented control, HF-high fat diet, HFBLF-5% Fagopyri herba supplemented high fat diet

	Control	**BLF-5%**	**HF**	**HFBLF-5%**
Protein (%)	21.738 ± 0.110^{b}	20.641 ± 0.234^{c}	17.11 ± 0.045^{a}	16.92 ± 0.032^{a}
Moisture (%)	7.220 ± 0.020^{b}	8.497 ± 0.255^{c}	6.84 ± 0.030^{a}	6.870 ± 0.020^{a}
Ash (%)	6.930 ± 0.100^{b}	6.897 ± 0.006^{b}	5.647 ± 0.150^{a}	5.647 ± 0.550^{a}
Cellulose (%)	4.863 ± 0.035^{c}	6.340 ± 0.010^{e}	2.193 ± 0.025^{a}	4.257 ± 0.025^{b}
Fat (%)	3.630 ± 0.190^{c}	2.910 ± 0.160^{a}	21.08 ± 0.160^{d}	20.19 ± 0.055^{e}
Reducing sugars (%)	5.070 ± 0.010^{d}	6.870 ± 0.120^{e}	4.460 ± 0.122^{a}	4.700 ± 0.121^{b}
Starch (%)	38.580 ± 0.001^{e}	33.690 ± 0.002^{c}	29.950 ± 0.001^{a}	30.52 ± 0.001^{b}
Calculated energy value*	1571	1547	1927	1925
Gross energy (kJ/100 g)	1752 ± 11.32^{ab}	1780 ± 2.42^{abc}	2231 ± 10.37^{bc}	2066 ± 1.56^{c}

* (kJ/100 g), calculated from the chemical composition by the calculator (Mullan, 2008)

^{a,b,c,d} Means in the same row not sharing a common superscript are significantly different ($p<0.05$) between groups.

Table 2. Body weight gain, total feed intake and feed efficiency of control and experimental rats

Group	**Weight at baseline (g)**	**Weight at 8 weeks (g)**	**Body weight gain (g)**	**Feed intake (g)**	**Feed efficiency (%)***
I	427.6 ± 23.6^{a}	504.0 ± 51.8^{ab}	76.4 ± 32.3^{a}	7295.6 ± 8.60^{a}	1.05 ± 0.15^{a}
II	400.0 ± 27.9^{a}	460.6 ± 42.9^{ab}	60.6 ± 16.91^{a}	6700.7 ± 11.14^{a}	0.90 ± 0.08^{a}
III	378.0 ± 1.9^{ab}	509.6 ± 46.7^{b}	131.6 ± 37.7^{b}	5546.7 ± 4.63^{b}	2.37 ± 0.21^{b}
IV	365.4 ± 56.2^{b}	451.2 ± 63.9^{a}	85.8 ± 14.9^{a}	5267.6 ± 6.02^{b}	1.63 ± 0.22^{b}
V	353.6 ± 29.1^{b}	461.6 ± 40.1^{ab}	108.0 ± 18.2^{b}	5182.6 ± 6.90^{b}	2.08 ± 0.19^{b}

* Feed efficiency (%) = (body weight gain/food intake) x 100

^{a,b,c} Means in the same column not sharing a common superscript are significantly different ($p<0.05$)

Net caloric intake, gross energy value, protein and lipid contents of faeces
Content of proteins in faeces significantly decreased starting with the group I to V. As far as protein content of the I group is concerned, a negative protein balance was noticed, meaning that the protein content of faeces was higher than the protein content of the control feed. According to Mahipala et al. [6], a large proportion of faecal protein is of bacterial and metabolic origin. The same authors concluded that faecal protein content does not accurately provide reliable quantitative prediction of the differences of the digestibility of dietary crude proteins. Contrary to the protein content, faecal fat content of the groups III and IV were similar, but significantly higher than of groups I and II, which corresponds with higher fat content of the diets. The highest faecal fat content and gross energy value and the lowest apparent fat absorption and net caloric intake was found in the V group, which indicates that postponed *Fagopyri herba* introduction into the high fat diet can ameliorate the effects of previous prolonged high fat diet consumption. Similar results were observed in a study of Lee et al. [5], who additionally demonstrated a hypolipidemic effect of *Fagopyri herba* in rats fed a high fat diet. Obtained results were connected with a synergistic effect of phenolic compounds and fibre present in *Fagopyri herba* by the authors.

Table 3. Gross energy value, protein and fat content of faeces of rats fed with control diet (I), BLF-5% buckwheat supplemented control (II), HF-high fat diet (III), HFBLF-5% buckwheat supplemented high fat diet (IV), HF-high fat diet for 7 weeks and with HFBLF-5% buckwheat supplemented high fat diet for 1 week

	I	**II**	**III**	**IV**	**V**
Protein (%)	23.6 ± 0.2^{e}	20.4 ± 0.2^{d}	16.92 ± 0.1^{b}	16.2 ± 0.29^{c}	14.9 ± 0.1^{a}
Fat (%)	1.81 ± 0.1^{a}	1.91 ± 0.2^{a}	9.1 ± 0.4^{b}	8.91 ± 0.1^{b}	11.8 ± 0.2^{c}
Gross energy value (kJ/100 g)	1402 ± 2^{b}	1353 ± 7^{a}	1821 ± 2^{d}	1807 ± 6^{c}	1833 ± 2^{e}
Net caloric intake (kJ/100 g)	350	426	410	259	233
Apparent fat absorption (%)	50.14	34.36	49.71	55.90	41.56

^{a,b,c,d} Means in the same row not sharing a common superscript are significantly different ($p<0.05$) between groups.

CONCLUSIONS

In conclusion, supplementation of the powdered *Fagopyri herba*, that is rich in phenolic compounds and fibre, seems to lower the weight gain in rats fed a high-fat diet without significantly suppressing the feed intake. Also, obtained results suggest that postponed *Fagopyri herba* introduction into the high fat diet can ameliorate the effects of prolonged high fat diet consumption in rats.

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LIMITATION OF FLAXSEED USAGE IN ANIMAL NUTRITION

Nikola Puvača[1], Vidica Stanaćev[1], Dragan Milić[2], Bojana Kokić[3], Ivana Čabarkapa[3], Vladislav Stanaćev[2]

[1]University of Novi Sad, Faculty of Agriculture, Department of Animal Science, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia
[2]Perutnina Ptuj - Topiko a.d. Petefi brigade 2, 24300 Bačka Topola, Serbia
[3]University of Novi Sad, Institute of Food Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

ABSTRACT

Because of its nutritive value and its economic cost, flaxseed regains nutritionist's attention. Flaxseed has the attributes of an oilseed that is being high in oil and protein content. Its relative lower price makes flaxseed a potential feed ingredient in animal nutrition. The other main consideration for flaxseed is its unique fatty acid composition. Flaxseed is the best source of the ω-3 fatty acids and especially α-linolenic acid. The health properties related to ω-3 fatty acids make it desirable to incorporate flaxseed in animal feed, with the expectation that flaxseed can alter the fatty acids composition of animal tissue. One possible commodity to offer the unique properties of flaxseed for human health could be broilers chickens. Broilers chickens are one of the major sources of meat for human. However, the inclusion of flaxseed in animal diets, especially for chicken is very often accompanied with depressed growth, when it is used in amount higher than 5 or 10%. The presence of antinutritional factors and the physical structure are the main limiting factors in animal nutrition. Having in mind the aforementioned, the aim of this study was to give a review of the main antinutritional factors such as cyanogenic glycosides, linatine, soluble nonstarch polysaccharides, phenolic acids and trypsin inhibitor, which representing the limiting factor of its usage in animal nutrition.
***Keywords**: Flaxseed, nutrition, broilers, cyanogenic glycosides, linatine*

INTRODUCTION

Flaxseed *(Linum usitatissimum L.)* is an oilseed that consists of seed coat, germ, endosperm and two cotyledons. Cotyledons make up 55% of the seed and are the storage tissue of flax oil. It is found that flax hull and fibrous contains little protein and oil [14]. There are four layers of flax hull. The outer one contains a mucilaginous carbohydrate material. Bhatty [4] reported the proportion of flaxseed meal being 37.5% hull and 62.4% flour, while for example rapeseed meal is 18.6 and 81.3% respectively. Flaxseed contains 24% protein, 41% ail, and 5% crude fiber [4]. Grossu et al. [9] reported that flaxseed contained 22.5% digestible protein. The available protein is 7.1%, while there is 6.0% retained protein. The digestibility of essential amino acids is between 74 and 87%, among them, lysine and methionine are 82 and 80%, respectively. Because of

its nutritive value and its economic cost, flaxseed regains nutritionist's attention. Flaxseed has the attributes of an oilseed, which content of protein and oil is high. The other main consideration for flaxseed is its fatty acid composition [8]. Flaxseed is the best non-marine source of the ω-3 fatty acid and especially α-linolenic acid [9]. The health properties related to ω-3 fatty acids makes flaxseed desirable in animal nutrition and in mixtures for animal feeding, because of flaxseed ability to alter the fatty acids composition of animal tissue. Consuming fatty acids enriched animal products, especially with ω-3 fatty acids, health benefits can be obtain. The main source of products enriched in fatty acids is chicken meat. One of the reasons for that is fact that the chickens are one of the major sources of meat for human. Broiler chickens are fast growers and efficient feed converters. Therefore, there are nutritional and economic benefits to explore the advantage of flaxseed in broilers diet [11]. However, the inclusion of flaxseed in animal diets, especially for chicken is very often accompanied with depressed growth, when it is used in amount higher than 5 or 10%. The presence of antinutritional factors and the physical structure are the main limiting factors. Cyanogenic glycosides, linatine and soluble nonstarch polysaccharides are the main antinutritional factors in row flaxseed. Non ruminants are less tolerant to these antinutritional factors and are therefore sensitive to dietary flaxseed inclusion [16]. For most oilseed crops, it is essential to properly process them before inclusion in animal diet. Having in mind the aforementioned, the aim of this study was to give a review of the main antinutritional factors such as cyanogenic glycosides, linatine, soluble nonstarch polysaccharides, phenolic acids and trypsin inhibitor, which representing the limiting factor of its usage in animal nutrition [13].

ANTINUTRITIONAL FACTORS

Presence of anti nutritional factor in flaxseed products has been associated with the usage of linseed meal in animal nutrition. Linseed meal in concentration higher than 5% in a broiler chicken diet would depress the growth of chickens, unless it was soaked in water for same period of time [12]. Feeding broilers with ground flaxseed increased LNA and DHA, slightly decreased EPA, and markedly reduced the ratio between ω-6 and ω-3 fatty acids. Ajuyah et al. [1] reported the effect of 0; 10 or 20% full-fat flaxseed on fatty acid profile of broilers meat fed over a 6-weeks period, as shown in Table 1, source and level of full-fat oil seed significantly modulate the fatty acids in white and dark meat. Anti nutritional factors in flaxseed could be part of the reasons that pigs fed a linseed meal in the diet had depressed growth, lower feed intake, and decreased weight of kidney, spleen, and pancreas but not liver [3]. Depressive effect of linatine on pig performance did not reduce addition of pyridoxine in amount of 3 mg/kg. Conclusion was that linseed meal is inferior to soybean meal as protein source. The probable reason, even though not established, could be the presence of anti nutritional factors. Adequate processing treatment could support the argument of the presence of anti nutritional factors in linseed meal. Madhusudhan et al. [13] reported that raw linseed meal significantly reduced broilers growth and caused

hstopathological changes, while water boiled linseed meal in amount of 13%, which was 50% protein replacement of expeller groundnut cake, had the same growth rate as compared to a yellow-ground peanut diet.

Table 1. Effect of full fat flaxseed on the fatty acid composition of broilers meat

Fatty acids, %	Full fat flaxseed, %					
	0.0	10.0	20.0	0.0	10.0	20.0
	Lipids in white meat, %			Lipids in dark meat, %		
C16:0	18.1	18.0	19.1	18.4	15.0	13.4
C18:0	12.5	11.0	12.4	10.9	11.0	14.4
C18:1ω6	33.5	28.9	19.0	37.4	28.6	21.1
C18:2ω6	18.4	20.6	23.8	18.5	22.1	26.9
C20:4ω6	8.0	4.8	3.4	5.7	4.9	3.4
C18:3ω3	1.2	4.1	7.0	1.2	6.9	10.3
C20:5ω3	0.8	2.1	3.6	0.5	1.3	2.2
C22:5ω3	2.0	3.1	4.7	1.5	2.7	2.9
C22:6ω3	2.5	4.8	3.9	2.3	4.7	2.8
SAT	30.8	29.3	31.7	29.6	26.3	28.0
MUFA	35.6	30.3	19.9	40.1	30.5	22.5
ω-3	6.4	14.1	19.2	5.4	15.6	18.2
ω-6	27.1	26.3	29.2	24.8	27.6	31.3
ω-6/ω-3	4.3	1.9	1.5	4.6	1.8	1.7

In spite of its high nutritional value, linseed has not been effectively exploited in animal nutrition, due to the fact that contains anti nutritional factors [10].

CYANOGENIC GLYCOSIDES

Glycoside of aldehyde or ketone cyanohydrin represents cyanogenic glycosides. They yield glucose, acetone and hydrogen cyanide on hydrolysis. Over thousand species of plant produce hydrogen cyanide [12]. Cyanogenic glycosides are not toxic as such, but only through their release of hydrogen cyanide, which occurs when they have been broken. Linamarin is an inhibitor of cytochrome oxidase and consequently a cellular respiration inhibitor. His absorption is very rapid, so is the toxic effect. The minimum lethal dose to man is quoted at between 0.5 to 4 mg/kg of body weight. There are four forms of cyanogenic glycosides in flaxseed: linamarin, linustatin, lotaustralin and neolinustatin. Flaxseed contains a very low level of linamarin, but a considerable amount of the diglycosides linustatin and neolinustatin [15]. Young flax contains mainly the monoglycosides, linamarin and lotaustralin, which could be over 90% of the total cyanogenic glycosides, whereas aider flax contains about 30% of the diglycosides linustatin and neolinustatin. The reported content of hydrogen cyanide in flaxseed varies, due to cultivar, differences in detection methods or as the expression in different compounds. Rosling [17] reported that the cyanide

content varied from 4 - 12 mmol/kg (104 – 312 mg/kg). Bhatty [5] concluded that the hydrogen cyanide content is largely affected by environments such as location and season. Bacala and Barthet [2] detected 124 – 196 mg of cyanide per kg flaxseed using HPLC system. Selective processing can significantly reduce the content of cyanogenic glycoside [6]. If methanol is used in flaxseed extraction system, it can reduce the cyanogenic glycosides content, as they are soluble in such a system [15]. An extraction system of hexane/methanol/water removed cyanogenic glycosides by 56, 80 and over 90% by 1, 2 and 3 times extraction, respectively. Soaking linseed meal by water with four times its weight reduces one half of its cyanogenic glycoside, while autoclaving under 10.5 kg/cm^2 for 15 minutes caused maximum reduction from 85 - 12 ppm hydrogen cyanide for linseed cake.

LINATINE

Other main anti nutritional factor in flaxseed is linatine. Broiler chicks fed with diets containing the linseed meal developed vitamin B6 deficiency symptoms which were overcome when 20 ppm of pyridoxine added to the diet. Klosterman et al. [11] revealed the toxic effect of flaxseed extract. They dissolved flaxseed extract into physiological saline solution and injected this mixture intraperitoneally into chicks. The chickens experienced mild to severe vitamin B6 deficiency symptoms and death at higher dosage [14]. The poor growth and the deficiency symptoms were alleviated by administration of pyridoxine. The authors suggested the vitamin B6 antagonist to be linatine.

SOLUBLE NONSTARCH POLYSACCHARIDES

One of the features of flaxseed is its high content of soluble non-starch polysaccharides, or mucilage. Its presence in chicken gut could lead to antinutritional effects. As any other non-starch polysaccharides, it may reduce the enzymatic action on other nutrients and increase fermentation in the gut. Oomah et al. [16] performed an in-depth analysis of over one hundred samples of flaxseed from over the world. They collected the flaxseeds from twelve geographical regions and included oil, fiber, to determine their composition of water-soluble polysaccharides. It was found that flaxseed contains about 3.6 to 8% water-soluble polysaccharides. The neutral monosaccharide of the water-soluble polysaccharides fraction is mainly glucose, xylose, galactose, and rhamnose. Glucose ranged from 21-40% and is the major monosaccharide. The Rhamnose to xylose ratio in flaxseed is an indicator of the viscous flaxseed gum [7]. For comparison, the non-starch polysaccharides content of some ingredients other than flaxseed reported by Smits and Annison [18] is between 2.4 to 13.9% for grains and oilseeds. Among them, wheat, rye, barley chickpeas, lupins, soybean meal, and rapeseed meal have soluble non-starch polysaccharides of 2.4, 4.6, 4.5, 3.3, 4.0, 5.7, 13.9, and 11.3%, respectively.

PHENOLIC ACID AND TRYPSIN INHIBITOR

Phenolic acids may form insoluble complexes with essential minerals, protein, and carbohydrate in feedstuffs and lower their nutritional value. Varga and Diosady [19] reported the polyphenols content in flaxseed to be 4.41 g/kg. The total phenolic acid of linseed meal was about 0.22% [20]. Oomah et al. [16] reported 8-10 g/kg of total, 5 g/kg of esterified, and 35g/kg etherified phenolic acids. They are affected mostly by season.
Lab prepared raw linseed meal contained 42-51 units of trypsin inhibitor activity, while commercially obtained samples contained 14-37 units, as reported by Bhatty [5]. For comparison, raw rapeseed and soybean meals contain 99 and 1650 units, respectively.

CONCLUSION

Flaxseed can be effectively used in enriching ω-3 fatty acids in chicken meat, while playing a role as an alternative feed ingredient. The production performance of this flaxseed fed animals is very often reduced, even more so with high amount of flaxseed. The usage of flaxseed and its products in animal nutrition is still limited due to presence of anti nutritional factors. Knowledge of its use in animal nutrition is still limited and requires further investigation.

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USE OF PHYTOGENIC PRODUCTS FOR PIG AND BROILER DISEASES

Ljiljana Kostadinović[1], Jovanka Lević[2]

[1]Megatrend University, Faculty of biofarming, Maršala Tita 3, 24300 Bačka Topola, Serbia
[2]University of Novi Sad, Institute of Food Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

ABSTRACT

The medicinal plants and herbs have been used for many years in the treatment and prevention of various diseases in animals and human beings. Now-a-days, utilization of these medicinal plants is increasing. This article summarizes the experimental knowledge on efficacy, possible modes of action, and aspects of application of phytogenic products as feed additives for treatment pig and poultry diseases. Phytobiotics is a term used to describe plant-derived natural bioactive compounds, which affect animal growth and health, and is often applied to essential oils, botanicals, and extracts derived from herbal plants. Some phytobiotics are known to have antimicrobial or antiviral activities. Selected herbs, however, are known to possess natural antibacterial activity and other characteristics that could be useful in value-added animal protein production. This area of investigation has not received substantive examination because of the relatively low costs, proven effectiveness, and ready availability of synthetic growth-promoting antibacterial products. Herbs and spices have always been helpful to cure diseases. In modern animal feeding, they are forgotten because of use of antimicrobial growth promoters (AGP). But due to the prohibition of most of AGP, plant extracts have gained interest in animal feed strategies. The risk of the presence of antibiotic residues in milk, meat, egg and their harmful effects on human health have led to their prohibition for use in animal feed in the European Union. Many plants also produce secondary metabolites such as phenolic compounds, essential oils and saponins They act as antibacterial, antioxidant, anticarcinogenic, antifungal, analgesic, insecticidal, anticoccidial and growth promoters. *Azadiracht indica, Zizyphus vulgaris, Ocimum gratissimum* and *Atlanta monophylla* have the strong antibacterial activity, whereas ocimum plant has strong antioxidant, anticarcinogenic, antifungal, analgesic and antipyretic properties. Leaves of *Azadirachta indica* are used for feeding and reducing the parasitic load of animals. The fruit of *Azadirachta indica* and *Artemisia annua* also has the anticoccidial activity for poultry. These plant extracts compete with the synthetic drugs. Majority of medicinal plants do not have the residual effect, because have an approved application in human medicine and wich can be added to animal feed for use in preventive and therapeutic treatment of different animal health disorders.

Keyword: *phytogenic product, pig, broiler, diseases.*

INTRODUCTION

The prevention of diseases and enhancement of growth, feed intake and feed efficiency are critical factors in modern animal production [57]. Current farm livestock production systems face challenges with a concept of 'clean, green and ethical' (CGE) animal production being promoted [6]. This concept involves limited use of drugs, chemicals and hormones, while reducing the impact of food production on the environment and considering animal welfare. The prophylactic use of antibiotics in poultry and pig nutrition to cause improvements in growth, feed consumption, feed utilization and decreased mortality from clinical diseases is well documented [28]. However, the growing concern over the transmission and the proliferation of resistant bacteria via the food chain has led to a ban of the feed use of antibiotic growth promoters (AGP) in livestock within the European Union since 2006. As a result, new commercial additives derived from plants including aromatic plant extracts and their purified constituents have been examinated as part of alternative feed strategies for the future. Such products have several advantages over commonly used commercial antibiotics since they are residue free and they are also, generally recognized as safe and commonly used items in the food industry (57). These botanicals have received increased attention as possible growth performance enhancers for animals in the last decade. Bioactive plants and plant compounds, when included as feed or food components, have a broad range of effects in the animal, from health promoting and beneficial for animal production to toxic or even lethal. There is interest in using plants and plant extracts as alternatives to synthetic drugs, because they can have potent properties and more complex bioactivity [52,53]. Bioactive plants and plant compounds may assist in some aspects of the proposed concept, as they are often inexpensive and considered to be environmentally safe [8]. Following the era of artificial synthesis, plant extracts and etheric oils have been increasingly used for their antimicrobial [38], antioxidative [9,10,32,33,34,40] and hypocholesterolemic effect [12,55] as well as for their stimulatory effect on the digestive system [21,50] and digestive enzymes production [36,18]. Also, herbal plant or their components have been shown to exhibit antiviral [7], antimycotic [42], antitoxigenic [24] antiparasitic [49] and insecticidal [26] properties as well as inhibition of odour and ammonia control [57]. Recent publications demonstrate renewed research interest in the use of medical plants as feed supplements for pig and poultry diseases [27]. These characteristics are possibly related to the function of these compounds in plants [41]. In contrast, many herbs and spices are popular food condiments. Etheric oils and oleoresins of garlic and capsicum as well as cinnamic aldehyde, carvacrol and pipperine (from black pepper) among others, have long been used due to their food flavor enhancement properties. Some researchers have found that many herbs and botanicals are able to improve growth rate through increased feed intake [29,30,31,39,59] nevertheless, others have reviewed the topic and found no clear evidence that herbs and spices improve palatability in farm animals [60]. Most studies investigate blends of various active compounds and report the effects on production performance rather than the physiological

impacts. In this context, the following provides an overview of recent knowledge on the use of phytogenic feed additives in piglet and poultry diets, possible modes of action, and safety implications [47,48].
Animal welfare is an important desired feature of livestock production. Besides health problems, the risk of zoonotic disease affecting food safety is an issue often mentioned in the literature. In the sections below we shall list the current literature on disease incidence in poultry and pig and collected information on traditional preparations (e.g herbs) with the objective of documenting existing plant material (herbs) and any other traditional preparations used for poultry and pig health management in intesive production systems.

POULTRY

The most common viral infections of poultry are: Chicken pox or fowl pox; epidemic tremor, infections bronchitis, Marek's disease; Newcastle disease and Avian influenza. The antiviral drugs fail to treat the infection due to viral resistance and viral latency which leads to recurrent infection in immunocompromised patients [15]. Recent success in using herbs/medicinal plants extract as antiviral agent has raised optimism about phyto-antiviral agents [22]. Plants contain a wide variety of diverse phytochemicals, such as alkaloids, tannins, saponins, flavonoids, terpenoids, lignans, cou-marins, and many other components. Plants like *Bergenia ligulata*, *Nerium indicum* and *Holoptelia integrifolia* showed significant antiviral activities against Influenza virus (RNA) and Herpes Simplex virus (DNA) [51]. The antiviral activity of *Azadirachta indica* (Neem) and *Ocimum sanctum* (Tulsi) against Newcastle Disease virus (NDV) is well known [35]. Ashwagandha /*Withania somnifera* (WS), a plant well known for its numerous medicinal properties is also used as antiviral herb for the treatment of genital disease caused by Herpes Simplex Virus among African tribes [25]. Providing chickens with access to an outdoor area may increase the risk of poultry becoming infected with *Salmonella* and *Campylobacter* due to contact with wild birds and other animals and their faeces. Earlier studies indicate that many plant extracts have antimicrobial actions *in vitro* against important pathogens, including fungi [1,11,54]. The active substances are largely the same as mentioned previously for antioxidative properties, with phenolic compounds being the principal active components [11]. Again, the plant family of *Labiatae* has received the greatest interest, with thyme, oregano, and sage as the most popular representatives [11]. The *Origanum vulgare* is described as containing more than 30 antibacterial chemicals [27, 31].The antimicrobial mode of action is considered to arise mainly from the potential of the hydrophobic essential oils to intrude into the bacterial cell membrane, disintegrate membrane structures, and cause ion leakage. High antibacterial activities are also reported from a variety of nonphenolic substances, for example, limonene and compounds from *Sanguinaria canadensis* [45,11]. According to Almas [5], the extracts of *Azadirachta indica* (neem plant) chewing sticks are effective against *Streptococcus mutans* and *Streptococcus faecalis.* Hayat *et al.* [17] studied the *in vitro* antimicrobial activity of *Zizyphus vulgaris* root extract against both gram

positive and gram negative organisms using *Staphylococcus aureus* and *Escherichia coli*, respectively. Three different concentrations of the ethanolic extract of the roots were used and the activity compared with the standard antibiotics. All the concentrations showed excellent inhibitory effect on the growth of gram positive and gram negative microorganisms. It is evident, however, that in practice most individual herb or spice extracts must be included at a high concentration to observe effects comparable to those of antibiotics. Akilandeswari *et al.* [2] tested aqueous neem extract prepared from the *Azadirachta indica* bark against the strain of bacteria *Proteus vulgaris* and fungi *Candida albicans*, to examine its efficacy as an antimicrobial agent. The growth of *Proteus vulgaris* and *Candida albicans* was inhibited remarkably due to aqueous neem bark extract. Out of these two organisms tested in the experiment, the bacteria *Proteus vulgaris* showed more susceptibility to neem bark extracts in comparison with fungi *Candida albicans*. Some studies with broilers demonstrated *in vivo* antimicrobial efficacy of essential oils against *Escherichia coli* and *Clostridium perfringens* [20,44]. In total, the available literature suggests that, at least for broilers, an overall antimicrobial potential of phytogenic compounds *in vivo* cannot generally be ruled out. Furthermore, some phytogenic feed additives have been shown to act against *Eimeria* sp. after experimental challenge [19, 46]. Another implication of the antimicrobial action of phytogenic feed additives may in be improving the microbial hygiene of carcasses [3]. Available data are still too limited to allow reliable conclusions on the possible efficacy of certain phytogenic feed additives to improve carcass hygiene.

Coccidiosis is the most common infectious disease of the digestive tract of poultry, causing a decrease in daily increment, prolonged fattening, poorer skin pigmentation, slower feed conversion and increased mortality [23, 43]. The disease is caused by protozoas from the genera of *Eimeria, Isospora* and *Cryptospora*, and it is manifested by the damage of the intestine epithelial cells, less frequently the bile duct and renal tubuli.The herbs especially *Azadirahta indica, Hobrrhena antidysentrica, Barberis aristata, Embelia ribes, Acorus calamus, Artemisia annua* and *Artemisia absinthium* have strong anticocidial activity (ostadinović). Zycox, a herbal product of India containing *Hobrhena antidyseatria, Barberis aristata, Embelia ribes* and *Acorus calamus,* is used as a prophylactic measure against coccidiosis. Tipu *et al.* [56] compared the anticoccidial efficacy of salinomycin sodium and neem fruit in boilers. They concluded that the addition of 0.3% ground neem fruit in boiler feed has tremendous efficiency in combating coccidiosis as compared to salinomycin sodium. They reported that neem fruit had compound margosate, responsible for the break down of Eimeria life cycle. Similarly, Allen *et al.* [4] investigated the effect of feeding dried *Artemesia annua* leaves and its components to birds infected with Eimeria *acervulina*, *E. tennela* or *E. maxima.* When fed at a dose rate of 1% for 5 weeks prior to infection, significant protection was noted for both *E. tenella* and *E. acervulina*. Artemesia contains artemisinin which protected weight gains and reduced oocyst yields for both *E. tenella* and *E. acervulina*. According to Youn-Hee Jeong *et al.* [61], the *Sophora flavescens* extract was

the most effective for survival rates, controlling bloody diarrhoea symptoms, lesion scores, body weight gains and oocyst excretion in the faeces.

PIGS

Parasites in swine have an impact on performance, with effects ranging from impaired growth and wasteful feed consumption to clinical disease, debilitation, and perhaps even death. It is particularly important to diagnose subclinical parasitism, which can have serious economic consequences and which should be treated with ongoing preventive measures. Internal parasitism is caused by nematode roundworms and coccidia in the gastrointestinal tract, lungworms in the respiratory tract, and by ectoparasites. The most commonly encountered gastrointestinal parasites are the large roundworm *Ascaris suum*, the threadworm *Strongyloides ransomi*, the whipworm *Trichuris suis*, the nodular worm *Oesophagostomum dentatum*, and the coccidia, especially *Isospora suis* and *Cryptosporidium parvum* and *Eimeria* spp.
In the treatment of ectoparasitoses and endoparasitoses a positive effect of a great number of plant species which used a singular or combined has been observed. White mugwort (*Artemisia absinthium* L., Asteraceae) and black mugwort (*Artemisia vulgaris* L., Asteraceae) had for centuries been used as anthelmintics (especially against oval and cylindrical worms) and in the treatment of animals infected by blood parasites (*Trypanosoma, Plasmodium* spp.). Today these plants are used also in various disturbances of gastrointestinal tract, diminished secretion of digestive enzymes, disturbed creation and secretion of bile and for strenghtening of the organism. *Artemisia absinthium* L. is administered as food supplement to improve appetite and food digestion [16]. Because of its exceptionally strong action even small doses can cause coma or death in adult animals so dried plant material is used instead of ether oil. In the treatment of diseases of digestive tract a great number of plants is used whose active principles include bitter substances (many *Asteraceae*), glucosides (for example salicine in *Salix alba* L., Salicaceae) essential oils and jelly (*Linum usitatissimum* L., Linaceae, *Malva sylvestris,* Malvaceae) [58]. In ethnoveterinary medicine the treatment of diarrhoea in pigs means the use of following plants: plantain (*Plantago major* L., Plantaginaceae), marigold (*Calendula officinalis* L., Asteraceae), nettle (*Urtica dioica* L.*,* Urticaceae), marsh mallow (*Althaea officinalis* L., Malvaceae), dill (*Anethum graveolens* L., Apiaceae), willow (*Salix alba* L., Salicaceae) [37] and seed of dock (*Rumex* sp., Polygonaceae) [13]. Sambucus leaves (*Sambucus ebulus* L,Sambucaceae), thanks to its antiinflamatory action the root and leaf of this plant are used in the treatment of burns, inflammations, oedema, eczema and urticaria [14]. In ethnoveterinary medicine the following plants having antiinflammatory and antiseptic action are used in healing of the wounds and they help forming of granular tissue and accelerate the wounds epithelization: yarrow (*Achillea millefolium* L., Asteraceae), marigold (*Calendula officinalis* L., Asteraceae) and aloe (*Aloe* sp., Liliaceae). The oily extract of Klamath weed (*Hypericum*

perforatum L., Hypericaceae) is used externally in various skin and mucous membrane injuries and wounds as well as in burns.

CONCLUSION

The increasing pressure on the livestock industry to reduce or eliminate feed-antibiotics as growth enhancers has initiated new research to find safe and efficient alternatives. This new generation of feed additives includes herbs and EOs. This research paper gives a review of the plants most frequently used in ethnoveterinary medicine, but the number of plant species which are successfully used in the prevention and treatment of poultry and pig diseases is far greater. Phytotherapy is one of the oldest and the most widely spread systems of therapy based on the use of plants regardless whether the healing properties of certain plants have been scientifically confirmed or not. Unfortunately, respective experimental results are available only from commercial products containing blends of phytogenic substances. Therefore, there is still a need for a systematic approach to explain the efficacy and mode of action for each of type and dose of active compound, as well as its possible interactions with other feed ingredients. Scientific findings on active ingredients, mechanisms of action and application of certain vegetable preparations are still incomplete, therefore it is necessary to intensify phytochemical, physiological and phytofarmacological research on insufficiently studied or less known plant species.

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OPTIMIZATION OF PORK MEAT REHYDRATION PROCESS USING SEQUENTIONAL QUADRATIC PROGRAMMING METHOD

Lato Pezo[1], Danijela Šuput[2], Biljana Dojčinović[3], Snežana Zlatanović[2], Olgica Kovačević[2], Ljubinko Lević[1]

[1]University of Novi Sad, Faculty of Technology, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia,
[2]University of Belgrade, Institute of General and Physical Chemistry, Studentski Trg 12, 11000 Belgrade, Serbia,
[3]University of Belgrade, Institute of Chemistry , Technology and Metallurgy, Studentski trg 12-16, 11000 Beograd, Serbia

ABSTRACT

Meat samples (1x1x1cm cubes) were osmotically treated in two solutions: (1) solution with 350g of NaCl and 1200g of sucrose diluted in 1 l of distilled water and (2) sugar beet molasses (80 ^{0}Brix) solution at 23±2°C for 1, 2, 3 and 4 hours. After being osmotically dehydrated (OD), meat samples were rehydrated by immersing meat cubes in water bath at constant temperature (20, 30 and 40 ^{0}C). The samples were removed after different immersion periods (15, 30, 45 and 60 min) and examined for mass changes. Process temperature was the most significant variable affecting final dry matter content and rehydration kinetics. Optimization was carried out using Sequential Quadratic Programming method, using the Response surface method's (RSM) models proposed to represent rehydration percentage and volume change in solution 1 and 2. A multiple optimization of the two response variables, for two OD solutions were accomplished, and the best conditions for meat rehydration were: temperature of 20 ^{0}C and time of 60 min.
***Keywords**: Rehydration, rehydration temperature, osmotic dehydration, pork meat, Optimization*

INTRODUCTION

The technique of dehydration is probably the oldest method of food preservation practiced by mankind [1]. Osmotic dehydration (OD) is a non-thermal process that consists in the immersion of a food material in a hypertonic solution. The difference of the chemical potential between the material and the solution promotes two main fluxes: the outcome of water from the material to the osmotic solution, and the income of soluble solids from the osmotic solution to the material. As osmotic agents are often used sugars (sucrose or glucose) and salts (sodium chloride).
In dehydration processes, heat and mass transfer flows can modify physicochemical properties of the material such as chemical composition,

mechanical properties [2], volume and porosity. The quality of the dehydrated product depends on the extension of these changes. Regarding to the changes in volume and porosity, high shrinkage and low porosity lead to products with poor rehydration capability [3]. Volume changes during OD are mainly due to compositional changes and mechanical stresses associated to mass fluxes. These changes have been analyzed as variations in the volumes of solid, liquid and gas phases of the food material during the process and have been correlated with changes in moisture content and weight reduction (WR) [4], or with water loss (WL) [5].
The objectives of here presented article were to investigate the effects of temperature and processing time on the mass transfer phenomena during rehydration of pork meat cubes, that were osmotically dehydrated in solution 1 or 2, to model rehydration percent (*R*) and volume changes (*dV*), as a function of the process variables.

MATERIALS AND METHODS

Fresh pork meat (Musculus brachii) was bought in local butcher store and transported to the laboratory where it was held at about 4°C for 1–2 h. The muscles were trimmed of external fat and connective tissues and manually cut into approximately 1x1x1 cm ($1cm^3$) cubes with shark sterile knives. Meat samples were osmotically treated in solution of sugar beet molasses (soluble solid content = 80 ^{0}Brix); sucrose-salt solution in distilled water (solution with 350g of NaCl and 1200g of sucrose diluted in 1 l of distilled water) at 23±2°C for 5 hours. Meat cubes were fully immersed and held in the solutions using wire mesh. Experiment was carried out using laboratory glasses (V=500 ml each). On every 5 minutes meat samples in osmotic solutions were mixed with hand-held agitator in order to induce sample - solution contact and provide better homogenization of the osmotic solution. After being removed from the osmotic solution, samples were gently blotted with a tissue paper in order to remove excessive solution from the surface and then analyzed. OD treated meat samples were rehydrated by immersing meat cubes in water bath at constant temperature (20^0C, 40^0C and 60^0C). The samples were taken from the bath at different immersion periods (15, 30, 45 and 60 min) and were weighted after being blotted with tissue paper in order to remove the excess water. Finally, rehydration percentage was calculated. Dry matter content of the fresh and treated samples was determined by drying the material at 105 ºC for 24h in a heat chamber (Instrumentaria Sutjeska, Croatia). Sample dimensions of meat cubes were measured before and after rehydration using digital caliper.
The RSM method was selected to estimate the main effect of solution type (solution 1 or 2) on mass transfer variables during the rehydration of pork meat cubes. The accepted experimental design was taken from [6]. The independent variables were rehydration time (X_1) of 1, 3 and 5h and temperature (X_2) of 40, 50 and 60°C, and the dependent variable observed were responces: rehydration percentage of solution 1 treated samples (Y_1), rehydration percentage of solution 2 treated samples (Y_2), samples volume

changes of solution 1 treated samples (Y_3), and samples volume changes of solution 2 treated samples (Y_4). The accepted experimental design included 12 experiments. A model was fitted to the response surface generated by the experiment design. The following second order polynomial (SOP) model was fitted to the data. Two models of the following form were developed to relate two responses (Y):

$$Y_k = \beta_{k0} + \beta_{k1}X_1 + \beta_{k2}X_2 + \beta_{k11}X_1^2 + \beta_{k22}X_2^2 + \beta_{k12}X_1X_2 \qquad (1)$$

where: β_{kn} are constant regression coefficients; Y, either rehydration percentage of solution 1 treated samples (Y_1), and rehydration percentage of solution 2 treated samples (Y_2); X either rehydration time (X_1), and temperature (X_2). The significant terms in the model were found by analysis of variance (ANOVA) for each response. An optimization was carried out using Matlab 5.3 (MathWorks, Natick, MA, USA). It was implemented a Sequential Quadratic Programming method, using the SOP models proposed to represent Y_1 and Y_2. The Sequential Quadratic Programming method is the most used technique to solve problems of minimization with constraints involving non-linear functions. These methods aim to solve a sequence of simple problems whose solutions converge to the solution of the original problem. A multiple parameters optimization of the two pairs response variables was accomplished in order to find the X_1 and X_2 values that give a higher value of rehydration percentage, and also volume change. The objective function (F) is the mathematical function whose minimum would be determined. It is a non-linear equation composed by the regression coefficients of the two pairs of models, according Eqn. (2):

$$F(X_1, X_2) = Y_1 + Y_2 \qquad (2)$$

Analysis of variance (ANOVA) and response surface regression method (RSM) were performed using StatSoft Statistica, for Windows, ver. 10 program (Statsoft Inc., Tulsa, OK, USA). The model was obtained for each dependent variable (or response) where factors were rejected when their significance level was less than 95%.

RESULTS AND DISCUSSION

The study was conducted to determine the rehydration conditions (rehydration percentage and volume changes) for pork meat cubes. The experimental data used for the analysis were derived from the Box and Behnken's 2 level-2 parameter design. Tab. 1 shows the response variables as a function of independent variables for the analysis. The analysis of variance (ANOVA) exhibits the significant independent variables as well as interactions of these variables.

Solution 1 treated samples were significantly affected by all process variables, temperature and treatment time, at 95% confidence level. It was noticed that rehydration percentage was most affected by linear term of processing temperature. The impact of temperature was dominant, as seen by temperature's quadratic term, and also the cross-product term, which were more influential then both rehydration time linear and quadratic term. The rehydration time quadratic term is significant at 90% confidence level.
Solution 2 treated meat samples rehydration percentage was most affected by linear term of processing temperature (significant at 95% confidence level). The quadratic terms for both temperature and rehydration time were found statisticaly insignificant, while cross product of rehydration time and temperature was found more influential than the linear term of rehydration time. Both of these terms were significant at 95% confidence level. Solution 1 treated samples volume change were significantly affected by cross product of temperature and processing time, and quadratic term of temperature, significantly at 90% level, while linear term affects volume change statistically significant at 90% level. All other sources were statistically insignificant. The temperture terms were found dominant, but mostly non-linear, which can be observed on the contour plots.
Solution 2 treated meat samples volume change were most affected by linear and quadratic terms of processing time (significant at 90 and 95% confidence level, respectively). The quadratic term of temperature and cross product term was found statistically significant at 95% level, while all others terms were found statistically insignificant. The analysis revealed that the linear terms for rehydration percentage contributed substantially in all cases to generate a significant SOP model. The SOP models for all variables were found to be statistically significant and the response surfaces were fitted to these models. The linear terms of SOP model were found significant, at 95% confidence level, and their influence were found most important in model calculation. On the other hand, non-linear terms in the SOP model for volume changes were found dominant, which is due to complexity of observed system. All SOP models had insignificant lack of fit tests, which means that all the models represented the data satisfactorily.
The r^2 values for rehydration percentage of solution 1 treated sample (99.171) and rehydration percentage of solution 2 treated sample (99.720) were very satisfactory and show the good fitting of the model to experimental results. Volume changes of solution 1 treated sample (91.467) and solution 2 treated sample (93.665) showed less confident model results, but also the good fitting of the model and the experimental results. Maximum rehydration percentage is achieved when processing time rises, while temperature is relatively low, for both solution 1 and 2 treated meat cubes, while volume changes seem to gain their maximum with mild temperatures and relatively low processing time, close to the center of contour plot. It seems that the upper left corner of contour plots showed on Fig.1 and 2, could produce an processing optimum, concerning low energy consumption, with long processing time, but also good rehydration percentage, and increase of sample volume. Upper right

processing conditions should be avoided, due to high energy cost, and also degradation of pork meat cubes structure.

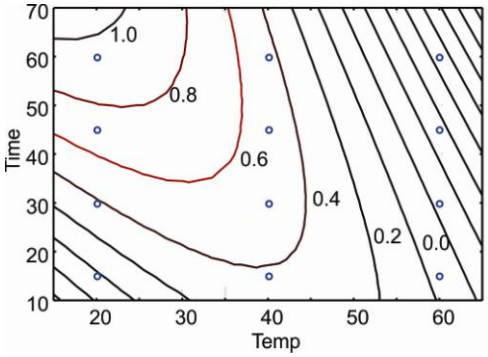

Figure 1. Objective function for solution 1 optimization

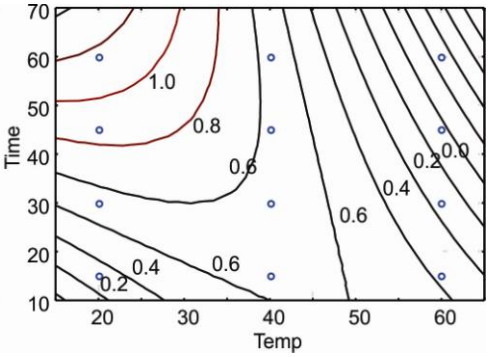

Figure 2. Objective function for solution 1 optimization

During rehydration process is essential to achieve high rates of rehydration and volume changes, depending on the final product application. If the product would be used for production of baby foods, it is essential to have a low incorporation of salts and sugars, for the maintenance of nutritional and sensory characteristics of the material. So the choice of the best process conditions for rehydration depends on the application that would be given to the product. An optimization with Matlab 5.3 (Sequential Quadratic Programming method implemented in the function *fmincon*) was carried out to determine the workable optimum conditions for the rehydration process of the pork meat. The goal is to achieve a higher value of rehydration percentage and volume changes.

CONCLUSION

The wide variety of dehydrated foods, which today are available to the consumer (snacks, dry mixes and soups, dried fruits, etc.) and the interesting concern for meeting quality specifications and conserving energy, emphasize the need for a thorough understanding of the operation and the problems related to the design and operation of dehydration and rehydration plants. The knowledge of physicochemical properties of food materials is important for an adequate design of food operations as well as for the control and improvement of the quality of the final product. Food shape is one of the main quality attributes perceived by the consumer. Drying not only causes volume changes but also may cause changes in shape. In this sense, product deformation is not fully described by the evaluation of volumetric shrinkage. Mathematical models of dehydration and rehydration operations are important in the design and optimisation of those operations. The RSM algorithm was used to model the rehydration percentage and volume change of pork meat cubes after osmotic dehydration in solution 1 and 2 solutions. SOP models for all system responses were statistically significant while predicted and observed responses correspond very well.

Solution 1 treated samples were significantly affected by all process variables, temperature and treatment time whereas the solution 2 treated meat samples rehydration percentage were most affected by linear term of processing temperature. In terms of volume change, in case of solution 1 treatment, volume changes were significantly affected by cross product of temperature and processing time, and quadratic term of temperature; while solution 2 treated meat samples volume changes were most affected by linear and quadratic terms of processing time. Rehydration percentage is most effective with the time increase at relatively low temperatures, for both cases of dehydration in solution 1 and 2. Volume change has its maximum at mild temperatures and at relatively low processing time.

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FOOD INDUSTRY PERSPECTIVE ON INNOVATION IN BOSNIA AND HERZEGOVINA

Radoslav Grujić[1], Mitar Perušić[1], Jasna Mastilović[2]

[1]University of East Sarajevo, Faculty of Technology, Zvornik, Bosnia and Herzegovina
[2]University of Novi Sad, Institute of Food Technology, Novi Sad, Serbia

ABSTRACT

Food industry, the largest manufacturing sector in Bosnia and Herzegovina has important role in the economic growth and employment. In Bosnia and Herzegovina the level of innovation in the food sector is not satisfactory to ensure competitiveness. The importance and benefits of innovation for food is not properly acknowledged by the society. The innovation of the food businesses, particularly of SMEs can be fostered by adopting agreed food innovation strategies, strengthening the capacity of the institutions through training on novel approaches, tools, organizing campaigns for improving the image of food sector innovation in the public. Food companies are constantly looking for new opportunities as respond on consumers' interests and demands. This paper presents an analysis of the current situation in the application of innovation in the food industry in Bosnia and Herzegovina. Situation analysis is done through working in two focus groups with 8-15 participants per groups. Participants included in discussion were representatives of industry, government, scientific and research institutions, universities and agencies). During the work SWOT and SOR analysis were carried out.
Keywords: *Innovation, Food industry, SWOT analysis, Bosnia and Herzegovina*

INTRODUCTION

In recent years the development of national innovation strategy led to a consensus on the impact of technology on economic growth and competitiveness. National innovation system is defined as a system of institutions that contribute to the development of innovation system and the established links between these institutions. It is widely accepted as the basis for government policy. In the development of national innovation systems, different strategies are used. The government policies most commonly used methods related to support of infrastructure development and technical assistance aimed at increasing of R&D performance. Policy innovation management has become a modern form of the defence of individual countries against globalization. Through a system of national innovation management two key objectives have been achieved: modelling of innovation processes and recommending of these models for wider use. In this way it aims to create conditions for a competitive advantage in certain areas of industrial production.

In the twentieth century innovations were the major driver of business success of companies in the U.S. (Omta and Fortin, 2009). Ren et al. (2006) conducted a study on the success of the implementation of technical innovations in the manufacturing industry of China. Based on these results, the authors believe that for the state-owned enterprises, which have their roots in the socialist economy, the sudden entry into the open market could not be the best solution. These companies have to become competitive and innovative.

Najib and Kiminami (2011) considered that the main problem in the application of innovation in SMEs in developing countries is their small budget for new product development and technology updating. The authors see the development of cooperation between SMEs as the solution for this problem. Najib and Kiminami (2011) analyzed the cooperation that exists between SME clusters in the food industry. They indicate that for the purpose of innovation, SMEs can develop cooperation with the other enterprises, with government and with the research institutions. Cooperation between the companies includes the interaction between different stakeholders, including suppliers, customers and competitors.

Omta and Fortin (2009) gave a definition of innovation as a process of creative destruction, where demand for profit forces the companies to constantly innovate, breaking the old and establishing the new rules. This includes not only the introduction of new products, but also the successful commercialization of new combinations based on the use of new materials and crucial components, the introduction of new processes, new markets or introducing of new organizational forms (Grujic and Grujic, 2011).

Food industry is faced with increasing competition and stringent market requirements, under pressure to increase the speed and quality of their innovations. Omta and Fortin (2009) cited a number of drivers and barriers to implementation of innovation in the food industry. Food industry can rely on the principles of innovation management, which are developed in high-tech industry, but it is needed to determine which factors act as major drivers and barriers to innovation in this industry. Clearly there is room for improvement, in particular, the possibility of "open innovation" with suppliers and customers as the innovation resources and capacities, which are not sufficiently exploited. Customers act as a powerful driver for innovation (Michael and Roger, 2011). In this sense, it is necessary to strengthened relations of R&D and marketing.

In the literature the research is reported in which the emergence of innovations was focused to the entire food industry or to its sector, but little number of researchers has explored the factors that affect the formation of innovation drivers and barriers down to the company level.

The influence of innovation on the business success of companies in the food industry can be compared with the impact of innovations in other industries. In the past, for the food industry, the focus was on cost reduction, with little attention to the benefit of the customers. The pressures on the companies, which have recently come from globalization and the requirements for food safety, nutritional quality of food and consumer needs satisfaction for variety and quality of products, combined with new capabilities that allows the biotechnology

revolution has led to a change in that paragraph; industry is more focused on creating products that meet consumer needs (Iliopuulos et al., 2012). In this process the key role plays the coordination of product design to consumer needs (Omta and Fortin, 2009). European Confederation of food and drink industry (CIAA) in its report believes that its member companies must increase their innovative potential, if they want to remain competitive in the upcoming years (CIAA, 2008).
The report of the project INCO.NET WBC (WBC INCO.NET, 2011), provides an overview of the infrastructure and the list of stakeholders in Bosnia and Herzegovina for innovation. In addition, links and government institutions responsible for innovation with programs and organizations (technology innovation centers, clusters, technology parks, business incubators for startup and other related organizations) have been identified. In Public report on the analysis of SMEs and stakeholders' needs, requirements and feedback needed for overcoming of barriers for research & innovation activities in Bosnia and Herzegovina (Dimitrijevic and Rodic, 2011), have been described. Although the above studies are related to SMEs, most of the conclusions can be viewed from the perspective of SMEs in the food industry. Authors cite the following advantages: knowledge benefits, benefits of networking, reputation benefits, economic benefits, benefits of internationalisation (total 24); and the following issues: administrative barriers, financial barriers, internal barriers in SMEs, external barriers (total 18).

METHODOLOGY

SWOT analysis is a strategic management technique by which the strategic choices being associated with strengths and weaknesses of the subject and with the opportunities and threats in the external environment can be recognized. SWOT analysis is a tool for exploring of the situation in the targeted area or organization. The data resulting from this analysis can be used for making of decisions that are strategically important for the formation of the organization's vision and future strategy, establishing a field advantage in the future. Analysis of strengths (S) and weaknesses (W) is the internal review of the subject, and analysis of the opportunities (O) and threats (T) is an external analysis of the environment in which the subject operates. In conduction of the SWOT analysis, it is important to keep in mind how and whether certain weaknesses of the subject or threats from the environment can be transformed into strengths or opportunities that can be used to achieve competitive advantage in the market. The Focus group approach has been used to draft and design SWOT and strategic orientation (SOR) analysis of current situation in Bosnia and Herzegovina. The main objective of the focus group discussion was the improvement of the environment for the development of innovation in food industry as a contribution to the national strategy for the development of innovation, but the objectives were also to analyze the environment for innovation development in the food industry and to design SWOT and SOR analysis of the subject (Fig. 1). On this basis, the relevant methodology has

been applied. Representatives of the institutions have been invited (8-12 participants - 2 focus groups) to the focus group discussions.
During the preparation of focus group meetings and discussions, all activities were carefully planned. The structure, contribution and selection of participants was defined including the equal representation of relevant geographical areas, key stakeholders, genders and SMEs from food industry sectors. Two focus group workshops and discussions were held, the first workshop with a main focus on support to innovation and the second workshop focused at application of innovation in practice.

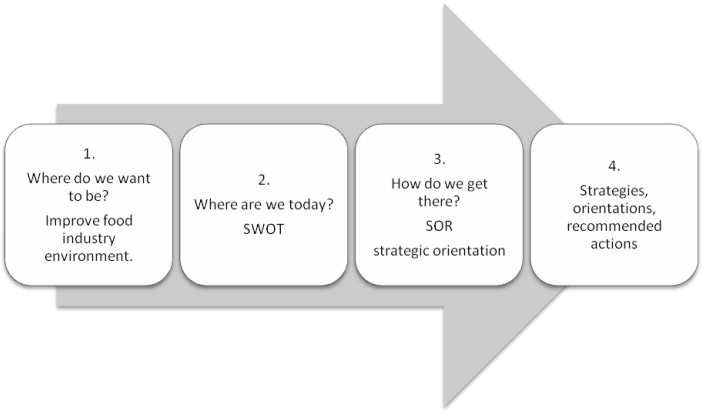

Figure 1. Focus group approach (1-inputs, 2 and 3-tasks and 4-outcomes)

RESULTS AND DISCUSSION

The research results obtained by SWOT analysis emphasize high human and technical potential for innovation as the basic strength. Among the weakness, seen as the obstacles for the utilization of identified potentials is a lack of sustainable cooperation between key stakeholders, which could enable the transfer of knowledge and innovation to the market. In the field of innovation and entrepreneurship the absence of mediators and consultants is also noticeable. The European integration process in this progress can be an opportunity to overcome this deficiency (Bratukhin, Treytl, 2011). Translational access to this effect could be quite useful, as beam research activities to generally accepted research agenda, creating the necessary critical mass, involving academic institutions and enterprises, and thereby improving visibility at the international level. Cooperation of existing networks, clusters, technology platforms and the public sector including financial aspects are also seen as the opportunity which could accelerate development of innovation and entrepreneurship.
On the basis of SWOT analysis, the focus group scored obtained matrix and designed SOR matrix providing the future strategic orientation in the field of innovation in the food industry. Results of the SOR matrix designed after the first meeting related to support to innovation (Fig. 2) indicate that the focus group

was primarily committed to the two focal points: using the own strengths in order to use opportunities (S/O-236 points) and using the opportunities to eliminate existing weaknesses (W/O-206 points).
Results of the SOR matrix designed after the second meeting of the focus group are related to future strategic orientation in the field of application of innovation in the food industry and practice (Fig.3). Results of the SOR matrix indicate that the focus group was primarily committed to the two focal points: using the own strengths in order to use opportunities (S/O-372 points) and using of opportunities to eliminate existing weaknesses (W/O-294 points). Interesting, the combination of the weaknesses-threats (W/T-259) was scored with significant number of points which can only be explained by the fact that the situation in the food industry in Bosnia and Herzegovina is difficult, but the upcoming threats can also make more alarming environment.

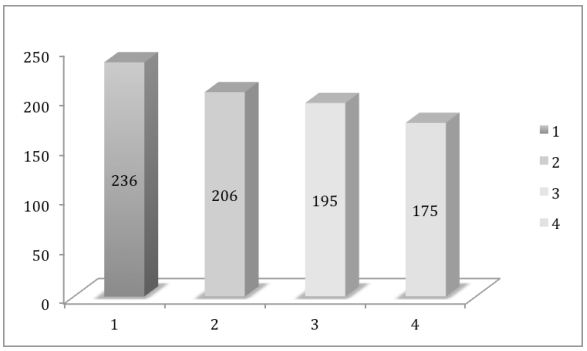

Figure 2.Results of strategic orientation (SOR) scoring matrix related to support to innovation (1-S/O; 2-W/O; 3-S/T; 4-W/T)

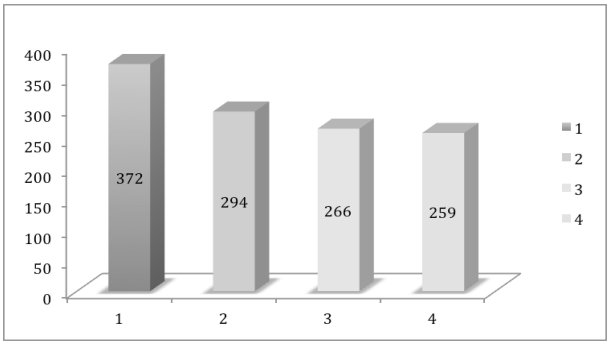

Figure 3.Results of strategic orientation (SOR) scoring matrix related to application of innovation in practice (1-S/O; 2-W/O; 3-S/T; 4-W/T)

Some research results have coincided with the results of Vermeire at. al (2011) who conducted SWOT and SOR analysis for different countries and regions in

the European Union. Vermeire et al. (2011) have found that certain regions within a country can specialize in certain sectors of the food industry. The ability of a specific regions to concentrate to the sub-sectors in the food industry brings the advantage for the regions, based on the fact that the governments under such circumstances are ready to support and lead producers of food products to the competitive position of the companies. According to these authors, regional specializing in food production is a prerequisite for the growth of local resources, which can potentially grow into a regional cluster.

CONCLUSIONS

In this research a number of measures that directly or indirectly encourage SMEs to undertake innovation of its processes and products have been identified. Cooperation with the government could improve innovation since the government supports and forms the public institutions and universities with the aim of increasing knowledge and innovation. It is believed that this kind of cooperation is the most effective tool to encourage innovation aimed at opening of new markets.

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VALIDATION METHODS FOR THE DETERMINATION OF β AGONISTS RESIDUES IN FEED

Risto Uzunov, Zehra Hajrulai-Musliu, Vlatko Ilieski, Biljana Stojanovska Dimzoska, Elizabeta Dimitrieska Stojkovic, Pavle Sekulovski, Velimir Stojkovski

Faculty of veterinary medicine, Lazar Pop-Trajkov 5-7, 1000 Skopje, Republic of Macedonia

ABSTRACT

Veterinary drugs are widely used in modern animal husbandry. Their usage is not only for therapeutic and prophylactic purposes but also for better breeding efficiency. A part of veterinary drugs are used as growth promoters in form of specified compounds or mixtures of compounds. Therefore, among other compounds, steroids and other substances that have similar pharmacological activity are used to improve efficiency of protein conversion. As a result of enhanced protein conversion the growth of animals is faster which lead to earlier slaughter. The aim of this study was to detect levels of β – agonist residues since they are used as additives in the feed. By using ELISA method level of β – agonist was determinate. Total of 49 feed samples were screened for the presence of β–agonist as a part of national monitoring residue plan. The feed samples were collected and delivered by the authorised veterinary inspectors within period of 1 year. The validation process was carried out according to Commission Decision 2002/657/EC criteria. Limit of detection (LOD) for β–agonist in feed was determined to be 0.63 ng/ml. The recovery was between 71.8% and 77.2%, a working range between 0.3 to 25.0 ng/ml. The regression equation of the final inhibition curve was: y=- 0.1143·Ln(x)+0.5906, R^2=0.9904. Additionally detection capability (CCβ) was 5.46 ng/ml. The levels of β – agonist residues were below the international allowable levels set by the Macedonian Residue Control Plan and the European Union. According to this study for β – agonist, feed in Republic of Macedonia is free and safe for animal consumption. However, it is necessary further monitoring of these chemicals as a food quality control measure. Shown data in this study will be helpful for screening of β – agonist residues and regulations on its illegal use in feed.
Keywords: *β – agonist, feed, ELISA, validation*

INTRODUCTION

β2-agonists or β2-adrenergic agonists have been illegally used to improve production performance and carcass condition of livestock. Pharmacologically, these compounds have been found to exert a repartitioning activity causing an increase in muscle accretion and decrease in fat deposition [7]. From many literary data we can say that beta agonists are transmitted in meat and meat products and present a risk for public health [6,7, 8]. Given orally or parenterally, β adrenergic agonists consistently decrease carcass fat and increase carcass

protein accretion in poultry, pigs, sheep and cattle [8]. Skeletal muscle protein accretion rate was increased by 130% during the first week of feeding the β - adrenergic agonist cimaterol to rapidly growing rats [6]. Although the chronic response was transient [6], skeletal muscle mass and protein content were increased by 20 to 30% after 3 week to 12 week treatment intervals [2,10,11]. Mexico and South Africa had approved use of β-agonists, including the feed additives zilpaterol hydrochloride and ractopamine hydrochloride, more than 10 year ago to improve feedlot performance. In 2003, ractopamine hydrochloride was approved for use in cattle in the United States, and zilpaterol hydrochloride was just approved in 2006 for increased rate of weight gain, improved feed efficiency, and increased carcass leanness in cattle fed in confinement for slaughter during the last 20 to 40 day on feed. In Mexico, consumption of viscera from animals fed with clenbuterol has caused acute toxicity in consumers, indicating an abuse in the use of this product, and therefore this β2-agonist was removed from the market [1]. However, the residues of β2- agonists may present health risk to public health [7].
The European Economic Community (EEC) banned the use of β-agonists and anabolic compounds as growth accelerators in feed while the United States Food and Drug Administration (USFDA) permitted limited use of some hormones with natural origin (such as oestradiol and testosterone) and some synthetic hormones such as trenbolone in animal husbandry [3,4]. The permitted limit values for all β-agonists are 50 ng/ml in feed [5]. The use of β-agonists as growth promoters is illegal in Republic of Macedonia, too. The Macedonian regulatory agencies implemented a monitoring surveillance program that uses only preliminary methods for determination of β-agonists. The examinations were carried out in the Faculty of veterinary medicine, Food Institute in Macedonia in five specialized veterinary diagnostic laboratories according to the requirements of the European Community.

MATERIAL AND METHODS

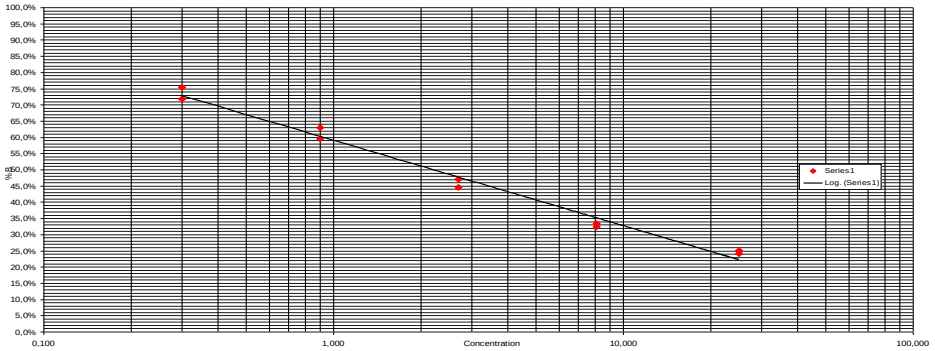
Sample collection: A total of 49 feed samples were screened for the presence of β–agonist as part of national monitoring residue plan. The samples were collected within period of 1 year as they were delivered by the authorised veterinary inspectors.
Enzyme-linked immunosorbent assay (ELISA): The concentrations of β-agonist in feed samples were determined using a commercial β-agonist ELISA kit (provided by R-biopharm Darmstadt, Germany). Each kit contained a microtiter plate with 96 wells coated with antibodies to rabbit IgG, clenbuterol standard solutions (0, 0.3, 0.9, 2.7, 8.1 and 25.0 ng/ml), peroxidase-conjugated clenbuterol, anti-clenbuterol antibody, substrate/chromogen solution, stop reagent, conjugate and antibody dilution buffer, and washing buffer. The extraction and clean-up procedures were those described by the ELISA kit manufacturer. The feed samples were minced and 1 g of ground feed was transferred into a suitable container, then 10 ml of 1 M HCl and 90 ml of distilled water were added and mixed vigorously for 15 min. Than samples were

centrifuged for 10 min at 4000 rpm on room temperature (20 - 25°C). The supernatant was transferred into a new vial and pH was checked and adjusted with 1 M NaOH (pH 8). Than samples were centrifuged for 10 min at 4000 rpm on room temperature (20 - 25 °C) and 20 µl of the supernatant was used per well in the assay. Data were analyzed using a special software RIDAWIN ELISA (R-Biopharm, Darmstadt, Germany).
Test procedure: All reagents in the kit had to be brought to room temperature (20 - 25 °C) before use. Standard used for β-agonist contain 0, 0.3, 0.9, 2.7, 8.1 and 25.0 ng/ml clenbuterol in 10% aqueous solution. We added 100 µl of diluted antibody to each well, mixed gently by shaking the plate manually and incubated for 15 min at room temperature (20 - 25°C). Liquid was poured out of the wells and after complete removal of the liquid; all wells were filled with washing buffer. Washing was repeated two more times. Then 20 µl of each standard solution or prepared sample were added, after that 100 µl of the diluted enzyme conjugate was added. The solution in the microplate was carefully mixed by shaking the plate manually. Plate was incubated for 30 min at room temperature (20 - 25°C). The liquid was poured out of the wells and after complete removal of the liquid all wells were filled with washing buffer. After rinsing, the water was also discarded; the washing was repeated two more times. Then, 100 µl of substrate/chromogen (tetramethylbenzidine) were added, and after mixing thoroughly and incubating for 15 min at room temperature and dark, 100 µl of stop solution (0.5 M H_2SO_4) was added. After mixing, the absorbance was read at 450 nm.
Method validation: The limit of detection (LOD) was obtained by analyzing 20 blank feed samples. The method recovery was determined at three levels by spiking feed samples (1, 2 and 5 ng/ml). For determination of repeatability, the same steps were repeated on two occasions in the same analytical conditions. Detection capabilities (CCβ) was evaluated by analyzing 20 spiked samples lover than MRPL level. The robustness of the method was determined with analyzing of spiking feed samples (1, 2 and 5 ng/ml) on two levels of pH (pH 8 and pH 5). In the latter case to determine the robustness had changed the procedure of extraction. Samples were dissolved in 10 ml 1M HCl and 90 ml distilled water in the first case and 1 ml of 1 M HCl and 9 ml of distilled water in the second case.

RESULTS

The calculation of the gained results was made by RIDAWIN Software. For construction of the calibration curve the mean of the absorbance values obtained for the standards was divided by the absorbance value of the first standard (zero standards) and multiplied by 100. The absorption is inversely proportional to the concentration of β-agonist. The obtained calibration curves for the ELISA method in the range 0.3-25.0 ng/ml in linear and exponential form are presented on Figure 1. The curve equation $y=-0.1143 \cdot Ln(x)+0.5906$, where y was relative absorbance (%) and x was clenbuterol concentration in ng/ml, was

utilized for determining β-agonist concentration in feed samples, obtaining high regression coefficient (R^2=0.9904).



Graph 1. Linearity of calibration curve for β-agonist standards

The results of method recovery (n=18) and repeatability (n=54) are presented in table 1.

Table 1. Recovery and repeatability of the method

Validation parameter	No. of replicates	Spiked concentra-tion ng/ml	Determinated concentration ng/ml	Mean recovery %	Coefficient of variation %
Recovery	6	1	0.718	71.8	0.42
	6	2	1.552	77.6	2.75
	6	5	3.860	77.2	1.18
Repeata-bility	18	1	0.801	80.1	4.37
	18	2	1.483	74.1	1.60
	18	5	3.972	79.4	3.04

Validation of the method used in β-agonist determination resulted in the mean recovery of 71.8%-77.6% and repeatability of 74.1%-80.1% with coefficient of variation (CV) of 0.42%-2.75% and 1.60%-4.37% respectively.
The results of method robustness are presented in table 2.
At pH 5 and pH 8 recoveries were from 53.5 to 60.8% and from 71.8 to 77.6% respectively. Extraction with 90 ml distilled water and 10 ml of 1 M HCl in our case gave better results than extraction that provides producers, where extraction is with 1 ml 1 M HCl and 9 ml distilled water. Recovery was from 71.8 to 77.6% with first extraction. With second extraction recovery was from 54.0 to 65.8%. Therefore created a modification of the method by the manufacturer and we use extraction with 90 ml distilled water and 10 ml of 1 M HCl. The estimated LOD for feed samples for β-agonist was 0.63 ng/ml. The CCβ for feed samples for β-agonist was 5.46 ng/ml.

Table 2. Robustness of the method

Validation parameter Robustness	No. of replicates	Spiked concentra-tion ng/ml	Determinated concentration ng/ml	Mean recovery %
pH 8	6 6 6	1 2 5	0.718 1.552 3.860	71.8 77.6 77.2
pH 5	6 6 6	1 2 5	0.608 1.070 2.907	60.8 53.5 58.1
Extraction with 10 ml HCl+90 ml distill. water	6 6 6	1 2 5	0.718 1.552 3.860	71.8 77.6 77.2
Extraction with 1 ml HCl+9 ml distill. water	6 6 6	1 2 5	0.658 1.225 2.698	65.8 61.3 54.0

Forty nine feed samples were tested applying the validated screening method. Eight of them (16.33 %) were with concentration less than LOD (< 0.63. ng/ml) and other samples were with concentration from 0.68 to 4.35 ng/ml. Feed samples with concentration above CCβ were not determined.

CONCLUSIONS

The European Economic Community (EEC) banned the use of β-agonist compounds as growth accelerators in feed. Elisa method was used as screening method for analyzing β-agonist in feed. ELISA method is rapid and practical method for residue detection in food products and is recommended by EU. It is mentioned that conducting recovery tests before the study would be useful for correct test result [9]. For this reason our test results are of importance as they give information about the use of β-agonist preparations in national animal husbandry and in the feed industry. A survey carried out in R. Macedonia demonstrated that the incidence of residues of β-agonist in feed is not a problem, which means that β-agonist gave no evidence of illegal use of them in R. Macedonia. The National Residues Control Plan guarantees fulfilment of the requirements which are of importance to health of both humans and animals as well as marketing of animals, feed and products of animal origin.

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SIGNIFICANCE OF PROTEOLYTIC PROCESSES IN SILAGE FOR MODERN NUTRITION OF RUMINANTS

Bora Dinić[1], Nenad Đorđević[2], Zoran Lugić[1]

[1] Institute for Forage Crops, Kruševac, 37251 Globoder bb, Serbia
[2] University of Belgrade, Faculty of Agriculture, Zemun – Belgrade, Serbia

ABSTRACT

This paper shows an overview of results from domestic and international studies on the problem of proteolytic activity in the silage. This issue is very important for the modern norms of ruminant nutrition.
Transformations of nitrogen compounds in silage are the result uncoordinated effects of proteolytic enzymes from plant cells and from present microorganisms. The result of this process is undesirable increase of the soluble protein, which is particularly evident and important for legume silages, predominately alfalfa silage. Solubility of proteins is positively correlated with degradability which can significantly reduce their utilization or lead to health and reproductive problems in polygastric animals.
The simplest procedure for the control of proteolysis in practice is the wilting of the plant matter prior to ensiling. This reduces the activity of enzymes from plant cells and microorganisms. The result is a reduction of both fermentation and proteolysis, which is considered to be positive not only for quality but also for the nutritional value of silage. In addition to this procedure, stimulation and induction of homofermentative lactic fermentation (inoculation) is used in modern ensilaging processes in order to make acidification better and faster which reduces proteolysis. In the ensiling technology the emphasis today is in the selection of legume to decrease proteolysis.
Keywords: *proteolysis, silage, legume, wilting, inoculation*

INTRODUCTION

Objective of various processes of food preservation for animals is maximum preservation of nutritional value in the long run [18]. Silage as a form of conserved food for ruminants in recent years is gaining importance because it provides a stable production of milk and meat, with minimal cost [19]. In addition, silage stored in optimal conditions can be stored for a long time, according to some literature data and up to 20 years [17]. However, during ensiling, and later, biochemical processes occur in the silage that lead to the transformation and degradation of nutrients. For nutrition of ruminants the most significant is the transformation of nitrogen matters, i.e. protein hydrolysis.
Silage or haylage of perennial and annual legumes is more common form of silage, which is saved and used in the world and Serbia, and an important and cheap source of protein for ruminants [40]. However, this feed is characterized by a high degree of hydrolysis of proteins, resulting in reduced nutritional value,

and thus the productivity of animals [15, 16]. In addition, there may be other problems, particularly in the health and reproduction, because of the high levels of blood urea [1]. One of the contemporary problems is increased emission of nitrogen in the environment [24]. Therefore, in this paper, a brief overview of the problem of proteolysis in ensiled feeds, as well as possible solutions for their control, are presented.

SPECIFICITIES OF NITROGEN FROM LEGUMES DURING ENSILING

Legumes are important material for silage, primarily because of their high protein content. In addition, perennial legume forage is characterized by a number of favorable biological characteristics of which the most significant are the longevity and the ability of nitrogen fixation [1998]. Among the perennial legumes are particularly prominent alfalfa, not only because of the nutritional value (high concentration of protein, calcium and beta carotene), but also because of the high yield (up to 20 or more tha^{-1}), the long operation period (5-7 years), more cuts during the growing season (up to 8 in conditions of irrigation) and various application possibilities: for green food, as hay, silage or haylage, alfalfa meal and protein concentrate [22].
In comparison to other legumes, alfalfa proteins are exposed to the greatest extent to hydrolysis [15]. These processes are most intense immediately after ensiling, although evident for months [23]. Products of enzymatic degradation of proteins are peptides, free amino acids and ammonia. In the rumen of ruminants these products decompose faster than real proteins, and the ultimate degradation product is ammonia, which may not be fully utilized for microbial protein synthesis. The above products are part of soluble, i.e. degradable proteins, whose content is very well defined in contemporary norms for dairy cow nutrition [25].
For these proteolytic processes in silage responsible are microorganisms present, and proteolytic enzymes in plant cells [29]. Tao et al. [37] studied the optimal pH and temperature for the activity of exo-and endopeptidase, in order to determine the importance of plant proteases during ensiling. The authors found a high proteolytic activity at pH 4 to 6, and temperatures from 20 to 40°C, which coincides exactly with the conditions prevailing in the silo mass of alfalfa. The alfalfa is characterized by a high buffer capacity, which arises from the large proportion of protein and calcium, and a small amount of fermentable sugar [9, 10]. Because of the high proportion of moisture, biomass of alfalfa is wilted always before ensiling, which reduces fermentation and reduces the activity of proteolytic enzymes [6, 8].
All green feed is characterized by increased content of soluble proteins. As stated by Slottner and Bertilsson [33] in living plants 75-90% of the total amount of nitrogen is in the form of true protein, while in silages it is only 30 - 50%. Experimentally significant differences are demonstrated in the degree of solubility between different legumes. According to Broderick [1] other legumes are characterized by a low degree of solubility of proteins compared to alfalfa

due to the higher presence of condensed tannins. Jones et al. [26] report that during ensiling in alfalfa 44-87% proteins are hydrolyzed, while in red clover only 7 - 40% of proteins are hydrolized. However, red clover contains no tannins, but still characterized by a low degree of proteolysis during ensiling [31]. Sullivan and Hatfield [35] found that the lower level of proteolysis in red clover silage compared to alfalfa, can be explained by the existence of buffer soluble proteins - polyphenolic oxidase. This enzyme in the presence of oxygen reacts with o-diphenol building the highly reactive O-quinone, which with other suitable molecules, such as proteins, build polymers.

CONTROL OF PROTEOLYTIC ACTIVITY IN LEGUME SILAGES

Proteolytic processes in silage can be successfully controlled by direct methods or induced acidification, as well as wilting, which leads to negative conditions for the enzyme activity of plants or microorganisms. The simplest and cheapest procedure is the practice of wilting, which is compulsory measure when ensiling legumes [7]. Increase in dry matter in ensiled material reduces the overall microbial activity, as well as enzymes. As a result, the increase of pH values occurs (as a result of lower production of lactic acid), and reduction of the amount of ammonia and soluble nitrogen (Table 1).The increase of dry matter in wilted material also enables greater consumption of dry matter, which is particularly important for high producing animals [13].
However, a material with a higher percentage of dry matter is difficult to compress. Residual oxygen in the silage mass enables more intensive and longer aerobic processes, and results in higher temperatures. In the experiment, Đorđević et al. [20] the significant effect of the degree of compaction on the share of ammonia nitrogen, soluble protein and true protein is determined. Muck and Dickerson [30] studied the effects of different temperatures (15, 25 and 30°C) in ensiling alfalfa with 40 and 55% dry matter and found that proteolysis increases with temperature, but that temperature has a greater influence on proteolysis, compared to the percentage of dry matter.
For maximum preservation of proteins and amino acids themselves, the most effective are chemical preservatives based on mineral acids with a high degree of dissociation, because their use provides quick change of pH and proteolytic inactivation. In order to investigate the effects of using chemical preservation, but also of botanical differences, Đorđević et al. [12] have set up two factorial experiment in which they monitored the effects of red clover silage and alfalfa cut at a similar stage of development, with the addition of sulphuric acid as a preservative (0, 3 and 6 ml of preservative to 1 kg fresh mass). The authors found that with increasing doses of preservatives, a significant reduction in proteolysis occurs. By using the same dose of preservatives red clover silage has always had a lower amount of ammonia and soluble nitrogen, and greater preservation of protein.

Table 1. Content of nitrogen fraction (gkg^{-1} N) and parameters of biochemical changes in alfalfa silages, gkg^{-1} DM [21]

Phenophase	Cut	Degree of wilting	pH	NH_3N	Soluble N	Lactic acid	Acetic acid	Butyric acid
A_1 10% flowers	B_1 II	C_1-Low	4.84	188.24	732.18	64.38	57.94	0.00
		C_2-Hihg	5.04	167.03	684.57	58.76	40.73	0.00
	B_2 IV	C_1-Low	4.86	153.95	715.29	56.45	48.30	0.00
		C_2-Hihg	5.14	142.57	698.38	50.81	44.88	1.05
A_2 50% flowers	B_1 II	C_1-Low	4. 68	136.32	674.27	42.78	37.23	0.32
		C_2-Hihg	4.75	108.62	660.32	40.31	42.61	0.00
	B_2 IV	C_1-Low	4.82	142.80	639.03	53.26	26.54	0.00
		C_2-Hihg	4.95	113.37	617.48	47.03	25.42	0.00
Average for A_1			4.97	162.95	707.60	57.60	47.96	0.26
Average for A_2			4.80	125.28	647.78	45.84	32.95	0.08
Average for B_1			4.83	150.05	687.84	51.56	44.63	0.08
Average for B_2			4.94	138.17	667.54	51.89	36.28	0.26
Average for C_1			4.80	155.33	690.19	54.22	42.50	0.08
Average for C_2			4.97	132.90	665.19	49.23	38.41	0.26
Significance for A			**	**	**	**	ns	ns
Significance for B			**	**	ns	ns	ns	ns
Significance for C			**	**	ns	**	ns	ns

ns - *no significance*; * (p<0,05); ** (p<0,01)

Despite the high efficiency and fast action, the negative side of the mineral acids is high aggression and corrosion. Therefore, these preservatives are abandoned, and instead, since the mid last century, organic acids are increasingly used. The principle of action of organic acids is based on the free ions or molecules dissociated. Accordingly, the organic acid to a lesser extent, affect the acidity of silage material, while the bactericidal, fungicidal and bacteriostatic effects are more expressed [3, 4, 5, 14].

In the last decade of the twentieth century, bacterial inoculants become current, based on lactic acid fermentation which intensify fermentation and rationally use fermentable sugars. The result of their use is faster fermentation, higher production of lactic acid and lower pH values. In such conditions, the proteolysis is reduced, or the amount of ammonia and soluble nitrogen. However, regardless of the significance of these differences, the pH value and the degree of proteolysis remain high, acting negatively on the nutritional value of alfalfa silage (Table 2). Some improvement is achieved by a combination of inoculants and carbohydrate supplements, but even that is not enough, especially in wilted material, when the lowest pH values are above 4.5 [11].

Table 2. Value of pH and content of ammonia, soluble and protein nitrogen (gkg^{-1} N) in alfalfa silage [20]

Compression (A)	Inoculation (B)	pH	NH_3N	Soluble N	Protein N
420 gkg^{-1} (A_1)	No inoculant (B_1)	5.55^{a}	220.86^{a}	762.71^{a}	262.56^{c}
	With inoculant (B_2)	5.15^{c}	197.71^{b}	723.41^{b}	293.76^{b}
560 gkg^{-1} (A_2)	No inoculant (B_1)	5.31^{b}	197.52^{bc}	707.04^{c}	307.88^{b}
	With inoculant (B_2)	4.99^{d}	189.34^{c}	686.06^{d}	324.26^{a}
Average for A_1		5.35	209.28	743.06	278.16
Average for A_2		5.15	193.43	696.55	316.07
Average for B_1		5.43	209.19	734.88	285.22
Average for B_2		5.07	193.52	704.74	309.01
Average for experiment		5.25	201.36	719.80	297.12
Significance for p	Factor A	0.1232	0.0198	0.0010	0.0011
	Factor B	0.0002	0.0219	0.0737	0.0868
	Interaction A×B	0.0000	0.0000	0.0000	0.0000

^{a,b,c,d}Values in the same column with different letters are statistically significantly different ($p<0,001$)

Further solutions to proteolysis problem should be sought in the selection. The main task of plant breeders for animal feed was once a yield increase [28] while the parameters of nutritional values were of secondary importance. In recent years, it is the selection of legumes in order to select varieties with high levels of protein stability in ensiling [2]. Such studies are now much more important when it comes to nutritional value (digestibility) of corn hybrids for silage [39]. So, for example, Tremblay et al. [38], examining the silage of alfalfa varieties found value for the degree of proteolysis in the range 612-717 g kg^{-1} N. Such a wide interval clearly provides significant choice of varieties with a lower degree of proteolysis. Then, a significant feature of red clover, that it contains polyphenol oxidase, could be used on the one hand for the creation of cultivars/varieties with increased activity of this enzyme [32], and on the other hand, for intergenus hybridization, primarily alfalfa [34]. There are other, under-researched and / or commercialized methods. Tabacco et al. [36] studied the effect of added tannins (from chestnut) in the amount of 0, 2, 4 and 6% of the dry matter on the degree of proteolysis in alfalfa silage and protein degradability in the rumen. The authors found that the addition of tannins leads to reduced proteolysis, and digestibility of organic matter, which is not good for production.

CONCLUSION

Inhibition of proteolysis in ensiled legumes is most successfully performed by direct acidification of biomass using mineral or organic acids. However, these methods are now abandoned for various reasons and the inoculation of silage

mass can not provide sufficiently low pH values. Past experiments have shown that there are significant differences in the degree of proteolysis between different varieties of the same species of legumes. For this reason, the task of selection in this area should be not only to increase the yield, but also the modification of nutritional value.

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PREDICTION OF METABOLISABLE ENERGY OF FEED FOR POULTRY BY USE OF NON-LINEAR MODELS

Radmilo Čolović[1], Dragan Palić[1], Kedibone Y. Modika[2], Penny Barnes[2]

[1]Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia
[2]Agricultural Research Council, Irene, South Africa

ABSTRACT

Energy value of animal feeds is one of the most important quality parameters. Portion of energy utilized by the animal, i.e. bio-available energy is called metabolisable energy (ME). The most accurate method for determination of ME is by *in vivo* trials. However, this method is often time-consuming and expensive. There has been a need for routine laboratory methods enabling *in vitro* prediction of the *in vivo* ME values of feeds. The aim of this study was to develop non-linear equations to predict the *in vivo* true metabolisable energy (TME_n) of poultry feeds based on *in vitro* determined organic matter digestibility.
Fifty seven samples of feedstuffs and sixteen samples of compound feeds for poultry were used. Adult rooster assay was used for *in vivo* determination of nutrient digestibility. Additionally, enzyme incubation-based laboratory procedure for estimating the enzymatic digestible organic matter (EDOM) was used for same feed samples. Second-order polynomial models were used for regression of EDOM values against the *in vivo* TME_n results. Following models were proposed for prediction of TME_n values: model 1, which includes EDOM and crude fat values, with R^2 (coefficient of determination) value of 0.82, model 2, which includes EDOM, crude fat and crude protein values, with R^2 value of 0.88 and model 3, which includes EDOM, crude fat and crude ash values, with R^2 value of 0.88. The conclusion was that in vivo TME_n of feedstuffs and compound feeds for poultry can be successfully predicted by use of enzymatic digestible organic matter using non-linear models.
***Keywords**: metabolisable energy, poultry feed, non-linear models*

INTRODUCTION

Quality control of animal feeds is commonly based on chemical analysis for determining the composition of the nutrients, e.g. gross energy, protein, etc. It is questionable to what extent the results of chemical analysis of feed reflect its real quality, as these are only slightly influenced by physical and chemical treatments of feed, such as milling, heat treatment, enzyme treatment, etc., which all greatly influence the digestibility and thus availability of nutrients to the animal [3].
One of the most important parameters of feed quality is its energy, since it is needed for execution of metabolitic processes and animal activity. Not all energy of the feed (gross energy) will be utilized by the animal, but only a bio-available

portion called metabolisable energy (ME). This parameter serves as an accurate indicator of feed quality, can be reliably used for feed quality control and is crucial for diet formulation [7]. Metabolisable energy is directly proportional to digestibility of nutrients, as it directly affects their availability and absorption [5].
The accepted method for direct determination of ME of feeds is by *in vivo* trials. These are often expensive and time-consuming. *In vitro* methods used for predicting ME are attractive because of rapidity and low cost [7]. Therefore, there has always been a need for reliable laboratory methods and related equations for prediction of the *in vivo* ME values of feeds, in order to implement an adequate system of quality control [1].
The aim of this study was to develop non-linear equations to predict the *in vivo* true metabolisable energy (TME_n) of poultry feeds based on *in vitro* determined organic matter digestibility.

MATERIAL AND METHODS

Feed material
A total of 73 different feed materials (16 commercial complete diets and 57 feedstuffs for poultry) were used in this study.

Proximate analysys
Standard methods of analysis [2] were used for determination of dry matter (DM), crude protein (CP), crude fiber (CF), crude fat (Fat) and crude ash (CA).

Determination of enzymatic digestibility of organic matter by *In vitro* method (EDOM)
Two-step enzymatic incubation of feed sample, obtained by modification of the three-step procedure [4], was used for EDOM determination. Pepsin incubation for 75 min was followed by incubation with pancreatin for 18 h. Sulpho-salicylic acid was used for precipitation of solubilised protein. After filtration, insolubilised and precipitated materials were collected, dried and finally ashed. EDOM was calculated based on the results from determined DM and CA in the sample and residue, respectively.

Determination of true metabolisable energy by *in vivo* method (TME_n)
For determination of TME_n a procedure for determining digestibility of nutrients ([10], [9], [8]) was used. In this procedure, 50 g of the test feed was introduced into the crop of an adult rooster by means of a stainless steel funnel and tube. Each test feed was replicated among six roosters. The collection period of excreta was 48 hours. Afterwards, the excreta were dried, weighed and analysed. For determination of endogenous energy or amino acid losses, glucose was fed in place of the test ingredient under the same conditions. The endogenous losses were used to calculate the true digestibility of the test nutrient.

Statistical analysis

Statistical software [12] was used for performing regression analysis of experimental data, and for generation of non-linear models for prediction of TME_n values. The TME_n (MJ/Kg DM, Y) was regressed to independent variables by second-order polynomial model. Independent variables in selected models were EDOM (%, x_1), Fat (%, x_2), CP (%, x_3), and CA (%, x_4). Coefficient of determination (R^2) was used for evaluation of adequacy of predicted models.

RESULTS AND DISCUSSION

Model No 1

TME_n (MJ/Kg DM) was correlated with EDOM (%) and Fat (%) with the second order polynomial model, which is expressed with equation 1:

$$Y = a + bx_1 + cx_2 + dx_1^2 + ex_2^2 + fx_1x_2 \quad (1)$$

Where:
Y – TME_n (MJ/Kg DM),
x_1 – EDOM (%),
x_2 – Fat (%).

Table 1. Regression parameter coefficients of model No 1

Parameter	Regression parameter coefficients
Intercept	-7.42744
EDOM (x_1)	0.37391
Fat (x_2)	0.89852
EDOM x EDOM (x_1^2)	-0.00122
Fat x Fat (x_2^2)	0.00303
EDOM x Fat (x_1 x_2)	-0.01006
R^2	0.82

Model No 2

TME_n (MJ/Kg DM) was correlated with EDOM (%), Fat (%) and CP (%) with the second order polynomial model, which is expressed with equation 2:

$$Y = a + bx_1 + cx_2 + dx_3 + ex_1^2 + fx_2^2 + gx_3^2 + hx_1x_2 + ix_1x_3 + jx_2x_3 \quad (2)$$

Where:
Y – TME_n (MJ/Kg DM),
x_1 – EDOM (%),
x_2 – Fat (%),
x_3 – CP (%).

Table 2. Regression parameter coefficients of model No 2

Parameter	Regression parameter coefficients
Intercept	-8.39905*
EDOM (x_1)	0.53975*
Fat (x_2)	0.04983
CP (x_3)	-0.11493
EDOM x EDOM (x_1^2)	-0.00270*
Fat x Fat (x_2^2)	0.00913
CP x CP (x_3^2)	0.00033
EDOM x Fat (x_1 x_2)	0.00298
EDOM x CP (x_1 x_3)	0.00018
Fat x CP (x_2 x_3)	0.00542
R^2	0.88

* $p < 0.05$

Model No 3

TME_n (MJ/Kg DM) was correlated with EDOM (%), Fat (%) and CA (%) with the second order polynomial model, which is expressed with equation 3:

$$Y = a + bx_1 + cx_2 + dx_3 + ex_1^2 + fx_2^2 + gx_3^2 + hx_1x_2 + ix_1x_3 + jx_2x_3 \qquad (3)$$

Where:
Y – TME_n (MJ/Kg DM),
x_1 – EDOM (%),
x_2 – Fat (%),
x_3 – CA (%).

Table 3. Regression parameter coefficients of model No 3

Parameter	Regression parameter coefficients
Intercept	-3.84102
EDOM (x_1)	0.36116*
Fat (x_2)	0.29955
CA (x_3)	-0.25929
EDOM x EDOM (x_1^2)	-0.00134
Fat x Fat (x_2^2)	0.00190
CA x CA (x_3^2)	0.00637*
EDOM x Fat (x_1 x_2)	-0.00314
EDOM x CA (x_1 x_3)	-0.00270
Fat x CA (x_2 x_3)	0.02143
R^2	0.88

* $p < 0.05$

DISCUSSION

Models 1-3 enable prediction of TME_n by combining EDOM and proximate chemical analysis results. Model No 1 includes two independent variables, EDOM (%) and Fat (%), while models No 2 and No 3 include three independent variables, EDOM (%), Fat (%) and CP (%), and EDOM (%), Fat (%) and CA (%), respectively. Therefore, proposed models enable fast prediction of TME_n by enzymatic and standard chemical analysis of two or three nutrients. Regarding the significance of parameters in equations, it can be seen that parameter "b" which stands in front of EDOM (x_1) is significant in two of three equations. This shows that EDOM value is most important data for prediction of *in vivo* true metabolisable energy. Coefficient of determination (R^2) of all three proposed models was above 0.8, which could be considered as a good data fit [11].

CONCLUSIONS

The conclusion was that all three proposed non-linear models could be successfully used in prediction of vivo TME of feedstuffs and compound feeds for poultry by use of enzymatic digestible organic matter.

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OCCURRENCE OF POTENTIAL MYCOTOXIN PRODUCING FUNGI ON MAIZE KERNEL IN HUNGARY

Beáta Tóth[1], Orsolya Török[1], Xénia Pálfi[1], Éva Toldi Tóth[1], Ákos Mesterházy[1], János Varga[2]

[1]Cereal Research Nonprofit Ltd., 6726 Szeged, Alsó kikötő sor 9, Hungary
[2]University of Szeged, Faculty of Science and Informatics, Department of Microbiology, 6726 Szeged, Közép fasor 52, Hungary

ABSTRACT

Maize is one of the most important ingredients of animal feed formulations. *Fusarium* species are important pathogens of maize, causing various diseases, and contamination of maize kernel by mycotoxins including trichothecenes, zearalenone and fumonisins. *Aspergillus* and *Penicillium* species and their mycotoxins including aflatoxins, ochratoxins, fumonisins and patulin are also frequently encountered on cereal products. We investigated the occurrence of these species and their mycotoxins on maize in various maize growing areas in Hungary in 2010 and 2011 years after harvest. Surface-sterilized cereal seeds were placed on selective media, and the isolated fungal strains were identified using morphological methods. 81.94% and 14.33% of the samples were found to be contaminated with potentially toxigenic isolates in 2010 and 2011, respectively. Species identifications of selected isolates have been carried out using sequence-based methods. Regarding *Fusarium* species, in 2010, when the weather was rainy, *F. graminearum* and *F. subglutinans* dominated, while in 2011 with hot and dry summer *F. verticillioides* was the predominant species identified. *F. culmorum* could not be detected in any of the samples. Regarding Aspergilli, several *Aspergillus flavus* isolates were identified, which are potential aflatoxin producers. Besides, other mycotoxin producer species were also isolated, including black Aspergilli which potentially produce ochratoxins and fumonisins, and *A. clavatus*, which produces patulin. Other genera (*Alternaria, Nigrospora, Epicoccum, Cladosporium*) were found in smaller proportions. Besides, the protective maize endophyte *Acremonium zeae* was also identified in some of the samples. Further studies are in progress to examine the mycotoxin producing abilities of the isolates, mycotoxin content of the maize samples, and the applicability of *Acremonium zeae* isolates for lowering fungal burden and mycotoxin contamination of maize.
Keywords: *maize, mycotoxins, aflatoxins, fumonisins, Fusarium, Aspergillus*

INTRODUCTION

Maize is among the most important ingredients of feed formulations worldwide. *Fusarium* species are among the most important mycotoxin producing pathogens of maize. These species cause several diseases on maize including

ear rot and stalk rot (Figure 1), and contaminate maize kernel with various mycotoxins including trichothecenes, zearalenone and fumonisins. The most important maize pathogens are *F. graminearum, F. culmorum, F. verticillioides, F. proliferatum* and *F. subglutinans*. Besides, although not considered to be major causes of plant disease, *Aspergillus* and *Penicillium* species may also be responsible for several disorders in various plants including maize, and most importantly cause mycotoxin contamination [10]. The most notorious plant pathogens are black Aspergilli and *A. flavus* which may cause ear rot and are also important as postharvest pathogens. In contrast with specialized plant pathogens such as powdery mildews, rusts or most *Fusarium* species, these species are opportunistic pathogens without host specialization as proved in *A. flavus* [10]. The most important aspect of food and feed spoilage caused by these organisms is the formation of mycotoxins, which may have harmful effects on human and animal health. Several mycotoxins produced by Aspergilli or Penicillia have been identified in maize in previous studies, the economically most important of which are aflatoxins, ochratoxins and fumonisins [10].
In this study, we examined the mycobiota of maize samples collected in 2010 and 2011 from various maize growing regions of Hungary using morphologhical and sequence-based identification methods.

Figure 1. Fusarium ear rot of maize

MATERIAL AND METHODS

Sample collection

The samples were collected in 9 and 10 maize growing regions in 2010 and 2011, respectively. Maize grains were surface sterilized using ethanol, and plated onto dichloran rose bengal agar media [1]. Plates were incubated at 25°C in darkness and monitored periodically for characteristic mycelium growing from the kernels. Single colonies were purified and transferred to malt extract agar

(MEA) media. Isolates were subcultured as single conidia on MEA, Czapek-yeast extract agar (CYA) and potato dextrose agar (PDA) plates [8].

Identification of fungal isolates

Morphological identification of fungal isolates came from maize grains have been done according to standard textbooks and monographs [2, 6, 8]. For sequence based identification, the cultures used for the molecular studies were grown on malt peptone broth for 2 days, and DNA was extracted from the mycelia using the Masterpure™ yeast DNA purification kit (Epicentre Biotechnol.) according to the instructions of the manufacturer. Parts of the ITS, Tri101 and calmodulin genes were amplified and sequenced as described previously [3, 5]. Sequences were compared using nucleotide-nucleotide BLAST (blastn) with default settings (http://blast.ncbi.nlm.nih.gov) to the Genbank database, and to our own sequence database. Species identifications were determined from the lowest expect value of the BLAST output.

RESULTS AND DISCUSSION

The overall fungal contamination rate of the samples was 81.94% in 2010, while only 14.33% of the samples were found to be contaminated in 2011 (Table 1). The lower contamination rate observed in 2011 is possibly due to the weather conditions. While the summer was rainy in 2010, it was dry and hot in 2011. Possibly due to the humid weather conditions, Penicillia were frequently isolated from the samples collected in 2010, while this genus was virtually absent in the samples collected in 2011. On the other hand, *Aspergillus* infection rates were higher in 2011 than in 2010. The higher prevalence of Aspergilli might have been caused by the warmer weather conditions in 2011, as these species (e.g. *A. flavus* or black Aspergilli) prefer higher temperatures. However, Fusaria were present in the samples in both years in large proportions (Table 1).

The number of primary isolates of each sample was restricted upon the grounds of colony and microscopic features and only the diverging ones were maintained for further investigations. 340 and 90 isolates were recovered in 2010 and 2011, respectively.

Table 1. Occurrence of mycotoxigenic fungi on maize in Hungary in 2010-2011

Year	Average infection of grains (%)	% of isolated fungal strains (based on sequence-based identification)			
		***Aspergillus* sp.**	***Penicillium* sp.**	***Fusarium* sp.**	**other genera**
2010	81.94	2.02	27.56	70.30	0.12
2011	14.33	8.27	0.00	60.25	31.48

Occurrence of Fusaria in maize kernel

In 2010, when the weather was rainy, *F. graminearum* and *F. subglutinans* dominated, while in 2011 with hot and dry summer *F. verticillioides* was the predominant species identified. *F. culmorum* could not be detected in any of the samples, in contrast with the results of the previous 1977 survey [4]. *F. graminearum* favors higher temperatures than *F. culmorum* and the observed shift might be an indication of climate change. However, *F. proliferatum* was detected in both years. Differences could not be observed in the species distribution of Fusaria in different locations (data not shown). Besides, *F. sporotrichioides* and *F. oxysporum* were also identified in some locations. Regarding the species distribution of *F. graminearum sensu lato* isolated from Hungarian maize, all isolates proved to belong to *F. graminearum sensu stricto* based on sequence analysis of the Tri101 gene of the isolates (data not shown). *Fusarium boothii* or *F. meridionale* could not be identified, in contrast with studies carried out in other countries including South Africa and Argentina [7].

Fumonisin and DON content of the maize samples was also analyzed using HPLC-MS. All samples were contaminated by these toxins under the EU limit (data not shown).

Occurrence of Aspergilli and Penicillia in maize kernel

Among the examined samples, several ones were found to be contaminated by members of section *Flavi* of the genus *Aspergillus* based on colony morphology and microscopic features. Although several *Aspergillus* species have been identified recently which are able to produce aflatoxins, *A. flavus, A. parasiticus* and *A. nomius* are the economically most important species regarding aflatoxin contamination of agricultural products [9]. These species can readily be distinguished using sequence analysis of part of their β-tubulin or calmodulin genes [9]. Species assignment of the isolates was carried out using partial sequence analysis of their calmodulin gene. All isolates assigned to section *Flavi* based on morphological features were found to belong to the *A. flavus* species based on sequence data.

Besides *A. flavus*, several other potential mycotoxin producers were identified in the samples. The patulin producer *A. clavatus* and black Aspergilli able to produce both ochratoxins and fumonisins were recovered from several samples. Among black Aspergilli, *A. niger* was identified most frequently (41 isolates), although *A. tubingensis* (10 isolates) was also isolated from some samples. Regarding Penicillia, several mycotoxin producers were identified (e.g. *P. crustosum, P. brevicompactum, P. chrysogenum* and *P. viridicatum*). Other potentially mycotoxigenic genera (*Alternaria, Nigrospora, Epicoccum, Cladosporium*) were found in smaller proportions (data not shown). Besides, the protective maize endophyte *Acremonium zeae* was also identified in some of the samples for the first time in Central Europe [11]. This species has been shown to inhibit infection and mycotoxin accumulation caused by Fusaria and *A. flavus* by producing the antibiotic compounds pyrrocidins [11].

Aflatoxin content of the samples was analyzed using HPLC-MS. None of the samples were found to be contaminated by any of the aflatoxins (B_1, B_2, G_1, G_2). Examination of ochratoxin content of the samples is in progress.

CONCLUSIONS

During a survey of mycotoxin producing molds in Hungarian maize samples in 2010 and 2011, 81.94% and 14.33% of the samples were found to be contaminated with potentially toxigenic isolates, respectively. Regarding *Fusarium* species, in 2010, when the weather was rainy, *F. graminearum* and *F. subglutinans* dominated, while in 2011 with hot and dry summer *F. verticillioides* was the predominant species identified. *F. culmorum* could not be detected in any of the samples. Among Aspergilli, several *Aspergillus flavus* isolates were identified, which are potential aflatoxin producers. Besides, other mycotoxin producing species, including black Aspergilli which potentially produce ochratoxins and fumonisins, and *Penicillium* species producing a range of mycotoxins have also been identified. The most contaminated samples came from 2010, possibly due to the rainy, humid weather conditions. Samples came from 2011 were found to be infected less severely, but the *Aspergillus* infection was higher than in the previous year. Further studies are in progress to examine the mycotoxin producing abilities of the isolates, mycotoxin content of the maize samples, and the applicability of *Acremonium zeae* isolates for lowering fungal infection and mycotoxin contamination of maize.

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EXTRUSION-COOKING IN FEED PREMIXES STABILIZATION

Leszek Mościcki

Lublin University of Life Sciences, Doświadczalna Str. 44, 20-236 Lublin, Poland

INTRODUCTION

The premixes (microelements, vitamins and chemotherapeutics) are the most expensive components and play important role in feed production. Usually added in small amounts, thay can be incorrectly mixed with other components because of its bulk density and suppleness to segregation. Moreover, the water absorbtion and chemical activation taking place during the storage of microelements can reduce the quality of the feed.
Using the extrusion-cooking technique we wanted to check how will it influence on physical and chemical products properties and to see how, the stabilization of feed premixes can be achieved with minimum energy costs and maximum quality effects.

MATERIALS AND METHODS

The typical mineral premix, applied usually to the production of fodder mixture for farm animals, was used in this research. It includes microelements as: Fe, Cu, Zn and Mn. Fifty kg samples of mineral premixmixed with a vegetable carrier as a corn (I variant) and faba beans (*vicia faba*, II variant) in the different proportions of the mineral to the carrier (30/70; 35/65; 40/60; 45/55; 50/50 and 55/45) were prepared for the experiments. Each sample of the mixture was exposed to extrusion-cooking to gain homogenic extrudates. The extrusion-cooking was conducted on the twin-screw extrusion-cooker of the 2S-9/5 Polish design (see fig. 1 and Table 1), with the application of thermal treatment from 140 to 170ºC, 6 mm die, the screw of the 1:3 c.r. and with a screw speed of 1,66 s^{-1}.

Table 1. Technical details of the equipment used

Specification	Extruder
	Z.M.Ch. Metalchem /2S-9/5/ Twin screw counter-rotating
Motor power, kW	30
Max screw speed, rpm	120
Max barrel temperature, ºC	200
Max capacity, kg/h	250
Residence time, s	30-60
L/D ratio	12 (conical)

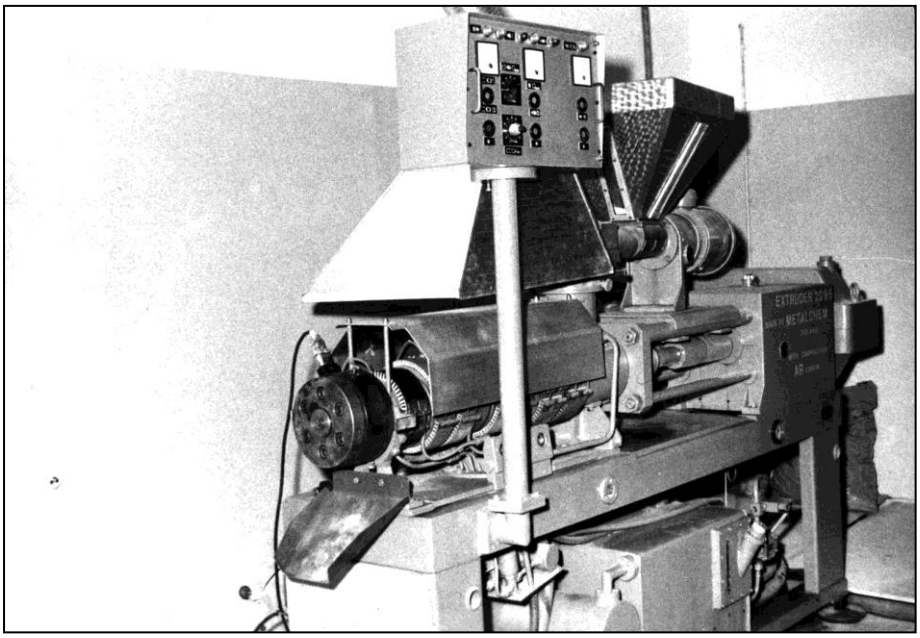

Fig. 1. Twin-screw extrusion-cooker 2S-9/5 (Polish design)

The prepared mixtures were extruded without the addition of water. The obtained product was ground to the size of 0,5 mm - the most common fraction of the typical component included in typical feed mixture.
Homogeneity of extrudates was estimated on the basis of the analysis of the Zn, Cu, and Co content in the sample using standard methods.

RESULTS

Table 2. presents the results of the Zn, Cu and Co designation, both in corn and faba bean extrudates. The data in the table indicate that the average content of the elements in the samples, taken in the initial, middle, and final phases of extrusion-cooking, did not differ significantly from the prescribed content ($P>0.05$) in the entire range of the examined relations of the mineral premixed with the vegetable carrier.
It needs to be emphasized that the variability of the content of the elements appeared to be extremely small and, in the case of Zn, it did not exceed 2%. The coefficient of variation of the concentration of Cu did not exceed 7% in any case, and Co did not exceed 8%.
Bearing in mind that these results were also influenced by the degree of blending before the extrusion-cooking, the obtained coefficients of variation V testifies to the high repeatability of the composition of the product. This was also confirmed by the stability of the composition of the corn extrudate after blending and fractionating with the sieves.

In the 50/50; 45/55 and 40/60 mixtures, the relationship of the mineral fraction to the organic one, the coefficients of variation V did not exceed 2% for Zn, 5% for Cu, and 5.5% for Co.
According to the above discussion we may conclude that during extrusion-cooking, the mineral fraction dilutes regularly in the whole mass of the organic carrier, stabilizing the product in this way. The blended extrudates, which, even after the self-segregation of the grain during the transport, prevents its primary mineral content.
The examination of the physical features of the stabilized feed premixes showed that their bulk density approximated the value of the typical cereal feed components. Moreover, the capability of absorbtion of water or the tractability of dissolution decreased in relation to the qualities of non-extruded materials. Accordingly, the process of extrusion-cooking, even on that scale, appeared to be promising.

Table 2. Zn, Cu, Co concentration in corn and horse bean extrudates [g/kg]

Mineral / Carrier	Compo-nents	Extrudates						Composition
		Corn			Horse bean			
		M	SD	V%	M	SD	V%	
30/70	Zn	39,100	0,245	0,62	38,310	0,802	2,09	39,420
	Cu	3,950	0,040	2,27	3,950	0,082	2,04	4,050
	Co	0,153	0,008	4,98	0,155	0,004	2,58	0,160
35/65	Zn	45,360	0,681	1,50	45,270	0,328	0,72	45,990
	Cu	4,670	0,999	2,14	4,630	0,311	6,71	4,720
	Co	0,177	0,011	6,29	0,184	0,006	3,48	0,187
40/60	Zn	51,630	1,118	2,16	52,410	0,336	0,64	52,560
	Cu	5,320	0,100	1,88	5,200	0,229	4,40	5,400
	Co	0,204	0,014	6,99	0,205	0,005	2,44	0,214
45/55	Zn	59,030	0,980	1,66	58,430	0,608	1,04	59,130
	Cu	6,030	0,100	1,65	5,920	0,142	2,39	6,070
	Co	0,232	0,005	2,21	0,229	0,018	7,96	0,240
50/50	Zn	65,490	0,499	0,76	65,180	0,724	1,11	65,700
	Cu	6,650	0,136	2,04	6,710	0,121	1,79	6,750
	Co	0,249	0,015	6,18	0,261	0,003	1,23	0,267
55/45	Zn	71,170	0,879	1,22	72,210	0,626	0,87	72,270
	Cu	7,220	0,204	2,83	7,310	0,373	5,10	7,420
	Co	0,280	0,013	4,72	0,293	0,006	2,07	0,294

Table 3. Zn, Cu, Co concentration in blended corn extrudates [g/kg]

Mineral / Carrier	Compo-nents	Fraction						Composition
		A	B	C	M	SD	V%	
50/50	Zn	64,80	63,30	62,30	63,47	1,2297	1,94	65,700
	Cu	6,43	6,65	6,48	6,52	0,1153	1,77	6,750
	Co	0,23	0,25	0,23	0,24	0,0115	4,88	0,227
45/55	Zn	57,60	58,30	58,20	58,04	0,3763	0,65	59,130
	Cu	5,98	6,10	6,00	6,03	0,0643	1,07	6,070
	Co	0,22	0,23	0,23	0,22	0,0061	2,69	0,240
40/60	Zn	49,50	50,50	51,20	50,43	0,8934	1,77	52,560
	Cu	5,59	5,32	5,07	5,32	0,2600	4,88	5,400
	Co	0,18	0,20	0,19	0,19	0,0104	5,43	0,214

CONCLUSION

- Extrusion-cooking technique can be applied for the preparation of stabilised, good quality feed premixes, which may have practical value especially during specified, costly bulk feed production.

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FATTY ACID COMPOSITION OF CARP MEAT: A REVIEW

Dušica Ivanov[1], Olivera Đuragić[1], Radmilo Čolović[1], Dragan Palić[1], Rade Jovanović[2], Jovanka Lević[1], Dragana Ljubojević[3]

[1]Institute of Food Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia
[2] Institute of Scientific Application in Agriculture, Bul. despota Stefana 68/b, Belgrade
[3]Faculty of Agriculture, University of Novi Sad, Trg Dositeja Obradovića 3, Novi Sad, Serbia

ABSTRACT

Fish provide significant amount of fatty acids (FAs) with beneficial effects on human health. Omega-3 FAs (n-3 FAs) are present in fish meat in high amounts, even in lean fishes. Long chain n-3 polyunsaturated fatty acids (PUFAs) are essential because mammals, and therefore humans, cannot synthesize them. The aim of this paper was to summarize data about FA composition of carp meat, as one of the most cultured freshwater fish in the world, and especially in Europe. Several studies showed that all carp species are generally rich in n-3 PUFAs, making them potentially attractive in terms of lipid content and composition. The paper also gives a review of factors that influence FA profile.
***Keywords**: carp, fatty acid composition, essential fatty acids*

INTRODUCTION

Potential health benefits attributed to fishes are related to presence of proteins, unsaturated essential fatty acids (FAs), minerals and vitamins. Special attention is paid to polyunsaturated fatty acids (PUFA), particularly omega 3 fatty acids (n-3 FAs). Long chain n-3 PUFAs are essential because mammals, and therefore humans, cannot synthesize them and must adopt them exogenously from dietary sources [5, 19]. They are derived from α-linolenic acid (ALA), which is the precursor of docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids [16]. These two FAs are known to decrease risk of cardiovascular diseases, especially coronary heart disease [11]. The biological effects of EPA and DHA are wide, and involve lipoproteins, blood pressure, cardiac function, endothelial function, vascular reactivity and cardiac electrophysiology, as well as potent antiplatelet and antiinflammatory effects [9].
Fish provide significant amount of FAs with beneficial roles in human health [1]. n-3 FAs are always present in fish meat, even in lean fish [2]. All fishes contain EPA and DHA, which quantities vary among species, environmental conditions, diet, etc [25]. On average, freshwater fish contain half as much EPA and DHA as marine fish [29]. Nevertheless, the differences between fish species may be much greater than between the average content in the two fish groups. It has been reported that the type and amount of FAs in fish tissues are mainly varied by fish feeding, but other factors also influence their FA composition. FA profile

of fish meat is influenced by temperature, geographic location, size or age of animal [3, 31, 26].
Carps represent one of the largest groups of cultured fish with around 70% of freshwater aquaculture production. The origin of carp is in central Asia, but it was spread east and west to China and the Danube [4]. In Asia and Europe, carp was domesticated as an ornamental and aquaculture species. Nowadays, carps are established on every continent except Antarctica. They are widely distributed throughout south-eastern Australia, with smaller populations in western Australia and Tasmania [24]. In these areas they are usually regarded as an exotic pest species. The majority of European carp production is placed in central Europe where it is produced in ponds using traditional semi-intensive techniques. In such conditions, fish growth is very dependent (50% and more) on natural food (plankton and benthos) (Vandeputte *et al.*, 2008). According to FAO report from 2008, the world and the European production of carp was 2987433 tons and 144747 tons, respectively.
This review summarizes the effects of several factors on FA composition of carp meat.

INFLUENCE OF FISH SPECIES ON FATTY ACID COMPOSITION

Species of the fish is the first factor which influences lipid composition in its meat. There are huge differences in muscle lipid content among fish species which also lead to differences in FA composition. Generally, lipids of marine fish contain more highly unsaturated FAs with higher n-3/n-6 ratio than the freshwater fish species [18].
Vujković et al. (1999) [32] investigated the differences in FA composition of meat between silver and bighead carp. According to their results, the muscle tissue lipids of these two species contained approximately the same amount of EPA (3.5 and 3.3%, respectively) and DHA (3.5 and 3.3%, respectively). Nevertheless, sum of DHA and EPA was significantly ($p < 0.05$) higher in bighead carp than in silver carp. Bighead carp also contained more ALA (0.141%, compared to the 0.089%).
Another similar investigation was conducted by Memon *et al.*, 2011[27]. The group of authors examined the differences between FA compositions of three farmed carp fish species raised under the same conditions. The analysis showed that there were considerable differences among the fish studied, although they were fed the same diet. Content of total saturated fatty acid (SFA) ranged from 27.87% (*C. mrigala* muscle) to 29.25% (*C. catla* muscle). In presented study, *C. mrigala* showed the highest percentage (25.08%) of oleic acid and *L. rohita* the lowest one (22.01%). The content of n-3 PUFA was the highest in *C. mrigala*, and n-6 PUFA content was the highest in *C. catla*. All three carp fish species contained arachidonic acid (20:4, n-6), which is the precursor for prostaglandin biosynthesis. This study showed that all carp species were generally rich in n-3 PUFAs, which is of great importance for human nutrition.

SEX AND MATURATION INFLUENCE ON FATTY ACID COMPOSITION

Sex and maturation have strong effect on fatty acid composition in all animals. The development of fatty tissues associated with growth of carp is stimulated by the use of lipid-enriched or high-energy diets. Fat is accumulated in specific adipose tissues, and accumulation of fat has either positive or negative consequences on nutritive value of carp meat, depending on source and composition of fat [14]. Percentage of particular FAs can be influenced by changes in live weight [15]. As Fajmonová *et al.*(2003) reported [13], the growth rate significantly influenced meat composition. The content of monounsaturated fatty acids (MUFAs) increased significantly ($p < 0.001$) with the increasing weight of fish of the same age, while PUFA content decreased ($p < 0.01$). The ratio of n-3 to n-6 PUFA significantly decreased with increasing of growth rate. The decrase in n-3 PUFA was more pronounced than that of n-6 PUFA.
There is not many data about influence of sex on FA composition of carp. According to the investigations of [23], females of Hungarian synthetic mirror carp were fatter than males probably due to later maturation. In study with four common carp hybrids Buchtova *et al.* (2007) [8] found only minor differences in lipid composition between males and females probably caused by different lipid content. Fajmonová *et al.* (2003) showed that females of common carp tended to be heavier than males (2164 g and 1923 g, respectively), but there were only minor differences in FA composition between the sexes [13].

INFLUENCE OF NUTRITION ON FATTY ACID COMPOSITION

Fish reflect the lipid pattern of its diet to a high extent. Carp is traditionally reared in earthen ponds and its nutrition is based on natural food with cereal supplementation [8]. Part of its diet include benthic animals [30]. Plankton and benthos naturally contain high levels of n-3 FA, including EPA and DHA [6, 7, 12]. Thus a proper pond management maintaining sufficient amount and appropriate structure of planktonic and benthic community is of great importance when improving carp FA composition. Cereals are usually used as a supplemental feeding for carp. Since they are rich in carbohydrates and have very low level of n-3 fatty acids, the flesh of the farmed carps generally contains a high level of oleic acid and low level of favorable n-3 HUFA [10]. An alternative approach to influence muscle lipid composition might be the use of biologically active compounds which modulate the fish metabolism to synthesize or deposit more n-3 HUFAs. Supplemental feeding which is rich in ALA could be the alternative way to increase n-3 HUFA content in carp flesh [28].
Ji *et al.* 2011 [20] reported that dietary provision of an appropriate amount of HUFAs (in their case 0.52%) enhanced growth performance and lipid metabolism in grass carp. HUFAs exert their effects by influencing the expression of lipid metabolism-related genes and the activities of their expressed enzymes. However, higher level of HUFAs impaired growth performance, lipid accumulation and immune organ development. They

concluded that grass carp is unable to effectively utilize high levels of these fatty acids. Their results are shown in Table 2.

Table 1. Effect of dietary highly unsaturated fatty acid content on the fatty acid composition of muscle in grass carp (% of total fatty acid) [20] *(Ji et al., 2011)*

Fatty acid	Group				
	0%	0.26%	0.52%	0.83%	1.13%
C14:0	1.40±0.11	1.01±0.02	0.95±0.06	1.19±0.14	1.22±0.05
C16:0	20.04±0.73	19.31±0.37	19.65±0.42	20.06±0.21	16.56±0.66
C18:0	5.84±0.90	6.52±0.11	5.65±0.29	6.46±0.99	4.43±0.07
Total saturated	27.28±1.73	26.84±0.25	26.26±0.16	27.72±1.07	22.21±0.62
C16:1 n-7	4.69±0.24	4.35±0.21	4.18±0.37	3.53±0.50	4.47±0.12
C18:1 n-9	30.78±0.23	28.81±0.34	25.36±0.35	25.32±0.48	25.23±0.78
C20:1 n-9	4.06±1.42	4.06±0.15	2.55±0.20	2.43±0.19	1.89±0.10
Total monounsaturated	39.53±0.95	37.23±0.28	32.08±0.07	31.28±0.82	31.59±0.71
C18:2 n-6	14.20±0.52	13.42±0.50	13.71±0.40	13.54±0.15	13.92±0.37
C18:3 n-3	6.72±1.48	6.64±0.15	8.40±0.17	6.55±0.99	8.97±0.15
C20:5 n-3	2.12±0.49	2.57±0.19	5.53±0.18	5.75±0.37	6.24±0.07
C22:5 n-3	1.45±0.08	1.20±0.02	1.03±0.05	1.22±0.27	2.13±0.01
C22:6 n-3	8.73±0.22	12.10±0.48	12.99±0.44	13.95±0.19	14.94±0.67
DHA+EPA	10.85±0.72	14.67±0.66	18.52±0.44	19.70±0.27	21.18±0.62
DHA/EPA	4.27±0.96	4.71±0.15	2.35±0.12	2.43±0.18	2.40±0.13
Total n-3 PUFAs	19.02±2.12	22.51±0.53	27.95±0.51	27.48±0.51	32.28±0.49
Total PUFAs	33.22±2.63	35.93±0.04	41.66±0.13	41.02±0.46	46.20±0.20
Total n-3 HUFAs	12.29±0.64	15.87±0.68	19.55±0.45	20.92±0.52	23.31±0.63
n-3/n-6	1.34±0.10	1.68±0.10	2.04±0.10	2.03±0.06	2.32±0.10

Results are presented as mean ± standard deviation, n = 3

INFLUENCE OF SEASONAL CHANGES ON FATTY ACID COMPOSITION

It was already mentioned that season have great impact on FA characteristics of carp meat. The FA profile is certainly influenced by temperature. Therefore, it is known that the biochemical contents of fishes undergo changes due to seasonal changes. The concentration of individual FA in different carp tissues changes within the year according to the living activities and fodder availability [22]. Several authors investigated these relations.

Investigations of Guler *et al.* (2008) proved that total lipid content in carp meat is the highest in winter [17]. According to their study, content of SFA was the lowest in spring (26.6%), and the highest in winter (29.6%). Content of ALA was also the highest in spring (1.09%), while it was the lowest in summer (0.38%). However, the highest content of n-3 FA was determined in summer. Results from this experiment are shown in Table 2.

Table 2. Seasonal variations on total fatty acid composition of fillets of carps (Cyprinus carpio) in Beysehir Lake [17]

Fatty acid	Spring	Summer	Autumn	Winter
C6:0	0.17	1.28	0.32	-
C8:0	0.06	0.02	0.12	0.58
C10:0	0.06	0.13	0.1	0.09
C12:0	0.05	0.06	0.1	-
C13:0	-	0.27	0.11	0.04
C14:0	2.24	1.5	1.75	2.09
C15:0	0.76	1.05	0.93	1.09
C16:0	14.6	16.6	15.7	15.8
C17:0	1.4	1.99	1.77	1.41
C18:0	4	5.2	4.17	4.29
C19:0	0.54	-	0.49	0.09
C20:0	1.58	-	0.16	2.4
C21:0	0.72	0.52	0.83	0.42
C22:0	0.36	0.3	0.09	0.34
C24:0	-	-	0.15	0.96
∑SFA	26.6	28.9	26.8	29.6
C14:1 n5	1.22	0.59	0.65	0.67
C15:1 n6	1.19	1.83	0.86	1.08
C16:1 n7	11.2	5.11	5.84	13.2
C17:1 n8	1.36	1.72	1.61	1.53
C18:1 n9	15.1	15.4	20.3	19.6
C18:1 n7	4.39	1.72	2.11	3.52
C20:1 n9	1.01	1.88	3.09	1.48
C22:1 n9	0.19	-	0.10	-
∑MUFA	35.7	28.3	37.3	41.1
C16:2 n4	2.77	3.16	1.64	1.77
C18:2 n6	7.83	8.32	10.5	3.64
C18:3 n6	4.38	2.66	3.52	1.57
C18:3 n3	1.09	0.38	0.66	0.68
C20:2 n6	-	-	0.23	0.15
C20:3 n6	0.92	0.71	1.31	1.15
C20:3 n3	0.7	0.39	0.51	0.57
C20:4 n6	5.38	6.99	5.57	4.38
C20:5 n6	5.69	4.72	4.10	4.82
C22:3 n3	-	-	-	0.13
C22:4 n6	0.71	0.33	0.72	0.95
C22:5 n6	0.46	1.79	0.55	0.98
C22:5 n3	2.89	2.33	2.23	2.11
C22:6 n3	4.94	11.0	4.32	29.3
∑PUFA	37.8	42.8	35.9	29.3
n3	15.3	18.9	11.8	14.7
n6	20.9	22.6	23.3	13.9
n3/n6	0.73	0.83	0.50	1.06

As presented by the results of Vujković *et al.* 1999, content of SFA in silver carp was higher in autumn than in spring (31% and 29.8%, respectively) [32]. They also recorded significant differences in content of MUFA in spring (44.72%) and autumn (47.79%), as well as in PUFA content (25.42% in spring, and 21.03% in autumn). The authors obtained similar results for bighead carp. Seasonal variations of MUFA were more pronounced than with the saturated ones, the content in both fish species being higher in autumn. As both investigated species contain more n-3 FA in spring, having at the same time a more favorable n-6/n-3 ratio, spring harvest fish had a higher biological value.
Another group of authors [21] showed that DHA was predominant FA in muscle lipids of carp in every season. High content of DHA in autumn increased the PUFA content. Their results showed that the content of PUFA was generally much higher than the SFA in spring, summer and autumn.

CONCLUSIONS

In conclusion, it can be said that all carp species have potential to become an attractive fish in terms of lipid content and composition. Since carps have biological predispositions for the n-3 HUFAs synthesis, it would be useful to explore how this synthesis is regulated and if it is possible to increase the effectiveness of this bioconversion. Higher levels of PUFA are mostly originated from the feed. Therefore appropriate diet might be solution for obtaining optimal FA composition of carp meat from the nutritional standpoint.

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AUTOCHTHONOUS BREED OF CHICKEN IN SERBIA: RESEARCH OR DEVELOPMENT

Zlatica Pavlovski, Zdenka Škrbić, Miloš Lukić

Institute for Animal Husbandry, Autoput 16, 11080 Zemun -Belgrade, Serbia

ABSTRACT

The results of the study of the quality eggs and chicken meat of native Naked Neck hen populations and hybrid chickens of hens from several experiments are presented. Chickens and hens were reared in the extensive system, on the experimental poultry farm of the Institute for Animal Husbandry, Belgrade-Zemun according to Institute's technology.
The aim of this study was to investigate whether the autochthonous breeds in Serbia represent only the basis for research or there is a realistic opportunity through product diversification for improvement of existing production.
***Keywords**: autochthonous breed, eggs, chicken meat, quality*

INTRODUCTION

Modern industrial production of eggs is based on the use of battery (cage) systems for keeping laying hens. Compared to the extensive, semi-intensive and floor system of housing hens, battery system has shown significant advantages (higher capacity, lower feed consumption, cleaner egg shells, better utilization of the production facility, facilitate mechanization and automation of certain business processes, increased worker productivity, etc.).
However, for several decades in many Western countries, the movements for protection of animal welfare strengthened, with the objective, among other things, to ban battery system as inhuman and unnatural. Therefore, in some European countries the system was banned in 2012, and in other countries ban of the battery system can be expected in the near or the distant future. Many participants in the chain of production of eggs and poultry meat, under the new legislation - Directive concerning the welfare of poultry, origin of eggs and meat, and consumer opinion, have developed a very serious strategy of production and sale of eggs and chicken meat. By polling consumers in the EU, and in our country, it was concluded that most consumers are willing to pay more for products from those industries that provide the welfare with the hope that this will satisfy the most demanding customers.
There is still no significant market production of table eggs in non-cage systems. This is due primarily to the fact that so far there were no regulations stipulating provisions obligating the producer to introduce changes in the housing. Current Animal Welfare Act [9] does not expressly prohibit keeping hens in battery cages. The law says only that "the owner or holder of the animals are required to provide adequate and safe housing for animals and micro-climatic conditions, hygiene, plenty of space, freedom of movement, food and water, which

corresponds to the type, race, gender, age and physical, biological, production needs and behavioural needs of the animal".
However, the recently adopted Regulation on conditions for the welfare of animals [10] closer defines the conditions of housing and determines this field entirely according to the model of the European Union. Thus, under Article 53 of this Ordinance "natural and legal persons and entrepreneurs can rear laying hens in not-enriched cages by 1 January 2012 [8]". The EU Directive which regulates this field was adopted in 1999, and the ban was designed in two stages - from 2003, the installation of battery cages prohibited, and from year 2012, housing of poultry in them is prohibited.
Of course, this development has motivated researchers in some countries to examine new alternative housing systems for layers and broilers that technologically and economically could be at least close to the conventional poultry keeping, and with ethological and ethical point of view to satisfy the requirements of animal protection activists for human and natural conditions in poultry housing.
Bearing all this in mind, the Institute for Animal Husbandry has developed a technology for the production of eggs and chicken meat in the free range rearing system [6, 12], which is based on the production of chickens of coloured plumage. In this sense reference is made to domestic Naked Neck chicken and Sombor Crested hen reared in our country for long time.
In this paper we present the results of our research of the quality of meat and eggs obtained from Naked Neck and Sombor Crested hen reared according to said technologies of the Institute in order to reach a conclusion whether the autochthonous breed in our country is only basis for research or is it possible to achieve improvement of the production.

MATERIAL AND METHODS

In the first part of the paper, the results of the table quality of eggs obtained from laying hens of two autochthonous breeds - Naked Neck and Sombor Crested hen, which are reared in Serbia, are presented and hybrid Hy Line and Lohman Brown reared in two production systems (extensive and battery) on the experimental farm of the Institute for Animal Husbandry, Belgrade -Zemun. The major external (egg mass, and eggshell color, shape index) and internal qualities of eggs (yolk color, albumen height, Haugh unit, egg white and yolk ratio) and shell quality traits (weight, thickness, deformation, shell breaking force) are examined.
The second part describes the meat quality traits of chicken genotypes: autochthonous breeds Banat Naked Neck and hybrids Arbor Acres, Farm Q and Red Bro. The tests were performed on the experimental farm of the Institute for Animal Husbandry, Belgrade-Zemun, where chickens were reared in extensive system, and the duration of fattening period lasted 50 [1] to 98 days [2, 3]. The body mass of chicks at the end of fattening, slaughter yields and shares of major carcass parts were examined.

RESULTS AND DISCUSSION

In Table 1, the traits of egg quality are presented.

Table 1. Some quality characteristic of eggs of different genotype

Trait/ Genotype	Pavlovski et al., 2010. *[5]		Škrbić et al., 2011.**[11]		Pavlovski et al., 2012.***		
	HL	NN	LB	NN	LB	NN	SC
Egg weight, g	64.54	53.77	63.69	60.13	65.24	57.45	59.17
Eggshell colour, point	3.37	2.25	3.43	2.03	3.86	2.03	2.06
Eggshell cleanliness, point	4.58	4.24	3.73	4.63	4.91	4.72	4.40
Eggshell deformation 0.001mm	21.57	24.82	21.17	27.23	20.21	26.85	24.74
Egg shape index	77.89	74.68	77.43	74.00	78.10	75.78	77.18
Eggshell breaking force, kg	2.68	2.39	2.66	2.21	2.57	2.23	2.39
Eggshell weight, g	8.87	6.97	8.96	7.59	8.91	7.33	7.87
Yolk colour, Roche	11.52	13.03	11.93	12.90	12.64	12.34	11.91
Albumen high, 0,1mm	84.20	68.83	74.57	68.00	82.06	69.81	67.92
Eggshell thickness, 0.01mm	34.88	24.82	35.93	31.60	34.45	27.18	28.87
Haugh Units	89.63	83.39	84.63	79.97	87.31	83.27	81.36

HL - Hy Line; LB - Lohmann Brown; NN - Naked Neck; SC - Sombor Crested
* Pavlovski et al. (2010) - the average value of traits for the production cycle presented
** Škrbić et al. (2011) - the average value of traits of layers at the age of 52 weeks
*** Pavlovski et al. (unpublished data) - the average value of traits of layers at the age of 50 - 54 weeks

Analysing data on the quality of eggs of different genotypes shown in Table 1 there is a relatively poor quality of eggs laid by Naked Neck compared to the hybrid chickens, actually lower weight, lower quality and poorer inner shell quality. The difference in the quality of the eggs was also identified between autochthonous breeds. Eggs obtained from Naked Neck had more intensive colour of egg yolks and better quality of egg whites, expressed by albumen height and number of Haugh Units, while eggs from the Sombor Crested hens had greater weight, thicker and tougher shell, and higher shape index. Shell colour of eggs of autochthonous hens was lighter and represents a genetic trait.
Based on obtained data in the study [7] on proximate composition, fatty acid profile and cholesterol content of eggs from two genotypes (hybrid, Naked Neck), it can be concluded that slight advantage in regard to the nutritional quality were demonstrated by eggs from Naked Neck hens.
From data presented in Table 2, significantly lower body weights and yields of New Hampshire and Amrok (pure breed), autochthonous breed (Naked Neck) compared to hybrids(Indian River, Arbor Acres, Red Bro, Farm Q) are observed. Naked neck had greater bone strength than hybrids, confirmed by the research [13]. Tibio-tarsal strength expressed through the breaking force was significantly higher in the Naked Neck varieties.

Table 2. Some quality traits of chicken meat different genotype

Trait/ Genotype	**Pavlovski et al., 1992.[1]**			**Pavlovski et al., 2009. [2]**			**Pavlovski et al., 2009.[3]**		**Pavlovski et al., 2009[4]***	
	NH	**A**	**IR**	**W**	**B**	**G**	**AA**	**RB**	**FQ**	**NN**
Body mass, kg	1.39	1.32	2.31	1.11	1.08	1.00	1.92	1.62	1.37	1.29
Yield, %										
Tradition. dressed carcass	79.9	80.9	83.0	75.1	75.1	76.3	83.4	83.1	79.4	75.4
Ready to cook	72.2	73.0	76.1	67.9	68.0	68.8	76.7	75.9	72.4	68.1
Ready to grill	61.3	62.1	66.7	58.4	58.5	59.0	67.3	65.8	62.6	58.7
Yield of most important parts of carcass, % of body mass										
Breast	-	-	-	13.6	13.2	13.4	-	-	12.6	13.6
Thigh	-	-	-	9.3	9.6	9.7	-	-	-	-
Drumstick	-	-	-	11.0	10.1	11.6	-	-	21.4	19.7

NH - New Hampshire; IR - Indian River; A - Amrok; W - white variety of Naked Neck; B - black variety of Naked Neck; G - gray variety of Naked Neck;
AA - Arbor Acres; RB - Red Bro; FQ – Farm Q; NN - Naked Neck
* Pavlovski et al. (2009) – presented data for thigh + drumstick

CONCLUSIONS

Presented results confirmed the presence of differences in the egg quality between autochthonous breeds in traditional prodution and eggs of hybrid layer hens from coventional prodution, as well as differences in changes of certain egg quality properies with the age of hens. In general, determined correlation coefficient between major egg qualityproperties and age of hens are in favour of longer efploation period of Naked Neck hens in condition of traditional production.
It can be concluded that slight advantage in regard to the nutritional quality were demonstrated by eggs from Naked Neck hens.
In general, chickens of autochthonous Naked Neck breed and hybrids in extensive production system and duration of fattening 98 days, do not realize the body mass which is adequate to present standards for fattening chickens. Also, their yields, conformation measures and shares of major carcass parts are significantly below minimum acceptable values. This indicates the need for further research of the quality which would confirm that investigated chickens have considerably better meat quality, which is suitable and in compliance to demands of the consumers which prefer natural food of specific and guaranteed quality for which they are ready to pay higher price.
Finally, we can conclude that the autochthonous breeds of chickens and hens are the basis for further research on the one hand, and on the other hand, the opportunity to introduce new products to the market thus realizing poultry product diversification and development of the existing production.

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SPECIFICS OF GOAT NUTRITION IN THE PRODUCTION CYCLE

Nurgin Memiši[1], Miroslav Žujović[2], Snežana Ivanović[3]

[1]AD Mlekara – Subotica, Tolminska 10
[2]Institut of Animal Husbandry, Belgrade – Zemun
[3]Institute of Veterinary Medicine of Serbia, Belgrade

ABSTRACT

This paper reviews current practices and procedures undertaken in the preparation of food for feeding goats and some specificity in their diet during the production cycle. For proper growth, development and high milk production and the proper balancing of meals in goats, it is important to know the changes in their metabolic cycle throughout the year. Otherwise, all these changes are primarily caused by latitude and climatic factors. As with cattle and sheep during the production cycle in goats during the year, followed by periods in which it creates in the body reserve nutrients in their body, compared to periods when they are consumed. Goats compared to sheep and cows show significant differences in pasture habits, physical activity, the needs in water, the choice of feed, milk composition, carcass composition, metabolic diseases and parasites.
Intensive and modern production in goat today is mostly based on the utilization of conserving nutrients in order that their prolonged use, and concentrated feed, which are various methods adapted to the needs and maximum use of the various categories of goats. When using such a prepared feed, based on precise standards and using software for preparing meals and mixtures can be achieved excellent results, with the genetic potential of the goat can reach maximum expression.
Goats are ruminants and well-utilized coarse forages: pasture, hay and silage, and therefore make it their primary food. Height of production, therefore, will depend largely on the quantity and quality of these nutrients.
***Keywords**: nutrition, goats, food preparation, standards, production cycle*

INTRODUCTION

For proper growth, development and high milk production and proper balancing rations for goats, it is very important to know the changes in their metabolic cycles throughout the year. Otherwise, all these changes are primarily caused primarily latitude and climatic factors. As with cattle and sheep during the production cycle of goats, during the alternate periods in which they create in the body reserve nutrients in your body, compared to periods when they consume [15,12,13]. Because of the seasonal sexual activity and start kidding goats in our conditions, which usually falls in the winter months, goats some time after birth increases milk production in that period are not able to consume enough nutrients to meet this production. It is a period in our country at the beginning of

spring and early summer. However, during the late summer, fall and early winter, before the new parturition, there is a decrease of milk, as well as a complete cessation of secretion. Because of this, the goats in this period can consume more food than is necessary for the production of milk and fruit growth (initial period of gestation), and thus can generate body resources necessary for the next lactation [7,8].

Metabolism of goats is the lowest level in early autumn, about three weeks before the start of the breeding season. During this period, the quantity of milk is significantly reduced. Food that is most appropriate at the time was rough and dry. As soon as the production is expected to stabilize at a lower level, appetite goats begins to increase. Nutrition during the next month, it is very important both for its fertility, and the creation of body reserves. The meal should be rich in cellulose, and energy, and for the most part should consist of dry forage, with the addition of some concentrates. Energy value meal should be like the best time of production [10].

When one breeding season is over, goat milk production and appetite gradually declining until the middle of winter. Meals at this time and should still be rich in cellulose and energy. From the middle of winter, metabolic processes are accelerated gradually. During this period, the goats should provide enough quality food.

POSSIBILITY OF DRY MATTER INTAKE IN GOAT NUTRITION

The data presented in Figure 1, it can be seen that in the first three months of pregnancy weight goats gradually increases from 2 to 4 kg. This is the period when the body resources begin to accumulate in the body. In the next 6 to 7 weeks that follow (high gestation period - the 4th and 5th month of pregnancy), weight of goats continues to grow and increased an average of 6 to 9 kg, and at the expense of increasing the mass of the uterus and its contents (fetus and membranes).

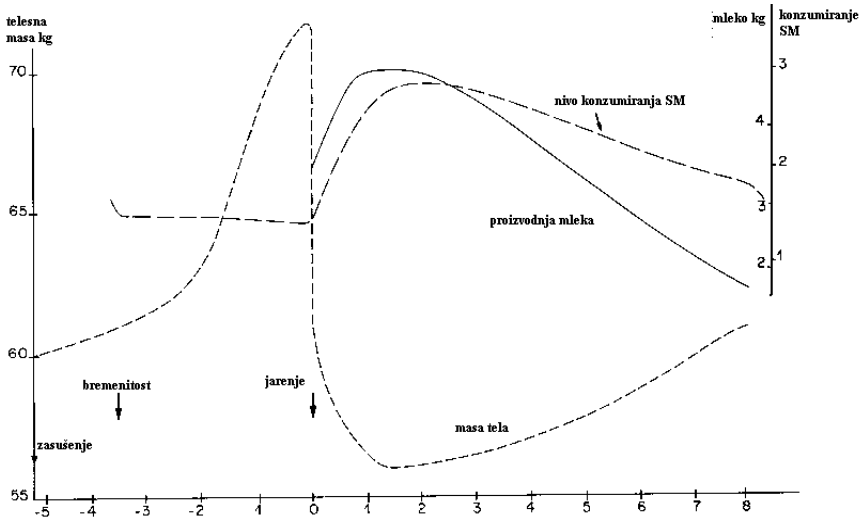

Figure 1. Changes in body weight, milk production and dry matter intake of food in goats in the production cycle [3]

Greater consumption of voluminous feed during periods of high pregnancy is considered good, because so fed animals continue with this type of diet and after birth, and this will have a positive influence on the consumption of concentrates. The result is higher production of milk. Also, with so fed goats rarely have problems with digestion, which can happen when a sharp increase in the consumption of concentrate in the first period of lactation.

After kidding, body weight of goats is decreasing due to insufficient intake of food, but also for the active mobilization of body reserves [11]. This reduction begins to stagnate at the end of the second month of lactation. In the first month of lactation, body weight animals is reduced by about 3 to 6 kg, and only in the last 5 or 6 weeks of lactation goats began to rebuild reserves and increase its mass to between 0.6 and 1.9 kg for the month days. This increase in body weight and body reserves accumulation as well as intensive as well as their mobilization in the initial period of lactation.

Ability dry matter intake of food was at a certain level in the initial period of gestation goats. After the breeding season, goats consume less food and produce less milk until the middle of winter, when the expected birth. In this period, the meals should continue to be rich in energy and cellulose [12].

Volume of food consumption DM decreased significantly in the period after birth. It is believed that the dry matter intake during the initial period of lactation, on average, smaller by about 15 - 20% as compared to the rest of lactation. That is why the production of milk in the first period of lactation, a significant part takes place on the basis of body reserves mobilized during peak breeding and the animals in this period is in negative energy balance. Maximum food consumption DM is achieved only a few weeks (about 3 to 4) after the peak reached in milk production [11]. In this period it is very important and should strive to be in the

shortest possible period increase the volume of food consumption in goats. This can largely be achieved primarily by proper balanced diets in the representation of all nutrients, then giving the meal quality forage (hay, alfalfa, red clover, ryegrass, cocksfoot, etc.), providing some parts of concentrated feeds in the diet, etc.
Dietary quality forages and concentrates, high production breed goats can consume up to 5 - 8 kg dry matter food per 100 kg body weight of goats, which is about two times higher than in sheep and cows (Table 1). If goats feeding a good quality forages, can consume between 2.5 and 3.0 kg of body weight DM/100 kg. The largest consumption of DM in goats in lactation period, are between 6 and 10 weeks of lactation, and a few weeks after reaching the peak in milk production. Consumption decreases significantly in the last 6 to 7 weeks of gestation high-goat, because considerable growth of mass fetus. In this period, ie. late gestation, goats can consume about 2.0 kg to 2.5 kg DM/100 kg body weight. Otherwise, goats that are in the second year of life, consuming up to 50% greater amount of food in the compared to those of the old year, while in goats in the third year of life increases the volume by 10% compared to the goats at the age 2 years.

Table 1. Average consumption DM of foods in dairy goats [14]

Consumption DM	Average	Min	Max
- Daily per head , kg	2.24	1.04	3.60
- Daily per 100 kg of body weight of goats	3.94	1.63	6.81
- Daily g / kg $BW^{0,75}$	108.12	47.14	181.18

Preparation and preservation of forage are very important and can significantly reduce the consumption of food if the procedure is not properly carried out. Phase in which the plant hair hay storage, significantly consumption of DM and the amount of milk production. Influence of roughage level on dry matter intake, as well as the dispersal, is shown in Table 2

Table 2. Influence of quality hay on dry matter intake and wastage of hay in nutrition of goats [9]

The quality of hay	consumption DM / kg day	Hay wastage% of the offered
good	1.7 – 1.9	15
medium	1.4 – 1.7	15 – 20
weak	1.0 – 1.4	20 – 25

GOAT NUTRITION IN THE PRODUCTION CYCLE

During the last weeks of pregnancy weight increases more slowly, and in some cases leads to the cessation of mass increase before birth. Period of gestation according to their dietary needs can be divided into two periods. The first period

covers the first 15 weeks of gestation, and the goats in that period dont have any special need for enhanced nutrition. If goats have access to good quality pasture, they meet all their needs. During this period, the amount of milk is significantly reduced, and also appetite of goats gradually decreases to the middle of winter. Need for nutrients during this period were at their maintenance requirements and are directly dependent on the body weight of the animals.

Table. 3. Requirements of goats in nutrients for maintenance [14]

Parameter	Body weight, kg	Dry matter intake, kg	Energy Requirements		Protein Requirements	
			TDN kg-d	ME Mcal-d	MP,g-d	DIP, g -d
Mature goats, early lactation, milk yield 2.06 – 3.22 kg						
	40	1.97	1.05	3.77	178	94
	50	2.30	1.22	4.41	205	110
Mature goats, mid lactation, milk yield 1.47 – 2.30 kg						
	40	1.96	1.04	3.74	156	93
	50	2.26	1.20	4.33	178	108
Mature goats, late lactation, milk yield 0.88 – 1.38 kg						
	40	1.69	0.89	3.23	121	81
	50	1.96	1.04	3.75	140	94
Mature bucks, prebreeding						
	50	1.36	0.72	2.59	58	65
	75	1.84	0.97	3.51	78	88

Depending on the growing system, for goat with body weights of 50 kg daily ration could consist of 5.0 kg pasture or 1.6 kg of hay. For goats with body weight of 60 kg, the daily ration was increased to 5.25 kg pasture or 1.75 kg hay.
The second period of pregnancy covers the last month and a half before the birth. In terms of nutrition, this is the most critical in the whole annual production cycle of goats. Requirements of goats in all nutrients significantly increased during this period (Table 3). During the period of pregnancy goats to increase their body weight for 10 to 25%. In the first half of pregnancy, weight changes are minor, and even undesirable, to the end of gestation increased rapidly. Otherwise, the goats requirements for maintentance with body weight of 50 kg, during this period are 40 NEL 5.04 g DCP, 3.5 g Ca and 2.5 g P [9].
Since the per unit body weight of goats can excrete up to 1.5 times more dry matter than dairy cows, their nutrient requirements are high. For high milk production, which is carried to the high necks, the importance of quality forages are more prominent *[2]*. Now it must take into account the dose using concentrated feed as forage crops are not enough to satisfy all the needs of animals. Mixture of concentrate are similar to the ones which obtained dairy cows with the proportion of protein from 12 to 18%. At the same time, the proportion of concentrate in the total ration is 40 - 60% [4,5]. Otherwise, goats requirements with body weight of 50 kg, for production of 1 kg of milk with 3.5%

milk fat is 7.91 NEL, DCP 96g, 8g Ca and 4.5 g P, and as the need for maintenance of 5, 04 NEL, 40g DCP, 8 g Ca and 4.5 g P [9], need for vitamins and minerals in the diet goats usually standardized by adding the premix in an amount of 1% (or 2% for goat in lactation). In the first month and a half days of lactation goats need supplemental feeding concentrate diets. After that, the amount of concentrate should be reduced gradually, so that 2 - 2.5 months after kidding, there is no need to use them, if of good quality hay. This is particularly the case if the goats are kept on good quality pastures.

Table 4. Nutrition program of goats with concentrate, green forages and hay [1]

Category	Concentrate	Green forages and hay
High Production goats	400 g/for one liter of milk and the addition of 150 g for maintenance	*ad libitum*
Buck	0,5 to 1,0 kg	*ad libitum*
Young goats	250 g	*ad libitum*
Pregnant goats	300 – 500 g	*ad libitum*
Lactating goats	300 to 400 g for each liter of milk	*ad libitum*

If milk production is high, the goat meal should include the maximum amount of high-quality hay (preferably legume hay), with the addition of concentrate mixtures containing sufficient amounts of protein, minerals and vitamins [6] to meet the high production and good health of animals (Table 5). Grass or legume hay are equally acceptable. With the increasing part of legumes in the diet, the need to proteins in the mixture of concentrate are reduced.

Table 5. Hay and grain rations for dairy herd [3]

Forages	The level of protein in the feed concentrate (%)	Mineral mixture to be used
Legumes or mixture of grass most legumes	14 to 16	Mixture with lots of phosphorus
Grasses or mixture	16 to 18	Mixture Ca:P 2:1

As a rule, in order to obtain the large amounts of goats milk in the next lactation period, with 6 - 8 weeks before kidding it is desirable that they had to dry. This is the period when the animals recovered their body on good resting mammary (udder) for the next lactation. The dry goats need more protein, energy, calcium and phosphorus than the goats that are not fertilized and do not produce milk, but less on the needs of lactation. Needs of dried of goats can often provide the only forages.

In this period of feeding is recommended to use meadow hay in the diet, since legumes such as alfalfa hay, contain large amounts of calcium, which can initiated in goats occurrence milk fever *[11].* If the goats during this period in a worse condition, the meal should be increased. Preparation of goats for kidding usually begins 10 to 15 days earlier. This is the period when requirements to make certain changes in goats diet, in order to adequately prepare for the kidding and subsequent lactation. These changes are made because of the adaptation of rumen microorganisms to meals high energy values, which are used in the initial period after kidding, and to ensure the increased need for nutrients goats. That means engaging grains in the diet goats. Meal cereal grains then needs to make half of the total amount of food that goats should be given in the first 15 days after giving birth. For high milk production in the post-kidding, should be added to the diets and minerals, and vitamins in the premix. The importance of minerals and vitamins is higher if milk production is higher *[8,16].*

CONCLUSIONS

For proper growth, development and high milk production and the proper balancing of meals in goats, it is important to know the changes in their metabolic cycle throughout the year. Otherwise, all these changes primarily are primarily caused by latitude and climatic factors. As with cattle and sheep during the production cycle in goats during the year, followed by periods in which it creates in the body reserve nutrients in their body, compared to periods when they are consumed. Goats compared to sheep and cows show significant differences in pasture habits, physical activity, the needs in water, the choice of feed, milk composition, carcass composition, metabolic diseases and parasites.
Intensive and modern production in goat today is mostly based on the utilization of conserving nutrients in order that their prolonged use, and concentrated feed, which are various methods adapted to the needs and maximum use of the various categories of goats. When using such a prepared feed, based on precise standards and using software for preparing meals and mixtures can be achieved excellent results, with the genetic potential of the goat can reach maximum expression.

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NUTRITIONAL VALUE OF RYE (*SECALE CEREALE L.*), TRITICALE (*TRITICO SECALE*) AND THEIR USE IN ANIMAL NUTRITION

Zuzana Formelová, Mária Chrenková, Ľubica Chrastinová, Mária Poláčiková

Animal Production Research Centre Nitra, Hlohovecká 2, 951 41 Lužianky, Slovak Republic

ABSTRACT

The objective of this work was to determine nutritive parameters of rye, variety Fernando (grown in three different localities in Slovakia) and triticale (variety Kendo). Results of chemical analyses show significant differences ($P<0.05$) in content of nutrients among the studied rye samples. Protoplasmic proteins in rye grain reached up to 40% proportion out of the total amount of proteins and higher concentration of lysine. Effective degradability of organic matter varied from 74.5% to 85.7% and crude protein was degraded in rumen to 80% on average. Intestinal digestibility reached the value 67.91%. We tested the suitability of rye and triticale for nutrition of monogastric animals on broiler rabbits. Complete feed mixtures with 22.5% portion of these cereals were tested. Higher content of crude protein, fibre, lower content of fat and nitrogen-free extract in triticale increased the value of digestible and metabolizable energy in feed mixture. We found significant differences in digestibility of dry matter, crude protein, fat, organic matter, neutral detergent fibre ($P<0.05$), and in acid-detergent fibre ($P<0.01$) among the studied feed mixtures.
We can summarize that rye and triticale represent cereals with higher portion of good quality proteins, high energy and nutritional value, and they can be incorporated into feed mixtures for ruminants and broiler rabbits (during the fattening phase) as a component of good quality. Nutrition-physiological specifics must be respected at feeding higher doses to individual animal species and categories.
***Keywords**: rye, triticale, nutritional value*

INTRODUCTION

Quality of feeds and their change in digestive tract influences the production of animal products, economy of production and environment as well. The quality of feed mixtures for farm animals depends on energy content and quality of protein components. In our country and in the world are mainly wheat and barley the plants mostly used for feeding. Less attractive cereal is rye, which is seldom used in feed-processing industry, although it is a cereal of good quality. It is possible to achieve high efficiency with it. Because of different opinions of rye utilization in animal nutrition it is necessary to inquire into this problem more deeply. Potential of triticale in feeding of non-ruminant animals is noticed by the

organism in full, due to the presence of non-starch polysaccharides, to which, first of all, belong pentosans, most part of which from arabinoxylans [Kairov et al., 2010]. This limits the utilization of these crops in feeding, and especially during intensive growth and fattening period [Semenov et al., 2009]. Rye is second only to wheat for flour production. As feed, rye is not relished by livestock, so rye grain is usually fed in mixtures with other cereals.
The objective of our work was to determine certain nutritional parameters of selected rye varieties for their utilization in nutrition of cattle, i.e. content of nutrients and nutritive value, effective ruminal degradability of crude protein and organic matter, and intestinal digestibility of by-pass crude protein and compare the quality of two grains rye variety Fernando, and triticale variety Kendo from the viewpoint of their suitability for nutrition of monogastric animals.

MATERIAL AND METHODS

Subject of testing was rye variety Fernando grown in different localities in Slovakia (Dobra Niva, Cerovo and Ružomberok). The content of nutrients in individual rye samples and in the remains after every feed incubation in the rumen were determined according to the Decree of the MA SK 1497/4/1997 - 100 Bulletin of the MA SK. We determined proteins by the method described by Barnstein. We used extraction ICC method in modification by Michalik et al. [1989] to determine proteinous fractions in rye. We determined degradation of organic matter and crude protein in the mentioned rye samples by the method in sacco [Ørskov and McDonald, 1979], acting upon Harazim and Pavelek [1999]. Three non-lactating cows (Black Pied breed, average live weight 550 kg) with large rumen canulas were used. The animals were fed the maintenance ration consisting of lucerne hay, maize silage and cereal meal (mixture of wheat and barley in ratio 1:1) two times a day. Roughage created 70 % dry matter in feed ration. The feeds were incubated 2, 3, 4, 6, 9, 16 and 24 hours (3 bags for each feed, animal and incubation). Bags with size 13x8 cm were made of Uhelon T 120; pore size 47µm. After incubation we rinsed the bags under running water to remove large impurities, washed them in automatic washing machine in cycle without spinning (3 x 5 min), and rinsed them in distilled water. We dried the bags at temperature 60^{o}C for 24 hours, weighed the non-degraded residues and mixed them for each feed and incubation. We used equations recommended by Ørskov and McDonald [1979] to calculate the effective degradability, taking into account washing losses by the programme Neway (Rowett Research Institute, Aberdeen, Great Britain).
Intestinal digestibility of crude protein was determined in rumen undegraded residues from the rye samples using mobile bags method [Van Straalen et al.,1993].
In the second part of the experiment, we used a total of 56 weaned rabbits (35th day of age, male sex, Hycole hybrid) divided into 2 experimental groups. The rabbits in 1st group were fed granulated mixture including 22.5% triticale variety Kendo. The rabbits in 2nd group were fed granulated mixture including 22.5% rye variety Fernando. The experiment lasted for 32 days. Rabbits were kept in

standard cages, 2 animals per cage. In fattening experiment were studied the growth of live weight and consumption of feed mixtures per unit of live weight growth. Between 65 and 70 days of age, 5 rabbits from each group were selected for digestibility tests using the balance method. The digestibility test was performed in accordance with the recommended methodology [Meartens and Lebas, 1989]. The samples of individual feeds were analyzed for content of nutrients according to procedures of the AOAC [1990], and starch according to the alpha-amyloglucosidase method. Metabolizable energy content was calculated by the equation of Wiseman et al., [1992]. Rabbits were fed ad libitum. The diet formulation (pellets of 3 mm diameter) for all groups is presented in Table 2. Data were treated by the one-way ANOVA. The means and standard deviation (SD) of the generated data per group were determined. For the subsequent statistical analysis the data were examined for significant differences using the Tukey-test.

RESULTS AND DISCUSSION

Average chemical composition of the studied variety of rye is in table 1. The data show differences in content of nutrients. We found significant differences ($P<0.05$) among surveyed localities. We found the highest level of crude protein (131.0 ± 0.87 $g.kg^{-1}$DM) in the variety Fernando from the locality Dobrá Niva, which is a significant difference compared with the other samples. Their variability is the result of environmental influences [Aman et al., 1995]. The grain of rye is marked by increased content of protoplasmic proteins of albumins and globulins; they represented up to 40% proportion out of the total amount of proteins in the tested rye. High content of albumins and globulins, which are richer in essential amino acids in general, suggests that the biological value of rye proteins is better than in wheat [Lásztity, 1984]. The highest content of prolamins (26.97%) and glutelins (18.78%), and lower content of albumins and globulins was determined in the rye Fernando from the locality Dobrá Niva. Content of lysine is in rye proteins on average by 40% higher than in wheat, and by 20% higher than in triticale. Content of lysine is a determinant factor for quality of cereal proteins [Čerešňáková et al., 1990]. Its content varied from 0.315 (Cerovo) to 0.344 $g.kg^{-1}$ DM (Dobrá Niva). High content of easily soluble saccharides, which determine the energy value of rye, reflected itself in the content of metabolizable energy the concentration of which represented on average 13.6 $MJ.kg^{-1}$ DM. Present systems of quality evaluation in feeds for ruminants necessitate besides content of nutrients also data about their degradation in rumen. We calculated parameters of degradation and effective degradability of organic matter and crude protein from values of disappearance in individual incubations. We found significant differences among the rye localities ($P<0.01$) in degradation speed of fraction "b" in crude protein, which was high in all tested samples of rye; equally high was also the speed constant (c) of organic matter degradation. High values of insoluble and degradable fraction "b" were determined in all studied rye samples, however, the potentially degradable fraction "a + b" was lower than 100. It appears from this that a part of

crude protein from rye is not degraded in rumen and passes to the small intestine. Crude protein from rye is degraded in rumen on average to 80 %. After 16 hours of rye incubation in rumen was degraded 90 % of crude protein from the original content. The remaining 10 % passes into duodenum (*by-pass* crude protein). Feeds with higher content of fibre have lower intestinal digestibility of crude protein [Chrenková et al., 2000]; our results proved it also, the intestinal digestibility of crude protein in studied rye was 67.91%.

Table 1. Content of nutrients in g.kg^{-1}DM in tested rye, and proportion of protein fractions out of total content of proteins

Locality / Item	Fernando			Significance of differences
	Dobrá Niva(1)	Cerovo(2)	Ružomberok(3)	
Crude protein	131.0	116.6	118.7	$1{:}2{,}3^{+++}$; $2{:}3^{++}$
Protein	83.3	91.4	83.3	$1{:}2^{+}$,$2{:}3^{+}$
Fibre	27.9	22.4	24.1	$1{:}2{,}3^{+++}$;$2{:}3^{++}$
Fat	17.5	19.4	20.6	$1{:}2^{++}$,3^{+++}; $2{:}3^{+}$
Starch	558.2	613.4	586.0	$1{:}2{,}3^{+++}$;$2{:}3^{+++}$
Total sugars	74.3	72.3	80.7	$1{:}2^{+}$,3^{+++};$2{:}3^{+++}$
Organic matter	978.9	982.5	981.8	$1{:}2^{+++}$,3^{++}
ME	13.5	13.6	13.6	-
Lysine	0.344	0.315	0.342	
Alb. + glob.(%)	36.52	40.20	39.98	-
Prolamins (%)	26.97	23.93	22.17	-
Glutelins (%)	18.78	16.96	17.34	-
N-residual (%)	17.36	18.28	19.56	-

$^{+}$ P<0.05; $^{++}$ P<0.01 and $^{+++}$ P<0.001

Differences in average content of individual nutrients were noticed not only in varieties of rye and triticale but also in complete feed mixtures. Higher content of crude protein, crude fibre and lower content of fat and nitrogen free extract in triticale variety Kendo increased the value of digestible and metabolizable energy in mixture (Table 2). Significant differences were in digestibility of dry matter, fat, organic matter (P<0.05) and neutral detergent fibre (P<0.01) in mixture containing rye (Table 3). Results of balance for nitrogen, calcium and phosphorus (% of retention N 68.12%; Ca 81.6%; P 60.1%; Mg 82.8%; Na 87.3% with feeding the feed mixture containing triticale compared with N 68.12%; Ca 81.9%; P 69.7%; Mg 78.7%; Na 88.87% retained at feeding the feed mixture with rye) confirmed the efficiency in nutrition of rabbits up to 56th day of age. With regard to average content of nutrients (crude protein 170 - 175 g, fat 18 - 26 g, fibre 154 - 158 g, starch 161 g and metabolizable energy 9.4 - 9.7 MJ.kg $^{-1}$) we recommend practical utilization of mixture containing triticale and rye for the finishing phase of fattening in broiler rabbits. This composition of nutrients in mixture suits better also the physiological demands of breeding rabbits.

Table 2. Content of nutrients and energy value in experimental mixtures, and triticale and rye in original matter (in g)

Item	Chemical analysis (g/kg)		Chemical analysis (g/kg)	
	mixture with included 22.5 % of triticale variety Kendo	mixture with included 22.5 % of rye Fernando	of the grain Triticale variety Kendo	of the grain Rye variety Fernando
Dry matter	906.32	897.85	866.18	869.49
Crude protein	174.8	170.45	130.35	104.81
Fat	21.42	18.10	14.28	19.18
Crude fibre	154.37	157.68	31.54	24.55
Nitrogen-free extract	488.86	485.33	669.82	702.02
Ash	66.86	66.29	20.18	18.93
Organic matter	839.46	831.56	846.00	850.56
Calcium	11.11	10.51	0.73	0.49
Phosphorus	3.67	4.25	2.72	2.17
[1]ADF	172.42	169.65	3.75	2.75
[2]NDF	304.74	293.4	1.15	3.13
[3]DE ($MJ.kg^{-1}$)	10.24	9.92	12.03	12.08
[4]ME ($MJ.kg^{-1}$)	9.73	9.43	11.43	11.47

[1]Acid-detergent fibre, [2] Neutral-detergent fibre, [3] Digestible energy, [4]Metabolizable energy

Table 3. Digestibility of nutrients in feed mixtures in %

Nutrients	Mixture with included 22.5 % of triticale variety Kendo	Mixture with included 22.5 % of rye variety Fernando
Dry matter	61.01 ± 0.76	63.33 ± 1.49 +
Crude protein	68.12 ± 2.16	72.60 ± 2.34+
Fat	64.04 ± 3.71	70.27 ± 2.74+
Crude fibre	19.64 ± 0.76	19.35 ± 2.21
Nitrogen-free extract	73.07 ± 1.11	74.76 ± 1.42
Ash	51.94 ± 3.66	54.85 ± 1.67
Organic matter	61.73 ± 0.94	64.01 ± 1.56+
Calcium	81.63 ± 3.12	81.92 ± 2.35
Phosphorus	60.08 ± 2.81	69.67 ± 3.20+
[1]ADF	13.31 ± 1.12	20.92 ± 3.96++
[2]NDF	28.52 ± 1.57	32.39 ± 1.71+

+P < 0.05; ++P < 0.01

CONCLUSIONS

Rye represents a cereal with higher content of good quality proteins, high energy and nutritive value. Rye is a source of disposable energy in the rumen of ruminants with respect to high degradability of organic matter. Feeding of triticale and rye to rabbits did not influence zootechnical parameters, as well as it had no negative effect on growth performance and did not influence negatively the health status of rabbits.

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TESTING OF CERTAIN CHEMICAL QUALITY CHARACTERISTICS OF VARIOUS SERBIAN MEAT PRODUCTS

Marija Vukašinović[1], Vladimir Kurćubić[2], Vesna Kaljević[1], Pavle Mašković[2], Milun Petrović[2], Slavica Vesković-Moračanin[3]

[1]Veterinary Specialist Institute, Žička 32, 36000 Kraljevo, Serbia
[2]Faculty of Agronomy, Cara Dušana 34, 32000 Čačak, Serbia
[3]Institute for Meat Hygiene and Technology, Kaćanskog 13, 11000 Belgrade, Serbia

ABSTRACT

The goal of our research was to examine the certain chemical quality parameters in samples of liver pate, canned meat pieces, bacon and dry meat products. The second objective was to compare values obtained in our study with reference values in Rule book on the quality of minced meat, semi-finished meat and meat products. Three reference chemical methods: determination of nitrogen, phosphorus and moisture content (ISO 937:1992, SRPS ISO 13730:1999, ISO 1442:1998), as well as determination of hydroxyproline by M050 "in house" method were used. We concluded that from total number of tested liver pate (n=15), 3 samples (20 %) does not match the quality requirements provided by Rule book, 2 samples (13.33 %) because lower total protein content (TPC) and 2 samples (13.33 %) because higher relative protein content of connective tissue (RPCCT). The most common chemical quality failure of examined canned meat pieces (n=47) is lower TPC (43 - 87.76 %). The values of total phosphate content was within the limits prescribed in the Rule book in all tested samples (n=8) of Bacon. From a total of 25 tested samples of dry meat products (beef prosciutto), 2 samples (8 %) had the higher water content than 60 %. All tested samples of Smoked sirloin (n=5) was in compliance with the Rule book to the values obtained for RPCCT.
***Keywords**: meat products, total protein content, relative protein content of connective tissue, total phosphorus content, total moisture content*

INTRODUCTION

Consumers are now much more aware of the impact of diet on their health and, consequently, the greater the interest in the nutritional value of food. Given the tendency for healthy eating, we set the task to examine some meat products, in order to gain insight into the nutritional value of these products. The importance of the link between nutrition and health becomes more and more a hot topic. We can consider 3 periods over the last 25 years with different emphases within a continuous growth of scientific knowledge of the ''Meat Processing Technology'':

(a) Period of quality (b) Period of quality and food safety (c) Period of quality, food safety and nutrition/health.
Quality of meat products became more standardized on a known level and growing knowledge of the meat processing technology resulted in an economically driven new product development towards products at a higher food safety risk [15].
Phosphates and polyphosphates are the most commonly used functional additives in the meat industry. As such, they deserve credit for the water-binding, improving the texture of products and stabilize the product [1]. Adding large quantities of additives, phosphates and polyphosphates may have consequences for human health. The impact on human health is not determined only by the amount entering the body, but also technological performance and quantity. The addition of phosphate and polyphosphate in excess of technological necessity changed the very nature and nutritional value of food.
Increased amounts of influence on the retention of large quantities of water in the product, which is directly proportional to reduced product quality [9].

MATERIAL AND METHODS

Samples

In this study, 15 samples of cooked meat products (Liver pate), 47 samples of Canned meat pieces, 8 samples of Bacon, 25 samples of Dry meat products and 5 samples of Smoked products originating from a number of manufacturers registred in the territory of four Serbian districts: Zlatibor, Moravica, Raska and Rasinski were analized.

Analytical Methods

Liver pate, Canned meat pieces, Bacon, Dry meat products and Smoked products were examined by standard (1-3) and modified (4) chemical methods in Chemical Laboratory of Veterinary Specialist Institute "Kraljevo":
1. Determination of total protein content (TPC) - SRPS ISO 937:1992 [10].
2. Determination of total phosphorus content - SRPS ISO 13730:1999 [12].
3. Determination of moisture content - SRPS ISO 1442:1998 [11].
4. Determination of relative protein content of connective tissue (RPCCT) - method M 050

Statistical Methods

Test results were statistically analyzed and presented in tables, as mean values ($\bar{x}$), standard error of mean values ($S_{\bar{x}}$), standard deviation (SD, percentage) and interval of variation (min/max). Descriptive statistics and determine the level of significance t-test was conducted using statistical software Statistica statistical software for Windows Release 5.0. [13].

RESULTS AND DISCUSSION

The protein content in cooked sausages - liver pate must not be less than 8-9 % and the relative protein content of connective tissue in meat protein should not exceed 25 %-30 %. The reason for the discrepancy in the two cases was lower TPC (13.33 %), while the other two cases (13.33 %) lower RPCCT.
According to the Regulations on the quality and conditions of use of additives in food and other requirements for additives and their mixtures [4], the maximum amount of phosphate that is added to meat products (diphosphate - E 450, triphosphates - E 451 and polyphosphate - E 452), singly or in combination, can be at most 5.0 g/kg, expressed as P_2O_5.
According to *Codex Alimentarius* standard for cooked cured ham 96, the maximum amount of total phosphate in this meat product is 8000 mg/kg and the maximum amount of added phosphate (mono-, di- and poly-sodium and potassium salts) is 3000 mg/kg, expressed as P_2O_5. Of the 14 respondent's sausage, one-half (50 %) did not meet the quality requirements of the *Codex*, due to lower TPC [6].
Stojković et al. (2010) was determined total phosphorus content by standard ISO (spectrophotometric) method, expressed as P_2O_5 (g/kg): canned between 0.19 g/kg P_2O_5 and 0.51 g/kg P_2O_5; in sausages of 0.42 g/kg P_2O_5 (fermented sausage) to 0.47 g/kg P_2O_5 (boiled sausages), the dry meat products of 0.53 g/kg P_2O_5 up to 0.58 g/kg P_2O_5 and bacon of 0.42 g/ kg P_2O_5.
The values of total phosphate content in all tested samples (n=8) of Bacon was within the limits prescribed in the Regulations [4] (Table 4.). Saicic et al. (2008) are determined by testing 41 samples of Bacon originating from domestic producers that the value of total phosphate was in the interval of variation from 1.31 to 6.64 g/kg, with an average value of 3.58±1.17 - all products were in compliance with the requirements of the Regulations [4], as well as in our study.

Table 1. Descriptive statistics of the cooked meat products (Liver pate) chemical composition

Name of product	Chemical composition	$\bar{x}$	$S_{\bar{x}}$	SD	Interval of variation	
					min	max
Liver pate (n= 15)	TPC	9.125	0.297	1.151	7.200	10.710
	RPCCT	19.461	2.487	9.631	6.360	47.970
	P_2O_5	2.139	0.210	0.812	1.440	4.860

From a total of 47 samples tested Canned meat pieces, 43 (91.49 %) was incompatible with Regulation, because in all samples value of TPC was lower than prescribed (Table 2.).

Table 2.Compatibility of the Canned meat pieces quality with the Rule book (Official Gazette of RS, no. 31/2012)

No.	Name of product	Compatibility			
		+	-	< TPC	> P_2O_5
Canned meat pieces					
1	Chicken breast in the gut (n= 17)	2	15	15	-
2	Ham in the gut (n= 28)	1	27	27	-
3	Pizza ham (n= 2)	1	1	1	-
	Σ = 47 samples	4 (8.51 %)	43 (91.49 %)		

Table 3.Descriptive statistics of canned meat pieces chemical composition

Name of product	Chemical composition	$\bar{x}$	$S_{\bar{x}}$	SD	Interval of variation	
					min	max
Chicken breast in the gut (n= 17)	TPC	13.592	0.298	1.228	12.200	16.220
	P_2O_5	4.476	0.203	0.836	3.010	6.560
Ham in the gut (n= 28)	TPC	12.786	0.270	1.431	8.130	16.230
	P_2O_5	4.635	0.146	0.773	3.050	6.500
Pizza ham (n= 2)	TPC	11.390	2.030	2.871	9.360	13.420
	P_2O_5	4.840	0.530	0.750	4.310	5.370

From Table 3. we can observe that the mean values for TPC for the first two tested products (Chicken breast in the gut and Ham in the gut) were below the limit, and the Pizza ham above the limit by Regulations. Total of 39 "cooked ham type products", products of pork and poultry meat, from Belgrade market, were examined by Živković et al. (2007). Basic chemical quality parameters were analyzed. Total protein content in pork meat products varied from 9.82 % to 16.82 %. In poultry meat products, total protein content was somewhat higher (11.38 % to 21.10 %). The greatest variations were detected regarding the connective tissue protein content.

Table 4. Descriptive statistics of Bacon chemical composition

Name of product	Chemical composition	$\bar{x}$	$S_{\bar{x}}$	SD	Interval of variation	
					min	max
Bacon (n= 8)	P_2O_5	4.651	0.315	0.890	4.651	5.870

From a total of 25 samples tested dry meat products (beef prosciutto), 2 samples (8 %) were the higher water content of 60 %, and as such are put out of trade (Table 5.). Saicic et al. (2010) observed that in the group of Dry meat products no significant differences ($p > 0.05$) in protein content between samples of pork ham and pork sausages (38.07 % and 38.2 2%, respectively), while in

samples of beef ham and pork neck protein content is significantly different ($p \leq 0.05$) and amounts to 39.85 % and 31.51 %, respectively). Moisture content is in the range of 38.99 % (pork ham) to 48.07 % (beef prosciutto) with a statistically significant difference in the obtained values ($p \leq 0.05$). The results for the beef prosciutto are consistent with the findings Radovanovic et al. (2003, 2004).

Table 5. Descriptive statistics of Dry meat products chemical composition

Name of product	Chemical composition	$\bar{x}$	$S_{\bar{x}}$	SD	Interval of variation	
					min	max
Beef prosciutto (n= 25)	H_2O	51.678	1.538	7.689	34.540	64.080

Table 6. Descriptive statistics of Smoked products chemical composition

Name of product	Chemical composition	$\bar{x}$	$S_{\bar{x}}$	SD	Interval of variation	
					min	max
Smoked sirloin (n= 5)	RPCCT	21.500	0.910	2.033	18.550	23.900
	H_2O	5.690	0.636	1.423	3.990	7.790

All tested samples of Smoked sirloin was in compliance with the Regulations to the values obtained for RPCCT (table 6.), and one sample (20%) was taken out of trade due to the higher content of total phosphate (7.790 g/kg).

CONCLUSIONS

Our results suggest that producers use very diferent recipes in attempts to produce "low cost" products, to get closer to customers. The most common chemical quality failure of examined canned meat pieces (n=47) is a lower content of total proteins, like nutritive most valuable substance (43 - 91.49 %).The values of total phosphate content was within the limits prescribed in the Rule book in all tested samples (n=8) of Bacon. From a total of 25 samples tested dry meat products (beef prosciutto), 2 samples (8 %) were the higher water content of 60 %. All tested samples of Smoked sirloin (n=5) was in compliance with the Rules to the values obtained for RPCCT.

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COMMON FOOD BORNE BACTERIAL DISEASES OF POULTRY

Ljiljana Suvajdzic[1], Jovanka Levic[2], Maja Velhner[3], Sava Pavkov[4], Aleksandar Potkonjak[5], Dubravka Milanov[3], Ivana Cabarkapa[2]

[1]Department of Pharmacy, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia
[2]Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, Novi Sad, Serbia
[3]Scientific Veterinary Institute "Novi Sad", Novi Sad, Serbia
[4]Institute Gosa, Milana Rakića 35, Belgrade
[5] Department of Veterinary Medicine, Faculty of Agriculture, University of Novi Sad, Trg Dositeja Obradovica 8, Novi Sad, Serbia

ABSTRACT

Though a variety of bacteria are responsible for numerous poultry diseases worldwide, only some of them cause enormous financial losses on daily basis. Those ubiquitous economic pests, which are transmissible via the feed, are addressed in this paper. Clinical entities encompass general septic state and infections and/or intoxications of the digestive system. Prevention of the infection in the flock is essential, overwhelming the effects of successful medicamentous therapy. Antimicrobials may reduce the mortality and morbidity; however, their application is not always economically justified. The problems are further aggravated by escalating and highly frequent multiple resistances to almost all known antibiotic categories. In that respect, global tendencies implicate reduction of massive antibiotic therapy and a shift towards effective prevention measures and introduction of plant extracts as feed supplementation. Such an approach could considerably reduce the incidence of leading bacterial infections in poultry caused by infective agents from the feed, thus decreasing the rate of disease outbreaks. Numerous plant preparations proved effective against *Salmonella* spp*., Escherichia coli, Pasteurella multocida, Staphylococcus aureus* and *Clostridium perfringens,* which are the most important pathogens causing enormous financial losses worldwide. It is necessary to formulate a combination of synergistic preparations effective against the entire spectrum of aforementioned organisms.
Key words: *food borne, bacterial, diseases, poultry*

INTRODUCTION

Bacteria are the oldest, the most manifold and most useful inhabitants of our Planet. Not only most of them are not detrimental for human and animal health, but also the living world would not survive without them and our planet would look much more different. Many microbes are valuable for their host, as well as in food and drink processing, fermentation process, and decay [19]. However,

some bacteria can cause fatal diseases in humans and in animals. Such diseases can cause considerable economical losses in cattle and poultry industry. Almost every bacterial taxon can cause disease in poultry [14]. Still, some genera and species are more frequently than others, whereas only few of them cause significant economical losses on daily basis all over the world[1]. Such ubiquitous economic pests, which are transmissible via the feed, are particularly addressed in this paper [2]. Once infected, the poultry manifests the poor general condition as well as the symptoms in the respiratory, digestive and locomotors organs. From the medical point of view, all clinical manifestations are important; however, economic pests are by definition bacteria that cause septic state or infections and/or intoxication of the digestive tract [3]. It is to be emphasized that prevention of the disease within the flock is more important than a successful and effective medicamentous therapy. Antimicrobial treatment results in reduction of the mortality and morbidity, but its application is not always economically justified. Besides adequate sanitary and hygienic measures, successful control of bacterial diseases includes prompt isolation and identification of the causative agent, as well as the prevention of its further transmission and spread within the flock.

Salmonellosis and Paracolon Infections - *Salmonellae* are Gram-negative rods belonging to the family *Enterobacteriaceae*. They cause diarrhea syndrome more frequently than other members of the family do. Unfortunately, they also cause toxic shock syndrome. The classification of salmonella is based on their antigenic composition. They have four types of antigens: somatic, flagellar, membrane and fimbrial, though the last two are rarely found. Salmonella serotype/species is determined by serotyping. Gender *Salmonella* encompasses over 2000 serotypes and most of them are potentially pathogenic for poultry [9]. Although they can cause systemic infection, *Salmonella* species are primarily intestinal pathogens, whilst members of the Arizona group induce paracolon infections. Because of their mutual similarity, these infections are addressed in the same chapter. Both diseases are widespread all over the world.

Fowl typhoid and Pullorum disease (*Salmonella gallinarum* and *Salmonella pullorum*)-Fowl typhoid and pullorum disease are septicemic diseases largely specific to avian species. They are caused by *S. gallinarum* and *S. pullorum*. Although they were first described more than one century ago, they still remain a serious economic problem to livestock in countries where measures of control are not efficient [12]. The infection has acute or chronic course. Chickens and

[1]*Campylobacter* spp. are frequently isolated from poultry (they are important cosative agents) and could be food borne, but they are not causing great losses in poultry industry. They are more important for human patology (human food borne disease) then for poultry patology. *Haemophilus pargallinarum, Mycoplasma gallisepticum, Mycoplama synoviae, Chlamydia psitaci, Borrelia anserina* etc. are important cosative agents of poultry diseases and dominantly transmited by aerosole or vector. Some other bacterial species (*Erysipelas insidiosa, Clostridium botulinum, Streptococcus spp, Arcanobacterium piogenes, Mycobacterium avian)* are not so frequently isolated, but we cannot qualified them as non important.

turkeys are primarily affected, but other domestic, wild birds and pets could be infected as well. The diseases are primarily transmitted via the eggs, but also by mechanical route, from bird to bird, via contaminated objects and the litter, as well via the feedstuff. Carrier birds are of highest epidemiological importance, as they continuously shed *Salmonella* and subsequently become a permanent source of infection. Major portals of entry are respiratory or digestive tract. **Causative agent** *S. pullorum* causes Pullorum disease, known as bacillary white diarrhea in chicks, transovarial transmission. *S. gallinarum* causes fowl typhoid at all age categories, mainly adult birds, egg transmission [13]. **Signs and lesions**: Pullorum disease is highly contagious (often fatal) for young chickens, pullets and pheasants, whereas adult birds are more resistant to the infection. Young birds could succumb to the disease without showing any symptoms (peracute form of the disease). An epidemic outbreak usually affects the birds younger than 3 weeks. The mortality rate could be as high as 90% if the infection remains unnoticed and the birds fail to receive a prompt and appropriate therapy. Survivors are rare. The following clinical symptoms may indicate the infection: the birds are falling down, feathers are ruffled, the birds tremble and manifest difficulties by breathing, the birds gather close to the heat and white viscous feces around cloaca is symptomatic. Fowl typhoid occurs primarily in young adult birds (mostly older than 12 weeks). The symptoms include sudden sporadic deaths within the flock, prostration, green or yellow feces around the cloaca, loss of appetite, increased thirst, and pale wattles and crest [3]. **Diagnosis**: Preliminary diagnosis relies on clinical signs. Isolation and identification of the pathogen is however, required for final diagnosis. In older birds, laboratory diagnosis is established by serology methods; however, additional bacteriological confirmation is desirable. Common bacteriology examination includes enrichment in Selenite - F Broth, inoculation onto Salmonella - Shigella (SS) agar or other selective solid medium, slide agglutination with poly- and monovalent sera and confirmation using biochemical parameters. **Prevention**: The only way to prevent the disease is to eradicate it from the flock. Only *S. pullorum* - free birds can be used in breeding and production. Presence of *S. pullorum* is monitored by special agencies, and poultry producers are obligated to purchase eggs, chicks and birds only from the certified flocks. **Treatment**: Antibiotics are administered in the feed. In spite that treatment could be highly effective and result in complete recovery of individual birds, such flocks are not recommended for further production [15].

Paratyphoid Infection (other *Salmonella*) - The term paratyphoid infection was introduced analogous to human diseases associated with febrile symptoms, which are caused by salmonellas. In chickens, paratyphoid encompasses any of over 2000 *Salmonella* species and serotypes, except *S. pullorum* and *S. gallinarum*. Majority of birds, reptiles and mammals cold be the carrier of one or more of the known serotypes. The infection occurs as an acute or chronic one. Acute infection occurs more frequently in young birds. Most of paratyphoid infections occur in birds younger than 4 weeks, except in pigeons and canaries who can suffer the acute stage of the disease with high mortality rate at any age.

The disease causes significant economic losses in poultry industry, particularly in turkey production. **Causative agent:** *S. enteritidis*, *S. typhimurium*, *S. hadar*, *S*. *infantis* and many other species. **Diagnosis**: The disease can be suspected according to anamnestic data, clinical symptoms in the field, and specific pathological changes in organs during necropsy. The final diagnosis is confirmed by isolation and identification of the agent in bacteriology laboratory. **Prevention and Treatment:** During the acute epidemic courses, the antimicrobial therapy can reduce the death rate during the acute stage of the disease. However, the antibiotic treatment cannot eliminate the infection from the flock after an epidemic outbreak. The carrier birds should thus be removed from the egg-production line. In that respect, prevention of salmonellosis and continuous veterinary monitoring and control (including bacteriology examination) is of outmost importance [10].

Paracolon Infections (*S. arizonae*) - *S. arizonae* cause severe infections, enteritis and septicemia in chicks and turkey poults and is transmitted via the eggs. Some serovars are associated with reptiles. It occasionally infects other animals. The causative agent of paracolon infections is similar to some species of the genus *Salmonella*, as well as to some commonly found coliform microorganisms. They are classified into the *Arizona* group, which can be distinguished from Salmonella only by the use highly sophisticated bacteriological methods. This large group of organisms is widespread in the nature, affecting same hosts / carriers as the *Salmonella* species. In that respect, the symptoms, signs, patho - anatomical changes as well as transmission routes and therapy is analogous to paratyphoid infections [16].

Colibacillosis - Colibacillosis encompasses a range of infections and/or intoxications caused by *Escherichia coli*. This is a common terminology for the large group of various diseases including omphalitis, reproductive disorder, peritonitis and sepsis. The infections are caused by *E. coli,* a bacterium that resides in the intestinal tract of all warm-blooded animals. The affected poultry can be treated with antibiotics. In the past few years, the organism has been identified as a major cause of morbidity, mortality and economic losses in poultry and turkey flocks. The incidence and severity of infections caused by *E. coli* and other coliform organisms' shows an increasing tendency, and the current trends suggest that these infections will become even bigger problem in the future (Alan et al.). The diseases caused by this agent are highly diverse, and may remain unapparent or extremely severe, associated with sudden outbreak and high mortality rate. The infection can have a subacute or acute, chronic or subchronic course, affecting the respiratory tract (droplet infection), digestive tract or enter the systemic blood stream (sepsis). The most frequent entities are acute septicemia, peritonitis, pericarditis, salpingitis, sinovitis/osteomyelitis, panophtalmitis, coligranuloma, swollen-head syndrome, avian cellulitis, and enteritis [4]. Any of the clinical entities may occur independently or combined. The disease frequently (but not necessarily) occurs as secondary infection to primary *Mycoplasma gallisepticum* or viral infection. All age categories can be

affected, but young birds are more susceptible to the infection and suffer much more severe course of the disease. Acute septicemia occurs more often in young turkeys, whilst chickens are more susceptible to droplet infection (air borne transmission). High mortality rate is usually associated with yolk sac infection leading to omphalitis. To prevent the spread of the infection, proper management of the waste material, adequate carcasses disposal, good hygiene and disinfection of the environment, feathers and equipment applying commercial disinfectants [1] is necessary. **Causative agent:** The disease manifested as infection or intoxication is caused by several serotypes of *E. coli*, which normally reside in the intestines of humans and other mammals, birds and reptiles. Moreover, it plays a very important role in the host's body. The colonization of the intestinal system after birth must not be prevented. However, many serotypes are toxin-producers and their uncontrolled multiplication in the body can result in severe disease, even death. The infection occurs commonly as a respiratory or intestinal. The infection of chicken embryo can occur from the egg yolk or if the bacteria penetrate the egg shell. **Signs and lesions**: Symptoms could be highly diverse, depending on the type of infection. The acute/septic course may result in sudden rapid death, without visible symptoms. In most cases, the symptoms occur at least as general signs of infection, such as ruffled feathers because of fever. Chronic stage of the disease is characterized by apparent stunting. The symptoms of the respiratory infection include sneezing and heavy breathing with crepitations. Enteritis is usually associated with diarrhea. High mortality in newborn chickens is mostly due to *E. coli* - induced omphalitis. **Diagnosis**: Different diagnostic criteria may lead to confusion with other infectious agents. Sole isolation of *E. coli* is not sufficient for establishing the diagnosis. Besides the precise biochemical identification, patho-anatomic changes, presence of other potential agents and the type of organ from which the organism was isolated should be taken into consideration. Diverse commercial strips for differentiation of genders and species within the family *Enterobacteriaceae* are obtainable at the market; however, the classical biochemical protocol including Kligler agar, Clark and Lubs media, adonite, inozit, urea by Christensen and SIM is much cheaper and readily available and thus is to be recommended [22]. **Prevention and treatment**: Strict sanitary monitoring and surveillance of the flock is the best method to prevent epidemic outbreaks of the disease. The response of the agent to antimicrobials is unpredictable and unreliable, and it is difficult to estimate the success of the therapy. In the everyday practice, the treatment is often disappointing, with unstable results, thus medicamentous therapy is not the method of choice. The susceptibility to antimicrobials varies from strain to strain. Some strains are susceptible, whereas others are completely resistant to common antibiotics. Resistotyping could be useful in determining the proper treatment, but displacement of birds into a clean space sometimes proved more successful than the therapy itself. In an infection outbreak on a turkey farm, the dislodgement and separation of infected and healthy birds was more effective than antimicrobial therapy [4].

Omphalitis, Embryo and Early Chick Mortality - Omphalitis could be defined as a navel infection. Usually, it occurs if the navel does not heal well. It is transmitted by contact. **Causative agent**: Besides the coliform bacteria, the disease is often caused by *Staphylococcus, Streptococcus, Pseudomonas* and other microorganisms than can induce mixed infection. The disease usually occurs as a consequence of poor hygiene, overheating or cold after hatch. The transport could also be a weak link in the chain. The importance of particular isolates is often questionable since the same organisms could be found in healthy birds after hatch as well as in the yolk sack. **Signs and lesions**: Infected chickens are somnolent, they have difficulties to stand and walk with the down being "puffed up", they are generally inferior, apathic, and they do not eat or drink and stream to be close to the heat. Diarrhea can occur as well. Death usually occurs within 24 hours, reaching the maximum rate between day 5 and 7. Characteristic lesions implicate an unhealed navel, subcutaneous edema, blue color around the navel, retention viteli of bad smell. Often yolks are ruptured, and subsequently peritonitis develops. **Diagnosis:** Preliminary diagnosis is established based on anamnestic data and typical appearance. Sometimes, various agents are isolated, thus requiring further bacteriology confirmation. **Prevention and treatment**: Appropriate management and sanitation procedures in the hatchery and during the first few days following hatching are the only reliable ways to prevent omphalitis. Dirty eggs should not be placed into the hatchery cabinet. Broad-spectrum antibiotics help reducing mortality and stunting in affected groups, but they do not replace sanitation [4].

Fowl cholera - The disease is widespread in all regions where chickens are raised. Since recently, it has become the leading infection in turkey production. The disease occurs in all age categories and in all birds' species including wild and ornamental birds (chickens, turkeys, pigeons, pheasants, waterfowl, sparrows and other flying birds). **Causative agent**: The disease is caused by *Pasteurella multocida*. It is a small Gram-negative rod with bipolar staining features, especially in primoisolates. Even though Gram-negative, the organism is oxidase positive and penicillin-sensitive. *P. multocida* survives 30 days in droplets and even 2-3 mounts in soil. It invades the host through digestive and respiratory tract. The vertical transmission, via the eggs, is not possible. The reservoirs of infection are sick birds and healthy carriers as well as infected carcasses. Some further infection sources include excretions of sick birds and carriers that contaminate water, food, equipment etc. Contaminated water sources such as open pulls, ponds, lakes and rivers are predilection sites for infection. Causative agents can be transmitted by mechanical means via contaminated footwear and equipment. Some recent studies demonstrated that other animals other then birds might have an important role in spreading the disease [26]. **Symptoms and lesions**: The disease is more common in turkeys under 4 months of age than in chickens of the same age. The incubation period usually lasts 4 to 9 days, and the epidemic course may wary between peracute and chronic. The peracute and some acute forms of the disease are not associated with apparent symptoms, and sudden death without any preceding

symptoms may occur in some birds during the acute stage. **Diagnosis**: Bacteriological diagnosis is established based on positive oxidase and catalase tests, biochemical features, lack of growth on McConkey agar and susceptibility to penicillin [21]. **Prevention and treatment**: An appropriately applied vaccination protocol might be useful in prevention of fowl cholera, especially in turkeys. However, vaccination per se is not sufficient. The hygiene-sanitary regimen on the farm is the key point in the prevention of the disease. The complete depopulation once a year, deratization, proper disposal of bird carcasses, clean drinking water, cleaning and disinfection of the environment and equipment is required at the time when chickens are particularly prone to infection (keeping such birds in closed systems is recommendable). The use of previously contaminated poultry houses should be avoided at least 3 months after they have been clean and disinfected. Even though antimicrobials reduce the disease course, the risk of the recurrence after cessation of treatment still exists. Therapy must frequently be extended using low doses administered in feed and drinking water.

Staphylococcus **infections** - (Synonyms: staphylococcal infection, staphylococcal septicemia, staphylococcal arthritis, bumblefoot)
The bacterium *Staphylococcus aureus* is widespread in nature and causes various opportunistic diseases in poultry. *Staphylococcus* could penetrate the eggshell. It is commonly manifested as foot abscess, infection of synovial fluid in joints, and dermatitis of the crest and wattles. The infection is treatable with antimicrobials administered in the feed. In case of particularly severe infection, culling of diseased animals is the most appropriate measure since full recovery is rare. The poultry house and environment must be disinfected. *Staphylococcus* infects all types of poultry but turkeys, chickens, game birds, and waterfowl are the most susceptible species. **Signs and lesions**: Infections appear in three forms: septicemia (acute), arthritis (chronic), and bumblefoot. The septicemic form resembles fowl cholera: the birds are listless, without appetite, feverish, and show pain during movement. Black rot may show up in eggs. The septic course is characterized by profuse watery diarrhea. Swollen joints and decrease in egg production are common. The arthritic usually follows the acute form. Birds show symptoms of lameness and breast blisters, as well as painful movement and they prefer to sit rather than stand. Bumblefoot is a localized chronic staph infection of the foot, usually unilateral. It is frequently caused by puncture injuries. The bird becomes lame from swollen footpads [12, 8]. **Causative agent**: *S. aureus* is a ubiquitous microorganism, which is pathogenic for all mammals including man, birds and reptiles. It is the most frequent Gram-positive isolate in human and veterinary medicine. The organism exhibits no host-selectivity, thus being easily transmitted both among the same species and between the different species. It causes infection, intoxication and mixed toxico-infection. The infection can be localized, general and metastatic but most often are purulent. According to reports of World Health Organization, each person becomes infected in one of the three aforementioned ways at least once in a lifetime. Also, every third person hosts the organism in the nose as a healthy

carrier [5, 20]. *S. aureus* resides in the soil. Epidemics among birds often occur after stormy weather because of subsequent contamination of water with the particles of the ground. **Diagnosis**: Definitive diagnosis is based on bacteriology examination. Most of the routine laboratories are well equipped to detect *S. aureus*. It is one of five coagulase-positive *Staphylococcus* species. Besides the coagulase tube test or slide coagulase test and mannitol fermentation an additional test, such as ornithine decarboxylase or lecithinase production, is performed [20]. **Prevention and treatment*:*** The wounds induced with the sharp objects usually present the route of infection and therefore objects that cause injury needs to be removed. Chronically affected birds have to be isolated. The litter has to be of good quality. Nutritionally balanced feed has to be also provided. Novobiocin (350 g/ton) can be given in the feed for 5-7 days. Erythromycin and penicillin can be administered in the water for 3-5 days or in the feed (200 g/ton) for 5 days. Other antibiotics and drugs are only occasionally effective.

Necrotic enteritis (rot gut, crud and cauliflower gut) - Necrotic enteritis is an acute infectious disease characterized by massive destruction of the intestinal mucosa. **Causative agent**: *Clostridium perfringens* is a toxin-producing sporulating rod that only grows in the absence of oxygen. It is the leading causative agent of myonecrosis and gangrene of the skin and mucosa in most mammals and birds. The spores can survive even several hours of cooking, so the organism is often responsible for food poisoning in both humans and animals*. C. perfringens* produces numerous enzymes and several toxins. According to its antigenic structure, the organism can be subdivided into 6 serogroups. Necrotic enteritis in birds is mostly caused by serogroups A and *C. Coccidiosis* frequently promotes the disease. The disease transmission route has not yet been elucidated, but droplet and alimentary routes are well known. The disease onset is sudden and massive. Apparently healthy birds become acutely depressed and die within several hours. The mortality rate during severe epidemics may reach even 30%, but common mortality ranges between 2 and 10%. Losses due to reduced growth and feed conversion may be more costly than flock mortality [23]. **Signs and lesions**: Lesions are visible mainly in the lower parts of the small intestines; however, the entire intestinal system may be affected. The intestines are dilated, filled with black content of bad smell and a pseudomembrane attached to the intestinal *mucosa.* Intestinal ulcerations are present on both mucosal and serous surface. Hyperemia and hemorrhage could be found around some ulcers. Ulcers could perforate the intestinal wall and cause focal peritonitis. **Diagnosis:** The suspect diagnosis is based upon history, symptoms and findings of characteristic lesions. Definitive diagnosis is established upon bacteriology confirmation. Usually, subsequently to anaerobic culture, small double-zone beta-hemolytic colony is isolated in pure culture, which grows only on the anaerobic plate. Blood agar plate, which is simultaneously incubated in aerobic conditions, remains sterile. The identity of *C. perfringes* is confirmed applying the reverse CAMP-test according to Gubaš. The test is used to confirm *C. perfringens* alpha-toxin, which in synergistic

hemolysis with *Streptococcus agalactiae* CAMP factor gives a crescent-shaped lytic zone [11]. **Prevention and therapy** include antibiotics and vitamin supplementation administered in the feed. Preventive application of antimicrobial therapy is sometimes reasonable, if early signs of infection are apparent. The antimicrobial therapy mainly includes bacitracin and antibiotics of the mycin-group. At the same time, coccidiosis should be excluded or treated (if detected).

CONCLUSION

In poultry industry, the importance and effectiveness of prevention greatly overwhelm that of the therapy. Proper housing and surveillance of the flock is crucial for good farm management and results. The aim of prevention is to reduce exposure to infectious agents through appropriate flock management, avoiding stress situation that may compromise the immunity status within the flock. Excessive bird density should be avoided; the housing premises should provide optimum heating (neither too hot nor too cold) and air circulation. Vaccination must not be performed during the critical periods, and strict control of feed and drinking water must be ensured. Regular cleaning and disinfection of the premises and equipment and appropriate removal of detritus and feces is critical. Strict compliance with hygiene-sanitation measures is particularly important in laying hens to prevent the infection of the eggs. If the epidemic occurs anyway and living birds manifest typical symptoms, prompt examination in a diagnostic laboratory is of highest importance. Such laboratories are equipped with necessary diagnostic tools and instruments to identify the infectious agent, as well as with well-educated and experienced personnel. Veterinarians in the field and in production plants, well-trained personnel on the farm and diagnostic laboratory could significantly reduce the losses. If the number of diseases and dead birds is high, it is advisable to consult a veterinarian before treatment. Drug administration should be performed by strictly complying with producers instructions. If the drug is to be mixed with drinking water or feed, make sure that the birds have no water or feed for a few hours prior to treatment to ensure that all birds drink or eat enough to get an adequate dose. If an antimicrobial treatment is inevitable, the prescribed withholding period must be strictly obeyed.

All drugs, including antibiotics and chemicals, which are used for the treatment of birds, and their yards and sheds, have the potential to form residues in eggs and/or in meat of treated birds. The residues may persist for some time in the birds and necessitate a withholding period before their meat or eggs are free of residue and safe for human consumption. Withholding periods vary. Directions on the container will indicate the necessary withholding period after treatment. Birds should not be killed for consumption during this period and any eggs laid during the period should be discarded. A withholding period also applies to medicinal feed additives.

In conclusion to this problem, we can say that bacterial diseases have been put under control by the discovery and wide application of antibiotics; however, they still are a leading problem in poultry industry. The problem is further aggravated

by escalating and highly frequents multiple resistances to almost all known antibiotic categories. The resistance is transmissible horizontally and vertically, and underlying genetic mechanism may be chromosomal or extra chromosomal [24]. Extra chromosomal transmission mainly implicates plasmid-mediated transmission of resistance associated with R-plasmids [25]. In that respect, global tendencies are aimed at reducing application of massive antibiotic therapy and shifting towards introduction of plant extracts as feed supplements. Numerous authors reported on effects of plant extracts on diverse bacteria [6, 17, 18, 7]. Such an approach could considerably reduce the incidence of leading bacterial infections in poultry caused by infective agents from the feed, thus decreasing the rate of disease outbreaks. Microbiological safety of feed and drinking water are *condition sine non* of a good farming practice. Numerous plant preparations proved effective against *Salmonella* spp*., Escherichia coli, Pasteurella multocida, Staphylococcus aureus and Clostridium perfringens.* It is necessary to formulate a combination of synergistic preparations effective against the entire spectrum of Gram-positive, Gram-negative and anaerobic pathogens that most frequently cause considerable financial damage in poultry industry.

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INFLUENCE OF *Kitaibelia vitifolia* EXTRACT ON COLOUR AND TEXTURE OF SREMSKA SAUSAGE

Djordje Okanović[1], Vladimir Kurćubić[2], Pavle Mašković[2], Marija Jokanović[3], Danijela Vranić[4], Slobodan Lilić[4], Natalija Džinić[3]

[1]Institute of Food Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia
[2] Faculty of Agronomy, University of Kragujevac, Cara Dušana 34, 32000 Čačak, Serbia
[3]Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia
[4]Institute for Meat Hygiene and Technology, Kaćanskog 13, 11000 Belgrade, Serbia

ABSTRACT

This paper presents the results of color and texture, instrumentally determined, of traditional fermented dry "Sremska" sausages, aiming to define the quality.
Fermented dry Sremska sausages (FDSS) were produced from mixture of 50% frozen pork shoulder, 30% of fresh pork meat with 25% of fat, and 20% of frozen pork back fat. The spices and additives (sweet pepper, hot red pepper, garlic, nitrite salt and sugar) were added to the PB I. PB II and PB III were prepared with the 0.3% and 3.0% *Kitaibellia vitifolia* extract as 1.8% v/v and without nitrite, garlic and hot red pepper. After fermentacion (twenty-six days) color and texture were determined, instrumentally ("Chromameter CR-400" and Texture Analyser.
Higher concentration of *Kitaibellia vitifolia* extract, characterized with stronger activities may be used in fermented dry sausage production providing safer products for the consumers, and at the same time with not significant affect on the color and texture of the.
Keywords: *Sremska sausages, Kitaibellia vitifolia, colour, texture*

INTRODUCTION

Fermented sausages are widespread, but Europe is still the major producer and consumer (Germany, Italy, Spain, and France), with annual production of 75 thousand tones in European Union [7]. These popular meat products can be declared as functional food [9, 15], when various herb extracts, containing primarily phenolic compounds which are potent antioxidants, are used in their production [8],
Sremska sausage is one of the most well-known Serbian traditional fermented sausage is, which is nowadays produced under industrial conditions with application of slow fermentation and ripening processes to simulate the traditional production process [5, 18].
Regarding meat products, dry-meat products quality is strongly affected by its texture and mechanical properties, which are mainly determined by the technological parameters and the characteristics of the raw material. On the

other hand the main quality attributes of dry-fermented sausages are developed during drying and ripening period [3].
Texture is an important factor in the process of selection and consumption of foods it is one of the most important components of organoleptic quality in meat products [4, 17]. Colour is one of the most important quality attributes of fermented sausages, since it influences consumer acceptance. In order to meet market demands and produce larger quantities of *Sremska sausage* with standard quality it is necessary to develop production of this fermented sausage in controlled conditions [16].
To our knowledge, no reports on the use of *K. vitifolia* for traditional sausages production, nor the previous studies on the biological activity of this plant are available. The aim of this work was to analyze the possibility of production of fermented dry meat products without nitrite salts, using ethanol herb extract from the overground part of *Kitaibelia vitifolia* as functional ingredient, and to analyze its impact on the texture and color of final product of Sremska sausage.

MATERIAL AND METHODS

Three production batches (PB) of fermented dry Sremska sausages (FDSS), about 20 kg of each, were produced from mixture of 50% frozen pork shoulder, 30% of fresh pork meat with 25% of fat, and 20% of frozen pork back fat. The spices and additives (sweet pepper, hot red pepper, garlic, nitrite salt and sugar) were added to the PB I. This original mixture recipe was used as control sample. To assess the influence of the various concentration of herb extract, nitrite, red hot pepper and garlic were replaced in the control batches by 0.3% in PB II and 3.0% (600 mL/20 kg of fillings) in PB III, extract of the *K. vitifolia* as 1.8 % v/v. Another modification in formulation of PB II and PB III sausages was addition of double amount of sweet pepper. In FDSS belonging to both modified PB because of their own antioxidant potential.
The examined variants of Sremska sausage were manufactured in a small processing plant in Central Serbia. All variants were produced on the same day and in an identical manner. The mixture was stuffed in natural casing (diameter 36-38 mm), hand-paired and were left to drain. Drying and ripening processes were under controlled conditions of temperature and relative humidity in industrial chamber.
Moisture content was carried out on the 26th day of production for all sausages. The analyses were carried out in duplicate. Moisture content was quantified according to the ISO recommended standard [6].
Color measurements of *Sremska sausage* were carried out using photo-colorimeter Minolta Chroma Meter CR-400 and colour characteristics were expressed by CIE *L*a*b** system (lightness - *L**, redness and greenness - *a**; yellowness and blueness - *b**). The colour measurements were performed on the fresh cut of the sausage at room temperature. Two measurements were taken on two fresh cut surfaces of sausages from each batch.
Texture profile analysis (TPA) was performed as described by Bourne [2] with an universal testing machine Texture Analyser TA XP (Stable Micro System,

Godalming, UK). For texture analysis samples of fermented dry Sremska sausages were prepared in cylindrical form (2 cm high, diameter of 2.54 cm), which after removing from casing were equilibrated to room temperature and compressed twice to 50% of their original height at a constant speed of 1 mm/s. The following parameters from the force–time curves were determined: hardness, springiness, cohesiveness and chewiness.
All data of colour and texture measurements of *Sremska sausage* are presented as mean values ± standard deviations. Analysis of variance (Duncan test) was used to test the hypothesis about differences among obtained results. The software package STATISTICA 8.0 [14] was used for analysis. Differences between average values are presented on the level of 95% ($P \leq 0.05$) and 99% ($P \leq 0.01$).

RESULTS AND DISCUSSION

Color characteristics of *Sremska sausage* samples, expressed in CIE *L*a*b** system, are shown in Table 1.

Table 1. Results of instrumental determination of cut surface colour of fermented sausages from three production batches

Characteristic	Sample		
	PB I	PB II	PB III
Moisture content, %	$21.82^{a} \pm 1.03$	$20.69^{ab} \pm 0.92$	$21.52^{a} \pm 0.60$
Light intensity, *L**	$38.07^{A} \pm 2.64$	$41.12^{B} \pm 1.92$	$37.84^{A} \pm 0.81$
Share of red, *a**	$22.00^{A} \pm 1.19$	$18.50^{B} \pm 1.80$	$13.64^{C} \pm 0.64$
Share of yellow, *b**	$20.57^{A} \pm 2.22$	$20.81^{A} \pm 2.33$	$12.82^{B} \pm 0.90$

Results are mean ± SD values
Different superscripts in the same row indicate significant difference
a, b - $P < 0.05$
A, B,C - $P < 0.01$

After 26 days of drying and ripening, the highest *L** value was determined on the cut surface of PB II (41.12) and the lower on cut surface of control group, PB I (38.07) and PB II (37.84). Different was statistically significant ($P < 0.01$)
The highest share of red color (*a**) was obtained for the sausages with addition of nitrite and higher pepper (PB I, 22.00), compared to the experimental groups of sausage, in which *Kitaibelia Vitifolia* extract was added (Pb II-18.50, PB III-13.64). The measured differences were significant ($P<0.01$).
For sausage samples of PB III group, in which higher concentration of *Kitaibelii Vitifolia* extract was added, statistically significant ($P < 0.01$) smallest proportion of yellow colour, *b** (12.82) was measured comparing with sausages of PB I (20.57) and PB II (20.81) group.
Such significantly different ($P < 0.01$) *L** values can be explained by added nitrite in control group (PB I) and lower ($P < 0.05$) moisture content in these sausages. With the moisture loss the concentration of myoglobin in product increases, and

on the other hand dehydrated muscle tissue absorbed a greater amount of light what result in a darker colour of the products, i.e. lower *L** value [1, 11]. It should be noted that the great influence on the *a** value in analyzed sausages had red hot paprika powder. Also, *b** values of analyzed sausages could probably be related to the presence of yellow carotenoids coming from paprika powder [4, 12].
Texture profiles of *Sremska sausages* (hardness, springiness, cohesiveness and chewiness), at the end of process are presented in Table 2.

Table 2. The results of TPA test of fermented sausage, from three production batches

	Sample		
Characteristic	PB I	PB II	PB III
Hardness, g	7372.15 ^{a} ± 574.71	9191.46 ^{b} ±2416.87	7846.22 ^{a} ± 807.44
Springiness	0.39 ^{a} ± 0.03	0.40 ^{ab} ±0.04	0.43 ^{b} ± 0.06
Cohesiveness	0.39 ^{a} ± 0.07	0.36 ^{b} ±0.02	0.40 ^{a} ± 0.03
Chewiness	1127.25 ^{a} ± 267.42	1279.63 ^{a} ±295.71	1346.51 ^{a} ± 281.64

Results are mean ± SD values
Different superscripts in the same row indicate significant difference
a, b - $P < 0.05$

At the end of drying process hardness of sausage PB II (9191.46 g) was significantly ($P < 0.05$) higher than for sausage of PB I (7372.15 g) and PB III (7846.22 g). Hardness of sausages is partly the result of protein coagulation at low pH, and also partly the result of decreasing moisture content [3]. Values of hardness, recorded for all groups of sausages, were lower comparing with chorizo de Pamplona [4], but similar to those of Italian low-acid [13] and slow fermented sausage analyzed by Olivares et al. [10].
Samples of PB III group had higher springiness (0.43) than in samples of PB II group (0.40), and significantly ($P < 0.05$) higher than for sausage control, PB I (0.39)
Similar relationships were obtained for cohesiveness: the highest was in the samples of PB III group (0.40), comparing with samples from group PB II (0.36) and group PB I (0.39), and this different was statistically significant ($P< 0.05$).
Chewiness values for analyzed samples did not differ significantly between groups, being higher for sausages of PB III (1346.51). Bozkurt and Bayram [3] also, found significant relation between moisture content and chewiness.

CONCLUSIONS

The present study confirmed validity of extract *Kitaibellia vitifolia* usage in the fermented dry sausages production. Examined extracts did not interfere in the colour and texture characteristics of the product. Therefore, higher concentration of extract determined with stronger activities may be used in fermented dry sausage elaboration to retard the oxidative rancidity and microbial growth,

providing safer products for the consumers, and at the same time having no significant affect on the color and texture of fermented dry Sremska sausages.

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WHEAT ADMIXTURES AND THE POSSIBILITY OF THEIR VALORISATION

Slavko Filipović[1], Šandor Kormanjoš[1], Vera Radović[2], Rade Jovanović[3], Nikola Đukić[3], Jelena Filipović[1], Nikola Hristov[4]

[1]University of Novi Sad, Institute of Food Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia
[2]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, 32000 Čačak, Serbia
[3]Institute for Science Application in Agriculture, Bulevar despota Stefana 68b, 11000 Belgrade, Serbia
[4]Institute of Field and Vegetable, Maksima Gorkog 30, 21000 Novi Sad, Serbia

ABSTRACT

Safe animal feed production requires feed components that are in accordance with the Regulations for the quality of animal feed [9]. In animal feed, grains comprise between 40 to 60% (cereals and legumes) and they have to meet quality demands cited in sections 8 and 11 of The Regulations for the quality of animal feed.
Quantities of admixtures separated during wheat seed processing and storing, as well as, their origin and type are presented in this work. Depending on their physical and chemical characteristics following technologies were applied: extrusion, pelleting or pressing (briquetting) for producing feed or energy (biobriquettes).
After harvesting the quantity of admixtures ranged from 2,32 % for cultivar Pobeda to 8,26% for cultivar Odisej. Concerning the structure of organic impurities so called "grain besatz", the share of shrunken and broken kernels, sprouted kernels, wheat bug infested and damaged kernels was either less than 1% or more than 2%, depending on the cultivar and applied techniques during cultivation and harvesting.
Keywords: *wheat, admixtures, extrusion, pelleting, briquetting, energy*

INTRODUCTION

In addition to the energy needs, food production in the world and our country is one of the priorities that each society must solve. The problem of food shortage in the world can be solved by more efficient use of existing resources in agriculture and by introducing new technological processes [6].
From the technological point of view, the world and our country have developed new technological procedures that aim to increase the nutritional value of food for human and animal consumption, particularly by-products from food industry and agriculture [2], [7], [11]. Many technological processes such as toasting, extrusion, microwave treatment etc. are applied in the world [4], [3], [5]. In feed production cereals along with meals of plant and animal origin occupy a central

place because their participation, depending on the feed type, ranges from 40 to 70%. The production of safe feed is regulated by Regulations for the quality of animal feed [9], and the quality of cereals is regulated by section 8. In terms of these Regulations, cereals have to meet the following requirements for quality:
1. Appearance and color should be characteristic for the type of grain.
2. Grain must be healthy, ripe, the inherent appearance, smell and taste with no signs of mold, not infected by crop pests and diseases. Not containing toxic substance in larger quantities than permitted.
3. Must not contain more than 3% of total admixtures, but no more than: 1% of inorganic impurities, 2% of organic impurities (parts of stems, leaves, seeds of other plants, etc.), of which up to 0.4% of mildew and ergot and up to 0.1% of weeds harmful to animals (*Lolium temulentum L, Datura stramonium L.*) and smutty grains.
4. Containing no more than 3% of defects of which up to 0.3% of moldy grain
5. Must not contain more than 4% of insect bored kernels and no live insects,
6. Containing no more than 5% broken, shrunken and sprouted kernels, of which up to 1% of the grains damaged by artificial drying except maize which is allowed up to 8% and 2% of broken and shrunken with sprouted kernels, respectively,
7. Must not contain more than 10% of ingredients from Section 3 and 6, except corn where is allowed up to 10% of admixtures,
8. Should not have a foreign taste or odor and in particular to: the pests, molds, weed seed, smut, inappropriate storage or transport, foreign substances, the plant protection product or substances for pests protection.
In order to provide grain for the production of safe food for humans and animals, after the harvest, grains have to be cleaned prior to storage.
This paper is analyzing the composition and quantity of wheat impurities after harvesting and before storage. The composition and the amount of impurities are tested, as well as, their energy value with the aim of evaluating them as a bioenergy fuel.

MATERIAL AND METHODS

Physico-chemical parameters of mercantile and seed wheat after the harvest before storing in silo bins were tested. Moisture content was determined according to the Rules of the methods of sampling and methods of physical-chemical and microbiological analyzes [10]. Bulk density was measured with a bulk density tester Tani indusrie, west and Goslar, Germany [1].
The energy value was determined according to: Serbian standards [12].
In commercial and seed wheat organic ingredients, particularly broken, shrunken, sprouted, insect bored, heat damaged and other cereal kernels, as well as weed seed were analyzed. The above analyses were done according to JUS E.B8.029, [8].

RESULTS AND DISCUSSION

The content of impurities in seed and commercial wheat in wheat samples after the harvesting collected after the cleaning process before storing in silo bins is shown in the tables 1, 2, 3, 4 and 5. Moisture content of wheat and wheat broken kernels after combine machine and receiving separator ranges from 9.69% concerning cultivar Dragana up to 12.17% concerning commercial wheat collected in "Jedinstvo" Apatin. Wheat broken kernels gathered at the receiving separator was 9.19% and 10.52% concerning cultivar Simonida, and commercial wheat in "Jedinstvo" Apatin, respectively. The moisture content of wheat and wheat broken kernels are influenced by high temperatures during wheat ripening. Table 2 shows the moisture content of the wheat impurities after purification in different devices and the results depend on the wheat cultivar, ranging between 8.57% and 9.98% which according to Stojanovic [13] corresponds to the modern technological method of storage commercial and seed wheat. Table 3 shows the quantity and types of impurities in the wheat cultivars.

Pertinent data show that the total impurities range from 2.32% to 5.32% for cultivar Pobeda and "Jedinstvo" Apatin, respectively. Concerning the structure, the most common organic impurities are shrunken and broken kernels and their amounts range from 1.09% for cultivar Pobeda to 1.66% for Simonida. Sprouted kernels ranged from 0.36% for Simonida to 1.66% for Odisej. Wheat bug infested and sprouted kernels were either less than 1% or up to 2.52% concerning cultivar Odisej. These data are significant due to the possibility of their safe evaluation like biobriquettes i.e. briquetting wheat by-products.

Tables 4 and 5 shows the energy values and bulk density of organic impurities separated by air flow in the cyclone and also the energy value and bulk density of wheat broken kernels separated at receiving separator. Organic impurities separated in cyclone have bulk density ranging from 0.136 to 0.685 kg/dm^3. Depending on the wheat cultivar, energy value of organic dust and tailings ranges from 16.8581 to 17.6294 MJ/kg. These data suggest that the pelleting or briquetting the chaff and organic dust are convenient processes for better evaluation of wheat by-products. Table 5 shows the bulk density and energy values of broken kernels separated from receiving separator in the process of wheat cleaning before storage. The values of bulk density for wheat broken kernels ranged from 0.710 to 0.800 kg/dm^3, while the energy value ranged from 16.5909 to 16.7933 MJ/kg. Pertinent energy values for either broken wheat pellets or briquettes valorize these ingredients into a valuable product i.e. bio-energy pellets which are of particular importance in the future in order to solve the deficit of energy in our country, but also positively contribute to solving the problem of environmental protection.

Table 1. Moisture content of wheat and broken wheat kernels below under the receiving separator, %

Wheat cultivars	Wheat after harvesting	Broken kernels from receiving separator
„Jedinstvo“	12,17	10,52
Odisej	9,85	9,73
Dragana	9,69	9,60
Pobeda	9,98	9,98
Simonida	9,76	9,19

Table 2. The moisture content in wheat fractions after cleaning (%)

Fractions	Wheat cultivars				
	Simonida	Odisej	Renesans	Dragana	Pobeda
Under screening separator	9,17	9,31	9,98	9,25	9,45
Under “Vib”sieve	9,37	9,49	9,53	9,38	9,63
Under cylinder separator	9,40	9,61	9,90	9,36	9,79
Paddy table over tails	9,41	9,43	9,96	9,21	9,83
Under cyclone	9,34	9,41	9,25	9,45	9,25
Under “Vib” sieve	8,90	8,88	8,57	9,30	8,86

Table 3. Quantities of organic impurities in the processing of seed wheat

Impurities %	Wheat				
	Simonida	Pobeda	Dragana	Odisej	„Jedinstvo“
Shrunken and broken kernels	1,66	1,09	2,09	1,23	1,63
Sprouted kernels	0,36	0,41	-	1,66	-
Bug wheat infested kernels	0,74	0,69	1,12	2,52	1,48
Damaged kernels	0,58	0,41	0,16	2,13	1,12
Impurities of organic origin	0,21	0,13	0,06	0,48	0,11
Total impurities	3,55	2,32	3,43	8,26	5,32
Total impurities (witout sprouted and/or wheat bug infested)	3,19	-	-	8,60	-

Table 4. Energy and bulk density of organic impurities separated by air flow in the cyclone in the process of wheat cleaning

Wheat cultivar	Cleaning device	Energy of chaff and organic dust MJ/kg	Bulk density kg/dm^3
Pobeda	Cyclon of the separator	17,0064	0,455
	„Vib" sieve	17,3547	0,260
Dragana	Cyclon of the separator	16,9745	0,685
	„Vib" sieve	16,9340	0,490
Renesansa	Cyclon of the separator	16,9714	0,410
	„Vib" sieve	17,6294	0,175
Odisej	Cyclon of the separator	17,0680	0,645
	„Vib sieve"	17,1068	0,395
Simonida	Cyclon of the separator	16,8581	0,425
	„Vib sieve"	17,1622	0,135

Table 5. Energy and bulk density of wheat broken kernels and through under the receiving sieve

Wheat cultivar	Energy, MJ/kg	Bulk density, kg/dm^3
„Jedinstvo"	16,5909	0,710
Odisej	16,6339	0,745
Dragana	16,6890	0,800
Pobeda	16,7002	0,740
Simonida	16,7933	0,770

CONCLUSIONS

In the storing process of either commercial wheat or wheat seeds, admixtures separated at various cleaning devices were analyzed with the aim to provide safe storage of wheat and also to find the possibility for the use of the by-product.

The moisture content in the samples depended on the wheat cultivars and varied in acceptable limits, ranging between 9.69% and 12.17%. Amounts and type of wheat admixtures were measured, which is of economic and environmental significance. Total admixtures content in certain wheat cultivars ranged from 2.32% to 5.32%. Concerning the admixtures structure, the share of shrunken and broken kernels was from 1.09% to 1.66%, while the share of bug wheat infested kernels was up to 2.52%.

In the study special importance have data concerning energy and bulk density of organic impurities separated in the technological process of cleaning. The energy amounted to 16.8581 MJ / kg to 17.6294 MJ / kg for organic cyclone dust, while the energy of broken wheat kernels ranged between 16.5909 to 16.7933 MJ/kg. Based on these results and due to the significant amount of impurities extracted in the processing of seed and commercial wheat solution to this problem is to convert them into either pellets or biobriquettes thus positively contributing both to environmental protection and energy sources.

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CHANGES IN EAR COLOR OF SPELT WHEAT AS A RESULT OF DIFFERENT INTENSITY OF *ALTERNARIA SPP.* INFECTION

Jovana Vučković[1], Jovana Brkljača[1], Tea Brlek[1], Jelena Ćulafić[2], Aleksandra Bočarov-Stančić[4], Milica Aćimović[3], Marija Bodroža-Solarov[1]

[1]Institute of Food Technology, Bul. cara Lazara 1, 21000 Novi Sad, Serbia
[2]Faculty of Medicine, Hajduk Veljkova 3, 21000 Novi Sad, Serbia
[3]Bio-Ecological Centre, Petra Drapšina 15, 23000 Zrenjanin, Serbia
[4]Scholar of the Ministry of Education, Science and Technological development

ABSTRACT

Spelt wheat (*Triticum aestivum* ssp*. spelta*) is becoming a highly attractive farming option due to valuable nutritional properties and possibility to grow in low input farming systems. *Alternaria* spp. are widely spread fungal moulds on cereal grains, which are possible mycotoxin producers harmfull for animal and human health. Aim of this work was to evaluate the discoloration of spelt ears in different inoculation treatments with *Alternaria* spp., fungicide and water as a control. The field trial was carried out on three spelt wheat genotypes. Color measurements included level of lightness (*L**) which is considered to be a reliable indicator of fungal infection intensity. It was found a significant difference between inoculation treatments related to lightness (*L**) of spelt ears, while interaction between inoculation treatment and genotype did not show significant difference. The efficiency of the fungicide was detected through the much lighter discoloration of treated ears compared to ears inoculated with *Alternaria* spp. The highest *L** value (*L**=69.67) has the genotype 1 inoculated with fungicide, while the lowest (*L**=58.38) was detected in treatment with *A.alternata* at genotype 2. A positive correlation between value *L** and yield parameters (weight and length of ears) was observed and the coefficient of correlation was r=0.57 and r=0.71, respectively. The intensity of fungal infection could be efficiently performed by instrumental colorimetric measurements. This method might be valuable in practice and further verification is needed.
Keywords: *spelt wheat, Alternaria spp., color, ears*

INTRODUCTION

Spelt wheat (*Triticum aestivum* ssp*. spelta*) is an old hulled sub-species of common wheat. It has been grown for hundreds of years in North and Central Europe, while it has been replaced by common wheat [8]. Nowadays, spelt wheat is becoming more widely used in the growing for feed and food on the world market. Ground spelt is used primarily as an alternative feed grain to oats and barley. Spelt is usually perceived as 'healthier' and more 'natural' than common wheat. It has been claimed to have more protein content, vitamins and minerals than common wheat [1, 3, 15].Agriculturally, spelt has advantages over common wheat as it is more resistant to harsh environmental conditions and can

grow without the use of pesticides and fertilization. It is considered suitable for growing in low-input systems and in marginal areas of cultivation [6]. Moulds of the genus *Alternaria* include cosmopolitan plant pathogenic and saprophitic species infesting a broad range of agricultural products. Beside losses in production, *Alternaria* spp. is well known post-harvest pathogen as well, which may cause spoilage of commodities during transport and storage [13]. The genus *Alternaria* is also known to be dangerous for human and farm livestock because some *Alternaria* species have a high toxigenic potential, producing mycotoxins such as alternariol (AOH), alternariol monomethyl ether (AME), altenuen (ALT), tenuazoic acid (TEA) and others. These toxins might pose a potential health hazard for the consumer if accumulated in food and feed [7]. Cereal grains are frequently infected by *Alternaria* spp., which can cause disease called „black point" which comprises of dark brown or blackish discoloration of the ear, germ and the seed due to mycelial and conidial masses. Black colour on infected plant tissues derived from dark pigment, melanin, which is characteristic for *Alternaria* genus [16]. With persistent rainfall and high moisture during cereal kernel development, black point disease may cause serious yield losses [10]. Economical losses are mainly refered to quality reduction due to decreased nutritive value, discoloration and off flavours [8]. Aim of this work was to assess the discoloration of spelt wheat ears infected by *Alternaria* spp. in different inoculation treatments.

MATERIAL AND METHODS

Field experiment-inoculation. Field experiment was carried out on the spelt wheat in the 2010/2011 growing season in the region of Vojvodina, north Serbia. Three spelt wheat genotypes were supplied from the local market. The trial was set up in block design with four replicates. The fungal inoculums consisted of *A.alternata* and two isolates of *A.tenuissima* were multiplied on potato dextrose agar (PDA) in dark at 25°C for 14 days. Conidial suspension was made in distilled water, filtrated through the plastic strainer and the concentration was determinated by haemocytometer. Concentration of *A.alternata* conidia was $0{,}247 \times 10^6$ infective particles per mL and two isolates of *A.tenuissima* had concentrations of $0{,}605 \times 10^6$ and $0{,}497 \times 10^6$ infective particles per mL. Inoculation was performed at the full flowering stage by spraying conidial suspension onto the spelt ears. Inoculated plants were immediately covered with polyethylene bags for 24 h. Treatments with fungicide and distilled water were used as two control objects. In the full ripeness stage spikes from each plot were cut by hands and used for next analysis.

Colorimetric measurements. Color properties were measured with a Chroma meter (CR-400, Konica, Minolta, Japan) which was calibrated against white calibration standard (CM –A70). The CIE *L** (lightness), was read using a D65 light source with the observer angle at 2 °C. *L** value is the brightness of the color in the range of values from 0 (black) to 100 (white); the higher the values,

the brighter the color. Samples were analyzed in triplicate, recording six measurements for each sample.

Statistical analysis. Statistica 10.0 Software (Statsoft Inc., 2010, Tulsa, Oklahoma) was used for statistical data processing using two-way ANOVA. The comparison of mean values was performed by Tukey- test. Differences were considered significant if $P<0.05$.

RESULTS AND DISCUSSION

Two-way ANOVA showed significant difference between inoculation treatments related to lightness (*L**) of spelt ears ($F=13.94$; $p<0.01$). In contrast, the lightness related to spelt genotype ($F=3.04$; $p=0.055$) and interaction between two factors (inoculation treatment*genotype) did not show significant difference ($F=1.48$; $p=0.184$) as presented in Table1.

Tabela 1. ANOVA of *L** (*ligthness*)

Effect	*Univariate Tests of Significance for L**				
	SS	*Degr. Of Freedom*	*MS*	*F*	*p*
Genotype	55.1	2	27.6	3.04	0.055133
Treatment	**503.3**	**4**	**126.3**	**13.94**	**0.0000001**
Genotype treatment*	107.1	8	13.4	1.48	0.184427
Error	543.6	60	9.1		

In Table 2 different *L** levels related to inoculation treatments and spelt genotypes were presented. It should be noted that in the inoculation treatments with *Alternaria* spp., the *L** value decreased, which means that the brightness decreased with fungal infection. The *L** value was the highest (*L**=69.67) at genotype 1 inoculated with fungicide, while the lowest (*L**=58.38) was detected in treatment with *A.alternata* at genotype 2. The efficiency of the fungicide was evidenced through the discoloration of treated ears, which were much lighter compared to ears inoculated with *Alternaria* spp. This observation applies to all three spelt wheat genotypes. Water treatment showed higher *L** level than ears inoculated with *Alternaria* spp., while slightly lower compared to fungicide treatment. This could be explained by weather conditions in 2010/2011 growing season which facilitated the colonization of wheat by fungi, including pathogens of the genus *Alternaria*. According to the Republic Hydrometeorological Institute' [14] a particularly high level of precipitation was reported in the end of May, which is the period of flowering for the spelt wheat in North Serbia. The average precipitation level in May amounted 57 mm which is 88% more compared to annual mean for this month. Variable weather and moderate temperatures with less precipitation characterized period until the full ripeness stage. In the last decade of June high temperatures which exceeded 33 ° in most areas resulted

in a reduction of the grain filling process and speed up the ripening of spelt wheat and caused earlier start of harvest of these crops.

Table 2. Changes in lightness (L) of ears due to different inoculation treatments on spelt wheat genotypes*

Spelt genotype	Treatment	*L**
1	F	$69.67^{d} \pm 3.69$
1	W	$66.38^{bcde} \pm 2.22$
1	Aa	$61.59^{abc} \pm 1.36$
1	At (1)	$60.34^{abc} \pm 3.92$
1	At (2)	$61.59^{abc} \pm 4.31$
2	F	$68.36^{de} \pm 1.62$
2	W	$66.64^{cde} \pm 2.46$
2	Aa	$58.38^{a} \pm 2.27$
2	At (1)	$62.58^{abcd} \pm 2.75$
2	At (2)	$61.36^{abc} \pm 3.71$
3	F	$65.60^{bcde} \pm 2.45$
3	W	$64.85^{abcde} \pm 2.43$
3	Aa	$59.88^{ab} \pm 2.37$
3	At (1)	$60.64^{abc} \pm 4.93$
3	At (2)	$60.16^{abc} \pm 3.92$

Abbreviations used in table: F-fungicide treatment, W-control with water, Aa- A.alternata, At (1)-A.tenuissima (1), At (2)-A.tenuissima (2); Data are the average values of concentrations with SD. Within column mean values followed by different superscript letters differ significantly at P < 0.05 (Tuckey- test)

Color properties were measured in cereal products based on spelt wheat such as spelt flour [2] brans [12] bread [4] and pasta [5,11] indicating baking quality of spelt products, but discoloration of spelt wheat ears infected with fungal inoculums has not been reported yet.

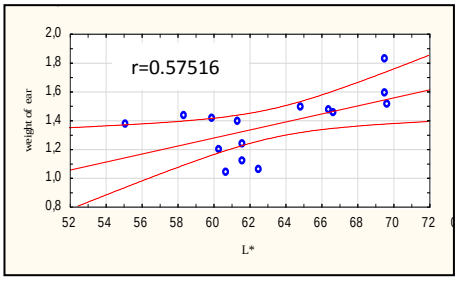

Figure 1. Correlation of weight of ear and L (lightness)*

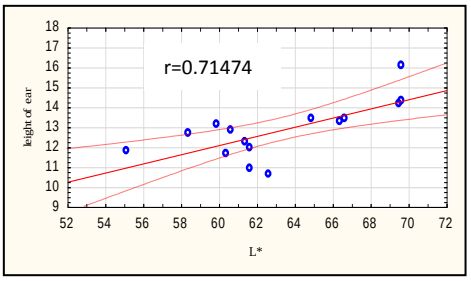

Figure 2. Correlation of length of ear and L (lightness)*

Since the fungicide and inoculation treatments had significant impact on yield parameters such as length and weight of ears, which has been proved in our previous work [17] the correlation between lightness and yield parameters is calculated. There is a significant correlation between value *L** and weight of ears (r=0.57) as presented in Figure 1. A positive correlation between lightness and length of ears was observed in Figure 2 (r=0.71). Higher *Alternaria* spp. infection resulted in lower weight and length of spelt ears, which could be expanded to color of ears.

CONCLUSION

Our study showed that different inoculation treatments resulted in distinguishable discoloration of spelt ears. Level of lightness (*L**) revealed that color may be a reliable indicator of level of fungal infection. Fungicides treatments indicated much lighter ears, while plants inoculated with *Alternaria* spp. had dark discoloration of ears. No difference in ear discoloration between spelt genotypes is registered. A positive correlation between level of *L** and yield parameters (weight and length of ear) is observed. The evaluation of intensity of fungal infection could be efficiently performed using such instrumental colorimetric method, which has been proved in this work. It could be concluded that this method might have great importance in practice and it should be improved through the verification in further researches.

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INFLUENCE OF STORAGE CONDITIONS ON QUALITY OF RAPESEED OIL CAKE FOR FEED

Vanja Jurišić, Tajana Krička, Ana Matin, Neven Voća

University of Zagreb Faculty of Agriculture, Svetošimunska c. 25, 10000 Zagreb, Croatia

ABSTRACT

During the past decade, there has been an increased exploitation of organic residues from different sectors of agriculture and industries. Years after the first-generation biodiesel has been introduced, the problem how to utilize large quantities of rapeseed oil cake/meal have arised in terms of their quantity and quality in feed for animals. Due to high free fatty acid content, which is increased with storage period, side-effects, such as those with gastro-intestinal tract can occur. The aim of this study was to determine the change in oil and free fatty acid content in oil cake of four cultivars (Alaska, Eurol, Lirajet, and Silvia) during a one-year storage period, as well as influence of different storage temperatures (4°C and 10°C) on parameters being monitored.
***Keywords**: storage, rapeseed oil cake, FFA content, feed*

INTRODUCTION

Rapeseed is one of the most important oil seeds for production of vegetable oil in the world, whereas rapeseed oil cake, as a solid residue remaining after extraction of rapeseed oil, is the most commonly preferred in animal feed because it contains 16–24% protein. Moreover, all parts of rapeseed (straw, stalk, seed, oil, and cake) are also used as biomass resource for production of biofuels and biodiesel [1].

Feedstock cost is the main factor affecting the competitiveness and profitability of biodiesel production, aside from its final cost on the market, and as such it is highly important to clearly identify and quantify current and potential feedstock [2]. The choice of feedstock also depends upon the specific conditions of a country concerned (climate, populations' consumption patterns, standard agricultural plant production, etc). Hence, in the Republic of Croatia, the most significant crops for vegetable oil production are rapeseed and sunflower [3].

Rapeseed oil (*Brassica napus L. ssp oleifera*) was originally chosen for transesterification experiments by biodiesel pioneers because of its low price compared to other readily available vegetable oils. However, it soon became apparent that thanks to its high content of monounsaturated oleic acid and the low levels of both saturated and polyunsaturated acids, this oil is practically an ideal raw material due to its combustion properties, oxidative stability and cold temperature behavior. Hence, rapeseed oil is still the feedstock of choice in most European countries [4].

However, biodiesel production by cold-press extraction of oil is intensifying and bringing increasing quantities of by-products, including rapeseed cake and glycerine, to the world market [5]. The protein content of rapeseed cake can make it a good substitute for soyabean meal, while glycerine can be a good source of energy [6]. Rapeseed is currently considered to be an adequate substitution of soya in cattle diets in some European countries because rapeseed cake is cheaper than soya or cereal concentrates, and feeding costs are lower [7]. On the other hand, due to its fatty acid composition, rapeseed cake is a feed that can be used to modify the composition of milk and meat fat [8]. Studies have also been conducted on feeding poultry with rapeseed oil cake [9-11].
The aim of this study was to determine the change in moisture, oil and free fatty acid content in oil cake of four "00" cultivars (Alaska, Eurol, Lirajet, and Silvia) during a one-year storage period, as well as influence of different storage temperatures (4°C and 10°C) on parameters being monitored.

MATERIAL AND METHODS

Material

Research was carried out on oil cakes of four "00" rapeseed cultivars (Alaska, Eurol, Lirajet, and Silvia), grown in Western and Eastern Slavonia (Republic of Croatia). Rapeseed was harvested in July 2010, and the cake was obtained by using cold-press extraction of rapeseed.

Methods

Moisture content was determined as received [12]. Samples were dried in a laboratory drier at 40 and 60°C, and stored at both, environmental and controlled conditions (4 and 10°C) during a one-year period. Air-dried sample was used as control. Oil and free fatty acid (FFA) content were determined according to protocols [13-14].

Statistical analysis

Statistical analysis was performed by using GLM procedure in SAS software [15].

RESULTS AND DISCUSSION

The press cakes are often used as feed [5, 7], because of the proteins which are usually present in quantities varying from 16-24% [1]; however, the free fatty acid content can deteriorate its quality and overall effect on the diet. Therefore, a systematic monitoring of its content, together with moisture and oil contents during storage of rapeseed cake is needed in order to provide a high-quality ingredient for feed. Therefore, a study of influence of storage conditions on quality of rapeseed oil cake for feed was conducted. The study included oil cakes of four "00" rapeseed cultivars (Alaska, Eurol, Lirajet, and Silvia), before and after a one-year storage period (4 and 10°C).

Statistical analysis showing the influence of cultivar and drying conditions on moisture, oil and free fatty acid (FFA) contents in rapeseed cake before storing is shown in Table 1.

Table 1. Moisture, oil and free fatty acid (FFA) content in air-dried and dried rapeseed cake before storage

Variable	Moisture (%)	Oil (%)	FFA (%)
Cultivar			
Alaska	5.744c±0.770	10.840b±0.382	0.404a±0.062
Eurol	6.011c±1.166	13.424a±0.494	0.503a±0.066
Lirajet	6.244b±0.658	12.991a±0.917	0.548a±0.049
Silvia	7.000a±0.973	10.806b±0.942	0.430a±0.060
Drying			
Air-dried	5.567a±2.431	11.855a±1.272	0.507a±0.099
40°C	6.275a±3.019	12.035a±1.479	0.458a±0.023
60°C	6.158a±2.761	12.159a±1.305	0.447a±0.051

Legend: mean values ± standard deviation are shown, where same letter refers to non-significant difference ($p<0.05$) accordint to Tukey HSD test

From the table, it can be observed that moisture content significantly differed between different cultivars, depending upon cultivar ($p<0.0001$). Moisture content varied from 5.74% in cultivar Alaska to 7.00% in cultivar Silvia; this was somewhat lower content than that observed in the investigation from [7], but similar to that from [5]. Lower moisture content ensures better quality of this material and as such is satisfactory. Furthermore, moisture content in the investigated samples didn't have significant difference when dried at temperatures of 40 and 60°C, in comparison to the control sample (air-dried). The same trend was present with oil content, where there was significant difference in oil content ($p<0.05$) between cultivars, with oil ranging from 10.81-13.42%. When speaking of FFA content, it ranged from 0.41-0.55 (Alaska) to 1.55% (Lirajet), depending upon the cultivar, with no significant difference between them.

Table 2 depicts statistical analysis of influence of cultivar, drying and storing conditions on moisture, oil and FFA content in rapeseed cake after storing at two different temperatures (4 and 10°C).

As it can be seen, there was no significant difference in moisture content depending upon cultivar and drying conditions; however, a significant difference was observed when storing at 4 and 10°C, where moisture ranged from 5.72-6.65%. From Tables 1 and 2 can be observed that moisture content didn't vary a lot depending on the storage; however, during a one-year storage, there were fluctuations in the moisture content (during winter time, it increased). Moreover, change in oil content is also shown in Table 2, where it is evident that there was a significant difference in oil content depending upon cultivar, with max. content of 11.86% in cultivar Eurol; significant difference was also found with FFA content, depending upon cultivar ($p<0.05$) and storing conditions ($p<0.05$).

Table 2. Moisture, oil and free fatty acid (FFA) content in air-dried and dried rapeseed cake after storage

Variable	Moisture (%)	Oil (%)	FFA (%)
Cultivar			
Alaska	6.280a±0.566	7.054a±0.018	0.556b±0.088
Eurol	6.409a±0.562	11.857b±1.118	0.648b±0.011
Lirajet	6.182a±0.457	10.739a±1.538	0.931a±0.181
Silvia	6.215a±0.485	8.884a±1.101	0.577ab±0.185
Drying			
Air-dried	6.187a±0.524	9.936a±1.027	0.756a±0.115
40°C	6.369a±0.533	10.120a±1.783	0.776a±0.047
60°C	6.258a±0.499	10.387a±1.573	0.727a±0.133
Storing			
Environment	6.650a±0.342	9.480b±1.853	0.889a±0.105
4°C	6.444b±0.444	10.264a±1.908	0.749ab±0.094
10°C	5.720c±0.147	10.695a±1.512	0.621b±0.055

Legend: mean values ± standard deviation are shown, where same letter refers to non-significant difference ($p<0.05$) accordint to Tukey HSD test

Similar decrease in oil content and increase in FFA content was obtained in the investigation conducted by Krička et al. [16], who stored rapeseeds at different conditions. The natural properties of oil, that is autooxidation of lipids and increase of the content of FFA during storage period are the main reasons for rapid deterioration of the material [17, 18]. However, the results of moisture, oil and FFA content, which were obtained in this study are still in the boundaries set by the Croatian legislative regarding the quality of feed [19], and as such can be used for feed.

CONCLUSIONS

Based on the investigation conducted on four "00" rapeseed cultivars (Alaska, Eurol, Lirajet, and Silvia), it can be concluded that rapeseed cake of all samples can be stored without significant deterioration in quality before utilization as feed ingredient for cattle and poultry. Moreover, drying the rapeseed cakes at 40 and 80°C caused no significant difference in monitored parameters. After a one-year storage at 4 and 10°C, it was determined that oil content decreased, whereas free fatty acid content increased with storage time; storage at 10°C was found to be optimal. This temperature is also suggested for storing because of the economical reasons. Results indicate that rapeseed cakes obtained from four "00" cultivars, dried and stored at controlled conditions can be used for feed.

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MONITORING OF RESIDUES OF PACKAGING MATERIALS IN BAKERY PRODUCTS USED AS FEED INGREDIENT

Leo W.D. van Raamsdonk, Victor G.Z. Pinckaers,
Jef J.M. Vliege, Harry J. van Egmond

RIKILT Wageningen UR, P.O. Box 230, 6700 AE Wageningen, the Netherlands

ABSTRACT

The current high standards for food production imply that certain food products are declared unfit for human consumption. Nevertheless, these so-called former food products can be used for other high-value purposes, such as animal feeding. The usual requirements of quality and safety for these products have to be assured, including a limited amount of remnants of packaging materials.
RIKILT Institute of food safety developed and published a validated method for this purpose.
The method is based on sieving the sample material, manual selection of particles that match the types of packaging materials (paper, board, plastic, clips, wires, etc.) from the appropriate fractions, defatting and dehydration of the selected material, weighing, and calculation of the percentage (w:w).
The method was successfully validated with a precision of 95.5% at a contamination level of 0.15% (w:w), a level of quantification of 0.01% (w:w) and sufficient levels for selectivity, sensitivity and robustness.
In a period of six years 160 samples of bakery products were investigated in the Netherlands. The annual average levels of residues of packaging materials ranged from 0.03% to 0.06% (w:w). Assuming a tolerance limit of 0.15% (w:w), only 7 out of those 160 samples (4.4 %) exceeded this limit.
It was shown that a validated method for quantification of packaging materials can be applied successfully in the feed production chain. Other labs can implement the method. RIKILT can assist in implementation or in outsourcing of monitoring activities.
Keywords*: feed ingredients, former food products, bakery products, packaging materials, quantification.*

INTRODUCTION

The current high standards for food production imply that certain food products are declared unfit for human consumption. This situation can be due to problems of manufacturing, over-production, packaging defects, limited shelf life, etc. Nevertheless, these so-called former food products can be used for other high-value purposes, such as animal feeding. These materials should meet the legal requirements such as microbiological standards and a range of chemical specifications.
Bakery products are a valued source of nutrition for animal feeding. However, residues of packaging materials can still be present after unpacking and

processing. Since standards for the presence of packaging materials are in force in the EU (1), monitoring with a method for screening and quantification is necessary in order to assure a safe application of these bakery products as feed ingredient.
The monitoring of residues of packaging materials in former food products in the Netherlands is carried out since 2005. Bakery products are the most imported category of former food products in the Netherlands, with an estimated annual volume of 300,000 metric ton.
In this framework a method for screening and quantification of packaging materials was necessary to be applied. RIKILT Institute of food safety developed and published a validated method for this purpose.

METHOD DEVELOPMENT

The method is based on sieving the sample material (Figure 1), manual selection of particles that match the types of packaging materials (paper, board, plastic, clips, wires, etc.) from the appropriate fractions, defatting and dehydration of the selected material (Figure 2), weighing, and calculation of the percentage (w:w). Values for selectivity, sensitivity, precision (recovery), robustness and level of quantification have been established.

Figure 1. Sieving a sample of bakery Products

Figure 2. Selected residues of packaging materials.

The method was validated according to Dutch guidelines for intralaboratory method validation (2). International guidelines are currently not available.

RESULTS AND DISCUSSION

The method was successfully validated with a precision of 95.5% at a contamination level of 0.15% (w:w), a level of quantification of 0.01% (w:w) and sufficient levels for selectivity, sensitivity and robustness (4).
In a period of six years 160 samples of bakery products were investigated in the Netherlands. The annual average levels of residues of packaging materials ranged from 0.03% to 0.06% (w:w), as shown in Table 1. Assuming a tolerance

limit of 0.15% (w:w), only 7 out of those 160 samples (4.4 %) exceeded this limit (3).
The materials found originated mainly from paper and board (fibres). A risk evaluation showed that these materials pose a limited risk for animal consumption. In general, the risks after consumption of the different types of plastic are also assumed to be limited (3). The types of this diverse category of packaging materials are not able to be distinguished by visual inspection. This is not necessary, since the current Regulation provides exclusively a general prohibition of packaging materials (1).

Table 1. Overview of the results of monitoring remnants of packaging materials in bakery products intended for animal feeding. Percentages in w:w.

	Years					
	2005	**2006**	**2007**	**2008**	**2009**	**2010**
Total number of samples investigated	25	39	19	21	24	32
Average level of remnants of packaging materials	0.04%	0.04%	0.04%	0.03%	0.04%	0.06%
Maximum level of remnants of packaging materials	0.21%	0.19%	0.22%	0.10%	0.23%	0.71%

It was shown that a validated method for quantification of packaging materials can be applied successfully in the feed production chain. Other labs can implement the method, based on the published detailed description (4). RIKILT can assist in implementation or in outsourcing of monitoring.

CONCLUSIONS

- A method for the screening and quantification of residues of packaging materials in bakery products intended as ingredient in animal feeds was successfully developed and validated.
- A limited number of batches of bakery products exceeded a level of 0.15% (w:w) residues of packaging materials (4.4% in six years), making this category of former food products a safe feed ingredient with high nutritional value for feeding purposes.

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ESTIMATION OF CHEMICAL AND FATTY ACID COMPOSITION OF SOME MARINE FISH SPECIES FROM RETAIL STORES

Dragana Ljubojević[1], Miroslav Ćirković[1], Nikolina Novakov[1], Vesna Đorđević[2], Dejana Trbović[2], Vesna Matekalo-Sverak[2], Danijela Vranić[2]

[1]University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia
[2]Institute of Meat Hygiene and Technology, Kaćanskog 13, 11000 Belgrade, Serbia

ABSTRACT

The aim of this paper was to estimate the chemical and fatty acid composition of representative marine fishes (European sea bass, Atlantic salmon, Gilt-head sea bream, European hake, Atlantic mackerel, European sprat and Atlantic blue fin tuna) which were collected from retail stores in area of Novi Sad. The amount of protein was the highest in Atlantic Blue fin tuna flesh (23.11%) and the lowest percentage of protein was found in European sprat fillets (15.09). Percentage of fat ranged from 1.62, in the muscles of European hake, to 23.29 in the meat of Atlantic mackerel. The total cholesterol content was the highest in European sprat fillets (around 80.25 mg/100g), and the lowest in Atlantic salmon (24.89 mg/100g). The amount of saturated fatty acids (SFA) was the lowest in Atlantic salmon (14.77%). The Atlantic mackerel contained the highest percentage of polyunsaturated fatty acids (PUFA) 47.33% and the lowest percentage was detected in Atlantic blue fin tuna (15.83%). PUFA/SFA, which is an indicator of the quality of fat was the most favorable in Atlantic salmon. Also, significant is the ratio of unsaturated (USFA) to saturated (SFA) fatty acids in fish lipids and it was the best in the fat of Atlantic salmon, and significant difference among species was observed ($p>0,05$), except for European sea bass and Atlantic mackerel. The chemical and fatty acid composition of fishes varies greatly between different species and within the same species

***Keywords**: chemical composition, fatty acid, marine fish species, retail stores*

INTRODUCTION

The knowledge about fat, protein and cholesterol content, as well as about quality of fat in fish meat is important because fish meat has been considered to have beneficial nutritional composition and favorable effects on human health. It represents rich source of protein with beneficial amino acids profile, unsaturated fatty acids, fat soluble vitamins, macro and microelements. Fish lipids are particularly rich of polyunsaturated fatty acids (PUFA) that are only slowly synthesized in humans which is the major difference between meat of fish and meat of farmed terrestrial animals [24]. These PUFAs can reduce the risk of cardiovascular diseases and they reduce mortality in patients with coronary diseases [7]; they decrease the contents of triacylglycerols, cholesterol, and low

density lipoproteins in the human serum, and inhibit the aggregation of blood platelets and the damage to blood vessels [8]; they are important to prenatal development of the human nervous system [1]; they also have a role in improvement of learning ability [22]. Further, polyunsaturated fatty acids of the n-6 and especially n-3, family prevent neural diseases and play a very important role in ontogenesis [2, 17]. Since n-3 PUFAs, such as α-linolenic acid (18:3 n-3, ALA), eicosapentaenoic (20:5 n3, EPA) and docosahexaenoic (22:6 n3, DHA), are effectively synthesized only by aquatic organisms, humans can receive these essential fatty acids by marine and freshwater fishes [21]. There is no available data concerning fatty acids and chemical composition of marine fish species accessible in Serbian market. The objective of this study was therefore to assess the chemical and fatty acid composition of commercial important fish (European sea bass (*Dicentrarchus labrax*), Atlantic salmon (*Salmo salar*), Gilt-head sea bream (*Sparus aurata*), European hake (*Merluccius merluccius*), Atlantic mackerel (*Scomber scombrus*), European sprat (*Sprattus sprattus*) and Atlantic blue fin tuna (*Thunnus thynnus*) which were collected from retail stores in area of Novi Sad.

MATERIAL AND METHODS

Seven species of marine fish of different geographical origin available on the Serbian market were collected randomly in the area of Novi Sad. Eight samples of each fish species were bought from the fish market and various supermarkets. The samples were collected in April and May of 2012 and stored at a temperature of –18ºC until analyses.

Fish fillets were blended (Braun Combi Max 600). The meat from dorsal muscles without skin was used for chemical analyses. Chemical composition of fish muscle tissue was determined using standard SRPS ISO methods.

Fatty acids determination was performed according to Spirić et al. [18] by capillary gas chromatography.

Cholesterol determination in fish fillets (from direct saponification) was performed by using HPLC/PDA system (Waters 2695 Separation module/Waters photodiode array detector, USA) on a Phenomenex Luna C18 (2) reverse/phase column, according to Maraschiello et al. [11].

The results obtained were statically treated by one way analysis of variance (ANOVA). Differences of means were evaluated for significance by the range test of Tukey HSD ($P < 0.05$). Calculations were performed with the STATISTICA 8.0 software package. The results were presented as means ± SE.

RESULTS AND DISCUSSION

Regarding nutrient composition of fishes flesh, it was expected to find some significant variations within the same species and among different species in percentages of the monitored chemical and fatty acid structure between tested fishes in the present experiment because the fishes were imported from various geographical regions, were of the different species and age. Table 1 presents

the results of chemical analysis and total cholesterol content in marine fishes, which were obtained from different stores. It was noted that fat content varies the most in European hake (1.22-1.78%), and the least in European sea bass (9.08-9.22%). Protein content also varied but less than fat content, and variation was the greatest in Atlantic blue fin tuna (22.89-23.34%). The varied content of fat was compensated by the content of water, which is in agreement with the results obtained by Zmijewski et al. [26] who found a reverse correlation between the fat and water contents, which is common for many fish species. Variation within the same species was noted in the amount of total cholesterol, which was the biggest in European sea bass (30.88-31.99mg/100g). The total cholesterol content of the animal tissues can be influenced by the composition of the feed [6], that could be explanation of variations in cholesterol content because examined fish were from different regions and fed different feed. In previous research conducted by Ćirković et al. [4] regarding chemical composition of fresh warm water fish from retail stores, fat content ranged from 1.8 in zander to 10.07% in common carp, and variation within the same species were notably higher which indicates that fresh water fishes from Serbian market also possess the potential for use in special low fat diets. Protein content in freshwater fish from supermarkets was in range from 14.73 in grass carp to 19.27% in zander [4], so in general it was similar with protein content in marine fish from the present examination.

Table 1. Chemical composition of seven marine fish species obtained from retail stores

Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Moisture content %	71,42± 0,09^{a}	62,66± 0,11^{b}	67,62± 0,24^{d}	78,25± 0,13^{e}	58,91± 0,11^{f}	76,3± 0,24^{f}	73,85± 0,2^{g}
Protein content %	18,29± 0,07^{a}	19,01± 0,09^{a}	18,89± 0,08^{a}	18,43± 0,08^{a}	17,3± 0,07^{d}	15,09± 0,07^{b}	23,11± 0,14^{e}
Fat content %	9,08± 0,07^{a}	18,23± 0,08^{b}	11,84± 0,13^{d}	1,62± 0,18^{e}	23,29± 0,12^{f}	6,62± 0,12^{h}	2,84± 0,13^{g}
Ash content %	1,08± 0,06^{a}	1,04± 0,03^{a}	0,82± 0,05^{d}	1,56± 0,04^{e}	1,11± 0,05^{a}	1,30± 0,05^{f}	1,11± 0,06^{a}
Cholesterol content mg/100g	31,38± 0,46^{a}	24,89± 0,07^{b}	75,98± 0,15^{d}	67,76± 0,15^{e}	51,71± 0,13^{f}	80,25± 0,11^{h}	31,66± 0,08^{a}

Values are means ± SD (n = 8); Values in the same row with different letter in superscript statistically significantly differ at $p < 0.05$

1- European sea bass; 2- Atlantic salmon; 3- Gilt head sea bream; 4- European hake, 5- Atlantic mackerel; 6- European sprat; 7- Atlantic blue fin tuna

Great variation within the same species and among species was noted by Ćirković et al. [4] in the amount of total cholesterol, who reported slightly lower cholesterol content in the most analyzed fish species (33.14-65.38mg/100 g) in comparison with pork or beef (45-84 mg/100 g) [14], which is in agreement with the present results for marine fish and amount of total cholesterol was in range 31.38-80.25%. Content of cholesterol in fish from a wild and fish from

aquaculture is different and depends on the species of fish [12]. According to Luzia et al., [9] the amount of total cholesterol in freshwater fish is lower in comparison with marine fish and this is partially confirmed in analysed samples of fishes from Serbian market.

Table 2. Fatty acid composition of seven marine fish species obtained from retail stores

Fatty acid%	1	2	3	4	5	6	7
$C_{16:0}$	18,24 ± 0,14^{a}	9,48 ± 0,1^{b}	15,74 ± 0,07^{d}	22,67 ± 0,13^{e}	15,01 ± 0,12^{f}	25,45 ± 0,51^{h}	36,24 ± 0,15^{i}
$C_{18:0}$	3,59 ± 0,1^{a}	2,29 ± 0,13^{b}	2,81 ± 0,16^{d}	3,62 ± 0,11^{a}	1,92 ± 0,09^{e}	2,59 ± 0,05^{g}	11,45 ± 0,19^{h}
$C_{18:1\ cis9}$	28,93 ± 0,86^{a}	37,94 ± 0,2^{b}	31,91 ± 0,04^{d}	12,05 ± 0,46^{c}	7,23 ± 0,11^{e}	31,29 ± 0,26^{g}	17,62 ± 0,17^{h}
$C_{18:2\ n6}$	10,19 ± 0,36^{a}	13,13 ± 0,05^{b}	10,93 ± 0,03^{d}	2,2 ± 0,05^{e}	2,15 ± 0,05^{e}	3,98 ± 0,1^{g}	1,36 ± 0,07^{h}
$C_{18:3\ n3}$	2,31 ± 0,07^{a}	4,26 ± 0,14^{b}	2,52 ± 0,11^{ad}	1,08 ± 0,08^{e}	2,68 ± 0,28^{d}	2,45 ± 0,19^{a}	0,88 ± 0,06^{e}
$C_{20:4\ n6}$	0,92 ± 0,06^{ab}	1,02 ± 0,09^{a}	0,98 ± 0,06^{a}	1,81 ± 0,2^{e}	2,21 ± 0,21^{f}	0,74 ± 0,03^{b}	1,69 ± 0,17^{e}
$C_{20:5\ n3}$	7,08 ± 0,15^{a}	3,35 ± 0,06^{b}	4,64 ± 0,07^{d}	8,17 ± 0,04^{e}	6,24 ± 0,06^{f}	4,96 ± 0,03^{h}	1,05 ± 0,09^{i}
$C_{22:5\ n3}$	1,77 ± 0,1^{a}	2,07 ± 0,06^{b}	3,6 ± 0,18^{d}	1,67 ± 0,09^{a}	1,28 ± 0,04^{e}	0,62 ± 0,03^{cf}	2,92 ± 0,1^{g}
$C_{22:6\ n3}$	6,21 ± 0,07^{a}	5,23 ± 0,06^{b}	5,86 ± 0,23^{a}	29,34 ± 0,31^{d}	12,6 ± 0,31^{e}	7,09 ± 0,08^{g}	6,9 ± 0,28^{g}
SFA	26,47 ± 0,31^{a}	14,77 ± 0,36^{b}	22,99 ± 0,26^{d}	29,84 ± 0,2^{e}	26,94 ± 0,15^{f}	37,76 ± 0,52^{h}	54,99 ± 0,26^{i}
MUFA	43,4 ± 0,96^{a}	50,95 ± 0,15^{b}	46,89 ± 0,32^{d}	23,32 ± 0,54^{e}	25,32 ± 0,31^{f}	42,89 ± 0,35^{a}	29,02 ± 0,31^{h}
PUFA	30,14 ± 0,3^{a}	34,26 ± 0,21^{b}	30,17 ± 0,23^{a}	46,82 ± 0,26^{d}	47,33 ± 0,57^{d}	20,38 ± 0,27^{e}	15,83 ± 0,36^{f}
n6	11,95 ± 0,3^{a}	15,71 ± 0,17^{b}	12,59 ± 0,07^{d}	4,27 ± 0,21^{e}	5,03 ± 0,28^{f}	5,04 ± 0,15^{f}	3,7 ± 0,24^{c}
n3	18,2 ± 0,12^{a}	18,55 ± 0,15^{a}	17,58 ± 0,25^{c}	42,55 ± 0,26^{d}	42,29 ± 0,6^{d}	15,34 ± 0,16^{f}	12,13 ± 0,3^{g}
n3/n6	1,52 ± 0,04^{a}	1,18 ± 0,02^{a}	1,40 ± 0,02^{a}	9,99 ± 0,53c	8,43 ± 0,52^{d}	3,04 ± 0,08^{e}	3,29 ± 0,23^{e}
n6/n3	0,66 ± 0,02^{a}	0,85 ± 0,01^{b}	0,72 ± 0,01^{d}	0,1 ± 0,01^{e}	0,12 ± 0,01^{e}	0,33 ± 0,01^{f}	0,31 ± 0,02^{g}
PUFA/SFA	1,14 ± 0,02^{a}	2,32 ± 0,06^{b}	1,31 ± 0,02^{d}	1,57 ± 0,01^{e}	1,76 ± 0,02^{f}	0,54 ± 0,01^{h}	0,29 ± 0,01^{i}
USFA/SFA	2,78 ± 0,08^{a}	5,77 ± 0,14^{c}	3,35 ± 0,05^{e}	2,35 ± 0,02^{f}	2,7 ± 0,02^{a}	1,68 ± 0,02^{h}	0,82 ± 0,01^{i}

Values are means ± SD (n = 8); Values in the same row with different letter in superscript statistically significantly differ at P < 0.05. 1- European sea bass; 2- Atlantic salmon; 3- Gilt head sea bream; 4- European hake, 5- Atlantic mackerel; 6- European sprat; 7- Atlantic blue fin tuna

Significant variations in distribution of various fatty acids were noted between and within species. Fatty acid compositions are shown in Table 2. The amount of saturated fatty acids was notably different in all examined species from around 14.77 in Atlantic salmon to 54.99% in Atlantic blue fin tuna and palmitic acid was the dominant saturated fatty acid and significant difference between species was observed (P>0.05). In freshwater fish which were examined by Ćirković et al. [4] the amount of total SFA was notably constant in all examined species at around 30 percent and palmitic acid was the dominant saturated fatty acid and no significance difference between species was not observed (P>0,01). The greatest deviation of total SFA was observed in European sprat flesh and of content of MUFA in European sea bass filets and PUFA in Atlantic mackerel. The lowest n-3/n6 ratio was found in one sample of Atlantic salmon (1.16), and the greatest in one sample of European hake (10.93). Wood et al [25] have suggested that ratio of PUFA/SFA should be above 0.4 and according that all examined fish species have had favorable (from 0.54 to 2.32) PUFA/SFA ratio except 0.29 in Atlantic blue fin tuna. All examined fresh water fish species from Serbian market [4] meet this recommendation. Scollan et al., [16] have advised that n-6/n-3 ratio should not exceed 4. All studied species meet this suggestion 0.1-0.85. It was also the case with the studied freshwater fish from Serbian market [4], despite the fact that freshwater fish had a slightly lower ratio of n3/n6 compared to the tested marine fish. The amount of C20:4 n-6, ARA was higher in fresh water fish from Serbian market [4] compared with examined marine fish species. It is consider that the main role of ARA is as a major precursor fatty acid of eicosanoids in fish [15]. According to Mahaffey [10], several species of salmon, mackerel and herring were high in n-3 fatty acids. Atlantic mackerel and Baltic sprats are rich sources of n-3 long chain polyunsaturated fatty acids (LC PUFA) [20] which is in agreement with the present results.

A high ratio of n-3/n-6 is beneficial for reducing the risk of coronary heart diseases [3] and this ratio is the highest in marine fish [23]. The n-3/n-6 ratio was found generally lower in cultivated than in wild fish [13].

Stansby [19] pointed out that fish should be included in diets for at least three reasons: as a general source of nutritional components; as low-fat, high protein food; and as source of polyunsaturated fatty acids, which is confirmed in the present study. At the same time, the recommended ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (PUFA/SFA) should be increased to above 0.4 [16]. Since some meats of terrestrial farmed animals naturally have a PUFA/SFA ratio of around 0.1 [25], meat has been implicated in causing the imbalanced fatty acid intake of today's consumers.

The ratio of n-6/n-3 is also a risk factor in cancers and coronary heart disease, especially the formation of blood clots leading to a heart attack [5]. The recommendation is for a ratio of less than 4 and again some meats of terrestrial animals are higher than this [25].

CONCLUSIONS

Based on the present study, it can be concluded that marine fish available on the Serbian market represent valuable nutritional component for human nutrition and an important source of n-3 fatty acids. Comparison with previous examined freshwater fishes indicates that meat of fresh water fish also represent valuable nutritional source for human, but it should be improved with better farming procedures on Serbian fresh water fish ponds. Further nutritional examination of aquaculture products is necessary and it represents an important step toward a quality certification process of aquaculture that leads to proper safety requirements and improvment nutritional quality of fish meat present on the market.

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THE INFLUENCE OF DIFFERENT OSMOTIC SOLUTIONS ON NUTRITIVE PROFILE DURING OSMOTIC DEHYDRATION OF PORK

Milica Nićetin[1], Vladimir Filipović[1], Biljana Ćurčić[1], Lato Pezo[2], Violeta Knežević[1], Jasmina Gubić[3], Tatjana Kuljanin[1]

[1]Faculty of Technology, University of Novi Sad, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia
[2]Institute of General and Physical Chemistry, University of Beograd, Studentski trg 12/V11000 Beograd, Serbia
[3]Institute of Food Technology, University of Novi Sad, Bulevar Cara Lazara 1, 2100 Novi Sad, Serbia

ABSTRACT

In order to determine the changes in nutritive profile of meat after osmotic treatment, samples of pork were osmotically dehydrated in three different osmotic solutions and compared to fresh sample. Process was studied in sugar beet molasses, aqueous solution of sodium chloride and sucrose (ternary solution) and combination of these solutions in range 1:1 (combine solution), under atmospheric pressure, at room temperature of 22^{o}C. Results of the chemical compositions of dehydrated meat indicate that all three solutions appeared to be satisfying osmotic mediums, but the best nutritive profile of final product was achieved using sugar beet molasses as osmotic agent. Unlike ternary solution that allows only the diffusion of salt and sugar into the meat during the process, molasses has complex chemical composition conducive to impregnation of desirable nutritional compounds and minerals in meat product. It was detected enrichment in analyzed minerals (K, Na, Mg, Fe and Ca), especially K (from 0.52g to 1.46g) in meat samples dehydrated in molasses.
Keywords: *osmotic dehydration, pork, osmotic solution, chemical composition*

INTRODUCTION

Presently, the food industry has tendency to cover the growing consumer demand for new functional products enriched in some nutritional valuable compounds [7]. Meat is a rich source of biologically and nutritionally valuable ingredients, but represents highly perishable foodstuff due to the high water content [10]. Traditional preservation methods have been very successful in slowing the rate of microbial spoilage, but these methods produce food products that are low in quality compared to their original fresh state [3]. The application of osmotic dehydration (OD) at mild temperature in meat preservation has many advantages as compared to traditional drying treatments. Meat is not exposed to high temperatures, minimizing, in that way, sensory characteristics changes, and preserving nutritional values of the fresh meat: vitamins, minerals, etc [6]. OD is

a process of the partial removal of water by direct contact of foods with a suitable hypertonic solution. Driving force for water removal is the concentration gradient between the surrounding solution and the intracellular fluid [8]. During OD, water, from the plant or animal tissue, flows out into the osmotic solution while osmotic solutes diffuse from the solution to the tissue. Simultaneously, third transfer process takes place, leaching of tissue's own solutes into the osmotic solution, but it is quantitatively negligible compared to the first two transfers [9]. Sugar beet molasses is an excellent medium for OD, primarily due to the high dry matter (80%) and specific nutrient content [8].
The objective of this research was to examine the effects of three different hypertonic solutions on nutritional profile of processed pork.

MATERIAL AND METHODS

For the experiment, fresh pork (M.triceps brachii) was purchased on the local butcher shop, shortly before use. Before the osmotic treatment, fresh meat was cut into cubes, dimension of approximately 1x1x1cm. Concentrated sugar beet molasses from sugar factory Pećinci was used as one osmotic solution. Ternary solution was prepared by mixing three components, commercial sugar in the quantity of 1200 g/kg water, NaCl in the quantity of 350 g/kg water and distilled water. Combine solution was obtained by mixing molasses and ternary solution in range 1:1. The material to solution ratio of 1:5 was used during experiments. The all experiments were carried out under atmospheric pressure at the room temperature of 22°C. The process was performed in laboratory jars. Samples of meat were dipped into all three solutions, and the immersion lasted for 5 hours. On every 15 minutes meat samples was manually agitated to provide better homogenization of the osmotic solutions. After 5 hours meat samples was taken out from osmotic solutions and then lightly washed with water and gently blotted with paper towels to remove excessive water from the surface.
The basic chemical composition of fresh and dehydrated meat samples was determined by examining: moisture content (dry matter*)* [15], protein-nitrogen content [1], free fat content [14], chloride content [13], sugar content by Luff-Schoorl-u [2], metal content (AAS) [5].

RESULTS AND DISCUSSION

Table 1 provides an overview of the basic chemical composition of initial meat sample. Table 2 shows the changes of chemical composition in samples of dehydrated pork after immersion in three different hypertonic solutions under the defined conditions.

Table 1. Chemical composition of meat sample before dehydration

Chemical parameter (g)	Fresh pork (100g)
Dry matter	28.45
Total Protein	20.36
NaCl	0.69
Sucrose	1.20
Fat	4.50
K	0.52
Na	0.25
Mg	0.11
Ca	0.0104
Fe	0.0046

Table 2. Chemical composition of meat sample after dehydration in three different solutions

Chemical parameter (g)	Pork after OD in molasses (70.03g)	Pork after OD in ternary solution (75.24g)	Pork after OD in combine solution (68.69g)
Dry matter	41.12	42.10	40.76
Total Protein	21.10	17.80	19.48
NaCl	0.81	5.26	2.46
Sucrose	6.19	8.39	7.27
Fat	4.75	4.35	4.55
K	1.46	0.36	1.01
Na	0.48	3.93	1.84
Mg	0.21	0.08	0.18
Ca	0.0608	0.0083	0.0390
Fe	0.0056	0.0022	0.0038

In this study were performed chemical analyzes of dehydrated and non-dehydrated meat, with the aim of better understanding of the mechanisms involved in three simultaneous flows that take place in the OD process. Based on mass balance of the chemical composition of meat before and after OD it is possible to quantitatively and qualitatively define the mass transfer occurring during the OD. Initial weight of meat sample before OD was 100g, and after 5 hours of treatment was measured weight of 75.24g for meat dehydrated in R2, 70.03g in R1 and 68.69g in R3. The content of dry matter (DMC) in dehydrated samples was increased compared to the fresh sample, from initial 28.45g to 42.10g, 41.12g and 40.76g when used R2, R3 and R1 as hypertonic solutions, respectively. Based on the measured weights obtained dehydrated products, and their DMC, it is possible to calculate the water loss and solid gain, for each samples, as the main indicators of the efficiency of the OD process. After OD in R2 increase of solids for 13.65g and reducing the amount of water for 38.41g

was observed. From meat submerged in R3 flow out 42.46g of water, while from molasses penetrated 12.67g of dry matter into the meat, during OD. After immersion in R3 dehydrated product had 43.62g of water less than in the initial sample, whereas 12.31g of dry matter more than in the initial sample. In all three cases process of dehydration has proved to be very efficient, but the best result in terms of the highest water loss and the least solid gain was achieved using R3. Protein is the most important nutritional component of the meat, and only OD with molasses proved to be useful in the preservation of this component. Unprocessed pork had protein content of 20.36 g, but after OD in R1 was reached 21.10g of proteins, indicating slightly increase of 0.74g. Reason for the increase the content of protein is that the molasses contains about 5% proteins[14], and it is possible that some of them diffuse into the meat dipped in molasses. On the other hand, after OD in the other two solutions an opposite mass transfer was occurred and causes reduction in protein content. Underway process of OD 0.88g of proteins were diffused from meat into the R3 and even 2.56g from meat into the R2. The amount of salt was ranged from initial 0.69g to 0.81g in meat dehydrated in R1, to 5.26g in meat dehydrated in R2, and to 2.46g in meat dehydrated in R3. The increase of salt was the most expressed using R2 as osmotic agent, about 7.6 times in comparison to the initial sample. Significant salt impregnation occurs because the R3 contains 23 g of salt as opposed to molasses, which has in its composition less than 1g of salt. High salt content in the final product is not desirable from the health point of view [4]. Therefore, the low enrichment of dehydrated product with salt gives advantage to the use of molasses in relation to the other two osmotic solutions that lead to penetration of greater amount of salt. Compared to the fresh state of meat which contained 1.20g of sucrose, sucrose content was increased about 5 times after OD in R1, about 7 times after OD in R2 and about 6 times after dehydration in R3. The changes in the fat content in all dehydrated samples are negligible compared to the initial fat content. The processes of OD with R1 and R3 slightly increase content of fat in meat, whereas OD with R2 slightly decreases fat content.

Minerals have irreplaceable significance for normal functioning of every organism and their role in maintaining health is very important [8]. It is known that sugar beet molasses represents a significant source of many minerals, especially potassium, calcium, iron and magnesium. Particularly significant is the fact that all the mineral components in the molasses are in dissolved state and the potassium is in much greater quantities than all other cations about 4g K/100g molasses [14]. For this reason, using molasses as osmotic agent during OD improves mineral composition of final products, unlike ternary solution that enriches the final product only with sodium ions. By analyzing the content of mineral components (K, Na, Mg, Fe and Ca) in the sample osmotically dehydrated in molasses increase the amount of all these mineral substances was observed. The most pronounced increase was observed in respect of K, from 0.52g to 1.64g, about 2.8 times in comparison to the meat in fresh state. The content of Na and Mg were slightly increased in treated meat product, for 0.23g and 0.1g, respectively. Ca and Fe are present in small amounts in meat,

but they are very important minerals for human health, and even their light increase after OD in molasses is significant. After OD in R2 was observed decrease in all analyzed minerals which diffused from meat into solution, except of Na. The content of Na was increase from initial 0.25g to 3.93g, which means that it has increased by about 16 times. Considering the fact that the value of recommended daily sodium intake amount is 2.4g, due to its relationship to hypertension, increase of salt in this large extent is not desirable [4]. After dehydration in R3 was observed the increase of K but to a lesser extent than after OD in pure molasses. The content of Na was increase to 1.84g, but still has a high value from health point. R3 was affected on slightly decrease of Mg, Ca and Fe in dehydrated meat.

CONCLUSION

Based on the presented results it can be concluded that all three solutions proved to be satisfying osmotic agents, but the best indicators of the efficiency of the OD process has a combine solution.
Sugar beet molasses is optimal solution in terms of improving the nutritional profile of dehydrated product in comparison with the other two solutions.
The presence of complex solute compositions in molasses maintains a high transfer potential favorable to water loss, and at the same time to penetration of desirable nutritional compounds and minerals in processed pork.
The use of molasses as an osmotic agent during dehydration enables preserving of protein and increasing salt and sugar in appropriate quantities, and thus has more positive effect on chemical composition of treated product compared to the effects of ternary and combine solutions.
On the basis of the analyzing changes in mineral content after OD is also evident considerable advantage of using molasses during the process. OD in molasses gives products with increased amount of mineral substances, especially K, while ternary solution increase only content of Na, but in excessive extent.
The nutritional improvement of the final product is the most important advantage of sugar beet molasses, but its application as osmotic agent also has many other advantages: it is sensory acceptable, always accessible in large quantities, cheap raw material and has an energy and environmental significance.

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SALMONELLA FROM ANIMAL FEED: BIOFILIM FORMING ABILITIES AND ANTIMICROBIAL SUSCEPTIBILITY

Dubravka Milanov[1], Dragica Stojanović[1], Bojana Prunić[1], Ivana Čabarkapa[2], Ljiljana Suvajdžić[3]

[1]Scientific Veterinary Institute „Novi Sad", Rumenački put 20, 21 000 Novi Sad, Serbia
[2]Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, 21 000 Novi Sad, Serbia
[3]Faculty of Medicine, Department of Pharmacy, University of Novi Sad, Hajduk Veljkova 3, 21 0000 Novi Sad, Serbia

ABSTRACT

Animal feed is frequently contaminated with different serotypes of *Salmonella* that can lead to infection or colonisation of animals used for food and to subsequently provoke the infection in the consumers. Sometimes, though rarely established, feed contamination with *Salmonella* strains resistant against antimicrobial agents of clinical relevance is possible.
Feed factory environment is one of potential sources of cross-contamination and recontamination of end-products with *Salmonella*. It is well known that some strains may persist on feed production sites for years. Such strains are commonly identified as „house-strain". It is hypothesized that their persistence is greatly enhanced by their ability to form biofilm on diverse abiotic surfaces. In biofilm, the bacteria manifest an increased resistance to disinfection and other stress factors commonly present on feed production sites.
The aim of this study was to investigate the ability of biofilm formation and antimicrobial susceptibility of 30 *Salmonella* strains isolated from feed in the period January-March 2012. Confirmation and serotyping of *Salmonella* was performed in the National Reference Laboratory. The ability of biofilm forming was examined using polystyrene (microtiter plate assay), and isolates susceptibility to various antibiotic groups by the standard disc diffusion test.
The obtained results confirmed ability of biofilm formation in different *Salmonella* strains isolated from feed. This suggests that biofilm can provide persistence of particular strains in feed factory environment. Because of susceptibility of all isolates towards main groups of antibiotics, feed was not identified as an important vector for resistant *Salmonella* strains.
Keywords: *Salmonella, feed, biofilm, antimicrobial susceptibility*

INTRODUCTION

Despite tremendous efforts to ensure production of healthy and safe food and continuous monitoring of microbial food safety, millions of people get infected and become ill of salmonellosis, and more than 95% of such infections are food borne. Animal feed is at the beginning of the food safety chain in the "farm-to-

fork" model [3]. The occurrence of *Salmonella* in feed ingredients and feed for production animals constitutes a considerable risk of *Salmonella* colonization or infection in animals, and subsequently in the consumers of animal products. In salmonellosis, the contamination tracing to the animal feed as its ultimate source is quite rarely possible; however, such association has been reported in several incidents [3, 20]. The common laboratory practice in our country implicates neither mandatory verification and serotyping of *Salmonella* strains isolated from animal feed nor testing their antimicrobial resistance. Thus, animal feed still represents an unclear source of human infection, as well as potential vector for clinically important *Salmonella* strains resistant to antibiotics.

Salmonellae may occur in the animal feed via the contaminated feed ingredients or through post-processing contamination [9, 13].Thermal processing of animal feed is mostly adequate to eliminate *Salmonella*. Two main risk factors in animal feed manufacturing involve insufficient heating and recontamination after the thermal processing. Feed factory environment being the potential source of post-processing contamination [5, 9, 20]. It is well known that some strains may persist on food production sites for years. Such strains are commonly identified as „house-strains". It is hypothesized that their persistence is greatly enhanced by their ability to form biofilm, alone or in coexistence with other common bacterial organisms [12, 13, 20]. Biofilms are defined as microbial sessile communities that are irreversibly attached to a surface, to an interface or else, enclosed in extracellular polymeric substances, which exhibit and altered phenotype in terms of the growth rate and gene transcription [4]. An initial step in biofilm formation is the attachment of bacteria to the surface, and it is strongly determined by a range of physicochemical properties of bacterial cell surface and the substrate. In a view of food safety, the major "benefit" of the biofilm environment for bacteria is their increased resistance to disinfectants, drying and other common stress factors in food and feed processing plants, as compared to the resistance established in some studies of planktonic cell populations in the laboratory [2,10,11,19].

Biofilms may create a persistent source of product contamination. Cross contamination occurs when cells detach from biofilm structure once food passes over contaminated surfaces or through aerosols originating from contaminated equipment [5, 14]. European Union legislation strictly requires adoption and compliance with Hazard Analysis and Critical Control Points (HACCP) procedures for control of *Salmonella* and other pathogens in feed by all manufacturers of animal feed [8].

The aim of the present study was to investigate the biofilm forming abilities on plastic surfaces and antimicrobial susceptibility of 30 *Salmonella* strains isolated from animal feed.

MATERIALS AND METHODS

Bacterial strains

A total of 30 *Salmonella* strains were used in this study. *Salmonellae* were isolated in the laboratory of the Scientific Veterinary Institute in Novi Sad in the

period January – March, 2012 from animal feed samples examined according to EN ISO 6579:2008. In National Reference Laboratory for *Salmonella, Shigella, Vibrio cholerae, Yersinia enterocolitica,* Institute of Public Health of Serbia "Dr Milan Jovanovic Batut", Beograd, Srbija, the following nine serotypes were identified: *S.Tennessee* (n=11); *S.Senftenberg* (n=5); *S.Stanleyville* (n=4), *S.Agona* (n=4), *S.enteritidis* (n=2), *S.Montevideo* (n=1), *S.Jerusalem* (n=1), *S.Thompson* (n=1) and *S.Mbandaka* (n=1). *Salmonella typhimurium* ATCC 13311 was used as a control strain.

Inoculum preparation

All strains were stored at -70^{o}C u Tryptone soy broth (TSB, CM129, Oxoid LTD, Basingstoke, UK) supplemented with 15% glycerine and recovered on Columbia blood agar (CM 331, Oxoid, Basingstoke, UK), supplemented with 5% sterile defibrinated sheep blood, at 37.0 ± 1.0°C for 24h. One characteristic colony of each strain was suspended in 3mL TSB and incubated statically overnight at 37.0±1.0°C. The obtained suspensions were employed as the inoculum in the microplate biofilm assay.

Microplate biofilm assay

Each well of a sterile 96-well polystyrene microplates (Cat. No.167008, Nunclon, Rosklide, Denmark) was inoculated with 200 µL TSB. 20 µL-aliquots of each *Salmonella* isolate and the control strain were inoculated into the 8 wells. The negative control wells contained TSB only. The plates were incubated for 48 h at 28^{o}C (without agitation). After the incubation period, the content of the plates was poured off and the wells washed three times with 300 µL of sterile distilled water. The plates were dried upside down at room temperature, and then stained with 250 µL per well of 0.3% Cristal violet (No. 42555, Sigma-Aldrich) during 15 minutes, at room temperature. Following staining, the plates were rinsed until there was no visible trace of stain. The stain bound to bacteria was dissolved by adding 250 µL of 95% (v/v) ethanol for 5 min. The optical density (OD) was measured spectrophotometrically (Labsystems Multiscan® MCC/340) using 595nm filter. Cut-off OD (ODc) is defined as three standard deviations above the mean OD of the negative control [17]. Strains were classified as follows: non-biofilm producers (OD ≤ ODc); weak biofilm producers (ODc < OD ≤ 2 x ODc); moderate biofilm producers (2 x ODc < OD ≤ 4 x ODc) and strong biofilm producers (4 x ODc < OD) [17].

Antibiotics susceptibility test

Susceptibility of *Salmonella* isolates was examined by the disc diffusion method using Mueller-Hinton agar (CM337, Oxoid, Basingstoke, UK). The following antibiotics were tested: amoxicillin + clavulanic acid (20/10 µg), ampicillin (10 µg), cefpodoxime (10 µg), ceftazidime (30 µg), cefotaxime (30 µg); ciprofloxacin (5 µg), chloramphenicol (30 µg), gentamicin (10 µg), nalidixic acid (30 µg), streptomycin (10 µg), sulphonamides (300 µg), tetracycline (30 µg), trimethoprim (5 µg), trimethoprim + sulfamethoxazole (1,25/23,75 µg) (antimicrobial

susceptibility discs *Bio-Rad Laboratories*). The plates were incubated for 18h at 37°C.

RESULTS AND DISCUSION

In the period from January to end March 2012, microbiological safety testing of 510 feedstuff samples was performed in the laboratory of the Scientific Veterinary Institute in Novi Sad, and *Salmonellae* were isolated from 30 (5.9%) samples. *Salmonella* were isolated mainly from premixed cattle, poultry and swine feed (26 isolates), yet it must be taken into consideration that such type of feedstuff is most commonly sampled and submitted for analysis. Four strains were isolated from feed ingredients: poultry meat meal (*S. Thompson*), soy pellets (*S. Tennessee*), corn gluten meal (*S. Seftenberg*) and soy grits (*S. Stanleyville*).

Table 1. Biofilm formation by Salmonella strains (n=30) incubated at 28°C for 48h

Serotypes	**Biofilm formation**							
	Strong biofilm producers		**Moderate biofilm producers**		**Weak biofilm producers**		**No biofilm producers**	
	No	OD±SD	No	OD±SD	No	OD±SD	No	OD±SD
S.Tennessee	1	0.657±0.06	10	0.616±0.12	-	-	-	-
S.Montevideo	-	-	1	0.46±0.08	-	-	-	-
S.Agona	-	-	1	0.375±0.02	3	0.232±0.02		
S.Jerusalem	-	-	1	0.366±0.04	-	-	-	-
S.Enteritidis	-	-	2	0.365±0.01	-	-	-	-
S.Mbandaka	-	-	-	-	1	0.215±0.02	-	-
S.Senftenberg	-	-	-	-	5	0.202±0.01	-	-
S. Thompson	-	-	-	-	1	0.198±0.02	-	-
S. Stanleyville	-	-	-	-	2	0.173±0.01	2	0.146±0.01
S.typhimurium ATCC 13311	-	-	-	-	1	0.165±0.01	-	-

Internationally, fishmeal, meat and bone meal, maize and soy products have been shown to have a relatively high prevalence of *Salmonella* [8]. Isolated *Salmonella* strains were tested in a microplate biofilm assay, with incubation for 48h at 28°C. The obtained results were classified according to the extinction rate and *Salmonella* serotypes. The results are displayed in Table 1.

The results obtained in the microplate biofilm assay confirmed the ability of different *Salmonella* serotypes isolated from animal feed to adhere to polystyrene and to form a biofilm that is more or less quantifiable. Our results are in agreement with other studies, which showed that *Salmonella* are able to form biofilm on plastic surfaces [1,14,17,18]. In the microplates, the examined strains attached predominantly to the surface at air-liquid interface, and to a

much lesser extent on the bottom of the wells. Examining the adherence of *S. enteritidis* revealed that air-liquid interface provided the best environment for biofilm formation on stainless steel [6] and plastic surfaces [16], whilst the colonization of the air-liquid interface is primarily due to overproduction of a cellulosic polymer [6].

The highest extinctions were yielded in serotype *Tennessee,* which were categorized as strong (n=1) and moderate (n=10) biofilm producers. *S.Tennessee* was the most commonly *Salmonella* serotype isolated from animal feed in the period January-March, 2012. According to literature data, this serotype is commonly found on the food-contact surfaces in food and feed production plants, being the most persistent strain identified as source of infection in salmonellosis epidemics in both humans and animals. Thus, during an epidemic outbreak caused by *S. Tennessee* in United States (2006) a peanut butter plant was identified as a source of contamination [15]. In spring 2009, a feed borne outbreak of *S. Tennessee* was reported in Finland, which was attributed to *S. Tennessee* contamination identified in the feed mill for an extended period of time [7]. Frequent isolation of this serotype and its well-established ability of biofilm production strongly advocate the importance of controlled and continuous monitoring of animal feed production plants in our country and the risk of existence of persistent strains. In our country, there are no data on persistence of this or other *Salmonella* serotypes in animal feed production plants.

Isolates of serotypes *Montevideo*, *Agona, Jerusalem* and *Enteritidis* were assessed as moderate biofilm producers. In feed and fishmeal factories, serotypes *Agona*, *Montevideo* and *Senftenberg* have been the ones most frequently isolated [13, 20]. Our isolates (n=5) of the serotype *Senftenberg* demonstrated weak ability of biofilm production. Vestby et al. reported that prolonged time of incubation (from two to four days) significantly increased the OD_{595} values in serotype *Senftenberg* in a microplate biofilm assay [20]. The lowest extinction values were observed in 4 isolates of *S. Stanleyville*. This serotype has been sporadically isolated from animal feed, in cattle and pigs. Moreover, there is a report on its isolation from human urinary infection. According to the available literature, there are not reports on persistence of this serotype at animal feed production plants.

Undoubtedly, the applied technique and conditions (time, temperature and nutritive medium) of a microplate biofilm assay strongly influence the evaluation of ability of biofilm formation in *Salmonella* strains; however, a standardized method for assessment of biofilm formation ability in bacteria is not yet available, thus making the evaluation of the obtained results extremely complex and difficult. Microtiter plate assay can be effectively used for the assessment of biofilm ability of *Salmonella* strains [1], a 48-hour incubation was found to be most efficient in bioflm formation by *Salmonella* [1, 11]. Microtiter plates are frequently used as a substrate, since plastic surfaces are particularly suitable owing to their hydrophobic non-polar nature with little or no surface charge. In the past few years, plastic materials have been widely used in the food and feed industry, as well as in the household. Application of plastic surfaces during food

preparation poses a high risk of cross-contamination [14]. A correlation between biofilm formation of *Salmonella* on polystyrene at room temperature and other materials, such as stainless steel was experimentally confirmed [20].
The established differences in biofilm-forming ability confirmed the belief that *Salmonella* adhesion is strongly strain-dependent [14]. The factors governing the bacteria adhesion to surfaces are still not well understood. Ability to form biofilm is important for the *Salmonella* persistence in feed factory environments. Common sites for the presence of *Salmonella* in food processing plants are filling or packaging equipments, floor drains, walls, cooling pipes, conveyors, collators for assembling product for packaging, racks for transporting products, hand tools or gloves, freezers, etc, which are usually made of plastics [1].
All 30 *Salmonella* isolates showed susceptibility to all antibiotics used in this research. Thus, animal feed encompassed by this examination has not been identified as an important vector for resistant strains. This, however, does not exclude the necessity of continuous monitoring of antimicrobial resistance of *Salmonella* isolates in animal feed, particularly in case of feedstuff of animal origin and imported ingredients.In our country, the surveillance of animal feed contamination with *Salmonella* strains is not integrated into the surveillance programs pertaining to animal health, microbiological control of food and human infections. All this obstructs and prevents real insight in the role of animal feed as a source of human and animal infections. Moreover, the existence, extent and distribution of *Salmonella* biofilms in animal feed production plants as a potential source of contamination of final products, still remain unclear.

CONCLUSION

Salmonellae isolated from animal feed can form biofilm on plastic surface, though significant differences in biofilm formation ability were established between particular serotypes. Plastic material in feed production plants may be a potential substrate for biofilm formation, and thus potential source of post-processing contamination with Salmonellas. Animal feed encompassed by this research has not been identified as a vector for introducing *Salmonella* strains into the food chain.

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TESTING CONCENTRATE FEED MIXTURES WITH INCREASED SELENIUM CONCENTRATION IN ORDER TO PRODUCE EGGS AS FUNCTIONAL FOOD

Nataša Tolimir[1], Rade Jovanović[1], Marijana Maslovarić[1], Marina Vukić Vranješ[1], Milanka Drinić[2]

[1]Institute for Science Application in Agriculture, Bulevar despota Stefana 68b, 11000 Belgrade, Serbia
[2]Faculty of Agriculture, University of Banja Luka, Bulevar Vojvode Petra Bojovića 1A, 78000 Banja Luka, Republic of Srpska

ABSTRACT

The goal of the research was to change egg nutritional content using concentrate feed mixtures with increased selenium concentration, and therefore to obtain selenium-enriched eggs as functional food and test egg quality and selenium concentration. The experiment was carried out with 240 laying hens of Isa Brown provenance, divided into two groups. Selenium was added in concentrate mixtures in the form of sodium selenite and selenized yeast. Selenium concentration in feed was 0.53 mg/kg in the control group and 3.18 mg/kg in the experimental group. The results indicate that adding selenium has no significant effect on the quality of table eggs, although there has been an increase in the egg mass and Houhg units. Selenium concentration in eggs was significantly higher in the experimental group (1.237 mg/kg) than in the control group (0.355 mg/kg). Adding selenium in feeds for laying hens is a very efficient method for producing selenium-enriched eggs, i.e. producing eggs as functional food.
***Keywords**: hens, eggs, selenium, egg quality, functional food*

INTRODUCTION

Selenium belongs to a group of essential nutrients and therefore it is a necessary ingredient in feed mixtures for laying hens [9]. Selenium in feed is important for improving poultry health and productivity. However, in the last decade, the importance of selenium from the aspect of functional food has been noted. Researches show that selenium-enriched eggs can be easily produced with adding selenium in feed mixtures for laying hens [16]. In their researches on selenium as a feed supplement and its different forms (organic and inorganic) and concentrations, many authors determined a positive correlation between the intake and the deposition of selenium in eggs [5], [12], [3]. Selenium-enriched eggs are functional food important for consumers, especially in areas of selenium-deficient soil. Plants grown on this soil have low selenium concentration, while people in these areas have low selenium level in their blood serum. Lack of selenium in human nutrition can result in poor health and

diseases [4]. Serbia is also classified as a country with low selenium concentration in soil, ranged 39-444 µg/kg [7]. One way to prevent selenium deficiency in human nutrition is to consume food with improved nutritive properties, i.e. food with higher selenium concentration. The daily selenium requirement of humans [17] can be met by consuming two selenium-enriched eggs containing 55-65 µg of selenium. Some authors [15] indicated that there is a need for improving the quality of urban life (characteristic for a high level of stress, lack of physical activity, unhealthy diet, and other negative factors jeopardizing human health), that is a need for preventing diseases and slowing down the aging process. According to these authors, functional food is therefore important for all categories of population, especially for the most vulnerable ones because of its supplemental ingredients that make it food of improved quality, important for human health [6]. The goal of the research was to change egg nutritional content using concentrate feed mixtures with increased selenium concentration, and therefore to obtain selenium-enriched eggs as functional food and test egg quality and selenium concentration.

MATERIAL AND METHODS

The experiment was carried out with 240 laying hens of ISA Brown provenance. The hens were divided into two groups – a control and an experimental group. Each group contained 120 hens, and the groups differed in the way of nutrition. The control group was given standard feed for laying hens, and the experimental group feed supplemented with selenium in an organic and an inorganic form (sodium selenite and selenized yeast). Selenium concentration in feed was 0.53 mg/kg in the control group and 3.18 mg/kg in the experimental group. The experiment lasted for 3 weeks. It started in the 57th week, while egg sampling was carried out after 21 days. Thirty eggs were separately tested on inner and external quality parameters: weight, shape index, shell colour, yolk content and colour, albumen height, Houhg units and shell thickness. Weight was measured using a technical balance (accuracy of 0.01 g), and albumen height with an AMES tripod micrometre (accuracy of 0.1 g). Yolk colour was determined with a Roche Yolk Colour Fan, and Hough units with a calculator of the American Instrument Co. Eggshell thickness was measured with a micrometre accuracy of 0.01 g. The chemical analysis of selenium concentration in homogenized yolks and albumens of ten individual egg samples was conducted according to the BSN method 15463:2009, ICP-MS technique. The analysis of obtained experimental data was done using a statistical package STATISTICA for Windows (StatSoft 2006), for a single-factor experiment ANOVA.

RESULTS AND DISCUSSION

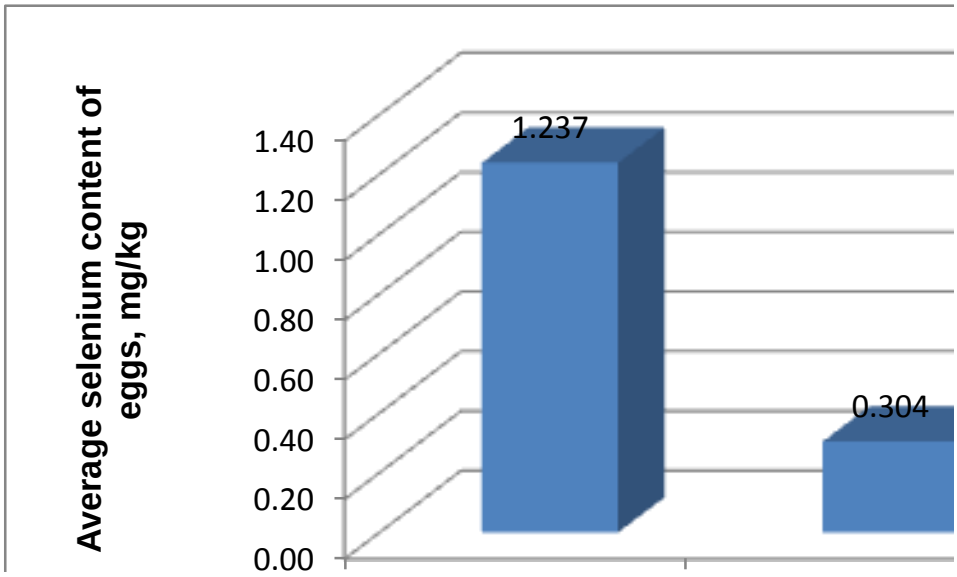
The effect of feed mixtures with increased selenium concentration on quality parameters of ISA brown hen eggs is shown in the Table 1.

Table 1. Effect of adding selenium in hen feed mixtures on egg quality parameters

Egg quality parameters	Diets*	
	Experimental group – Selenium added	Control group
Egg weight, g	65.65±4.42	63.70±3.76
Egg length, mm	56.43±2.01	56.00±1.66
Egg width, mm	45.33±0.99	45.27±1.39
Shape index	80.40±2.55	80.89±3.11
Shell colour	3.17±0.46	3.17±0.59
Yolk colour (Rouche)	11.97±0.18	12.10±0.48
Yolk weight, g	16.32±1.23	16.28±0.95
Yolk content, %	24.93±2.00	25.61±1.59
Albumen content,%	63.04±2.21	61.82±1.90
Hough units (HU)	74.03±8.62	71.40±12.61
Shell weight, g	7.90±0.95	7.99±0.63
Shell content, %	12.03±1.25	12.56±0.89
Shell thickness, mm	0.39±3.43	0.40±2.26

*Mean value ± SD, n=30 eggs per group

The analysis of the obtained results determined that differences between the control and the experimental group in egg quality parameters were not at the level of statistical significance, which indicates that adding selenium in hen feed mixtures has not had any effect on egg quality.
However, when it comes to egg quality parameters, it can be noted that the experimental group had bigger egg weight, which may have something in common with the researches in which adding selenium had resulted in a significant increase in egg weight [1], [5]
Moreover, although no significance was determined, the experimental group had higher values of Hough units, which was in accordance with one of the previous researches [10]. Significant effect of adding sodium selenite and selenized yeast in hen feed mixtures on Hough units was confirmed in one of the previous researches [1], while another research [13] showed positive effect of adding selenium on Hough units
The effect of adding selenium on shell thickness was not determined, which is in compliance with one research [11], but not with some others [8] and [14] that showed positive effect of selenium on this egg property.
The effect of adding selenium-enriched mixtures in feed for laying hens on selenium concentration in table eggs is shown graphically (Graph 1) and tabular (Table 2).



Graph 1. Selenium concentration in eggs

The analysis of obtained results of selenium concentration in eggs showed statistically significant difference between the control and the experimental group (Table 2).

Table 2. Effect of adding selenium in hen feed mixtures on selenium concentration in eggs

Property	Diets*	
	Experimental group – Selenium added	Control group
Selenium concentration, mg/kg	1.237±0,038[a]	0.304±0.009[b]

*Mean value ±SD, n=10 eggs per group
a,b – Values in the same line are significantly different ($p<0.01$)

Determined significantly higher selenium concentration in table eggs as a result of adding selenium in hen feed mixtures was also confirmed in some researches [2] that used organic and inorganic selenium as a feed supplement. Moreover, a significant linear increase in selenium concentration eggs was determined [12] after applying different selenium forms and concentrations. Increased selenium concentration in a yolk after adding mineral and organic selenium is also determined in one research [5].

CONCLUSIONS

Based on the obtained experimental results, it could be concluded that adding selenium in feed for laying hens has no significant effect on egg quality parameters, although there has been an increase in the egg mass and Houhg units. Adding selenium in feed mixtures resulted in a significant increase in selenium concentration in eggs. Adding selenium in feed for laying hens is a very efficient method for producing selenium-enriched eggs, i.e. producing eggs as functional food. Consummation of selenium-enriched eggs, as food with better nutritive properties, could be especially important for areas of selenium-deficient soil, as well as for preventing diseases and improving human health.

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SHARE OF *PENICILLIUM* SPECIES IN MYCOPOPULATIONS ISOLATED FROM FEEDS

Marija Škrinjar[1], Mira Ač[1], Slavica Vesković-Moračanin[2], Vladislava Šošo[1], Nevena Blagojev[1]

[1]Faculty of Technology, University of Novi Sad, Bul. cara Lazara 1, 21000, Novi Sad, Serbia,
[2]Institute of meat hygiene and technology, Kaćanskog 13, 11000, Belgrade, Serbia

ABSTRACT

In this work, mycological investigations of different feed samples (117) (concentrate – 21 samples, dried clover – 21, fresh clover – 3, hay – 12, corn silage – 23, corn germ – 10, dried cornstalks – 6, corn dent – 1, pelleted sugar beet pulp – 10, fresh sugar beet pulp – 3, fresh rape leaf – 1, malt spent grains – 6) were conducted. Samples were isolated on the dairy cattle farms in the Vojvodina province, with special focus on frequency of *Penicillium* species in isolated mycopopulations. Investigations were carried out during one research year, all four seasons. During summer, 33 samples were examined, during autumn 27, during winter 30 and during spring 27 samples.
It was observed that during summer all examined samples were contaminated with moulds, while during autumn, winter and spring 85%, 90% and 93%, respectively. Diverse mould species isolated from feed samples belonged to 29 genera ((*Absidia, Acremonium, Alternaria, Aspergillus, Aureobasidium, Botrytis, Chaetomium, Cladorrhinium, Cladosporium, Eurotium, Fusarium, Geotrichum, Gilmaniella, Paecilomyces, Penicillium, Phoma, Rhizopus, Scopulariopsis, Stachybotrys, Stemphilium, Trichoderma, Trichothecium, Ulocladium and Verticillium*). The biggest share in isolated mycopopulations had genus *Penicillium*, which was presented with 31 different species (*P. aurantiogriseum, P. brevicompactum, P. camemberti, P. canescens, P. capsulatum, P. charlesii, P. citrinum, P. clavigerum, P. commune, P. cyaneum, P. echinulatum, P. expansum, P. frequentans, P. funiculosum, P. griseofulvum, P. islandicum, P. italicum, P. janthinellum, P. lividum, P. nigricans, P, purpurogenum, P. roqueforti, P. rugulosum, P. tardum, P. variabile, P. velutinum, P. viridicatum, P. verrucosum, P. verruculosum, P. vulpinum* and *P. waksmani*). The most frequent species was *P.aurantiogriseum*, which was isolated from 69 (59%) of samples. Its presence was observed during the whole research year at nearly the same level. The next highest frequency had *P. camemberti, P. echinulatum* and *P. purpurogenum,* respectively, which was detected in the samples only during the summer period, and *P.waksmani* which was isolated from feeds only in autumn.
The most of *Penicillium* species, isolated within this work, are reported to produce different toxic metabolites.
Keywords: *feed, fungal contamination, Penicillium spp.*

INTRODUCTION

Moulds are ubiquitously distributed microorganisms which can be found in all climate regions. As such they are often contaminating food and feed and also raw materials used in food and feed production. Organic origin of feed is considered as a very favourable substrate for growth of different mould species therefor presence of pathogenic and toxigenic fungi is of great importance [1]. Numerous fungal species are able to produce harmful metabolites – mycotoxins, principally ones belonging to the *Aspergillus, Penicillium and Fusarium* genera.
The most common representatives of plant pathogenic species ("field fungi") belonged to the genera *Fusarium* and some from the *Hyphomycetes Dematiaceous* group (*Alternaria* and *Cladosporium*), while *Aspergillus* and *Penicillium* presenting storage organisms [1,2]. Prevention is essential since there are few ways to completely overcome problems once mycotoxins are present [3].
A number of *Penicillium* species, which are very commonly found in the region of South-East Europe, are reported to produce different toxic metabolites. The main species of *Penicillium* genus that produce mycotoxins are *P. aurantiogriseum, P. brevicompactum, P. camemberti, P. chrysogenum, P. citrinum, P. commune, P. expansum, P. griseofulvum, P. roqueforti, P. verrucosum, P. viridicatum* and others. The main toxic metabolites produced by toxigenic *Penicillium* species are: penicillic acid (*P. aurantiogriseum*, *P. roqueforti*), citrinin (*P. citrinum, P. expansum*), cyclopiazonic acid (*P. camemberti, P. commune, P. griseofulvum)*, patulin (*P. expansum, P. griseofulvum, P. roqueforti*), ochratoxin A (*P.verrucosum*), and others. Concerning the importance and diversity of their toxic effects – carcinogenic, teratogenic, mutagenic, immunotoxic, neurotoxic, nephrotoxic and hepatotoxic – the occurrence of mycotoxinogenic moulds in foods constitutes a high risk for human and animal health.
Aim of this work was to investigate level of fungal contamination of feed used for diary cattle feeding, during one year (in all four seasons) and to identificate isolated moulds, with a special aspect on distribution of species from genus *Penicillium* and their share in isolated mycopulations.

MATERIALS AND METHODS

In this work, mycological investigations of various feed samples (117) were done, with special focus on frequency of *Penicillium* species from isolated mycopopulations. Samples (concentrate – 21 sample, dried clover – 21, fresh clover – 3, hay – 12, corn silage – 23, corn germ – 10, dried cornstalks – 6, corn dent – 1, pelleted sugar beet pulp – 10, fresh sugar beet pulp – 3, fresh rape leaf – 1, malt spent grain – 6) originated from farms for milking cows breeding, from area of Vojvodina, Serbia. Investigations were carried out during one year in all four seasons, whereby during summer 33, autumn 27, winter 30 and spring 27 samples are tested.

Total count of moulds is determined in all tested samples and thereafter isolation and identification of present fungi. According to Koch dilution method total mould count expressed on 1 g of feed (cfu/g) is determined, using Sabouraud maltose agar (SMA) as isolation medium with addition of streptomycin (30 ppm). Inoculated agar plates are incubated for 7 to 10 days on temperature of 25 °C. Identification of isolated moulds is done according to Raper and Thom [4], Samson and Frisvad [5] and Samson et al. [6].

RESULTS AND DISSCUSION

Obtained results are showing that during summer all tested feed samples were contaminated with moulds. The lowest contamination per 1 g was observed in concentrate ($1.4x10^5$) and highest in corn germ ($8.4x10^6$). Contamination degree during other seasons varied, but yet were very high. In autumn, 85% of all tested samples was contaminated with moulds, and total mould counts in contaminated samples were in range from $2.4x10^4$/g (concentrate) to $9.6x10^4$/g (fresh rape leaf). During winter, 90% of all tested samples was contaminated with moulds, and total mould counts in contaminated samples varied from $1.2x10^3$/g (malt spent grain) to $5.4x10^6$/g (dried cornstalks). The highest contamination was observed during spring, 93% of tested samples was contaminated with moulds and total mould count varied from $5.1x10^4$/g (pelleted sugar beat pulp) to $2.4x10^8$/g (dried cornstalks).

From investigated feed samples used for feeding of dairy cattle, numerous moulds are isolated and classified in 29 genera: *Absidia, Acremonium, Alternaria, Aspergillus, Aureobasidium, Botrytis, Chaetomium, Cladorrhinium, Cladosporium, Eurotium, Fusarium, Geotrichum, Gilmaniella, Humicola, Monilia, Monodyctis, Mortierella, Mucor, Paecilomyces, Penicillium, Phoma, Rhizopus, Scopulariopsis, Stachybotrys, Stemphilium, Trichoderma, Trichothecium, Ulocladium* and *Verticillium.*

The higest diversity in isolated species (30) had genera *Penicillium* with: *P. aurantiogriseum* Dierckx, *P. brevicompactum* Dierckx, *P. camemberti* Thom, *P. canescens* Sopp, *P. capsulatum* Raper and Fennell, *P. charlesii* Smith, *P. citrinum* Thom, *P. clavigerum* Demelius, *P. commune* Thom, *P. cyaneum* (Bain. And Sart) Biourge, *P. echinulatum* Fassatiová, *P. expansum* Link, *P. frequentans* Westling, *P. funiculosum* Thom, *P. griseofulvum* Dierckx, *P. islandicum* Sopp, *P. janthinellum* Biourge, *P. lividum* Westling, *P. nigricans* (Bainier) Thom, *P. purpurogenum* Stoll, *P. roqueforti* Thom, *P. rugulosum* Thom, *P. tardum* Thom, *P. variabile* Sopp, *P. velutinum* van Beyma, *P. verrucosum* Dierckx, *P. verruculosum* Peyronel, *P. viridicatum* Westling, *P. vulpinum* (Cooke & Massee) Seifert & Samson and *P. waksmani* Zaleski.

From mentioned species, the most ubiquitous one was *P. aurantiogriseum*, which was contaminating around 54% of tested feed samples in summer, 63% in autumn, 53% in winter and 67% in spring (Table 1). It is interesting to point out the presence of *P. camemberti, P. echinulatum and P. roqueforti,* which had important share in isolated mycopopulations in summer season.

Table 1. Number of feed samples contaminated with Penicillium spp. during one-year investigations

Species	Summer	Autumn	Winter	Spring
P. aurantiogriseum	18	17	16	18
P. brevicompactum	2	2	2	—[a]
P. camemberti	9	—	—	—
P. canescens	4	—	—	—
P. capsulatum	—	—	—	1
P. charlesii	—	—	3	—
P. citrinum	—	—	—	1
P. clavigerum	—	1	3	3
P. commune	—	—	—	1
P. cyaneum	—	1	—	—
P. echinulatum	6	—	—	—
P. expansum	1	2	—	2
P. frequentans	—	1	—	2
P. funiculosum	1	—	—	1
P. griseofulvum	—	—	1	—
P. islandicum	—	1	—	1
P. janthinellum	—	2	4	1
P. lividum	—	—	—	3
P. nigricans	1	—	—	—
P. purpurogenum	6	—	—	—
P. roqueforti	—	—	—	1
P. rugulosum	—	—	—	2
P. tardum	—	1	—	—
P. variabile	3	—	—	—
P. velutinum	2	—	—	—
P. verrucosum	—	—	1	—
P. verruculosum	—	1	—	—
P. viridicatum	—	1	—	—
P. vulpinum	1	2	2	—
P. waksmani	—	7	—	—

During investigation year presence of certain *Penicillium* species in feed significantly varied. The highest number of different species from this genus was isolated from concentrate (10) and dried clover (7) in spring, hay in autumn (7) and corn germ in summer (7) (Table 2). *P. aurantiogriseum* was constantly present and had the highest share in mycopopulations isolated from contaminated feed samples, except from corn silage in autumn and fresh sugar beet pulp in winter period.

Table 2. Feed samples contaminated with Penicillium spp. during one-year investigations

Feed	Summer	Autumn	Winter	Spring
Concentrate	*P. aurantiogriseum* *P.brevicompactum* *P.camemberti* *P. canescens*	*P. aurantiogriseum* *P.commune* *P.expansum* *P.janthinellum*	*P. aurantiogriseum* *P.clavigerum* *P.janthinellum* *P.verrucosum*	*P. aurantiogriseum* *P.capsulatum* *P.citrinum* *P.expansum* *P.frequentans* *P.islandicum* *P.janthinellum* *P.lividum* *P.roqueforti* *P.rugulosum*
Dried clover	*P.aurantiogriseum* *P.camemberti* *P.canescens* *P.echinulatum* *P.purpurogenum*	—	*P.aurantiogriseum* *P.charlesii* *P.clavigerum* *P.griseofulvum* *P.janthinellum* *P.vulpinum*	*P.aurantiogriseum* *P.clavigerum* *P.frequentans* *P.funiculosum* *P.lividum* *P.purpurogenum* *P.velutinum*
Fresh clover	*P.aurantiogriseum* *P.camemberti* *P.purpurogenum*	—	—	—
Hay	*P.brevicompactum* *P.camemberti* *P.vulpinum*	*P.aurantiogriseum* *P.brevicompactum* *P.expansum* *P.frequentans* *P.janthinellum* *P.vulpinum* *P.waksmani*	—	*P.aurantiogriseum* *P.velutinum*
Corn silage	*P.aurantiogriseum* *P.funiculosum* *P.purpurogenum*	*P.islandicum*	*P.aurantiogriseum* *P.vulpinum*	*P.aurantiogriseum*
Corn germ	*P.aurantiogriseum* *P.camemberti* *P.canescens* *P.echinulatum* *P.expansum* *P.purpurogenum* *P.variabile*	*P.aurantiogriseum* *P.brevicompactum* *P.waksmani*	*P.aurantiogriseum* *P.charlesii* *P.clavigerum*	*P.aurantiogriseum* *P.expansum*
Dried cornstalks	—	*P.aurantiogriseum* *P.verruculosum*	*P.aurantiogriseum*	*P.aurantiogriseum*
Corn dent	—	—	—	*P.aurantiogriseum* *P.vulpinum*
Pelleted sugar beet pulp	*P.aurantiogriseum* *P.nigricans* *P.purpurogenum*	*P.aurantiogriseum* *P.tardum* *P.viridicatum*	*P.aurantiogriseum* *P. brevicompactum* *P. italicum* *P. janthinellum*	*P.aurantiogriseum*
Fresh sugar beet pulp	—	—	*P.janthinellum*	—
Fresh rape leaf		*P.aurantiogriseum* *P.cyaneum* *P.waksmani*	—	—
Malt spent grains	*P.aurantiogriseum*	—	—	—

Great share, about 47%, of isolated *Penicillium* species are potential producers of different toxic metabolites [5]. Considering the high level of feed contamination with these fungal species and harmful effects caused by toxic metabolites, it should be appointed to high risks for animal and human health.

CONCLUSION

Isolated moulds belonged to 29 genera. Genus *Penicillium* was presented with the highest number (31) of different species. The most frequent one was *P. aurantiogriseum.* Concentrate was contaminated with the highest diversity in species (10) in summer.

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GELATINIZATION OF STARCH AND METHODS FOR ITS DETERMINATION

Bojana Kokić, Dragan Palić, Olivera Đuragić, Nedeljka Spasevski, Tea Brlek, Dušica Ivanov, Radmilo Čolović

University of Novi Sad, Institute of Food Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

ABSTRACT

Starch is formed of granules of variable size and is composed of amylose and amylopectin macromolecules. When a suspension of starch is heated in the presence of excess quantities of water, an irreversible order–disorder transition called gelatinisation takes place. During gelatinisation, starch granules take up water, swell, lose crystallinity and leach amylose. Several analytical techniques and methods can be used to study different aspects of the gelatinization process: birefringence, enzymatic methods, viscosity measurements, differential scanning calorimetry, ultrasonic spectroscopy, colorimetrically, conductance measurements etc. In this paper a review of methods used for determination of starch gelatinization was presented.
***Keywords**: starch, gelatinization, determination, methods*

INTRODUCTION

Starch is formed of granules of variable size and is composed of amylose and amylopectin macromolecules. The size, form and structure of starch granules vary with the botanical source.

Amylose is a linear molecule composed of D-glucose units linked by α-1,4 glucosidic bonds with a small number of branches [19]. Amylose has a helical structure where the interior of the helix contains hydrogen atoms, while the hydroxyl groups remain on the outside [6]. The presence of hydrogen atoms in the interior of the helix makes amylose hydrophobic and enables it to form complexes with free fatty acids, component glycerides of fatty acids, iodine and some alcohols [29].

Amylopectin is a highly branched macromolecule with linear chains of shorter length linked by α-1,4 glucosidic bonds containing 10–60 glucose units and side chains with 15–45 glucose units with an average of 5% of links α(1–6) in branched points [26]. Amylopectin chains are arranged radially within the granule with their non-reducing terminal ends oriented towards the surface, and these are arranged with alternating crystalline areas (as a double helix) and amorphous areas (with regions of branching points).

Starch is semicrystalline in nature with varying levels of crystallinity. The crystallinity is exclusively associated with the amylopectin component, while the amorphous regions mainly represent amylose [22]. According to *Billiaderis (1991)*, the crystalline areas of starch maintain the structure of the granule,

control its behaviour in the presence of water and make it more or less resistant to chemical and enzymatic attack. The amorphous zone of starch granules is the least dense, is more susceptible to enzymatic attacks and absorbs more water at temperatures below the gelatinization temperature [29]. When viewed under the polarising microscope, native starch granules show a dark birefringence cross ('Maltese cross') which is characteristic of crystalline substances whose index of refraction varies depending on the direction that a light ray travels through the substance [25].

When starch is heated with excess of water it undergoes a transition from ordered to disordered that is called gelatinization [13]. Mechanism of starch gelatinization is shown in Figure 1. *Svihus et al. (2005)* describe gelatinization as a swelling driven process. Swelling occurs along the amorphous regions, and since the crystalline regions do not expand during swelling, stress increases at the interface between the crystalline and amorphous regions, where amylopectin in the crystalline regions and amylose in the amorphous regions are bonded. Thus, at a certain point of swelling process the crystalline regions are rapidly and irreversibly broken and gelatinization is initiated. With excess water content, this onset of the gelatinization usually occurs between 50 and 70 °C. Swelling causes nearly all amylose in the starch granule to leach out [12]. Viscosity increases during gelatinization, and is caused by swollen granules and gels consisting of solubilized amylose [13].

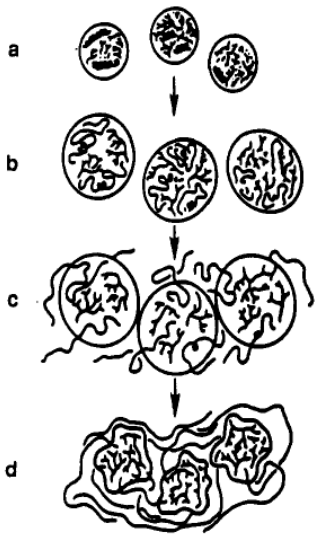

Figure 1. Mechanism of starch gelatinization. (a) Raw starch granules made up of amylose (helix) and amylopectin (branched). (b) Addition of water breaks up amylose crystallinity and disrupts helices. Granules swell. (c) Addition of heat and more water causes more swelling. Amylose begins to diffuse out of granule. (d) Granules, now containing mostly amylopectin, have collapsed and are held in a matrix of amylose forming a gel.

Baks et al. (2007) explained the difference between the gelatinization process when water is abundant and limited. The semi-crystalline, concentric growth rings on the outside of the granule are the first growth rings that have access to the available water. This part of the granule will gelatinize and hold the available water. As a result, there is an excess of water at the outside of the granule at both high and limiting water concentrations during the onset of the gelatinization process. For this reason, the same process can take place initially in both cases resulting in the same starting temperature for gelatinization. Because all water has been absorbed and bound by the gelatinized starch fraction in the concentric growth rings on the outside of the granule, there is no water available to induce swelling and disruption of the remainder of the starch granule at high starch–water ratios. For this reason, a heterogeneous mixture is formed that consists of amorphous, gelatinized starch and a part that has remained semi-crystalline. Complete loss of crystallinity can only be achieved by further increasing the temperature resulting in melting of the remaining semi-crystalline starch fraction [28].
According to *Donovan (1979)*, the loss of crystallinity of the granule, the uptake of heat accompanied by the conformation change of starch, take up of water resulting in swelling of the granule and a decrease in the relaxation time of the water molecules occur simultaneously (or nearly so) during starch gelatinization in presence of excess of water. Each method that can be used to study the gelatinization of starch focuses on one or several of these specific aspects of the starch gelatinization. Because all these phenomena occur at the same time, it is not important which method is used for the determination of the degree of starch gelatinization with excess of water. On the other hand, the phenomena that take place during the gelatinization process at higher starch concentrations do not occur simultaneously and differences between measurements using different methods become significant.

METHODS FOR DETERMINING THE DEGREE OF STARCH GELATINIZATION

Several analytical techniques and methods can be used to study different aspects of the gelatinization process.
Birefringence is used to follow the structure in the granule at approximately 500 nm [28]. The total number of granules (n_t) and the number of granules that had not lost their polarization or Maltese crosses (n_m) is determined. The ratio n_m/n_t can be used as a measure for the degree of gelatinization of the sample [4,18].
After starch has been gelatinized, it is susceptible to hydrolytic enzymes [24]. For this reason, enzymatic methods have also been used to investigate the gelatinization process indirectly [20]. In enzymatic analysis, the gelatinized starch is digested by glucoamylase, and the resulting glucose can be detected by either reacting with o-toluidine to form a green chromogen in glacial acetic acid or by other glucose measurements. Glucoamylase is an exosplitting enzyme that can split off glucose units from the nonreducing terminal end of starch, and its action is not prevented by the α-1,6-glucosidic linkage in

branched molecules such as amylopectin. However, damaged or degraded starch has also been shown to be easily attacked by enzyme action [10]. According to *Svihus et al. (2005)* enzymatic method is difficult to reproduce and is based on an assumption of linearity between gelatinization and enzymatic degradation that may not be true.
The viscosity of the starch–water mixture changes during gelatinization due to swelling of the granules, and therefore the viscosity can also be used to monitor the gelatinization process, typically by Rapid Visco Analyser (RVA) or amylograph [21]. The viscosity method is based on changes in torque during gelatinization, which can be measured by using the amylograph. The amylographic value (ΔV) is usually used as a rough indication of the degree of gelatinization. The degree of gelatinization is defined [16] as:

$$\Delta V = (V_F - V_i)$$

where V_F is the final viscosity of starch suspension, using Brabender units, at a temperature (a typical temperature is 95 °C) higher than the threshold temperature for gelatinization and V_i is the initial viscosity of the starch suspension at 30 °C. A decrease in the amylographic number indicates an increase in the degree of gelatinization [16]. In addition to measuring the gelatinization temperature range, information on initial viscosity, maximum viscosity, and time of cooking can also be provided by the amylograph [15].
Liu et al. (1991) have found a quantitative correlation between crystallinity loss and thermal transitions during the gelatinization of starch, since melting is a first order transition accompanied by a heat effect that can be measured well. Therefore, differential scanning calorimetry (DSC) measurements can also be used to monitor the loss of structure that takes place during the gelatinization process. The DSC-method consists of heating the sample mixed with water at a fixed rate and then measuring the heat absorbed by the sample in comparison to a reference sample. When starch gelatinises it absorbs energy, which can be quantified by the area of the curve plotted against the temperature. In addition to giving the extent of gelatinization, this method also determines: gelatinization temperature (T_G, corresponding to that where half of the granules have lost their birefringence or to the midpoint of the DSC transition curve), initial or onset temperature (T_{onset}, where birefringence loss starts or the intercept of the baseline with the tangent to the gelatinization DSC peak's first half), final or endset temperature (T_{endset}, where 90% of the granules have lost their birefringence or the intercept of the baseline with the tangent to the gelatinization DSC peak's second half) [1]. Example of DSC thermogram is shown in Figure 2. DSC has become the dominating method for measuring extent of gelatinization [1,23].

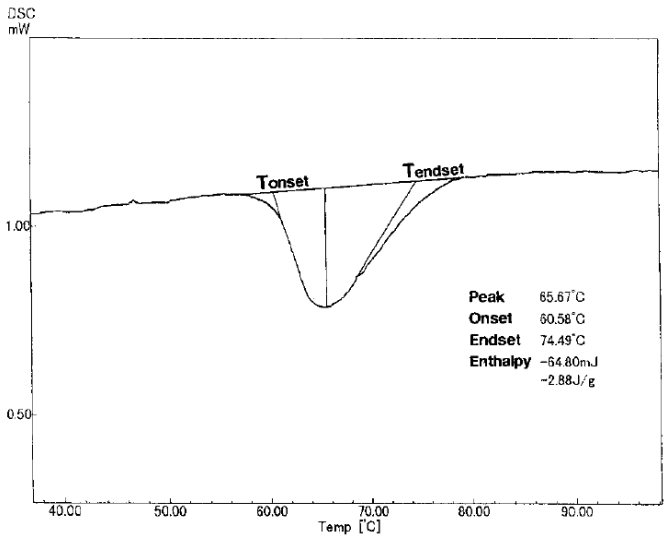

Figure 2. Example of DSC thermogram (300 g kg^{-1} potato starch suspension)

According to *Aparicio et al. (2009)* non-invasive ultrasound monitoring can be advantageously used for several proposes, such as following gelatinization processes in a variety of laboratory and industrial environments and situations, including reactors, continuous production processes, transport pipes, etc. The ultrasound-derived parameters, being directly related to viscosity, can be employed to obtain information on the mechanical properties of starch at a given stage of the process and also shed light on the different processes occurring during the overall gelatinization. This method is based on the measurements of parameters of acoustical (ultrasonic) waves propagating through the analysed sample. Scattering of ultrasonic waves on micro non-homogeneities allows analysis of the microstructure of the sample. Measurements of ultrasonic velocity provide information on microelasticity and hydration of starch components. Overall, ultrasonic spectroscopy presents the advantage of providing access to both structural and thermodynamic information [17].

Iodine staining of granular starch is a routine procedure to distinguish between the red-staining waxy varieties and their normal blue-staining counterparts. Although only the amylose fraction of starch can react with dilute iodine solution, amylopectin will also react if the concentration of iodine is brought within the range of 10^{-4} M. This minimum concentration of iodine, though adequate for complete reaction of the dissolved starch components, is not high enough to break the associative forces within starch granules. The iodine complexing method is based on the fact that gelatinized starch could solubilize in water more easily and uptake iodine faster than the ungelatinized starch to form an iodine-starch complex [15].

Short angle X-ray scattering (SAXS) and wide angle X-ray scattering (WAXS) can be used to follow, respectively, short-range order (crystalline double helices)

and long-range order (alternating crystalline and amorphous lamellae) [14]. Where X-ray scattering probes the double helices packed in regular arrays, solid state NMR detects the double helix content at a molecular order level [8,11]. IR spectroscopy can also be used to follow the gelatinization of starch on a short-range molecular level, because the IR spectrum of starch is affected by changes in structure such as starch chain conformation, helicity and crystallinity [27]. When a starch–water mixture gelatinises, the water distribution and manner at which water is bound to the starch matrix changes. These changes affect the dielectric properties of the starch–water system and for this reason conductance measurements can be used to monitor the gelatinization process [21].

COMPARISON OF METHODS FOR DETERMINING THE DEGREE OF STARCH GELATINIZATION

Baks et al. (2007) compared 4 different methods that are used for measuring the degree of gelatinization in 10 w/w % and 60 w/w % starch suspension in water. Their conclusion was that measurement of the degree of gelatinization of starch in a 10 w/w % wheat starch–water mixture as a function of the treatment temperature based on birefringence, DSC, X-ray or amylose–iodine complex formation measurements gave similar curves, because the physical–chemical processes involved occur simultaneously. When a 60 w/w % wheat starch–water mixture is used, the curves of the degree of gelatinization vs. temperature were affected by the method used for calculation of the degree of gelatinization. The highest values were obtained with birefringence measurements. Calculations based on DSC and X-ray measurements resulted in slightly lower values. Finally, amylose–iodine complex formation resulted in the lowest degree of gelatinization. These differences were explained by the phenomena that take place during the gelatinization at limited water conditions.
Aparicio and co-workers (2009) assessed starch gelatinization by ultrasonic and calorimetric techniques. They concluded that the thermal parameters of starch gelatinization obtained by means of ultrasound amplitude analysis show a good agreement with those obtained, for the same samples, by DSC.
Bastajian and co-workers (2003) compared enzymatic and colorimetric methods for the determination of degree of starch gelatinization on 23 wheat and rice products. The correlation coefficient (R) between values obtained by the two methods was 0.97. They concluded that the colorimetric procedure is easier to execute, needs less time and is relatively less expensive.
Chaiwanichsiri and co-workers (2001) measured electrical conductivity, differential scanning calorimetry and viscosity of starch and flour suspensions during gelatinization process. T_i and T_f were determined as the beginning (T_i) and ending (T_f) temperatures of the steep increase in electrical conductivity gradient. Good correlation (R=0.868) between T_{onset} from DSC with T_i from electrical conductivity showed that the initiation of ion release from starch granules strongly relates to the beginning of the thermal event in gelatinization. No correlation was found between T_{endset} with T_f suggesting that the end of the thermal event and the completion of ion release in gelatinization were

independent of each other. Furthermore, the gelatinization temperature measured by viscosity, T_{vis}, was compared with T_f, showing a good correlation (R=0.865). This suggested that the completion of ion release corresponded to the collapse of starch granules, which caused a drastic increase in viscosity.
Paola et al. (2003) proposed a new enzymatic method to evaluate the degree of starch gelatinization in starchy food and feed. The parameter selected to quantify the degree of starch gelatinization was the initial velocity value of the enzymatic reaction (Vi). The new method was compared with DSC and viscosity measurements in order to check its efficiency. A good correlation was observed between the degree of starch gelatinization calculated by Vi and by DSC (R= 0,97), and a lower correlation between Vi and viscosity measurements (R=0.78).

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IMPROVING THE NUTRITIVE VALUE OF CHICKEN MEAT BY THE ADDITION OF KOMPO OMEGA SUN AS FUNCTIONAL COMPONENT ENRICHED WITH LINSEED OIL

Đuro Vukmirović[1], Dušica Ivanov[1], Radmilo Čolović[1], Jovanka Lević[1], Rade Jovanović[2], Olivera Đuragić[1], Nedeljka Spasevski[1]

[1]University of Novi Sad, Institute of Food Technology in Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia
[2]Institute of Scientific Application in Agriculture, Bul. despota Stefana 68/b, Belgrade

ABSTRACT

The objective of this experiment was to increase the content of polyunsaturated fatty acids and to improve the ratio of ω-6 to ω-3 acids in broiler chicken meat by the addition of commercial functional component enriched with linseed oil (KOMPO OMEGA SUN). A total of 480 day-old Ross 308 Broilers were assigned to two treatments (control and experimental) with six replicates. Control and experimental diet had the same composition, but with the addition 5% of KOMPO OMEGA SUN in experimental diet. A starter diet was given until day 21, followed by a finisher diet, until slaughter at the day 35. The adverse effects of the addition of KOMPO OMEGA SUN on broiler performance were not observed. On the other hand, there were obvious positive effects on content of PUFAs, α-linolenic acid, PUFA/SFA ratio and ω-6/ω-3 ratio in chickens fed the experimental diet, which suggest better nutritive quality of chicken meat from experimental group compared to control group.
***Keywords**: broilers, linseed oil, ω-fatty acids, ω-6/ ω-3 ratio*

INTRODUCTION

Beneficial effects of consumption of ω-6 and ω-3 polyunsaturated fatty acids (PUFA) on human health have been known for years [11, 12]. These PUFA are essential because mammals, and therefore humans, cannot endogenously synthesize them and must adopt them exogenously from dietary sources [3]. One way to increase content of ω-6 and ω-3 PUFAs, and to improve their ratio in human diet is by modifying fatty acid composition in meat, which is a natural supplier of fatty acids [16]. Addition of fish meals and oils in animal feed is the most effective method [4, 17] but it entails a number of organoleptic problems that adversely affect the meat's acceptability [5, 15].
Recently there has been a growing interest in linseed oil due to the high concentration of linoleic (LA, 18:2, n-6) and especially α-linolenic acid (ALA, 18:3, n-3) [14]. Furthermore, linseed is the richest oilseed source of ALA [9]. On the other hand, inclusion of linseed oil involves a less degree of off-flavours [1] compared to inclusion of fish oil in diets for broilers.

The aim of this study was to investigate the influence of the addition of linseed oil in the form of commercial functional component, in diet for broiler chickens on performance and fatty acid composition in raw meat and abdominal fat samples. The overall aim was to improve the nutritional value of chicken meat using linseed oil enriched diet, thus making it a good source of beneficially ω-3 fatty acids.

MATERIAL AND METHODS

Experimental design and diets

A total of 480 day-old Ross 308 Broilers were assigned to two treatments with six replicates. Each replicate consisted of 40 as-hatched birds per pen. The size of each cage was 2.5 m^2 which resulted in stocking density of 16 birds per square meter. Air temperature was adjusted in accordance to the technological demands. Lighting program provided 23h of light + 1h of dark. Birds were vaccinated against Newcastle disease (NCD) and infectious bursal disease (IBD) as per commercial recommendations.

Broiler chickens were fed with mash diet and had *ad libitum* access to water and feed. A starter diet was given until day 21, followed by a finisher diet, until slaughter at the day 35. Two different diets based on corn and soybean meal were assessed: control and experimental diet. Control diet was composed according to the technological demands of the concerned hybrid. Experimental diet had the same composition, but with the addition 5% of functional component of feed, commercially named KOMPO OMEGA SUN. The chemical compositions of diets are shown in Table 1. Calculated metabolisable energy of starter and finisher diets were 12.65 MJ/kg and 13.40 MJ/kg, respectively.

Table 1. Chemical composition of diets

	Control		**Experimental diet**	
	Starter, 1 - 21 d	**Finisher, 22 - 35 d**	**Starter, 1 - 21 d**	**Finisher, 22 - 35 d**
Dry matter (%)	90.31	90.46	90.82	90.21
Crude protein (%)	21.83	17.78	22.48	18.30
Ash (%)	6.02	4.67	6.86	5.13
Crude fiber	3.21	3.95	3.58	4.38
Crude fat (%)	6.40	9.29	7.94	9.94
Sodium (%)	1.85	2.30	2.51	2.60
Phosphorus (%)	0.90	0.80	0.91	0.72
Calcium (%)	1.10	1.07	1.78	0.86

All the animals were weighed at the day 21 and before slaughtering at the day 35. Animals were slaughtered in a licensed abattoir, and samples of leg and breast meat, and abdominal fat were obtained. Meat and abdominal fat samples from twelve birds per treatment (three birds per box) were frozen and sent to Institute of Food Technology (FINS) in Novi Sad, Serbia, to assess fatty acid

composition and intramuscular fat content. All birds were chosen as an average representative of their experimental group and box, taking into account average mass of 30 birds from each box.

Fat extraction for fatty acid analysis

Supercritical fluid extraction with CO_2 was used for preparation of fat extracts, as recommended for fatty acid analysis. Extractions were performed on a LECO TFA2000 fat analyzer with methods developed in the laboratory. Temperature, pressure and extraction flow rates were adopted from existing LECO procedures [13] for meat samples, and for linseed oil and mash feed samples, extraction parameters were taken from previous experiments conducted on mentioned samples [8]. Cell temperature and heated variable restrictor (HVR) temperature were set at 100 °C. The collection vials on the instrument are not temperature-controlled and remained near room temperature of approximately 25 to 30 °C. Infusorial soil (flux calcined infusorial soil, up to 54% crystalline silica, cristobalite < 50%, quartz < 4%, produced by LECO Corporation, MI, USA) was used as absorbent to remove traces of water from samples. The preselected samples were homogenized. 1.0 g of homogenized sample was weighed into glass beaker with accuracy of ±0.001 g. 2.2 g of absorbent was added to the beaker and the sample was vigorously dispersed with a glass rod. This way prepared mixture was transferred into a metal extraction thimble (12 cm length and 10 mm diameter). Filled extraction thimbles were closed with approximately 0.5 g of glass wool on the top and appropriate cap. Glass scintillation vials (Wheaton, NJ, USA) were used as vessels for collecting extracted fat. After finishing the extraction step, the instrument was depressurized. Finally, extracts in collection vials were de-gassed for ten minutes, until achieving constant weight of extracts.

Fatty acid determination

From the extracted lipids, fatty acid methyl esters were prepared by transmethylation method that uses 14% wt. boron trifluoride/methanol solution (Sigma Aldrich, MO, USA), as recommended method for this type of samples [10]. Nitrogen gas (99.99%, Messer, Germany) was used for removing boron trifluoride/methanol solution and n-heptane (99.99%, J.T. Baker, NJ, USA) from fatty acid methyl esters. Obtained samples were analyzed by a gas chromatograph Agilent 7890A system (Agilent Technologies, Santa Clara, CA, USA) with flame ionization detector (GC-FID), auto-injection module for liquid, equipped with fused silica capillary column (DB-WAX 30 m, 0.25 mm, 0.50 um). The oven temperature was programmed at 90 ºC for 4 min, increased from 70 to 150 ºC at rate of 15 ºC/ min and then held at 190 ºC for 5 min. Carrier gas was helium (purity > 99.9997 vol %, flow rate = 1.26 ml/min, produced by Messer, Germany). The fatty acids peaks were identified by comparison of retention times with retention times of standards from Supelco 37 component fatty acid methyl ester mix and with data from internal data library, based on previous experiments and fatty acid methyl ester determination on GC-MS. Results were expressed as percentages of total fatty acids.

Statistical analysis
STATISTICA software version 10 (StatSoft, Tulsa, OK, USA) was used for analyzing variations (analysis of variance – ANOVA) and for Fishers LSD comparison of means of samples. Differences among means with probability (p) ≤ 0.05 were accepted as representing statistically significant differences, and differences among means with 0.05 ≤ p ≤ 0.10 were accepted as representing tendencies to differences.

RESULTS AND DISCUSSION

Significant differences of productive parameters are entirely absent throughout the experimental period, with results slightly better for control feed (Table 2). This implies that the addition of KOMPO OMEGA SUN does not adversely affect productive parameters of chickens.

Table 2. Productive parameters of broilers[1,2]

		Day 0 - 21	Day 22 - 35	Day 0 - 35
Feed intake (g/bird/day)	C	56.60 ± 3.42[a]	126.68 ± 4.07[a]	84.29 ± 2.23[a]
	E	55.70 ± 2.07[a]	123.22 ± 9.32[a]	81.20 ± 6.44[a]
Weight gain (g/day)	C	35.10 ± 2.45[a]	68.29 ± 2.36[a]	48.12 ± 0.88[a]
	E	33.74 ± 0.94[a]	66.70 ± 5.13[a]	45.96 ± 3.03[a]
Feed conversion (g of feed/g of weight gain)	C	1.65 ± 0.04[a]	1.83 ± 0.01[a]	1.74 ± 0.03[a]
	E	1.65 ± 0.04[a]	1.85 ± 0.14[a]	1.78 ± 0.10[a]

[1] Values are means of all birds in treatment
[2] C = Control diet: E = Experimental diet
[a,b] Means in the same row for each productive parameter, with no common superscript differ significantly ($P < 0.05$)

Fatty acid compositions of abdominal fat, breast and leg muscles of control and experimental group of broilers are shown in table 3.

Table 3. Fatty acid composition of abdominal fat, breast and leg muscles for control and experimental group[1]

	% of fatty acid in total fatty acids					
Fatty acid	**Control group**			**Experimental group**		
	Abdominal fat	Breast muscles	Leg muscles	Abdominal fat	Breast muscles	Leg muscles
C14:0	0.37±0.02	0.47±0.07	0.39±0.01	0.36±0.02	0.47±0.09	0.36±0.02
C16:0	20.07±0.20	20.38±0.53	19.35±0.28	18.34±0.63	19.59±0.17	18.87±0.98
C16:1	2.74±0.28	2.49±0.03	2.74±0.20	3.37±0.45	3.14±0.26	3.60±0.54
C18:0	7.06±0.17	7.99±0.13	7.77±0.59	5.91±0.15	7.03±0.83	6.50±0.25
C18:1ω-9	31.18±0.58	28.68±0.29	29.13±0.24	31.14±0.81	30.12±0.66	31.76±0.18
C18:2ω-6	34.08±0.73	33.86±0.50	23.62±0.08	30.72±0.22	29.79±0.97	29.08±0.38
C18:3ω-6	0.23±0.01	0.25±0.02	11.76±0.92	0.18±0.01	0.20±0.02	0.19±0.02
C18:3ω-3	3.38±0.04	3.54±0.34	3.37±0.12	9.01±0.18	7.76±0.56	8.24±0.41
C20:0	0.12±0.01	0.11±0.02	0.13±0.02	0.10±0.02	0.12±0.03	0.14±0.01
C20:1	0.20±0.04	0.19±0.03	0.20±0.01	0.21±0.02	0.21±0.03	0.21±0.02
C20:2	0.21±0.01	0.38±0.03	0.29±0.01	0.18±0.03	0.27±0.04	0.23±0.03
C20:4ω-6	0.23±0.01	1.29±0.24	1.10±0.29	0.20±0.05	0.73±0.19	0.50±0.14
C20:3 ω-3+ω-6	ND	0.08±0.01	ND	0.08±0.01	0.13±0.01	ND
C20:5ω-3+ C22:6ω-3	0.11±0.02	0.29±0.02	0.16±0.04	0.22±0.03	0.45±0.03	0.33±0.63
SFA[1]	27.50±0.10	28.83±0.66	27.50±0.63	24.60±0.56	27.09±0.06	25.73±0.54
MUFA[2]	33.92±0.60	31.17±0.27	31.87±0.54	34.51±0.25	33.26±0.92	35.36±0.99
PUFA	37.46±0.69	37.41±0.36	26.99±0.99	39.73±0.72	37.55±0.21	37.33±0.11
PUFA/SFA	1.36±0.03	1.30±0.04	0.97±0.11	1.61±0.10	1.39±0.17	1.45±0.16
ω-6	34.54±0.73	33.86±0.50	36.48±0.53	31.10±.022	30.72±0.98	29.77±0.53
ω-3	3.49±0.04	3.54±0.34	3.53±0.41	9.23±0.98	8.21±0.54	8.57±0.41
ω-6/ω-3	9.90±0.32	9.62±0.98	10.33±0.47	3.37±0.69	3.84±0.81	3.49±0.47

[1] Values are presented as mean ± standard deviation, n = 12
[2] Saturated fatty acids
[3] Mono-unsaturated fatty acids

Looking at table 3, it is obvious that experimental diet had positive effect on fatty acid composition of broiler muscles and abdominal fat. Content of PUFA significantly ($p < 0.05$) increased, especially in leg muscles (37.33% in comparison with 26.99% for experimental and control group, respectively). Content of α-linolenic acid was approximately three times higher in all tissues for experimental group, in comparison with control group.

One of the most important parameter in analysis of fatty acid composition of meat is PUFA/SFA ratio. The average ratio of PUFA/SFA recommended by the British Department of Health is more than 0.45, and WHO/FAO experts have reported guidelines for a "balanced diet" in which suggested ratio of PUFA/SFA should be above 0.4 [7, 19, 20]. Both groups showed positive results regarding PUFA/SFA ratio. Nevertheless, experimental group had higher values of mentioned ratio for all investigated tissues, inducing better nutritive quality in comparison with control group.

As it can be seen from the results, ω-6/ ω-3 ratio is approximately three times lower for all tissues of experimental group in comparison with control group. As previously reported, ω-6/ω-3 ratio lower than 4 is recommended as optimal for human nutrition [6, 18]. Therefore, all tissues of experimental broilers can be considered as more favorable from the nutritional standpoint.

CONCLUSIONS

From the results of productive performance of broilers it can be concluded that there are no negative effect of the addition linseed oil in the form of commercial functional component (KOMPO OMEGA SUN) on broiler performance. On the other hand, increased content of PUFAs, α-linolenic acid, and especially high values of PUFA/SFA ratio indicate improved nutritional value of chicken meat from experimental group. Also, the value of ω-6/ω-3 ratio was in the range which is optimal for human nutrition. Therefore, the addition of KOMPO OMEGA SUN as a functional component of feed for chicken can be recommended. Further investigation of optimal quantity of KOMPO OMEGA SUN in the feed is necessary.

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QUALITY OF WHEAT BY-PRODUCTS AIMED FOR ANIMAL FEEDING

Šandor Kormanjoš[1], Slavko Filipović[1], Ivana Čabarkapa[1], Vera Radović[2], Jelena Filipović[1], Nikola Hristov[3]

[1]University of Novi Sad, Institute of Food Technology in Novi Sad, Bul cara Lazara 1, 21000 Novi Sad, Serbia
[2]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, 32000 Čačak, Serbia
[3]Institute of Field and Vegetable, Maksima Gorkog 30, 21000 Novi Sad, Serbia

ABSTRACT

Prior to drying and storing, in the wheat seed processing technology, significant quantities of wheat by-products are separated. In this research chemical composition, microbiological safety and content of mycotoxins in wheat broken kernels separated in the processing of wheat seed is investigated with the aim to find out if this raw material can be used as animal feed. Complete mixes for animal feeding, as well as, feed meals have to be hygienically safe and meet the regulations for the quality of animal feed. Wheat broken kernels are characterized by a high starch content (cca 60%) and microbiology proved to be a safe raw material concerning its hygiene. In all tested samples aflatoxin content was <0.001 mg/kg. These data point, that wheat broken kernels, as a by-product in wheat seed processing technology, are convenient raw material for animal feed preparation.
Keywords: *wheat, seed processing, broken kernels, extrusion, quality*

INTRODUCTION

Wheat (*Triticum sativum*) is primarily grown and used for human consumption, and far less as a feed. Wheat as animal feed is used in following cases: price is lower than the price of corn; wheat is characterized by poor technological quality or as a by-product in wheat processing (broken and shrunken kernels). It is believed that about 20-28% of the total wheat produced quantity is used for animal nutrition [1]. Complete feed mixes must fully satisfy the nutritional needs of individual species and categories of animals as well as energy requirements. The energy levels of a feed mix can be achieved by adding appropriate amounts of cereal grain and fat. Animals like to eat wheat, which is a tasty feed. However, it is necessary to take into account the share of wheat in the diet, especially in poultry diets. Regarding wheat milling products, the nature of the protein and particle size lead to sticking around the beak of poultry and may causes major problems, especially regarding younger categories. According to Jokić et al. [8] the share of wheat in diets for chickens aged 1 - 4 weeks should be a maximum of 20%, and for animals older than 4 weeks up to 25%. Jovanović et al. [9] state that wheat may be the only ingredient in the diet for all types of farm animals,

especially pigs. In this case, it is important to pay attention to the particle size of ground material, i.e. it should not be fine, due to dough-like structure caused by fine particles that may cause indigestion, ulcers or reduced feed consumption. In the technological procedure of seed wheat cleaning, before the drying and storage a significant amount of broken kernels is separated. Broken kernels are susceptible to mold contamination, particularly to those aflatoxin producers thus having a negative impact on the growth and development of animals. In the case of the use of microbiologically spoiled wheat as a part of a feed mix, the product gets the low quality and may be a major threat to the health of animals.

MATERIAL AND METHODS

This paper investigates chemical analyses, microbiological and aflatoxin content of wheat by-products intended for animal feed, caused by seed wheat processing.
Moisture, crude protein, crude fiber, ash and starch content of wheat by-products were determined according the regulations on methods of sampling and methods of performing physical, chemical and microbiological analysis of animal feed [12].
The total number of bacteria was determined by the method EN ISO 4833:2008 [13], *Coagulase positive staphylococci* by method EN ISO 6881-1:2008 [4], *Clostridium perfringens* by method EN ISO 7937:2008 [15], *Salmonella* spp. by method EN ISO 6579:2008 [14], the total number of yeasts and molds using EN ISO 21527-2:2008 [3] the total number of *Escherichia coli* using EN ISO 16649-2:2008 [15], and *Sulphite-reducing clostridia by* method EN ISO 15213:2003 [2]. The content of aflatoxins (B1 + B2 + G1 + G2) was determined by ELISA test using a kit Aflatoxin Veratox 8031 High Sensitivity Test.

RESULTS AND DISCUSSION

The results of the quality of by-products from the seed wheat processing intended for animal feed are presented in Tables 1, 2 and 3. Data from above mentioned tables show that wheat broken kernels regarding their nutritional value are very similar to corn, but contain more protein. Through the "Vib" sieve contains the highest protein share ranging from 12.21 to 14.06% depending on the variety of wheat. Regarding the content of starch (about 60%) wheat broken kernels separated in the process of seed treatment are a high-energy food convenient for feeding the animals. In the tested samples (Table 1, 2 and 3): *Salmonella* spp., *Sulphite-reducing clostridia*, *Escherichia coli*, *Clostridium perfringens* and *Staphylococcus aureus* are not isolated. Based on the total number of bacteria and the total number of yeasts and molds in 1 g of the sample all samples meet the requirements of the Rules of the quality of the feed [11] and can be used in the preparation of either complete or supplemental feed mixes. Aflatoxin content in the tested wheat by-products is lower than the maximum permitted level prescribed by the Regulation on the quality of the feed [11].

Table 1. Characteristics of broken wheat kernels

Quality parameters	**Wheat cultivars**				
	Pobeda	Dragana	Renesans	Odisej	Simonida
Chemical content, %					
Moisture content	9.45	9.25	9.98	9.31	9.17
Crude protein content	13.93	13.00	13.45	12.80	12.08
Ash content	1.57	1.41	1.49	1.55	1.50
Crude celullose content	2.13	2.00	2.13	2.41	2.33
Starch content	60.61	60.90	60.04	60.76	61.04
Microbiological analyses					
Salmonella spp. (50g)	-	-	-	-	-
*SRC (*cfu/g)	<10	<10	<10	<10	<10
*E. coli (*cfu/g)	<10	<10	<10	<10	<10
TVCYM *(*cfu/g)	<10	10 000	2 000	1 000	8 000
TVAC (cfu/g)	50 000	21 000	150 000	120 000	250 000
*C.perfringens(*cfu/g)	<10	<10	<10	<10	<10
*S. aureus (*cfu/g)	<100	<100	<100	<100	<100
Mycotoxins					
Aflatoxins B1+B2+G1+G2 (mg/kg)	<0.001	<0.001	<0.001	<0.001	<0.001

cfu- colony forming units
SRC- *Sulphite-reducing clostridia*
TVCYM - Total viable count yeasts and moulds
TVAC- total viable aerobic count

Wheat broken kernels are susceptible to mold contamination, particularly to aflatoxin producers, thus having a negative impact on the animal growth and development.
Improvement of hygienic, nutritional, physical and chemical characteristics of wheat broken kernels, as well as, their sustainability can be achieved by the process of extrusion.
Cereal extrusion process leads to reduction in the number of microorganisms which provides improved hygiene of nutrients obtained [10]. Regardless relatively low extrusion temperature (105°C) and a very short extrusion time (6-10 sec), extrusion process leads to the reduction of microorganisms which can be explained by very high pressure of extrusion ranging from 30-40 bar.
Extrusion process also leads to changes in the grain complex carbohydrates. Starch is partly gelatinized and degraded to the dextrin. Gelatinization provides better availability to enzyme degradation. These changes bring about an increase *in vitro* and *in vivo* digestibility of starch, and parallel to the above

mentioned, extrusion parameters lead to the inactivation of amylase inhibitors [6].
Extrusion significantly reduces the moisture content of the starting material, which is very important in terms of storage and preservation of extrudates. This fact is confirmed by the results of Filipović et al. [7] and Filipović et al. [5].

Table 2. Fractions of broken wheat collected from receiving separator

Quality parameters	**Wheat cultivars**				
	Pobeda	Dragana	Renesans	Odisej	Simonida
Chemical analyses, %					
Moisture content	9.98	9.60	10.52	9.73	9.19
Crude protein content	10.55	11.49	9.65	12.37	12.03
Ash content	1.52	1.50	1.45	1.62	1.56
Crude celullose content	2.14	2.56	1.84	2.20	2.22
Starch content	63.35	63.54	63.35	58.83	63.71
Microbiological analyses					
Salmonella spp.	-	-	-	-	-
SRK	<10	<10	<10	<10	<10
E. coli	<10	<10	<10	<10	<10
TVCYM *(*cfu/g)	1 000	1 000	1 000	7 000	1 300
TVAC (cfu/g)	500 000	300 000	400 000	1 200 000	1 500 000
C. perfringens	<10	<10	<10	<10	<10
S. aureus	<100	<100	<100	<100	<100
Mycotoxins					
Aflatoxins B1+B2+G1+G2 (mg/kg)	<0.001	<0.001	<0.001	<0.001	<0.001

cfu- colony forming units
SRC- *Sulphite-reducing clostridia*
TVCYM - Total viable count yeasts and moulds
TVAC- total viable aerobic count

Table 3. Fractions of broken wheat kernels collected from "Vib"sieve

Quality parameters	Wheat cultivars				
	Pobeda	Dragana	Renesans	Odisej	Simonida
Chemical analyses, %					
Moisture content	9.63	9.38	9.53	9.49	9.37
Crude protein content	14.06	13.26	13.35	13.25	12.21
Ash content	1.56	1.44	1.48	1.53	1.54
Crude celullose content	2.70	2.14	1.87	2.53	2.06
Starch content	61.04	60.32	58.74	61.04	60.90
Microbiological analyses					
Salmonella spp.	-	-	-	-	-
SRK	<10	<10	<10	<10	<10
E. coli	<10	<10	<10	<10	<10
TVCYM *(*cfu/g)	3 000	14 000	1 000	2 000	38 000
TVAC (cfu/g)	150 000	22 000	220 000	220 000	640 000
C. perfringens	<10	<10	<10	<10	<10
S. aureus	<100	<100	<100	<100	<100
Mycotoxins					
Aflatoxins B1+B2+G1+G2 (mg/kg)	<0.001	<0.001	<0.001	<0.001	<0.001

cfu- colony forming units
SRC- *Sulphite-reducing clostridia*
TVCYM - Total viable count yeasts and moulds
TVAC- total viable aerobic count

CONCLUSIONS

- After the treatment of seed wheat, broken wheat is highly valuable energy food suitable for animal feeding.
- On the basis of microbiological testing, fractions of broken wheat kernels meet the requirements of the Regulations on the quality of feed and can be safely used in animal feed production.
- The content of aflatoxins in the tested by-products is lower than the maximum permitted level prescribed by the Regulation on the quality of feed and components and therefore this by-product is suitable for the production of animal feed.

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THE EFFECT OF CONCENTRATION OF MOLASSES ON TECHNOLOGICAL AND MICROBIOLOGICAL PARAMETERS OF OSMODEHYDRATED MEAT

Vladimir Filipović[1], Biljana Ćurčić[1], Milica Nićetin[1], Lato Pezo[2], Violeta Knežević[1], Dragana Plavšić[3]

[1]Faculty of Technology, University of Novi Sad, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia
[2]Institute of General and Physical Chemistry, University of Beograd, Studentski trg 12/V11000 Beograd, Serbia
[3]Institute of Food Technology, University of Novi Sad, Bulevar Cara Lazara 1, 2100 Novi Sad, Serbia

ABSTRACT

Recent research has shown that use of sugar beet molasses as hypertonic solution improves osmotic dehydration processes. The objective of this research was to evaluate the effect of the reducing concentrations of the molasses as a hypertonic osmotic solution for the osmotic dehydration of pork meat on a technological and microbiological parameters. Decrease of the concentration of the molasses has lead to the statistically insignificant decrease of the dry matter content, water loss and solid gain, while a_w values have increased. Decrease of the concentration of the molasses have exerted statistically insignificant increase of the total number of microorganisms in osmodehydrated meat, while tested samples of meat from all osmotic dehydrations have satisfied food safety and hygiene process criteria. Results of the change of microbiological profile of osmotic solutions after the process of osmotic dehydration have shown that the increase of total number of bacteria is statistically significant, but still in acceptable limits. The conclusion of this research is that concentration of osmotic solution statistically insignificantly affects the technological and microbiological parameters which gives the opportunity of reusing the molasses as a osmotic solution, reducing environmental risk of the process of osmotic dehydration.
Keywords: *osmotic dehydration, pork meat, sugar beet molasses*

INTRODUCTION

Osmotic dehydration (OD) is an environmentaly acceptable, material gentle drying method, which received considerable attention due to the low processing temperature, waste material and low energy requirement [6]. Driving force for water removal is the concentration gradient between the surrounding solution and the intracellular fluid [4].

In comparison to other drying treatments main advantages of OD process are water is removed in liquid form, at mild temperatures and osmotic solution can be reused [1].
Recent research has shown that use of sugar beet molasses as a hypertonic solution improves OD processes [2]. Sugar beet molasses is an excellent medium for OD, primarily due to the high dry matter (80%) and specific nutrient content [3]. The presence of complex solute compositions maintains a high transfer potential favorable to water loss, and at the same time by the presence of sugar, salt impregnation is hindered [8].
Preliminary sensory analysis have shown that meat processed in this manner have satisfactory sensory characteristics.
The objective of this research was to examine the influence of reducing concentrations of molasses in proces of OD of pork meat on technological and microbiological parameters, as an indication of the possibility of reusing molasses in consecutive OD.

MATERIAL AND METHODS

Prior the treatment all working areas and tools were thoroughly washed, cleaned and disinfected with the pharmaceutical ethanol 70% vol. Fresh meat, purchased shortly previous the experiment, was cut into cubes dimension of approximately 1x1x1cm. Sugar beet molasses was obtained from the sugar factory Pećinci, Serbia. Initial dry matter content in sugar beet molasses was 85.04%, and molasses was diluted to the specific concentrations with distilled water. In all experiments, a weight ratio of solution to meat sample of 5:1 was used. Every 15 minutes meat samples in osmotic solutions were stirred with the same intensity, duration and frequency for purpose of removing quantity of water that diffunded from the center of the meat cube to its surface and allowing better homogenization of the osmotic solutions.
The experiments were conducted under atmospheric pressure at the temperature 20°C, during 5 hours. After the process of OD, meat samples were washed with sterilized water and gently blotted to remove excessive water. The meat samples were weighted and part of the samples were kept in an oven (Instrumentaria Sutjeska, Croatia) at 105°C until constant weight was attained, and dry matter content calculated from the samples weights before and after drying. Values of dry matter content (DM), water loss (WL), and solid gain (SG) were calculated as described by Mišljenović et. al [5], and presented as a mean values and standard deviations of 6 parallel runs.
Water activity (a_w) of the osmotically dehydrated samples was measured using a water activity measurement device (TESTO 650, Germany) with an accuracy of ±0.001 at 25ºC.
Determination of the *Salmonella* spp., *Escherichia coli*, the total number of bacteria and *Enterobacteriaceae* was done by the SRPS EN ISO 6579 [10]; SRPS ISO 16649-2 [11]; SRPS EN ISO 4833 [9] and the SRPS ISO 21528-2 [12], respectively, and presented as a mean values and standard deviations of 3 parallel runs.

Analysis of variance (ANOVA) and post/hoc Tukey's HSD test were performed using StatSoft Statistica, for Windows, ver. 10 program.

RESULTS AND DISCUSSION

DM of the fresh pork meat was 25.46±1.29%, table 1. and Tukey's test of the values of DM has shown that there was significant statistical difference ($p<0.05$) between DM values of fresh and osmodehydrated meat, indicating that process of OD has statistically significant effect on the increase of the DM values. However, according to Tukey's test, there was no significant statistical difference between DM values of the samples of the osmodehydrated meats in descending concentrations of the molasses, indicating that the decreasing concentration of the molasses as an osmotic solution (OS) has statistically insignificant effect on the decreasing of the DM values of the dehydrated meat.
The higest value of the WL of the osmodehydrated meat was achived in the process with the most concentrated molasses (0.4703±0.0025 g/g i.s.), and the lowes value of WL was achived in the process with the lowest concentracion of molasses (0.4188±0.0223 g/g i.s.), table 1.

Table 1. Technological parameters of osmotic dehydration of meat in molasses of different concentrations

Conc. of molasses	DM (% d.m.)	WL (g/g i.s.)	SG (g/g i.s.)	a_w
Osmodehydrated meat				
60% d.m.	53.87±2.18[a]	0.4188±0.0223	0.1248±0.0082	0.886±0.003[a]
70% d.m.	56.29±2.07[a]	0.4567±0.0098	0.1402±0.0059	0.884±0.008[a]
80% d.m.	56.53±0.37[a]	0.4703±0.0025	0.1467±0.0059	0.872±0.006[a]
Fresh meat	25.46±1.29[b]	-	-	0.926±0.013[b]

[ab] different letters in the superscript in the same column indicate significant statistical difference between the values, at level of significance $p<0.05$

The same trends were determined for the values of SG, as for the values of the WL for the osmodehydrated meat, where the highest concentration of the molasses as an osmotic medium has lead to the highest transfes of mass, therefore gaining the highest values of SG, table 1.
Tukey's test of a_w values have shown that process of OD has statistically significant effect on the decrease of the a_w values (a_w values of the fresh and all dehydrated meats), while there was no significant statistical difference between a_w values of dehydrated meats in different concentrations of osmotic solution. This indicates that decrease of the concentration of molasses does not have statistically significant effect on increase of achieved a_w values of dehydrated meats. Achieved a_w values of the meat dehydrated in all concentrations of the OS are lower than a_w values that inhibit growth of the most microorganisms except molds, [13].

In table 2. results of the change of microbiological profile of meat after the process of OD in OS of different concentrations are shown, while in the table 3. the results of the change of microbiological profile of OS after the process of OD are shown.

Table 2. Change of microbiological profile of meat after the process of osmotic dehydration

Conc. of molasses	*Food safety criteria*	*Production hygiene criteria*		
	Salmonella spp (negative/10 g)	Total number of bacteria $\cdot 10^5$ (CFU[1]/g)	*Enteroba-cteriaceae* (CFU/g)	*Escherichia coli* (CFU/g)
60% d.m.	0±0 [a]	0.51±0.08 [a]	0±0 [a]	0±0 [a]
70% d.m.	0±0 [a]	0.12±0.01 [a]	0±0 [a]	0±0 [a]
80% d.m.	0±0 [a]	0.12±0.03 [a]	0±0 [a]	0±0 [a]
Fresh meat	0±0 [a]	4.6±0.57 [b]	440±30 [b]	0±0 [a]

[ab] different letters in the superscript in the same column indicate significant statistical difference between the values, at level of significance $p<0.05$

[1] CFU – Colony Forming Units

In the neither of the analyzed samples of meats and OS *Salmonella* spp., was found. This indicates that the produced dehydrated semi-product from meat and used molasses after the process of osmotic dehydration is in accordance with the requirements for Food safety criteria of the Serbian National Regulation [7].

Total number of bacteria in the fresh meat cut and prepared for the OD in laboratory was $4.6 \cdot 10^5 \pm 5.7 \cdot 10^4$ CFU/g. Total number of bacteria in the molasses prior the osmotic dehydration was from 470±20 CFU/g to 520±30 CFU/g.

Tukey test's showed that there was significant statistical difference between the total number of bacteria in all dehydrated meats and the fresh meat, while there was no significant statistical difference between the total number of bacteria in the meat dehydrated in different concentrations of osmotic solutions. This indicates that the process of OD has a significant influence on lowering total number of bacteria in the osmotically dehydrated meat, while decreasing concentration of osmotic solution have insignificant statistical effect on increase of the total number of bacteria of osmodehydrated meat.

Tukey's test of the total number of bacteria of the osmotic solutions prior and after OD, table 2., have shown that the process of OD has statistically significant effect on the increase of the total number of bacteria in osmotic solutions, and also that the higher concentration of the molasses has also statistically significant effect on the decrease of the total number of bacteria.

Number of *Enterobacteriaceae* in the fresh meat was 440±30 CFU/g, table 2, but after the process of OD, none was detected. In the osmotic solutions of all concentrations, before and after the OD, none of the *Enterobacteriaceae* was detected. These results indicate that process of osmotic dehydration, and molasses as an osmotic medium, has statistically significant effect on the

reduction of the number of *Enterobacteriaceae* in the osmotically dehydrated meat.

Table 3. Change of microbiological profile of osmotic solutions after the process of osmotic dehydration

Conc. (d.m.)	OD	*Food safety criteria*	*Production hygiene criteria*		
		Salmonella spp. (negat./10 g)	Total number of bacteria $\cdot 10^3$ (CFU/g)	*Enterobacteriaceae* (CFU/g)	*Escherichia coli* (CFU/g)
60%	Prior	0±0 [a]	0.52±0.03 [a]	0±0 [a]	0±0 [a]
	After	0±0 [a]	3.6±0.4 [b]	0±0 [a]	0±0 [a]
70%	Prior	0±0 [a]	0.52±0.03 [a]	0±0 [a]	0±0 [a]
	After	0±0 [a]	2.17±0.06 [c]	0±0 [a]	0±0 [a]
80%	Prior	0±0 [a]	0.47±0.02 [a]	0±0 [a]	0±0 [a]
	After	0±0 [a]	1.4±0.36 [d]	0±0 [a]	0±0 [a]

[abcd] different letters in the superscript in the same column indicate significant statistical difference between the values, at level of significance $p<0.05$

Serbian National Regulation [7] doesn't determine reference values for the total number of bacteria or number of *Enterobacteriaceae* for the meat pieces, but by tracking the change of these parameters it can indicate the level of hygiene of the process and the sustainability of the produced semi product. The results of the reduction of these microorganisms in any dehydrated meat in comparison to the fresh meat indicate that the process of OD is hygienically safe.
In all tested samples of meats and osmotic solutions *Escherichia coli* was not detected, table 2. and 3., which, again, confirms high production hygiene of the process of OD.

CONCLUSION

Based on the presented results it can be concluded that concentration of molasses as an OS statistically insignificantly affects the technological parameters of osmotic dehydration of pork meat, providing the best results of DM, WL, SG and a_w values in the most concentrated osmotic solution.
Testing of the microbiological profile of the osmodehydrated semi-product of meat has shown that all the samples have satisfied the Food safety criteria, so the semi-products are ready for further technological finalization.
Molasses as a osmotic medium has shown excellent results in reducing total number of bacteria and *Enterobacteriaceae* in dehydrated meat regardless of the concentration and therefore has satisfied Production safety criteria.
Increase in total number of bacteria after the process of OD is expected, and it is the only issue that has to be addressed in purpose of reusing the molasses for consecutive dehydrations.

With the proper heat treatment dual goals would be achieved, concentration of the molasses would be increased and also total number of bacteria would be reduced to the initial level. The reuse of the molasses in ODs would have great impact on lowering environmental risk by reducing volume of waste OSs.

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OPTIMIZATION OF PORK OSMOTIC DEHYDRATION PROCESS USING FUZZY SYNTHETIC EVALUATION

Biljana Ćurčić[1], Vladimir Filipović[1], Milica Nićetin[1], Lato Pezo[2], Gordana Koprivica[1], Tatjana Kuljanin[1], Danijela Šuput[1]

[1]Faculty of Technology, University of Novi Sad, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia,
[2]Institute of General and Physical Chemistry, University of Beograd, Studentski trg 12/V11000 Beograd, Serbia

ABSTRACT

This paper presents the optimization of osmotic dehydration (OD) process of pork (*M. triceps brachii*) cubes, shaped as 1x1x1 cm, at atmospheric pressure, in sugar beet molasses + NaCl + sucrose water solution (OD solution). The effects of different process temperature (20, 35 and 50^{o}C), immersion time (1, 3 and 5 hours) and the concentration of osmotic solution were examined, and the influence of different parameters on the mass transfer kinetics during osmotic treatment was determined. The observed system's responses were: water loss (*WL*), solid gain (*SG*), final dry matter content (*DM*) and water activity (a_w). The optimum osmotic conditions were determined using standard fuzzy synthetic evaluation (FSE), coupled with Response Surface Methodology (RSM), and the optimum process parameters were: temperature of 40^{o}C, treatment time of 3.7h and concentration 63%, while the system responses were: *DM*=58%, *WL*=0.45, *SG*=0.15, and a_w=0.86.
Keywords*: Osmotic dehydration, pork, sugar beet molasses, ternary osmotic solution, Fuzzy synthetic evaluation*

INTRODUCTION

During OD partial removal of water from plant or animal tissue is achieved by osmotic pressure difference between product and hypertonic solution, which are in direct contact. Mass transfer is caused by a difference in osmotic pressure: water outflow from product to solution, solute transfer from solution into the product, and leaching out of the products own solutes [1]. OD is an environmentally acceptable method, with its ultimate aim for keeping the initial characteristics of the final product, also material gentle drying method, which received considerable attention because of the low processing temperature, low waste material and low energy requirements [2, 3].Water removal in liquid form, usage of mild temperatures and osmotic solution reusing are main advantages of OD process in comparison to other drying treatments [3]. Mass transfer mechanism and quality of final product are affected by many factors such as composition and concentration of osmotic agents, immersion time of the product in the solution, agitation /circulation of osmotic solution, operating temperature, solution to sample ratio, nature and thickness of food

material and pre-treatment [4]. The kinetics of water removal and solid gain is greatly influenced by the type of osmotic agent. Ternary aqueous solutions containing salt and sugar are usually used as osmotic agents for meat dehydration. Sugar beet molasses is an excellent medium for OD, primarily due to the high dry matter and specific nutrient content. The application of OD in food industry has many advantages: improvement of texture, flavor and color, no chemical pretreatment, energy efficiency, providing stable and quality product [2]. The focus of this study was to determine the optimal process parameters, depending on a final product quality. Non-linear optimization problems were constrained by a fuzzy synthetic evaluation (FSE) method. FSE process all the parameters on the basis of predetermined weights and decrease the fuzziness by using the membership function, giving quite high sensitivity compared to other index evaluation techniques.

MATERIAL AND METHODS

Initial moisture content of the fresh meat was 72.83%. Sugar beet molasses solution, with inital dry matter content of 85.04%, was obtained from the sugar factory Pećinci, Serbia. Distilled water was used for dilution of solutions. Osmotic solution was prepared from: sucrose in the quantity of 1.200 g/kg water, NaCl in the quantity of 350 g/kg water and distilled water, and mixed with molasses in ratio 1:1. This mixture was diluted to concentrations: 52.5, 61.25 and 70% w/w. The sample to solution ratio was 1:5 (w/w). The process was performed in laboratory jars at temperature of 20, 35 and 50^{o}C. After 1, 3 and 5 hours the samples were taken out from osmotic solutions to be lightly washed with water and gently blotted to remove excessive water. *DM* of the fresh and treated samples was determined by drying the material at 105 ºC in a heat chamber until constant weight was achived (Instrumentaria Sutjeska, Croatia). Water activity (a_w) of the osmotic dehydrated samples was measured using a water activity measurement device (TESTO 650, Germany) with an accuracy of ±0.001 at 25ºC. Soluble solids content of the molasses solutions was measured using Abbe refractometer, Carl Zeis Jenna at 20 ºC. The RSM method was selected to estimate the main effect of the process variables on mass transfer variables, during the OD of pork meat cubes. The accepted experimental design was taken from [5]. The independent variables were temperature (X_1) osmotic time (X_2) and X_3 is the concentration of osmotic solution, and the dependent variables observed were the response: *DM* (Y_1), *WL* (Y_2), *SG* (Y_3), and a_w (Y_4). The experimental data, used for the optimization study, were obtained using a central composite full factorial design (3 level-3 parameter) with 27 runs (1 block). The variables osmotic temperature, treatment time, and solution concentration were coded as X_1, X_2, and X_3, respectively and the responses *DM, WL, Sg* and a_w as Y_1, Y_2, Y_3, and Y_4. A model was fitted to the response surface generated by the experiment. The following second order polynomial (SOP) model was fitted to the data. Two models of the following form were

developed to relate four responses (Y) such as WL and SG to four process variables (X):

$$Y_k = \beta_{k0} + \sum_{i=1}^{4} \beta_{ki} X_i + \sum_{i=1}^{4} \beta_{kii} X_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{kij} X_i X_j, \; k=1\text{-}4, \qquad (1)$$

Where: β_{kn} are constant regression coefficients; *Y,* either *DM* (Y_1), *WL* (Y_2), *Sg* (Y_3) and a_w (Y_4); X_1, osmotic temperature; X_2 treatment time and X_3, solution concentration.

Analysis of variance (ANOVA) and response surface regression method (RSM) were performed using StatSoft Statistica, for Windows, ver. 10 program. The model was obtained for each dependent variable (or response) where factors were rejected when their significance level was less than 95%.

Optimization was performed according to FSE algorithm, using MicroSoft Excel 2007 to determine the workable optimum processing conditions. The fuzzy composite operator is critical factor that will affect the final optimization results. Two fuzzy composite operators are widely used in a variety of evaluation systems. The supremum – infinum operator M (Λ, V) and the multiplication – summation operator M (•, +) determine the fuzzy algorithm of the comprehensive relative importance sets. In fuzzy synthetic evaluation models "Λ" and "V" denote the supremum and the infinum operator respectively, while "•" and "+" are the notations for the algebraic multiplication and summation separately. According to study [6], the fuzzy model operator M (Λ, V) will lose more information then M (•, +), and therefore the optimization function chosen is O= M (Λ, V). The objective function (F) is the mathematical function whose maximum would be determined, by summing the FSE results for of the four response surface models, according to Eq. (1). Each group of response parameters have the same influence (i. e., weight) on the function F:

$$F(Temp., Time, Conc.) = \frac{\overline{WL} - \overline{SG} - \overline{a_w} + \overline{DM}}{4} \qquad (2)$$

RESULTS AND DISCUSSION

The OD process leads to an increase in *DM* of all meat samples. *DM* increased from initial 27.17 to 69.54% in OD solution concentrated to 70% w/w, after 5 hours of experiment. The huge difference in osmotic pressure between hypertonic solution and the meat tissue causes the vast initial loss of the water at the beginning of the dehydration process. The maximum value of water loss 0.55 was achieved, after 5 hours at maximum concentration 0.70% w/w. Solid gain (*SG*) value indicates the degree of penetration of solids from hypertonic solution into the meat sample. The aim of OD is the achievement of as low as possible solid uptake, and the most acceptable results were achieved by using OD solution concentrated to 70% w/w (0.14 g/g i.s.w.), after 3 hours of osmotic process. During the OD process, total mass of the meat samples was evidently reduced.

To determine optimal conditions for the OD water loss/solid gain ratio must be considered. High value of *WL*/*SG* ratio is the most important indicator of the effectiveness of OD treatment. The highest value of *WL*/*SG* ratio (3.85) was achieved by using OD solution concentrated to 70% w/w, after 3h at room temperature. The RSM study was conducted to determine the optimum OD conditions for pork meat cubes dehydration process.

The analysis revealed that the linear terms contributed substantially in most of the cases to generate a significant SOP model. The SOP models for all variables were found to be statistically significant and the response surfaces were fitted to these models. The linear terms of SOP model were found significant, at 95% confidence level, and their influence were found most important in all model calculation. ANOVA test was used to show the significant effects of the independent variables to the responses and which of responses were significantly affected by the varying treatment combinations. *WL* was significantly affected by all process variables, treatment time, temperature, and concentration, at 95% confidence level, for all osmotic solutions. The main influential variable seems to be the treatment time, while temperature and concentration term is also very influential and statistically significant at 95% confidence level. Quadratic terms for treatment time and temperature were also found significant. The quadratic terms of concentration were found not significant, for all three experimental solutions. *DM* is most significantly affected by treatment time, and the impact of temperature and concentration terms were also found significant. The influence of treatment time quadratic terms were also significant at 95% confidence level. All SOP models had insignificant lack of fit tests, which means that all the models represented the data satisfactorily.

The values of coefficient of determination, r^2, for all three concentrations of OD solution, for *WL* (98.8-99.2), *SG* (88.3-95.7), a_w (89.4-94.4) and *DM* (98.8-99.3), were found very satisfactory and showed the good fitting of the model to experimental results.

Optimization of the dehydration process is performed to ensure rapid processing conditions yielding an acceptable product quality and a high throughput capacity. Coordinates of optimized point in temperature, time concentration graph, shown on Fig. 3, were: 40°C, 3.7 h and 63% w/w. These coordinates represent the optimum conditions for OD process for pork meat, in OD solution.

FSE is commonly used technique to solve problems with constraints involving non-linear functions. These methods aim to solve a sequence of simple problems whose solutions converge to the solution of the original problem. The maximum of function F represents the optimal parameters for major processing parameters, and also the optimum *WL*, *SG*, a_w and *DM*. The graphs of the dependent variables with significant parameters were determined using objective function to determine optimum production conditions, plotted on optimization graphic. If the value of function F is close to 1, it shows the tendency of tested processing parameters of being optimal. In this work, F reached the value of 0.67.

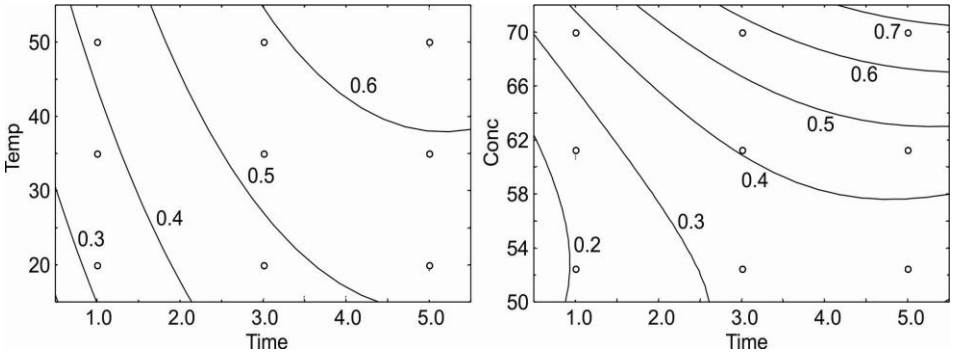

Figure 1. Objective function

To determine the adequacy of the SOP models, independent experiments were performed at optimum conditions for validation.

Table 1. Predicted and observed responses at optimum conditions

Responses	Predicted	Observed	Standard deviation	Coeff. of variation
DM	58.00	57.72	1.39	2.43
WL	0.45	0.46	0.4	7.06
SG	0.15	0.14	0.01	7.14
a_w	0.86	0.85	0.06	7.06

Table 1 shows the model validation results. As shown in the previous ANOVA Tables, the predicted values were comparable to the actual values in the experiment. Very good coefficients of variation (CV) of less than 10% for all process variables were calculated. CV values higher than 15% for response variables show great influence to the statistically minor significance of its SOP model. The low CV values for response variables *DM, WL*, *SG* and a_w indicated the adequacy of these models.

CONCLUSIONS

The experimental data used for the optimization study were obtained using a Box and Behnken's full factorial design (3 level-3 parameter), 27 runs. The RSM algorithm was used to optimize the OD of pork cubes, utilizing *WL, SG*, a_w and *DM,* as responses. SOP models for all system responses were statistically significant while predicted and observed responses correspond very well. The values of coefficient of determination, r^2, for all three concentrations of OD solution, for *WL* (98.8-99.2), *SG* (88.3-95.7), a_w (89.4-94.4) and *DM* (98.8-99.3), were found very satisfactory and showed the good fitting of the model to experimental results. Optimal conditions for OD in OD solutions were: 40°C, 3.7 h and 63% w/w, while system responses were: *WL* 0.45% w/w, *SG* 0.15, a_w 0.85 and *DM* 58%.

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OSMOTIC DEHYDRATION IMPACT ON MICROBIOLOGICAL PROFILE OF PACKED PORK MEAT

Danijela Šuput[1], Vera Lazić[1], Lato Pezo[2], Milica Nićetin[1], Vladimir Filipović[1], Biljana Ćurčić[1], Nevena Krkić[1]

[1]University of Novi Sad, Faculty of Technology, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia
[2]University of Beograd, Institute of General and Physical Chemistry, Studentski trg 12, 11000 Beograd, Serbia

ABSTRACT

Fresh meat samples were cut into 1x1x1cm cubes. One half of samples were osmotically dehydrated in sugar beet molases solution for 5 hours prior to packaging in order to reduce water content. Samples were packed under modified atmosphere (MAP) (40%CO_2 + 60%N_2) in highly barrier materials and examined for microbiological profile during 20 days. The purpose of this study was to investigate the effects of osmotic dehydration (OD) on microbiological profile of pork meat. The change of the microbiological profile between the fresh and dehydrated meat, both packed under MAP was observed. It was noticed that the process of OD does not cause deterioration of the initial microbiological profile of the pork meat, but rather improve it due to increasing of dry matter content. In all processed meat samples, decreased microbial load was detected. Better results of the reduction of the present microorganisms in pork meat were obtained in the process of OD+MAP rather that MAP packed pork.
Keywords: *packaging, osmotic dehydration, pork, microbiological profile*

INTRODUCTION

From the consumer's point of view, food appearance (size, shape, form, colour, condition and absence of defects) is decisive in their choice of purchase. Consumers expect food to look fresh, taste natural, be microbially safe, have no additives and have an extended shelf-life [1,2]. In response to consumer demand, research in the food industry aims to develop mild food preserving methods.

Meat spoilage is characterized by undesirable changes in color, texture, flavor, and odor. Major parameters are qualitative composition of microflora, water activity of samples, and atmosphere in the packs. The high moisture and nutrient contents make fresh meat a highly perishable food. Water activity (a_w) is the most important factor that affects the stability of dehydrated and dry products during storage. Water activity is determinant for microbial growth and can be associated with most degradation reactions of a chemical, enzymatic and physical nature [3]. Microbial flora and lipid oxidation are limiting factors, which determine shelf-life and safety of meat. Knowledge about the a_w levels at which microbial growth stops is essential for food preservation purposes. The lowest

limit for growth in foods or any other item is 0.6, but most bacteria can be inhibited at aw of 0.8. To stop yeast and moulds growing aw must be as low as 0.75 to 0.7. Insausti [4] established that microbial levels of 6–7 log CFU cm^{-2} or g^{-1} are critical levels for the spoilage of meat.

A promising treatment method for meat preservation is osmotic dehydration (OD). Osmotic dehydration (OD) is becoming an attractive complementary processing step in the chain of integrated food processing [5]. The main advantages of OD are improvement and/or preservation of nutritional and functional properties [6,7]. Osmotic treatment (OT) of fruits, vegetables and meats is performed by immersion of a food sample in an aqueous solution containing at least one of the osmotic agents: salts, sugars, phosphates, acids and others [8,9]. Many works have reported results on the osmotic dehydration of meats [10,11,12,13].

Osmodehydration results in extended shelf-life, fewer aroma losses in dried and semidried foodstuffs, reduction of the freezing load and/or the possibility to freeze the food without causing unwanted textural changes and dripping during thawing [14]. The purpose for which OD products are used will depend on their degree of stability. OD products that lose about 70 % of their water content are ready to eat. If a fresher appearance is required, dehydration must be about 30%.

Modified atmospheres (MAs) are commonly used for preserving fresh meat [15]. Modified atmosphere packaging (MAP) at refrigeration temperatures has been widely shown to delay the growth of spoilage aerobic bacteria and considerably prolong the shelf-life of red meats. The use of carbon dioxide as a bacteriostatic increases this shelf-life. Modified atmosphere containing high concentrations of CO_2 may act synergistically with lowered temperature to increase cell death rate. Highly barrier material application is necessary to maintain necessary atmosphere [16].

Common food spoilage microorganisms and pathogens vary considerably in their oxygen requirements [17]. The effect of MAP varies widely in different groups of microorganisms. Pseudomonas, enterobacteriaceae, lipolytic, and proteolytic bacteria are more strongly inhibited than LAB. High level of CO_2 has generally been found to have an inhibitory effect on Staphylococcus aureus, Salmonella spp., E. coli, and Y. Enterocolitica [18]. The degree of inhibition increases as temperature decreases.

Aim of this work is evaluation of OD and MAP on shelf-life of fresh meat in terms of microbiological profile.

MATERIAL AND METHODS

Sample preparation

Fresh pork (Musculus brachii) of normal pH (6.05) was bought in local butcher store. The muscles were trimmed of external fat and connective tissues and manually cut into approximately 1x1x1 cm ($1cm^3$) cubes with shark sterile knives.

Modified atmosphere packaging
Atmosphere modification and package sealing were performed using laboratory packaging machine. Samples received gas treatment: 40% CO_2 + 60% N_2. Samples were packed in highly barrier materials. Packaged meat samples were stored at 4^0C and sampled during 20 days for microbiological analyses.

Osmotic dehydration
One half of the samples were osmotically dehydrated prior to packaging. Meat samples were osmotically treated in solutions of sugar beet molasses (80 ^{0}Brix) at 22°C for 5 hours. The solution to sample ratio was 5:1 (w/w) to avoid significant dilution of the medium by water removal. On every 5 minutes meat samples in osmotic solutions were mixed with hand-held agitator in order to induce sample - solution contact and provide better homogenization of the osmotic solution. After treatment, samples were removed from the osmotic solution and gently blotted with a tissue paper in order to remove excessive water from the surface.

Microbiological analysis
Total Viable Counts (TVC) was determined in accordance with ISO 4833:2003 [19], and Enteroabcteriaceae in accordance with ISO 21528-2:2004 [20].

Statistical analysis
Descriptive statistical analyses for calculating the means and the standard error of the mean were performed using MicroSoft Excel software (MicroSoft Office 2007). All obtained results were expressed as the mean ± standard deviation (SD). Regression analysis was performed using StatSoft Statistica 10.0.

RESULTS AND DISCUSSION

TVC value didn't exceed value of 7 log CFU·g^{-1}, which is considered as the upper microbiological limit for good quality fresh poultry meat, as defined by the ICMSF [21]. As soon as spoilage can be detected by odour, taste or appearance, most foods have more than 6 log CFU·g^{-1} of bacterial load. It is accepted that counts of 7 log CFU·g^{-1} is the approximated point from which the meat would be unacceptable [22]. MAP had a clear effect on the inhibition of TVC, due to its higher concentration of CO_2.
The decrease of Enterobacteriaceae throughout storage could be attributed to the initial pH lowering of the meat as microorganisms normally compete better in meat of high pH (>6.0) which is accordance with findings of Leygonie et al. [23].
The number of Entherobacteria and TVC of experimental obtained data were fitted to the exponential regression model by using non-linear least squares regression solved by a Levenberg–Marguardt numerical method. The fitting function were evaluated using parametric regression and the results were examined using standard statistical error tests, i.e., coefficient of determination (R^2), the mean relative percent error (*MPE*), the root mean square error (*RMSE*) and the reduced chi-square (χ^2). The higher the values of R^2 and the lower the

values of *MPE*, *RMSE* and χ^2, the better is the goodness of fit. These parameters can be calculated as follows:

$$MPE = \frac{100}{N} \cdot \sum_{i=1}^{n} \frac{\left| y_{MAP,i} - y_{no,i} \right|}{y_{MAP,i}}, \qquad RMSE = \left[\frac{1}{N} \sum_{i=1}^{n} \left(y_{MAP,i} - y_{no,i} \right)^2 \right]^{1/2},$$

$$\chi^2 = \frac{\sum_{i=1}^{n} \left(y_{MAP,i} - y_{no,i} \right)^2}{N - n}$$

where, $y_{MAP,i}$ is the i^{th} experimentally number of Entherobacteria and TVC under MAP preservation, y_{no}, is the i^{th} number of Entherobacteria and TVC with no pr, *N* the number of observations and *n* is the number model constants.
Obtained statistical coefficient for regression for number of Entherobacteria were: R^2= 0.917; MPE=$1.19 \cdot 10^{-2}$; *RMSE*=$2.67 \cdot 10^{-2}$, and χ^2=$2.93 \cdot 10^{-2}$, while the statistical coefficient for TVC value: R^2= 0.456; MPE=$-7.46 \cdot 10^{-1}$; *RMSE*=$1.67 \cdot 10^{0}$, and χ^2=$4.12 \cdot 10^{0}$.

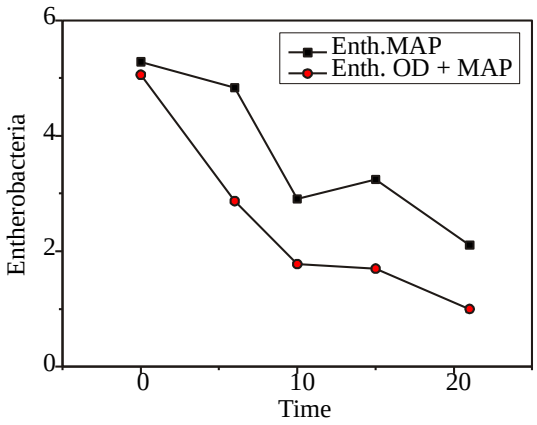

Figure 1. Number of Entherobacteria during experiment

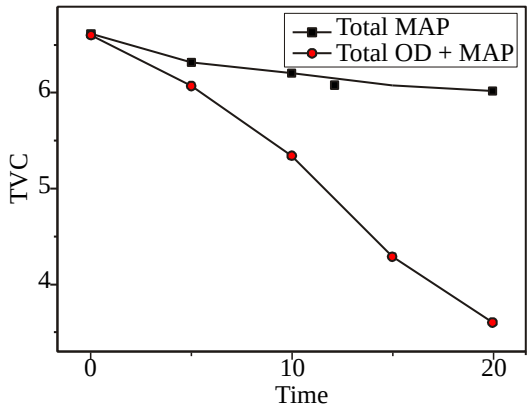

Figure 2. Total number of bacteria during experiment

CONCLUSION

The results of this study indicate that the shelf life of fresh pork meat stored at 4°C can be extended by packaging the product under anaerobic conditions (MAP) in particular those saples that were previously OD treated. In both group samples, microbial load decreased during time. This increase in shelf life is due to the reduction of microbial spoilage. Enterobacteriaceae and total viable counts during whole storage period constantly decreased. Better results of the reduction of the present microorganisms in pork meat were obtained in the process of OD+MAP rather that MAP packed pork because OD treated samples have initial dry matter content increased.

ACKNOWLEDGEMENT

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ASSESSMENT OF MINCED AND GRILL MEAT MICROBIOLOGICAL SAFETY IN YEAR 2012

Ana Varga, Dragana Plavšić, Bojana Kokić, Tatjana Tasić, Ljubiša Šarić, Jasmina Gubić, Bojana Šarić

Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

ABSTRACT

In this study microbiological safety of minced and grill meat in year 2012. was assess on 200 samples. Fifty of samples belong to type of minced meat products, while other 150 samples, equally divided, belong to three type of semi-finished meat products: grilled meat, grilled meat type "ćevapčići" and raw sausage ready to grill.
In the majority of minced meat samples (39) total number of microorganisms was in the range from 10^4 to 10^5 cfu, while total number of microorganisms in all three type of semi-finished meat products was higher than 10^5 cfu, 44 samples for grill meat, 39 samples for grilled meat type "ćevapčići" and 36 samples for raw sausage ready to grill.
Total number of *Escherichia coli* was lower than 10 cfu/g in most of the analyzed samples, in 38 samples for minced meat, 20 samples for grill meat, 27 samples for grilled meat type "ćevapčići" and 35 samples for raw sausage ready to grill.
Keywords: *microbiological safety, minced meat, semi-finished meat products*

INTRODUCTION

Meat provides a lot of necessary nutrients for all ages and is very important in human nutrition, but at the same time it is delicate food. It´s quality depends on numerous factors, such as race, gender, method of animal feeding, procedures during the slaughtering process and primary processing, manner of cooling, postmortem processes, etc. [4,5,10,11].
Taking into account the fact that meat belongs to a group of easily perishable foods, it is necessary to emphasize the importance of microbiological quality of meat and meat products monitoring [1,3,7,13].
Although meat is rich in all essential nutrients, it is not an ideal environment for development and reproduction of microorganisms. The muscle fibers are wrapped in connective-tissue which forms a natural barrier to microorganisms. Unfortunately, during the handling and processing of meat, conditions for more intensive contamination are created [2,5]. Meat mincing creates favorable conditions for microbial growth. The meat surface is greatly increased by tearing of the fascia and aponeurosis (connective-tissue involucre of muscle fibers) and thus stops the biological barrier against the penetration of microorganisms into the depths of meat. The juice released from damaged muscle fibers increases the moisture content of minced meat [2].

In production of grill meat various additives and spices are used to enhance the sensory properties, primarily smell and taste. Even many of them have antibacterial properties and are added in small quantities, additives and spices are often contaminated with numerous microorganisms, and therefore can be a source of secondary contamination. Another potential source of secondary contamination in minced and grill meat processing can be equipment, such as machine for mincing, shredding, filling etc [12].
According to the Regulations on the quality of minced meat, semi-finished meat and meat products [6], minced meat is meat product obtained by fresh meat grinding and it can be produced and placed on market packed or it can be minced in front of the consumer. Semi-finished meat products are semi prepared fresh meat products, including meat that is minced, in which other foodstuffs, spices or additives can be added or that is exposed to process sufficient to modify the internal structure of muscle fibers of meat and thus eliminate the characteristics of fresh meat.
The aim of this study was to assess microbiological safety of minced and grill meat in year 2012.

MATERIAL AND METHODS

A total of 200 samples were analyzed in this study. Fifty of samples belong to type of minced meat products, while other 150 samples, equally divided, belong to three type of semi-finished meat products: grilled meat, grilled meat type "ćevapčići" and raw sausage ready to grill. Presence of the total number of microorganisms and *Escherichia coli* were determined in all samples.
Presence of the total number of microorganisms was determined according to the reference test method SRPS EN ISO 4833:2008 [9]. Presence of *Escherichia coli* was determined by the reference test method SRPS EN ISO 16649-2 [8].

RESULTS AND DISCUSSION

Results for microbiological safety of minced meat and three types of semi-finished meat products are presented in Table 1. In the majority of minced meat samples (39) total number of microorganisms was in the range from 10^4 to 10^5 cfu, while total number of microorganisms in all three type of semi-finished meat products was higher than 10^5 cfu, 44 samples for grill meat, 39 samples for grilled meat type "ćevapčići" and 36 samples for raw sausage ready to grill.
Total number of *Escherichia coli* was lower than 10 cfu/g in most of the analyzed samples, in 38 samples for minced meat, 20 samples for grill meat, 27 samples for grilled meat type "ćevapčići" and 35 samples for raw sausage ready to grill.
Total number of microorganisms in minced meat ranged from 10^4 to 10^5 cfu/g in 78% of the samples, from 10^3 to 10^4 cfu/g in 12% of the samples and was higher than 10^5 cfu/g in 10 % of the samples. For all three types of semi-finished meat products, total number of microorganisms ranged from 10^3 to 10^4 cfu/g was 0%. This parameter was in the range from 10^4 to 10^5 cfu/g in: 12 % of grill meat

samples, 22 % of grilled meat type "ćevapčići" samples and 28 % of raw sausage ready to grill samples, while was higher than 10^5 cfu/g in: 88 % of grill meat samples, 78 % of grilled meat type "ćevapčići" samples and 72 % of raw sausage ready to grill samples.

Table 1. Microbiological safety of minced meat and three type of semi-finished meat products in year 2012

Sample	No	Total number of microorganisms in 1g (cfu)			Total number of *Escherichia coli* (cfu/g)		
		10^3-10^4	10^4-10^5	>10^5	<10	10-100	>100
Minced meat	50	6	39	5	38	6	6
Grill meat	50	0	6	44	20	9	21
Grilled meat type "ćevapčići"	50	0	11	39	27	13	10
Raw sausage ready to grill	50	0	14	36	35	5	10
		Share in total number (%) of microorganisms in 1g			Share in total number (%) of *Escherichia coli*		
Sample	**No**	10^3-10^4	10^4-10^5	>10^5	<10	10-100	>100
Minced meat		12	78	10	76	12	12
Grill meat	50	0	12	88	40	18	42
Grilled meat type "ćevapčići"	50	0	22	78	54	26	20
Raw sausage ready to grill	50	0	28	72	70	10	20

Total number of *Escherichia coli* in minced meat was less than 10 cfu/g in 76 % of the samples, in the range from 10 to 100 cfu/g in 12% of the samples and was higher than 100 cfu/g in 12 % of the samples. For three type of semi-finished meat products, total number of *Escherichia coli* was less than 10 cfu/g in 40 % of grill meat samples, 54 % of grilled meat type "ćevapčići" samples and 70 % of raw sausage ready to grill samples, in the range from 10 to 100 cfu/g in 18 % of grill meat samples, 26 % of grilled meat type "ćevapčići" samples and 10 % of raw sausage ready to grill samples and higher than 100 cfu/g in in 42 % of grill meat samples, 20 % of grilled meat type "ćevapčići" samples and 20 % of raw sausage ready to grill samples.

Comparison of microbiological image of minced and grilled meat in terms of contamination with total number of microorganisms and contamination with ß-glucuronidase positive Escherichia coli, indicates the extent to which

manipulation and processing of meat creates conditions for more intensive contamination with microorganisms.

CONCLUSION

Total number of microorganisms in the majority of minced meat samples was in the range from 10^4 to 10^5 cfu, while total number of microorganisms in all three type of semi-finished meat products was higher than 10^5 cfu in most of the samples. Total number of *Escherichia coli* was lower than 10 cfu/g in most of the analyzed samples.

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COMPARISON OF THE NUTRITIONAL PROFILE OF GM MAIZE MON 89034 × NK603 AND CONVENTIONAL MAIZE

Mária Polačiková, Mária Chrenková, Zuzana Formelová, Ľubica Chrastinová, Silvia Pomikalová

Animal Production Research Centre Nitra, Hlohovecká 2, 951 41 Lužianky, Slovak Republic

ABSTRACT

Genetically modified (GM) crops, which have been developed, offer a wide variety of benefits to producers including resistance to insects, diseases and herbicides. The aim of this study was to determine the composition and nutritional value of genetically modified maize (MON 89034 x NK 603), isogenic maize (DKC 5143) and three reference hybrids of maize growing on allotment in CVRV Piešťany. GM maize MON 89034 × NK603 was produced by crossing of two genetically modified parental lines MON 89034 and NK603. Like MON 89034, MON 89034 × NK603 produces the Cry1A.105 and Cry2Ab2 insecticidal proteins, which impart protection against damage caused by the European corn borer (ECB, *Ostrinia nubilalis*) and other lepidopteran insect pests. Like NK603, MON 89034 x NK603 expresses the CP4 EPSPS proteins, derived from *Agrobacterium* sp. strain CP4, which provides tolerance to glyphosate. Samples were analysed for dry matter, crude protein, crude fibre, ash, fat, starch, sugar, amino acid, macro and microelements, mycotoxines, *in sacco* crude protein (CP) degradability and intestinal digestibility (using mobil bag technique). All tested samples of maize had similar chemical composition, a low level of Ca (0.10 - 0.16 g·kg^{-1} DM) and the essential amino acid lysine (2.05 - 2.74 g·kg^{-1} DM) were observed. Increased content of mycotoxins was observed only in a conventional maize (Zearalenone 43 µg.kg^{-1}, Deoxyvalenol 339 µg.kg^{-1}). GM and isogenic maize were equal in effective crude protein degradability (59.57% and 58.74%) and in intestinal digestibility (94.42% and 94.21%).
The results of compositional analyses demonstrated that the grain of maize MON 89034 x NK603 is comparable in composition with those of the isogenic control and conventional maize.
Keywords: *genetically modified crops; substantial equivalence; nitrogen digestibility*

INTRODUCTION

With the increasing population and the decreasing area of land available for food production the development and use of genetically modified crops is considered as an important tool to ensure global food security. The cultivation of genetically modified plants (GMP) increased worldwide from 1,7 (in 1996) to nearly 148 milion ha (in 2010), representing about 10% of total arable land [9]. While North America and Argentina were responsible for the vast majority of the area grown,

in the European Union growing transgenic feed crops on large areas is limited. Slovakia ranks among other seven EU countries, which have practical experience in GM maize cultivation; these are: Spain, France, Romania, Portugal, Germany, the Czech Republic and Poland. Composition analysis of feeds from GMP is the starting point for nutritional assessment. The aim of this study was to determine the composition and nutritional value of genetically modified maize (MON 89034 x NK 603), isogenic maize (DKC 5143) and three reference hybrids of maize by chemical and biological methods.

MATERIALS AND METHODS

Tests were carried out on genetically modified maize (MON 89034 x NK 603), isogenic maize (DKC 5143) and three reference hybrids of maize growing on allotment in CVRV Piešťany. GM maize MON 89034 x NK603 was produced by crossing of two genetically modified parental lines MON 89034 and NK603.Like MON 89034 parental line, hybrid MON 89034 x NK603 produces the Cry1A.105 and Cry2Ab2 insecticidal proteins, which impart protection against damage caused by the European corn borer (ECB, *Ostrinia nubilalis*) and other lepidopteran insect pests. Like NK603 parental line, hybrid MON 89034 x NK603 expresses the CP4 EPSPS proteins, derived from *Agrobacterium* sp. strain CP4, which provides tolerance to glyphosate (the active ingredient of a broad spectrum Roundup® herbicide).

Chemical methods
The feeds were analysed for Dry matter (DM), Crude protein (CP), crude fibre, fat, starch and sugars according to Decree of The Ministry of Agriculture of The Slovak Republic [5]. Starch was determined by polarimetric method on Polarimeter ADP 220 (Bellingham&Stanley Ltd., UK). For macro and micro elements analysis samples were ashed at 550°C, the ash was dissolved in 10 ml of HCl (1:3) and minerals were determined by AAS iCE 3000 (Thermo, UK), phosphorus content was determined by molybdovanadate reagent on Camspec M501 (Spectronic Campes Ltd, UK) [5]. The amino acid composition after acid hydrolysis by 6 M HCl and sulphuric amino acids after oxidation hydrolysis [5] was analysed by ion-exchange chromatography on the AAA 400 (Ingos Prague, Czech Republic). Metabolizable energy was calculated according to the equations for metabolizable energy [13].

Biological methods
Effective degradation and degradation parameters (a,b,c) will be determined by in sacco method [8]. Three rumen fistulated cows (feed twice a day by experimental diet consist of 70 % forage and 30% concentrate on dry matter basis) will be used for 0,3,6,9,12,16, 24 and 48 hour of incubation time of samples of maize (with a minimum of three bags per animal, incubation and feed). The experimental bags will be inserted into rumen immediately before feeding. The parameters of CP degradation and effective degradation will be calculated using the equations described by Ørskov and McDonald [10]. In the

calculation of effective CP degradation an outflow rate of 0.06.h^{-1} will be used. Intestinal digestibility of by pass protein will by determined by method mobile bags [15] on three cows with duodenal T - cannula.

RESULTS AND DISCUSSION

Compositional analysis results for corn grain are presented in Table 1. These results demonstrate that the levels of proximate components (protein, ash, fiber, fat and minerals) were comparable to those in the grain of the nontransgenic control.

Table 1. Content of nutrients and mycotoxins in GM maize (MON 89034 x NK 603), isogenic maize (DKC 5143) and three reference hybrids of maize (g.kg^{-1} DM)

	PR 36V52	**LG 3475**	**NK Columbia**	**MON 89034xNK 603**	**DKC 5143**
Dry matter	900.12	895.39	897.24	897.15	897.20
Crude protein	94.17	90.14	93.63	83.23	86.16
Crude fibre	24.06	28.86	22.26	24.36	19.02
Fat	37.03	33.39	34.4	36.72	35.21
Ash	14.28	13.41	15.69	14.40	14.66
N-Free Extract	830.46	834.20	834.02	841.29	844.96
Organic matter	985.72	986.59	984.31	985.6	985.34
Starch	697.90	727.40	726.6	739.66	728.93
Sugar total	17.49	15.75	24.16	26.15	24.02
Calcium	0.12	0.10	0.10	0.16	0.10
Phosphorus	2.06	1.86	1.68	2.31	2.28
Magnesium	0.85	0.97	0.93	0.89	0.93
Natrium	0.15	0.10	0.15	0.10	0.16
Potassium	2.49	2.52	2.76	2.42	2.57
Iron	35.59	24.46	22.88	23.84	24.79
Manganese	5.56	6.01	6.62	5.44	5.16
Zinc	32.43	31.47	20.16	30.46	26.05
Copper	4.08	4.94	3.44	3.87	3.57
ME (MJ.kg^{-1})	14.14	14.07	14.09	14.19	14.19
Fumosidines Total (µg.kg^{-1})	-	-	-	-	-
Zearalenone (µg.kg^{-1})	<LQ (=42)	43 (+/-28,38%)	<LQ (=42)	<LQ (=42)	<LQ (=42)
Deoxyvalenol (µg.kg^{-1})	<LQ (=183)	339 (+/-8,71%)	<LQ (=183)	<LQ (=183)	<LQ (=183)

LQ = limit of quantification

There were only very slight differences between the basic components of GM maize and nontransgenic maize. GM maize (MON 89034 x NK 603) and isogenic maize (DKC 5143) have a high nutritional value, high starch contents (739.66 g·kg^{-1} DM and 728.93 g·kg^{-1} DM) and thus also nitrogen-free extract (841.29 g·kg^{-1} DM and 844.96 g·kg^{-1} DM). The fat and CP levels reached 36.72 - 35.21 g·kg^{-1} and 83.23 - 86.16 g·kg^{-1} DM, respectively, whereas the share of fibre was low (24.36 - 19.02 g·kg^{-1} DM). As to the energetic value no significant differences were observed (14.19 and 14.19 MJ ME.kg^{-1} DM). One of the typical insufficiencies of maize is its low level of mineral elements, mainly Ca. Increased content of mycotoxins was observed only in a conventional maize (Zearalenone 43 µg.kg^{-1}, Deoxyvalenol 339 µg.kg^{-1}).The existing studies which compare the substantial equivalence between conventional and transgenic plants are focused on soybean [11,14]. Some studies compared also conventional and Bt maize [6,7,1,2] or conventional and RR maize [12], but also other crops like sugar beet [3,4]. In this work were not found statistically significant differences between transgenic and isogenic crops. The content of the amino acid in GM maize was comparable to that observed in the nontransgenic control (Table 2). Maize is one of the most important cereal crops, providing between 50% and 70% of the dietary protein for humans. It is also one of the major crops used for feeding farm animals, particularly poultry and swine. Maize seeds are very low in lysine. GM maize (MON 89034 x NK 603) and isogenic maize (DKC 5143) have low levels lysine (2.56 and 2.31 g·kg^{-1} DM).

Table 2. Amino acid composition in GM maize (MON 89034 x NK 603), isogenic maize (DKC 5143) and three reference hybrids of maize (g.kg^{-1} DM)

	PR 36V52	**LG 3475**	**NK Columbia**	**MON 89034xNK 603**	**DKC 5143**
Asparatic acid	5.69	5.38	5.43	5.00	5.21
Threonine	3.17	3.09	3.24	2.84	2.99
Serine	4.51	4.09	4.29	3.89	3.99
Glutamic acid	15.26	13.92	14.59	13.09	13.76
Proline	8.60	7.98	8.91	7.85	8.42
Glycine	3.09	2.89	3.12	2.92	2.81
Alanine	6.29	5.50	5.65	5.33	5.34
Valine	3.48	3.36	3.42	3.20	3.42
Isoleucine	2.43	2.21	2.29	2.22	2.33
Leucine	10.83	9.44	9.65	9.07	9.41
Tyrosine	3.08	2.91	3.08	2.69	2.92
Phenylalanine	4.10	3.76	3.95	3.57	3.73
Histidine	2.52	2.26	2.52	2.36	2.21
Lysine	2.64	2.05	2.74	2.56	2.31
Arginine	3.99	3.84	4.13	3.70	3.76
Methionine	0.99	1.01	1.20	1.09	1.19
Cystine	1.22	1.52	1.50	1.35	1.49

GM and isogenic maize were equal in effective crude protein degradability and in intestinal digestibility (Table 3).

Table 3. In sacco degradability and intestinal digestibility of crude protein in GM maize (MON 89034 x NK 603), isogenic maize (DKC 5143) and three reference hybrids of maize (%)

	PR 36V52	**LG 3475**	**NK Columbia**	**MON 89034xNK 603**	**DKC 5143**
Effective degradability ($k=0,06h^{-1}$)	53.86	53.93	60.41	59.57	58.74
Intestinal digestibility	94.11	94.90	94.74	94.42	94.21

CONCLUSION

In conclusion it can be stated that the genetically modified maize MON 89034 x NK 603 used in our experiments revealed both matter and nutritional equivalence with commercial maize. Only small differences were found between the individual components which corresponded with the results of the other authors.

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INFLUENCE OF SLAUGHTER AGE ON MEAT QUALITY OF GOAT

Snežana Ivanović[1], Zoran Stojanović[2], Boris Pisinov[3], Ksenija Nešić[1], Ivan Pihler[4], Nevena Maksimović[5], Nikola Stanišić[5]

[1]Institute of Veterinary Medicine of Serbia, Vojvode Toze 14, 11000 Beograd, Serbia
[2]Serbian Environmental Protection Agency SEPA, Ruze Jovanovica 27a, Belgrade, 11000, Serbia
[3]Dairy Institute, Atoput br 3 11070 Beograd, Serbia
[4]Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia
[5]Institute of Animal Husbandry, 11080, Beograd-Zemun, Serbia

ABSTRACT

Goat meat in Serbia is increasingly consumed because of its characteristic taste and desired chemical composition. The quality of goat meat, as well as in other animal species, includes technological, nutritional and sensory aspects. These aspects are affected by many factors before and after slaughter. One factor that has significant impact on the quality of meat is the age of animal. The aim of this study was to compare some meat quality parameters of goats of various ages and to determine the differences between them. Goats were of Bunte Deutsche Edelziege race, aged three and six years. Goats were grown in semi-intensive fattening system in the region of Stara Planina. From each group we used twenty samples of meat for testing. Chemical composition (moisture, protein, total fat, ash, fatty acids) and pH value were determined by standard ISO methods. Tenderness, cooking loss and fatty acids composition were measured instrumentally. After testing we found that there was a statistically significant difference between the samples of goat meat ($P < 0.05$) in relation to: weight (%), fat (%), protein (%), ash (%) and between 10 of 15 tested fatty acids. Statistically significant differences between the groups did not existed regarding the pH value and remaining 5 fatty acids. These results suggest that the age of animal has an impact on meat quality.

Keywords: *goat, meat, chemical composition, quality*

INTRODUCTION

Goat and kid meat in many countries, especially in Asia and Africa, is of great importance in human nutrition. Because of its high biological value, goat meat is increasingly required in high-income countries [13, 11, 2]. Because of the long ban on goat raising since 1954 [1], in the Republic of Serbia nobody paid attention to the production of goat and kid meat, nor was performed statistical analysis of their number and production. It is estimated that the territory of the Republic of Serbia today raise about 318,000 goats [15]. However, in recent years there has been a noticeable increase of interest for goat raising, so their

number from day to day increase [4]. Goat meat is less esteemed than other kinds of meat because its strong flavor, but on the basis on nutritive and biological value it is not inferior to other types of meat. The quality of goat meat, as well as in other animal species, includes technological, nutritional and sensory aspects [3]. These aspects are affected by many factors before and after slaughter. One factor that has significant impact on the quality of meat is the age of animal. The aim of this study was to compare some meat quality parameters of goats of various ages and to determine the differences between them.

MATERIAL AND METHODS

Material was meat of Bunte Deutsche Edelziege (BDE) race, female, aged three and six years. Goats were grown in semi-intensive fattening system in the region of Stara Planina. Goats were raised in the same time. The floor was of stuffed soil and covered by thick layer of wheat straw. Watering was *ad libitum*. Diet of goats during the winter consisted of hay collected from natural pastures (3.5 kg per day) and concentrate (0.25 kg per day). In the summer months, goats were pastured and fed with concentrate in the amount of 0.25 kg per day. Concentrate was made of maize meal, wheat bran with added sodium chloride and premix. Goats were slaughtered in experimental slaughter house in the Institute for animal husbandry. From each group twenty samples of meat from *m. longissimus dorsi* were taken for testing.

Moisture content was determined by ISO 1442:1998 [6], fat content by ISO 1443:1992 [7] and ash contet by ISO 936:1999 [9]. Protein content was calculated from nitrogen content multiplied with 6.25 using ISO 937:1992 [10] and pH value by ISO 2917:2004 [8]. Chemical parameters and pH were measured in meat 24 hours after slaughter.

For the lipid extraction from abdominal fat tissue Folch-Lees method was applied. After the lipid hydrolisis, the esterification of faty acids to methyl esters was performed. FAMEs analysis was performed by gas chromatography technique (Agilent GC6890N) with external standard method using standard FAMEs mix 37 ("Supelco", USA).

Determination of heat treatment loss, as a mass loss of the sample due to heat treatment, was done by measuring the weight of the samples before and after the completion of the heating and subsequent cooling of the sample. Goat meat was heat treated in a boiling water bath at 90^{o}C for 60 minutes. Heat treatment loss was expressed in % of mass. Tenderness was determined by Warner-Bratzler instrument. It was done by measurement of force, expressed in lb and converted into N, which was required to cut a cylindrical sample obtained by drill 0.5 inches in diameter in the direction of muscle fibers from heat-treated and cooled sample. The result was expressed as the mean value of 8 to 10 measurements.

Data obtained in this study were analyzed by descriptive and analytical statistical parameters: mean value (SV) and standard deviation (SD) by using MS Excel

2003 and analysis of variance ANOVA. The differences between the averages were compared by t-test at the level of significance of 99 and 95%.

RESULTS AND DISCUSSION

Table 1 shows the body weight of goats before slaughter and the results of analyses of *m. longissimus dorsi* of these goats. There was statistically significant difference ($p<0,05$) between the values of body weight before slaughter [kg], moisture [%], fat [%], ash [%], cooking loss [%] and tenderness, related to different age of goats, while the pH value did not significantly differ ($p>0.05$). The results obtained in this study were similar to the results obtained in previous studies [3]. It was difficult to compare these findings with the findings of other authors, because the animals differed in race, diet, age, mode of holding. Our findings were not consistent with the findings of Wattanachant et al. [14]. These authors examined the meat of goat breeds Anglonubian x Thai native, aged 1, 3 and 7 years, with a diet that was different from ours. The pH results of these authors for 3 years old goats were 6.68 and for 7 years old goats 6.62; moisture % in goats 3 years old was 77.73 and in 7 years old goats was 78.63; protein % in 3 years old goats was 19.02 and in 7 years old goats was 17.47; percentage of fat at 3 years old goats was 2.01 and in 7 years old goats 3.16; ash % in 3 years old goats was 1.16 and in 7 years old goats was 0.57; Cooking loss in 3 years old goats was 31.73 %, while in 7 years old goats it was 27.39 %.

Table 1. Live weight, chemical composition, pH, tenderness and cooking loss in Bunte Deutsche Edelziege race

Goats 6 years old		**Goats 3 years old**
n=20	M ± SD	M ± SD
Live weight, kg	50.65 ± 3.41^{b}	40.10 ± 1.41^{a}
Moisture,%	75.32 ± 0.32^{b}	74.96 ± 0.21^{a}
Fat,%	3.74 ± 0.65^{a}	4.78 ± 0.82^{b}
Protein,%	19.89 ± 0.76^{b}	19.24 ± 0.66^{a}
Ash, %	1.05 ± 0.02^{b}	1.00 ± 0.02^{a}
pH, after 24h	5.71 ± 0.05^{NS}	5.71 ± 0.04^{NS}
Tenderness	75.55 ± 8.39^{b}	64.07 ± 8.02^{a}
Cooking loss, %	39.80 ± 2.08^{a}	44.68 ± 3.70^{b}

NS - no significant difference; ^{a,b} - Means within the same column with different superscripts differ significantly ($p<0.05$).

Table 2 presents the results of the fatty acid composition in *m. longissimus dorsi* of goats slaughtered at the age of three and six years. Comparison between them showed that the lauric, myristic, pedtadecanoic, palmitic, palmitoleic, margaric, heptadecenic, stearic, elaidic and oleic fatty acids statistically significantly differed ($p<0.05$), while between the pentadekanoic, linelaidic, linoleic, alpha linolenic and eicosenoic fatty acids there was no statistically significant difference ($p>0.05$). The ratio of unsaturated/saturated fatty acids in

meat of goats 6 years of age was 0.40 and in goats 3 years old was 0.38. These results also could not be fully comparable with the findings of other authors. Comparing unsaturated/saturated fatty acids ratio, shown in the Table 2, with the findings of Stydom and Tshabala [12], who got the result 0.82 for Boer Goat breed and 0.86 for Indigenous Goat race, it is obvious that they were not in compliance. Our results also did not agree with the results of Mushi et al. [5]. These authors examined the composition of fatty acids in meat of castrated SEA goats 14 months old. The ratio of unsaturated and saturated fatty acids in their study was 4.86.

Table 2. Fatty acid composition in Bunte Deutsche Edelziege race

Goats 6 years old		**Goats 3 years old**
n=20	M ± SD	M ± SD
Lauric acid (C12:0)	1.80 ± 0.21^{a}	2.04 ± 0.35^{b}
Myristic acid (C14:0)	14.99 ± 0.30^{a}	17.29 ± 1.44^{b}
Pedtadecanoic acid (C15:0)	1.46 ± 0.19^{a}	1.60 ± 0.15^{b}
Pentadekanoic acid (C15:1)	0.36 ± 0.03^{NS}	0.36 ± 0.03^{NS}
Palmitic acid (C16:0)	37.28 ± 1.34^{a}	38.01 ± 0.88^{b}
Palmitoleic acid (C16:1)	2.64 ± 0.17^{a}	2.95 ± 0.13^{b}
Margaric acid (C17:0)	1.52 ± 0.21^{b}	1.21 ± 0.22^{a}
Heptadecenic acid (C17:1)	0.65 ± 0.19^{a}	0.86 ± 0.08^{b}
Stearic acid (C18:0)	11.33 ± 1.11^{b}	9.95 ± 0.85^{a}
Elaidic acid (C18:1n9t)	1.12 ± 0.11^{b}	1.01 ± 0.06^{a}
Oleic acid (C18:1n9c)	20.07 ± 1.08^{b}	19.12 ± 0.99^{a}
Linelaidic acid (C18:2n6t)	0.64 ± 0.13^{NS}	0.60 ± 0.08^{NS}
Linoleic acid (C18:2n6c)	1.57 ± 0.22^{NS}	1.54 ± 0.14^{NS}
Alfa Linolenic acid (C18:3n6**)**	0.48 ± 0.09^{NS}	0.52 ± 0.08^{NS}
Eicosenoic acid (C20:1)	0.16 ± 0.04^{NS}	0.22 ± 0.04^{NS}
SFA	68.38	70.10
USFA	27.69	27.18
USFA/SFA	0.40	0.39

NS - no significant difference; ^{a,b} - Means within the same column with different superscripts differ significantly (p<0.05); SFA: Saturated fatty acid; USFA: Unsaturated fatty acid

CONCLUSIONS

Based on the obtained results it could be concluded that slaughter age of goats had an effect on body weight, moisture, fat, ash, cooking loss and tenderness.
Age of animals also had influence on the fatty acid composition in *m. longissimus dorsi:* lauric, myristic, pedtadecanoic, palmitic, palmitoleic, margaric, heptadecenic, stearic, elaidic and oleic fatty acid.

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HYDROLYTIC AND OXIDATIVE CHANGES OF UNPACKED AND PACKED BEEF

Danijela Vranic, Lazar Turubatovic, Slobodan Lilic, Zoran Petrovic, Milan Milijasevic, Radivoj Petronijevic, Dejana Trbovic

Institute of meat hygiene and technology, Kacanskog 13, 11000 Belgrade, Serbia

ABSTRACT

Parameters examined in this experiment (acid number, peroxide value and TBARs) are very important factors for shelf life of fresh meat and show lipid changes in meat during the storage. Even at low temperature, tissue enzymes from meat as well as microbial enzymes stay active and cause hydrolytic and oxidative rancidity that are one of essential parameters significant for shelf life of meat. Rancidity of meat depends on temperature, light and duration of storage. Material in this experiment was beef meat obtained from beef carcasses that were after chilling cut into main parts and for examination is used *m. quadriceps femoris*. Meat was divided into two groups and stored at temperature of 0-4°C. Beef from the first group (unpacked meat) was not packed than just covered by plastic foil and meat from the second group (packed meat) was packed under vacuum conditions in multilayered combined biaxially oriented thermoshrinkalbe polimer foil. During the storage acid number determined by standard method SRPS EN ISO 660:2011 [11], peroxide value by standard method SRPS EN ISO 3960:2011 [12] and TBARs by method according to [13] and [4]. Examinations were performed each 7 days. Unpacked meat was shelf stable for 15 days, while meat packed under vacuum conditions was shelf stable for 21 days.
***Keywords**: beef, hydrolytic and oxidative changes, packing*

INTRODUCTION

Meat is one of the most perishable foods present in commerce. Its susceptibility to degradative modification of microbiological and physical-chemical nature is tied up with its nourishing composition [1]. It represents an ideal substratum for the growth of spoilage microorganisms (*Pseudomonas*, *Acinetobacter*/*Moraxella*, *Lactobacillus*, *Brochothrix thermosphacta*, etc.) and potential pathogens (*Escherichia coli* O157:H7, *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, etc.), [7]. Further, lipid oxidation causes a rancid off-flavour and off-odour in meat and it is initiated in muscle systems at the membrane level in the intracellular phospholipids fractions. Many factors affect lipid oxidations: light, temperature, oxygen concentration, degree of unsaturation of the fatty acids and the presence of enzymes [10]. Among these factors, fatty acid structure of muscle is the most important because it affects the number and the proportion of the produced hydroperoxides [3]. According to some authors [15], lipid oxidation has slower increase than microbial growth and

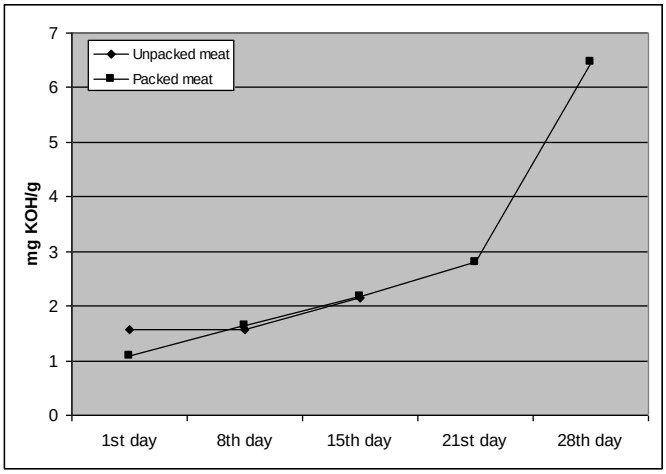
discoloration and it is not considered to be a limiting factor for shelf life of aerobic packed meat. Increased lipid oxidation has been reported for meat stored at elevated oxygen concentrations [5]. Lipid oxidation does not only contribute to off-flavour but it is also essential to the typical aroma for many meat products [8].
The aim of this paper was to examine the hydrolytic and oxidative changes of fresh beef meat during the storage, in unpacked meat and meat packed under vacuum conditions.

MATERIAL AND METHODS

As material in this experiment is used beef meat (*m. quadriceps femoris*). The muscles were removed from the animal carcasses after two days of chilling. Meat were trimmed of external fat and cut into suitable shape for packing, aproximately 1 kg per piece. Meat from different animals was used under the same conditions of chilling and trimming. Meat was divided into two groups and stored at the temperature 0-4°C. Meat from the first group was not packed than just covered by plastic foil (unpacked meat) and meat from the second group was packed under vacuum conditions in multilayered combined biaxially oriented thermoshrinkable foil (packed meat).
During the storage were determined parameters that show hydrolytic and oxidative rancidity. Acid number was determined by standard method SRPS EN ISO 660:2011 [11], peroxide value by standard method SRPS EN ISO 3960:2011 [12], and TBARs by method according to [13] and [4]. Examinations were performed each 7 days during the storage in three cycles of testing. Each parameter was determined in six replications and results are expressed as average value with standard deviation.

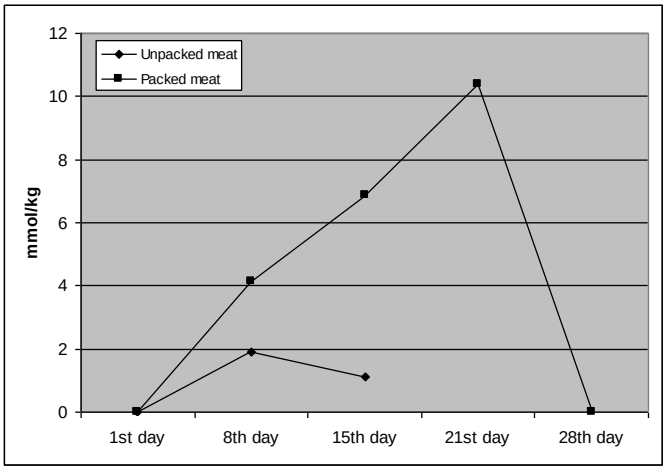
RESULTS AND DISCUSSION

Acid number (Graph. 1) slightly increased during unpacked meat storage and ranged from 1.56 ± 0.23 mg KOH/g at the first day of storage (1st cycle) up to 2.15 ± 0.57 mg KOH/g at the 15th day of storage (3rd cycle). In packed meat, acid number permanently increased during the first 21 days of storage and ranged from 1.08 ± 0.68 mg KOH/g at the first day of storage (1st cycle) up to 6.58 ± 0.82 mg KOH/g at the 21st day of storage (3rd cycle). Between 21st to 28th day of storage, acid number in packed meat rapidly increased due to long storage of meat. Acid number is the parameter that shows the first step in degradation of meat lipids and sign hydrolytic changes of lipids. It can not be used as only one indicator for meat rancidity and its increasing during the storage is common appearance. Value of acid number is linked with the moisture content in meat which contributes to lipolysis reactions [9].



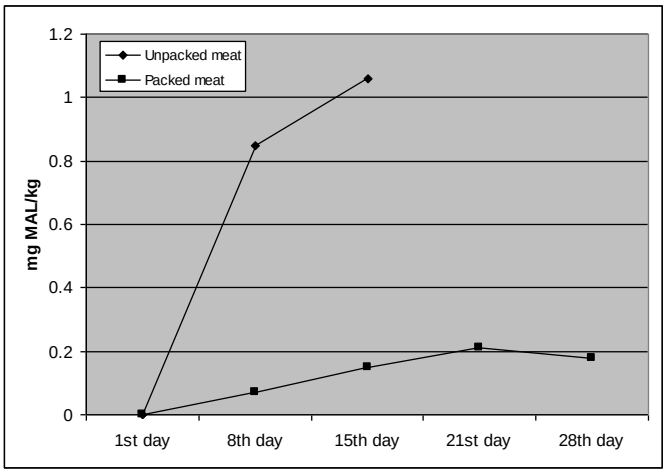
Graph 1. Acid number in meat during the storage

Peroxide value (Graph. 2) was not detected at the first day of examination in both unpacked and packed meat in all three cycles of examination (0 mmol/kg). The highest peroxide value was detected at 15th day of storage of unpacked meat from the second cycle of examination (1.92 ± 0.42 mmol/kg). In packed meat, the highest peroxide value was detected in meat from the 2nd cycle of examination; 10.37 ± 0.25 mmol/kg, at 21st day of storage. Peroxide value is connected mostly with pH value of meat. If pH value is closer to neutral point, it is favourable conditions for oxidation [14]. According to [6], the amount of hydroperoxide increased more rapidly at pH=6.8 than pH=3.



Graph 2. Peroxide number in meat during the storage

In both unpacked and packed meat, TBARs were not detected at the first day of examination in all cycles (Graph 3). The highest content of TBARs of 2.36 ± 0.01 mg MAL/kg in unpacked meat was detected in beef meat from the 3rd cycle at 15St day of examination. The highest value for TBARs of 0.39 ± 0.01 mg MAL/kg in packed meat was determined at 21st day of examination in the first cycle. TBARs is one of the products derived from peroxide decomposition and has the potential for reaction with other components [2]. Changes of TBARs are related to peroxide value. Simultaneous increase of TBARs and peroxide value at 21st day was probably duo to the partial decomposition of peroxide beside its formation, which results in increase of TBARs that is in accordance to [6].



Graph 3. TBARs in meat during the storage

According to obtained results for peroxide value and TBARs and sensory evaluation of meat, it has shelf life for 15 days (unpacked meat) and 21 day (packed meat).

CONCLUSIONS

Acid number increased during unpacked meat storage and ranged from 1.56 ± 0.23 mg KOH/g at the first day of storage (1st cycle) up to 2.15 ± 0.57 mg KOH/g at the 15th day of storage (3rd cycle). In packed meat, acid number permanently increased during the 21 days of storage and ranged from 1.08 ± 0.68 mg KOH/g at the first day of storage (1st cycle) up to 6.58 ± 0.82 mg KOH/g at the 21st day of storage(3rd cycle).

Peroxide value was not detected at the first day of examination in both unpacked and packed meat in all three cycles of examination (0 mmol/kg). The highest peroxide value was detected at 15th day of storage of unpacked meat from the second cycle of examination (1.92 ± 0.42 mmol/kg). In packed meat, the highest

peroxide value was detected in meat from the 2nd cycle of examination, 10.37 ± 0.25 mmol/kg, at 21st day of storage.
In both unpacked and packed meat, TBARs were not detected at the first day of examination in all cycles. The highest content of TBARs of 2.36 ± 0.01 mg MAL/kg in unpacked meat was detected in beef meat from the 3rd cycle at 15st day of examination. The highest value for TBARs of 0.39 ± 0.01 mg MAL/kg in packed meat was determined at 21st day of examination in the first cycle.
Acid number, peroxide value and TBARs are important factors that influence meat shelf life and their highest values shows the moment when fresh meat both unpacked and packed is not more shelf stable, according to contribution of sensory evaluation.

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ASSESSMENT OF ANTIMICROBIAL ACTIVITY OF EIGHT ESSENTIAL OILS AGAINST *SALMONELLA* ENTERITIDIS

Ivana Čabarkapa[1], Jovanka Lević[1], Slavica Sredanović[1], Bojana Kokić[1], Dubravka Milanov[2], Ljiljana Suvajdžić[3], Sava Pavkov[4]

[1]Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, Novi Sad, Serbia
[2]Scientific Veterinary Institute "Novi Sad", Rumenački put 20, Novi Sad, Serbia
[3]Department of Pharmacy, Faculty of Medicine, University of Novi Sad, Novi Sad, Serbia
[4]Institute Gosa, Milana Rakića 35, Belgrade

ABSTRACT

In recent years, occurrence of antibiotic resistance among bacteria isolated from animal feeds and the environment lead to increased interest in evaluating natural alternatives for antibiotics.
Medicinal plants constitute valuable phytogenic sources, wherefore are more popular as animal feed supplements. Essential oils (EOs) extracted from herb and spices are a complex mixture of various compounds, which consists of aromatic and volatile substances. Generally recognized as safe admitted by the Food and Drug Administration (FDA), EOs inhibit microbial growth in the gut and enhance nutrient digestibility. Majority of medicinal plants and their EOs do not have the residual effects.
The aim of the current investigation was to study the antimicrobial activities of eight EOs against *Salmonella* Enteritidis. The plants used in this study were oregano (*Origanum vulgare* L.), wild oregano (*Origanum heracleoticum* L.), thyme (*Thymus vulgaris* L.), wild thyme (*Thymus serpyllum L.*), summer savory (*Satureja hortensis* L.), basil (*Ocimum basilicum L.*), rosemary *(Rosmarinus officinalis) and sage* (*Salvia officinalis* L.). All the tested oils showed antimicrobial activity against *S.* Enteritidis. Minimal inhibitory concentrations (MICs) of the tested EOs were in the range from 0.39-12.5 µl/ml, while minimal bactericidal concentrations (MBCs) of the tested EOs were in the range from 0.78-25.0 µl/ml. The highest activity against *S.* Enteritidis exhibited EOs of wild oregano and summer savory (MIC/MBC=0.39/0.78 µl/ml). The results of the antimicrobial activity assays indicated that sage EO had the weakest antimicrobial propeties against *S.* Enteritidis (MIC/MBC=12.5/25.0 µl/ml).
***Keywords**: Salmonella, essential oils, antibiotic resistance*

INTRODUCTION

Salmonella is the most important bacterial pathogen in feed which frequently occurs in a large number of feed ingredients of animal or plant origin and also in compounded feed. Contaminated feed is an important source for infecting animals with *Salmonella* and also human infections of *Salmonella* have been

traced back to contaminated feed [24]. The prophylactic use of antibiotics in animal nutrition to cause improvements in growth, feed consumption, feed utilization and decreased mortality from clinical diseases is well documented. However, the growing concern over the transmission and the proliferation of resistant bacteria via the food chain has led to a ban of the feed use of antibiotic growth promoters (AGP) in livestock within the European Union since 2006. As a result, new commercial additives derived from plants including aromatic plant extracts and their purified constituents have been examinated as part of alternative feed strategies for the future. Such products have several advantages over commonly used commercial antibiotics since they are residue free and they are also, generally recognized as safe and commonly used items in the food industry [4]. The phytochemical treatment except the antimicrobial activity has a residual protective effect in feed, which helps reduce recontamination and also helps reduce contamination of milling and feeding equipment and the general environment [15].
Essential oils (EOs) are aromatic oily liquids obtained from plant material (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots). Chemical analysis of different EOs revealed the presence of several ingredients, most of which have important antioxidant, antibacterial and antifungal properties [4,5,31]**.** The chemical composition of the EOs which is responsible for the antibacterial properties is highly depended on various factors like the climatic and geographical conditions as well as harvesting, isolation techniques and storage [19].
This study was undertaken in order to investigate the effectiveness of eight EOs in inhibiting *S.* Enteritidis using broth micro-dilution susceptibility assay.

MATERIAL AND METHODS

Plant Material

The plants used in this study were: oregano (*Origanum vulgare* L.), wild oregano (*Origanum heracleoticum* L.), thyme (*Thymus vulgaris* L.), wild thyme (*Thymus serpyllum L.*), summer savory (*Satureja hortensis* L.), basil (*Ocimum basilicum L.*), rosemary *(Rosmarinus officinalis)* and sage (*Salvia officinalis* L.).

Isolation of the essential oil

The EOs were isolated from dried plant material by hydro-distillation according to the standard procedure reported in the Sixth European Pharmacopeia [16]. Distillation was performed using Clevenger type apparatus, for 2.5 hours. The resulting EO was dried over anhydrous sodium sulfate and stored at 4ºC.

Antimicrobial activity assay

The antimicrobial activity of EOs was evaluated using laboratory control strain, *Salmonella* Enteritidis ATCC/13076/, obtained from the American Type Culture Collection. Broth microdilution method was used to determine the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) according to the National Committee for Clinical Laboratory Standards [25]. The

bacterial inoculates were prepared using overnight cultures and suspensions were adjusted to 0.5 McFarland standard turbidity.
All tests were performed in Mueller Hinton broth (MHB, Himedia). Propylene-glycol (2-(2-hydroxypropoxy)-1-propanol) was used to dissolve the EOs and then diluted to the concentration (500-0.25 µl/ml). Twenty microliters aliquots of the EOs were added to 96-well microtitre plates, in geometric dilutions ranging from 500-0.25 µl/ml. After that, aliquots of 160 µl of MHB were added into each microplate well. As the final step, 20 µl of 2×10^6 cfu/ml (according to 0.5 Mc Farland turbidity standards) of standardized microorganism suspension was inoculated into each microplate well. The test was performed in a volume of 200 µl with final EO concentrations of 50-0.025 µl/ml. Plates were incubated at 37 °C for 24 hours. The same tests were performed simultaneously for growth control (MHB + test organism) and sterility control (MHB + test oil). The MIC was defined as the lowest concentration of EO at which microorganisms show no visible growth. The microbial growth was determined by absorbance at 620 nm using the universal microplate reader (ThermoLabsystems, Multiscan EX, Software for Multiscan ver. 2.6.). Referring to the results of the MIC assay, the wells showing complete absence of growth were identified and 5 µl solutions from each well was transferred to agar plates (PCA-plate count agar) and incubated at 37 °C for 24 hours. The MBC is defined as the lowest concentration of the EO at which ≥ 99.9% of the inoculated microorganisms were killed (complete absence of bacterial growth).

RESULTS AND DISCUSSION

Various publications have documented the antimicrobial activity of EOs including oregano, wild oregano, thyme, wild thyme, summer savory, basil, rosemary and sage [6,20,21,29].
Using the broth microdilution method, all of the tested EOs showed antimicrobial activity against *S.* Enteritidis. The results showed variation in the antimicrobial properties of plant EOs (Table 1). MICs of the tested EOs were in the range from 0.39-12.5 µl/ml, while MBCs of the tested EOs were in the range from 0.78-25.0 µl/ml.
The highest activity against *S.* Enteritidis exhibited EOs of wild oregano and summer savory (MIC/MBC=0.39/0.78 µl/ml). The obtained value for MIC of the tested wild oregano EO is slightly lower compared with previously published results of antimicrobial activity of wild oregano EO against *S.* Typhimurium in which the EO of wild oregano exhibited MIC value of 0.5 µl/ml [26].
The obtained value for MIC of the tested summer savory EO was in agreement with previously published results of antimicrobial activity of this oil (MIC=MBC=0.39 µl/ml) [22], while the MBC value obtained in our study was slightly higher compared to results of Mihajilov-Krstev et al. [22] and amounted 0.78 µl/ml. In the research of Oussalah et al. [26], MIC value of summer savory EO against *S.* Typhimurium was 0.5 µl/ml.
MIC and MBC values of the tested thyme, wild thyme and oregano EOs were the same and amounted 0.78 and 1.56 µl/ml, respectively. MIC and MBC values

of the tested thyme and wild thyme obtained in our study were slightly lower compared to results of Lević et al. [20] (MIC/MBC=1.56/3.125 µl/ml against *S.* Choleraesuis. In the same study oregano EO was more efficient against *S.* Choleraesuis based on values for MIC and MBC (0.39 and 0.78 µl/ml, respectively).

Table 1. Minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) of tested EOs against Salmonella Enteritidis

Common name (Latin name)	**MIC (µl/ml)**	**MBC(µl/ml)**
Oregano (*Origanum vulgare* L.)	0.78	1.56
Wild oregano (*Origanum heracleoticum* L.)	0.39	0.78
Thyme (*Thymus vulgaris* L.)	0.78	1.56
Wild thyme (*Thymus serpyllum L.*)	0.78	1.56
Summer Savory (*Satureja hortensis* L.)	0.39	0.78
Basil (*Ocimum basilicum L.*)	1.56	3.125
Rosemary (*Rosmarinus officinalis*)	3.125	6.25
Sage (*Salvia officinalis* L.)	12.50	25.0

Basil EO was efficient in the following range of concentrations MIC/MBC=1.56/3.125 µl/ml. Bajpai et al. [2] reported slightly higher MIC and MBC values for basil EO against S. Typhimurium (2 and 5 µl/ml, respectively).
According to the results shown in Table 1, higher concentrations of rosemary EO were required in order to achieve the MIC and MBC (3.125 and 6.25 µl/ml, respectively). Jordan et al. [18] examined antimicrobial activity of different chemotypes of rosemary EOs (eucalyptol; camphor; α-pinene) and reported significantly lower values for MICs and MBCs against *S*.Thyphi. MICs were in the range <0.5-1 µl/ml and MBCs ranged from <0.5-5 µl/ml.
The results of the antimicrobial activity assays indicated that sage EO had the weakest antimicrobial properties against *S.* Enteritidis (MIC/MBC=12.5/25.0 µl/ml). In the research of Hammer et al. [17] MIC value of sage EO against *S.* Typhimurium was 20 µl/ml.
The antimicrobial action of EO components is determined by the lipophilicity of their hydrocarbon skeleton and the hydrophilicity of their major functional groups. The antimicrobial activity of EO components has been ranked as follows: phenols > aldehydes > ketones > alcohols > ethers > hydrocarbons.
According to literature reviews the main components of the tested EOs from genus *Origanum* (wild oregano, oregano), *Thymus* (thyme, wild thyme) and *Satureja* (summer savory) are carvacrol and thymol, which are known to have antimicrobial activity [3,6,9,12,14,22]. The strong antibacterial activity of these EOs has been attributed to the phenolic monoterpenes carvacrol and thymol, which have similar, synergistic, and non-selective antimicrobial activity. Additionally, there is also a possible synergistic effect with other minor components such as the monoterpene hydrocarbons γ-terpinene and *p*-cymene

[5], which are biosynthetic precursors of thymol and carvacrol [32]. For example, *p*-cymene is a very weak antibacterial compound but it swells bacterial cell membranes to a greater extent than carvacrol does. By this mechanism *p*-cymene probably enables carvacrol to be more easily transported into the bacterial cell so that a synergistic effect is achieved when both compounds are simultaneously present [32].

The main constituents present in basil EO are methyl eugenol, α-cubebene, nerol and ε-muurolene [27], in rosemary EO α – pinene, 1,8-cineole, borneol, verbenone, and camphene [1,11] and in sage EO are limonene, α-pinene, α-thujone, menthone, carvone, 1,8-cineole and camphor [13,23]. Considering that the major components of these EOs are located much lower on a scale of antimicrobial activity in comparison to carvacrol and thymol, therefore their MIC and MBC values are higher. Weak activity of sage EO is consistent with its chemical composition, characterized by the presence of monoterpene hydrocarbons (limonene, α-pinene and α-thujone) and oxygen containing monoterpenes (menthone, carvone, 1,8-cineole and camphor). These compounds have shown weaker antimicrobial activity compared with phenolic monoterpenes [13,23].

Given the note that large number of different groups of chemical compounds are present in EOs, it is most probably that their antibacterial activity is not attributable to one specific mechanism but that there are several targets in the cell [7,30]. An important characteristic of EOs and their components is their hydrophobicity, which enables them to partition in the lipids of the bacterial cell membrane, disturbing the structures and rendering them more permeable. Leakage of ions and other cell contents can then occur [7,30]. Although a certain amount of leakage from bacterial cells may be tolerated without loss of viability, extensive loss of cell contents or the exit of critical molecules and ions will lead to death.

Comparison of the data obtained in this study with previously published results is problematic. First, the composition of plant oils is known to vary according to local climatic and environmental conditions as well as harvesting, isolation techniques and storage of EOs [19]. Secondly, the method used to assess antimicrobial activity and the choice of test organism(s), varies between publications.

CONCLUSIONS

Based on the described data, it can be concluded that tested EOs from Serbia showed high antibacterial activity against *S.* Enteritidis. Especially active oils were wild oregano, summer savory, thyme and wild thyme which can be explained by high content of monoterpens carvacrol and thymol in their structure. Therefore, their EOs or particular antimicrobial components can be used as multifunctional feed supplements for animals or for the prevention of sallmonelosis.

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IMPORTANCE OF CLINICAL AND PATHOLOGICAL DIAGNOSTICS OF MYCOTOXICOSIS IN FATTENING TURKEYS CAUSED BY T-2 TRICHOTHECENE

*Miloš Kapetanov, Dubravka Potkonjak, Igor Stojanov,
Milica Živkov-Baloš, Sandra Jakšić*

Scientific Veterinary Institute "Novi Sad", Rumenački put 20, 21000 Novi Sad, Serbia

ABSTRACT

The most relevant mycotoxin from the trichothecene group termed T-2 causes prominent cytotoxic effects. The toxin is a secondary product of fungi from the genus *Fusarium* that contaminates feed. Orally intaken, T-2 is absorbed fast in the upper digestive system and only 3 to 4 hours later reaches liver, kidneys and muscle tissue. Clinical and pathological changes are sometimes not obvious.
A case of mycotoxicosis in two flocks of fattening turkeys, here presented, is aimed to underline the significance of clinical and pathological diagnosis supported with laboratory analysis, that resulted in objective causative diagnosis.
On the farm, the disease occurred suddenly and with total cessation of feed consumption. First cases were recorded in the flock at the age of 32 days and mortality multiplied until day 4 with total mortality rate of 26,16%. In the other flock the disease started at the age of 42 days with similar dynamics, but the mortality rate of 8,72% was significantly lower. Turkeys showed signs of grouping, intensive breathing and lying with overstretched legs and extended neck. Evident necrosis of beek tips and painful multi-focal necrosis in oral cavity were present on clinical examination. On section, dark unclothed blood was first observed. Other *postmortem* findings included: filled gizzard with mucosal erosions and easy-removable cuticle, enlarged congested liver with multi-focal necrosis and subcapsulary bleeding.
Culture of the complete diet resulted in approximately 50000 colonies per 1g of *Fusarium*. The T-2 was detected using ELISA in concentration of 280 µg/kg that corresponded to the upper limit of maximum permitted concentration for chickens, according to national legislations. This bylaw interpretation of "tolerable" concentrations of mycotoxins provokes controversy among experts and public concerns.
Keywords*: clinical and pathological diagnostics, turkeys, mycotoxins, T-2, legislative*

INTRODUCTION

Mycotoxin-caused diseases are noncontagious and usually feed related. They quite resemble the conditions of some avitaminosis, but can not be treated with

antibiotics or other drugs in general. Due to the small molecular weight mycotoxins do not provoke immune response. In practice, chronic cases occur more frequently. Small concentration intake in prolonged time and *vice versa* the intake of higher concentration of mycotoxins in short time have the same effects [5,9].
Based on the mechanism of action, mycotoxins are classified as hepato-, nephro-, neuro- and cyto-toxins [11]. Their biological effects are diverse: cancerogenous, mutagenous and theratogenous, immunomodulation and inhibition of protein synthesis [10]. The toxicity of mycotoxins depends primarily on the group, concentration and duration of exposition, the species, gender and age of poultry, general health and immune status, ambiental factors (zoohygiene and zootechnology), nutritional requirements and feeding regimen [1].
In the group of trichothecenes, the T-2 that expresses prominent cytotoxic effect is of most significance for the veterinary medicine. Fungi from the genus *Fusarium* produce T-2 that contaminates crops on field and feed during storage. The microorganisms grow under different environmental conditions and produce the toxin on lower temperatures from 4 to 8^0C, but not at 32^0C [3].
Orally intaken, T-2 is quickly absorbed in the upper digestive tract and 3 to 4 hours later reaches liver, kidneys and muscle tissue [10]. In compare to chickens, turkeys and geese are more sensitive to T-2 [12]. Residual concentrations of T-2 can be detected in eggs and meat.
In the acute intoxication of poultry with trichothecenes, dysfunctions of digestive and nervous system occur. The hyperpnoe with lethargy and loss of balance are present. Hemorrhage is a regular finding in the digestive tract and muscle tissue, also prominent focal necrosis and ulcerations in oral cavity and stomatitis. During the chronic course, lower feed consumption, decreased body weight, and oral lesions and irregular moving are evident.
For several decades, mycotoxin related problems are quite challenging for the researchers, veterinarians and farmers. Some confusion exists in relation to the clinical and pathomorphological diagnostic features of the disease complex caused by mycotoxins [6]. In the paper, a case of mycotoxicosis in fattening turkeys was presented in order to point how important clinical and pathological diagnosis supported with laboratory analysis, is to form the final, objective causative diagnosis.

MATERIAL AND METHODS

The one day old broiler turkeys of hybrid line *Big BUT-6* originated from the Republic of Slovakia. In a seven day interval total of 5000 day old turkeys were placed on a farm with two separate houses, on the ground (flock A) and the first floor (flock B), each containing 2500 birds. The starter feed ratio for fattening turkeys was purchased from one supplier.
The outbreak of the disease was simultaneous in both flocks at the age of 35 (flock A) and 42 days (flock B), respectively. Because of the sudden occurrence, acute course and clinical signs it was suspected that mycotoxins were involved. The carcasses and complete feed mixtures were sampled for further

investigation. Approximately 50% of carcasses were submited to necropsy. The feed sampling procedure was in accordance to the Official Directive of the European Union [14].
The samples of feed were prepared for microbiological analysis and cultured on the Saburo agar. After the incubation period, grown colonies of fungi were purified and identified.
The content of T-2 was determined in feed samples using ELISA test, Ridascreen® (Art.No. R:3801, R-Biopharm, Germany), with detection limit 3.5 pp [15]. The readings were processed with software package Softv Rida®Soft Win (Art. No. Z9999, R-Biopharm, Germany). The interpretation of the results was done according to the instructions provided by the manufacturer.

RESULTS AND DISCUSSION

Weak feeding activity and grouping along the side walls in flocks was first observed by the workers in the early morning. A few hours later, a veterinary clinical observation revealed the following:

- at first, the turkeys had their necks retracted and were mostly located in groups near the bulkhead made of wire, and the central part of houses containing feeding and drinking lines was almost empty,
- the feed consumption completely stopped,
- most of birds were lying with extended neck and overstreched legs,
- opened beak and accelerated breathing were recorded,
- a minor part of the standing turkeys had difficulties to move by helping with extended wings,
- beak and painful multy-focal necrosis in oral cavity were obvious,
- yellow to yellowish-green feces of almost liquid consistance was observed on the litter.

Pathological changes found on necropsy were identical in all carcasses obduced. They were all found in a characteristic position with extended neck, overstretched legs and with filty feathers on abdomen and around the cloaca due to the yellowish to green diarrhea. Unclothed, dark coloured blood was first observed at necropsy. Other *postmortem* findings included: gizzard filled with feed and with mucosal erosions and easy-removable cuticle enlarged congested liver with multi-focal necrosis and subcapsulary bleeding.
The dynamics of mortality in both flocks was presented in Table 1. The disease lasted for seven days in both flocks. Total of 561 (22.44%) and 218 (8.72%) turkeys in flocks A and B died, respectively. Additionally, 93 turkeys in poor state were excluded from flock A and culled, expressing the overall loss of 654 (26.16%). In both flocks, the number of death cases was increasing rapidly until the day 4 (flock A) or day 3 (flock B). The decreasing tendency in number of death outcome was observed until the day seven.

Table 1. The dynamics and the mortality rate in two turkey flocks during the seven day period of the disease

Flock	Age (days)	Number of recorded deaths by days							Number of dead + culled birds	Mortality rate (%)	Total mortality rate (%)
		1	2	3	4	5	6	7			
A	35	23	51	139	146	96	62	44	561+ 93	22.44	26.16
B	42	16	39	91	46	16	6	4	218 + 0	8.72	8.72

The microbiological analysis - culture of complete diet, declaired for nutrition of fattening turkeys (starter), was positive only to *Fusarium*, in the calculated quantity of aproximately 50000 colonies per gram. This result is on the upper limit of maximum permitted number of colonies for young categories of poultry [7]. The T-2 concentration of 280 ± 17 µg/kg was detected in ELISA, that is also on the upper limit of maximum permitted concentration for chickens [7].

Sudden clinical disease on the farm with high mortality and drastic pathological changes, pointed to the acute alimentary intoxication with T-2. In the literature, similar *postmortem* findings were described and was noted that in compair to chickens, species including turkey and goose are more susceptible to the toxin [12].

Numerous reports exist on the death outcome in domestic animals due to the consumption of contaminated feed [8]. One of the most massive toxicosis was recorded in the year 1960, when 100000 turkeys died on a farm located in England. The cause of the disease was unknown and termed „Disease X" at the time [4].

In the time gap until laboratory confirmation, preliminary clinical and pathological diagnosis had iniciated the following procedures must be undertaken: First was to empty the feeding lines and replace the existing diet with the reliably safe; in the older group of turkeys (flock B) it was not possible to obtain the needed contigent of safe feed and was decided to leave the feeders empty for 30 hours. The second action was to add commercial product *Evitaselen* containing vitamine E and selenium (flocks A and B). The replacement of diet and a day and a half of „starvation", aimed to eliminate the toxin and detoxicate turkeys faster, both efficiently moderated the disease. The summarization of losses in the presented case of mycotoxicosis revealed significant difference between the two flocks e.i. two age groups. Although in compair to adults, neonates are more susceptible and the disease takes more severe course, even outcome, after exposure to mycotoxins [12], age-related difference of the mortality rates seems unlikely due to only 7 days interval of age. Perhaps the reason should be looked for in the operations undertaken after the disease was suspected. The restriction of feed was maybe more efficient than the exchange, because of the later being more time-consuming and also, some residual feed may have been left in feeding lines and mixed with the new one. Early and beforehand detection of mycotoxins in feed and exclusion of particular diet may help to mitigate the detrimental effects [5].

The calculated values for the number of *Fusarium* colonies in the complete diet and the detected T-2 concentration of 280 µg/kg were on the upper limit of maximum permitted concentration for chickens, according to the national regulations [7]. Such interpretation provokes strong controversy among experts. For such type of substance there is no limit for the negative effects and clearly, daily tolerable concentration is impossible to determine [2,11,13]. The regulations on the content of harmful substances in feed need to be corrected, particularly for more susceptible species and categories of poultry.

CONCLUSIONS

Clinical and pathological diagnostics is of utmost importance in the veterinary medicine due to efficient means to note and suspect the disease, direct investigations toward specific laboratory tests and moderate the outcome. The results of numerous mycotoxine tests indicate that in most cases the determined concentrations in feed do not exceed values regulated by the law. However, because of the cumulative effects and chronic exposure to mycotoxins, even in low doses, prompt expertise and action undertaken by the veterinarians are inevitable, especially in more susceptible poultry species and categories.

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TOTAL PHOSPHORUS, PHYTATE AND PHYTASE ACTIVITY OF SOME CEREALS GROWN IN ALBANIA AND USED IN NON RUMINANT FEED RATIONS

Etleva Delia[1], Myqerem Tafaj[1], Klaus Männer[2]

[1]Department of Animal Production, Agricultural University of Tirana, Albania
[2]Institut für Tierernährung, Freie Universität, Berlin, Germany

ABSTRACT

A total of 16 cereal samples: 5 maize (*Zea mais*), 5 wheat (*Triticum. Sp*), 3 barley (*Hordeum sativum*) 2 rye (*Secale cereale*) and 1 oat (*Avena sativa*), used in Albanian feed mills were quantitatively analyzed for phytase activity, phytate and total phosphorus. It was concluded that the number of feedstuffs showing significant phytase activity more than 100 FTU kg^{-1} is rather limited. Of the cereals analyzed, only rye (5384.3 ± 384.3) FTU kg^{-1}, wheat (1156.8 ± 282.9) FTU kg^{-1} and barley (755.8 ± 134) FTU kg^{-1}, are rich in phytase activity. All other feedstuffs analyzed: maize (44.3 ± 13.08) FTU kg^{-1} and oat (33.9 ±19.7) FTU kg^{-1} show a moderate phytase activity. Our results showed that phytase activity is not related to total phosphorus or phytate content. As expected a significant relationship was found between phytate and total phosphorus which confirms the overall opinion that phytate is the principal storage form of phosphorus in bran and seeds.
***Keywords**: Cereals, phytase activity, phytate, total phosphorus*

INTRODUCTION

Cereals with oilseeds and their by-products are the main ingredients for poultry, swine and high productive ruminant diets. Nutritive ration of non ruminant animals contains 65-70% cereals seed (corn, wheat, barley, rye, and oat). Total P concentration in cereals (%) ranges from 0.35 to 0.45 and between 0.65 and 1.12 in oilseeds and by-products (7). In these ingredients, phosphorus (P) is present mainly in the form of phytates, which represent 50 to 80% of total P (11). Phytate content in cereals, legumes and oilseeds varies, depending upon cultivars and soil types, climatic and irrigation conditions, processing and locations (4). Phytates are poorly utilized by non ruminant animals, due to a low activity of phytase, the enzyme that hydrolyzes these compounds in the digestive tract of poultry and swine (14). Some plant feedstuffs (such as wheat or triticale) and some fungi (such as *Aspergillus sp*) contain powerful phytases, capable of hydrolyzing phytic acid in (bioavailable) inorganic P and inositol within the animals' digestive tract (10, 15, 5, 6). In addition, there is an endogenous phytase activity in cereals, oilseeds and by-products, which varies according to grain and type of by-product. Thus, this activity (16, 7) is (FTU/kg) high in wheat (1200), rye (2700) and triticale (1100), intermediate in barley (580) and low in

rice (120), corn (12), sorghum (24) soybean (31) and oat (42). To reach a better understanding of P bioavalability in vegetable ingredients used in animal feeding, knowledge of phytate content and endogenous phytase activity of these materials is required.

MATERIAL AND METHODS

Clean, uncontaminated 16 samples were taken at different Albanian feed mills and sent to laboratory of analysis in Institut für Tierernährung, Freie Universität, Berlin.

Method for analyzing phytase activity in feedstuff
Determination of phytase activity based on the estimation of inorganic orthophosphate released on hydrolysis of phytic acid was routinely performed at 37 ^{0} C following the method described by Engelen et al (8). One unit of enzyme activity was defined as the amount of enzyme that liberates 1 µmol of inorganic orthophosphate per minute under assay conditions. All measurements were performed in duplicate and repeated in some cases if there was discrepancy in duplicate values.

Preparation of samples
The feedstuff sample was ground with the mill (1.5 mm sieve) for sample preparation. 2 x 5 g ground feedstuff was weighted into a 100 ml-conical flask. It was added 50 ml buffer solution with Triton X-100. It was extracted by shaking for 60 min in the shaker and filtered supernatant into a 50 ml GREINER®- tube. Then it was centrifuged for 10 min and 3000 rpm, pipette an amount of the supernatant into test tubes and was diluted with buffer solution with Triton X-100 to a phytase activity of 0.01-0.07 FTU/2ml.

Calculation
Calculate the dilution factor for the sample:
For example for 1:1- dilution (1.0 ml buffer + 1.0 ml sample):
(1x50x1000) / 5 = 10000

Formula:
FTU/kg = dilution factor (e.g. 10000) x {[(absorption of sample-absorption of blind) axis intercept of standard curve] / gradient of standard curve

Method for determination of phytic acid or phytate
Phytic acid was determined according to the method of Wheeler and Ferrel (21). Two grams of sample were weighted into 125 ml conical flask. The sample was extracted with 50 ml of 3% trichloroacetic acid (TCA) for 3 h with shaking. The suspension was centrifuged for 5 min at 2500 rpm. 10 ml aliquot of the supernatant was transferred to 40 ml tube. 4 ml $FeCl_3$. ($FeCl_3$ solution containing 2 mg Fe^{+3} ion/ml 3% TCA) were added to the aliquot. The tube was heated in a boiling water bath at 45 ^{0} C. One or two drops of 3% Na_2SO_4 in 3% TCA were

added to develop precipitate. Then the tube was cooled and centrifuged for 10-15 min at 2500 rpm. The clear supernatant was decanted and the precipitate was washed twice by dispersing well in 25 ml 3% TCA, heated for 10-15 min in boiling water bath, then cooled and centrifuged. The precipitate was washed one or two times with water and was dispersed in a few ml of water. 3 ml of 1.5 N NaOH were then added and the volume completed to 30 ml with water. The tube was heated in a boiling water bath for 30 min and hot filtered using Whatman No. 1 filter paper. The precipitate was washed with 60-70 ml of water and washings were decanted. The precipitate was dissolved from the filter paper with 40 ml hot 3.2 N HNO_3 into 100 ml volumetric flask and the paper was washed again with water in the same flask and completed to volume with water.
A volume of 0.5 ml of the above suspension was transferred into 10 ml volumetric flask. 2 ml of 1.5 N KSCN were added and completed to volume with water, then immediately the absorbance was read using spectrophotometer (DR3 spectrophotometer) at 480 nm.
A standard curve of different $Fe(NO_3)_2$ concentrations was plotted to calculate the ferric ion concentration. The phytate phosphorus was calculated from the ferric ion concentration assuming 4:6 irons: phosphorus molar ratio.

$$\text{The phytate} = \frac{6}{4} \times \frac{A \times \text{mean } k \times 20 \times 10 \times 50 \times 100}{1000 \times 2} \text{ mg/100 g sample}$$

A= optical density.

Total phosphorus was performed according to the standard procedures (1).
Data are presented as arithmetic means with standard deviations (Mean ± SD). One-way analysis of variance and Student's t-test ($P< 0.05$) were performed to test the differences between levels of the phytase activity, phytate and total phosphorus contains in these cereal samples.

RESULTS AND DISCUSSION

The data presented in Table 1 and Table 2 were classified in the following manner:

1. Cereals (rye, wheat, barley) with high phytase activity (more than 100 FTU kg^{-1})
2. Cereals (maize, oat) with very low phytase activity (less than 100 FTU kg^{-1})

Table 1. Phytase activity, phytate and total phosphorus of wheat, rye and barley, grown in Albania.

Cereals	**Phytase activity FTU kg^{-1}**	**Phytate %**	**Total phosphorus %**
Wheat	**Mean ± s.d.**	**Mean ± s.d.**	**Mean ± s.d.**
Wheat Dajti Korce	1046.45 ± 512.66	1.071 ± 0.100[a]	0.360 ± 0.002
Wheat LSV Lushnje	1339.25 ± 95.00	1.814 ± 0.177[b]	0.428 ± 0.002
Wheat Progres Lushnje	846.19 ± 64.53	1.767 ± 0.066[c]	0.399 ± 0.009
Wheat Fier	1356.99 ± 23.30	0.868 ± 0.254[d]	0.319 ± 0.002
Wheat Centauro R-2	1195.26 ± 277.84	1.634 ± 0.144[e]	0.381 ± 0.006
Mean	**1156.83 ± 282.99**	**1.431 ± 0.425**	**0.377 ± 0.038**
Rye	**Mean ± s.d.**	**Mean ± s.d.**	**Mean ± s.d.**
Rye Kruje	5574.50 ± 358.51	1.243 ± 0.100	0.382 ± 0.003
Rye Korce	5194.25 ± 412.28	0.938 ± 0.088	0.355 ± 0.001
Mean	**5384.37 ± 384.31**	**1.091 ± 0.192**	**0.369 ± 0.0164**
Barley	**Mean ± s.d.**	**Mean ± s.d.**	**Mean ± s.d.**
Barley Korca	757.97 ± 215.10	1.657 ± 0.133	0.428 ± 0.008
Barley Fier	789.66 ± 80.66	0.844 ± 0.022	0.355 ± 0.007
Barley polistic Korce	719.94 ± 179.25	1.000 ± 0.00	0.295 ± 0.001
Mean	**755.86 ± 134.00**	**1.201± 0.427**	**0.359 ± 0.060**

a:b; b:d; c:d; d:e; pairs values with different superscripts within columns are significantly different ($p<0.05$).

Table 2. Phytase activity, phytate and total phosphorus of maize and oat grown in Albania

Cereals	**Phytase activity FTU kg-1**	**Phytate %**	**Total phosphorus %**
Maize	**Mean ± s.d.**	**Mean ± s.d.**	**Mean ± s.d.**
Maize Fier	59.95 ± 4.48	1.313 ± 0.133	0.328 ± 0.005
Maize Kruja	32.70 ± 16.13	0.758 ± 0.276[a]	0.241 ± 0.001
Maize Alisea	39.36 ± 13.89	0.923 ± 0.088	0.366 ± 0.004
Maize R-111	44.11 ± 14.34	1.240 ± 0.293	0.401 ± 0.007
Maize Korca	45.38 ± 7.17	1.822 ± 0.055[b]	0.363 ± 0.002
Mean	**44.30 ± 13.08**	**1.214 ± 0.403**	**0.341 ± 0.05**
Oat	33.97 ± 19.72	0.876 ± 0.022	0.313 ± 0.001

a:b values with different superscripts within columns are significantly different ($p<0.05$).

The present study showed that rye has by far the highest phytase activity, followed by wheat and barley. Our estimates for rye, wheat and barley phytase activity are similar to those obtained by Eeckhout and De Paepe (7). These authors concluded that among the cereals, there seems to be no naturally occurring phytase in maize, oats and grain sorghum capable hydrolyzing phytic acid in appreciable amounts under the test conditions used.
Although the bioavailability of phosphorus in high moisture ensiled maize is three to four times higher than that of phosphorus in dry maize (19), practically no phytase activity was noted.
Although variation in phytase activity within a single feedstuff is important, it should be noted that the average values for the five groups of cereals are very different and there is a little or no overlapping. In this point of view, statistical differences for phytase activity between cereals and between cultivars were not significant.

Phytate and Phosphorus
Phytic acid concentrations in seeds from a given species vary because of many factors including cultivars (17, 2, 13), soil conditions, fertilizer applications (3) and moisture plus other climate factors (9). Lott et al. (12) concluded that the range for phytic acid in cereal grains is 0.86-1.06%, while Reddy et al. (18) give values between 0.50-1.89%. Our results about the phytate and phosphorus content in cereals are similar with the results declared by Eeckhout and De Paepe (7). Belong to their results for each feedstuff class studied; total phosphorus content is positively correlated with phytate-P content. However, from a practical standpoint, the predictability of phytate-P content from total phosphorus is only justified for two feedstuff classes, wheat and wheat by-products and maize and maize by-products (5). According to our results, there was statistical significance in phytate content between Maize Kruja and Maize Korca, due to the very different growth conditions, climate and soil. There were also statistical significance between Wheat Dajti Korce and Wheat LSV Lushnje, Wheat LSV Lushnje and Wheat Fier, Wheat Progres Lushnje and Wheat Fier, Wheat Fier and Wheat Centauro R-2. Statistical significances for phosphorus content between cultivars were not documented.
Simons et al (20) found good linear relationship between total P and phytate –P for several classes of vegetable feedstuffs, notwithstanding the fact that phytate-P data were from three different laboratories involving three different methods. Eeckhout and De Paepe (7) also concluded for each feedstuff class studied, total P content is positively correlated with phytate-P content. According to our analysis, the cultivar Wheat LSV Lushnje has the highest phytate and total P content, between wheat samples.

CONCLUSIONS

This paper provides a first estimate of phytase activity, phytate and total phosphorus of some cereals grown in Albania and used in the non ruminant nutritive rations. It is only an estimate, because the magnitude of the "phytate

problem" in terms of nutrient management in agricultural and livestock production is very important. According to the results of this paper Albanian cereals were classified like cereals in all over the world. The results of this study showed that phytase activity was not related to total phosphorus or phytate content, otherwise phytate was related with total P content.

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ECONOMIC FACTORS WITH INFLUENCE ON FEED MANUFACTURING

Jasna Stevanovic[1], Aleksandar Drljacic[2], Biljana Miljkovic[3]

[1]Association of Agriculture, Food-processing and Tobacco and Water Industry, Chamber of Commerce and Industry of Serbia, Belgrade, Serbia
[2]MagnaVita Hol.a.d., Superprotein A.D., Pančevačka 70, Zrenjanin, Serbia
[3]Scientific Institute of Veterinary of Serbia, Vojvode Toze 14, Belgrade, Serbia

ABSTRACT

Technological development of industrial feed production is intended to achieve a standardized and sustainable livestock production, and must be economically justified to provide a safe and permanent placement of animal products at market. Moreover, going along with price stability, products of animal origin must be safe for use in order to protect consumers.
It is known that feed industry promotes technology development of other food processing industries, by giving "a new nutritional value" to their by-products, and using them as their raw materials. From the economic point of view, optimal use of by-products of vegetable or animal origin is in improvement of efficiency of animal feed, controlled use of resources and reduction of nutrients losses.
In this manner, waste management and "solving the problem" is more promote at the very source of their origin. Speaking in ecological terms, any waste that can be to used is considered as a resource, in order to sustained industrial feed production.
The data that we used for analyzing production, prices and terms of use of vegetable or animal origin by-products in animal nutrition, were taken from Statistical Office, official reports, trading informants, and up-to-date regulations in Serbia, accompanied with EU legislative.
The aim of this paper is to evaulate the legitimate need of using the by-products in slaughter industry, in accordance with the regulations that defining their use, beside the plant by-products, and in accordance with current legislative and new acknowledgement of their safety.
***Keywords**: by-products, animal nutrition, raw materials*

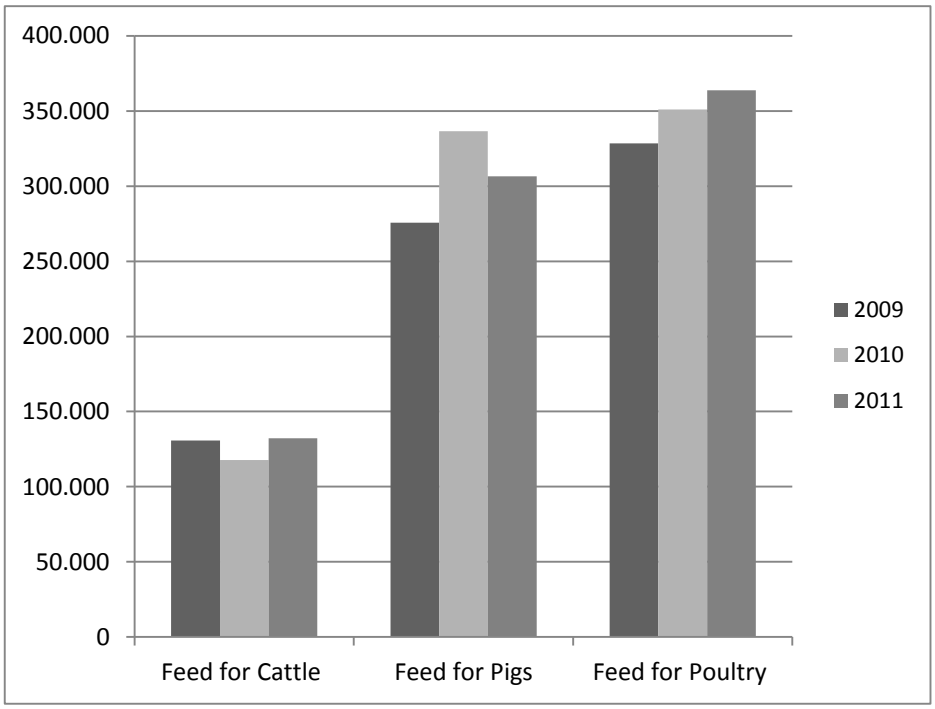
INTRODUCTION

The interest of financial markets in agricultural commodities has taken a new turn after the global financial crisis. This was triggered the need for fostering the introduction of financial risk management tools to farmers and grain users which can be used for protection of their supplies and income. It is necessary to systematically consider the legal loads and regulatory burden and to remove measures which hamper the fluidity of markets for agricultural raw materials, without compromising feed and food safety standards.

Farmers agree that the price of inputs affect the prices of finished product and expenses for final consumer. Establishing a sustainable development of livestock in a large percentage depending on the price of raw materials for feed, and it is expected that the ability to stabilize the production could lead to stability in raw material prices, which are included in feed.

THE INDUSTRY PRODUCTION OF FEED AND ANIMAL NUTRITION IN SERBIA

In terms of market access and food safety, feed industry is considers, in terms of the practices in EU, that access to market of competitive raw materials for use in animal feed represent the key to maintain permanent livestock production in Serbia. Serbian feed industry manufactures about 850 000 tonnes of feed, rich with protein primarily of vegetable origin, domestic origin (4). Share of industry production feed in animal nutrition, in Serbia, for selected species is shown in Chart 1.



The data source: Statistical Office of the Republic of Serbia

Chart 1. The industry production feed for selected species, in tones, period 2009-2011

Compound feed in Serbia are manufactured from a mixture of raw materials designed to achieve pre-determined objectives in animal nutrition. These raw materials are obtained from a wide variety of sources. In domestic market mainly cereals, oilseeds and pulses are available. Some raw materials are obtained from the by-products of the food industry. Other important ingredients which cannot be production in Serbia are imported from EU and other countries. These diverse sources of raw material are an important factor in the industry's ability to manufacture feeds of both high quality and at competitive prices for livestock farmers. In Serbia, as well in other countries, the problems in priority of raw materials supply for feed production still remains. The domestic industry of feed sees the potential for the development of livestock in high technological requirements setting of production, that can meet the farmers demands.
On the basis of the number of animals and intermediate consumption feed in Serbia, share of industry manufactured feed in that animal nutrition in 2010 is about 27%. The industry for feed should be the largest market for produced grains and oilseed meals in Serbia. In a case of deficit raw materials (plant origin), feed- product represents the highest input cost for livestock farmers.
Despite the markedly favorable natural conditions, livestock production in Serbia has long been in crisis. According to official statistics, from year to year number of livestock is declining,as well as the production and consumption of meat (4). At the same time the price of basic livestock products (meat, milk, eggs) is increasing. In the National Programme of agriculture in Serbia 2010-2013. is stated that the median goods producers, who mostly process the food by themselves along with crop farming and animal husbandry, are significant from the perspective of the agricultural policy of Serbia, because of they have the most of beef, pork, chicken and lamb meet production. It is also known that that median goods producers, with their technology of feed production and farms sizes are behind the larger companies. Also, they are in the minority compared to the total number of small producers (3). Beside the prices, the establishment of control over compliance with regulations the EU, before or after placing the raw materials, animal feed and livestock products on the market,are tightly embroidered for market conditions. It is not precisely specified in Serbia by national legislation yet (9).

THE USE AND IMPORTANCE OF USE OF BY-PRODUCTS IN ANIMAL NUTRITION

It is important to point out that those by-products of crops, as a feedingstaff, are an integral part of the daily ration of animals, whereas the quantity and quality of nutrients are crucial for the nutrient quality. It is well known that the by-products of oils industry, representing the feedingstaff of high nutritive and biological value, are in the structure of meals between 15% - 20%, depending on the species and categories of animals (6). Also, by-products incurred during cereals processing, have a relatively high content of dry matter, fiber, and high energetic value (6). Furthermore, the by-products of confectionery industry can be a source of energy for young animal categories. The by - product of animal origin

which are not suitable for human food have a variety of use (5)(6). For example, animal fats and milk powder are used for nutrition of animals grown on farms or for pet food. Animal skins and fur are used in production of leather and textile industries, while the blood of animals, is used in pharmaceutical industry or as a diagnostic means (8).
It is understandable that the wide range of animal waste, securely use for different purposes on a sustainable manner, under the condition of minimal health risks (8). Regardless of the investment for the establishing the system of management with industrial waste, the gain is sure,and the system can not be understand as an act of of goodwill, but as a manner leading to the survival of the entire economy (10). From the manufacturer are therefore expected to be responsible for his actios, and each activity in the course of production plan and implement in a manner which does not affect on the environment, respecting the principle of "polluter pays" (2).

SOME POSTULATES ABOUT ANIMAL BY PRODUCT AND FEED FROM THE PRACTICE OF EU

As a result of the BSE crisis by the European Commission, first were introduced restrictions (gradually from 1988. Limited use of certain animal protein), and then is ban (2001). use of meat meal and bone meal in animal feed in animal nutrition. Regulation (EC) 1069/2009, the EU Council and Parliament define ways of handling, the purpose of categorization and by-products and derived products originating from animals that are not intended for human consumption, according to the potential risks that they pose (8). By abolition the prohibition principle for use of these products, defined by Regulation (EC) 1774/2002, the ban remains on protein of the same species in animal nutrition. Also, each possibility of a risk to public health, can have the opposite wider effect on society as a whole, by its impact on the socio-economic situation of farmers and concerned industrial sectors as well as consumer confidence in the safety of products of animal origin. The outbreak of the disease could also have negative consequences for the environment, not only because of problems with the disposal of waste that is imposed, but also in a manner of biodiversity.
A more flexible approach in the new European legislations, permitting imports of all categories of animal by-products originating from animals for scientific research and development (8). Acording to that, technical details, specific hygiene standards, methods of processing and import requirements (monitoring in traceability), are left to measures of the European Commission (SCoFCAH). For Animal by – product (ABP) slaughtered animals, there is a program of risk analysis, which implies the results of ante-mortem and post-mortem inspection (1)(8).
Below are shown the protein values, which are related to the quality products from some species of animals:

- Poultry: 60 – 70 % Protein
- Pork: 45 – 55 % Protein from By-products

- Pork: 60 – 75 % Protein from Fat Melting
- Blood: 90 – 95 % Protein
- Feather: 75 - 85 % Protein

It is important to consider that deviations may occur in applying the Animal by - product regulation on industrial production of feed, in part related to monitoring of traceability. According to this, the traceability of full animal feed produced from ABP/derived products, as defined in section 3 (h) of Regulation 767/2009. refers only to the initial point. For a complete feed mixture is not necessary to specify and identify the ingredient (ABP-derived product) or keep a commercial document during transport and follow of records (8)(2)(7).

FINAL CONSIDERATIONS

Proceeding from the fact that significant share of the people nutrition are products of animal origin, the task of national agricultural expert services, should be based on measures for establishing sustainable development of animal husbandry. It is questionable, whether it is economically justified to dismiss the possibility of using meat and bone meal as a high quality raw material, in a diet of other (different) species animals. From the trading market point of view, it is necessary to monitor the quality and economic viability of the feed at the same time. However, one must be cautious of appearance of some new epizootic form by introducing the meat and bone meal. This leads to the need for developing new methods of identification and disregards of specific proteins intended for animal nutrition.
Moreover, the confusion can be made for the objects approved in the EU for use of processed animal protein (PAP) and safe export of PAP. The question is if this product includes processed protein derived from ruminants, whether will such PAP also be safe to use. It is significant to define and enable the safe treatment of animal origin by-products in order to obtain of safe raw materials for the production of animal feed. By that, it means the application of such manufacturing procedures on an input (or previously used) materials, which could reduced or decrease to an acceptable level the risks for public and animal health. It is necessary to ensure, by testing the final product, that derived product do not represent risks for public and animal health. Safety use of derived products means no risks or danger for public and animal health under any circumstances.

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APPLICATION OF NIR TECHNOLOGY FOR THE QUALITY CONTROL OF SUNFLOWER AND SOYBEAN MEAL AVAILABLE ON THE SERBIAN MARKET

Marijana Maslovarić, Rade Jovanović, Snežana Janković, Nataša Tolimir, Marina Vukić – Vranješ

Institute for Science Application in Agriculture, Bulevar despota Stefana 68b, 11000 Belgrade, Serbia

ABSTRACT

Near Infrared Spectroscopy (NIR) is used for a number of years in the feed industry as a fast and accurate analytical method for determining quality parameters of raw materials and compound feed. The application of NIR technology significantly improves the quality control, thereby enables increasing production competitiveness and profitability. Sunflower and soybean meal are the most commonly used raw materials in animal feed production. However, the quality of these raw materials on the Serbian market varies significantly. For this reason global calibration packages require appropriate settings and adjustment. The aim of this study was to develop customized calibration models for protein content in soybean and sunflower meal which are being used in animal feed factories in Serbia.

Standard chemical analysis of crude protein content was performed for 56 samples of soybean meal and 38 samples of sunflower meal. In order to develop calibration models for protein content, the samples were scanned by NIR Perten Diode Array 7200 scanning spectrophotometer in the reflectance mode (spectral range 950 to 1650 nm). Pretreatment of the spectra was performed by Savitzky–Golay followed by Standard Normal Variate transformation. The calibration models were developed by using Partial Least Square regression (PLS) in the CAMO Unscrambler 9.7. software package. The following parameters were used for the calibration models' evaluation: the Coefficient of Determination of Calibration (R_C^2), Standard Error of Calibration (SEC), Coefficient of Determination of Cross Validation (R^2cv) and Standard Error of Cross Validation (SECV). The obtained values of the evaluation parameters showed high accuracy for both calibration models. The obtained customized calibration models for protein content in sunflower and soybean meal are significantly less robust than the global calibration packages, but also highly accurate for a certain range of protein content, and they can be used successfully in the process of quality control in the feed production in Serbia.

***Keywords**: NIR, feed, sunflower, soybean, meal, calibration, protein content*

INTRODUCTION

Soybean and sunflower meal are the most commonly used protein supplements in the animal feed industry. Protein content is an important quality parameter of the soybean and sunflower meals and those are generally priced by the protein content. [5] Soybean and sunflower meal used in animal feed factories in Serbia do not have constant quality since crude protein content may vary considerably within the broad range. On the other hand, strong competition in the field of feed production requires higher efficiency of the production process, which among other things, implies fast and constant monitoring of the chemical composition, i.e. quality of raw materials entering the production process. Therefore, it is necessary to introduce fast analytical methods such as near infrared spectroscopic methods (NIR). Nearinfrared (NIR) spectroscopic methods have become a widely spread technology for qualitative and quantitative analysis in the chemical, pharmaceutical and agro-food industries because it is a fast, simple, cheap and non-destructive technique with nearly no sample preparation required. [1] Like any other spectroscopic technique, NIR requires calibration samples to build models that can later be used to identify or predict properties of new samples. [8] Calibration procedure includes application of certain mathematical and statistical techniques (chemometrics) for the purpose of obtaining empirical equation which connects spectrum data with data obtained by chemical analysis [9]. Leading manufacturers of NIR devices have developed global calibration packages, which enable a single calibration to be used in different locations around the world. [2] Global (starter) calibration packages are useful to have a fast and easy implementation of the NIR technology in the feed production, but they also have certain limitations: most often these are protected data that can be used only with a paid license, and they cannot be extended with additional data in order to be adjusted to the user's specific needs. Therefore, it is recommended to develop your own customized calibration models in compliance with the specific raw materials used in a particular production process and available on the particular market and geographic location. [6]
The aim of this study was to develop calibration models for crude protein content in soybean and sunflower meals that are available on the Serbian market.

MATERIAL AND METHODS

In order to develop calibration models for crude protein content in soybean and sunflower meals, standard chemical analysis of crude protein content was carried out on 56 samples of soybean meal and 38 samples of sunflower meal from different oil mills in Serbia. These samples were used in the process of feed production in the animal feed factory "Komponenta" from Ćuprija in the period December 2011 - March 2012. Chemical analysis of the crude protein content was done according to Kjeldahl method and the Rulebook on methods of sampling, physical, chemical and microbiological analysis of feed, 1987. [7] Samples were analyzed in a NIR Perten Diode Array 7200 scanning spectrophotometer in the reflectance mode (950 to 1650 nm), using rotating dish

with a diameter of 75mm. Samples were analyzed as they were, with no grinding or other sample preparation. Each sample was repacked three times in order to reduce the random error from inhomogeneous sample distribution. [10] The following transformations were carried out on the spectra of the soybean and sunflower meal, in order to remove all irrelevant information from the spectra, and to interpret the results easier: Savitzky-Golay – to obtain the first derivative of the spectra and SNV – Standard Normal Variate. The calibration model was developed using PLS – Partial Least Squares regression. The following parameters were used for the calibration models' evaluation: the Coefficient of Determination of Calibration (R_C^2), Standard Error of Calibration (SEC), Coefficient of Determination of Cross Validation (R^2cv), Standard Error of Cross Validation, (SECV). For transformation of the main spectra of the soybean and sunflower meal samples and development of calibration model, the software package CAMO® Unscrambler 9.7. was used.

RESULTS AND DISCUSSION

Calibration data sets of the sunflower and soybean meal showed uneven distribution of the protein content. The cause of the protein content variation in the sunflower and soybean meal can be the result of the differences between the processing methods of the oil mills, but more often it is the result of producers getting the part of the hulls back into the production process in order to achieve extra profits. This increases the crude fiber content and decreases the crude protein content in sunflower and soybean meal. Distributions of protein content in soybean and sunflower meals are shown in Figures 1 and 2.

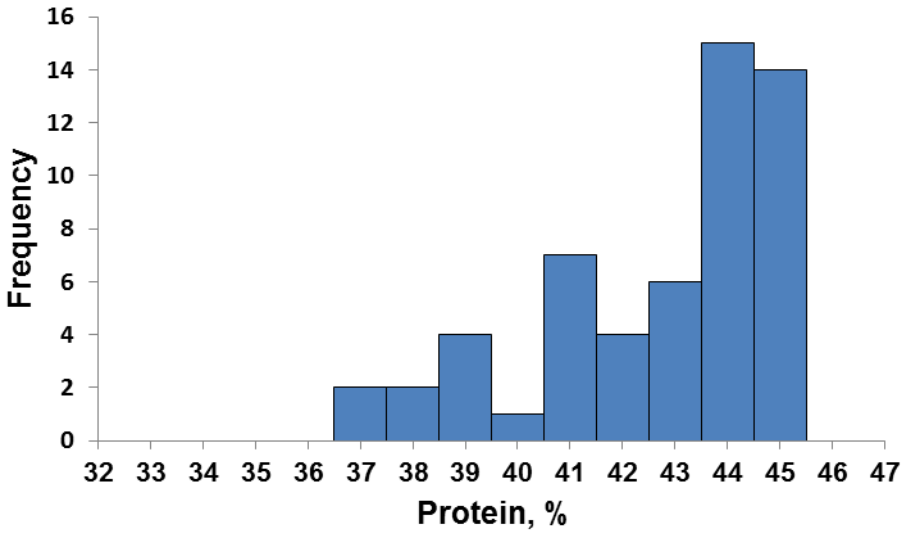

Figure 1. Histogram plot of the crude protein content distribution within the calibration set of the soybean meal samples

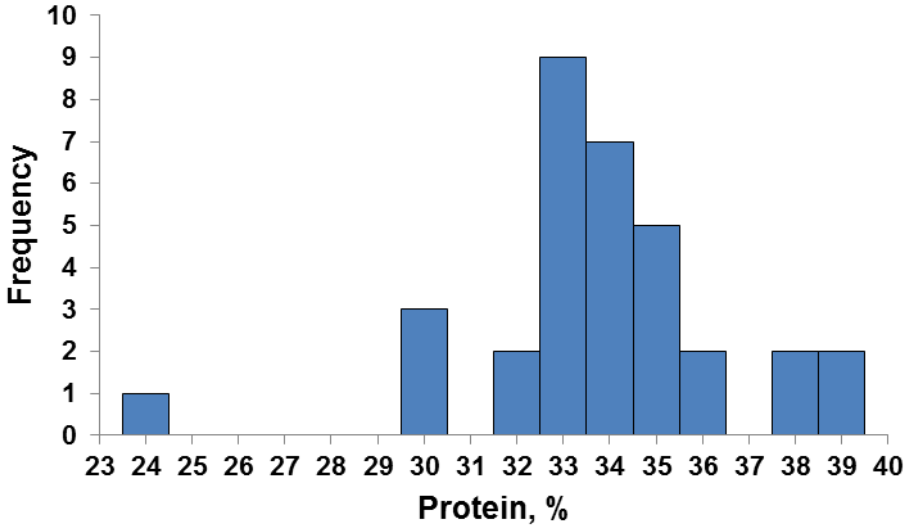

Figure 2. Histogram plot of the crude protein content distribution within the calibration set of the sunflower meal samples

Protein content varied widely within the sample sets of both soybean and sunflower meal (Table 1).

Table 1. Descriptive statistics for the protein content in the calibration sample sets of sunflower and soybean meals

	n	Mean	SD	Range, %	SEL
Soybean meal	56	42.29	2.3	36.90 – 44.96	0.21
Sunflower meal	38	33.32	2.7	24.00 – 38.85	0.17

SD: Standard Deviation; SEL: Standard Laboratory Error

Due to uneven distribution of the protein content and great variations in the calibration data set, some samples (outliers) had to be excluded in order to achieve better accuracy of the model. As a consequence, the number of calibration samples, as well as protein content range, was reduced. This implies that spanning the calibration data space and updating the model when more new samples become available is necessary to enable predicting of the variabilities that may appear in the future. [11]
Table 2 summarises statistical values of the obtained calibration model. High values of the coefficient of determination of calibration (R_C^2) and cross validation (R^2_{CV}) were obtained, reaching values close to 1.

Table 2. Calibration statistics

	Mean	SD	Range, %	R_C^2	SEC	R^2_{CV}	SECV
Soybean meal	43.83	0.91	41.6–44.96	0.98	0.13	0.93	0.24
Sunflower meal	33.22	1.51	29.99-35.33	0.99	0.14	0.98	1.19

Apart from coefficients of determination, low values of SEC were obtained for both sunflower and soybean meal. Evaluation of the predictive ability of the calibration model was performed by cross-validation. Cross–validation was performed as there were not enough samples for a separate test set and it is routinely used to determine the usefulness of NIRS equations. [3] The SECVs values were low and they meet the commonly accepted criterion that SECV should be less than two times the SEL. [4] This indicates that the useful calibration models were obtained in both cases. Nevertheless, external validation of the model is recommended, which requires additional samples for further verification of the model.

CONCLUSIONS

Based on the values of the evaluation parameters of the calibration models R^2cv and SECV we can assume that calibration models obtained as a result of this research highly reliable for the protein content range from 41.60 to 45.00% in soybean meal and from 30.00 to 35.30% in sunflower meal.
Global (starter) calibration packages enable a fast start when implementing NIR technology, but they almost always require adjustments to the particular production process and geographical region. Therefore, it is recomended to build custom NIR calibrations that fit to a preferred raw material from the local supplier on the particular location. Obtained calibration models should be improved over time in order to become more robust. Calibration range should be extended with additional samples, which would result in a reliable calibration model for a wider protein content range in soybean and sunflower meals available on the Serbian market.

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MOLASSES AS A FEED SUPPLEMENT

Rada Jevtić-Mučibabić[1], Jasna Grbić[1], Bojana Filipčev[1], Vojislav Banjac[1], Tatjana Kuljanin[2], Jasmina Živković[1]

[1]University of Novi Sad, Institute for Food Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia
[2]University of Novi Sad, Faculty of Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

ABSTRACT

Molasses is important raw material in many processing industries owing to its composition. Therefore, the world sugar industry emphasizes regular monitoring of molasses quality. Molasses composition mainly depends on the quality of sugar beet (cultivar, vegetation conditions, agro-technical measures, root storage, etc.). Molasses, obtained under adequate conditions of sugar beet processing, has about 80% of dry matter content (about 50% is saccharose and about 30% non-saccharose matter). The molasses containes concentrated non-sugar components that have not been removed in the purification process, non-sugar components that are formed during the evaporation and crystallization, as well as the remains of the auxiliary materials used in the sugar beet processing.
This paper presents the results obtained by continuous monitoring of the quality of molasses produced in domestic sugar factories during campaign.
***Keywords**: molasses, quality, chemical composition*

INTRODUCTION

Sugar beet molasses, due to its composition, is a valuable raw material for the production of technically and economically highly important products from biotechnological processing such as baker's yeast, ethanol, lactic acid, citric acid, acethone, butanol, glycerol and a range of secondary metabolites (antibiotics, amino-acids, etc.) [1].
In Serbia, molasses is mostly used for the production of ethanol and baker's yeast, of which the ethanol production is primary. Molasses has important role in agriculture where it has been used as a component in feed for all categories of animals [2]. Amounts recommended for poultry and cattle range from 5-10% whereas slightly larger amounts are applicable for pigs nutrition. Molasses exerts slight laxative effect; it improves the taste of food and can be used as a binder during feed pelleting [3].
In molasses, besides carbohydrates as a main energy source, substantial amount of minerals is present.
Molasses is a rich source of Ca, K, Na as well as microelements (Ni, Co, Fe, Pb, Al, Mg, Mn) and B group vitamins (riboflavin, holine, panthotenic acid and niacin). The content of nitrogen compounds in molasses depends on their content in beet root and transformation of some compounds during processing.

Volatile organic acids present in molasses (formic, acetic, propionic, butyric, isobutyric, valeric and caprylic) do not originate from sugar beet but could be transferred to molasses with water during processing or could be formed by microorganism fermentation in extraction juice before alkalisation [4,5].

MATERIAL AND METHODS

The investigations were conducted with samples of molasses produced from sugar beet in domestic sugar factory. Basic quality parameters of molasses, were determined according to methods described in handbook for the laboratory control of sugar processing [6]. The methods are in accordance with the regulations guided by the International Commission for Uniform Methods of Sugar Analysis [7]. The obtained data were processed using StatSoft Statistica, for Windows version 10. Basic statistical descriptors were calculated [8].

RESULTS AND DISCUSSION

The average values and statistical analysis of the molasses basic quality parameters are presented in Table 1. Variations of this group of indicators are influenced by sugar beet technological quality and characteristics of the processing conditions, used in the sugar factories. The largest coefficients of variation were observed in the content of reducing substances which imply to the fact that this parameter is largely dependent of nonsucrose compounds content in sugar beet. Statistically significant variation in this parameter shows that sugar plants use different processing conditions with different efficacy.

Table 1. Basic quality parameters of the molasses

Parameter (%)	Minimum	Maximum	Average	Standard deviation	Coefficient of variation
Dry matter	78.60	87.00	82.77	3.55	4.29
Polarization	46.70	52.30	49.27	2.27	4.60
Purity	58.58	60.11	59.52	0.63	1.07
pH value	6.10	7.80	7.18	0.66	9.13
Reducing substances	0.304	1.015	0.530	0.28	52.47

Table 2 shows the content of mineral matters. Mineral matters present around one third of total mass of molasses nonsucrose compounds. The potassium/sodium ratio of molasses is 2.10 : 1 and it is different in related to ratio in sugar beet, because of NaOH or Na_2CO_3 introducing in sugar beet processing with the aim of correction of the intermediate products alkalinity. The average content of magnesium in molasses is 0.07 % and it is insignificant value. Significant quantities of magnesium were eliminated during purification of

extraction juice. During the processing of deteriorated sugar beet content of soluble calcium compounds in molasses is increased.

Table 2. Mineral matters of molasses

Parameter (%)	Minimum	Maximum	Average	Standard deviation	Coefficient of variation
K	2.19	2.98	2.57	0.27	10.56
Na	0.96	1.48	1.22	0.22	18.23
CaO	0.34	1.02	0.65	0.28	42.72
MgO	0.03	0.17	0.08	0.06	76.04

The contribution of determined nitrogen compounds in total nitrogen content of examined molasses is presented in Figure 1.
The content of nitrogen compounds in molasses ranges from 1.4 to 2.1% and depends on their content in beet root and transformation of some compounds during processing. Around one third of nitrogen compounds is present in betaine and the rest includes amino-acids, proteins, amides, melanoidines and negligible amounts of ammonium salts.
The content of SO_2 and total volatile acid expressed as acetic acid are presented in Table 3.

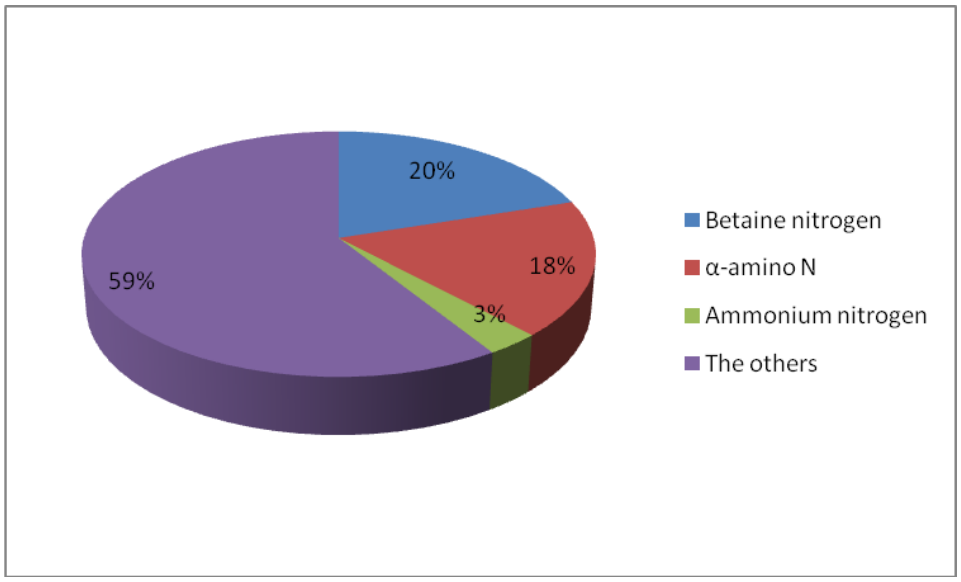

Figure 1. Contribution of single nitrogen matters in molasses total nitrogen content

Table 3. Volatile matters of molasses

Parameter (%)	Minimum	Maximum	Average	Standard deviation	Coefficient of variation
SO_2 content	0.001	0.007	0.004	0.002	50.00
Volatile acids	0.46	0.90	0.65	0.17	26.33

The contents of sulphur-dioxide measured in the samples are all below the regulated limits for molasses [9]. Values are within the boundaries of 0.001 to 0.007%. Sulphur-dioxide in molasses originates from the sulphur-dioxide used for acidification water for extraction of sugar beet cossettes. Higher levels of sulphur-dioxide could be the result of sulfitation of purified juice.

According to the studies of Schiweck [10], from all of the volatile acids in molasses, the concentration of acetic is the highest – around 60%, then formic acid – around 36%. The average value of volatile acids is 0.65% expressed as acetic acid.

CONCLUSION

The feeding value of molasses is based on the fact that it contains approximately 50% sugars in the form of sucrose and reducing substances. It is therefore a source of readily available energy. Molasses contains 1.4 to 2.1% nitrogen compounds. Molasses can supply the carbohydrate in a very readily available form and in combination with urea, can provide the protein as well.
It also a good source of mineral matters.
On the basis of the obtained results it can be concluded that molasses produced in domestic factories conform to the criteria of current standard which regulates the required quality of molasses in processing, fermentative and feed industry.

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THE INFLUENCE OF PHYTOGENIC ADDITIVES AND ORGANIC ACIDS SUPPLEMENTATION TO DIET ON BROILER CARCASS COMPOSITION AND MEAT YIELD

Tatjana Tasić[1], Predrag Ikonić[1], Đorđe Okanović[1],Natalija Džinić[2], Jasmina Gubić[1], Dragan Milić[3], Marina Vukić - Vranješ[4]

[1]Institute of Food Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia
[2]Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia
[3]Perutnina Ptuj – Topiko AD, Petefi Brigade 2, 24300 Bačka Topola, Serbia
[4]Institute for Science Application in Agriculture, Bulevar despota Stefana 68b, 11000 Belgrade, Serbia

ABSTRACT

The influence of phytogenic additives and organic acids addition in broiler diet on carcass composition and meat yield were examined in present paper. The experiment was conducted on broilers hybrid ROSS (n = 24000) divided in three groups. First group (control - C) was fed with commercial broiler feed mixture, while in second (E1) and third group (E2) phytogenic additives and organic acid were included, respectively. Fattening of broilers lasted for 40 days. Food and water were provided *ad libitum* in the floor fattening system.
Mass of cold carcasses and breast mass was not significantly ($P > 0.05$) affected by inclusion of phytogenic additives and organic acids in broiler diet, while mass of whole leg was significantly higher in E1 (486.0 g) than in C (459.9 g) group. Differences of meat yield in breast and whole leg between examined groups were not significant ($P > 0.05$).
Based on the obtained results it can be concluded that inclusion of phytogenic additives and organic acids in broiler diet did not influence significantly carcass composition and meat yield.
Keywords: *broiler, feeding, phytogenic additives, organic acid, carcass quality*

INTRODUCTION

The extensive use of antibiotics in animal production has increased the risk of development of resistance in human and animal pathogens [21]. Because of concerns about potential negative human health consequences, as well as satisfying consumer demand for a food chain free of drugs [14, 1], use of antibiotics as growth promoters is forbidden in the European Community [3]. The ban on antibiotic usage in Europe lead to increasing researchers interest in finding alternatives to antibiotics for poultry production such as enzymes, organic or inorganic acids, herbs, essential oils, immunostimulators, microelements, probiotics and prebiotics [1, 11, 13, 19].

Phytogenic additives are a group of natural growth promoters, derived from herbs, spices or other plants [7, 11, 13, 20]. In recent years, the use of phytogenic compounds has increased because their potential role as natural alternatives to antibiotic growth promoters in animal nutrition [12]. Phytogenic additives enhance broiler performance and health, and have beneficial effects on: feed intake, broiler growth performance, digestive function, feed conversion, gut health parameters, body weight gain [7, 12, 13]. Also, may have a beneficial effect on carcass and stored meat quality [4, 10].
Organic acids have been used for a long time as food additives to prevent food deterioration and extend the shelf life of perishable food ingredients [16]. The supplementation of organic acids in the diet of broilers enhanced nutrient utilization, growth, and feed efficiency [5], and can prevent bacteria and fungal growth [9, 11]. Organic acid supplementation have been reported to decrease colonization of pathogens and production of toxic metabolites, improve digestibility of protein and minerals like Ca, P, Mg and Zn. Dietary supplementation of organic acids increases the body weight and feed conversion ratio in broiler chicken [1, 17], as well as increased growth performance, reduced diseases and management problems [2].
Thus, the aim of this study was to determine the influence of phytogenic additives and organic acids in diet on broiler carcass composition and meat yield.

MATERIAL AND METHODS

The experiment was carried on 24000 broilers, hybrid ROSS. Broilers were divided in three groups, control group (C) and experimental groups (E1) and (E2) and fed under the same conditions in the period of 40 days. Broilers from control group were fed with commercial mixtures, while in broilers diet from experimental group E1 phytogenic additives Biomin P.E.P were added and in broilers diet from experimental group E2 organic acids biotronic SE forte were added. During whole broiler growing period water and feed were provided *ad libitum*.
In order to determine an average mass, 3.75% of broilers from each group were selected and weight. Investigation of carcass composition and meat yield were carried out on 12 animals of average weight from each group.
After growing and 12h starving period, representative broilers from each group were slaughtered and processed by bloodletting, scalding, plucking, ~~and~~ evisceration and chilling. Then chickens carcasses "ready to grill" from each group were transfered into laboratory and cut in the basic anatomical parts [15]. Cutting and deboning of breast and whole leg were applied in order to determine the breast and whole leg meat yield. Analysis of variance (Duncan test) was used to test the hypothesis about differences among obtained results. The software package Statistica 9.1 for Windows, Stat Soft, Tulsa, Oklahoma, USA, 2009.[18] was used for analysis.

RESULTS AND DISCUSSION

The results of broilers carcass composition from control and both experimental groups are presented in Table 1. Maximum weight (1702.2 g) of cold carcass was determined in the experimental group E1 comparing to control (1667.7 g), and experimental group E2 (1631.6 g). The values were significantly different only between experimental groups E1 and E2 (P<0.05). Value of breast mass was the highest (653.0 g) in the control group, while the highest value of whole leg mass (486.0) was observed in group E1. The differences between values of breast mass were not significantly different (P > 0.05), but the values of whole leg mass were significantly different between groups C and E1. Like chilled carcass mass and mass of whole leg, the highest mass of back portion and tail end (362.3 g) and mass of wings (179.8 g) were also determined in group E1. Mass of back portion and tail end were significantly different (P<0.05) between C and E2, as well as between E1 and E2, while in mass of wings there was no significant differences between groups. Mass of abdominal fat ranged from 14.4 g (E1) to 14.9 g (E2), with no significant differences (P<0.05) between observed values. Mass of cold carcasses and breast mass was not significantly (P > 0.05) affected by inclusion of phytogenic additives and organic acids in broiler diet, while mass of whole leg was significantly higher in E1 (486.0 g) than in C (459.9 g) group. Although, values of carcass mass between groups were not significantly different, numerical value was the highest in experimental group (E1), which can confirm the fact that phytogenic additives can enhance body weight gain in broilers [7, 12, 13].

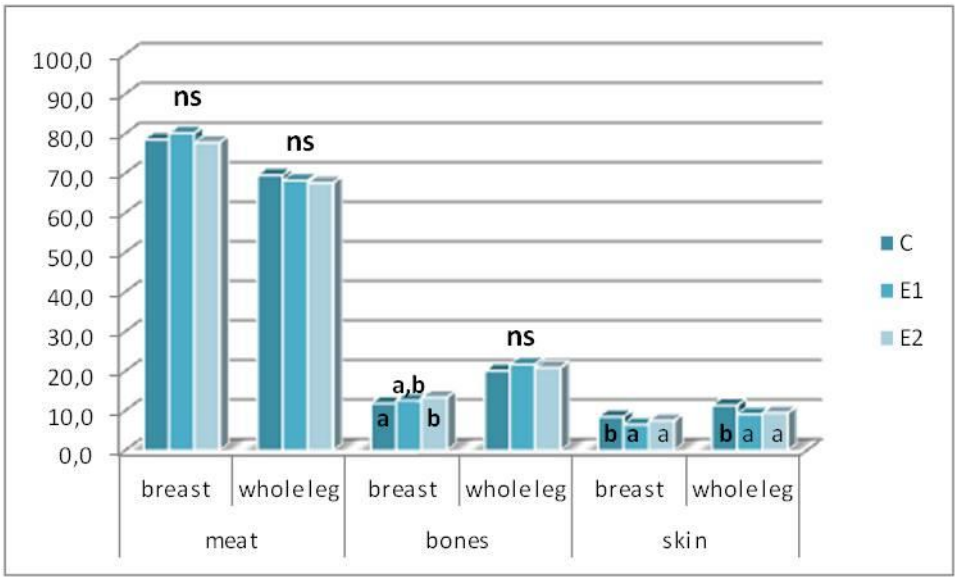
Table 1. The results obtained by weighting (g) the main parts of chilled carcasses of control and experimental chicken groups (mean values ± standard deviation)

Group	Chilled carcass mass^{ns} (g)	Breast mass^{ns} (g)	Mass of whole leg^{ns} (g)	Mass of back portion and tail end^{ns} (g)	Mass of wings^{ns} (g)	Mass of abdominal fat (g)
C	1667.7^{a,b} ± 60.1	653.0^{ns} ± 43.0	459.9^{a} ± 22.5	357.4^{a} ± 27.0	173.1^{ns} ± 7.2	14.6^{ns} ± 11.1
E1	1702.2^{b} ± 47.3	648.6^{ns} ± 26.9	486.0^{b} ± 21.6	362.3^{a} ± 24.5	179.8^{ns} ± 8.5	14.4^{ns} ± 6.7
E2	1631.6^{a} ± 80.0	625.9^{ns} ± 40.2	474.3^{a,b} ± 21.7	334.5^{b} ± 26.0	173.2^{ns} ± 13.0	14.9^{ns} ± 10.3

In the same column, different letters means that values are significantly different (P < 0.05)

Share (%) of meat, bones and skin in total breast and total whole leg mass are presented on Graph 1. Share of meat in breast within groups was in the range from 77.8 % (E2) to 80.2 % (E1), while share of meat in total whole leg mass

was in the range from 67.6 % (E2) to 69.6 % (C). Differences between groups in share of meat were not significant ($P > 0.05$) for both, breast and whole leg, but numerical values of meat yield was the lowest in experimental group with organic acid inclusion, for both breast and whole leg.
Share of bones in breast mass ranged from 12.0 % (C) to 13.4 (E1) and in whole leg mass from 20.1 % (C) % to 21.8 % (E1), while share of skin was in range from 6.6 % (E1) to 8.5 % (C) in breast mass and in the range from 9.2 (E1) to 11.4 % (C) in whole leg mass.



Graph 1. Share (%) of meat, bones and skin in total breast and total whole leg mass
Different letters means that values are significantly different ($P < 0.05$)

CONCLUSIONS

The diet with inclusion of phytogenic additives and organic acids did not affect significantly ($P > 0.05$) mass of chilled carcass, but numerical value was the highest in group with phytogenic additives addition.
Differences between groups in share of meat were not significant ($P > 0.05$) for both, breast and whole leg. Numerical values of meat yield was the highest in group with addition of phytogenic additives for breast and in control group for whole leg, while numerical values of meat yield was the lowest in experimental group with organic acid inclusion, for both breast and whole leg.

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EFFECT OF HEMPSEED *(CANNABIS SATIVA sp.)* SUPPLEMENTATION TO DIET ON PERFORMANCE, CARCASS AND INTESTINAL ORGAN TRAITS IN JAPANESE QUAIL *(Coturnix coturnix japonica)*

Yusuf Konca, Selma Büyükkiliç Beyzi

University of Erciyes Faculty of Agriculture, Department of Animal Science, 38039 Kayseri-Turkey

ABSTRACT

This study was carried out to determine the effect of hempseed *(Cannabis sativa sp.)* on performance and carcass traits in Japanese quail (Coturnix coturnix japonica). A total of 192 quail 7-days of age were divided into four experimental groups with four replicates in randomized design. The treatments were; (1) Basal diet (control=C); (2) 5% hempseed in diet (H5); (3) 10% hempseed in diet (H10); and (4) 20% hempseed in diet was added (H20). The experimental diets were offered to respective quail for 5 weeks. The body weight and feed intake were determined at 7, 21, and 42 d. The carcass and internal organ traits were determined at end of the experiment in eight male and female quails for each group (in total 64 samples). In the C and H10 groups' body weight was higher than H20 at 42 d ($p<0.05$). The feed intake and feed conversion ratio were not influenced by the treatments. The carcass, liver, gizzard, intestine and heart weight and percentage to carcass were influenced by the treatments ($p<0.05$). The heart and gizzard weight and percentage were not affected by the treatments. However, carcass yield of hempseed included groups were higher than C groups ($p<0.05$). According to this result, up to 10 % hempseed may use in quail diet without negative effect on performance traits and hempseed inclusion may increase carcass yield.
***Keywords**: quail, hempseed, performance, carcass, internal organs*

INTRODUCTION

In Turkey, hempseeds have been used and are imported as bird feed. The cultivation of hemp has been banned in Turkey due to psychoactive drug. Also, many countries have been prohibited its cultivation but its usage free for nutrition and industry [2]. However, in 2003 industrial hemp with low levels (<2 g/kg) of the narcotic substance was once again permitted for cultivation in all EU countries [4]. Hempseed contains about 30% oil and 25% protein, 34% carbohydrates, dietary fibre, vitamins and minerals [1, 6]. Hempseed oil is over 80% in polyunsaturated fatty acids (PUFAs), and is an exceptionally rich source of the two essential fatty acids (EFAs) linoleic acid (18:2 *omega*-6) and *alpha*-linolenic acid (18:3 *omega*-3). Yalcin et al. [11] reported that hempseed inclusion to quail diets 20% may increase egg yolk and meat omega-3 fatty acid content.

Although it is valuable contents for thousands of years in old cultures have not been studied extensively for their nutritional potential in recent years, nor has hempseed been utilized to any great extent by the industrial processes [1].
It is reported that hempseed contains cannabidiols which has antibacterial, anti-inflammatory effects [10]. Khan et al. [5] reported that 20 % hempseed in broiler rations increased body weight gain (BWG) and decreased feed consumption (FC) and better feed conversion ratio (FCR) but did not positively affect on dressing percentage at 42 d.
The aim of this study was to evaluate the effects of hempseed in quail diets on performance and carcass and internal organ traits.

MATERIAL AND METHODS

Animal and diets

A total of 192 unsexed quail chicks with 7-day-old were individually weighed, wing banded and distributed into 4 treatment groups with 4 replicates and 12 chicks per cage. Each cage was furnished with a heater, two waterier and feeder. The rearing cage dimensions were 50x90x20 cm. The replicates were designated as the experimental units, and randomized with respect to the dietary treatments. The treatments were; (1) Basal diet (control=C); (2) 5% hempseed *(Cannabis sativa sp.)* in diet (H5); (3) 10% hempseed in diet (H10); and (4) 20% hempseed in diet was added (H20). The experimental diets were offered to respective quail for 5 weeks. Maize-soybean based diets were utilized and all formulated on similar level of nutrient composition. The hempseed has bought from a commercial shop. All diets compositions were prepared according to NRC [7] recommendations. The nutrient compositions of experimental diets used in this study were given in Table 1.

Measurements

The individual body weights (BW) of birds and FC for each subgroup were measured at 7, 21 and 42 days of age. Feed conversion ratio (FCR) was calculated for 7 to 21, 21 to 42, and 7 to 42 days. Feed and water were offered *ad libitum*. A 24-h light cycle was throughout the experiment. Mortality was recorded daily and considered to calculate FC and FCR.
For carcass evaluation 16 birds (8 male and 8 female) in each group were randomly selected at 42 d of age and slaughtered. Their feathers were plucked, and the carcasses were eviscerated by hand. The small intestine, large intestine, and gizzard were removed, the contents were expelled. The carcass, liver, heart, proventriculus, gizzard, and empty intestine (duodenum+ileum+ jejunum+cecum+colon) were recorded individually and part yields were obtained as part weight: carcass weight X 100. Cold carcass weight was recorded after the carcasses had been stored at +4°C for 18 h.

Statistical analysis

The data were subjected to one-way Anova using General Linear Models SPSS computer program [9]. The model included hempseed level of diets. The means

were separated using Duncan's multiple range tests. The results of statistical analysis were shown as mean values and standard error of means (SEM) in the Tables. Statistical significance was considered at P<0.05.

Table 1. Diets feedstuff and nutrient composition

Feedstuffs	Hempseed level, %			
	0 (C)	5 (H5)	10 (H10)	20 (H20)
Corn	391.40	400	400	385.11
Wheat	100.00	55.60	22.58	0.00
Soybean meal	353.94	345.41	337.33	339.20
Sunflower meal	100.00	93.82	84.84	27.23
Vegetable oil	25.76	26.00	26.00	18.37
Sodium chloride	3.46	3.53	3.59	3.64
Di-calcium phosphate	6.94	7.42	7.90	9.03
Limestone	13.48	13.37	13.26	13.20
Vitamin-mineral premix[1]	2.50	2.50	2.50	2.50
DL-Methionine	1.20	1.09	0.80	0.83
L-Lysine	1.32	1.26	1.20	0.89
Hempseed	0.00	50.00	100.00	200.00
The calculated values				
Dry matter, %	88.29	88.74	88.11	88.69
Crude protein, %	24.39	24.48	24.52	24.60
Calcium, %	0.80	0.80	0.80	0.80
Available phosphorus, %	0.30	0.30	0.30	0.30
Lysine, %	1.30	1.30	1.30	1.30
Methionine, %	0.50	0.50	0.50	0.50
Metabolizable energy, kcal/kg	2860.3	2865.1	2889.0	2899.6

[1]Vitamin-mineral premix per kilogram of the diet, Vitamin A, 15,000 IU; Vitamin D3, 2000 IU; Vitamin E, 40.0 mg; Vitamin K, 5.0 mg; Vitamin B1 (thiamine), 3.0 mg; Vitamin B2 (riboflavin), 6.0 mg; Vitamin B6, 5.0 mg; Vitamin B12, 0.03 mg; Niacin, 30.0 mg; Biotin, 0.1 mg; Calcium D-pantothenate, 12 mg; Folic acid, 1.0 mg, Choline chloride, 400 mg, Manganese, 80.0 mg; Iron, 35.0 mg; Zinc, 50.0 mg; Copper, 5.0 mg; Iodine, 2.0 mg; Cobalt, 0.4 mg; Selenium, 0.15 mg assures.

RESULTS AND DISCUSSION

The body weight, BWG, FC and FCR values were given in Table 2. There were no statistically differences among the treatment groups at 7 and 21 days of age. However, inclusion of 20 % hempseed decreased BW of quail at 42 days of age ($p< 0.05$). Similar trend was observed in the BWG values of groups. Feed intake and FCR were not influenced by the hempseed inclusion. Eriksson and Wall [3] reported that hempseed cake inclusion did not affect production performance of broilers. Also Silversides and Lefrancois [8] showed that including up to 20 % hempseed meal in diets for laying hens did not affect BW, FC and FCR.

However, Khan et al., [5] found that 20 % hempseed inclusion to diets increased BW and decreased FC of broilers. When hempseed ratio was increased BW of quail was decreased. Hempseed contained at high percentage of crude ash (8.8%, not shown in tables) and cellulose. This ratio is so high compared to some traditional feedstuffs such as corn, wheat, soybean meal. Therefore, high crude ash and cellulose levels may cause low BW. On the other hand, there is no enough study in poultry to compare to these results.

Table 2. Effects of treatments on the body weight (BW), weight gain (BWG), feed consumption (FC) and feed conversion ratio (FCR) in quail

	Treatments					
Day	C	H5	H10	H20	SEM	p
BW						
7	21.26	22.34	21.75	22.58	0.51	NS
21	110.9	114.6	114.2	115.2	2.39	NS
42	183.6[a]	175.3[ab]	183.4[a]	168.5[b]	3.94	*
BWG						
7 to 21	89.64	92.26	92.45	92.62	0.115	NS
21 to 42	72.7	60.7	69.2	53.3	0.161	NS
7 to 42	162.34[a]	152.96[ab]	161.65[a]	145.92[b]	0.180	*
FC						
7 to 21	8.24	7.92	8.84	7.41	0.670	NS
21 to 42	25.58	23.77	27.33	22.82	1.51	NS
7 to 42	18.64	17.43	19.93	16.63	1.26	NS
FCR, g feed/g BWG						
7 to 21	1.19	1.33	1.34	1.26	0.567	NS
21 to 42	5.80	8.19	6.10	9.83	0.948	NS
7 to 42	2.88	3.53	2.98	3.81	0.082	NS

[a,b,c]: Values with different superscript in a row differ significantly *:p<0.05. C: control, H5, 10, 20: hempseed ratio in diet as 5, 10, 20 %), SEM: standard error of means. NS: non significant.

Effect of hempseed inclusion on carcass and intestinal organ traits and their relative incidence were given in Table 3. In the H10 group's carcass and heart weights were higher than other treatment groups (p<0.05). The liver and intestine weight in the C group was higher than others (p<0.01) but carcass yield was lower than all hempseed groups (p<0.05). The gizzard weight and gizzard and heart relative incidence were not influenced by the treatments. Intestine weight and relative incidence of H20 groups was lower than other groups (p<0.01). The heart weight and relative incidence were not influenced by sex. The carcass, liver, gizzard, intestine weights and liver, gizzard and intestine relative incidence were lower in male quail (p<0.05 and p<0.01). Hempseed level and sex interactions were not influenced by the treatments. In general, 20 % hempseed inclusion decreased the carcass and internal organ weight and relative incidence. Khan et al. [5] reported that 20 % hempseed in broiler rations did not affect positively dressing percentage at 42 d. Callaway [1] reported that hempseed has some beneficial effect on health in human and animals. However, in this study superior results were not observed in performance traits but increased carcass percentage.

CONCLUSIONS

In conclusion, according to this result, up to 10 % hempseed may use in quail diet without negative effect on performance traits and hempseed inclusion may increase carcass yield. Further experiments should need to be conducted to determine the effect of hempseed at poultry.

Table 3. Effect of hempseed in quail diet on carcass and intestinal organ traits

	Weight, g					Relative incidence, %				
	Carcass	Liver	Gizzard	Intestine	Heart	Carcass	Liver	Gizzard	Intestine	Heart
Treatment										
C	125.6^{b}	4.19^{a}	3.97	9.16^{a}	1.54^{b}	67.08^{b}	3.33^{a}	3.15	7.27^{a}	1.22
H5	124.9^{b}	3.55^{b}	3.74	7 .40^{b}	1.46^{b}	70.85^{a}	2.84^{b}	3.00	5.91^{b}	1.17
H10	133.9 ^{a}	3.86^{ab}	3.87	7.68^{b}	1.74^{a}	70.60^{a}	2.89^{b}	2.89	5.72^{bc}	1.29
H20	122.5^{b}	3.16^{b}	3.71	6.14^{c}	1.51^{b}	72.00^{a}	2.59^{b}	3.03	5.05^{c}	1.23
Sex										
Male	124.0^{b}	2.86^{b}	3.41^{b}	6.28^{b}	1.55	72.91 ^{a}	2.31^{b}	2.75^{b}	5.07^{b}	1.25
Female	129.5^{a}	4.52 ^{a}	4.23 ^{a}	8.91 ^{a}	1.57	67.36^{b}	3.51^{a}	3.27^{a}	6.90^{a}	1.21
Interaction										
C*M	124.9	3.12	3.62	7.24	1.50	71.19	2.51	2.91	5.82	1.20
C*F	126.4	5.26	4.31	11.07	1.58	62.97	4.15	3.38	8.72	1.24
H5*M	118.7	3.00	3.24	6.23	1.43	72.68	2.52	2.74	5.23	1.20
H5*F	131.0	4.10	4.23	8.56	1.50	69.01	3.15	3.25	6.60	1.14
H10*M	129.5	2.84	3.56	6.38	1.74	74.07	2.21	2.76	4.96	1.34
H10*F	138.3	4.88	4.17	8.98	1.74	67.13	3.58	3.02	6.47	1.24
H20*M	122.9	2.48	3.20	5.25	1.53	73.69	2.02	2.60	4.29	1.25
H20*F	122.2	3.85	4.23	7.03	1.48	70.32	3.16	3.45	5.82	1.22
SEM										
Hempseed	2.62	0.18	0.16	0.40	0.06	1.08	0.14	0.11	0.29	0.04
Sex	1.86	0.13	0.11	0.29	0.05	0.76	0.10	0.08	0.21	0.03
Hempseed*sex	3.71	0.25	0.22	0.57	0.09	1.53	0.20	0.15	0.42	0.05
P										
Hempseed	*	**	NS	**	*	*	**	NS	**	NS
Sex	*	**	**	**	NS	**	**	**	**	NS
Hempseed*sex	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

$^{a.b.c:}$ Values with different superscript in a column differ significantly *:p<0.05, **: p<0.01. C: control, H5, 10, 20: hempseed ratio in diet as 5, 10, 20 %), SEM: standard error of means. NS: non significant.

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DETERMINATION OF THE EFFECTS AND TYPE OF LIQUID ADDITIVES ON MIXTURE HOMOGENEITY IN VARIOUS MIXERS

Olivera Đuragić, Radmilo Čolović, Bojana Kokić, Jovanka Lević, Đuro Vukmirović, Slavica Sredanović, Dušica Ivanov

Institute of Food Technology, University of Novi Sad, Bul, cara Lazara 1, 21000 Novi Sad, Serbia

ABSTRACT

This paper presents results for mixture homogeneity and optimal mixing times obtained by method "Microtracer" in three different type of mixers as well as determining of mixture homogeneity after adding different type and quantity of liquid components. Homogeneity was tested with addition of 1% and 3% of soybean oil and molasses. Addition of 1% and 3% of soybean oil did not influence homogeneity of mashes in twin shaft mixers and generally had a positive effect on stability of mixture. In the vertical mixer, homogeneity was not achieved after 3% of oil addition. Addition of molasses significantly influenced homogeneity of mashes in all cases by increasing of coefficient of variation (CV) under level of 10%, except in small twin shaft mixer on the level of 1% of added molasses were CV was below 10% (6.3%).
Keywords: *mixers, mixing time, homogeneity, soybean oil, molasses*

INTRODUCTION

Complete feed mashes can be characterized as dispersible multi-component mixtures wherein particles differ in their size, density and shape and tend to segregate [5]. To avoid segregation, uniformity of particle size and particle density are mostly required which is hardly achievable in the practice. The size uniformity of the various ingredients that comprise the finished feed can directly impact final ingredient dispersion [1]. If all the physical properties are relatively the same, then mixing becomes fairly simple. As the physical characteristics of ingredients begin to vary widely, blending and segregation problems are compounded. Another factor that influences uniformity of dry mash is mixing time which should be determined depending on type of mixer used in production. The mixer plays a vital role in the feed production process and efficient mixing is the key for good feed production. If feed is not mixed properly, ingredients and nutrients will not be properly distributed and it means that the feed will not have same nutritional benefit according requirements for the animals that are feeding. Quality of mixture depends on mixer properties and mixing condition [6]. There are a number of different type of mixers used in the feed industry differing depending on construction, position of working part as well as purpose, efficiency and time to achievement of homogeneity [1].

Use of liquids such as oil, molasses, enzymes, aminoacids, etc. in animal nutrition is constantly rising.The main objectives are to improve nutritional quality of feed, to improve mash characteristics such as flow properties and reduce of dustiness. Liquid distribution among primary particles is considered as a key manufacturing parameter, since it gives rise to particle interactions through liquid bridges and can reduce mixing uniformity [7, 8]. Most of them can be aded into a main mixer but with limitation regards to the amount and sensitivity to other processes in production line. But liquid components in the main mixer can cause insufficient homogeneity by formation of agglomerates and deposits on the mixing tools and walls [2, 3]. So, maximal quantity of liquids that can be added into main mixer without decereasing of homogeneity should be determined for each mixer separately.
In this paper are shown results for mixture homogeneity and optimal mixing times obtained by method "Microtracer" in three different types of mixers as well as determining of mixture homogeneity after adding different type and quantity of liquid components.

MATERIALS AND METHODS

All tests were done using corn meal obtained by hammer mill with sieve opening of 3mm. Granulometric analysis were determined by method of Test sieving [4]
Three types of mixers were used in experiment: vertical mixer (50 kg charge), small scale twin shaft mixer (3 kg charge capacity) and large scale twin shaft mixer (100 kg charge).
Two types of liquids were added into mixers: soybean oil and molasses in the concentration of 1% and 3%.
In vertical mixer liquids were added manually, whereas in twin shaft mixers liquid addition was executed by nozzles installed in the mixers. Soybean oil was added under room temperature and molasses was tempered to 27° C.
Optimal mixing times before and after liquid addition were determined by method of Microtracer, using Microtracer RF blue, with spectrophotometric analysis of color after sample extraction [9].
At the end of the mixing process, 10 single samples of about 50 g sample size were taken from the mixer. The indicator (iron particles colored by food dye) was extracted by Rotary detector (magnetic device), dissolved by 7% Na_2CO_3. The concentration was photometric determined and statistically evaluated by the coefficient of variation (CV) which is s expressed in %. The criteria for mixture homogeneity (homogeneous random mixture) for complete mashes were CV ≤ 10 %.

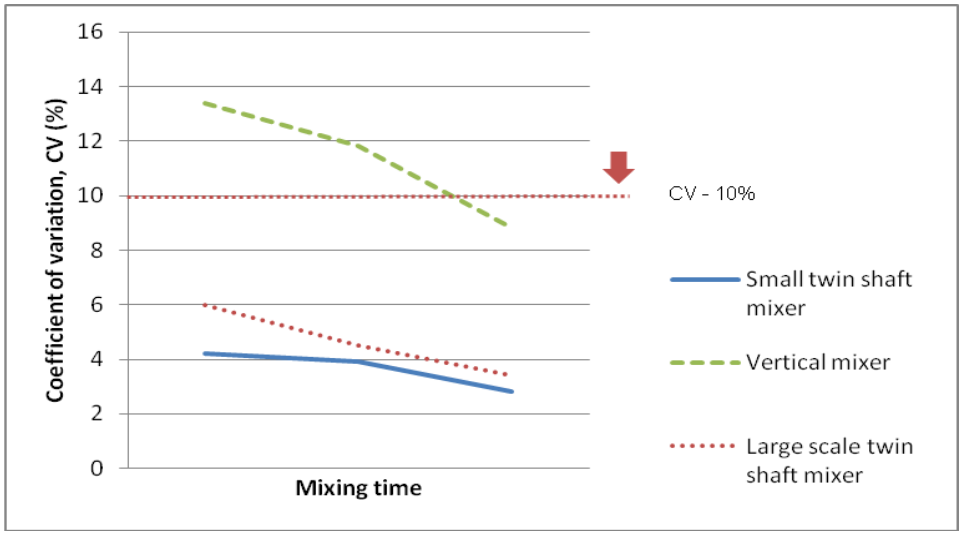
RESULTS AND DISCUSSION

Results of granulometric analyse performed by sieve test and are shown in the table 1.

Table 1. Granulometric profile of corn meal (hammer mill sieve opening 3 mm)

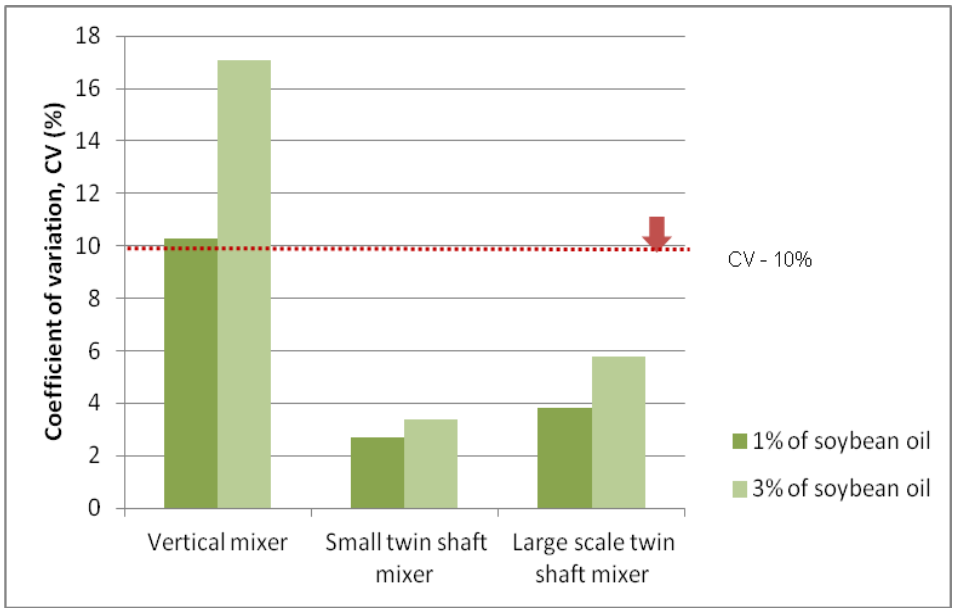
Sieve opening (µm)	Sieve remains (gr)	Sieve remains (%)	log(d)	m*log(d)
bottom	0.00	0.00		
63	0.40	0.40	1.80	0.72
125	7.02	7.01	2.10	14.72
250	26.50	26.47	2.40	63.55
630	10.00	9.99	2.80	27.99
800	6.00	5.99	2.90	17.42
1000	14.68	14.67	3.00	44.04
1250	30.12	30.09	3.10	93.28
2000	5.00	5.00	3.30	16.51
2500	0.38	0.38	3.40	1.29
	100.10	100.00		279.51
Geometrical diameter (µm)				619.92

Mean geometrical diameter of corn meal is about 600 microns and this value is considered as acceptable size for obtaining good mixture. Results of homogeneity test and determination of optimal mixing time has shown on the graph 1.



Graph 1. Mixing time of different types of mixers

Optimal mixing time for vertical mixer was achieved after 12 minutes, comparing with other two types of mixers (small and large scale) where homogenous mixture has accomplished after 90 sec and increased with prolongation of mixing time. After addition of 1% soybean oil, CV (coefficient of variation) was decreased in twin shaft mixers (2.7% in small mixer and 3.8% in large scale mixer comparing to 4.2% and 6% without oil respectively. In the vertical mixer, homogeneity was slightly disturbed (10.1% comparing to 8.8%). After adding of 3% of soybean oil homogeneity in vertical mixer was unacceptable (17.1%) and in both twin shaft mixers was better than the mixture without oil addition.(graph 2). It can be noticed that the achievable mixture homogeneity was improved in comparison with the dry mixture when liquids were added.



Graph 2. Addition of different level of soybean oil in three type of mixers

Graph 3. Addition of different level of molasses in three type of mixers

Comparing to results of soybean oil addition, addition of molasses caused unacceptable homogeneity of mashes. Only in the case of 1% of molasses in the small twin shaft mixer homogeneity was good (CV = 6.3%), other results were under the level of good homogeneity (CV>10%).

CONCLUSION

It can be concluded that type of liquids, concentration as well as mixing time depending on mixer type, influenced homogeneity of mashes. Addition of 1% and 3% of soybean oil did not influence homogeneity of mashes in twin shaft mixers, but on the contrary had a positive effect on stability of mixture. In the vertical mixer, homogeneity was not achieved after 3% of oil addition.
Addition of molasses remarcably influenced homogeneity of mashes in all cases (increase of CV above level of 10%) except in small twin shaft mixer on the level of 1% of added molasses where homogeneity was not significantly influenced.

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QUALITY MANAGEMENT

Ružica Milovanović

Faculty of economics, University of Kragujevac, Đure Pucara 3, 34000 Kragujevac, Serbia

ABSTRACT

Strategic goal of every company is to dominate in the market. Within the framework of such efforts, there are processes of achieving excellence or unique competitiveness, which helps to emphasize its advantages.
In selection between different opportunities, enterprises choose business excellence in which the key role has quality of products and services. That is why the introduction of the system of management quality and usage of adequate tools and procedures within it, as well as respect of adopted regulations for the control are essential.
So, the concept of quality management is becoming a part of management in running businesses, defining market position of enterprise.
Also, one of the possible ways for achieving ascendant place in the market is new products ideas development. Responsibility for this activity lies in the part of strategic planning of marketing activities.
When the organization perform structural division of market and select target consumers, identify their needs and define strategy for positioning in the market, it is better equipped for new products development.
Reasons for these procedures are increase of sales and profits, which is the core of competitiveness.
Keywords: *quality, system management, tools, procedures, environmental norms, marketing*

INTRODUCTION

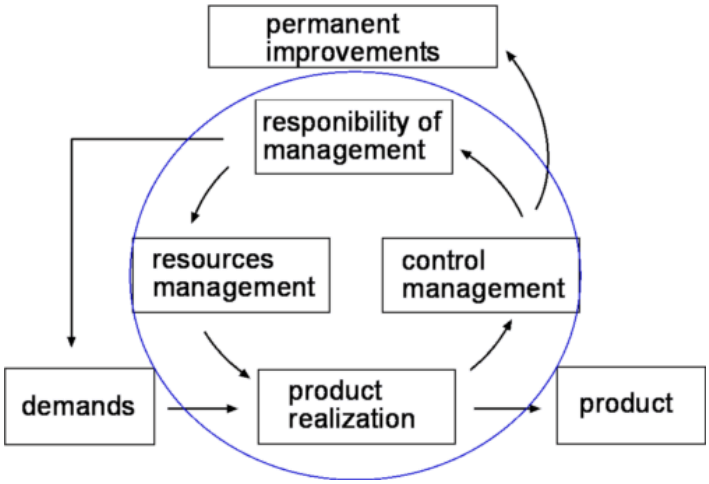
In modern business conditions, quality management and standardization are necessity [4]. Organization with improving quality of their products and services, realize competitive advantage on the basis of better market position, lower costs and greater profitability. The concept of quality management is becoming an important process in conducting the manufacturing system [3]. Quality Management System encourages organizations to analyze user requirements and define processes that contribute in achieving acceptable products. In this way confidence is gained, that it is possible to deliver products which is constantly meeting set requirements [6].
Satisfied users with greater buying, influence the increase in market participation of organizations, allowing growth of income. Increase of market share in the combination with the quality of products contributes to costs reduction of the new products introducing [2].

Marketing experts have the primary role in the process of developing new products, because they are identifying and assessing ideas regarding the new product [1].
Product life cycle concept is an extremely important tool in running marketing policy. Having knowledge about the concept core, and with help of proper computer software support, the organization is able to timely respond to all the changes [2]. New products are important because they can to encourage different advantages, lead toward the technological development and create big profit.
That is why organizations have to continually develop new products, introduce innovations and investigate needs of customers in order to meet users requirements.

STARTEGY OF MARKETING

Achieving business excellence and the new products creation are the basic prerequisites for survival and development of companies. It is the reason for increasing number of companies that apply Total Quality Management (TQM). Management system means actively determining the needs and demands of the customers, incorporating quality in the working processes, education of employees and continuous improvement of business.
System is a process of management, who is facing toward maximum quality. It is based on engagement of all members and is aimed to long-term success, by satisfying customers.
Quality is a function of user`s level satisfaction and the price of products. The highest value is an optimal combination of quality and price. Such a criterion, often called an engineer's criterion, is a tendency of production without mistakes. It is base for the idea of building image and brand recognition of particular products and companies.
Modern concept of the system management model is based on process's approach, which ensures maximum quality.
Application of such a system, above all, should be a strategic decision of an organization.
Also, system needs to demonstrate and ensure readiness and stability of organizations to continuously meet needs of the customers.
Sense of process's approach is based on the fact that process creates the result that should be operated with.
Such an approach allows organization to be observed as a system of interrelated wholes, where, first, processes must be identified and defined, as well as mutual links.
Successful management of processes means full control, which rely on measurement, analysis, and improvement of key elements.
In view of the requirements for improving the system management quality, process approach is based on basic factors:

- defining responsibility of management,
- resource management,
- new products realization and
- permanent improvement process (picture 1) .



Picture 1. Structure of the process's approach

Application of the process's approach provides:

- systematic definition process,
- establishing responsibility,
- permanent control and
- orientation on resources.

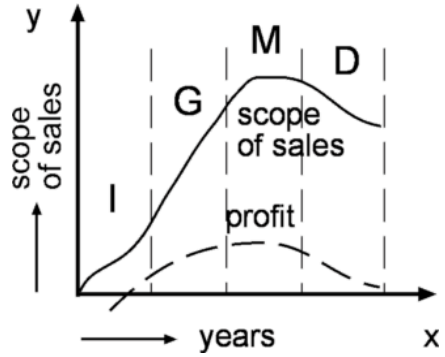
Also, significant advantages are achieving:

- reduction of total costs,
- rational use of resources,
- evident results and
- permanent improvements.

PRODUCT LIFE CYCLE CONCEPT

Research shows that for the company is primarily to appoint and to develop practice of management of quality.
Defined procedures are activities which lead, directly or indirectly, toward quality improvement and creation of competitive advantage. Also, measuring tools should define and incorporate them into the quality management system and establish mutual links. Process's marketing provides clear labeling of critical

activities and repetition of existing set of routines. Company gains possibility for gradually increasing of efficiency in product development cycle and response to consumers needs more quickly.
Quality Management encourages business results through increase in the operational capacity within companies.
Reduce costs, and on the other side is growing sales and market participation. Also, reduces the amount of waste products in production, and encourages efficiency through profit magnification, which increases and profitability.
Lower prices can increase market participation and the overall sale. The appearance that the company sells quality products and services may affect the demand, so that companies can increase their prices, and increased profits. Improvements in the quality encourage satisfaction of buyers and their loyalty, the total sale as well as competitive position.
Organizational potency for the management of processes is very important. Also, control of measured results and alignment of disagreements in the critical procedures is necessary for leading and creating radical innovations.
Statistical methods in combination with the introduction of modern information technology are necessary resource that enables the implementation of system for quality control, and which leads to better positions companies on the market.
Statistical methods in combination with the introduction of modern information technology, is a necessary resource that enables the implementation system for quality control, and which leads to better positions companies on the market. System for quality control, more and more is the goal toward better business.
So that activities in the operations, marketing and engineering functions, presented with living cycle of products, can be of assistance in the process of planning, monitoring and analysis of applied improvements.
Each individual product is going through its life cycle which can have a direct impact on organizational organization of company or on its survival. Life cycle of products is based on the overall demand for products that belong to the same company, for a longer period of time (picture 2).



Picture 2. Life cycle of a product

Clearly four-stage life cycle of product is revealed.

1. Introduction - moment of products introducing in commercial market,
2. Growth - phase in which product increase its presence in the market,
3. Maturity - phase when leverage up product reaches its maximum
4. Drop - moment when comes to decrease in interest of consumer for product.

Full line is the total volume of selling certain product categories, while dashed line presents actually profits.
Maturity is a phase in which product reaches its maximum representation in the market and it is a signal to company that innovations are necessary, with redesigning or with full innovation.
Research studies show that there is a trend toward shortening life cycle of products, which is the result of:

- increased spending on the development of new products and
- operational strategy with using computer process,

enable companies to quickly respond for purpose of redesigning products.
Given the short life of products, it is necessary to continuously introduce innovations. New products encourage different advantages, lead the technological development, maintain growth of sales and create big profits.

MANAGEMENT OF NEW PRODUCTS

Development of new product is one of the greatest challenges in the area of strategic planning. Basis for success, according to the requirements of modern business, are innovations. Be innovative is one of the ways for answering everyday challenges of growth and development changes.
Product innovations are observing not only as a tool for successful business insurance and achieving competitive advantage, but as a tool for the extension of living products cycle. New solutions require significant financial resources primarily for research, development and winning market. However, this is a very small investment because of the results that can be achieved.
Regardless of the difficulties, uncertainty, and risks that may arise in the process of innovation, it should be persistent, because it is a condition for survival. With new product appearing on the market and proper customer's reaction, many dilemmas disappear and cease to exist.
Reaction from buyers greatly depends upon the market strategy which will be applied.
The nature of process of introducing new products on the market is different, if the products of daily use or home appliance good. For these reasons one must have in mind numerous factors that influence the success.

Strategy for development of new products requires increased co-operation among functions of marketing, production, and development. In the strategy are important several areas:

- development of manufacturing program,
- product development
- costs of quality.

Analysis of manufacturing program has goal to indicate advantages and disadvantage of production, which are not easily recognizable in individual review. The primary goal of product development, presents company's ability to produce product with acceptable costs. Development of new products is extremely complex. It is conditioned with uncertainty, how will market accept new idea. That is why is used elaborated methodological approach, which is based on an efficient organization of entire work.
Significant place in the process strategy belongs to the area that defines quality costs. They were generated in order to provide that product fulfill established standards of quality.
They are categorized in several group:

- costs of prevention - insurance from malfunction,
- control costs - quality planning, control, information systems,
- internal costs - expenses from new product before shipment control
- external costs - expenses from selling nonstandard products.

The essence of cost quality measurement is elimination of external costs, reduction in internal costs, as well as efficient investment in prevention.

CONCLUSION

High level of competition in the market imposes radical change of existing understanding of and relations toward quality. Modern concept presents maximum of planning and prevention. Implementation of quality system of management brings the number of positive effects. Standards give reference base which allows easier establishment of relations among producers, buyers, and logistics.
Standards allow expanding of production and creation of the additional value, which acts positively to trade balance and profitability.
Success of every organization depends and on their possibilities for adjustment to new market conditions and the ability for constant improvement.
One of more efficient form of management is process approach.
By optimization and management processes, emphasis is placed on elements that add value to the end user.
Greater satisfaction of users is achieving, as well as their loyalty and notable increase of competitiveness.

Organization should have in mind that standard products, no matter how successful they are, can not maintain a high level of sales and profits. Therefore, the product life cycle concept is used, which points out the moment of withdrawal of existing product and start of promotion of new one. Given the short life cycle of product, it is necessary to innovate, because only a new product can provide competitive advantage.
By knowing the essence of this concept, with available software application support, the organization is ready to timely respond to all the changes.

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QUALITY PARAMETERS OF MAIZE BIOMASS AS A FEED RAW MATERIAL

Dušanka Terzić, Milica Radosavljević, Marija Milašinović-Šeremešić, Goran Todorović, Zorica Pajić, Milomir Filipović

Maize Research Institute, Zemun Polje, Slobodana Bajića 1, 11185 Belgrade, Serbia

ABSTRACT

Maize is one of the most important crops, and as such, one of the most significant feed raw materials. As a high-yielding carbohydrate plant, maize is very competitive compared to other cereals in animal feeding. Furthermore, maize is the most important forage plant by the amount of yield, biomass quality, its suitability for silage, versatility of use for animal feed. In order to define more fully the quality parameters of maize biomass, as well as, its nutritive value, it is necessary to study the structure of cell walls of the whole plant. Analyses of the fibre or cell walls in forages is of major concern in ruminant nutrition because diets often contain large amounts of forage, and the fibre fraction affects both feed intake and animal performance.

The objective of this study was to observe quality parameters of biomass of eleven ZP maize silage hybrids of different genetic background and to determine the relationship of these parameters, as well as, their effects on the digestibility of maize biomass dry matter. The hybrids of the FAO maturity groups 200-800 (ZP 209, ZP 388, ZP 427, ZP 588, ZP 600, ZP 606, ZP 623, ZP 677, ZP 725, ZP 789 and ZP 873) were used in the study. The contents of the lignocelluloses fraction were determined by the modified Van Soest detergent method, while *in vitro* digestibility of the whole plant was established by the Aufréré method.

Obtained results showed that the NDF, ADF and ADL contents in the whole maize plant of the observed ZP maize hybrids varied from 42.6% to 52.7%, 19.3% to 26.6%, and 1.5% to 2.4%, respectively. The difference in the digestibility of the dry matter of the whole plant among hybrids amounted to 11.8%. Moreover, the differences in the contents of the lignocelluloses fraction affected the differences in dry matter digestibility.

***Keywords*:** *Maize hybrids, yield, lignocellulose fibres, digestibility*

INTRODUCTION

Maize is one the most important crops and one of the most significant feed raw materials. Due to high yields, quality of biomass, suitability for silage and diversified utilisation as feed, maize ranks first among forage plants. The yield of the total dry matter of silage maize ranges from 12 to 26 t ha^{-1} in dependence on genetic potential and agroecological conditions in the full waxy maturity stage for silage (dry matter content of the whole plant ranges from 35 to 42%) [1]. It has been considered that yielding maize hybrids good for the grain production were

also good for the silage production [2]. Later research within this field has indicated some changes [3,4,5].
It is proven that there are significant changes in the content of lignocellulose fibres in the whole maize hybrid plant, which considerably affect the digestibility of the dry and organic matter, and therefore on the yield of digestible dry matter [6,7,8]. All carbohydrates in plant nutrients are grouped into: I - structural carbohydrates (carbohydrates of cell walls), which include NDF (neutral detergent fibres – hemicelluloses + cellulose + lignin), ADF (acid detergent fibres – cellulose + lignin), ADL (lignin) and II - nonstructural carbohydrates - NFC (carbohydrates present in the plant cell content) that are made of starch, sugars and pectin [9].
In vitro digestibility of maize morphological fractions are particularly important because there are significant differences in digestibility since digestibility of cellulosic plant parts depends on the genetic background [1]. Considering all stated, it is necessary to study the content of lignocellulose fibres and the dry matter digestibility of the whole maize plant as a primary indicator of silage biomass quality.

MATERIAL AND METHODS

The hybrids of the FAO maturity groups 200-800 (ZP 209, ZP 388, ZP 427, ZP 588, ZP 600, ZP 606, ZP 677, ZP 725, ZP 789 and ZP 873) were used in this study. The two-replicate trail was set up according to the randomised complete-block design in the experimental field of the Maize Research Institute, Zemun Polje. The experimental plot size amounted to 21m^2, while sowing density was 60,000 plants ha^{-1}. Plants of each replicate were harvested at the full waxy maturity stage from the area of 7m^2 (two inner rows), and yields of fresh biomass of the whole plants, plants without ears and ears were estimated. Five average plants per replicate were selected for further tests. Samples of the whole plants were cut and dried at 60^oC for 48h. In order to determine the content of dry matter, the whole plant samples were ground in the 1-mm mesh mill. Then, the analysis of the absolute dry matter was done on the oven dry basis (105^oC for 12 h) in order to estimate the total dry matter. Moreover, the analysis of the content of forage fibres (NDF, ADF, ADL, cellulose, hemicellulose) was performed by the modified Van Soest detergent method [10]. The method was modified by Mertens [11]. *In vitro* digestibility of the whole maize plant was done by the Aufréré method [12]. This method is based on the hydrolysis of proteins of the whole plant in the pepsin acid solution (Merck 2000 FIP u/g Art 7190) at 40^oC for 24 h, and then on the hydrolysis of carbohydrates in the cellulase solution (cellulase Onozuka R10) in duration of 24 h.
Data reported for quality parameters of ZP maize hybrids biomass were assessed by the analysis of variance (ANOVA) and the LSD multiple test was used for any significant differences at the $P<0.05$ level between the means. All the analyses were conducted using statistical software package STATISTICA 8.1. (StatSoft Inc. USA).

RESULTS AND DISCUSSION

Table 1 shows the structure of the dry matter yield of investigated ZP maize hybrids. The dry matter yield of the whole maize plant for silage at physiological maturity stage was in the range from 16.7 to 25.2 t/ha while with dry matter content of the whole plant varied from 31.67 to 37.53% for tested ZP maize hybrids. The yield of digestible dry matter biomass for tested hybrids ranged from 11.0 to 15.4tha^{-1}. The hybrid that had the highest yield of digestible dry matter per hectare (ZP 388) did not achieve the highest yield of dry matter per hectare. The participation of the ear dry matter in the yield varied from 52.48 to 57.14%. For this tested parameter there are no significant differences among the hybrids ($p < 0.05$). Differences in dry matter yield of whole hybrid plants amounted to 8.5tha^{-1}, digestible dry matter to 4.4tha^{-1}, while the participation of ear in the dry matter yield of the whole plant amounted to 4.66%.

Table 1. Yield Structure of ZP Maize Hybrids

Hybrid	Dry matter content (%)	Dry matter yield (t ha^{-1})			Participation of the ear dry matter in the yield (%)	Yield of digestible dry matter of the whole plant (%)
		Whole plant	Whole plant without ear	Ear		
ZP 209	35.62	16.7^{e}	7.7^{e}	9.0^{f}	54.00	11.0^{c}
ZP 388	34.19	22.3^{cd}	10.6^{abc}	11.7^{bcd}	52.48	15.4^{a}
ZP 427	31.67	18.6^{e}	8.5d^{e}	10.1^{ef}	54.30	12.1^{c}
ZP 588	34.49	18.5^{e}	8.0^{e}	10.5^{de}	56.98	12.3^{c}
ZP 600	35.78	22.8^{bc}	9.8^{cd}	13.0^{ab}	57.14	14.4^{ab}
ZP 606	37.19	22.6^{cd}	10.4^{abc}	12.2^{bc}	53.62	15.3^{ab}
ZP 623	36.82	25.2^{a}	11.5^{ab}	13.7^{a}	54.48	14.4^{ab}
ZP 677	34.28	20.8^{d}	9.6c^{d}	11.2^{cde}	53.98	12.2^{c}
ZP 725	32.69	24.7^{ab}	11.7^{a}	13.0^{ab}	52.75	15.0^{ab}
ZP 789	36.02	22.1^{cd}	9.8^{bc}	12.3^{bc}	55.45	14.1^{b}
ZP 873	37.59	23.0^{bc}	10.9^{abc}	12.1^{bc}	52.91	14.1^{ab}
LSD $_{0.05}$		2.0	1.5	1.4	-	1.3

Means in the same column with different superscripts differ significantly ($p<0.05$)

In order to define more fully the quality parameters of maize biomass, as well as, its nutritive value, it is necessary to study the structure of cell walls of the whole plant. Data on the content of NDF, ADF, ADL, hemicelluloses, cellulose and digestibility of the whole maize plant are presented in Table 2. The results show that the NDF, ADF, ADL, hemicelluloses, cellulose contents and digestibility of dry matter in the whole maize plant of the observed different ZP maize hybrids varied from 42.57 (ZP 388) to 52.71% (ZP 873), 19.32 (ZP 388) to 26.60% (ZP 623), 1.54 (ZP 427) to 2.39% (ZP 725), 22.24 (ZP 606) to 26.23% (ZP873),

17.70 (ZP 388) to 24.27% (ZP 623) and 57.22 (ZP 623) to 69.04% (ZP 388), respectively. The differences in the contents of NDF, ADF, ADL, hemicelluloses, cellulose and digestibility among observed ZP hybrids were 8.42%, 7.28%, 0.85%, 3.94%, 6.57% and 11.82%, respectively.

Table 2. Whole Plant Lignocellulose Fibres Content and Digestibility of ZP Maize Hybrid

Hybrid	Content (%)					Dry matter digestibility (%)
	NDF	ADF	ADL	Hemicellulose	Cellulose	
ZP 209	44.03^{e}	20.60^{g}	2.01b^{cd}	23.43^{c}	18.59^{ef}	65.84^{abc}
ZP 388	42.57^{f}	19.32^{h}	1.63^{ef}	23.25^{c}	17.70^{f}	69.04^{a}
ZP 427	47.97^{cd}	23.08^{e}	1.54^{f}	24.89^{b}	21.54^{d}	64.91^{abcd}
ZP 588	44.71^{e}	21.94^{f}	1.64^{ef}	22.78^{cd}	20.30^{d}	66.34^{ab}
ZP 600	47.20^{d}	23.87^{d}	1.97^{cd}	23.33^{c}	21.90^{cd}	63.34^{bcd}
ZP 606	42.91^{f}	20.67^{g}	1.65^{ef}	22.24^{d}	19.02^{e}	67.54^{bcde}
ZP 623	52.71^{a}	26.60^{a}	2.33^{a}	26.11^{a}	24.27^{a}	57.22^{f}
ZP 677	50.83^{b}	25.74^{b}	2.12^{abc}	25.09^{b}	22.87^{ab}	58.68ef
ZP 725	48.39^{c}	25.19^{bc}	2.39^{a}	23.20^{cd}	22.80^{bc}	61.00^{def}
ZP 789	48.29^{c}	23.53^{de}	2.29^{ab}	24.76^{b}	21.24^{d}	63.71^{bcd}
ZP 873	50.99^{b}	24.76^{c}	1.84^{de}	26.23^{a}	22.92^{bc}	61.51^{cdef}
LSD $_{0.05}$	0.82	0.63	0.29	0.96	1.04	4.37

Means in the same column with different superscripts differ significantly ($p<0.05$)

According to results obtained by Deinum and Bakker [3] and Andrieu and Demarquilly [13], the difference in the digestibility of the whole maize plant varied from 2% to 3%, while Terzić [6], Terzić et al. [7] and Terzić et al. [8] established the highest difference among observed hybrids of 11.52% and 8.56%, 14.4%, respectively. The low digestibility may alter the assessment of silage maize hybrids if the dry matter yield is used as a criterion instead of the digestible dry matter yield. Among the tested hybrids, hybrid ZP 388 had the highest yield of digestible dry matter of the whole plant (15.4tha^{-1}), and the highest dry matter digestibility of the whole plant (69.04%) and the lowest NDF (42.57%), ADF (19.32%) and cellulose (17.70%). The highest contents of NDF, ADF and cellulose were determined in ZP 623, which was reflected in very low dry matter digestibility of the whole plant. Among all the tested hybrids ZP 623 had the lowest digestibility.

Considering the crucial effect of the lignocellulose fibres on the digestibility of the whole maize plant dry matter, the correlation dependence between the content of these components and the dry matter digestibility was observed (Table 3).

Table 3. Correlation Dependence between Whole Plant Digestibility and Lignocellulose Fibres of ZP Maize Hybrids

	NDF	ADF	ADL	Hemicellulose	Cellulose
Digestibility	-0.77**	-0.82**	-0.58**	-0.50*	-0.77**
NDF		0.95**	0.56**	0.86**	0.92**
ADF			0.64**	0.66**	0.97**
ADL				0.30	0.52*
Hemicellulose					0.64**

** and ** - significance at 0.05 and 0.01 probability levels, respectively.*

A very significant negative correlation was determined between the digestibility and NDF, ADF, ADL and the cellulose content (r=-0.77, r=-0.82, r=-0.58, r=-0.77), and a significant negative correlation between the hemicelluloses content and the dry matter digestibility (r=-0.50). A highly significant correlation between NDF and ADF, ADL, hemicelluloses and cellulose (r=0.95, r= 0.56, r=0.86, r=0.82) and between ADF and hemicelluloses and the cellulose content (r=0.64, r=0.66, r=0.97) of the whole maize hybrid plants and a significant correlation between the content of ADL and the cellulose content (r=0.52) were established. A very significant correlation was determined between the contents of hemicelluloses and cellulose (r=0.64). The results gained in this study are in agreement with ones previously published [6, 7, 8, 14, 15, 16].

CONCLUSION

Obtained results showed that the NDF, ADF, ADL, hemicellulose and cellulose contents in the whole maize plant of the observed ZP maize hybrids varied from 42.57 to 52.71%, 19.32 to 26.60%, 1.54 to 2.39%, 22.29 to 26.23% and 17.70 to 24.27%, respectively. The digestibility of the whole ZP maize hybrid plant dry matter ranged from 57.22% to 69.04%. The difference in the digestibility of the dry matter of the whole plant among hybrids amounted to 11.82%. A very significant negative correlation was determined between the digestibility and NDF, ADF, ADL and the cellulose content (r=-0.77, r=-0.82, r=-0.58, r=-0.77), and also a significant negative correlation was established between the hemicelluloses content and the dry matter digestibility (r=-0.50).
Among the tested maize hybrids, the hybrid ZP 388 had the highest yield of digestible dry matter of the whole plant, and the highest dry matter digestibility of the whole plant, while the hybrid ZP 623 had the lowest digestibility.

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CHANGES IN THE TECHNOLOGICAL QUALITY AND SAFETY OF WINTER WHEAT INFECTED WITH MYCOBIOTA

Marija Bodroža-Solarov[1], Jovana Brkljača[1], Jovana Vučković[1], Tea Brlek[1], Ferenc Balaž[2], Maja Manojlović[2]

[1]Institut of Food Technology, University of Novi Sad, Bul. cara Lazara 1, 21000 Novi Sad, Serbia
[2]Faculty of Agriculture, University of Novi Sad, D. Obradovića 8, 21000 Novi Sad, Serbia

ABSTRACT

Aim of this work was to assess the changes in technological quality and safety of flour obtained from wheat infected with mycobiota compared to flour obtained from wheat treated with fungicide. Significant improvement in the protein and starch part was detected in the flour obtained from wheat treated with fungicide. Mixolab® parameter of sample treated with fungicide showed less protein weakening (C2) which was 0.57 nm compared to sample infected with mycobiota in amount of 0.39 nm. Flour obtained from fungicide treated wheat showed significant higher starch retrogradation (C5) which was 2.45 nm during the cooling period in comparison to the wheat infected with mycobiota (1.95 nm) Fungicide treatment caused significant decrease in the content of mycotoxin DON which was less than 0.25 mg/kg, while in the sample infected with mycobiota it was much higher (1.3 mg/kg). Reduction in technological quality and mycotoxin occurrence in wheat flour caused by fungal infection indicate that application of fungicides in field production is recommended.
Keywords: *wheat, mycobiota, technological quality, DON*

INTRODUCTION

In climatic conditions of Serbia the main causal agents of wheat kernel damage include *Fusarium* as a major pathogen, and other moulds from genera *Alternaria, Aspergillus and Penicillium* [2]. Fungal contamination causes significant yield decrease, but the losses are even greater because of mycotoxins produced by these fungi [5]. In the work of Balaž et al. [3] high share of kernels infected by moulds caused a significant decrease in 1000 kernel weight and test weight of wheat sample. Fungal infection influences significant reduction of yield parameters, such as length and weight of spikes [11]. There are different reports on the correlation between the *Fusarium* infection grade and deoxynivalenol (DON) content. Some authors did not confirm positive correlation between the infection grade and DON content [4,7], while others found a high positive significant correlation [10]. Nevertheless, strong infections evoked by artificial inoculation suppose high DON content in the inoculated samples. Papoušková et al. reported the highest average content of DON in different grain samples with the highest intensity of infection [8].

Increasing intensity of Fusarium spp. contamination worsened rheological quality and hence took up a negative effect on protein and mainly on the starch part of Mixolab curves [8]. The consequences of field mould attack, especially by Fusarium spp. but also Alternaria spp. are yield losses and a significant decrease in technological wheat quality that can go so far as to render its useless for processing. By comparing the results of infected samples with those at the localities where there were no severe mould infections, a significant decrease in total technological wheat quality was proved, which caused also the impossibility of bread production out of such kinds of wheat [9].
Despite the relatively large number of studies on the impact of fungal infestation on parameters of yield and safety, information on the effect of mycobiota infection on technological parameters is rather scant.
Aim of this work was to assess the changes of technological quality of flour obtained from wheat infected with mycobiota compared to flour obtained from wheat treated with fungicide.

MATERIAL AND METHODS

The experiment was conducted in 2010 on the location of Bački Petrovac using wheat variety Renesansa. During vegetation period standard agro technical measures were applied. Chemical treatments were applied in flowering stage using TwenJet 11004 nozzle. In the research, the following fungicides were used: Prosaro 1 l/ha (tebuconazole+prothioconazole). Plots of each treated wheat variety were established randomly in four replications with untreated control. Each parcel was 5 m^2.
Mycotoxin DON was determined from both treated and untreated wheat cultivars by ELISA (enzyme-linked immunosorbent assay) method. Screening method for analysis was done using Neogen Veratox® testing kits with limits of detection of 0,25 mg/kg for DON (342 Veratox DON-a 5/5).
Rheological properties of wheat flour samples were analyzed in a Brabender farinograph according to method No. 54-21 (AACC, 2003) and Brabender extensograph according to method No. 54-10 (AACC, 2003). The wheat flour samples were analyzed for termomechanical behavior by Chopin Mixolab® (Tripette et Renaud, Paris, France) according to "Chopin +" procedure
Statistica 10.0 Software (Statsoft Inc., 2010,Tulsa, Oklahoma) was used for statistical data processing using one-way ANOVA. The comparison of mean values was performed by the LSD- test.

RESULTS AND DISCUSSION

Table 1 showed changes in the dough rheological properties obtained by farinograph and extensograph analysis. Flours from wheat treated with fungicide showed significant higher water absorption and extensograph resistance compared to the wheat infected with mycobiota These results are in accordance to the investigations of Šarić et al.1997 [9], where mould infection caused a considerable deterioration of protein quality

Table 1. The effect of fungicide treatment on farinograph and extensograph parameters

Treatment	Farinograph parameters			Extensograph parameters		
	Water abs. value (kg kg^{-1})	15-minute drop (BU)	Flour Quality Number	Exten. area (cm^2)	Extensibility (mm)	Resistance to extension (BU)
Mycobiota infestation	56.8^a	120^c	36,9^a	43^a	250^b	118^a
Fungicide treatment	59.2^b	30^a	74,3^c	84^c	290^c	147^b

Mean values in the same column followed by different letters of the same case are significantly different ($P < 0.01$).

Table 2. The effects of fungicide treatment on mixolab® parameters and content of DON

	Mixolab®parameters				DON
	C2 (nm)	C3 (nm)	C4 (nm)	C5 (nm)	(mg/kg)
Mycobiota infestation	0.39^a	1.91^a	1.69^b	1.95^a	1.30^b
Fungicide treatment	0.57^b	1.99^b	1.64^a	2.45^b	<0.25^a

Mean values in the same column followed by different letters of the same case are significantly different ($P < 0.01$).

The effect of fungicide treatment on mycotoxin content of wheat is reported in the work of Bagi et al. [1], which has been proven in our study. Results presented in Table 2 showed that fungicide treatment caused a significant decrease in the content of DON which was less than 0.25 mg/kg compared to the untreated sample infected with mycobiota (1.3 µg/kg)
The Mixolab® instrument is a useful tool to evaluate the quality of flour samples [6]. High sensitivity of Mixolab® system for monitoring the changes in rheological characteristics of winter wheat with different intensities of mycobiota contamination was confirmed in our study.

The obtained results showed that in the flour from wheat treated with fungicide protein weakening (C2) was 0.57 nm, while in the sample infected with mycobiota it amounted 0.39 nm. Significant changes in the starch part were detected in the flour obtained from fungicide treated wheat. Starch retrogradation during the cooling period (C5) was 2.45 nm in the flour obtained from wheat treated with fungicide which is significantly higher than that in the flour from wheat infected with mycobiota. These findings are in congruence with Papoušková et al. 2011 [9] showing that fungal contamination evidently worsened the rheological quality and had negative effects on the protein and mainly on the starch part of the grain.

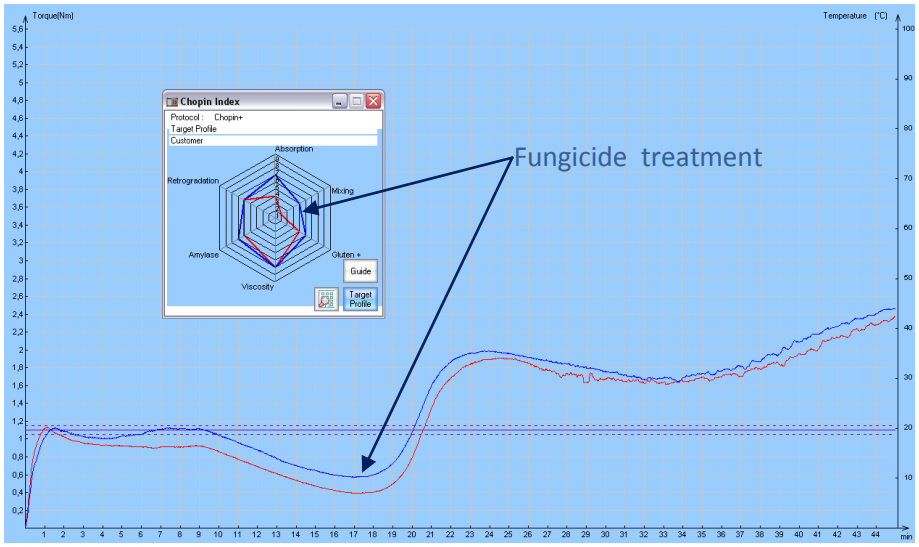

Figure 1. Mixolab® graphs of different flour obtained from wheat treated with fungicide and infected with mycobiota

CONCLUSIONS

Flour obtained from wheat treated with fungicide showed higher water absorption and stability during mixing and less protein weakening during heating in comparison to the flour from wheat infected with mycobiota. Fungicide treatment caused significant decrease in the content of DON. There is evidence that fungicide application is important measure in order to improve the technological quality of wheat.

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COWS AND WETHERS NITROGEN EXCRETION DEPEND ON NUTRITION

Mariana Petkova

Institute of Animal Science, Kostinbrod, Bulgaria

ABSTRACT

In ruminants nitrogen is absorbed from 2 major sites - the reticulum and the small intestine. Crude protein intake often exceeds the ruminant`s nutritional demand. Moreover, the quality of dietary protein and specific ration components such as fodder beet could affect the efficiency of nitrogen utilization. It can have an effect on the environment. Recently nutritionists have been facing a new challenge - to quantify the animals' response in terms of nutrition. This response is expressed as efficiency in the utilization of nitrogen, in other words a minimum loss of nitrogen to the body with the urine. This requires a good knowledge of the distribution of protein intake between the one which is output and the other which is lost with the products of excretion and is danger for the environment because of pollution. Our aim was to verify the hypothesis that nutrition influences rumen degradability of protein (RDP) and N excretion and there is a relationship between them in regard to environmental protection. A total of 22 physiological trials with two rations A and B with 4 cows with rumen fistulae and 4 wethers were carried out. Three nutritional factors were examined: diet protein levels (in % of DM) were 8, 10, 12 and 14 (Ration A) and 8, 12, 16 (Rations B); different types of protein (natural and non-protein sources, NPN); and influence of fodder beet presence in basal diet with NPN. Meadow hay and concentrate mixture (Ration A) and corn silage, alfalfa hay, wheat straw and concentrate mixture (Ration B) were used as daily ration components of. We estimated RDP for 24 h incubation and nitrogen excretion with urine. A standard statistical procedure was applied to the obtained results supplemented by correlation analysis. The results show that rumen microorganisms degrade approximately 66-88% of the dietary protein. The excreted urinary nitrogen, as percent of intake, is between 24.73 and 43.54%. In regard to environmental protection excreted urinary N is correlated to the ruminally degradable protein fed. We made the conclusion that the feeding of diets with higher protein levels, the presence of NPN and fodder beet increase both RDP and urine excretion of N into the environment when corn silage, alfalfa hay, straw, conventional compound feeds were used as components of the daily rations.
Keywords: *cows, wethers, nutrition, RDP, N excretion*

INTRODUCTION

Crude protein consumption often exceeds the ruminant`s nutritional demand. Moreover, the quality of dietary protein and specific ration components such as fodder beet, could affect the efficiency of nitrogen utilization. It can have an

effect on the environment. Recently nutritionists have been facing a new challenge - to quantify the animals' response in terms of nutrition. This response is expressed as efficiency in the utilization of nitrogen, in other words a minimum loss of nitrogen to the body with the urine. This requires a good knowledge of the distribution of protein intake between the one which is output and the other which is lost with the products of excretion and is danger for the environment because of pollution. When protein is overfed a significant amount is lost to the environment as fecal, urinary, and gaseous N because its efficiency of utilization for production sharply decreases [5, 8]. Dietary protein concentration is perhaps the most important on-farm variable that can be controlled relatively easily and practically, and it can have a significant and immediate, measurable effect on nitrogen excretion [6]. As the conversion of dietary N to urine N increased exponentially as N intake increased [2] and urinary N excretion is the main contributor to ammonia emission from livestock facilities than fecal N [7, 11, 13] reducing ration CP would effectively reduce volatile N losses with urine [3, 4, 10]. A similar effect would be expected from reducing dietary RDP concentration [12, 14]. Fodder beet is potentially the best buffer crop available, producing significant yields of highly digestible dry matter. Key fodder beet fact is that it cold increase efficiency of utilization of dietary nutrients.
The aim of our study was to verify the hypothesis that nutrition influences rumen degradability of protein (RDP) and urine N excretion and there is a relationship between them in regard to environmental protection.

MATERIAL AND METHODS

Experimental design

A total of 22 physiological trials in two main rations A and B were carried out. Three nutritional factors were examined: diet protein levels (in % of DM) were 8, 10, 12 and 14 (Rations A) and 8, 12, 16 (Rations B); different types of protein (natural and non-protein sources, NPN); and influence of fodder beet presence in basal diet with NPN. Meadow hay and concentrate mixture (Rations A) and corn silage, alfalfa hay, wheat straw and concentrate mixture (Rations B) were used as components of daily rations.

Chemical composition of the feeds

Dry matter (DM), crude protein (CP), crude fibre (CF), crude fat (CF) and crude ash (CA) were determined according to AOAC official methods [1].

In sacco determination of Rumen Degradability of Protein (RDP)

We estimated RDP after 24 h incubation by using three non-lactating and non-pregnant cows with rumen fistulae according to European standard procedure for artificial fiber bad estimates of protein degradability [9]. The samples for incubation were representative to the fed diets.

In vivo determination of nitrogen balance

A total of 22 trials on balance of nitrogen were carried out on four wethers. They received daily rations, identical in composition to those of the cows but reduced by 10%.

Statistical analysis

STATISTICA software version 9 (Statsoft, Tulsa, OK, USA) was used for performing one-way analysis of variance – ANOVA and Tukey HSD test for comparison of sample means, in order to analyze variations of the values obtained. Differences between the means with probability $P \leq 0.05$ were accepted as statistically significant differences. The level of confidence was set at 95%.

RESULTS AND DISCUSSION

Chemical composition of the feeds

The nutrient content of the main components of experimental daily rations is shown in Table 1. The results for the chemical composition of the main roughage feeds – alfalfa hay, meadow hay, straw, corn silage and fodder beet as well as components of concentrate mixtures - provide information on the quality of the feeds we work with. The composition of feedstuffs is similar to conventional, which is an indicator for good quality of the components of the experimental rations.

Table 1. Actual chemical composition of main components of experimental rations

Components	**DM, %**	**OM***	**CP***	**EE***	**CF***	**Ash***
Meadow hay	82.42	91.85	9.87	2.37	34.53	8.15
Corn silage	22.04	88.70	9.00	3.26	26.54	11.27
Alfalfa hay	80.29	91.88	16.17	2.64	28.61	8.12
Wheat straw	80.98	91.00	5.12	0.78	41.04	9.00
Barley Ration A	84.05	96.79	12.29	2.17	5.97	3.21
Barley Ration B	82.86	96.83	11.27	1.99	5.37	3.17
Corn	81.25	98.38	10.73	3.65	2.88	1.62
Wheat bran	81.93	94.32	17.89	4.69	9.49	5.68
Sunflower meal Ration A	85.54	92.24	42.28	3.52	17.61	7.76
Sunflower meal Ration B	84.22	93.16	38.92	2.41	20.19	6.84
Fodder beet - Ration A	11.94	90.90	11.07	0.75	9.92	19.10
Fodder beet - Ration B	1082	91.82	8.32	1.29	9.02	8.18

in % of DM

In sacco determination of Rumen Degradability of Protein (RDP)

The influence of the chosen ration factors on the process of rumen degradability is demonstrated by the obtained results for RDP (Table 2. These values ranged between 66.46 to 88.51 for Rations A and 80.37 to 87.52% for Rations B. In other words, Rations B cause higher degradation. Corn silage and fodder beets as components of rations cause high degradation. Increased consumption of protein also leads to increased degradation in both rations: from 73.46 to 76.28% (Rations A) and from 82.20 to 87.52% (Ration B). The rations with the addition of non-protein nitrogen in various forms and fodder beets have the highest values for degradability.

Table 2. Rumen Degradability of Protein (RDP_{24}), x±SE

Experiments	**Ration A**	**Ration B**
CP 8	73.46 0.5	82.20 0.7
Cp 10	73.8 0.3	-
CP 12	74.02 0.3	84.41 0.9
CP 14	76.28 0.4	-
CP 16	-	87.52 0.6
Control – without NPN	66.46 0.9	84.52 1.9
NPN1	67.34 1.1	85.47 1.78
NPN2	75.33 0.7	-
NPN3	76.98 0.6	82.69 1.6
Control without fodder beet (FB)	75.28 0.5	80.37 1.1
FB	88.51 0.4	87.52 0.6
FB+NPN1	87.35 0.4	85.56 0.7
FB+NPN2	86.16 0.8	-
FB+NPN3	79.00 1.2	-

In vivo determination of nitrogen balance

With respect to the efficiency of nitrogen utilization, both urinary and faeces N excreted is higher in trials with Ration B in general (Table 3). It is correlated to the higher RDP in the same variants. Analysis of experimental data from trials with wethers for quantification of the nitrogen excretion in urine and faeces showed that increased nitrogen consumption leads to increased excretion in the urine. This applies up to the CP 12. The following average levels of nitrogen excretion with urine were obtained: 24.73 to 32.00 (Rations A) and from 34.87 to 43.24% (Ration B). The amount of N excreted into the environment is also dependent on the presence and type of NPN.

Table 3. Nitrogen excretion with urine and faeces, % from intake, x±SE

Experiments	Ration A		Ration B	
	Urine	Faeces	Urine	Faeces
CP 8	24.73	62.23	34.87	56.76
Cp 10	31.25	46.72	-	
CP 12	32.00	38.09	41.65	40.27
CP 14	29.28	30.72	-	
CP 16	-		43.24	29.34
Control – without NPN	32.00	38.89	36.66	36.02
NPN1	34.85	41.43	41.65	40.27
NPN2	31.97	35.43	-	
NPN3	38.15	33.87	39.10	29.66
Control without fodder beet (FB)	32.00	38.87	41.65	40.27
FB	28.64	38.49	43.54	30.51
FB+NPN1	39.05	29.64	41.95	32.28
FB+NPN2	26.97	34.91	-	
FB+NPN3	29.30	34.68	-	

CONCLUSIONS

The feeding of diets with higher protein levels, the presence of NPN and fodder beet increase both rumen degradability of protein and urine excretion of N into the environment when diets with corn silage, alfalfa hay, straw, conventional compound feeds were used as components of daily rations.

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MINERAL ADSORBENTS AND ENZYMES IN POULTRY PRODUCTION

Vera Radović[1], Slavko Filipović[2], Šandor Kromanjoš[2]

[1]University of Kragujevac, Faculty of Agronomy, Cara Dušana 34, 32000 Čačak, Serbia
[2]University of Novi Sad, Institute of Food Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

ABSTRACT

Deterioration of foods for animals considers variation from the normal quality, considering the changes of organoleptic traits, nutritive values and for hygienic accuracy of food. The bad effects of adulterated food are shown in achieving worse production results in animals, incidence of metabolism disorder and illness, as well as losing animal life.
Besides the bad influence of adulterated food, on the less utilization of nutritions ingredients it has bad effects on the fattening as well, antinutritions ingredients: phytate, oxalate, some structure carbohydrates, pentosan, etc.
Considering all these facts, the aim of our examinations was that with addition of different additives into the diets for animals, we have influence on improving hygienic accuracy of food as well as improving utilization of feeding substance from food.
The examination were done during the experiments of feeding poultry: laying hens, turkeys, chickens. Mineral adsorbents (zeolites) and enzymes (phytasa) were used as additives.
The main results of examinations presents following: laying hens had better carryning capacity and better eggs quality, with addition of 0,3 or 0,5% zeolites into diets; turkeys with 0,2% zeolites gained better body weights and had better results in surviving; chickens also had better production performances with addition of 0,3% zeolites into the diets. Addition of 0,1% enzymes of phytase into the diets for fattening chickens had well influence to all observed traits.
We conclude, that addition of examined additives into the diets for animals is totally warrantable and the reason for that are the results of our examinations.
Keywords: *diet, additives, poultry, quality, production*

INTRODUCTION

Feeding of domestic animals has great influence to the production and quality of animals products. The most significant of them in human diets are: milk, meat and eggs, and according to that great attention is payed onto their quality.
Legal regulations of many countries all over the world, and more to begun, to express the request that all meutioned products do not contain residues of pesticides, suphonamides, antibiotics, mycotoxines or any other substances which could harm human health (Lions, 2000).

Safety of food, from the viewpoint of human and veterinary medicine, presents significant problem, so the great attention is payed onto diseases which are tightly connected to the various mycotoxicoses. The report of the world Helth organization (WHO, 1985) presents that the presence of mycotoxins, toxic metabolites of mildew metabolism in human diets is not decay. Considering that, increasing the number of diseases which are in integration with mycotoxins as etiological factors, the great efforts and their elimination to be done.

The examinations confirms that mycotoxins are the cause of: respiratory and nervous system disorders, cancer on different target organs (Davidoff, 1994). Also, Alchaimer disease, multiplysclerosis, arteriosclerosis are getting in connection with mycotoxin as etiological factors.

Considering all those facts, new approaches in diets of domestic animals are suggesting using of natural minerals, enzymes, probiotics etc, as additions to animal feed (Radović, 2006, 2012).

Among the additives are zeolites, which are used as addition to feed, but also as correctors of ambiental conditions. Zeolites are crystals, hydrated alumosilicates. They have volcanic origin. They have big power of chemioresorption, especially towards aflatoxins, and partly onto ochratoxin A and zearalenone.

Zeolites are because for theirs non-toxic traits classified as safe meaning GRAS (Generally Regarded As Safe) compound according to the conclusion EU 21 CFR PART 5822729.

It is totally non-resorptive, it does not leave residues in animal products.

Besides absorption of mycotoxins, zeolites absorb bacteriological toxins, undesirable decomposed diet products, bacterium and gas. Because of thise traits, zeolites had its effects on improvements of food utilization and increasing of production even in the the case when the food in not contaminated with mycotoxins (Radović, 1997).

Also, one of the risks with whom we are refered to considering feeding domestic animals is using the mineral source of phosphorus. They are determined as one of the greatest reason of pollution of life environment, and for the reason that animals through faeces pollute ground. Besides that, using of raw phosphates, whose regular escort is fluor, canses disease of fluoroses, at human and animals (Živković et al. 2001).

Contemporary approaches in feeding domestic animals suggest using of phytase enzyme, with increasing or totally expulsion of mineral source of phosphorus. Phosphorus is essential mineral element and without suitable ammounts of it production, surviving, reproduction is imposible. In grain feed, which is ment for poultry and pigs there is enough phosphorus, but it is connected into complex organic form-phytate. With addition of enzyme phytase, hydrolitical decomposition of phytate is done, so non-organic phosphorus could be adapted by the side of animals.

The aim of this work was to present results gained by application of zeolites and phytase enzyme, added as additives into feed for: laying hens, fattening turkeys and fattening chickens. Observed criterions were poultry production and quality of products.

MATERIAL AND METHODS

Influence of mineral adsorbent (zeolites), added into feed, was observed during feeding experiment while the poultry were fed:

- laying hens (Isabrown SSL) with 1000 laying hens, divided into 4 groups (control and 3 experimental; each group had 250 laying hens).experiment lasted for 21o days. Feeding was different only in level of added zeolites (K-0,0; O-I – 0,2; O-II – 0,3 and O-III 0,5%). For the experiment was used zeolites under the trade mark name „Minazel"
- fattening turkeys (hybrid Bigg 6), totally 100 turkey divided into 2 group (each group had 50 turkeys). Control group (K – 0,0 and O-I group 0,2% „Minazel Plus" added into feed. The feeding experiment lasted for 3 months). Minazel Plus was gained in cation exchange of superficial exchangable cation, mineral zeolites, with organic cation (exchange in superficial electricity and hydrophobic) and that has influence on efficiency of adsorption of organic molecules (Tomašević-Čanović et al. 2001).
- Fattening chickens (Cobb 500 strain), 400 chickens, divided into 4 groups, considering levels of added „Minazel" or „Minazel Plus" into feed: K – 0,0; 0-I – 0,5% „Minazel"; 0-II – 0,2% and 0-III – 0,3% „Minazel Plus". Feeding experiment lasted for 42 days.

Influence of added phytase, into diet for fattening chickens, at lower levels of mineral source of phosphorus (DKF and MKF) was examined during the experiment of feeding fattening chickens:

- Fattening chickens (Arbor Acres strain) totally 440 chickens, divided into 4 groups (each group had 110 chickens) considering the diet composition (K group – 2% DKF – 0,0% phytase; 0-I – 1,4% MKF – 0,0% phytase; O-II – 1% DKF + 0,1% phytase; O-III – 0,7% MKF +0,1% phytase). Feeding experiment lasted for 42 days.

During mentioned experiments, control measuring of head of animals were done (every 7th day chickens; every 30th day turkey). Food consumation was evidented, health condition was observed as well as mortality.
At laying hens eggs for analyzes were taken for samples, in determined period (5 periods). Chickens were takes for samples, after sacrificing, for the cause of determination of slaughtering traits and meat quality. In the experiment with phytases, blood analyzes of chicken as well as bones quality were done.
Chemical, mycrobiological and mycotoxical analyzes of food were done.
Statistical significance of difference was done with Microsoft Statistica ver.5.0 Stat.Soft.Inc.1995.

RESULTS AND DISCUSSION

The results of examinations done on the experiments of feeding poultry (laying hens, fattening turkeys and fattening chickens), which gained by diet different

levels of zeolites and fattening chickens which gained by diet enzyme phytase at lower levels of mineral sources of phosphorus are shown in following table.

Table 1. Feed conversion and production of egg weight per used diet unit for the whole feeding period

Traits	Groups of hens							
	K	index	O-I	index	O-II	index	O-III	index
Total utilization for whole feeding period (kg)	7279.66	100	7227.23	99.28	7180.36	99.63	7122.65	97.84
Total number of produced eggs	43826.0	100	43908.0	101.19	44299.0	101.08	45034.0	102.76
Average weight of examined eggs (g)	67.08	100	68.03	101.42	69.73	103.95	68.57	102.22
Total produced eggs weight (kg)	2940.02	100	2987.06	101.61	3088.97	105.07	3087.98	105.03
Feed conversion food egg weight kg/kg	2.476	100	2.419	97.70	2.324	93.86	2.306	93.13
Produced egg weight/feed utilization kg/kg	0.404	100	0.413	102.22	0.430	106.43	0.433	107.18

The best food utilization at laying hens, during whole examination period, was by O-III group with (0,5% added zeolites to the feed); feed conversion 2,306 and egg weight production 0,433kg/kg feed consumption. The worst feed utilization, was at K-group (0% zeolites in diet); feed conversion 2,476 and production 0,404kg of egg weight at 1kg of feed consumption. Our results are in accordance with the results of many authors Lon Wo (1993), Andronikasvili et al. (1994), Radović et al. (2006) and they emphasize decreasing of feed conversion, at chickens ad laying hens, into whose diet zeolites was added. Andronikasvilli (1994), summon that zeolites added into diets, keeps back breaking through of digest, through digestion tract for 2-2,5 hours and therefore impoves adsorption of nutritions ingredients.

Differences among groups had statistical significance (P<0,05) and statistical highly significance (P<0,01) on behalf of the groups which gained in diet zeolites.
Also, we perceive that the average weight of sampled eggs was bigger at O-II group (0,3% zeolites) 69,72g, and the least at K-group (0% zeolites) 67,09g larger than eggs of K-group. Velikanov et al. (1983), Radović et al. (2003), quate good effects of zeolites added into diets onto increasing of average egg weight.
The effects of zeolites application in feeding fattening turkeys (Table 2) show that O-I group of turkeys which gained by diet 0,2% „Minazel Plus" accomplished: better growth, better finishing body weight (18,36kg) for 0,670g per bird, and had les food conversion (3,16) in comparison to K-group (0% „Minazel Plus"), finishing body weight (17,69kg) conversion 3,44kg/kg.

Table 2. Production results for the whole examination period (3 months)

Item	Groups	
	Control group	O-I
Feed utilization, average (kg) (n=50)	1701	1684.3
Index (%)	100	99.02
Difference		-0.98
Body weight of the strart (kg)	7.805	7.695
Body weight of the end (kg)	17.693	18.36
Index (%)	100	103.79
Difference		+3.79
Conversion (kg/kg)	3.44	3.16
Index (%)	100	91.86
Difference		-8.14
P<0,05 (for the item BW)		
P>0,05 (for the item feed utilization)		
P<0,05 (for the item conversion)		

In literature we do not find enough data about application of mineral adsorbents into feeding of turkeys, but we can recall on the results of other authors who presents the facts about positive influence of zeolites into the feeding of laying hens Radović et al. (2011), chickens Karović (2009) and ducks Lon Wo et al. (1993) on the increasing of production, with addition of this additive in food.
The influence of addition of zeolites products (Minazel and Minazel Plus), for fattening chickens show, the following the most important results are the promoters of production index which is calculated in order to receive inspection in complete production. This criterion comprehend inside itself essential and the most important production results: body weight, feed conversion and mortality (table 3).

Table 3. Productional index

	Groups of chickens			
Traits	C	O-I	O-II	O-III
Number of chickens at the begin of experiment	100	100	100	100
Productional index	174.39	287.09	216.63	239.08

The meat quality of chickens, as well as slaughtering traits as well as chemical, was better with addition of Minazel. As the one of the presenters we quote increasing of proteins content in white meat (22,90%) (O-III group 0,3% Minazel) in comparison to K-group (22,27% with 0% Minazel) (Radović et al. 2011).
These results also confirm, already mentioned results from the literature.
* We remark that during analyzing feed for laying hens, was confirmed presence of aflatoxin G 3,19mg/kg, and aflatoxin A 0,24mg/kg. In diets for turkeys content of aflatoxin was 0,37mg/kg air dried substance of diet.
The results of examinations which refer to adding of phytase enzyme into diets for fattening chickens at lower levels of mineral sources of phosphorus (DKF MKF), show folloving; the chickens which gained by diet phytase, gained better meat quality. They also had bones of better quality, and that is also the most significant criterion considering measuring of provision food with phosphorus during the period of feeding chickens. In Table 4, productional results of chickens are shown.

Table 4. Feed utilization per chickens (g) and conversion of food during whole experimental period

Traits	Groups (n= 110)			
	C	0-I-	0-II-	0-III-
Feed utilization (g)	3545	3563	3573	3564
Index % difference	100.00	100.51 +0.51	100.79 +0.79	100.53 +0.53
Body weight (g)	1875.63	1730,65	2006.50	2035.21
Index % difference	100.00	92.27 -7.73	106.98 +6.98	108,51 +8.51
Conversion	1.89	2.06	1.78	1.75
Index % difference	100.00	108.99 +8.99	94.18 -5.82	92.59 -7.41
*	P < 0,05 (for the trait BW) P > 0,05 (for feed utilization) P > 0,05 (for the conversion)			

Shown results, which refer to consumation and utilization of food (conversion), are in comprehension with the results of many authors, who have confirmed that enzyme phytase, added into diet, increase adaption of phytinic phosphorus and other components of animal feed (Lević, 1998, Radović et al. 2006).

In Table 5 shown the results of ash content, Ca and P in chickens femur.

Table 5. Ash content Ca and P and relation Ca:P in femur of chickens

Parametar	Group	Source of P	$\bar{X}$	Sd	CV%
Ash % femur	K	DKF	54.62	0.94	1.72
	0-I-	MKF	54.90	1.01	1.84
	0-II-	DKF+phyt.	56.09	1.24	2.22
	0-III-	MKF+phyt.	56.57	1.14	2.01
Ca % femur	K	DKF	25.97	1.30	5.03
	0-I-	MKF	26.18	0.66	2.47
	0-II-	DKF+phyt.	26.18	1.68	6.43
	0-III-	MKF+phyt.	26.88	1.60	6.12
P % femur	K	DKF	9.11	0.64	7.02
	0-I-	MKF	9.09	0.52	5.78
	0-II-	DKF+phyt.	9.14	1.12	12.32
	0-III-	MKF+phyt.	9.18	0.45	5.24
Ca: P femur	K	DKF	2.85:1		
	0-I-	MKF	2.88:1		
	0-II-	DKF+phyt.	2.86:1		
	0-III-	MKF+phyt.	2.92:1		
	* ash * P < 0.05 **P < 0.01				

Considering all these results, we conclude that they are in comprehension with data of many authors who also gained greater ash bone content by application of phytase in feedings chickens at lower lever of non-organic P (Harter-Dennis 2000, Radović et al. 2005).
In our examination O-III and O-II group (lover lever of mineral sources of phosphorus with phytase addition) had statistically significant and highly bigger percentage od ash bone (P<0,05; P<0,01).

CONCLUSION

Considering the results of our examinations done with feeding experiments on diffferent species and poultry categories, which gained by diet various additives (mineral adsorbents – zeolites or enzymes – phytase) we can conclude:
Added additives shown positive influence to improvement of productional performances of animals also to the eggs quality, turkeys and chickens meat. They also improve hygienic accuracy of food, by elimination of mycotoxins and other harmful as well as antinutritive materies.
Mineral adsorbents contribute to the wellness of animals and human heath, from the danger that could mycotoxins cause.
Phytase enzyme, besides its positive effects to animal production, had also its influence to the protection of life environment, by decreasing of use mineral

sources of phosphorus. According to this, their application has its justification in ecological sense.

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PERFORMANCE CHARACTERISTICS OF WEANED RABBITS FED GRADED LEVELS OF DRY CASSAVA PEEL FORTIFIED WITH SOYBEAN RESIDUE BASAL DIET

Olayeni Victor Olutayo

University of Ibadan, Oyo State, Nigeria

ABSTRACT

The experiment was conducted to assess the performance of weaned rabbits fed cassava peel fortified with residue (CSR). The dry cassava peel plus dry soybean residue were mixed in ratio 3:1.Thirty-two cross breed of New Zealand and White California weaned rabbits were allotted to four dietary treatments (D1, D2, D3 and D4). Each treatment was replicated four times with two animals per replicate in a completely randomized design. Four diets were formulated in which maize fraction of the diet was replaced by CSR at 0, 50, 75 and 100%. Completely randomized design was used for the study. The parameters measured were feed intake and weight gain. Efficiency of feed utilization, cost per weight gain and dressing percentage were calculated. The trial lasted for twelve weeks. The mean weekly weight gain ranged between 78.18 and 78.47g and showed no significant (P>0.05) differences among the treatment means. The efficiency of feed utilization was relatively similar among the treatments. The lowest cost per weight gain (4.10/kg) was recorded in while the highest cost per weight gain (103.11/ kg) was recorded in the control diet (D1). No significant (P>0.05) differences were observed in the mean dressing percent, liver, heart and kidney weights. Zero mortality was recorded in all the treatments. It could be concluded that maize fraction of the diets of weaned rabbits could be replaced by CSR up to 100% without any deleterious effect.
***Keywords**: Weaned rabbit, cassava peel, soybean residue, performance, cost benefits*

INTRODUCTION

Rabbit production is a good source of animal protein. One of the major problems facing rabbit and livestock production in general is the availability of quality feed at affordable price [2].

Research has been conducted to replace the conventional feed of livestock like maize with cassava tuber, sweet potato, and biscuit waste and mango seed kernel as one of the source of energy in livestock diets [3,5,7]. Leaf meal and *Cajanus cajan* had been used to replace and groundnut cake in broiler's diet. Cassava peel is the left-over material after the pulp has been removed while soybean residue is the left over material when the milk has been removed manually from the soybean mash by sieving [4,6]. This study was designed to

assess the performance of weaned rabbit fed cassava peel fortified with soybean residue as replacement for maize.

MATERIALS AND METHODS

The experiment was carried out at the Rabbitry Unit, agricultural farm of University of Ibadan, Nigeria. Thirty-two cross breed of New Zealand and White California weaned rabbits were allotted to four dietary treatments (D1, D2, D3 and D4). Each treatment was replicated four times with two animals per replicate in a completely randomized design. Cassava peel and residues were collected from 'gari' processing and crop utilization units of University respectively. Soybean milk residue was obtained after soybean milk has been extracted from a blanched mixture of 5:1 ratio of blanched cotyledons and fresh green maize respectively [1]. The dry cassava peel and soybean residue (CSR) were mixed together in ratio 3:1 before incorporating with other feed ingredients. Four diets (D1, D2, D3 and D4) were formulated in which maize fraction of the diets was replaced at 0, 50, 75 and 100% respectively (Table 1). The diets were formulated to contain 15–16% crude protein and energy of 2500–2600 kcal ME/kg. The feed intake was taken on daily basis by subtracting the left over from the feed offered with the use of weighing balance. The weight gain was taken on a weekly basis. Feed conversion ratio and cost per weight gain were calculated. Cost differential was calculated by deducting cost/weight gain of the test diet from cost/weight gain of control diet while relative cost advantage was calculated by dividing cost differential by the cost/weight gain of the control diet in percentage. Four animals per treatment were selected and housed individually making one animal per replicate. The animals were given the same type of feed given during the feeding trial. Faecal samples were collected daily and oven dried at 100°C for 24 hours and stored inside refrigerator for further chemical analysis.

Four rabbits were randomly selected from each treatment. The rabbits were starved over night before slaughtering. The fur and viscera were removed. The dressing percent was calculated as the ratio of dressed weight to live weight. Organs like kidney, lung and heart were removed and weighed individually.

Table 1. Gross composition of the experimental diets

	PRICE #/KG	D1	D2	D3	D4
MAIZE	24	32	16	8	0
CSR	7	0	16	24	18
WHEAT OFFAL	12	25	22	20	32
PALM KERNEL CAKE	3	12.0	12.0	12.0	12.0
MAIZE BRAIN	16	14	17	19	21
GROUNDNUT CAKE	26	7	7	7	7
MEAL	34	3.55	3.55	3.55	3.55
FISH MEAL	120	0.5	0.5	0.5	0.5
OYSTER MEAL	6.0	4.2	4.2	4.2	4.2
BONE MEAL	26	1.25	1.25	1.25	1.25
PREMIX	300	0.25	0.25	0.25	0.25
SALT	20.0	0.25	0.25	0.25	0.25
TOTAL		100	100	100	100
COST/KG CALCULATED ANALYSIS		18.28	15.68	14.4	13.12
CRUDE PROTEIN		15.51	15.68	15.93	16.11
ENERGY KCAL ME/KG		2582.42	2562.1	2530.4	2510.2

Table 2. Chemical composition of cassava peel, residue meal (csr) and maize on dry matter basis

PARAMETER (%)	CASSSAVA PEEL	RESIDUE	CSR	MAIZE
DRY MATTER	92.56	90.38	89.78	90.18
CRUDE PROTEIN	3.34	18.95	10.39	9.72
CRUDE FIBRE	11.98	7.56	9.84	4.23
ASH	3.34	6.08	9.85	3.98
ETHER EXTRA	1.23	4.12	4.42	2.25
NITROGEN FREE EXTRA	80.11	63.29	70.5	79.82

Table 3. Chemical composition of the experimental diets

PARAMETER (%)	D1	D2	D3	D4
DRY MATTER	90.15	90.61	91.01	90.91
CRUDE PROTEIN	15.33	15.38	15.71	15.90
CRUDE FIBRE	9.89	10.12	10.18	10.21
ASH	7.14	7.26	7.30	7.33
ETHER EXTRACT	3.86	3.91	3.94	4.10
NITROGEN FIBRE EXTRACT	63.78	63.33	62.87	62.46

Table 4. Summary of performance characteristics of the weaned rabbits fed experimental diets

	D1	D2	D3	D4	± SEM
NO. OF ANIMALS USED FOR THE EXPERIMENT	8	8	8	8	_
DURATION OF THE EXPERIMENT	12	12	12	12	_
MEAN TOTAL FEED INTAKE(G)	5291.28[a]	5286.48[a]	5299.33[a]	5320.32[a]	30.8
MEAN WEEKLY FEED INTAKE(G)	440.94[a]	440.54[a]	441.61[a]	443.36[a]	14.71
MEAN INITIAL BODY WEIGHT (G)	551.0[a]	553.42[a]	552.1[a]	555.18[a]	11.84
MEAN FINAL WEIGTH (G)	1498.12[a]	1492.36[a]	1493.35[a]	1492.86[a]	23.5
MEAN WEEKLY WEIGTH GRAIN (G)	78.18[a]	78.25[a]	78.44[a]	78.47[a]	4.18
FEED CONVERSION RATIO	5.64[a]	5.63[a]	5.63[a]	5.65[a]	0.93
CRUDE PROTEIN DIGESTIBILITY (%)	74.56	74.57	74.61	74.82	4.18
CRUDE FIBRE DIGESTBILITY (%)	67.9	67.68	67.62	67.59	3.97
MORTALITY (%)	0	0	0	0	0

Table 5. Cost analysis of weaned rabbit fed experimental diets

PARAMETER	D1	D2	D3	D4	± SEM
COST/KG(#/KG)	18.28[a]	15.68[ab]	14.4[b]	13.12[c]	1.46
MEAN TOTAL FEED COST	96.72[a]	82.89[ab]	76.31[b]	0.942[a]	3.94
MEAN TOTAL WEIGHT GAIN (KG)	0.938[a]	0.939[a]	0.941[a]	0.942[a]	3.94
COST/WEIGTH GAIN (#/KG)	103.11[a]	88.28[bc]	81.097[c]	74.10[d]	4.28
COST DIFFERENTIAL	_	14.83[c]	22.02[b]	29.01[a]	1.38
RELATIVE COST ADVANTAGE (%)	_	15.81[c]	23.45[b]	30.80[a]	1.05

means with different superscript along the same row are significantly different ($p<0.05$)

Table 6. Carcass analysis of weaned rabbits fed experimental diets

Parameter	D1	D2	D3	D4	± SEM
Mean live weight (g)	1478.5[a]	1484.9[a]	14.81.08[a]	1479.9[a]	30.1
Fur weight (g)	101.2[a]	101.7[a]	100.3[a]	103.5[a]	10.13
Dressed weight (g)	1066.0[b]	1081.01[a]	1079.56[a]	1088.91[a]	22.15
Dressing percent (%)	72.1[a]	72.8[a]	72.89[a]	73.58[a]	5.28
Liver weight (g)	32.1[a]	32.3[a]	32.89[a]	32.99[a]	3.41
Heart weight (g)	5.51[a]	5.50[a]	5.54[a]	5.56[a]	0.5
Lung weight (g)	15.24[a]	15.87[a]	15.89[a]	15.91[a]	1.89
Kidney weight (g)	14.50[a]	14.61[a]	14.76[a]	14.79[a]	1.79

Means with different superscripts along the same row are significantly different ($P < 0.05$)

RESULTS AND DISCUSSION

Cassava peel has lower protein and higher fibre content than corn residue (Table 2). Mixture of the two test ingredients resulted in increase in protein and reduction in crude fibre. CSR had higher crude protein content than maize while the crude fibre content of CSR was higher than that of maize. The chemical composition of the experimental diets (Table 3) indicated the diets were balanced in nutrients i.e. protein, fibre, etc. as recommended for growing rabbit 4, [4].

There were no significant ($P>0.05$) differences in the mean weekly feed intake of weaned rabbit fed experimental diets (Table 4). The value ranged between 443.36 and 440.94 g which was relatively similar to what was reported by Awosanya and Akinyode. The mean weekly weight gain in all the treatments were relatively similar ($P>0.05$), an indication that maize can be substituted with CSR up to 100% without any adverse effect on body weight gain. The improvement in body weight gain recorded in D4 over the other treatments, though no significant differences were observed could be as a result of inclusion of CSR at highest level which is richer in protein than maize (Table 2). The efficiency of feed utilization in all the treatments were not significantly ($P>0.05$) affected by the dietary treatments, indicating that any of the formulated feed could be given to rabbit without affecting the conversion of feed to edible meat. The zero mortality recorded in all the treatments showed that CSR has no deleterious effect on performance characteristics of rabbit. Moreover, the relatively similar values recorded in weight gain, efficiency of feed utilization and zero mortality recorded in all the treatments also suggest that the antinutritional factors present in *(trypsin* inhibitor) and cassava *(hydrocyanide*) must have been minimal in the feed composition to the extent that the effects of these antinutritional factors could not be felt on performance characteristic of rabbit. The crude protein and fibre digestibility were not significantly affected by varying levels of CSR in the diets.

The results of cost analysis (Table 5) showed that cost per kg feed reduced from #18.28/kg in D1 to #13.12/kg in D4 ($P<0.05$). There were significant ($P<0.05$) differences in mean cost per weight gain. The lowest cost per weight gain (#74.10/kg) was recorded in D4 where maize fraction of the diet was replaced by 100% CSR. The highest cost per weight gain (#103.11/kg) was recorded in the control diet (D1). This is due to high cost of maize (#24/kg) at the time of this study compared with low cost (#7/kg) of CSR. As the level of CSR in the diet increased, the feed cost and cost per weight gain reduced as shown in Table 5. The mean cost differential and relative cost advantage were better as the level of CSR in the diets increased from D1 to D4. No significant ($P>0.05$) differences were observed in the mean dressing percent, liver, heart and kidney weights (Table 6) thus indicating that inclusion of CSR in the diets of rabbit did not have any adverse effect on the organs mentioned.

CONCLUSION

In conclusion, considering the weight gain, efficiency of feed utilization, cost per weight gain and dressing percentage which were relatively similar in all the treatments, maize fraction of the diets of weaned rabbits could be replaced by CSR up to 100%without any deleterious effect.

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INFLUENCE OF DIFFERENT PROTEIN SUPPLEMENTS ON SHEEP MILK QUANTITY AND QUALITY

Marin Yossifov

Institute of Animal Science, 2232, Kostinbrod, Bulgaria

ABSTRACT

Animal milk productivity depends mainly on the quantity and quality of feedstuffs. In our study was analyzed and discussed the influence of different protein supplements on quantity and quality of sheep milk at the pick of lactation (27 – 72 lactating day). Sixteen lactating dairy sheep of Synthetic Bulgarian Dairy Population (SBDP) were used in 60-d feeding trial (7-d preparatory + 45-d experimental period + 8-d closing periods). Animals were randomly divided into two diet treatments: 1./ control diet (CD) with roughage (75 %), corn (8 %), supplement (0.65 %), wheat (8.79 %) and sunflower meal (SFM) (8.26 %), and 2./ experimental diet (ED) with replacing part of the wheat and whole SFM with rapeseed meal (RSM) (10 %). Diets were iso-caloric and equal in protein truly digestible in small intestines (PDI), Ca, P and approximately equal in crude protein (CP). Analyzed 960 milk samples showed that in this segment of lactation period the high lactopoiesis is related to source of dietary protein. No significant differences were found in average daily milk yield (+ 0.73 %) and milk composition: solids non fat (SNF) + 0.82; fat content of milk + 2.30 and milk protein content – 0.53 % per sheep for ED, compared with CD. RSM- based diet significantly ($p<0.01$) increased 6.5 % fat corrected milk yields (+ 3.05 %) from one sheep per day.

***Keywords**: lactating dairy sheep, rapeseed meal (RSM), consumption, feed efficiency, dry matter intake (DMI), feed conversion, milk yield, composition*

INTRODUCTION

After Bulgarian admission into EU in 2007, sheep- breeding continue be largest branch of Bulgarian animal husbandry. Total count of sheep is 1.4 million [16]. With average daily yield 81.2 L per sheep [16], annual sheep milk production is 0.82 million tons [16]. Thus, sheep milk production is important raw material for Bulgarian traditional animal products, such as yoghurt, cheese and other dairy products. So, sheep milk is an important part of the economy in small and middle-sized farms and means of livehood for many families.

Papers about effects of sheep nutrition on milk quality are published by some authors [25, 18]. Sheep milk production depends on level of intake and quality of feed. At segment of lactation period with high lactopoiesis is very important source of dietary protein. Milk fat content depends on the indirect effect of dilution and supply of dietary fatty acids (FA) and rate of their rumen biohydrogenation (rumen protected/inert FA supplements). Milk protein content depends on dry matter intake (DMI) [5] and supply of dietary crude protein (CP)

[7] and its degradability (rumen degradable (RDP) and undegradable protein (RUP) ratio) [13].
The aim of this study was to estimate effect of RSM as a protein source in diets of SBDP sheep on some physicochemical characteristics and nutritive and technological qualities of raw sheep milk.

MATERIAL AND METHODS

Experimental animals
At the Experimental Base of Institute of Animal Science Kostinbrod, Bulgaria (BG) was conducted 60-d feeding trail (7-d preparatory + 45-d experimental period + 8-d closing periods). Sixteen lactating dairy sheep (n=8) of Synthetic Bulgarian Dairy Population (SBDP) in early lactation (27 – 72 lactating day) were randomly (by age, lactation, milk production, % milk fats, % milk protein) divided into two diet treatments: control (CD) and experimental diet (ED).

*Table 1. Experimental design**

Item	SFM- based diet	RSM- based diet
		Roughage:
Meadow hay	13.32	13.38
Corn silage	61.26	61.55
		Concentrate mixture:
Rapeseed meal	-	10.44
Sunflower meal	8.26	-
Wheat	8.79	6.69
Corn	7.72	7.76
		Supplement:
Limestone	0.20	0.11
$(NH_4)_2SO_4$	0.45	0.07

*as DM basis

Dietary Treatments
Experimental design was placed in table 1. Daily rations (as DM basis) were consisted of 75 % roughage (meadow hay + corn silage) and 25 % concentrate mixture and were formulated to meet and exceed all nutrient requirements of lactating dairy sheep [29]. CD' concentrate mixture was consisted of corn (7.72 %), supplement (0.65 %), wheat (8.79 %) and sunflower meal (SFM) (8.26 %). In ED was replaced part of the wheat and whole SFM with 10.44 % rapeseed meal (RSM). Diets were iso-caloric and equal in protein truly digestible in small intestines (PDI), Ca, P and approximately equal in crude protein (CP). The supplement provided Ca (limestone), ammonium sulfate and vitamin-mineral mix (per kg of diet: Mg - 60.0 mg, Fe - 1.3 mg, copper - 1.0 mg, I - 1.6 mg, Zn - 60.0 mg, Co - 1.0 mg, Vit. A – 5000 IU, Vit. D - 2000 IU, Vit. E -10.0 mg). The diets were fed twice daily – 7.00 AM and 6.00 PM throughout the experimental period.

Feed intake was adjusted daily. Animals were provided *ad libitum* access to fresh water and salt blocks.

Forage samples collection and Analyzes
Diet' ingredients were sampled in each 15-d period and composited for analysis. The residua were collected and weighed daily and analyzed also twice a month. Samples were analyzed for DM by drying in a forced-air oven at 65°C for 48 h and then 105°C. Dried feed samples were ground to pass through a 1-mm screen and analyzed for Crude Protein (CP) (Kjeldahl N x 6.25), Ether Extract (EE), Crude Fibers (CF), Ash, Calcium (Ca) and Phosphorus (P) [1].

Milk samples collection and Analyzes
Milk yield was controlled twice a day – individual per sheep, during the morning and evening milking. Milk samples were taken and analyzed weekly per sheep in accordance to the regulations for milk sampling (country AC method). Physicochemical characteristics of the raw milk samples were analyzed with apparatus EcoMilk (Milkana KAM 98-2A – Bultech Company). It was established milk composition: solids non fats (SNF), dry matter (DM), milk fats (MF) and milk protein (MP). To evaluate nutritive and technological values of raw sheep milk were established following ratios: MP/MF, MP/DM and MF/DM.

Statistical Analyses
Feed intake and dry matter intake (DMI) (average per sheep), average daily milk yield, milk/forage ratio (M:F), nutrient efficiency (kg/L milk), nutritive and technological milk ratios (MP/MF, MP/DM and MF/DM) and other parameters were analyzed using MS Office 2007 and Student t-test.

RESULTS AND DISCUSSION

Diet composition
Chemical composition of feedstuffs is presented in table 2. Tested protein source (RSM) was lower in protein content (- 6 %) than SFM. These data corresponded with other authors [11, 12, 21], but were lower than our previous analyzes [31] and those reported by [24, 32]. Lower values of CP were found by [2, 9, 33]. Content of fats (EE) was twice as much in RSM as SFM. This value corresponded with previous our studies [31]. Similar values were reported by [2, 33]. Contrary, significantly lower values were found by [24, 32]. Other authors [9, 11, 17] reported higher values. Percentage of fibers (CF) in RSM was half as mush as SFM. These values corresponded with these reported by [2], but were lower than those found by [9, 11, 21]. Our previous studies [31] found higher values. The chemical composition of compound feeds (table 3) was similar in DM (2.1 kg) and ensured iso- nitrogenous (in average 0.39 kg CP), iso- caloric (in average 0.1 kg EE), iso- fibrogenous (in average 0.6 kg CF) and equal in Ca (0.016 kg) and P (0.008 kg). Feeding values (table 3) also were similar in both diets according to Bulgarian Feed Evaluation System [28] about following parameters: Feed Units for Milk (FUM: 2.4 and 2.3); Protein truly Digestible in

small Intestines (PDI: 0.18 and 0.17 kg); Balance of Protein in Rumen (BPR: - 0.001 and + 0.001 kg) for CD and ED, respectively.

Table 2. Chemical composition of diets' feedstuffs[1] (as % of DM)

	MH	Corn silage	SFM	RSM	Wheat	Corn
Dry matter	80.68	39.58	84.62	85.19	86.88	86.29
Crude protein	6.63	6.86	36.55	34.42	11.70	9.28
Ether extract	1.65	2.66	1.37	2.76	2.35	3.42
Crude fiber	30.66	16.74	21.96	12.39	2.62	3.84
Ash	6.34	5.21	7.14	6.78	1.96	1.47
Ca	0.38	0.41	0.41	0.62	0.07	0.06
P	0.09	0.11	1.04	0.87	0.33	0.24

MH- Meadow hay, SFM - Sunflower meal, RSM- Rapeseed meal

Table 3. Chemical composition and feeding value of RSM and SFM-based diets

	SFM- based diet (CD)	**RSM- based diet (ED)**
		Chemical composition[1] (g):
Dry matter	2134.69	2119.50
Crude protein	391.77	385.00
Ether extract	91.35	95.99
Crude fiber	626.18	604.33
Ash	184.40	187.14
Ca, %	15.71	15.51
P, %	7.87	7.79
		Feeding value[2,3](g)
FUM[4]	2.37	2.28
PDI	179.37	168.39
BPR	- 2.19	+ 3.19
BPR/FUM	- 0.92	+ 1.40

PDI- Protein truly digestible in small intestines, BPR- Balance of protein in rumen, FUM- Feed units for milk

[1] As DM basis (except DM)

[2] Our own data (Yossifov et al., 2011)

[3] As fed basis

[4] According to Bulgarian feed evaluation system

Intake

Average daily intake (ADI) of forage and dry matter (DMI) and consumption of nutrient ingredients from total diets are presented in figure 1. Sheep fed ED consumed higher levels of total diet as fed basis (+ 0.5 %), DM from roughage (+ 1.3 %), DM from concentrate mixture (- 1.9 %) and DM from total diet (+ 0.1). Thus, results of some studies that RSM depressed feed consumption (e.g. DMI) weren't proved in this trail. It was established negligible difference about average daily intake (ADI) of CP (- 1.9 %), Ash (+ 3.4 %), Ca (- 2.0 %) and P (- 0.7 %) for ED compared with CD. Higher EE intake from ED (+ 50 %) equilibrated

lower consumption of fibers (- 4.3 %) and didn't increase intake of feed units for milk (- 3.1 %). Consumption of PDI was lower in ED (- 5.6 %) and balance of protein in rumen varied between CD (- 1.39) and ED (+ 3.49%).

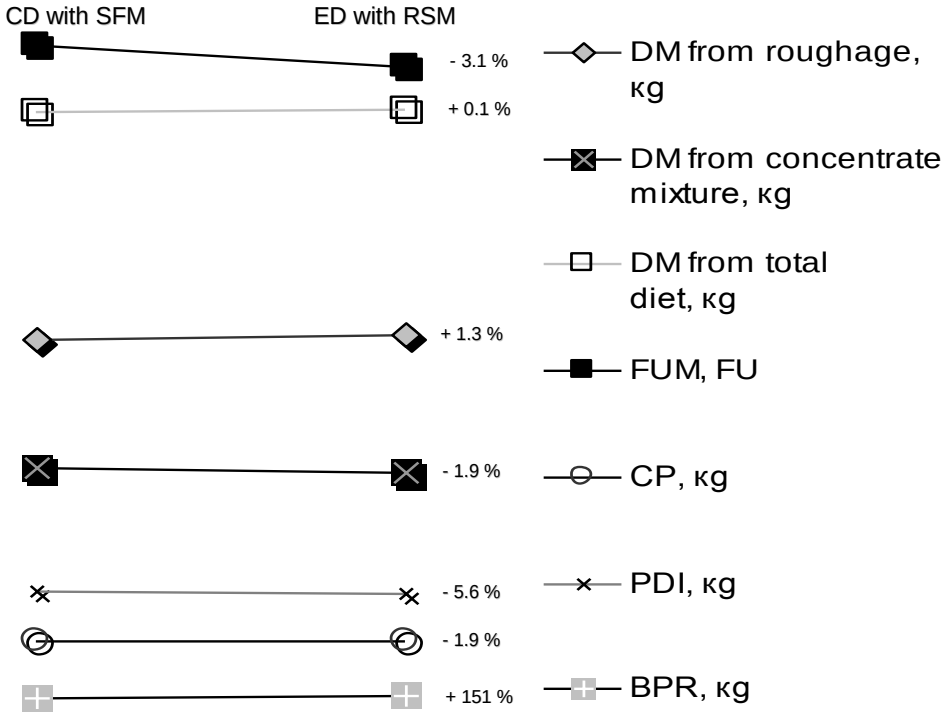

Figure 1. Average daily intake of forage, DM and nutrient ingredients

Animal performance

Performance of sheep was shown in table 4. The average daily milk yield for studied segment of lactation curve was 1.32 and 1.33 L for animals fed CD and ED, respectively. Differences between treatments weren't significant and statistically proved. When corrected milk to 6.5 % milk fat, differences between means for ED >> CD (+ 3.1 %) were proved ($p<0.01$).

Table 4. Yield, composition and properties of sheep milk

ITEMS \ GROUP		SFM- based diet	RSM- based diet
			Productivity:
Average daily milk yield, ml	Actual	1315.75 ± 196.90	1325.38 ± 204.21
	6.5 % fat corrected	1437.44 ± 215.11[c]	1481.29 ± 228.23[c]
			Physicochemical parameters:
Solids non fats		10.93 ± 0.32	11.02 ± 0.39
Dry matter		18.03 ± 0.93	18.29 ± 1.53
Protein		5.65 ± 0.279	5.68 ± 0.344
Fat		7.10 ± 0.801	7.26 ± 1.169
			Ratios:
Protein /Fat		0.80 ± 0.05	0.79 ± 0.07
Protein /Dry matter		0.314 ± 0.005	0.311 ± 0.009
Fat /Dry matter		0.393 ± 0.024	0.395 ± 0.028
[cc] p<0.01.			

Milk analyses

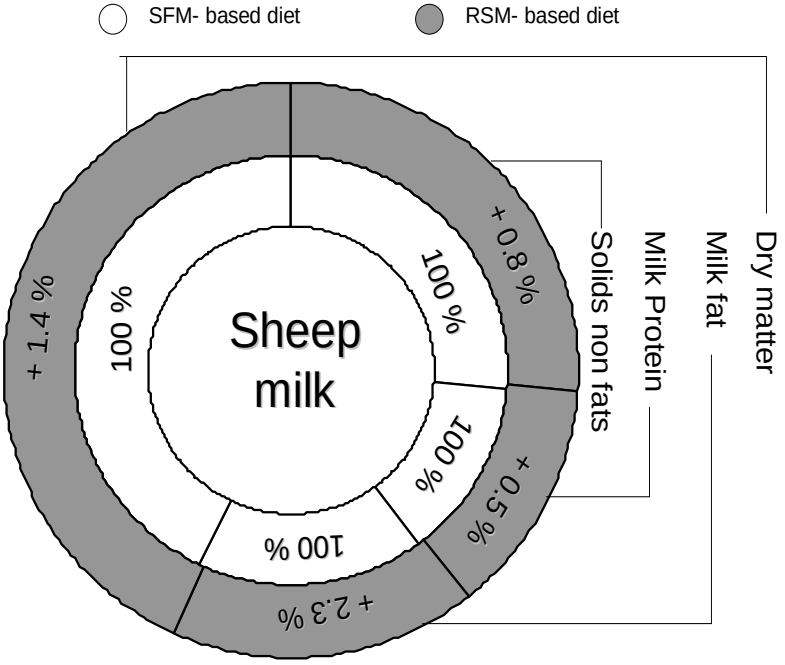

Figure 2. Physicochemical composition of sheep milk

In figure 2 is presented milk composition. Differences between controlled parameters weren't significant. Our data corresponded with results to other authors [4, 8] for milk content at dairy sheep and standards for SBDP [10, 23,

26]. To evaluate comprehensively tested raw milk analyses were carried out into two aspects: physicochemical analysis and nutritive and technological parameters. Percentage of SNF and DM (fig. 2) was in norms among the both groups (10.93 – 11.02 and 18.03 – 18.29 %) and differences were not significant (CD << ED). Similar values reported [3]. Lower % published [26], and higher - [8]. Content of milk fat (fig. 2) showed upward tendency – CD << ED (- 2 %), but results were in norms and corresponded with results found by [26]. Lower % found [8], and higher – [27]. Lactic proteins were actual the same among the groups (5.66 %) and exceeded values found by other authors [3, 8, 27]. To characterize nutritive and technological parameters of raw sheep milk were used following ratios (fig. 3): MP/MF, MP/DM and MF/DM. All values were in recommended standards (0.80, 0.31 and 0.39). MP/MF and MP/DM were lower in ED (- 1.2 and 0.8 %) and corresponded with values published by [26], but were lower than values found by [8, 27]. MF/DM ratio was higher in ED (+ 0.6 %). Higher values were found by [8, 27].

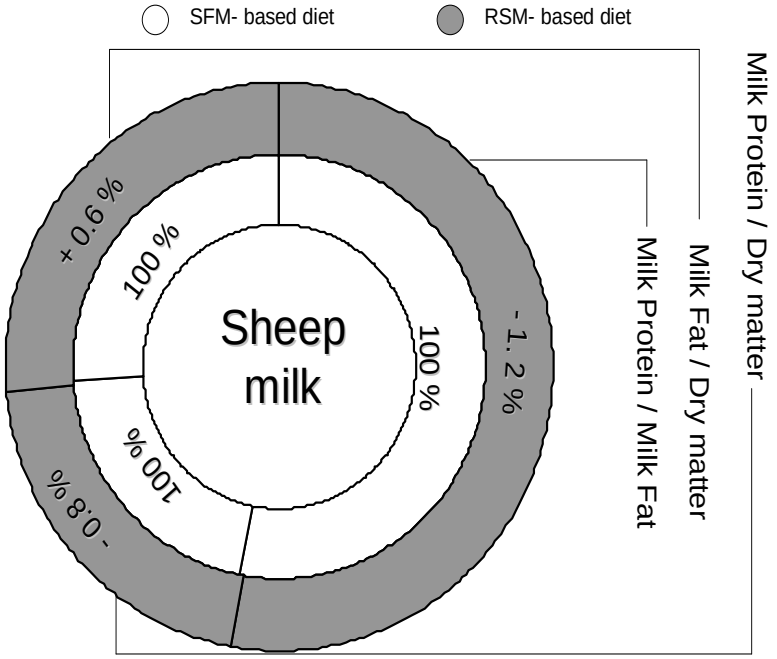

Figure 3. Nutritive and technological quality of sheep milk

Feed efficiency

To evaluate utilization of nutrient ingredients and their biotransformation into milk production was made fig. 4. The conversion of nutrient ingredients into 1 L milk was more effective from animals consumed RSM- based diet, compared with CD: Total diet (- 2.5 %), DM (- 2.9 %), FUM (- 6.3 %), PDI (- 8.4 %). Only consumption of CP was negligible higher (+ 0.3 %), but this may be as a result

of protein quality. The feed efficiency, presented as milk/feed (M/F) ratio, also was advantaged of RSM-based diet: + 8 % (fig. 4).

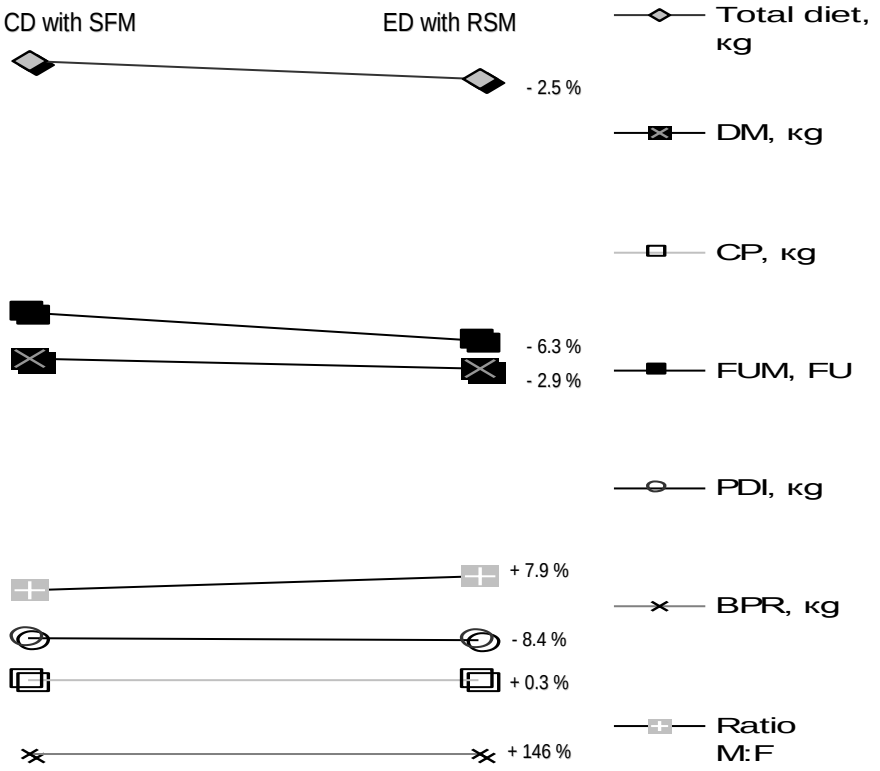

Figure 4. Feed conversion (g.L[1]milk) and feed efficiency (M/ F)

Nitrogen as a limiting factor in high productive dairy animals was used to established effect of dietary protein source (SFM vs. RSM) on milk production (fig. 5). Animals fed with CD consumed (253 g) higher % dietary CP (e.g. N), compared with ED (- 1.91 %). Percentage of N deposited in milk rised in order ED >> CD (+ 1.3 %). Thus, % of N utilization was higher in RSM- based diet (+ 3.23 %).

The results of this study confirmed positive effect of RSM as protein source in ruminant diets' – benefits proved in cow diets' [6, 14, 15, 19, 20] and slowly investigated in ewe diets' [30]. Established data about milk yield, milk composition, nutritive and technological parameters of raw milk, feed efficiency, N utilization, feed conversion and so on manifested future place of this vegetable protein source from biodiesel industry into practice.

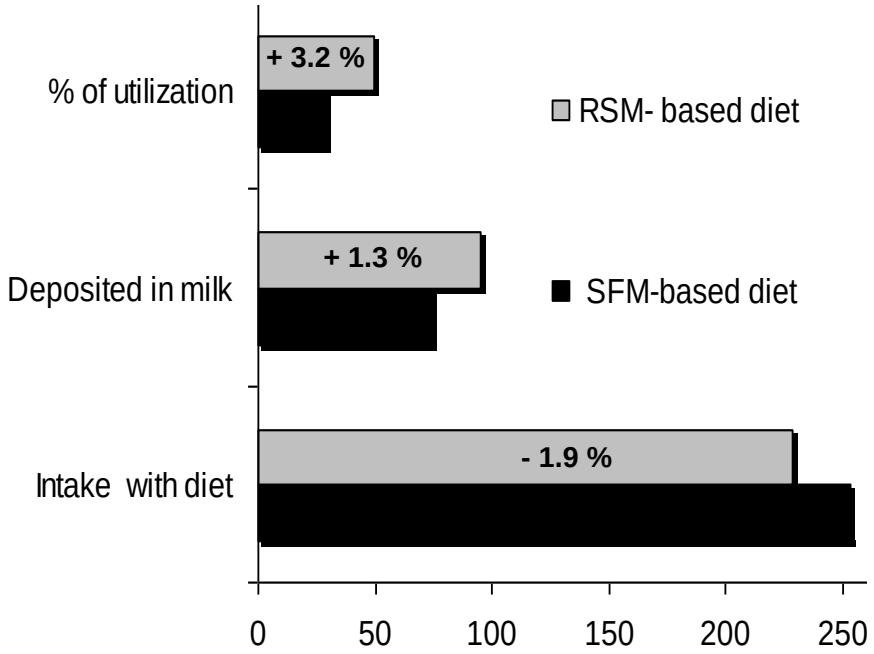

Figure 5. Percentage of Nitrogen utilization

CONCLUSION

- The data obtained on the chemical composition of RSM were as follows: DM – 85.19 %; CP – 344.20 g/kg DM; EE – 27.60 g/kg DM; CF –123.90 g/kg DM; Ash – 67.80 g/kg; Ca – 0.62 g/kg DM and P – 0.87 g/kg DM;
- Sheep fed ED consumed higher levels of total diet as fed basis (+ 0.5 %), DM from roughage (+ 1.3 %), DM from concentrate mixture (- 1.9 %) and DM from total diet (+ 0.1). Differences about average daily intake (ADI) of CP, Ash, Ca and P were negligible (- 1.9, + 3.4, - 2.0 and - 0.7 %) for ED compared with CD. Higher EE intake from ED (+ 50 %) compensated for the lower consumption of fibers (- 4.3 %) and didn't increase the intake of feed units for milk (- 3.1 %). Consumption of PDI was lower in ED (- 5.6 %) and balance of protein in rumen varied between CD (- 1.39) and ED (+ 3.49%);
- Average daily milk yield for the studied segment of lactation curve was higher in ED >> CD (+ 0.73 %). The differences between treatments were significant and statistically proved as 6.5 % fat-corrected milk – CD << ED ($p<0.01$);
- Differences between controlled physicochemical parameters in milk composition (solids non fats (SNF), dry matter (DM), milk fat (MF) and milk protein (MP)) and nutritive and technological parameters (MP/MF, MP/DM, MF/DM) were within the recommended range and were not affected by treatments;

- The conversion of nutrient ingredients into 1 L milk production was more effective from animals consuming RSM- based diet, relative to CD: Total diet (- 2.5 %), DM (- 2.9 %), FUM (- 6.3 %), PDI (- 8.4 %). Only consumption of CP was negligible higher (+ 0.3 %);
- The feed efficiency, presented as milk/feed (M/F) ratio was more effective at RSM- vs. SFM- based diet (+ 8 %).

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DISCONTINUITY IN GRAIN DRYER OPERATION AS A CAUSE OF HIGHER ENERGY CONSUMPTION

Mirko Babić, Ljiljana Babić, Ivan Pavkov, Milivoj Radojčin

University of Novi Sad, Faculty of Agriculture, Department of Agricultural Engineering, Trg Dositeja Obradovića 8, Novi Sad

ABSTRACT

Soybean and maize are usually dried after harvesting. Flow dryers with a continuous process are often used for grain drying in agricultural production. The best energy efficiency in drying by means of these devices is achieved when there is no change in process parameters, and when there are no interruptions in operating. However, under normal operating conditions, certain disruptions occur which lead to discontinuity in operation. The most common disorders are: abrupt changes in moisture content, changes in types of hybrids (or varieties), plant outages caused by different factors, and changes in drying regime. This paper analyses quantitative effects of these disorders on drying energy efficiency and shows an increase in fuel consumption required for drying (*DB*):

$$\Delta B = \Delta B_1 + \Delta B_2 + \Delta B_3 + \Delta B_4$$

where DB_1 is the increase in fuel consumption as a result of changes in grain moisture content, DB_2 is the increase in fuel consumption as a result of changes in types of hybrids, DB_3 is the increase in fuel consumption as a result of operating interruption, and DB_4 is the increase in fuel consumption as a result of the change in drying regime. Drying cost reduction, in the case of discontinuity, can be obtained on the basis of these results. Another result of this analysis is the proposal of a series of technological, organizational and technical activities in order to eliminate the discontinuities in drying as much as possible.

Keywords: *grain drying, drying parameters, continuity in dryer operation, maize, fuel consumption*

INTRODUCTION

The analysis of heat consumption reduction in grain drying processes is a permanent challenge for researches and grain dryer designers. Fuel consumption during grain drying poses an important economic issue in countries which are major crop producers. The development of novel methods and enhancement of existing technical systems constantly contribute to energy consumption reduction (Kudra, Mujimdar). However, scientists pay insufficient attention to organisational factors and their effects on heat consumption during drying. Based on several years of experience in grain drying in domestic environment, it can be concluded that specific heat consumption is higher under normal operating conditions than in stationary operating regime. This is a consequence of the disruption of operation during a grain drying season.

Seasonal indicators of fuel consumption show that the mean specific fuel consumption during the whole season of corn grain drying is higher than the specific fuel consumption during stationary operating regime (Ivanišević, Tepić). The mean specific fuel consumption under normal operating conditions can be up to 30% higher than the specific fuel consumption during stationary operation. This is a large amount of fuel, and its studying and reduction pose a challenge for researchers. Recent personal researches have confirmed the differences in fuel consumption due to disruptions in the stationariness of dryer operation (Babić, 1988).

MATERIALS AND METHODS

The scientific methods of analysis and synthesis were applied. Potential practical cases of disruptions in operating stationariness of a flow grain dryer were analysed. The effects of certain disruptions were analysed as well. The following factors were selected as important indicators of the deviation from stationariness:

1. Discontinuous changes in grain moisture content;
2. Changes in types of hybrids and/or varieties of grain material at the entrance to a dryer;
3. Operating disruptions in drying facilities due to various causes;
4. Changes in dryer operating regime (parameters).

Regarding continuous (flow) grain dryers, the causes of operating stationariness disruption were selected on the basis of scientific literature, practical records, and personal experience. The synthesis of the results was based on the conducted analysis. A unique mathematical model was made for the calculation of fuel consumption based on parameters obtained during grain drying.

RESULTS AND DISCUSSION

Analysis

Change in moisture content

Raw materials of various manufacturers arrive at drying facilities. Therefore, moisture content changes discontinuously at the entrance to a dryer. This fact could verify the assumption that materials of various moisture content can be found at the entrance to a dryer. Volumes of different initial moisture contents indicate different curves of drying kinetics (Fig. 1). In order to dry volumes with higher moisture content, the drier volumes ought to be overdried. This causes excessive heat consumption. Redundant heat consumption is proportional to the mass of a drier volume and different drying duration:

$$\Delta\tau = \tau_1 - \tau_2 \qquad (1),$$

where ***Dt*** is the difference in drying duration to the storage moisture content, t_1 is the drying duration of the drier volume, and t_2 is the drying duration of the moister volume.

In such cases, dryer material will be overdried as follows:

$\Delta w = w_{21} - w_{22}$,

where ***D****w* is the difference in moisture content between volumes at the end of drying, w_{21} is the drying duration of the drier volume, and t_2 is the drying duration of the moister volume. A relative increase in energy consumption is proportional to an increase in drying duration and it can be expressed by the factor k_2:

$$k_2 = \frac{\tau_1 - \tau_2}{\tau_2} \quad (3)$$

where k_1 equals 0.

The total redundant energy consumption in case of *n* volumes of materials with different initial moisture content is:

$$Q = \sum_{i=1}^{n} k_i m_{2i} q_o \quad (4)$$

where m_{2i} is the amount of dried *i* volume (kg/s), and q_o is the specific heat consumption during stationary operation. The increase in heat consumption of *i* volume is

$$k_2 = \frac{\tau_{\max} - \tau_i}{\tau_i} \quad (5)$$

where t_{max} is the drying duration of the moistest material.

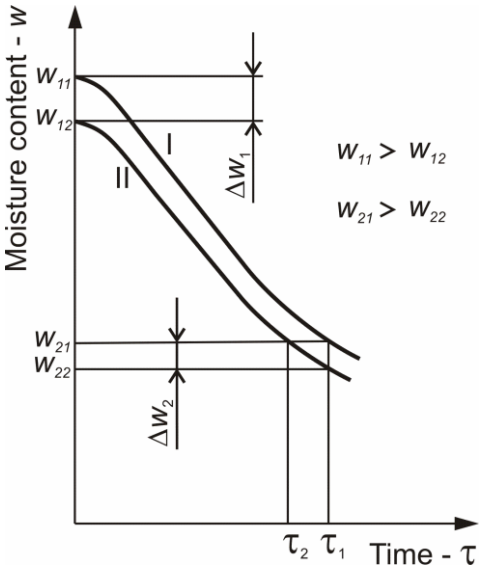

Fig 1. Kinetic curves of simultaneous drying of two materials with different initial moisture content

In order to use the expressions 3, 4, and 5, it is necessary to know the following function:

$$\tau_i = \tau_i(w_{1i}) \qquad (6)$$

for a certain real technical system. Since A.V. Likov and thereafter, a great number of empirical and semiempirical models have been formulated for drying kinetics of various materials (Babić and Babić, 2012). However, it is possible to conduct a statistical analysis on the basis of a case study. Therefore, every center or facility for drying and storing grains should record inlet grain volumes. A fairy accurate assumption can be made that the moisture distribution of raw material is approximate to normal moisture distribution. The testing method χ^2 can confirm a deviation from normal moisture distribution with a certain probability. Graphic representation of moisture content distribution within the grid is acceptable as well.
In practice, charts indicating the dependency between an increase in drying duration and final moisture content can be used. Most producers use well-known Campbell (USA) charts. Consequently, a correlation obtained from the chart analysis can be made. The increase in fuel consumption caused by uneven moisture content at the exit of a dryer:

$$\Delta B_1 = 0{,}1366(14 - \overline{w})B \qquad (7)$$

where **D***B*$_1$ (kg) is the total increase in fuel consumption within a season due to uneven moisture content of raw material, and $\overline{w}$ ($\%_{wb}$) is the mean moisture value of dried material.
A practical example has confirmed the normal moisture distribution of dried material. The mean moisture value of dried material was 12.31%$_{wb}$. Then, an increase in fuel consumption was calculated on the basis of the ideal conditions of the expression 7, and the fuel consumption increase of 23.1% was obtained. Regarding massive amounts of fuel utilised for corn grain drying, it can be concluded that the increase in fuel consumption is massive as well. Although ideal conditions cannot be assured, better organization would decrease the percentage of redundant fuel consumption.

Changes in types of hybrids

A majority of authors (Babić Ljiljana, 1989, Kemp et al, 2001, Katić, 1985) conclude that different hybrids demonstrate different kinetic characteristics during drying. Therefore, some hybrids dry faster under the same conditions (parameters) of drying fluids. This scientific fact is derived from morphological differences of various hybrids. The change in the type of hybrids will entail the need for overdrying because unfavourable hybrids ought to be dried to desirable moisture content. Unfortunately, there are no comprehensive data which could be utilised for the prediction of fuel consumption increase. Empirical assessments indicate that the change in the type of hybrid can cause overdrying

by 1% of moisture content. If this fact is acknowledged, then it could be concluded that:

$$\Delta B_2 = \sum_{i=1}^{n} 2 * 0{,}1366 B_i \,, \qquad (8)$$

where DB_2 is the increase in fuel consumption caused by the change in the type of hybrids, i is the individual disorder within a season, and B_i is the fuel consumption during a stationary operating regime, and *n* is the total number of disorders.
Operating disruptions can be of various (temporal) duration depending on the cause. During certain disruptions, dryers are emptied. Operating disruptions cause an increase in fuel consumption for:

$$\Delta B_3 = \frac{c_z M_{zs}}{H_d \eta_t} \sum_{j=1}^{m} \Delta t_{zj} \qquad (9)$$

where c_z is the mean specific heat of grains, M_{zs} is the mass of grains during drying season, H_d is the lowest thermal power of fuel, h_t is the thermal efficiency of a thermal power unit, Dt_{zj} is the difference between the mean grain temperature at the beginning and at the end of a dryer operating disruption, and *m* is is the total number of disorders.

Changes in operating regimes
The change in operating regime implies a discontinuous change in drying fluid temperature. This disorder affects energy consumption by affecting the exit grain moisture. Personally conducted analyses (Babić, 1988) have resulted in the following expression:

$$\Delta B_4 \cong \sum_{s=1}^{p} 0{,}05 * 0{,}1366 \Delta t_{1s} B_{si} \qquad (10)$$

where *s = 1...p* is the individual abrupt temperature change, *p* is the total number of disorders within a season, B_s is the fuel consumption during stationary operating regime which was established after disorders.

Synthesis
The synthesis of a mathematical model which encompasses all abovementioned discontinuities in a dryer's operation is:

$$\Delta B = \Delta B_1 + \Delta B_2 + \Delta B_3 + \Delta B_4$$

or

$$\Delta B = 0{,}1366(14-\overline{w})B + \sum_{i=1}^{n} 2*0{,}1366B_i + \frac{c_z M_{zs}}{H_d \eta_t} \sum_{j=1}^{m} \Delta t_{zj} + \sum_{s=1}^{p} 0{,}05*0{,}1366\Delta t_{1s} B_{si} \quad .(11)$$

This semiempirical model can be used for the assessment of seasonal indicators of a dryer's operation. The practical application of this model and other analyses indicate that disorders in the operating stationariness of a grain dryer within a corn drying season can cause an increase in fuel consumption up to 30% in comparison with stationary operating regime.

CONCLUSION

During the use of continuous grain dryers under normal operating conditions many disorders occur causing discontinuity. These disorders cause a significant increase in fuel consumption reaching the amount of 30%. Special attention should be paid to the organisation of operations in order to minimise these disorders. The quantification of fuel consumption can be obtained by the application of the equation 11.

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BIODEGRADATION OF AFLATOXIN B1 BY FUNGAL SPECIES

Aleksandra Bočarov-Stančić[1], Marija Bodroža-Solarov[2], Ferenc Bagi[3], Vera Stojšin[3], Jovana Vučković[2], Vladimir Pantić[1]

[1]Bio-ecological center DOO, Petra Drapšina 15, 23000 Zrenjanin, Serbia
[2]Institute for food technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia
[3]Faculty of agriculture, Trg Dositeja Obradovica 8, 21000 Novi Sad, Serbia

ABSTRACT

The ability of 37 nontoxigenic fungal isolates to degrade aflatoxin B_1 (AFB_1) was investigated in controlled laboratory conditions. AFB_1 was added to the test medium to the final concentration of 0.6 µg ml^{-1}. The presence of AFB_1 residues in the Vogel's medium was determined after 7 and 14 days of fungal cultivation at 27±1 °C. Biotransformation of aflatoxin B_1 (20-85%) was detected by the majority of analyzed representatives of the genus *Aspergillus*: *A. clavatus* (1/2), *A. flavus* (2/5), *A. fumigatus* (5/7), *A. nidulans* (1/1), *A. niger* (8/10), *A. terreus* (1/1) and *Aspergillus* spp. (2/3). From other investigated fungal cultures highest *in vitro* biodegradation capability of AFB_1 demonstrated isolates *Cladosporium* sp. (95%) and C*ephalophora tropica* (95%).
***Keywords**: aflatoxin B_1, microbial degradation, fungi*

INTRODUCTION

Among naturally occurring mycotoxins aflatoxins (primarily aflatoxin B_1 – AFB_1) are most harmful toxic metabolites of fungi because of their hepatotoxic, teratogenic, immunosuppressive and mutagenic nature. The International Agency for Research on Cancer classified AFB_1 in a group I as human carcinogen [10]. These difuranocoumarin derivatives are produced by fungal species *Aspergillus flavus*, *A. parasiticus* and *A. nomius* [3]. Due to the ubiquitous occurrence of aflatoxins (cereal grains, oil seeds, nuts, spices, milk etc.) preventive and remedial measures are necessary, including detoxification procedures. Since physical and chemical decontamination strategies are often inconvenient, biodegradation of AFB_1, either by the whole cell or an enzyme system, is very promising strategy.

Fungi play very important role in AFB_1 biodegradation: Zygomycota (*Rhizopus* sp. and *Mucor* sp.), Ascomycota (*Aspergillus flavus, A. niger, A. parasiticus, Neurospora* sp. and *Trichoderma viride*), plant pathogens (*Peniophora* sp., *Phoma* sp. and *Alternaria* sp.) as well as Basidiomycota (*Armillariella tabescens*, *Pleurotus ostreatus* and other white rot fungi) [16, 1, 2].

Detoxication of aflatoxin molecule occurs after removal of double bond of the terminal furan ring or opening of lactone ring [1]. The structural changes result in loss of fluorescence, and changes in toxicity and mutagenicity. Different enzymes are involved in microbial degradation of AFB_1: F_{420} – dependent

reductase in the case of *Actinomicetales* [11] or laccase in the case of *Peniophora* sp. and *Pleurotus ostreatus* [2].
Therefore this study explored, by rapid screening method, the potential of different fungal isolates to biodegrade AFB_1 in laboratory condition.

MATERIAL AND METHODS

Microorganisms. Thirty-seven isolates of nontoxigenic fungi were selected for the present *in vitro* investigation. The largest number of test microorganisms belonged to the genus *Aspergillus*: *A. clavatus* (2), *A. flavus* (5), *A. fumigatus* (7), *A. nidulans* (1), *A. niger* (10) *A. terreus* (1) and 3 unidentified isolates. The remainder of the analyzed fungal microorganisms were: *Cephalophora tropical* (1), *Cladosporium* sp. (1), *Eurotium amstelodami* (1), *Fusarium* sp. (1), *Purpureocillium lilacinus* (1), *Penicillium* sp. (2), and *Rhizopus* sp. (1). The majority of tested fungi originated from samples of feed and its components that were not mycotoxin-contaminated (18) or from the air in Microbiological laboratory (11) and were isolated during 2009-2012 period. Fungal species were determined according to Domsh *et al.* [8] and Luangsa-ard *et al.* [12]. Fungal cultures were maintained on potato dextrose agar (PDA) at 4-6 ºC.
Production of toxin. AFB_1 was produced employing solid substrate fermentation with *A. flavus* isolate GD-2 per the method of Bočarov-Stančić *et al*. [4]. AFB_1 was isolated from the substrate on which the fungus was cultivated and purified according to the TLC method [14], evaporated to a dry residue and dissolved in ethanol (100 µg ml^{-1}).
Experimental procedure. Obtained AFB_1 was added to the modified Vogel's medium [17] to the final concentration of 0.6 µg ml^{-1} (VAFLA). The presence of AFB_1 residues in the test medium was determined after 7 and 14 days of fungal cultivation at 27±1 °C by the screening method of Bočarov-Stančić *et al*. [6]. Discs, cut out of the central part of the fungal colony as well as control discs (with no test fungal culture), were directly placed on TLC plates coated with Kieselgel G (thickenss of 2.5 mm) and wetted with 10-20 µL of a chloroform-methanol (2:1, v/v) mixture. Several seconds later discs were removed from the TCL plates and chromatography plates were developed together with different volumes of working AFB_1 standard solution (concentration of 0.0005 µg $µl^{-1}$).
Thin layer chromatography was performed in a saturated tank of t*oulene-ethyl acetate-formic acid* mixture (5:4:1, v/v/v). After the plate development and natural drying in a darkened digester, plates were examined under long wavelength UV rays (366 nm). All analyses were done in three replicates. Detection limit (LoD) of the applied TLC method amounted to 1.33 µg kg^{-1}.
Degradation rate (%) was calculated by the following formula, where C_o is AFB1 quantity in control disc and C AFB_1 quantity in disc with fungal growth:

$$Degradation\,rate = [\frac{C_0 - C}{C_0}]\,x\,100$$

RESULTS AND DISCUSSION

Fungal isolates that biodegraded AFB_1 *in vitro* are presented in Tables 1 and 2.

Table 1. Biodegradation of aflatoxin B_1 by Aspergillus spp.

No.	Species	Isolate origin	Isolate designation	Degradation (%)
1.	*A. clavatus*	Air	PR-43/11	85.0
2.	*A. flavus*	Sunflower meal	932-1/12	50.0
3.	*A. flavus*	Feed mixture	523-1/12	85.0
4.	*A. fumigatus*	VAFLA	Inf.-1/11	75.0
5.	*A. fumigatus*	VAFLA	Inf.-2/11	80.0
6.	*A. fumigatus*	Air	PR-45/12	85.0
7.	*A. fumigatus*	Feed mixture	523-2/12	80.0
8.	*A. fumigatus*	Air	PR-43/12	65.0
9.	*A. nidulans*	Sunflower meal	149-A/12	60.0
10.	*A. niger*	Feed mixture	47-2/10	80.0
11.	*A. niger*	Cob	506-2/10	65.0
12.	*A. niger*	Sunflower meal	653-2/12	25.0
13.	*A. niger*	Cob	1292/09	50.0
14.	*A. niger*	Air	D1-1/10	70.0
15.	*A. niger*	Air	D1-2/10	85.0
16.	*A. niger*	Air	D1-3/10	75.0
17.	*A. niger*	Soil	Rb-gr/10	65.0
18.	*A. terreus*	Feed mixture	523-3/12	80.0
19.	*Aspergillus* sp.	Sunflower meal	187/12	85.0
20.	*Aspergillus* sp.	Sunflower meal	653-3/12	50.0
21.	*Aspergillus* sp.	Sunflower meal	932-2/12	25.0

In all cases biotransformation was observed after 7 days of cultivation; prolonged cultivation did not change the results.
Majority of 29 tested *Aspergillus* spp. isolates biotdegraded AFB_1 during the growth on modified Vogel's agar supplemented with 0.6 µg ml^{-1} of aflatoxin B_1: *A. clavatus* (1/2), *A. flavus* (2/5), *A. fumigatus* (5/7), *A. nidulans* (1/1), *A. niger* (8/10), *A. terreus* (1/1) and *Aspergillus* spp. (3/3)(Table 1). The reduction of

AFB_1 in VAFLA varied from 25.0-85.0%. Most efficient aspergilli in AFB_1 degradation (85.0%) were fungal cultures isolated from the air during routine control of hygiene in microbiological laboratory (*A. clavatus* PR-43/12, *A. fumigatus* PR-45/12, *A. niger* D1-2/10) and two isolates from feed (*A. flavus* 523-1/12, *Aspergillus* sp. 187/12). Obtained results that *A. flavus* and *A. niger* can biotransforme AFB_1 are not surprising because other investigators have been reported simillar results [1, *16*].
It is interesting to point out that five tested *A. niger* cultures, besides AFB_1, can degraded also other mycotoxins *in vitro*: T-2 toxin, and ochratoxin A (20.0-50.0%) [6, 7]. These results indicate that *A. niger* is a promising candidate for environmental remediation.

Table 2. Biodegradation of aflatoxin B_1 by different fungal species

No.	Species	Isolate origin	Isolate designation	Degradation (%)
1.	*Cephalophora tropica*	Sunflower meal	400/12	95.0
2.	*Cladosporium* sp.	VAFLA	INF-3/12	95.0
3.	*Eurotium amstelodami*	Air	PR-6/11	20.0
4.	*Fusarium* sp.	Sunflower meal	426/12	75.0
5.	*Penicillium* sp.	VAFLA	INF-3/11	90.0
6.	*Penicillium* sp.	VAFLA	INF-4/11	80.0
7.	*Purpureocillium lilacinus*	Air	D-6/11	75.0
8.	*Rhizopus* sp.	Feed mixture	681/12	65.0

Contrary to the results of Shantha *et al.* [15] who reported that isolates of *Cladosporium* sp. and *A. terreus* were by far least efficient in AFB_1 biotransformation, our own isolate of *Cladosporium* sp., that represented infection of uninoculated Petri plates with Vogel's agar supplemented with AFL B_1, degraded almost all aflatoxin added in the test medium (95.0%). The same result ig. 95% $AFLB_1$ reduction, was achieved by C*ephalophora tropica* obtained from sunflower meal (Table 2). Although this mainly coprophilous fungal species is known principally from tropical and subtropical countries, we isolated it during routine microbiological analysis of sunflower meal from Serbian province Vojvodina.
Very efficient fungi in $AFLB_1$ biodegradation were also *Penicillium* isolates (80.0 and 90.0%, respectively). *Fusarium* sp. and *Purpureocillium lilacinus* (former *Paecillomyces lilacinus*) demonstrated somewhat smaller reduction of $AFLB_1$ in the growht medium (75.0%) (Table 2). Last mycobiota is common saprobic filamentous fungus that has been isolated from a wide range of habitats. *P. lilacinus* has shown promising results for use as a biocontrol agent for growth control of destructive root-knot nematodes [12]. Besides $AFLB_1$ some isolates of

this species are capable for ochratoxin A (OTA) detoxication. Bočarov-Stančić *et al.* [5] demonstrated that co-cultivation of *P. lilacinus* with OTA producing strain of *Aspergillus ochraceus* resulted in inhibition of OTA biosynthesis by 99.8%.
Rather high $AFLB_1$ biotransformation capability observed by *Rhizopus* sp. (65.0%) was similar with the results obtained by El-Shiekh et al. [9]. These authors showed that *P. lilacinus, Penicillium griseofulvum* and *Rhizopus nigricans,* when grown in co-culture with *Aspergillus parasiticus* natural $AFLB_1$ producer, decreased total aflatoxin concentration 52.4%, 52.0% and 35.4%, respectively.
The smallest biodegradation activity (20.0%) showed *Eurotium amstelodami* (Table 2). There are no data in avalilable literature on the ability of this mycobiota to decontaminate mycotoxins, but it is known that *E. amstelodami* is capable to biotransform anti-malarial drug artemisinin [13].

CONCLUSIONS

78.4% of tested fingal species biodegraded *in vitro* $AFLB_1$. Most of the tested aspergilli transformed more then 50% of $AFLB_1$ added to the cultivation medium. The highest *in vitro* biodegradation capability of AFLB1 (95%) demonstrated isolates of *Cladosporium* sp., and C*ephalophora tropica*. Obtained results indicate that *A. niger* and *P. lilacinus* are the most promising candidates for environmental remediation because they can, besides AFLB1, degrade and other mycotoxins (OTA and T-2 toxin).

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DSM NUTRITIONAL PRODUCTS – YOUR PARTNER FOR EUBIOTIC SOLUTIONS

Matthias Wiemann[1], Christophe Paulus[1], Zoran Grbic[2]

[1]DSM Nutritional Products – Animal Nutrition and Health, Post Box 2676, CH-4002 Basel, Switzerland; matthias.wiemann@dsm.com
[2]DSM Nutritional Products – Country Representative ANH (SRB, BiH, MTE), V. Kacanskog 8, 21000 Novi Sad, Serbia: zoran.grbic@dsm.com

ABSTRACT

DSM Nutritional Products is a leading supplier of feed additives. One of their product categories covers Eubiotics aiming at an optimizing of intestinal microflora for better animal performances.
Added to pig diets VevoVitall (benzoic acid) results in a strong reduction of E.coli at intestinal level. In a number of 12 scientific studies on weaned piglets the inclusion of 0.5% VevoVitall resulted in 10.6% better daily weight gain and 2.6% lower feed conversion.
For application to broiler diets the product CRINA Poultry plus (combination of benzoic acid and essential oils) is dedicated to control Clostridium perfringens and E.coli. A series of 14 broiler studies under field conditions using 300 ppm CRINA Poultry plus showed 2.3% higher live weight and 2.7% lower feed conversion.
Overall DSM Nutritional Products aims via their Eubiotic product portfolio at offering optimal solutions for modern animal production systems.
***Keywords**: feed additive, benzoic acid, essential oils, piglets, broilers*

ABOUT DSM NUTRITIONAL PRODUCTS

DSM Nutritional Products is a leading supplier of additives for the human and animal nutrition business. In Animal Nutrition the products cover feed additives and are categorized in groups of vitamins, carotenoids, enzymes and eubiotics. Via a global network DSM Nutritional Products is present in each country and sells its product via direct sales offices, premix sites or local distributors. DSM focuses strongly on innovation and therefore steadily launches new products facing the upcoming challenges in the industry.

ABOUT EUBIOTICS

Among the Animal Nutrition product portfolio the Eubiotics represent the youngest product category and as well an excellent examples for innovative solutions for the last 10 years. Eubiotics are defined as products which are modulating the gut microflora for improving health and performance of the animals. In the DSM Eubiotics portfolio it covers organic acids (VevoVitall),

essential oils (CRINA) and probiotics (CYLACTIN) After the final ban of antibiotic growth promotors in 2006 the search for alternative solutions to maintain a good gut health status increased the application of such additives especially in diets for piglets and broilers. DSM refers to this market needs via their key products VevoVitall for pigs feed and CRINA Poultry plus for broilers feed.

ABOUT VEVOVITALL

VevoVitall is a product containing 99% pure benzoic acid of food quality origin. Benzoic acid is well known for its antimicrobial efficacy especially against E.coli, Salmonella and yeasts. Due to low solubility VevoVitall can act up to the small intestine of the pig and control especially at this stage the growth of E.coli which under pathogenic conditions can cause severe cases of diarrhoea. Based on the improved health status the pigs eat better and show higher performances.
In a number of studies DSM Nutritional Products tested VevoVitall at recommended dosage of 0.5% in diets for weaned piglets compared to a negative control. The average of all those trials resulted in improved daily weight gain of 10.6% and reduced Feed conversion ratio by 2.6%

Table 1. Performance parameters from studies with weaned piglets testing 0.5% VevoVitall with negative control

	Daily weight gain (g)			Feed conversion ratio		
Trials (Location)	**Vevo Vitall**	**Control**	**Diff. (%)**	**Vevo Vitall**	**Control**	**Diff. (%)**
Belgium (KUL, 2005)	394	428	+8.6	1.64	1.66	+1.2
Denmark (DMA, 2003)	317	347	+9.4	2.02	1.91	-5.5
France (CRNA, 2003)	425	477	+12.2	1.50	1.42	-5.3
France (CRNA, 2005)	335	379	+13.1	1.65	1.55	-6.1
Germany (FUB, 2004)	480	503	+5.0	1.36	1.38	+1.1
Germany (MLU, 2003)	338	374	+10.7	1.58	1.55	-1.9
Netherlands (2001)	287	315	+9.8	1.63	1.58	-3.1
Spain (IRTA, 2004)	357	390	+9.2	1.53	1.50	-2.0
Brasil (Sao Paulo, 2004)	564	621	+10.1	1.75	1.69	-3.6
UK (field trial, 2005)	522	595	+14.0	1.33	1.30	-2.3
Average 12 studies			**+10.6**			**-2.6**

Further trials testing VevoVitall in comparison to competitive solutions (mainly blends of organic acids) showed improved performance of 0.5% VevoVitall by 6.1% on daily weight gain and 2.3% on feed conversion ratio. As well in many trials the feed intake of piglets was higher in groups fed with VevoVitall.

About CRINA Poultry plus

Especially for broiler feeds DSM launched 4 years ago the product CRINA Poultry plus which is a combination of benzoic acid with essentials oils (main compounds thymol, eugenol, piperine). The combination of both elements

broadens the range of antimicrobial activity against E.coli, Clostridium perfringens, Campylobacter and Salmonella. In a first set of scientific trials the product was tested at 300ppm in broiler feed and resulted in 1.7% higher body weight and 0.9% reduced feed conversion ratio. It has to be considered that all these trials had been done with modern broiler genetics at high performance levels. Additionally a series of field trials had been conducted in several European countries. Here again the supplementation of 300ppm CRINA Poultry plus showed 2.3% higher body weight and 2.7% improved feed conversion ratio. Setting the performance data into an economic calculation it can result into an additional margin of 40-60€/1000 broilers

Table 1. Performance parameters from studies with broilers testing 300ppm CRINA Poultry plus (CPP) with negative control

	Final body weight			**Feed conversion ratio**		
Trials (Location)	**CPP**	**Control**	**Diff. (%)**	**CPP**	**Control**	**Diff. (%)**
Spain (10/2009)	2497	2596	+4.0	1.70	1.68	-0.9
Germany (06/2009)	2189	2195	+0.3	1.69	1.66	-1.8
Germany (10/2010)	2519	2520	0.0	1.71	1.63	-4.9
Denmark (03/2010)	2495	2488	-0.3	1.74	1.67	-4.0
Netherlands (03/2010)	2444	2463	+0.8	1.57	1.56	-0.6
France (06/2010)	1880	1900	+1.1	1.87	1.85	-1.1
Poland (06/2010)	2823	2853	+1.1	1.69	1.62	-4.1
Spain (08/2010)	2865	2902	+1.3	1.77	1.75	-1.2
UK (SAC, 07/2010)	2483	2614	+5.3	1.63	1.56	-4.2
UK (MPP, 07/2011)	1960	2040	+4.1	1.62	1.61	-0.6
UK (MPP, 09/2011)	1880	1920	+2.1	1.67	1.62	-2.4
Poland (06/2010)	2823	2853	+1.1	1.69	1.62	-4.1
Greece (07/2011)	2661	2898	+9.1	1.75	1.71	-2.3
Spain (04/2012)	2644	2709	+2.5	1.63	1.54	-6.5
Average 14 studies			**+2.3**			**-2.7**

CONCLUSIONS

The data shown on VevoVitall and CRINA Poultry plus demonstrates very well that DSM Nutritional Products offers perfect solutions for todays pig and poultry business. The usage of both products results in better performance and finally in economic benefits of the farmer.

For further information on DSM Nutritional Products and its animal nutrition products please contact the above mentioned authors via e-mail.

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