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LIVESTOCK PRODUCTION: RECENT TRENDS AND FUTURE PROSPECTS

Abstracts

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CEREAL BY-PRODUCT VALORIZATION BY APPLYING ENZYMATIC TREATMENT AND FERMENTATION MODELS

Elena Bartkienė^{1,2}, Vita Lėlė^{1,2}, Vytautė Starkutė^{1,2}, Eglė Zokaitytė^{1,2}, Vadims Bartkevics³

¹Department of Food Safety and Quality, Veterinary Faculty, Lithuanian University of Health Sciences, Mickevičiaus str. 9, LT-44307 Kaunas, Lithuania,

²Institute of Animal Rearing Technologies, Faculty of Animal Sciences, Lithuanian University of Health Sciences, Mickevičiaus str. 9, LT-44307 Kaunas, Lithuania,

³Institute of Food Safety, Animal Health and Environment "BIOR", Lejupes iela 3, Zemgales priekšpilsēta, Riga, LV-1076, Latvia

Corresponding author: elena.bartkiene@lsmu.lt

The by-products of the cereal industry are potential resources for functional compounds. They can also be used as a solid substrate for the cultivation of biomass of desirable beneficial microorganisms. In addition to degradation properties (compared to pure enzymes), selected viable microorganisms may possess desirable antimicrobial and microbiota modeling properties *in vivo*. Wheat and barley processing generates a large amount of by-products, and the latter are very good producers of enterolignans *in vitro*, compared to other cereals. In addition to lignans and enterolignans, alkylresorcinols are also present in the outer layer of the cereal. Finally, the outer layer is a good source of many phytochemicals, but the highly complex insoluble structure of bran reduces the accessibility of these valuable compounds. Therefore, the application of enzymatic treatment and fermentation can be considered potentially useful to effectively utilizing cereal by-products. The objective of this study was to apply enzymatic treatment and lactic acid fermentation models to the valorization of cereal by-products. In addition, to increase the sustainability of the process, the possibility of using the potato juice for the propagation of technological microorganisms was tested. It was found that the potato juice is a suitable substrate for the cultivation of *P. acidilactici*, as the juice has more than $9.6 \log_{10}$ CFU/mL of viable lactic acid bacteria cells. The fermented juice powder (stabilized by spray drying) also had a number of viable lactic acid bacteria greater than $7.0 \log_{10}$ CFU/g after 12 months of storage. The changes in the microbial profile and the chemical composition of treated cereal by-products showed that microbial treatment increases biosafety and reduces mycotoxin content of grain by-products. In addition, the use of selected lactic acid bacteria strains for the fermentation of cereal by-products increases the concentration of matairesinol and secoisolariciresinol. Finally, fermentation of cereal by-products with selected technological strains can be recommended as an effective method for more sustainable and higher value raw material production.

Keywords: fermentation, enzymatic treatment, cereal outer layer, functional compounds.

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EVALUATION OF MILK YIELD AND MILKABILITY TRAITS OF DIFFERENT GENOTYPE COWS DURING LACTATION

Lina Anskienė¹, Renata Japertienė¹, Sigita Japertas²

¹Department of Animal Breeding, Lithuanian University of Health Sciences, Faculty of Animal Husbandry Technology, Tilžės str. 18, LT-47181, Kaunas, Lithuania,

²Public Institution Practical Training and Trial Center of Lithuanian University of Health Sciences, Akacijų str. 2, Giraitės village, LT-54310, Kauno distr., Lithuania

Corresponding author: lina.anskiene@lsmu.lt

Dairy herds in Europe and the United States of America were selected for high milk production under intensive farming conditions. Even under optimal management conditions, selection for the increase of milk yield has reduced dairy cattle health and reproductive efficiency worldwide [1]. This raises doubts whether these high-cost genotypes are suitable for organic farming systems [2], [3]. The evaluation of milk production and milkability traits of different genotype Lithuanian black and white cattle population ($n = 528$) was carried out by the data of state enterprise Agricultural Information and Rural Business Center and in a herd of an organic farm. The aim of this study was to investigate the milk yield and milkability traits during lactation of Lithuanian Black and White and Holstein cows with cows of different genotypes. The milk yield, milking speed, the highest milk flow, and milking time were investigated during the study. Evaluated traits were measured with DeLaval electronic milk meter, “Apro Windows” software. All records were obtained during 305 days of lactation. Statistical data analysis was conducted using SPSS 25.0 (SPSS, Inc., Chicago, IL, USA) software. The data were presented using descriptive statistics and normal distribution analysis methods, Kolmogorov–Smirnov test. The Pearson correlation (r) was determined to define the linear relationship between investigated traits from Apro Windows software. Multiple comparisons were calculated using the Tukey test. We observed that the highest milk yield and the highest milk flow rate were detected in cows with Lithuanian Black and White breed genotype (Lithuanian Black and White, Lithuanian Black and White \times Holstein, Lithuanian Black and White \times Holstein \times Lithuanian Black and White) during all stages of lactation. Milking time of these cows during the first two stages of lactation was longer, compared to cows of other genotypes ($P < 0.05$). An average positive correlation was estimated between the stage of lactation and milk yield ($r = 0.397$), high milk flow ($r = 0.331$), ($P < 0.001$). We estimated that of all fixed effects (stage of lactation, genotype, interaction of stage of lactation with genotype) the biggest influence on milk yield, highest milk flow, milking speed, milking time was produced by the stage of lactation ($P < 0.001$); a genotype showed the highest impact only on milking time ($P < 0.01$)

Keywords: genotype, milk yield, milk flow, milking time.

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CANNABIDIOL MEDIATE ASSOCIATION BETWEEN CAECA BACTERIAL ACTIVITY AND BREAST MEAT VOLATILE COMPOUNDS IN CHICKENS

Paweł Konieczka^{1,2}, Iwona Wojtasik-Kalinowska³, Andrzej Poltorak³, Misza Kinsner¹, Dominika Szkopek¹, Bartosz Fotschki⁴, Jerzy Juśkiewicz⁴, Joanna Banach⁵, Monika Michalczuk⁶

¹The Kielanowski Institute of Animal Physiology and Nutrition Polish Academy of Sciences, Jabłonna, Poland,

²University of Warmia and Mazury in Olsztyn, Olsztyn, Poland,

³Department of Technique and Food Development, Warsaw University of Life Sciences, Warsaw, Poland,

⁴Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland,

⁵Institute of Natural Fibres and Medicinal Plants – National Research Institute, Poznań, Poland,

⁶Department of Animal Breeding, Institute of Animal Sciences, Warsaw University of Life Sciences, Warsaw, Poland

Corresponding author: p.konieczka@ifzz.pl

Currently, great attention has been put to investigating the use of bioactive substances in poultry diet to improve birds' welfare and ensure final product safety and quality. One phytochemical substance that manifests the potential to beneficially modulate gut health and functionality is cannabidiol (CBD) obtained from hemp, which has recently attracted increasing attention. Therefore, in the present experiment, we investigated the effects of CBD on meat volatile organic compounds (VOCs) and gut microbiota activity in chickens reared under optimal (with no induced inflammation) conditions or subjected to *C. perfringens*- or *E. coli*-induced inflammation. Samples of breast meat were obtained from chickens (Ross 308 male broilers) allocated considering average body weight into 6 treatments, each containing 34 birds. The birds in the control (CON) group were fed a basal diet over the entire experimental period. The birds in the CBD treatment received diet as CON but supplemented (on top) with 30 g/kg *C. sativa* extract, while the birds in the CON+*C. perfringens* and CON+LPS groups (positive control groups) were fed the CON diet but they were subjected to a *C. perfringens* and LPS challenge, respectively. Chickens in the CBD+*C. perfringens* and CBD+LPS groups received the same diet as the birds in the CBD treatment group in addition to being subjected to the respective challenge factor (*C. perfringens* or LPS). Results indicated that CBD supplementation counteracted the formation of breast meat spoilage VOCs, including alcohols, trimethylamine and pentanoic acid, in the challenged birds, partly by decreasing caecal putrefactive SCFA production. Meat VOC/caecal SCFA relationships differed according to the applied challenge; however, CBD attenuated the effects of *C. perfringens* infection better than the effects of the LPS challenge on meat VOCs. The present study demonstrated the existence of a close association between caecal bacterial activity and chicken meat VOCs based on the production of SCFAs. CBD supplementation decreased the formation of putrefactive fatty acids, which resulted in decreased production of spoilage VOCs in the meat. Due to the complexity of pathogen–host interactions, more research is needed to investigate CBD potential in modulating the health status of the host.

Keywords: cannabidiol, *C. perfringens*, *E. coli*, chickens.

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COMPARATIVE ANALYSIS OF TWO CONFORMATION SCORING SYSTEMS OF POLISH WARBLOOD MARES ASSIGNATED AS JUMPING AND DRESSAGE TYPE

Alicja Borowska¹, Dorota Lewczuk²

¹Department of Genetics and Animal Breeding, Poznań University of Life Science,
ul. Wojska Polskiego 28; 60-637 Poznań, Poland,

²Department of Biotechnology and Nutrigenomics, IGAB PAS, Jastrzębiec, ul. Postępu 36A, 05-552 Magdalenka, Poland

Corresponding author: alicja.borowska@up.poznan.pl

The selection of horses is based primarily on conformation evaluation. Over the years, the evaluation methods have been improved, and finally, in many sport horse breeding populations, linear scoring is used. This study aimed to compare differences between jumping and dressage types of warmblood horses according to two systems used simultaneously in Poland (point evaluation on the scale 0–100 pkt [1] and linear profiling [2]). Additionally, a comparison of basic measurements (height at withers, circumference of the trunk, and cannon circumference) was conducted. The data coming from 1500 mares were used. The point scaling for 10 traits was analyzed separately and as a general result. The linear scoring was transformed into the 1–40 points scale for the statistical analysis with a mean value of 20 points [3]. The analysis of variance using the MIXED procedure of the SAS program was conducted with the statistical model including the following fixed effects: sport type, breeding district, and breed. The sire effect was used as a random effect. Correlations between different traits were also calculated using the CORR procedure. Horses of different sports types differ in the circumference of the trunk ($P = 0.0013$), forelimbs point evaluation ($P = 0.0274$), and walk point evaluation ($P = 0.0368$). Dressage horses were stronger in the trunk, with a higher evaluation of forelimbs and walk points. Linear conformation profiling analyses showed differences between both types of horses at the position of the neck ($P = 0.0373$), the position of the shoulder ($P = 0.0036$), the line of loins ($P = 0.0142$), the shape of the croup ($P = 0.0054$), the stance of the pastern ($P = 0.0266$) and quality of legs ($P = 0.044$). Differences observed for these traits were for both types of horses on the same side of the mean (< 20 , > 20). Linear movement profiling showed differences between both types of horses only for the trait balance of the canter ($P = 0.0103$). This trait was less balanced for jumping horses. The traits repeatabilities calculated from the sire effect were low for the basic measurements (0.13–0.30), very low for point evaluation (0.02–0.11), and divergent for linear profiling – general (0.12–0.51) and descriptive (0.03–0.12) – traits. The Pearson correlations between the general marks of the linear evaluation and the 100-point scale were positive and showed a similar direction of the assessment, while the correlations between the 100-point assessments and detailed linear traits of conformation were mostly low negative. According to obtained results, the diversification of horses in jumping and dressage types is small and should be investigated further to obtain the required horse specialization.

Keywords: horse, conformation type, linear profiling, scaling.

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THE EFFECT OF A LOW OR HIGH-FAT DIET AND SUPPLEMENTATION WITH VARIOUS FORMS OF CHROMIUM ON IMMUNOLOGICAL PARAMETERS IN THE RAT'S ORGANISM

Anna Stępniewska¹, Krzysztof Tutaj¹, Przemysław Solek¹, Bartosz Fotschki², Jerzy Juśkiewicz², Paulius Matusevicius³, Katarzyna Ognik¹

¹*Department of Biochemistry and Toxicology, Faculty of Animal Science and Bioeconomy, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland,*

²*Division of Food Science, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Tuwima 10, 10-748 Olsztyn, Poland,*

³*Lithuanian University of Health Sciences, Tilžės 18, 47181 Kaunas, Lithuania*

Corresponding author: paulius.matusevicius@lsmu.lt

The aim of the study was to determine how the administration of a low-fat or high-fat diet supplemented with various forms of chromium to rats affects the immune response in blood plasma. The study schema consisted of two periods, i.e. initial and experimental, 9 weeks each. During the initial 9-week period, the rats aged 6 weeks were randomly assigned to the control group (n = 12) fed standard low-fat C diet and the HF group (n = 72) fed high-fat diet. After the initial period, the rats from the control group were fed the same standard diet for subsequent 9 weeks of the experimental period. The HF rats were then randomly divided into 6 groups with n = 12 per one group. The M group was subjected to the standard low-fat diet, the F group was further fed for subsequent 9 weeks the high-fat diet, the MP group was fed a standard low-fat diet with supplementation of chromium picolinate, the MN group was fed a standard low-fat diet with supplementation of chromium nanoparticles, the FP group was subjected to a high-fat diet with chromium picolinate supplementation, and the FN group was fed a high-fat diet with nanoparticle chromium supplementation. The amount of chromium administered to each rat was 0.3 mg/kg body weight (BW), selected according to the EFSA NDA Panel [2014]. Plasma levels of interleukins (IL-2, IL-6), immunoglobulins (IgA, IgG, and IgM), as well as TNF- α , acute phase protein CRP and ceruloplasmin-Cp were determined. Compared to the control diet (C), the use of a high-fat diet (group F) in the feeding of rats resulted in lower plasma levels of CRP, IgA ($P < 0.001$, respectively). Shortening the duration of the high-fat diet (group M) resulted in an increase in plasma IL-2 levels and a decrease in plasma CRP levels ($P < 0.001$, respectively). Two-way ANOVA analysis showed interactions of diet x chromium addition for IL-2, CRP and Cp ($P < 0.001$, respectively) and IL-6 ($P = 0.032$). The occurrence of the interaction indicates that the studied parameter was influenced by both diet and chromium addition. However, it was found that chromium addition in the form of nanoparticles resulted in lower levels of IgG ($P < 0.001$) and TNF- α ($P = 0.003$). In conclusion, it was found that in the case of prolonged use of a high-fat diet, no elevation of inflammatory parameters was observed, but the addition of chromium in the form of nanoparticles to a high-fat diet, although it did not increase plasma CRP levels, caused an adverse increase in the pro-inflammatory cytokine IL-6. Such an effect was not observed in the case of a shorter use of a high-fat diet with the addition of chromium nanoparticles. On the other hand, in the case of a long-term use of a high-fat diet, the addition of chromium in the form of picolinate favorably reduced plasma levels of CRP and Cp.

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SOIL-DERIVATED PRODUCTS IN POULTRY PRODUCTION

Agila Dauksiene^{1,2}, Dovile Klupsaite¹, Romas Gruzauskas⁴, Vytaute Starkute^{1,3}, Vita Lele^{1,3}, Paulina Zavistanaviciute^{1,3}, Erika Mozuriene¹, Egle Zokaityte^{1,3} and Elena Bartkiene^{1,3}

¹*Institute of Animal Rearing Technologies, Faculty of Animal Sciences, Lithuanian University of Health Sciences, Tilžės str. 18 Kaunas, Lithuania,*

²*Department of Anatomy and Physiology, Faculty of Veterinary Medicine, Lithuanian University of Health Sciences, Tilžės str. 18 Kaunas, Lithuania,*

³*Department of Food Safety, Faculty of Veterinary Medicine, Lithuanian University of Health Sciences, Tilžės str. 18 Kaunas, Lithuania,*

⁴*Department of Food Science and Technology, Faculty of Chemical Technology, Kaunas University of Technology, Radvilėnų str. 19 Kaunas, Lithuania*

Corresponding author: agila.dauksien@lsmu.lt

In recent decades, various zootechnical feed additives, that affect good health, improve digestibility and productivity of broiler chickens have been formulated and applied [1]. In the frame of sustainable poultry production, solid-derived substances as feed additives for poultry have become more promising. Humic acids (HAs) are a naturally occurring component of soil, which is formed by the decomposition of organic matter, especially plants [2]. HAs are involved in metabolic processes in animals, interact with many compounds and structures (organic and inorganic molecules, minerals), thereby promoting the better absorption of minerals necessary to the host [3; 4]. The following study was performed to investigate the effect of dietary HAs supplementation on growth performance, blood indices and *Tibia* development of broiler chickens. The broilers were assigned to 2 treatments: 1 (control group) = basal diet, 2 (HAs group) = basal diet with 2 g/kg of feed HAs. Chicks were raised from 1 to 42 days of age. A corn-soybean meal-based diet (basal diet) was formulated according to the nutritional requirements prescribed in the Ross nutrition specification (2019) and NRC (2004). SPSS software version 15.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. Differences were classified by the Duncan multiple comparison test. Results were considered statistically significant at $P \leq 0.05$. The final body weight (FBW) of broilers increased by 1%, feed conversion ratio (FCR) by 0.6% and mortality by 2% were decreased ($P \geq 0.05$). The blood cholesterol, HDL-cholesterol and LDL-cholesterol concentrations were lower ($P \geq 0.05$) in broiler chickens with HAs. The results showed that the addition of HAs had a positive impact on the broilers' blood Ca, P, Fe levels, compared with the control group ($P \geq 0.05$). The *Tibia* length, weight and strength showed responses to HAs addition to the feed of broiler chickens ($P \geq 0.05$). The results of this study indicate that HAs can be used in broiler feeds. It showed tendencies to increase productivity, blood Ca, P and Fe concentrations and better *Tibia* development of broiler chickens.

Keywords: humic acids, productivity, blood indices, bone development, broiler chickens.

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DOES 43RF-AMIDE MODULATE THE PITUITARY GONADOTROPHE CELLS SECRETORY ACTIVITY IN SHEEP? PRELIMINARY RESULTS

Kinga Pałatyńska, Bartosz Jarosław Przybył, Michał Szlis, Bartłomiej Wysoczański,
Anna Wó

The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences; Jabłonna, Poland

Corresponding author: b.przybyl@ifzz.pl

Peptides involved in the regulation of food intake, synthesized in the central nervous system, are often involved in the controlling of the gonadotrophic axis activity. Premises exist that 43RF-amide (43RFa) may be simultaneously engaged in the functioning of the complex neurohormonal network responsible for the maintenance of the organism energy homeostasis and the regulation of reproductive processes. The aim of this study was to verify the research hypothesis, which assumes that 43RFa (orexigenic neuropeptide, belonging to RF-amide peptides) can be engaged in the modulation of the pituitary gonadotrophes secretory activity in sheep. The experiment was performed on sexually mature Polish Merino sheep (n = 48). Animals were divided into three groups. The following intracerebroventricular infusions were performed: control group (Ringer-Locke solution), group I (43RFa in dose 10µg/480µL/day), and group II (43RFa in dose 50µg/480µL/day). After the experiment, the animals were slaughtered: the pituitaries were stored for Real Time RT qPCR or immunohistochemistry and plasma samples were stored for radioimmunoassay analysis. Preliminary results showed that central infusion of 43RFa at dose of 50µg/480µL/day increase *fsHβ* mRNA expression in pituitary cells. At the same time, no changes in FSH blood concentration were noted after 43RFa treatment in any groups of sheep. Furthermore, received results showed no changes in *lhβ* gene expression as well as LH concentration in blood plasma in all investigated groups of sheep. Based on the presented results it can be concluded that 43RFa can modulate the FSH, but not LH secretory activity in pituitary cells in sheep but further studies, especially immunohistochemical determinations, need to be done to confirm those observations.

Keywords: sheep reproduction, FSH, LH, orexigenic neuropeptide.

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FACTORS DETERMINING GOAT MILK AMINO ACIDS AND FATTY ACIDS

Evaldas Šlyžius¹, Lina Laučienė², Lina Anskienė¹, Birutė Šlyžienė³, Renata Bižienė⁴

¹Lithuanian University of Health Sciences, Faculty of Animal Science, Department of Animal Breeding, Kaunas, Lithuania,

²Lithuanian University of Health Sciences, Faculty of Veterinary Medicine, Department of Food Safety and Quality, Kaunas, Lithuania,

³Small Community "Research Gamma", Kaunas, Lithuania,

⁴Lithuanian University of Health Sciences, Faculty of Animal Science, Institute of Biology Systems and Genetic Research, Kaunas, Lithuania

Corresponding author: evaldas.slyzius@lsmu.lt

The aim of this study was to estimate the influence of factors on milk amino acids and fatty acids of the dairy goats. The research was carried out at the Lithuanian dairy goat farm with Saanen and Alpine goat breeds ($n = 97$). Analyses of the amino acids and fatty acid composition of goat milk were carried out with SHIMADZU gas chromatographer. Statistical analysis was conducted with statistical package SPSS 25.0 (SPSS, Inc., Chicago, IL, USA). Results were considered to be reliable when $P < 0.05$. The present study showed that the milk yield was higher in Saanen goats, but their milk was significantly lower in fat and protein content than that of Alpine goats ($P < 0.05$). The quantity of all individual amino acids and their groups (essential (EAA), nonessential (NEAA), total (TAA), and branched-chain (BCAA)) was significantly higher in Alpine compared to Saanen goat milk ($P < 0.01$). An analysis of amino acids in milk from different seasons showed that the highest content of both essential and nonessential amino acids was found in the grazing season ($P < 0.05$), except for arginine and alanine acids the content of which was slightly higher during the housing period and for glycine acid the content of which was the same in both seasons. The individual fatty acids ranged between goat breeds while the total amount of SFA, UFA, and MUFA was not significantly different between the breeds ($P < 0.05$). An analysis of fatty acids in milk from different LPL genotype showed that the highest content of fatty acids was determined in the CG genotype of the LPL gene (on average 11.77% higher than in the CC and GG genotypes) of goats ($P < 0.01$). The milk of CC and GG genotypes was similar for MCFA, LCFA, SFA, UFA, MUFA and PUFA percentages. Meanwhile, the CG genotype milk had significantly more MCFA and SFA, and less UFA, MUFA and PUFA than the CC and GG genotype milk ($P < 0.05$). The highest content of short-chain and medium-chain fatty acids was found in the grazing season, and that of long-chain fatty acids – in the housing season goat milk ($P < 0.05$). Analysis of variance revealed that the goat breed, LPL genotype and season showed a statistically reliable impact on amino acid and fatty acid content ($P < 0.01$).

Keywords: goat, milk, amino acids, fatty acids.

TRENDS IN THE COURSE OF PARTURITION OF POLISH HOLSTEIN-FRIESIAN COWS

Dariusz Piwczyński, Beata Sitkowska, Magdalena Kolenda

*Bydgoszcz University of Science and Technology, Faculty of Animal Breeding and Biology,
Department of Animal Biotechnology and Genetics / 85-084 Bydgoszcz, Poland*

Corresponding author: darekp@pbs.edu.pl

The ease of the course of labour contributes to low calf mortality, thus improving the economic performance of dairy farms. In Poland, the course of labour is expressed on a 6-point scale: CE1 – independent delivery (performed by natural forces), CE 2 – easy delivery (completed with little human interference), CE3 – difficult delivery (completed with the use of more force than normally), CE4 – complicated delivery (related to surgery, damage to a cow or calf), CE5 – miscarriage, and CE6 – caesarean section. In numerous studies, however, a simplified classification is used, i.e., easy (CE1 + CE2) and difficult (CE3 + CE4, CE6) [1]. The aim of the research was to analyse the trends in the delivery of Holstein-Friesian cows (from 2014 called Polish Holstein-Friesian) of the Black and White variety used in 9 highly productive herds. A total of 39,346 cows calving in 2008–2021 were included in the study. Cows were controlled in terms of the course of the delivery expressed in the bipartite scale (easy, difficult). Moreover, data on the milk yield of cows for full lactation, the body weight of calves after birth with division into pregnancy size (1–3), and perinatal mortality (the number of stillborn calves and those who died in the perinatal period) were recorded. The statistical analyses were performed separately for the group of heifers/primiparous (37.317 cows) and multiparous cows (28.779 cows). Development tendencies in terms of the controlled traits were established using regression analysis. Based on the research carried out in 2008–2021, it was shown that the share of easy births among heifers was 94.65%, and the perinatal mortality of their offspring was 5.29%. The corresponding rates for multiparous cows were 96.97% and 2.77%, respectively. It should be emphasised that the level of both these features in 2008–2021 was stable ($P > 0.05$). Moreover, a favourable trend was found in the scope of milk yield: in the group of primiparous cows, it increased annually by over 34 kg, and in multiparous cows by 82 kg. At the same time, the increase in the body weight of calves from single pregnancies born to heifers (by 0.11 kg/year) and multiparous cows (by 0.07 kg /year) was observed. It was found that in 2008–2021, the share of easy deliveries increased insignificantly, which was accompanied by a systematic increase in the lactation milk performance of heifers and multiparous cows.

Keywords: reproduction, delivery type, mortality, calf body weight.

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GENE AND HORMONE IMMUNOCASTRATION IN SMALL RUMINANTS

Özdal Gökdal

Aydın Adnan Menderes University, Çine Vocational School, Department of Plant and Animal Production, Aydın, Türkiye

Corresponding author: ogokdal@adu.edu.tr

Castration is carried out in order to stop the production of male hormones and spermatozoa production by removal of testicles or by in situ destruction of testicular function. Nevertheless, physical castration has negative effects on some characteristics such as feed efficiency, growth rate and carcass characteristics. Besides, its painful effects and different levels of stress in animals are questioned in terms of animal welfare. Immunization against reproductive hormones could be an alternative means of surgical castration methods for various practical and experimental reasons [1–5]. The hormone immunocastraton is based on inducing antibodies against gonadotropin releasing hormone (GnRH). Immunization against GnRH has been described as one of the effective means to reduce reproductive functions in farm animals [6]. Gene immunization is a novel immunization method that is performed by constructing a plasmid encoding an exogenous gene and injecting it into an animal [7]. In this review, gene and hormone immunocastration in sheep and goats were evaluated in terms of reproductive characteristics, growth, carcass, meat quality and feed efficiency.

Keywords: DNA, GnRH, castration, sheep, goat.

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EFFECT OF EARLY ADMINISTRATION OF AN ANTIBIOTIC OR FEEDING A DIET CONTAINING A COCCIDIOSTAT ON THE LEVELS OF MINERALS IN YOLK SAC AND BLOOD IN TURKEYS

**Radosław Smagiel¹, Ewelina Cholewińska¹, Magdalena Krauze¹, Anna Stępniewska¹,
Dariusz Mikulski², Katarzyna Ognik¹, Jan Jankowski²**

¹University of Life Sciences in Lublin, Department of Biochemistry and Toxicology, Akademicka 13, 20-950 Lublin,

²University of Warmia and Mazury in Olsztyn, Department of Poultry Science and Apiculture,
Oczapowskiego 5, 10-719 Olsztyn, Poland

Corresponding author: radoslaw.smagiel@up.lublin.pl
magdalena.krauze@up.lublin.pl

The study verified the hypothesis that that early administration of the antibiotic or vaccinations may affect the levels of minerals in the yolk sac and blood plasma of turkeys. The experiment was conducted on female turkeys in a two-factor design (2×4), which included 2 groups of birds (vaccinated, unvaccinated) and 4 treatments (CON, MON, ENR, DOX). Half of the birds were vaccinated against aMPV and NDV on day 1 and against ORT on day 28. MON turkeys were given the coccidiostat monensin in the feed for 56 days. ENR and DOX turkeys received enrofloxacin or doxycycline for the first 5 days of life. Birds in the control group (CON) received no coccidiostat or antibiotics. On days 1, 3 and 5 of the birds' lives, yolk sacs were collected post mortem from 21 birds in the group (3 birds from the repetition). On days 1, 3, 5, 7 and 56, blood was collected from 21 birds in the group to assess Ca, P, Mg, Cu, Zn, and Fe levels. Two-way ANOVA showed antibiotic x vaccine interactions for levels of Ca, P, Mg, Zn, Cu and Fe ($P < 0.001$, respectively) in yolk sacs taken from both 3 and 5-day-old turkeys. The antibiotic x vaccine interaction was also found for Ca, Cu levels ($P < 0.001$ respectively) in blood plasma collected from 3, 5 and 7-day-old turkeys. For P levels ($P < 0.001$) in blood plasma, antibiotic x vaccine interactions were found in 3, 5 and 56-day-old turkeys. For Fe levels ($P < 0.001$) in blood plasma, antibiotic x vaccine interactions were found in 5, 7 and 56-day-old turkeys, while for Zn levels ($P < 0.001$) in blood plasma, antibiotic x vaccine interactions were found in 3, 7 and 56-day-old turkeys. The recorded interactions indicate that the tested levels of mineral indicators in yolk sacs and blood were affected by both the antibiotic and vaccine used. Only the application of MON resulted in a reduction of Ca levels ($P = 0.031$) in the blood plasma of 56-day-old turkeys. In contrast, vaccination of turkeys resulted in an increase in plasma P levels ($P = 0.025$) in 7-day-old turkeys, and Ca levels ($P < 0.001$) in 56-day-old turkeys. Short-term administration of the antibiotic has no significant effect on the levels of Ca, P, Mg, Cu, Zn, and Fe in the yolk sac and blood plasma of growing turkeys. On the other hand, long-term administration of MON may result in decreased Ca levels in the blood plasma.

Keywords: antibiotics, yolk sac, blood, minerals.

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RELATIONSHIP BETWEEN B-CASEIN GENOTYPES (A1A1, A1A2, AND A2A2) AND COAGULATION PROPERTIES OF MILK AND THE FATTY ACID COMPOSITION AND SENSORY CHARACTERISTICS OF DAIRY PRODUCTS (SOFT CHEESE, SOUR CREAM, AND BUTTER)

**Kristina de Vitte¹, Sigita Kerzienė², Jolita Klementavičiūtė³, Marius de Vitte⁴,
Vaida Dilbiene¹, Rolandas Stankevičius¹**

¹Department of Animal Nutrition, Lithuanian University of Health Sciences, Faculty of Animal Husbandry Technology,
Tilžės str. 18, LT-47181, Kaunas, Lithuania,

²Department of Animal Breeding, Lithuanian University of Health Sciences, Faculty of Animal Husbandry Technology,
Tilžės str. 18, LT-47181, Kaunas, Lithuania,

³Institute of Animal Rearing Technologies, Faculty of Animal Sciences, Lithuanian University of Health Sciences,
Mickevičiaus str. 9, LT-44307 Kaunas, Lithuania,

⁴Coventry University, Priory St, Coventry CV15FB, England, United Kingdom

Corresponding author: kristina.de.vitte@lsmu.lt

Milk products are a source of lipids, carbohydrates, proteins, minerals, and vitamins. Although milk is a staple in the diets of many cultures, the demands for specific milk products differ between consumers (some are allergic to cow's milk proteins or incapable of digesting lactose). However, such consumers are not without options, and multiple studies suggest that milk containing only A2 beta-casein may be an alternative. [1] concluded that people with lactose intolerance reported less abdominal pain while consuming milk containing only A2 beta-casein. Furthermore, a review by [2] concluded that A2 beta-casein milk is a potential remedy for people with a milk protein allergy. We hypothesized that because the *CSN2* genotype (A1A1, A1A2, and A2A2) influences milk's biochemical composition, it should also influence the final product. The study aimed to investigate the viability of breeding crossbred dual-purpose Simmental cows exclusively towards the A2 variant beta-casein by investigating the influence of the *CSN2* genotypes, with a focus on the A2A2 genotypes' influence on the coagulation traits, fatty acid composition, and sensory characteristics of different milk products (soft cheese, sour cream, and butter). We assigned 15 crossbred cows (1/2 Swedish red X 1/4 Simmental X 1/2 Holstein Friesian) into three groups (A1A1, A1A2, and A2A2) of five cows per group. Milk samples for milk products (soft cheese, sour cream, and butter) production were taken following the ISO 707:2008 protocol [3]. Milk coagulation properties were determined by exposing milk samples to rennet enzyme solution. Rennet coagulation time and curd-firming rate were fastest in A2A2 milk and slowest in A1A1 milk. Accordingly, curd firmness was highest in A2A2 milk and lowest in A1A1 milk. A1A1 milk products had higher levels of monounsaturated fatty acids and lower levels of saturated fatty acids. Polyunsaturated fatty acids were mainly found in A2A2 milk products and were lowest in A1A2 products. Although *CSN2* genotypes influenced milk coagulation traits and fatty acid content of milk products, they did not influence the latter's sensory traits.

Keywords: cow milk, MUFA, PUFA; SFA, β -casein, *CSN2*.

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BOVINE COLOSTRUM EXERTS IMMUNOMODULATORY ACTIVITY ON INNATE IMMUNE SYSTEM AND INTESTINAL EPITHELIUM BY PASSIVELY TRANSFERRED PROTEINS

Ramunė Grigalevičiūtė^{1,3}, Rita Plančiūnienė², Vilma Zigmantaitė¹, Audrius Kučinskas¹, Paulius Matusevičius³, Povilas Kavaliauskas^{1,4,5,6}

¹Biological research center, Lithuanian University of Health Sciences, Kaunas, Lithuania,

²Institute of Microbiology and Virology, Lithuanian University of Health Sciences, Kaunas, Lithuania,

³Department of Animal Nutrition, Lithuanian University of Health Sciences, Kaunas, Lithuania,

⁴Joan Sanford I. Weill Cornell Medical College, New York, United States,

⁵Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, United States,

⁶Institute of Infectious Diseases and Pathogenic Microbiology, Prienai, Lithuania.

Corresponding author: ramune.grigaleviciute@lsmu.lt

Bovine colostrum (BC) is the first milk produced by lactating cows after parturition. BC is rich in numerous bioactive components that ensure the nutrition of a neonate calf, confers passive protection against pathogens and promotes the gut colonization with beneficial microbiota. Despite the numerous efforts to understand BC effects on the development of neonates, very little is known about the complex protein composition of BC. In order to better understand the BC proteome and changes at different time points, BC was collected at 1–4 hours postpartum. BC samples collected from animals (n = 10) at each time point were pooled together. The BC serum was produced by gradient centrifugation and total proteins were digested into peptides using trypsin, alkylated and subjected to mass spectrometry. Proteins were identified using the reference genome of *Bos taurus*. In total, 553 proteins were successfully identified in BC samples collected over 4 hours postpartum. Identified proteins represented coagulation factors (factor V–XII), growth factors (IGF, EGF, GMS), immune markers (CD177, CD44, CD5, CD81), enzymes (MPO, ENO1) and others. Carboxipeptidase 2 (CPN2) was only detected 4 hours BC and was absent in the 1–3 hour samples. Moreover, collagen alpha-1 was only detected at 1 hour and was absent in 2–4 hour BC samples. Finally, BC was highly enriched by numerous innate immune response proteins, such as antimicrobial peptides (Cathelicidin 1-9, complement), lipocalin, TNF receptor superfamily proteins and others. Bovine colostrum secreted during the first four hours postpartum is rich in numerous nutritious and immunomodulatory proteins. The high amounts of antimicrobial peptides, immunoglobulins and protective factors confer passive protection for calves. Further studies are needed to better understand these proteins role during calves growth and development.

Keywords: bovine colostrum, intestinal homeostasis, immunomodulation.

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INFLUENCE OF LACTIC ACID BACTERIA AND ROSEMARY ESSENTIAL OIL ON ILE DE FRANCE LAMB BREED MEAT (*MUSCULUS GLUTEUS*) QUALITY PARAMETERS

Vilija Buckiuniene, Dovile Klupsaite, Paulina Zavistanaviciute, Vytaute Starkute, Vita Lele, Jolita Klementaviciute, Elena Bartkiene

Institute of Animals Rearing Technology, Faculty of Animal Science, Lithuanian University of Health Sciences, Kaunas, Lithuania

Corresponding author: Vilija.Buckiuniene@lsmu.lt

The aim of this study was to evaluate *Lactobacillus paracasei* (LUHS244), rosemary essential oil (0.1% v/v) and their combination to treat Ile de France lamb meat. Changes in the microbiological profile and physiochemical parameters of meat were evaluated after 24 h of treatment at 4°C. The experiment resulted in significantly lower mold/yeast ($P < 0.05$), water-holding capacity (by 49–63%), and coking loss (by 9.05–11.31%) in all trial groups. Moisture content in all groups had a tendency to decrease from 7.09% to 8.49%. Polyunsaturated fatty acid content increased in all experimental groups. The content of malondialdehydes increased in all experimental groups from 4.7 to 10.1 times ($P < 0.05$) compared to the control group. For the cholesterol content in lamb meat, the highest effect was determined where meat was marinated only with rosemary essential oil (it decreased by 22% ($P < 0.05$)). Treatments significantly affected some biogenic amines. Sensory analysis showed that raw lamb meat colour was more acceptable after all treatments, while the odour acceptability was ranked highest after combined treatment. In conclusion, this treatment showed that it could be used to improve microbiological safety and some quality characteristics, increasing PUFA content of lamb meat.

Keywords: lamb meat quality, lactic acid, essential oil.

LAYING HENS' AGE INFLUENCE ON EGG QUALITY AND CONSUMER ACCEPTABILITY

Šarūnė Aranauskaitė, Asta Racevičiūtė-Stupelienė, Monika Nutautaitė, Vilma Vilienė

*Institute of Animal Rearing Technologies, Veterinary Academy, Lithuanian University of Health Sciences,
Tilžės str. 18, LT-47181 Kaunas, Lithuania*

Corresponding author: Sarune.Aranauskaite@lsmu.lt

From a nutritional perspective, eggs are particularly important since they include essential lipids, proteins, vitamins, minerals, and trace elements. They also provide a moderate calorie source (about 140 kcal/100 g), have a wide range of culinary applications, and are affordable as they are the second-cheapest animal source of zinc and calcium and the lowest-cost source of proteins, vitamin A, iron, vitamin B12, riboflavin, and choline [1]. However, egg producers should be aware of the age at which laying hens produce eggs of the finest quality. Therefore, the aim of this study was to compare the nutritional value of eggs produced by laying hens of different age groups. Research was performed on eggs produced by these laying hen age groups: 1-year-old (LH1), 2-year-old (LH2), and 3-year-old (LH3). Laying hens were raised under the same feeding and housing conditions. The following indicators were determined in fresh and stored (for 28 days) eggs: height of egg, yolk, and albumen; Haugh unit; yolk colour intensity; eggshell thickness; yolk and albumen pH; dry matter (DM), fat, ashes, and protein contents in the yolk; the sensory profile of fresh eggs. When compared to LH1 fresh eggs, the LH2 fresh eggs were found to be 16% higher and to have substantially redder (a^*) egg yolks ($P < 0.05$). The weight of the egg yolk was found to be highest in LH3 and lowest in LH1 ($P < 0.05$). Comparing LH2 to LH1, the ratio of egg yolk to egg mass was 42% higher ($P < 0.05$). LH1 had the lowest egg albumen index, while LH3 had the highest. Compared to LH3 eggs, the albumen index in LH1 eggs was even 49% lower ($P < 0.05$). However, as compared to LH2 eggs, the egg yolk index for LH1 eggs had the greatest value (26%) ($P < 0.05$). Meanwhile, fresh egg albumen's height was 31% higher in LH2 than in LH3 ($P < 0.05$). When comparing distinct age groups of laying hens, no significant variations in the chemical composition of the eggs were discovered. After storage, egg weight in LH2 was 24% higher than it was in LH1 ($P < 0.05$). The egg yolk index after storage was 9% higher in LH1 than in LH3, and the egg mass to yolk ratio was 37% greater in LH2 than in LH1 ($P < 0.05$). When analysing eggshell strength, it was found to be 19% higher in LH1, compared to the eggs of LH3 ($P < 0.05$). After evaluating the sensory profile and consumer acceptability of the egg albumen, it was found that the albumen in LH1 was 10% harder, compared to LH2 ($P < 0.05$); the general flavour intensity of the yolk was most noticeable in the eggs of LH3, and the least in LH1 ($P < 0.05$). However, LH2 yolk residual flavour intensity was 13% lower than in LH3 ($P < 0.05$). According to the recent study's findings, in order to produce fresh, high-quality eggs that consumers would be satisfied with, it is appropriate to regularly replace the flock of farmed laying hens with younger ones.

Keywords: laying hens, influence of age, egg quality, comparative analysis.

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REDOX STATUS OF RATS FED A DIET CONTAINING VARIOUS SOURCES OF DIETARY FIBER AND COPPER NANOPARTICLES

Aleksandra Marzec¹, Radosław Smagiel¹, Ewelina Cholewińska, Magdalena Krauze, Bartosz Fotschki², Jerzy Juśkiewicz², Paulius Matusevicius³, Katarzyna Ognik¹

¹Department of Biochemistry and Toxicology, Faculty of Animal Science and Bioeconomy, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland,

²Division of Food Science, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Tuwima 10, 10-748 Olsztyn, Poland,

³Lithuanian University of Health Sciences, Tilžės str. 18, 47181 Kaunas, Lithuania

Corresponding author: Paulius.Matusevicius@lsmu.lt

The aim of this study is to verify the statement that the metabolic effect of copper nanoparticles (Cu-NPs) is strongly reliant on the physiological function of different dietary fibre types. We hypothesized that a dietary combination of Cu-NPs with either a control inert (cellulose) or a prebiotic (inulin) or a viscous (pectin) or a bulking (psyllium) fibre would stimulate immune response of rats. Rats were fed for 6 weeks a standard diet with dietary addition of two Cu-NP dosages (recommended and two times higher; 6.5 and 13 mg/kg diet) and combined with different types of dietary fibre; 6% of a diet (control – cellulose, inulin with a prebiotic effect, psyllium with a bulk effect, pectin with a viscous effect; 10 groups, n = 10 per group). Blood was collected from 10 rats from the group for the determination of immunological parameters. Nanoparticles increase the stimulation of the immune system, but determining whether this effect should be considered beneficial requires a wider range of research. Plasma levels of superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), glutathione (GSH and GSSG) were determined in the blood plasma of rats by Elisa assays. It was found that compared to a diet containing cellulose, replacing it with pectin or inulin or psyllium resulted in lower SOD levels ($P < 0.001$). A fiber x Cu-NP dose interaction was noted for MDA, GSSG ($P < 0.001$ both). The interaction for MDA was due to the fact that in diets containing cellulose and pectin, increasing Cu from 6.5 mg/kg to 13 mg/kg in the diet had no effect on MDA levels, while in diets containing inulin resulted in an increase in MDA levels, and in diets containing psyllium in a decrease of this parameter. The interaction for GSSG was due to the fact that in diets containing cellulose or pectin or psyllium, increasing the Cu dose from 6.5 mg/kg to 13 mg/kg had no effect on the level of oxidized glutathione, while in diets containing inulin, increasing the Cu dose resulted in a decrease in the level of this parameter. It was established that it is beneficial to use fiber in the diet of rats in the form of inulin, especially with an increased dose of copper nanoparticles (13 mg/kg), as lipid oxidation can be reduced in this way.

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DIVERSE DIFFERENTIATION PATTERNS BETWEEN RABBIT PREIMPLANTATION EMBRYOS DEVELOPED IN VITRO VERSUS IN VIVO

Anna Chołoniowska^{1,2}, Jan Chołoniowski³, Elżbieta Wenta-Muchalska¹, Katarzyna Filimonow¹, Anna Piliszek¹

¹Department of Experimental Embryology, Institute of Genetics and Animal Breeding of the Polish Academy of Sciences, Jastrzębiec, Poland,

²Department of Neurogenetics and Functional Genomics, Mossakowski Medical Research Institute of the Polish Academy of Sciences, Warsaw, Poland,

³Faculty of Physics, Center of Excellence for Complex Systems Research, University of Technology, Warsaw, Poland

Corresponding author: a.piliszek@igbzpan.pl

Proper differentiation and segregation of the first three cell lineages in the mammalian embryo – epiblast (EPI), primitive endoderm (PrE) and trophoctoderm (TE) – play a crucial role in embryo development. The knowledge about specification processes and timing is mostly based on the mouse model studies. Here, we focus on non-rodent model: rabbit. Previously, we analyzed specification of rabbit EPI, PrE and TE at different time points in *in vivo* preimplantation rabbit embryos. Here we compare the timing and distribution of these lineages in *in vitro* cultured embryos with embryos obtained *in vivo*, to determine how culture conditions affect the differentiation potential of a rabbit embryo. Popielno breed rabbit zygotes (19 hours *post coitum*, hpc) were cultured *in vitro* in commercially available sequential culture media: 1) G-1™ PLUS/G-2 PLUS™ (Vitrolife); 2) ORIGIO® Cleav/ORIGIO® Blast (CooperSurgical); and single step media, 3) Global®Total®, and 4) SAGE 1-step™ (CooperSurgical). Embryos cultured in single step RDH medium served as *in vitro* culture control. After culture, 120 hpc embryos (5 dpc) were fixed and immunostained. Cell number and specific lineage differentiation were assessed based on localization of lineage-specific transcription factors: CDX2 for TE, SOX17 for PrE and SOX2 for EPI. Cell lineage distribution in *in vitro* cultured embryos was compared to *in vivo* embryos from stage 3 dpc to 5 dpc. In all analyzed culture media, more than 80% of the rabbit zygotes reached the blastocyst stage. Nonetheless, the pattern of differentiation significantly varied between the analyzed media. Moreover, *in vitro* cultured rabbit embryos had significantly fewer cells and fewer differentiated cells compared to *in vivo* ones. Our results show that 5 dpc *in vitro* cultured embryos are developmentally delayed. After culture in Global medium, the embryos show a similar distribution of trophoctoderm and primitive endoderm lineages as 3.25 dpc *in vivo* derived embryos. Culture in ORIGIO or RDH medium results in the expression pattern more typical for 3.75 dpc *in vivo* embryos. All the presented results were statistically significant.

Keywords: rabbit, preimplantation embryo, differentiation, *in vitro* culture.

DOES CORRELATION BETWEEN LENGTH OF INFECTION AND ACUTE PHASE PROTEINS GENE EXPRESSIONS IN SOMATIC CELLS FROM MILK OF SRLV-INFECTED DAIRY GOATS EXIST?

Daria Urbańska¹, Justyna Jarczak², Michał Czopowicz³, Jarosław Kaba³, Emilia Bagnicka¹

¹Institute of Genetics and Animal Biotechnology PAS, Postępu 36A, Jastrzębiec, Poland,

²Nencki Institute of Experimental Biology, PAS, Ludwika Pasteura 3, Warszawa,

³Warsaw University of Life Sciences, Institute of Veterinary Medicine, Nowoursynowska 159 C, Warsaw, Poland

Corresponding author: Daria.m.urbanska@gmail.com

Caprine arthritis-encephalitis (CAE) is a result of a small ruminant lentivirus (SRLV) infection. One of the CAE symptoms can be mastitis. Thus, CAE is an economic problem [1]. Acute-phase proteins (APPs) participate in immune defense during various infections. Their activation is very important to the reinstatement of homeostasis. In goats, seven proteins belong to APPs: serum amyloid (SAA), haptoglobin (*Hp*), ceruloplasmin (*Cp*), C-reactive protein (*CRP*), alpha-1 acid glycoprotein (*AGP*), fibrinogen (*Fb*), and α -lactalbumin (*LALBA*) [2, 3, 4]. The goal of the study was to estimate the correlation between the length of infection and expressions of APPs genes in milk somatic cells (MSC) of SRLV-seropositive goats as well as correlation with milk traits being indicators of mastitis such as MSC count (MSCC), total protein and lactose contents. The study was conducted on 12 dairy goats (n = 6 Polish White Improved, n = 6 Polish Fawn Improved) being between 2nd and \geq 4th parity, naturally infected with SRLV and confirmed at least twice using ELISA test, but without clinical CAE symptoms. The milk was collected five times during lactation (day 1, 30, 60, 120, 180 day). The gene expression was measured using the qPCR method with *cyclophilin A* as a reference. Pearson correlation with PROC CORR of SAS package was used to estimate the associations. The duration of the SRLV infection was not correlated with expressions of any studied APPs gene. However, the longer the infection lasts, the higher the MSCC (0.47, $P < 0.0001$) and total protein (0.39, $P = 0.0006$) were, but not lactose contents. Moreover, correlations between *AGP* and *Cp* (0.41, $P = 0.0019$), *AGP* and *CRP* (0.66, $P < 0.0001$), *AGP* and *Hp* (0.63, $P < 0.0001$), *Cp* and *CRP* (0.36, $P = 0.0048$), *Cp* and *Hp* (0.41, $P = 0.0010$), *Cp* and *LALBA* (-0.57, $P < 0.0001$), and *CRP* and *Hp* (0.65, $P < 0.0001$) gene expressions were found. No correlation between the length of infection and APPs gene expressions may suggest that the length of infection in asymptomatic goats does not trigger an acute immune response in the udder. However, increased MSCC and total protein content in parallel to infection may, however, indicate inflammation but probably subclinical and mild. The relationship between expressions of some APPs gene probably indicated synergism or antagonism (*Cp* and *LALBA*) in their activity.

Keywords: SRLV, goat, milk somatic cell, acute phase proteins genes, correlations.

INFLUENCE OF PUERPERAL METRITIS ON THE RECOVERY OF THE ESTROUS CYCLE AFTER CALVING IN MODERN DAIRY COWS

Vilmantas Juodžentis^{1,2}, Giedrius Palubinskas², Jūratė Šiugždaitė³, Vytuolis Žilaitis¹, Henrikas Žilinskas¹

¹Large Animals Clinic, Faculty of Veterinary, Veterinary Academy, Lithuanian University of Health Sciences, Tilžės str. 18, Kaunas, Lithuania,

²Animal Breeding Department, Faculty of Animal Sciences, Veterinary Academy, Lithuanian University of Health Sciences, Tilžės str. 18, LT-47181, Kaunas, Lithuania,

³Department of Veterinary Pathobiology, Faculty of Veterinary, Veterinary Academy, Lithuanian University of Health Sciences, Tilžės str. 18, LT-47181, Kaunas, Lithuania

Corresponding author: vilmantas.juodzentis@lsmu.lt

The aim was to determine how puerperal metritis influences the recovery of estrous cycle in modern dairy cows. The study was carried out on lactating Holstein cows from a commercial dairy farm located in Lithuania. The cows were housed in freestall barns with access to fresh water ad libitum and were fed a total mixed ration supplemented with the concentrate based on milk yield. The cows were selected between day 5 to 14 after calving (day 0 = day of calving). The cows were divided into two different groups: multiparous cows after puerperal metritis treatment (M (n = 34)) and multiparous cows without signs of puerperal metritis (H (n = 38)). All 72 cows were divided into groups after their first ovulation: HSO (n = 29) – cows without signs of puerperal metritis and with a single ovulation; MSO (n = 21) – cows after puerperal metritis treatment with a single ovulation; HDO (n = 9) – cows without signs of puerperal metritis and with a double ovulation; and MDO (n = 13) – cows after puerperal metritis treatment with a double ovulation. The changes in ovaries were examined using a digital diagnostic ultrasound scanner (Dramiński iScan, Dramiński S.A., Olsztyn, Poland) at a frequency of 7.5 MHz, using a linear rectal transducer. The first dominance of the follicle postpartum was recorded when at least one of the follicles reached 8.5 mm in diameter. To detect follicle ovulation, the cows were monitored by ultrasound machine three times a week (Monday, Wednesday, Friday). Ultrasonography was started on day 5 postpartum and was continued until the follicle ovulation was diagnosed. The statistical analysis was performed using computer software (SPSS Inc., Chicago, IL, USA) SPSS 22.0. A probability below 0.05 was considered reliable.

The mean time to the first follicle deviation postpartum was longer in the MSO group compared with the HSO group, 8.9 ± 1.6 and 6.8 ± 1.8 days postpartum, ($P = 0.002$). The same tendency was observed in the MDO and HDO groups, 9.5 ± 1.3 and 7.0 ± 1.4 days postpartum, ($P = 0.002$). We found that in the HDO group the first dominant wave follicle's ovulation was more frequent when in the MDO group, 55.6% and 23.1%, ($P = 0.027$). Moreover, the HDO group cows ovulated their follicle during the first follicular wave faster compared to the MDO group (11.4 ± 2.7 day and 20 ± 1 day, ($P = 0.01$). Also, cows of the HDO group had a smaller diameter of the ovulatory follicles compared to the cows of the MDO group (15.3 ± 1.9 mm and 17.3 ± 1.7 mm, $P = 0.04$).

In conclusion, dairy cows which have had puerperal metritis need more time until the first follicle deviation postpartum. Also, healthy cows have a higher frequency for double ovulation in the first dominant wave postpartum.

Keywords: metritis, postpartum, ovulation, follicle.

RELATIONSHIP BETWEEN LACTATION NUMBER, MILK YIELD AND COMPOSITION IN DAIRY COWS

Jūratė Rudejeviene^{1*}, Ramutė Mišeikienė²

¹Dr. L.Kriauceliunas Small Animal Clinic, Veterinary Academy, Faculty of Veterinary Medicine, Lithuanian University of Health Sciences, Tilžės str. 18, Kaunas, Lithuania,

²Institute of Animal Rearing Technologies, Veterinary Academy, Lithuanian University of Health Sciences, Tilžės str. 18, Kaunas, Lithuania

Corresponding author: jurate.rudejeviene@lsmu.lt

Milk production increases 30% from the first to fifth lactation, but the percentage increase progressively decreases with age. The milk yield are increased with an increasing lactation number, which may be due to increasing development and size of the udder with a resulting increase of secretory cells. Other reasons for high milk yield may be increased parity, which plays a significant role in the control of the tissue mobilization between the primiparous and multiparous cows, and includes increasing body weight of dairy cattle over that of first lactation. The lower milk yield in first lactation cows, because their mammary gland and mammary vein not well developed [1]. Milk composition also changes over lactation [2]. The aim of this work was to investigate the relationship between lactation with milk yield and composition in dairy cows. The study was carried out on 80 Holstein lactating dairy cows, in accordance with the Law on the Care, Keeping and Use of Animals of the Republic of Lithuania. Milk production was determined by control milking. Milk composition (milk proteins, milk fat, lactose concentration and somatic cell count) was determined in the laboratory at Joint Stock Company "Pieno tyrimai". According to the lactation number, cows were divided into 5 groups: 1st lactation (n = 15), 2nd (n = 17), 3rd (n = 14), 4th (n = 18) and 5th (n = 16). The statistical analysis was performed using computer software SPSS 22. Data was statistically significant when $P < 0.05$. During the study, results showed that the fattest milk was in 4th lactation cows, which was $4.49 \pm 0.05\%$. The leanest milk was in the milk of 1st lactation cows, $4.31 \pm 0.05\%$ ($P < 0.05$). Milk whiteness differed slightly in all lactation cows. The highest milk protein content was found in lactating milk of 2nd and 5th lactation cows ($3.28 \pm 0.03\%$). The highest amount of lactose in milk was found in the milk of 1st and 4th lactation cows ($4.73 \pm 0.02\%$). The highest amount of milk was milked from 4th lactation cows (31.77 ± 1.19 kg), and the smallest in 5th lactation cows (28.21 ± 2.03 kg, $P > 0.05$). Examination of the influence of lactation on the number of somatic cells demonstrated that the SLS in the milk of 1st lactation cows was the lowest in comparison with other lactations of cows and amounted to 232.40 thousand / mL. ± 44.33 thousand / mL, which is 11% less than in the milk of 2nd lactation cows. The milk of 5th lactation cows had the highest number of somatic cells in comparison with other lactations and amounted to 328.38 thousand / mL ± 53.83 thousand / mL ($P < 0.05$).

Keywords: milk yield, composition, lactation, cows.

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IDENTIFICATION AND PREVALENCE OF MILK PROTEIN B-CASEIN A1 AND A2 GENETIC VARIANTS IN THE DAIRY CATTLE POPULATION

Kristina Morkūnienė, Renata Bižienė, Simona Birutė Ašmonaitė, Nijolė Pečiulaitienė, Ramutė Mišeikienė, Laimutis Kučinskas

Institute of Biology Systems and Genetics, Veterinary Academy, Lithuanian University of Health Sciences, Tilžės str. 18, LT-47181 Kaunas, Lithuania.

Corresponding author: kristina.morkuniene@lsmu.lt

The aim of this work was to identify prevalence of milk protein β -casein A1 and A2 variants in dairy cattle population. β -casein is encoded by exon 7 of the CSN2 gene. The protein encoded by the gene consists of 209 amino acids (molecular weight ~24 kDa) and has a high degree of polymorphism [1, 2]. Mutation of proline (CCT) at position 67 to histidine (CAT) in the original A2 genetic variant of the *Bos genus* 5-10 thousand years ago gave rise to the A1 variant [3]. The structural change between the A1 and A2 variants resulted in the two β -casein variants being hydrolyzed differently in the human gastrointestinal tract. The peptide bond between proline and isoleucine in the A2 variant is more resistant to enzymes than the bond between histidine and isoleucine in the A1 variant [2]. This means that milk containing variant A1 is much more easily hydrolyzed by enzymes in the gastrointestinal tract and secretes higher levels of opioid protein β -casomorphin-7 (BCM-7), which can adversely affect human health [4, 5]. During this work, in total, 208 samples were tested from eight dairy cattle breeds: Danish Black-and-White (22), Danish Red (18), Holstein (32), Lithuanian White Back (34), Lithuanian Black-and-White (31), Lithuanian Grey (32), Lithuanian Red (28), and Swedish Black-and-White (11). Genetic testing was done using the allele specific oligonucleotide primer PCR (ASO-PCR) method. All three possible genotypes of β -casein A1 and A2 variants – A1A1, A1A2 and A2A2 – were detected in the studied breeds, except for the Danish Red and the Lithuanian Black and White. Comparing all eight studied breeds, the highest frequency of the A2 allele was in the Danish Red and the lowest in the Lithuanian Grey. The highest frequency of the heterozygous A1A2 genotype was found in the Lithuanian Grey, and the lowest in the Danish Red. Due to the high frequencies of the A1 allele, homozygous A1A1 and heterozygous A1A2 genotypes, the consumption of milk from Lithuanian Grey cattle may increase or promote the risk of the development of diseases due to the release of BCM-7 during digestion. Finally, it can be stated, that Lithuanian Black and White cattle mostly give milk containing A2 variant, which after hydrolysis produces four times less opioid-like protein β -casomorphin-7 (BCM-7) and thus is safer for human consumption.

Keywords: β -casein, dairy cattle, CSN2 gene, ASO-PCR.

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EFFECTS OF AGE AND GENDER OF HORSE AND RIDER IN HIGH LEVEL SHOW JUMPING COMPETITION

Katarzyna Becker¹, Dorota Lewczuk²

¹Bydgoszcz University of Science and Technology (Politechnika Bydgoska), Mazowiecka 28, 85-084 Bydgoszcz, Poland,

²Department of Biotechnology and Nutrigenomics, PAS, Jastrzębiec, ul. Postępu 36A, 05-552 Magdalenka, Poland

Corresponding author: d.lewczuk@igbzpan.pl

In many countries, women are constituted a large majority of riders, especially in recreation riding or low-class competition. In higher classes, the gender structure is quite different [1]. The financial success is also lower in higher classes in horses ridden by women. On the other side, the horse's age and gender are significant effects on show jumping results [2]. According to the literature, the relationships between horses and people depend on gender [3]. The stated hypothesis is that the results in high-class show jumping will be comparable between people's gender after corrections for additional influences like the number of starts or horse age and gender. The data were collected on the final jump-off during the Polish Show Jumping Championships during the years 2016–2020. Because of the limited amount of riders in the jump-off, the number of observations was 50. The observations of 29 riders and 40 horses were divided according to gender and age. The analyzed observations belong to stallions (30%), mares (24%), geldings (46%), men (92%) and women (8%). The following age groups were created: people below 30 years (36%), 30–39 years (46%), 40 years and above (18%); horses below 10 years (28%), 10–12 (38%), and above 12 (34%). Preliminary analysis showed that almost all subclasses had observations. Analysis of the results overall showed that, among general points, place and time, the general point trait has to be transformed by the SQR function (root extraction) to be normally distributed. The statistical analysis of variance was conducted using the MIXED procedure from the SAS program with the model including a random rider-horse pair effect and fixed effects of rider and horse age and gender effects, as well as the year of competition. Horse age and gender did not affect competition results. The rider's age was statistically significant for location ($P = 0.003$) and points ($P = 0.04$), but not the time of jump-off. The placing is better with the rider's age, and the amount of points is lower for the oldest group of riders (however, differs only with the youngest age group). The rider's gender was statistically significant for placing in competition ($P = 0.01$) probably because of some differences in timing as differences in points were not noted. The hypothesis on equal results between the results of people of different genders is not supported. However, it is worth noting that 75% of women's records were noted for the oldest horses. This percentage was much lower for men (30%). Such characteristics may be gender-specific. Because of the limited amount of data, the study should be continued with a larger amount of data.

Keywords: horse, show jumping, age, gender.

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AQUAPORINS EXPRESSION AND SPERM METABOLOME FOR THE EVALUATION OF BULL SEMEN QUALITY

Katarzyna Michałek¹, Elena Bartkiene², Patrycja Oberska¹, Maciej Murawski³,
Dariusz Gączarzewicz⁴, Marta Marynowska¹, Andrzej Syczewski⁵

¹Department of Physiology, Cytobiology and Proteomics, West Pomeranian University of Technology in Szczecin, Poland,

²Department of Food Safety and Quality, Lithuanian University of Health Sciences, Kaunas, Lithuanian,

³Department of Animal Nutrition, Biotechnology and Fisheries, University of Agriculture in Krakow, Poland,

⁴Department of Animal Reproduction, Biotechnology and Environmental Hygiene, West Pomeranian University of Technology in Szczecin, Poland,

⁵Genetic and Animal Husbandry, Szczecin, Poland

Corresponding author: kmichalek@zut.edu.pl.

It is well known that standard semen analysis is a basic test that is used to determine male fertility in farm animals, including cattle. However, sensitivity of this test does not meet challenges associated with the modern animal reproduction, and it leads to economic loss, especially, when cryopreserved sperm is used [1]. In the view of the above, new fertility-related biomarkers are urgently needed for diagnosis and prognosis of male fertility potential [2]. In our project, we hypothesize that aquaporins (AQPs) and related *in situ* metabolic processes can be integrated as sensitive markers for bull fertility and production of high quality semen. The present study includes a total of 20 cryopreserved ejaculates, each coming from a separate healthy and sexually mature Polish Holstein-Friesian, black and white bull. The ejaculates will be classified into two groups: high-quality semen (n = 10) and low-quality semen (n = 10), based on sperm motility, viability, morphology, DNA defragmentation and sperm apoptosis. Subsequently, semen samples will be processed (i) for analysis of the location and expression of AQPs, and (ii) for analysis of the selected physicochemical markers (free amino acid and fatty acid profile, biogenic amines, cholesterol and malondialdehyde concentration). Research carried out to date has confirmed the presence of AQP3 and AQP7 in bovine sperm. Both proteins belong to the subfamily of AQPs called aquaglyceroporins and are probably involved in the transport of glycerol into sperm cells for energy production, elimination of reactive oxygen species and regulation of sperm volume.

Keywords: water channel, metabolomics, male reproduction, sperm, biomarker.

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IMPROVED CHROMATOGRAPHIC METHOD FOR QUANTIFYING FATTY ACIDS IN PIG ERYTHROCYTES AS A BIOMARKER OF DIETARY FAT SUPPLEMENTATION

Marian Czauderna¹, Monika Karpińska¹, Jarosław Woliński¹, Kamil Zaworski¹,
Małgorzata Białek¹, Stefan Pierzynowski^{2,4} and Kateryna Pierzynowska^{1,2,3,4}

¹The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, 05-110 Jabłonna, Poland,

²Department of Biology, Lund University, Sölvegatan 35, 22362 Lund, Sweden

³Department of Biology, Institute Rural Medicine, ul. Jaczewskiego 2, 20-090 Lublin, Poland

⁴SGP + Group Consortium, Alfågelgränden 24, 23132, Trelleborg, Sweden

Corresponding author: m.bialek@ifzz.pl

Erythrocytes (red blood cells, RBCs) are one of the major cellular components of blood. They are small, round, and biconcave, they lack nucleus and contain haemoglobin, which binds oxygen, and to the lesser extent carbon dioxide. RBCs are covered with a membrane containing nearly equal amounts of proteins and lipids, which are either phospholipids or neutral lipids, mostly unesterified cholesterol (Chol) located in between phospholipids [1]. Changes in lipid composition of membranes result in an impairment of their deformability and, thus, affect their functional characteristics. RBCs are particularly suitable for studies on membrane lipid composition, as due to the lack of internal organelles, the majority of their lipids are attributed to the membrane. Factors such as a diet, lifestyle, aging, and diseases may alter the fatty acid (FA) composition of the RBC membrane, and, thus, the FA profile may be used as an indicator of dietary interventions and various states [2]. In animal sciences, membrane lipidomics studies are still scarce [3], partially due to the some methodological issues. RBCs membranes are very fragile, and both chemicals and sample preparation conditions (time, temperature) may alter the lipid profile and hence reduce the usefulness of the RBC as a biomarker. The aim of this study was to improve methods of saponification and methylation of FA present in RBCs membrane of pigs' blood samples, followed by gas chromatography-mass spectrometry analysis (GC-MS). Blood samples (~2 mL) were collected from crossbred ((Polish Landrace × Yorkshire) × Hampshire) male piglets (*Sus scrofa domestica*), fed high fat diet supplemented with 5 g of fish oil (FO). RBCs were collected by centrifugation of blood collected into heparinized tubes and thrice saline rinse of the residue. RBCs samples were subjected to saponification for 2, 10 and 20 minutes at 95°C to choose a suitable time period, then to mild base and acid methylation followed by GC-MS analysis. The shortest time (2 min) of saponification resulted in significantly higher concentrations of arachidonic, eicosapentaenoic, docosapentaenoic, docosahexaenoic, C24:5 n-3 and C24:6 n-3 FAs in RBCs. The longest time (20 min) of saponification significantly reduced concentrations of all FA whereas time of saponification shorter than 2 minutes did not ensure visible dissolution of RBCs. Moreover, they were also visible after mild base and acid methylation. The improved saponification method (95°C for 2 min) is an appropriate analytical procedure of sample preparation for GC-MS analysis of FA profile in RBCs.

Keywords: fatty acids, gentle saponification, mild methylation, erythrocytes, piglet.

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LIQUID CHROMATOGRAPHIC METHOD FOR ANALYSIS OF CANNABINOIDS LEVEL IN HEMP SAMPLES AND EGG YOLKS

Marian Czauderna¹, Věra Skřivanová², Milan Marounek², Małgorzata Białek¹
and Agnieszka Białek^{1,3}

¹The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, 05-110 Jabłonna, Poland,

²Institute of Animal Science, Department of Nutrition Physiology and Animal Product Quality, CZ-104 00, Praha-Uhřetěves, Czech Republic,

³University of Economics and Humans Sciences, Okopowa 59, 01-043 Warsaw, Poland

Corresponding author: m.czauderna@ifzz.pl

The diet composition, especially added supplements, can be an effective factor in increasing the nutritive value of animal products from the aspect of human health. Currently, cannabinoids (CBDs) derived from hemp could be used as physiologically valuable dietary supplements for farm animals (like poultry, ruminants, pigs or rabbits) [1]. CBDs have a wide spectrum of biological activity, including anti-oxidant and anti-inflammatory activity and anti-necrotic protective effects, as well as displaying a favourable safety and tolerability profile in humans. Interestingly, hemp seeds added to tap water and feed have increased bone strength of farm animals, which is of great practical importance. Thus, hemp rich in CBDs (with trace levels of psychoactive tetrahydrocannabinol (THC)) added to diets appears to be of particular interest for farm animal feeding [2]. It is important to monitor the CBD profile in animal products due to the possible presence of psychactive CBDs (like Δ^9 -THC and Δ^8 -THC). Thus, the aim of our study was to develop the reversed-phase (RP) high performance liquid chromatographic method (HPLC) with photodiode array detection (DAD) for the determination of cannabinoids in hemp supplements, the diets enriched in CBDs and yolk and white of eggs of hens fed these diets. CBDs are lipophilic substances, so their transition to egg yolks and adipose tissues is possible. The determination of CBDs in animal products is important because some CBDs (like cannabidiol) alleviate the psychoactive effects of THC. The chromatographic separation of twelve non-psychoactive CBDs, two psychoactive CBDs (Δ^9 -THC and Δ^8 -THC) and free cholesterol in these samples was conducted using a Shim-Pak C18-column (2.2 μm ; 75 mm \times 3 mm) and two Phenomenex C18-columns (2.5 μm ; 100 mm \times 3 mm). The preparation of egg yolks for HPLC analysis consisted of four extractions with hexane. All hexane layers were combined, and hexane was removed under a stream of argon at $\sim 45^\circ\text{C}$. The residue in a vial was dissolved in 1 mL of acetonitrile (ACN). The preparation of fine powdered hen diets, hemp and flax seeds for chromatographic analyses consisted of four extractions with hexane and four extractions with methanol. Hexane and methanol layers were combined, and solvents were removed under a stream of argon at $\sim 45^\circ\text{C}$. The residue in a vial was dissolved in 1 mL of ACN. Then, biological samples and CBDs standards were analysed using a ternate gradient of ACN with formic acid (solvent A), solvent B consisted of water with formic acid, while solvent C was methanol; the maximum system pressure was 29.5 MPa. After detailed studies of the influence of column temperature, mobile phase compositions and the pH of methanol on resolution of CBDs, the use of three columns, 40°C elution temperature and methanol was chosen as optimum separation conditions for analysis of all assayed CBDc and cholesterol in egg yolk samples, feed, hemp seeds and flax seeds.

Keywords: Cannabinoids, liquid chromatography, hemp, farm animals, egg yolks.

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BEEF AND DAIRY BULL SEMEN QUALITY PARAMETERS IN RELATION TO AMBIENT TEMPERATURE AND HEAT STRESS

Monika Veikutyte, Audrone Rekesiute, Rokas Macijauskas, Neringa Sutkeviciene

Lithuanian University of Health Sciences, Veterinary Academy, Tilžės str. 18, Kaunas, LT-47181, Lithuania

Corresponding author: neringa.sutkeviciene@lsmu.lt

High ambient temperature is considered to be one of the most important factors for subfertility in cattle in tropical or subtropical countries [1]. Bull spermatozoa are very susceptible to heat stress, so the current increase in global temperature is of concern for future livestock production [2]. Some differences in sperm quality between dairy and beef bulls are also described [3]. A further aim was to identify where potential differences in sperm quality occur between dairy and beef bull semen in relation to ambient temperature and heat stress. In this study, 233 fresh raw ejaculated bull semen samples (107 of dairy breeds and 126 of beef breeds), collected over a period of three years, were analyzed. After semen collection, the volume of ejaculate was fixed and NewBauer chamber was used to measure sperm concentration. Progressive as well as total sperm motility were measured via Sperm Class Analyzer software. The average age was 68.80 ± 8.92 months for beef and 49.77 ± 6.93 months for dairy bulls.

The data on the average monthly ambient temperature were obtained from the Lithuanian Hydrometeorological Service. The highest average ambient temperature was detected on summer season: June ($17.84 \pm 1.06^\circ\text{C}$), July ($18.42 \pm 0.74^\circ\text{C}$) and August ($17.67 \pm 2.71^\circ\text{C}$). Our results showed that the type of the bull has a significant influence on sperm motility parameters for the whole tested period. Dairy bulls showed a higher average of the total and progressive sperm motility rates ($79.39 \pm 15.02\%$ and $59.31 \pm 16.00\%$) ($P < 0.01$), while the average of the volume of the ejaculate (7.37 ± 2.26 mL) ($P > 0.05$) and sperm concentration (1836.12 ± 791.50 cells/mL) ($P < 0.01$) were higher in beef bull samples. During the summer, sperm motility parameters in the beef bull samples decreased significantly while the motility in dairy bull samples remained constant and did not differ from other seasons ($P < 0.01$). An increase in ejaculate volume and a decrease in concentration in dairy bull ejaculates were observed during the summer. Meanwhile, the beef bull samples in summer were more concentrated without a rise in the volume of the ejaculate. The analysis of influence of ambient temperature and heat stress on bull semen parameters showed that the spermatozoa of the beef bulls to compare with dairy bulls were more sensitive for high ambient temperature by decreasing their motility, which could be caused by higher sperm concentration in the ejaculate and an increase in oxidative stress.

Keywords: heat stress, cattle, fertility.

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STAT5 GENE (6853C>T) POLYMORPHISM AND ITS INFLUENCE ON THE CARCASS QUALITY IN BEEF CATTLE

Nijolė Pečiulaitienė, Ramutė Mišeikienė, Kristina Morkūnienė, Renata Bižienė, Laimutis Kučinskas

*Institute of Biology Systems and Genetics, Veterinary Academy,
Lithuanian University of Health Sciences, Tilžės str. 18, LT-47181 Kaunas, Lithuania.*

Ccorresponding author: Nijole.Peciulaitiene@lsmu.lt

Signal transducer and activator of transcription 5 (STAT5) is known as a main mediator of growth hormone (GH) action on target genes [1]. The STAT5 transcription factors are members of the somatotrophic axis. They initiate the growth process in the target cells, a process mediated by the pituitary growth hormone [2]. Owing to its mediator role in the effects of the prolactin and growth hormones, it is suggested that the STAT5A gene is a potential quantitative trait locus for the quantitative traits of livestock, such as meat yield [3, 4]. The aim of this study was to investigate the prevalence of STAT5 gene (6853C>T) polymorphism and to determine its influence on the carcass quality in beef cattle. Samples of cattle hair follicles were collected from 85 bulls consisting of Angus (41), Limousin (19), Galloway (19) and Simmental (6) cattle. Hair samples and the data on carcass quality records were obtained from Šilutė control bulls feeding station. Bovine genomic DNA was extracted from hair follicles using Chelex DNA extraction method. Polymorphism of STAT5 locus was identified using a PCR-RFLP method. PCR product of STAT5 gene was digested with *AvaI* (*Eco88I*) restriction nuclease. Both C and T alleles of the STAT5 gene were detected in Lithuanian beef cattle population. Frequency of C allele was found the highest and that of T allele the lowest. The C and T allele frequencies were 0.959 and 0.041, respectively. The STAT5 gene CC genotype was the most common in the studied population (91–92%) followed by the CT genotype (9–8%), while the TT genotype was not found in the analyzed population. Evaluation of the observed and expected heterozygosity in the investigated group of animals demonstrated that the observed heterozygosity was found to be lower than expected, indicating an insufficient amount of genetic diversity in the loci studied; the difference was not statistically significant. According to SEUROP carcass classification system, most of the CT and CC genotypes bovine carcasses were classified as R (good) carcass conformation class and the 3rd (average) fat coverage class. However, results were not statistically significant (P value – 0.334, $P > 0.05$); thus, STAT5 gene polymorphism has no influence on bovine carcass conformation and fat coverage. In conclusion, after evaluating the polymorphism of the STAT5 gene (6853C> T) in the studied population of beef bulls bred in Lithuania, it was found that the frequencies of alleles and genotypes are unevenly distributed. Therefore, the low frequency of some of the obtained genotypes of polymorphism makes it difficult to determine the influence on the carcass quality traits of cattle. An increased sample size is required to obtain more confident results.

Keywords: cattle, STAT5 gene, polymorphism, PCR-RFLP.

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THE INFLUENCE OF RATION CHANGES IN THE DYNAMICS OF THE ACTIVITY OF BLOOD BIOCHEMICAL INDICATORS AND LIVER ENZYMES IN HEIFERS

Ruta Budreckiene^{1*}, Filip Samveljan¹, Jurgita Dailidaviciene², Sigita Kerziene³

¹Department of Biochemistry, Medical Academy, Lithuanian University of Health Science, Tilžės str. 18, Kaunas, Lithuania,

²Department of Anatomy and Physiology, Veterinary Academy, Lithuanian University of Health Science, Tilžės str. 18, Kaunas, Lithuania,

³Department of Animal Breeding and Nutrition, Veterinary Academy, Lithuanian University of Health Science, Tilžės str. 18, Kaunas, Lithuania

Corresponding author: Ruta.Budreckiene@lsmu.lt

Cattle, like all other animals, need five main components in their diet: carbohydrates, proteins, minerals, vitamins and water (although the water is not a nutrient material, any diet or vitality is impossible without it) [1, 2]. Sometimes, it is difficult to ensure a proper diet and to match the ration exactly as required by the animals. In this case, feed additives are used to ensure a proper diet that fully meets the needs of the animal. Feeds may also be fortified with supplements if clinical symptoms appear to be present due to a lack of some materials in the diet. Proper selection of the supplement and its dose is very important, because too much of it can be harmful to animals: decreased productivity, impaired metabolism, the onset of pathological processes may occur [3]. The main tasks of this investigation were determination of the effect of dietary changes on the hepatic activity of the liver enzyme; analysis of the most important biochemical parameters of the blood, which are the most sensitive indicators for changes in the diet and how they relate to changes in liver enzymes; and calculation of the interactions and relationships between the obtained biochemical parameters. A particularly pronounced change was observed in GGT and ALP enzymes. The mean GGT concentration at the beginning of the study was 19.07 U/L, and at the end of the study (90 days), it reached 22.41 U/L (norm: 6–17.4 U/L), while the ALP at the beginning of the study was 160.07 U/L, and at the end, it was 224.31 U/L (norm: 0–488 U/L). The correlation coefficient of these enzymes is very strong – 0.97. Hypocalcaemia and hypomagnesaemia, which occurred throughout the study irrespective of changes in the diet, were investigated for other biochemical parameters, determining the cause and relationship between changes in liver enzymes. The calcium concentration on day 0 of the experiment was 5.61 mg/dL and on day 90, it was 6.2 mg/dL doses (norm: 8–11.4 mg/dL), magnesium concentration 0.98 mg/dL and 1.1 mg/dL, respectively (norm: 1.5–2.9 mg/dL). However, a significant increase in phosphorus concentration was observed throughout the study, hypophosphataemia was found to be 5.36 mg/dL on day 0 and increased to 8.22 mg/dL on day 90 (rate: 5.6–8.0 mg/dL). A positive correlation was found between the increase in protein concentrations and the increase in urea and protein uptake and creatinine change, respectively, by a factor of 0.97 and 0.64. It is also likely that due to the increased protein content and the resulting ammonia in the liver, GGT serum levels were also increased due to liver toxicity of ammonia, as the GGT and urea correlation coefficient is very strong and equal to 0.97.

Keywords: ration, proteins, minerals, liver enzymes, heifers, dynamics.

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INVESTIGATION OF *PIT1* GENE POLYMORPHISMS AND THEIR INFLUENCE ON MILK TRAITS IN DAIRY CATTLE

**Renata Bižienė, Kristina Morkūnienė, Gintarė Kolaitytė, Ramutė Mišeikienė,
Nijolė Pečiulaitienė, Laimutis Kučinskas**

*Institute of Biology Systems and Genetics, Veterinary Academy, Lithuanian University of Health Sciences,
Tilžės str. 18, LT-47181 Kaunas, Lithuania*

Corresponding author: renata.biziene@lsmu.lt

Investigation of gene polymorphisms is very useful in determining the genetic potential of an animal [1]. The use of genomic or marker selected analysis in cattle breeding can effectively improve the quality of dairy products and better results of quantitative traits [2]. Previous studies in dairy cattle have shown that polymorphisms in the pituitrin-specific transcription factor (*PIT1*) gene are associated with traits of cattle milk productivity. The pituitrin-specific transcription factor (*PIT1* or *POU1F1*) gene is involved in the transcription of the growth hormone (GH) gene, the prolactin (*PRL*) gene, and the thyroid stimulating hormone- β (*TSHB*) gene. Pituitrin-specific transcription factor is also important for the differentiation and growth of adenohypophyseal somatotropic, lactotropic, and thyrotropic cells, pituitary development, and maintenance of function [3]. Blood samples were taken from 101 dairy cattle from various dairy farms in Lithuania. Data sets for second lactation production period from dairy cattle were analysed. In this study, two *PIT1* gene polymorphisms (c.1178A/G and c.545G/A) were analysed. Genotyping of polymorphisms was achieved using the PCR-RFLP method. There were three genotypes detected at *PIT1* gene polymorphism c.1178A/G, i.e., BB, AB and AA. The observed frequencies were 64.4% (BB), 27.7% (AB) and 7.9% (AA). The observed allele frequencies were B 78.2% and A 21.8%, respectively. In this polymorphism, heterozygosity was insufficient. There were three genotypes detected at *PIT1* gene polymorphism c.545G/A, i.e., GG, GA and AA. Their observed frequencies were GG 62.4%, GA 27.7% and AA 9.9%. The mutated allele A frequency was 23.8% and the wild type allele G frequency was 78.2%. In this polymorphism, heterozygosity was also insufficient. The investigational analysis of *PIT1* gene polymorphism c.1178A/G shows that B allele determines higher milk yield, while *PIT1* gene polymorphism c.545G/A allele A determines higher milk and protein yield. In conclusion, results from the analysis show that *PIT1* gene polymorphisms c.1178A/G and c.545G/A have a significant impact on the quantity of milk and protein yield.

Keywords: dairy cattle, *PIT1* gene, RFLP.

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EVALUATION OF MILK YIELD AND MILKABILITY TRAITS OF LITHUANIAN RED COWS WITH DIFFERENT GENOTYPES DURING LACTATION IN ORGANIC FARMS

Renata Japertienė¹, Lina Anskienė¹, Sigitas Japertas²

¹Department of Animal Breeding, Lithuanian University of Health Sciences, Faculty of Animal Sciences,

Tilžės str. 18, LT-47181, Kaunas, Lithuania,

²Practical training and trial center of Lithuanian University of Health Sciences, Akacijų str. 2, Giraitės village, LT-54310, Kauno distr., Lithuania

Corresponding author: renata.japertiene@lsmu.lt

Holstein cattle have been used for many decades for the breeding of other dairy cattle in order to increase milk production. Productivity of cows differs between organic and conventional herds; therefore, the ability of cows to adapt to an organic production environment has been questioned, whether these high-cost genotypes are suitable for organic farming systems. Organic dairy farming has grown, farmers have realised that many available conventional breeds of cows are not well adapted to new situations and that more robust cows are able to function well in the organic environment.

The aim of this study was to investigate the milk yield and milkability traits during lactation of Lithuanian red cows with different genotypes. The research was carried out in an organic farm in 2020 with dairy cows ($n = 248$) of Lithuanian red cattle population. The milk yield (MY), milking speed (MS), highest milk flow (HMF), and milking time (MT) were evaluated. Investigated traits were measured with DeLaval electronic milk meters, "Apro Windows" software. All records were between 5 and 330 days of lactation, with average 2.26 ± 0.44 of lactation. All cows had two milk-recording events per test day (morning and evening). Lactation of cows was divided into stages: (stage 1 – ≤ 60 ; stage 2 – 61–150; stage 3 – 151–240; an stage 4 – 241–330 days of lactation). The statistical analysis of data was performed using the SPSS 25.0 (SPSS Inc., Chicago, IL, USA) software.

The highest number of cows was with LRxRHxRH genotype, which accounted for 37.83% ($\chi^2 = 53.540$, $df = 1$, $P < 0.001$) of all investigated cows. We observed that the highest MY and HMF in the organic farm was detected in cows with genotypes LRxAIxRH, LRxHxH and LRxRHxRH in the first and the second stages of lactation ($P < 0.05$). MT of LRxHxH, LRxRHxDR and LRxAIxRH genotype cows during all stages of lactation was longer, compared to cows of other genotypes ($P < 0.05$). We estimated that of all fixed effects the biggest influence on MS, HMF, MT ($P < 0.001$) and MY was by a genotype of cows ($P < 0.01$), while the stage of lactation showed the highest impact on MY, HMF, MT ($P < 0.001$) and MS ($P < 0.01$). Analysis of different genotypes of cows revealed that local breeds are well-adapted and more suitable for organic farming.

Keywords: genotype, milk yield, milking speed, milking time, stage of lactation.

INFLUENCE OF THE FIRST CALVING AGE ON THE COW PRODUCTIVITY INDICATORS

Ramutė Mišeikienė¹, Saulius Tušas¹, Jūratė Rudejvienė², Agata Balcevič¹

¹*Institute of Animal Rearing Technologies, Lithuanian University of Health Sciences, Lithuania,*

²*Dr. L.Kriauceliunas Small Animal Clinic, Veterinary Academy, Lithuanian University of Health Sciences, Lithuania*

Corresponding author: ramute.miseikiene@lsmu.lt

Age at first calving is considered an important predictor of subsequent milk yield and is an extremely important economic trait determining the profit of cow milk production [1, 2]. Cow longevity and lifetime performance traits are good indicators of breeding effectiveness and animal welfare. They are also interrelated with the economics of dairy herd [3]. Our study aimed to analyze and to determine the influence of the first calving age on cow productivity. In the investigated farm, the cows were raised under loose-housing conditions in a modern cold-type barn throughout the year. Cows were fed a complete mixed diet that met their physiological needs. For the study, 362 cows with their completed first lactation were selected. According to the age of the first calving, the cows were divided into 4 groups according to the calving age: up to 22 months, 22.1–24.0 months, 24.1–26.0 months, and over 26.1 months. For the analysis of the data, statistical indicators (arithmetic mean, standard error of mean and statistical reliability of the data (P)) were calculated for each evaluated trait. The obtained results were considered statistically significant when $P < 0.05$. The first calving age of cows ranged from 20 to 29 months, with an average of 23.7 months. We found that 1483.16 kg ($P < 0.05$) more milk was produced in cows of 22.1–24 months of age compared to the cows up to 22 months of age, and 300.35 kg milk more compared to the cows over 26.1 months of the first calving age. The milk fat content of the first-calved cows that calved before the age of 22 months was 0.16% ($P < 0.05$) higher than in the 22.1–24-month-old group of cows with the lowest milk fat content of all groups of cows. Milk protein content was the highest in milk of cows of calving under 22 months and in cows from 26.1 months of age; it was 0.1% ($P < 0.05$) higher than in the group of cows aged 22.1–24 months and 0.06% higher than in cows aged 24.1–26 months. The number of somatic cells in milk increased with the increasing first calving age. The number of somatic cells in the milk of cows that calved at age from 26.1 months and over was 137.71 thousand/mL ($P < 0.05$) higher than in the group of cows up to 22 months of age.

Keywords: dairy cows, age at first calving, productivity.

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CHOLESTEROL DEFICIENCY HAPLOTYPE IMPACT ON MILK PRODUCTION IN LITHUANIAN HOLSTEIN COWS

Ramutė Mišeikienė, Nijolė Pečiulaitienė, Renata Bižienė, Kristina Morkūnienė, Laimutis Kučinskas

Institute of Biological Systems and Genetic Research, Lithuanian University of Health Sciences, Lithuania

Corresponding author: ramute.miseikiene@lsmu.lt

Cholesterol deficiency (CD) is an autosomal monogenic recessive defect in Holstein cattle [1]. Carriers of cholesterol deficiency haplotype (CDH) have been reported to have a significantly higher genetic merit for milk protein and fat, somatic cell score than non-carriers [2]. Our study examined the distribution of CDH in the *APOB* gene in Lithuanian Holstein cows and its impacts on milk production. A total of 80 cows were checked for the *APOB* mutation. Genomic DNA was isolated from the blood samples. All cows were diagnosed by the test described by Menzi et al. [3]. In this test, a combination of three allele-specific primers was allowed for genotype differentiation: a reverse common primer starting from a wild sequence and two forward primers discriminating the wild sequence from a mutant. The wild forward primer ensured amplification of 249 bp, and the mutant forward primer produced larger amplicon of 436 bp. Genotypes were determined using RFLP-PCR and detected by performing 2% agarose gel electrophoresis of PCR-RFLP samples and evaluating fragment sizes according to the molecular marker in UV light. Statistical analysis was performed using the statistical program SPSS. Using the Kolmogorov-Smirnov test, we found that the values of the studied symptoms were distributed. To evaluate the differences between genotypes, the means of the studied traits and the arithmetic errors of the means were calculated, and the Student criterion for independent samples was calculated to assess the statistical significance of the differences. Differences were considered statistically significant at $P < 0.05$. The average carrier frequency of analysed cows was 8.77%. Non-significant differences were found between milk production traits of carriers and non-carriers. The cholesterol deficiency haplotype status had a non-significant ($P > 0.05$) effect on milk fat ($P = 0.577$), milk protein ($P = 0.337$), milk lactose ($P = 0.82$) and milk somatic cell count ($P = 0.710$). Still, the analysis showed that the milk of heterozygous cows compared with non-carrier homozygous cows had about 0.16% less fat, 0.05% less protein, and 0.01% less lactose. The somatic cell count in carrier milk was about 30.9 thousand/mL less than in non-carrier Holstein cows.

Keywords: *APOB* gene, cattle, productivity.

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BIOCHEMICAL PARAMETERS OF LIVER FUNCTION IN GUINEA PIGS FED WITH DIFFERENT DIETS

Judita Zymantiene, Rasa Zelvyte, Vaidas Oberauskas, Ugne Spancerniene, Kristina Musayeva, Antanas Sederevicius

Department of Anatomy and Physiology, Faculty of Veterinary Medicine, LSMU VA, Tilžės str. 18, Kaunas, Lithuania

Corresponding author: judita.zymantiene@lsmu.lt

Guinea pigs have a close resemblance to humans, including comparable plasma lipoprotein metabolism enzymes, a requirement for dietary vitamin C, similar gender plasma lipoprotein differences and equivalent responses to dietary interventions, exercise and drug treatment, toxicity tests, and can be used as a convenient animal model for human hepatic injury, hepatic steatosis and ultimately hepatic cirrhosis without undergoing any genetic manipulations [1, 2]. They are also used as models to investigate the auditory system, kidney function, osteoarthritis, nutrition, infectious diseases, and reproductive biology [1]. It is noteworthy that diet plays a vital role in maintaining animal health and could have a meaningful impact on liver functions. The present study aimed to assess the changes of some biochemical serum parameters associated with the liver function and to find out the correlation between these parameters using two different diets. Housing conditions and the experimental procedures of 12 short-haired guinea pigs served in this study were in line with EU Directive 2010/63/EU. Guinea pigs of group 1 ($n = 6$) were fed for six months with commercial pellets (free of genetically modified organisms sourced ingredients), whereas animals of group 2 ($n = 6$) got a diet containing genetically modified soybeans. The reductions in aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) of 16.59%, 6.60%, 23.72% and 32.00% were fixed in group 2, respectively, as compared with group 1. However, the albumin (Alb) value in group 2 showed an increment of 16.00% in comparison to the group 1. No marked changes between the groups were observed in total protein (T-Pro) values. A significant strong positive correlation was noted between AST and ALT ($r = 0.80$), AST and LDH ($r = 0.88$), ALT and LDH ($r = 0.87$) activity values, as well as between Alb and T-Pro values ($r = 0.88$) in group 1 ($P < 0.01$). In group 2, a significant ($P < 0.05$) positive correlation was determined between ALP and LDH ($r = 0.48$), ALT and LDH ($r = 0.73$) activity values, as well as between Alb and T-Pro values ($r = 0.51$). The correlations between ALT and ALP, ALP and LDH activity values in group 1, AST and ALT, AST and LDH, ALT and ALP activity values in group 2 were found to be statistically insignificant ($P > 0.05$). Based on these results, we concluded that liver enzymes activity declined from 6.60% to 32.00% in group 2, but corresponded to the established reference intervals for healthy guinea pigs; therefore group 2 may be fed the diet without restrictions for guinea pigs used in experimental studies.

Keywords: guinea pig, diet, serum parameters, liver.

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EFFECTS OF DIFFERENT TUNNELS ENRICHMENT ON BEHAVIOR AND PHYSIOLOGICAL PARAMETERS OF MOUSE

Judita Žymantiėnė¹, Vaidas Oberauskas¹, Rasa Želvytė¹, Antanas Sederevičius¹,
Kristina Musayeva¹, Ugnė Spancernienė¹

¹Department of Anatomy and Physiology, Faculty of Veterinary Medicine, LSMU VA, Tilžės str. 18, Kaunas, Lithuania

Corresponding author: judita.zymantiene@lsmu.lt

There are many incongruencies between the natural adaptations of mice (*Mus musculus*) and the laboratory conditions in which they are typically housed [1]. Therefore, environmental enrichment maybe used to increase sensory/motor stimulation, increase natural and facilitate species-typical behavior, and provide the animals with some degree of control over their environment [2]. Recent evidence indicates that even the addition of simple forms of enrichment to standard laboratory cages may enhance mice well-being, including behavioral, physiological and neurochemical parameters, as shown by reduced abnormal repetitive behavior, reduced measures of anxiety, and/or reduced aggression and mortalities [3, 4]. A broad variety of enrichment items and materials (various innovative elements as tunnels, toys, lily pads, mouse wedge, tent, mezzanine, boulds, etc.) are available, but there is quite a high knowledge gap (whether they do not deteriorate the quality of scientific data) and further investigations are essential. There is mounting evidence that enrichment can differ in its effects on physiology and behavior between species and strains; therefore, it remains a major area of interest. Thus, we aimed to evaluate the effects of tunnel enrichment on several mouse physiological parameters and behavior. Eight-week-old mice of BALB/C strain (a total of 40 mice) were randomly selected and divided into 4 groups of 10. The holding facilities were kept at a relatively constant temperature ($24 \pm 2^\circ\text{C}$), humidity ($55 \pm 5\%$), and a 12-hour light-dark cycle. The mice were fed with commercial rodent diet containing 19.90% of crude protein, 12.50% of crude fat, 1.70% of crude fiber, cereals, trace elements and vitamins; they also received *ad libitum* water. We varied cage enrichment across four levels: (group 1, control) without enrichment object (no tunnel); (group 2) with an iron tunnel; (group 3) with a plastic tunnel; (group 4) with iron and plastic tunnels. A thermal imaging camera (FLIR T640) was used for measuring eyes and noses temperatures. A 15-minute period was chosen for assessment of behavioral patterns (rearing (R), grooming (G), heading up and rearing (HR), sitting (S), hunching (H), heading down (HE), and stopped huddled (ST)). The eye temperature of mice was $35.23 \pm 0.53^\circ\text{C}$, $34.98 \pm 0.71^\circ\text{C}$, $35.45 \pm 0.58^\circ\text{C}$ and $35.40 \pm 0.47^\circ\text{C}$ in groups 1, 2, 3 and 4, respectively. Cage enrichment with plastic tunnels increased eye temperature by 0.47°C comparing with cage enrichment of an iron tunnel. The nose temperature was $24.79 \pm 1.78^\circ\text{C}$, $25.17 \pm 1.74^\circ\text{C}$, $26.48 \pm 2.09^\circ\text{C}$ and $24.65 \pm 1.21^\circ\text{C}$ in groups 1, 2, 3 and 4, respectively. Statistically significant R behavior in mice was noticed among groups ($\chi^2 = 14.09$; $P = 0.0028$). The distribution of other behavioral patterns between the groups exhibited to be statistically insignificant. To summarize, we may state that cage enrichment with tunnels does not cause stress because temperature of eyes and noses (as indicative of the mice emotional state) remained stable, and enrichment did not arouse abnormal behavioral pattern.

Keywords: mouse, enriched environment, tunnels, temperature, behavior.

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NFKB AND NFKBIA GENE EXPRESSION ANALYSIS IN DAIRY CATTLE UDDER PARENCHYMA WITH CHRONIC MASTITIS CAUSED BY STAPHYLOCOCCAL INFECTION

**Adrianna Szprynca¹, Ewelina Kawecka-Grochocka¹, Magdalena Zalewska²,
Magdalena Rzewuska³, Tomasz Ząbek⁴, Emilia Bagnicka¹**

¹*Institute of Genetics and Animal Biotechnology PAS, Jastrzębiec, Poland,*

²*University of Warsaw, Faculty of Biology, Warsaw, Poland,*

³*Warsaw University of Life Sciences, Institute of Veterinary, Warsaw, Poland,*

⁴*The National Research Institute of Animal Production, Balice, Poland*

Corresponding author: a.szprynca@igbzpan.pl

The expression of cytokines, the main agents regulating inflammation, may be regulated by a transcription factor *NFκB* [1], which, in turn, regulates numerous innate and adaptive immunological processes and increases the expression of several pro-inflammatory genes. *NFκB* high activity in milk somatic cells (MSC) derived from udders with both acute (high activity) and chronic (wide range of activity from low to high) mastitis, but not from healthy ones, has been observed [2]. The study aim was to compare the expression of the *NFKBIA* and *NFκB* genes in dairy cow udder parenchyma infected with coagulase-positive (CoPS) or coagulase-negative staphylococci (CoNS) with healthy cows. A total of 36 samples were taken from udder quarters infected with CoPS (n = 12) or CoNS (n = 12) and healthy one derived from the whole healthy udders (control, H = 12). Animals were culled at the end of lactation (286 days; SD, 27) and showed either reproductive difficulties but had healthy udders, or chronic asymptomatic mastitis. RNA was extracted from frozen tissue samples. Only RNA samples with the integrity number (RIN) > 7.0 (the Bioanalyzer 2100, Agilent, Santa Clara, USA) were selected for further analysis. The RT-qPCR method was used to measure gene expression with *GAPDH* and *HPRT* as reference genes. The *NFKBIA* gene was higher expressed in CoPS than in the H group, whereas the *NFκB* gene was discovered to be the least expressed in this group. Both genes in the CoNS group were moderately expressed. An expression of *NFKBIA* gene is always associated with the expression of *NFκB*. *NFKBIA* gene had an increased expression probably to reduce the expression of *NFκB* to protect tissue from destruction, but further study is needed. Yang et al. (2008) [3] showed that during *E. coli*-induced mastitis *NF-κB* signalling pathway is strongly activated, with elevated expression of this factor. The inhibition of *NFKB* expression by *NFKBIA* in subclinical mastitis caused by CoPS is evidenced by the lower level of *NFκB* mRNA and higher *NFKBIA* in the CoPS group than in H. These phenomena taking place in CoPS-infected tissues may represent the activation of tissue-protection mechanisms during chronic and subclinical staphylococcal mastitis.

Keywords: dairy cow, subclinical mastitis, expression.

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QUALITY OF CHEESE MADE FROM EWE'S MILK DURING STORAGE

Raquel P. E. Guiné and Paula M. R. Correia

CERNAS-IPV Research Centre, Polytechnic Institute of Viseu, Campus Politécnico, Viseu, Portugal

Corresponding author: raquelguine@esav.ipv.pt

The extension of shelf-life is pivotal in the cheese industry, and the test of different conservation methods is, therefore, necessary. In this context, this work aimed at evaluating the effect of storage temperature and packaging material in ewe's cheeses quality along a period of 4 months' storage. Cheeses were produced from raw ewe's milk produced by animals of two Portuguese breeds (Bordaleira Serra da Estrela and Churra Mondegueira). They were stored under different conditions: under refrigeration and in a chamber with controlled temperature and RH, packed in vacuum and using two different plastic films. The ewe's cheeses were analysed in three different moments: on day 1 and again after 2 and 4 months. The samples were evaluated for moisture, water activity, colour, texture, and also sensory characteristics, involving a descriptive test (appearance, aroma, taste, texture, global appreciation) made by 25 untrained panellists.

The results showed that the cheeses stored in polyolefin film developed moulds after 20 days, because the moisture content and water activity were high at the beginning of storage, although they decreased along storage time, particularly for the cheeses that were not packed with plastic films. With respect to colour, the vacuum packed cheeses stored in the chamber presented a uniform colour along storage, with high lightness and low yellowness. The texture results revealed that the harder cheeses were those stored in the chamber after 4 months, while the stickier were the ones packed in plastic after the same 4 months. The sensorial tests showed that the refrigerated cheeses were the most appreciated, followed by those stored in the chamber with vacuum package. Because these cheeses are a soft paste cheese type, and this characteristic is pivotal for their appreciation and valorisation by consumers, the buttery characteristics were analysed and they showed high differences according to the storage conditions or type of package. In conclusion, the storage conditions and duration, as well as the packaging material, exert a great influence on the physical characteristics and sensorial properties of ewe's cheeses.

Keywords: texture, sensory quality, colour, storage conditions.

A COMPARATIVE ANALYSIS OF THE EXTERIOR, HORSE TEMPERAMENT, AND KEEPING CONDITIONS OF ŽEMAITUKAI HORSES RAISED ON DISTINCT (X AND Y) STUD FARMS

Vilma Vilienė, Asta Racevičiūtė-Stupelienė, Monika Nutautaitė, Jurgita Noreikaitė

Institute of Animal Rearing Technologies, Veterinary Academy, Lithuanian University of Health Sciences, Tilžės str. 18, LT-47181 Kaunas, Lithuania

Corresponding author: vilma.viliene@lsmu.lt

Each breed of horses has its own set of evaluation standards, and body composition has long been an essential indicator of working capacity. Aspects of body shape and size correlate directly with movement characteristics, making it easier to select horses for certain use [1, 2]. Therefore, this study was designed to perform a Žemaitukai horse breed comparative analysis between X and Y studs by evaluating the horses' exteriors, body measurement indices, temperaments, and keeping conditions. Research was carried out with a total of 12 horses, each stud farm consisting of 6 Žemaitukai breed horses ($n = 6$ horses/stud farm), which were selected by sex (4 mares and 2 stallions were selected from each stud) and age (the average horse age was 10 years old). The horses were assessed on their exterior according to a certain feature, 24 body parts measurements and their indices, temperament according to positive and negative traits, and keeping circumstances. The exterior of the horses showed that statistically significantly higher measurements were obtained for Žemaitukai horses bred in the Y stud farm: a significantly longer average forearm length by 1.8 cm, an almost 10 cm wider pelvis, and 5.4 cm longer foreleg length from wrist joint to hoof, compared to horses bred in the X stud farm ($P < 0.05$). Horse body size indices (foreleg length from heel to hoof; large body format; small body format) revealed that the X stud farm had 5.5, 2.8, and 6.6 cm lower indices than the Y stud farm ($P < 0.05$). Compared to the exterior of the horses according to the calibre type, the horses of the Y stud farm scored 0.8 points more than the X horses ($P < 0.05$). However, the horses from the X stud farm had a higher ossification of the fore cannon and the hind cannon ($P < 0.05$). After assessing the temperament of Žemaitukai horses, it was discovered that the horses from the X stud farm possessed a greater number of positive character qualities. These horses displayed features of peace, comfort, and energy for the most of the time. The Y stable had generally peaceful horses, but after evaluating the negative attributes, more of them were discovered on the X stud farm, primarily impatience and challenging management. The horses were more difficult to manage in the Y stud farm, even though the total number of negative features was lower. As an outcome, both studs possessed more positive than negative temperament traits. Examining the conditions of keeping Žemaitukai horses in X and Y stud farms revealed that horses are kept in different types of stables: the X stud farm had a pen system ($n = 1$ horse/pen), and the Y stud farm had a fencing system. However, the housing systems of both stud farms met the requirements. Overall, while the research provided relatively comparable findings, only a few significant differences in the exterior, body measurements, and indices of X and Y stud farm horses were detected. Despite varied horse-keeping practises, there were not enough significant differences in the traits studied, and no clear trend was revealed.

Keywords: Žemaitukai breed, horse, exterior, temperament, body measurement indices.

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ANALYSIS OF CHANGES IN RADIOLOGICAL IMAGE MEASUREMENTS OF TARSAL JOINT OF YOUNG HORSES DURING PERFORMANCE TESTS

Roma Buczkowska¹, Mateusz Hecold¹, Dorota Lewczuk²

¹Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Nowoursynowska str. 159,02-776 Warsaw, Poland,

²Department of Biotechnology and Nutrigenomics, PAS, Jastrzębiec, ul. Postępu 36A, 05-552 Magdalenka, Poland

Corresponding author: d.lewczuk@igbzpan.pl

The study aimed to assess the effect of training on skeleton dimensions to understand changes connected with the horse OCD status of horses. Muscle and bone tissue respond to early training to varying degrees [1], and the lack of exercise delays the development of the musculoskeletal tissue of horses [2]. Young warmblood horses trained under controlled conditions on performance riding tests were investigated (19 stallions and 50 mares). Horses were 1249 days old (SD, 114.6). The average height at withers was 164.5 cm (SD, 3.02) with a circumference of the chest 189.8 cm (SD, 4.04) and 20.77 (SD, 0.78) for the fore limb (cannon bone). The average quality of their conformation was 78.15 (SD, 1.13) on a scale of 0–100 points. Lateral x-ray images of each hock joint were performed twice at the beginning and the end of the training. To characterize the changes in the dimensions of the hock joint area, 14 measurements of the hock joint were made (VetRay Vision 4.4.7 Vet Xp / 2000 software). The influence of investigated factors on the joint measurements was analyzed using Proc Mixed from the SAS program for all 191 observations. The statistical model included a random effect of the horse (69), fixed effects of training center (Bogusławice, Biały Bór), sex (stallion, mare), limb (left, right), training (investigations before and after training) and regression on age in days. The post-hock test for LSMs was used to evaluate differences between individual classes of the effects. The associations between measured parameters of hock bones structures were achieved using partial correlation analysis. The MANOVA statement in the GLM procedure of the SAS program corrected calculations for factors influencing the results (training center, sex, limb, training, age). Partial correlations between investigated measurements are presented for all *P* values levels. The training effect has the most significant influence on changes in the bone dimensions of young horses. It was statistically significant for all parameters on the level of $P < 0.001$. Gender turned out to be another important factor being significant for 11 of 14 measured parameters. The age effect was significant for two measured parameters. The right–left limb dimensions were different for five parameters, and the training center for one. The parameters characterizing the calcaneus were the most strongly correlated with each other and the other parameters. Obtained significant correlations are mainly between 0.28 to 0.5. Based on conducted research, it can be stated that training has a significant influence on the horse hock joint dimensions, so it should be taken into account by radiographic evaluation of the horse's health status.

Keywords: horse, tarsal joint, dimensions, training.

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THE POSSIBILITY TO USE BOTH HEALTHY AND RECOVERED COWS AS OOCYTE DONORS FOR THE OPU PROCEDURE

Renalda Juodžentytė¹, Vytuolis Žilaitis², Giedrius Palubinskas¹

¹Veterinary Center for Continuing Education and Counseling, LSMU, Tilžės str. 18, Kaunas, Lithuanian

²Large Animals clinic, Veterinary academy, LSMU, Tilžės str. 18, Kaunas, Lithuanian.

Corresponding author: renalda.juodzentyte@lsmu.lt

The main goal of successful animal husbandry is to quickly raise healthy, good offspring of a genetic breed. This can be achieved by applying innovative advanced technologies. Superovulation and embryo transplantation are one of the reproductive technologies capable of increasing animal productivity [1]. Nowadays, embryo transplantation technology is considered a key technique required to achieve good results in various artificial insemination technologies, especially *in vitro* fertilisation and animal cloning [2]. However, with innovative methods of improving reproduction, the success rate in farm practice is relatively low. There are many limitations associated with the internal and external factors that influence the success of these methods. Such factors include the choice of oocyte production methods, donor variety, and reproductive status. The influence of ovarian dysfunction on oocytes production and fertilization is increasingly being investigated. Finding ways to successfully obtain oocytes from donors without compromising their ovarian function and without prolonging recovery time is an increasingly widespread procedure around the world, ovum-pick-up (OPU), in which oocytes are obtained from secondary follicles [3]. The aim of this study was to investigate the influence of the reproductive status of cows on the success of the OPU procedure and embryo yield. For oocytes aspiration the donor cows (n = 20) were selected of the reproductive status (healthy group – cows without functional reproductive disorders, recovered group – cows after treatment of functional reproductive disorders, i.e., cysts, hypofunction). For this study, the Holstein cows (n = 20) in the 1st to –4th lactation, 60–100 days after calving with an average annual milk yield of 7.000–7.500 kg were selected. Oocyte aspiration was performed using the OPU procedure after hormonal stimulation. After oocytes aspiration, they were classified according to the expansion of the cumulus cells into classes. Quality grading (A, B, C, D) of the oocytes was performed on the basis of cumulus cell development and homogeneity of cytoplasm according to Chaubal et al. (2006) [4]. A total of 81 COCs were aspirated from ovaries. Only normal COCs (Class A and Class B) were used for maturation and fertilization. *In vitro* matured COCs were fertilized with frozen-thawed unsorted sperm. After fertilization, the embryonic cleavage was evaluated within 48 hours (cleavage rate). In the group of healthy cows, 31.91% of Class A oocytes were aspirated and 25.53% of Class B oocytes, of which 45.45% of Class A matured, and 27.27% of Class B matured. In the group of treated cows, 32.35% Class A oocytes and 23.32% Class B oocytes were aspirated of these, and 37.5% of Class A and 25% of Class B matured. In the assessment of oocyte fertilisation in the groups, we found that in the group of healthy cows, Class A oocytes were fertilised by 9.09% more (P < 0.05) in the group of healthy cows. In conclusion, this study shows that the OPU procedure can be performed on both healthy cows and cows with reproductive dysfunction, but the OPU procedure should only be performed on healthy cows to achieve higher embryo yields through hormonal stimulation.

Keywords: fertilization, *in vitro*, OPU, oocytes.

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