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Days of veterinary medicine 2015
Struga, Macedonia
September 24-26, 2015*

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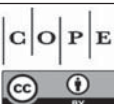
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of the type of the animal feed, which is in accordance with the Regulation for Quality of animal feed. Adding minerals in the feed is usually required at the time of high production and pregnancy, its content is between 4.4-7.2%, the moisture concentration is between 9.5-13.2% and the fat content is between 2.1-3.4%, which shows that each fodder mixture fulfills the requirement for quality with the Regulation for Quality of feed (Official gazette of Republic of Macedonia, No.54/2014).

P18

Effect of dietary eicosapentaenoic and docosahexaenoic acid supplementation during last month of gestation on colostrum fatty acids composition in Charolais cows

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Introduction: Eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids are essential nutrients. Their significance is especially important in the perinatal period of the calf for the development of central nervous and immune system. The aim of the study was to explore whether it is possible to alter cow colostrum fatty acid composition with a low level of fat supplement, high in EPA and DHA.

Material and methods: In feeding trial were included a total of 20 Charolais cows during the last month of their gravidity. Cows were divided into 2 groups: a control group (Control) and an experimental group (DHA + EPA), each group consisting of 10 animals. All the animals were fed a basal diet, consisting of haylage and corn concentrate, and had constant access to drinking water. For the period of one month before expected calving, cows in experimental group (DHA + EPA) were supplemented with fat supplement, consisting of 9.1g/cow/day of EPA and 7.8 g/cow/day of DHA. Milk samples were collected on the 1st day (6 hours after calving, colostrum). Analysis of fatty acid composition was performed by gas chromatography.

Results: Fatty acid composition of colostrum in experimental group (DHA + EPA) was significantly altered: we found higher concentrations for fatty acids: EPA, DHA, docosapentaenoic, oleic and stearic acid. Summed profile of saturated fatty acids (SFA),

monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) was not altered but summed profile of n-3 long chain PUFA was significantly higher in experimental group. Also, addition of fat supplement significantly decreased concentration of myristic acid in colostrum.

Conclusion: These results showed that fat supplement, high in DHA and EPA, modified the fatty acid profile of colostrum milk fat and increased the proportion of fatty acids beneficial for health of calf in perinatal period.

P19

Optimization and validation of UHPLC tandem mass spectrometry method for determination of multi-class pesticide residues in bovine liver applying QuEChERS sample preparation

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Introduction: The determination of pesticide residues in matrices of animal origin is a matter of high necessity in view of the proven toxicity and stability thereof. Traditional sample preparation techniques usually are time-consuming, labor-intensive, complicated and expensive. Lately, it is a common opinion that many chemically different compounds have to be determined with one method, rather than single class of compounds. The QuEChERS (Quick, Easy, Cheap, Easy, Effective, Rugged, and Safe) approach for sample preparation offers unique possibility for multi-residual and multi-class determination of pesticides applying single method of analysis. For the purpose of monitoring pesticides within the National control program for food of animal origin, we optimized and validated UHPLC-tandem mass spectrometry method for determination of carbamates, pyrethroids and organophosphorus pesticides in bovine liver.

Material and methods: The UHPLC-mass spectrometry method in ESI+ mode was optimized for the following pesticides: carbaryl, carbofuran, fenvalerate, permethrin, cypermethrin, deltamethrin, malathion, parathion, dichlorvos and diazinon. The initial extraction with acetonitrile was followed by matrix-dispersive sample purification with 1.2 g of mixture from magnesium sulfate, PSA and C18 in ratio 6:1:1. Validation parameters were determined in accordance to the SANCO 12571/2013 document requirements. The established linearity was in

the range from 5 to 250 µg/kg. The LODs and LOQs were estimated at concentration levels with S/N ratio of at least 3:1 and 10:1, respectively. Accuracy was tested spiking blank bovine liver at two concentration levels, 10 and 50 µg/kg (n=5).

Results: The linearity determination revealed regression coefficients values higher than 0.99 for all pesticides tested. The estimated LODs and LOQs were lower than 10 µg/kg, with exception of diazinone, for which, LOQ value of 12.4 µg/kg was obtained. However all values estimated are lower than the established maximum residue levels for liver. Recoveries obtained were in the range 71.9 -115.9 % and 70.2 – 98.8 %, for spiked levels of 10 µg/kg and 50 µg/kg, respectively. The determined precision for all pesticides within the method scope was lower than 20%. Validation parameters estimated are in line with the respective legislative requirements, proving that the method is suitable for performing official pesticides control.

Conclusion: It was presented that QuChERS sample preparation, with some modifications, may be successfully applied for pesticides determination in liver samples with UHPLC-Tandem mass spectrometry. In accordance with the legislation requirements, the method proposed may be used for pesticide analysis within the Monitoring program for residues in animal products.

P20

Aflatoxins occurrence in peanuts and products containing peanuts

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Introduction: Aflatoxins are highly toxic, cancer causing metabolites of certain strains of the fungi *Aspergillus flavus* and *A. parasiticus*, that cause immune-system suppression, growth retardation, liver disease and death in both humans and domestic animals throughout the world. They are a group of closely related compounds with small differences in chemical composition. Aflatoxins of concern are designated B1, B2, G1, G2 and are usually found together in various foods and feeds in various proportions, of which aflatoxin B1 is considered the most prevalent and the most potent form. In accordance to EU Commission Regulation (165/2010) groundnuts (peanuts) intended for direct human consumption or use as an ingredient in foodstuffs, should comprise maximum concentration of 2.0 µg/kg of AFB1 i.e. 4.0 µg/kg of sum of B1, B2, G1 and G2.

Material and methods: HPLC method with fluorescence detection was used for investigation of quantitative

determination of aflatoxins in peanuts and products containing peanuts, after their clean-up on immunoaffinity columns. This method is in accordance with ISO 16050 and AOAC Official Method 991.31. In duration of 21 consecutive months (2013, October – 2015, June), 38 samples of peanuts and products containing peanuts were analyzed. Among them: 19 samples raw peanuts, 7 samples roasted peanuts, 6 samples salty sticks with peanut butter, 4 samples peanut flips, 1 sample peanut paste and 1 sample peanut skins. Limit of detection (LOD) is 0.005 µg/kg.

Results: Aflatoxins concentration was below LOD in 26 (68.42%) samples: in 21 out of 26 peanuts samples and in 5 out of 12 products containing peanuts. Aflatoxins were detected in 12 (31.58%) samples, in a concentration range between 3.21 µg/kg and 61.42 µg/kg. Aflatoxins concentration detected was: 3.21 µg/kg, 10.6 µg/kg, 12.8 µg/kg and 14.2 µg/kg in 4 samples raw peanuts; 2.12 µg/kg in a roasted peanuts sample; 61.42 µg/kg in a peanut paste; 4.98 µg/kg and 5.7 µg/kg in 2 samples salty sticks with peanut butter; 16.83 µg/kg, 21.11 µg/kg, 22.68 µg/kg and 37.2 µg/kg in 4 samples peanut flips. Ten of the samples exceeded maximum limit of aflatoxins concentration i.e. 26.31% of total investigated samples.

Conclusion: Relative high percent of aflatoxin occurrence in investigated samples points out that further examinations on this topic are necessary. Thus it is highly recommended to increase the monitoring of aflatoxins in commodities. Beside this, proper agricultural and agronomic practices have to be imposed to reduce susceptibility and exposure of commodities to fungal invasion during pre-harvest, storage and processing periods.

P21

Comparison of extraction methods in HPLC-FD analysis of ochratoxin-A in swine kidney

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Introduction: OchratoxinA (OTA) is mycotoxin which is produced by fungi *Aspergillus* and *Penicillium* species. OTA is found in food commodities and several animal products. The toxin is known as etiologic agent of Balkan Endemic Nephropathy disease. OTA was classified in Group 2B as a possible carcinogen in humans by The International Agency for Research on Cancer. The aim of this study was to make comparison between three extraction methods (immune-affinity column extraction (IAC), liquid-liquid extraction (LLE) and solid phase