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**LIVESTOCK PRODUCTION:
RECENT TRENDS AND FUTURE PROSPECTS**

Abstracts

Comparison Between Pork Quality Traits When Pigs Were Reared Under Intensive and Extensive Conditions

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Pig production is one of the most important livestock sectors globally (1). Primarily, domesticated livestock has been farmed extensively. However, the overall objectives of the development of intensive livestock production have been improved: animal nutrition, feed efficiency, health management, environmental control, reproduction management, genetic selection for better performance, and consistency of product quality and delivery to the marketplace (2). So, this study was aimed at performing a comparative analysis of pork quality from pigs raised under intensive and extensive conditions. From each group, 6 pigs were selected, for a total of 12 pigs. After slaughter, the samples were collected post-mortem and taken from the longest back muscle (*Longissimus dorsi*) between the 12th and the last rib. The following analyses of pork quality were performed 48 hours after slaughter: physical and chemical characteristics, biogenic amine and fatty acid profiles, cholesterol and malondialdehyde concentrations were determined. When analysing pork colour characteristics, it was found that pork reared under extensive conditions had 6% lower brightness (L^*), but 15% higher redness (a^*) and 39% higher yellowness (b^*), compared with pork reared intensively. Pork from extensively reared pigs showed 1.9% higher water binding, 0.94% higher tenderness and 16.66% higher water content, but 11.41% lower cooking losses and 3.26% lower pH compared with pork raised under intensive conditions. However, the results obtained for physical properties, as well as chemical features, were not statistically significant ($P > 0.05$). Assessing the profile of biogenic amines, the meat of extensively reared pigs showed a 35.54% lower phenylethylamine content than the meat of intensively reared pigs ($P < 0.05$). Pork from pigs reared under extensive conditions was found to have 3.52% lower cholesterol and 0.1 $\mu\text{mol/kg}$ lower malondialdehyde, as well as higher levels of linoleic, elaidic and total monounsaturated and polyunsaturated fatty acids contents compared with meat from intensively grown pigs. Nevertheless, the results obtained were found to be not significant ($P > 0.05$). In conclusion, we can claim that the meat of pigs reared under both intensive and extensive circumstances is of similar quality because we observed no significant differences between analysed pork reared under different conditions.

Keywords: intensive, extensive, pigs, pork quality, comparative analysis.

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Possibility to Use Mechanical Vibrations for Prevention and Treatment of Bovine Mastitis

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The aim of the study is to develop a mechanical vibration excitation device for the improvement of udder and teat blood flow in cattle (cows), prevention of subclinical mastitis and treatment of clinical mastitis.

There are known devices developed by foreign scientists that expose the udder by acoustic or electromagnetic waves of a certain frequency (1). This induces harmonic and directional oscillations of different frequencies directed to a certain area of the excited object, e.g. local udder location (2) (Device "ARMENTA", Israel, 2018)). The udder and teat contain an extensive network of blood vessels and capillaries, which reduces the efficiency of the milking process and increases the risk of developing mastitis. The mechanical vibration device developed by LSMU and KTU researchers is mounted on the milker, and while operating during the milking process, it analyses input parameters such as temperature, amplitude of oscillations and pulse using the oximeter; the system is able to adapt the vibration frequency and duration of action according to measured parameters. This device is designed to improve the blood flow to the udder and teats of cows, reduce the incidence of mastitis and increase the effectiveness of therapy. The device has a feedback control system, by which the data can be stored in a database, which allows changing the technological parameters of the device with the help of artificial intelligence.

Experimental studies were performed with a cow udder training model and Laval milkers, each of which was excited by low-frequency directional harmonic oscillations (frequency 10–45 Hz, amplitude 2–5 mm). Vibrations were measured with a laser vibration sensor (KEYNCE LK-G82) both on the milking machine body and on the teats and at various locations in the udder. The mechanical vibrations caused by the vibrator in the milker propagate through the teats to the entire udder and produce a physiotherapeutic effect, activating its blood circulation. Tests were performed when the test udder model was filled with fluid. In this way, the dynamic characteristics of the udder subjected to vibrations change, thus approaching the real conditions when the cow's udder is full of milk. Further research will be done with real animals, thus confirming the results of our training model.

Keywords: mechanical vibration, shockwave therapy, mastitis

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Effect of Humic Substances Supplement on Growth Performance, Gastrointestinal Tract and Meat Quality Parameters of Broiler Chickens

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The rapid spread of antimicrobial-resistant bacterial communities is a threat to human, animal, and environmental health. Humic substances (HS) are a promising feed additive as an alternative to reduce the use of growth promoter antibiotics in poultry feed (Domínguez-Negrete et al., 2019). Active ingredients of HS consist of humus, humin, humic acid, ulmic acid, fulvic acid, and certain microelements (Arif et al., 2019). The objective was to evaluate the growth performance, caecum microbial profiles and physico-chemical meat quality parameters of broiler chickens (BC). A total of 60 ths. Ross 308 cross broilers were randomly divided into two groups and fattened for 40 days. The dietary treatments consisted of the basal diet feed (control group n = 30 000) without supplementation and the diet supplemented with HS at the level of 2 g/kg of feed (experimental group (HS group), n = 30 000). A corn-soybean meal-based diet was formulated according to the nutritional requirements prescribed in the Ross nutrition specification (2019) and NRC (1994). 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 results showed that the addition of HS had a positive impact on the broilers' growth performance: the body weight by 1% and the feed conversion ratio (FCR) by 0.6% were decreased ($P \geq 0.05$). It was found that the caecum of 1-day old BC was colonised with bacteria; however, the variety of microorganisms was low, with the most prevalent *Escherichia*, *Clostridium*, and *Enterococcus*. In the gut of 40-day old BC, predominant bacteria were *Bacteroides*, *Bransielia*, *Lactobacillus*, *Faecalibacterium*, and *Blautia*. As the results showed, the addition of HS increased the amount of caecal probiotica bacteria (particularly *Bifidobacterium* spp.). An addition of HS caused an increase in breast meat yield by 1.5% and leg meat yield by 0.8%, compared with the control group ($P \geq 0.05$). Meat tenderness, cooking and drip loss, protein and fat content of the HS group was higher, but differences in these indicators were not statistically significant ($P \geq 0.05$). HS group meat samples showed lower dry matter, water holding capacity, redness, and pH (24 h), compared with the control group ($P \geq 0.05$). Overall, these results show that HS had positive tendencies on poultry production, caecal probiotica bacteria colonisation and meat quality.

Keywords: humic substances, productivity, microbiota, meat quality, broiler chickens.

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Impact of Extruded and Fermented Wheat Bran on Production Quality of Broiler Chickens

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Wheat bran (WB) is a cereal by-product of the milling industry, which can be used as valuable feed ingredient in feed of poultry (1). WB is also considered to be a source of dietary fibers or non-starch polysaccharides, which cannot be directly utilized by broiler chickens (2). However, WB can be converted into value-added products and also animal feeds by using the process of extrusion and fermentation. To study the impact of extruded and fermented WB on meat quality of broiler chickens, a total of 42 ths. broilers (1-day old, Ross 308 cross) were divided into two groups for 40 days of fattening. The control group (CON group) was fed the basic compound feed. The 3% of basal were replaced by WB extruded and fermented by *Lactobacillus casei* and *Lactobacillus paracasei* in the diet of treatment group (WB group) and fed for 15 days of fattening. After 15 days, the broilers of the WB group received the basic compound feed. A soybean meal and corn based diet was formulated according to NRC (1994) and the Ross nutrition specification (2019). 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 $P \leq 0.05$. Results showed that the breast and leg meat yield was improved, compared with the control ($P \geq 0.05$). There was a significant ($P \leq 0.05$) increase in breast meat water binding capacity (WHC), cooking losses (CL), amount of fat and ashes, compared with the control. The leg meat CL, lightness and redness were increased, compared with the control group ($P \leq 0.05$). The results of meat sensory attributes showed that WB had positive tendencies on breast meat overall acceptability, leg meat total odour intensity, juiciness, fatness and softness, compared with the control ($P \geq 0.05$). It was found that WB in the feed of broilers had an effect on chicken meat emotional acceptability: predominant emotions of taste were happy, surprised and neutral, compared with the control ($P \geq 0.05$). In summary, the use of extruded and fermented WB in the feed of broiler chickens had no adverse effect on broiler meat physical and chemical parameters.

Keywords: valorization of wheat bran, meat quality, broiler chickens.

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Royal Jelly Supplementation Can Improve Boar Semen Motility and Viability Parameters during Liquid Storage

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Royal jelly is gluten secreted by hypopharyngeal and submandibular glands of young worker bees and has different types of biological activity in various cells and tissues of animal models, and serves as an antioxidant source (1, 2, 3). A successful effect of royal jelly supplementation on sperm quality and fertilizing ability has been reported in domestic animals (4, 5, 6). The current study was carried out to investigate the protective effects of the royal jelly supplementation on the sperm kinematics and plasma membrane functionality during the liquid storage of boar semen at 16°C and 4°C, at various periods of time (0, 24, 48, 72 and 96 h). Semen samples were collected from 11 boars, diluted with a long-term extender and supplemented with different concentration of raw royal jelly (control – 0%, 0.5%, 1% and 2%) at a final concentration of 50×10^6 sperm/mL. Only those samples having more than 75% motility and more than 75% normal sperm were used for further experiments. In the laboratory, the semen was assessed for sperm morphology, viability (eosin-nigrosin staining), subjective motility and objective sperm motility by the sperm class analyzer (SCA). Sperm viability and motility in two ways were checked after 24, 48, 72 and 96 h of incubation. In total, 396 tests for sperm viability and motility were performed. The longer storage time and the lower incubation temperature showed the lower sperm motility and viability results in the all treated and non-treated samples. The results showed that royal jelly supplementation at lower concentration (0.5% and 1%) for storage time at 16°C temperature resulted in a protective effect on cell membrane integrity; however, the liquid storage of semen supplemented with 2% royal jelly had a negative effect on sperm plasma membrane integrity. The highest viability values were found in 1% royal jelly concentration at all storage times at 16°C temperature. Sperm subjective and objective motility SCA results in the samples stored at 4°C decreased with a higher royal jelly concentration and a longer storage time and differed significantly compared with results in the samples stored at 16°C ($P < 0.05$). Our data showed that the royal jelly supplementation at lower concentrations can improve boar semen motility and viability parameters during liquid storage at 16°C for 96 h and no protective effect was observed for sperm functionality and kinematics at 4°C temperature.

Keywords: royal jelly, boar, sperm viability, sperm motility.

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Effect of Extenders on Stallion Epididymal Sperm Motility and Viability after 72 Hours of Storage

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Collection of epididymal stallion sperm offers the opportunity to retain and use genetic material from the males after elective castration or even post mortem (1, 2). Epididymal semen can be used either cryopreserved or fresh, and stored for some days according to the extender used (3). The aim of the present study was to evaluate the effect of the extender on stallion epididymal sperm motility and viability during liquid storage for 72 h at 5°C in EquiPlus extender and Ringer B. Braun solution. For this study, 8 stallions (3–10 years old) of different breeds were used. Sperm from the cauda epididymis was harvested immediately after routine castration and diluted in a prepared EquiPlus semen extender and in Ringer B. Braun solution. The semen was assessed for primary (immediately after collection) semen quality: sperm viability (eosin–nigrosine staining), progressive subjective motility and motility by sperm class analysis (SCA). The samples were stored at 5 ± 1°C for 72 h. Sperm viability, progressive subjective motility and SCA motility were checked after 24, 48 and 72 h of incubation of the samples at 5 ± 1°C. The motility assay showed that the longer the storage time the lower the sperm motility results in both diluents. Results of subjective sperm motility in EquiPlus after 72 h of storage were 12.2% ± 3.61% higher than those in Ringer solution ($P < 0.05$), although subjective sperm motility 1 h after dilution was 2.5% ± 1.49% higher in Ringer solution ($P > 0.05$). SCA motility results after 72 h were higher in EquiPlus (37.05% ± 2.01%, $P < 0.001$). Analysis of sperm viability during liquid storage at 5°C in the different diluents showed better results in EquiPlus extender after 24 h (4% ± 6.03%), 48 h (4.35% ± 2.45%) and 72 h (10.15% ± 0.92%), although primary sperm viability results were better (90.8% ± 5.57%) in Ringer solution than those in EquiPlus (88.45% ± 7.05%, $P > 0.05$). We found statistically significant differences in epididymal sperm motility results in the two extenders after 72 h liquid storage. The highest percentages of progressive motile epididymal spermatozoa (22.00% ± 10.59%) and viable sperm (80.35% ± 11.2%) after 72 h were found in sperm diluted with EquiPlus extender ($P < 0.05$); meanwhile, for short term storage (1–24 h), epididymal semen can also be stored in Ringer B. Braun solution.

Keywords: stallion, semen storage, semen quality, Ringer B solution.

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Micro- and Macroelements in Seminal Plasma Influence Diluted Boar Semen Quality for Seven Days

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Growing evidence shows that macro- and microelements in the seminal plasma of domestic animals are of great importance due to their roles in sperm metabolism, function, survival and oxidative stress (1, 2). The current study was carried out to investigate the effect of micro- and macroelements in fresh boar seminal plasma and their association with sperm quality parameters after 7 days of liquid storage at 16°C. Semen samples were collected from 40 boars and diluted with a long-term extender at a final concentration of 35×10^6 sperm cells/mL. In the laboratory, the semen was assessed for sperm morphology, viability (eosin-nigrosin staining), pH, subjective and objective sperm motility by sperm class analyzer (SCA). Only those samples having more than 75% motility and more than 75% normal sperm were used for further experiments. Sperm viability, motility and pH were checked after 24, 48, 72 and 168 h of storage at 16°C. Seminal plasma was separated and the concentration of macroelements (Na, K, Ca, Mg, P) and microelements (Cu, Zn, Fe) was determined. The longer storage time showed the lower sperm motility and viability results in all samples. Motility measured subjectively dropped by 21% ($P < 0.01$) and by 12.58% ($P < 0.01$) measured objectively. Viability decreased by 21.98% ($P < 0.01$) and pH value dropped by 0.21 ($P < 0.01$) after 7 days of incubation. Elements correlated with sperm quality parameters. Iron and copper negatively correlated with sperm tail abnormalities ($P < 0.05$) and showed a positive effect on sperm motility and viability results ($P < 0.05$) after 7 days. With the increase of potassium, sperm viability after 7 days declined ($P < 0.01$). After 7 days of storage, pH values strongly correlated significantly with sperm viability ($P < 0.01$). Our data showed that micro- and macroelements in seminal plasma have an effect on diluted boar semen quality parameters for 7 days of incubation and could be as a predictive value of boar semen fertility.

Keywords: boar, semen plasma, microelements, macroelements, semen quality.

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The Effect of Diets with Different Ratios of Arginine and Lysine on Immune Status, Oxidative and Epigenetic Changes in Tissues of Turkeys

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The results of our long-term feeding trials indicate that the inclusion levels of methionine (Met) in turkey diets should be higher than those recommended by the National Research Council (NRC 1994). In the present experiment, it was assumed that the appropriate dietary ratio of arginine (Arg) to lysine (Lys) can improve the immune status and growth performance of turkeys. The aim of this study was to evaluate the effects of two inclusion rates of Arg relative to Lys (95% and 105%) in turkey diets with Lys content consistent with NRC recommendations (1994) or 10% higher on the immune status of birds and indicators of protein and DNA damage due to oxidation, nitration or epigenetic changes. Another goal was to determine which dietary Arg:Lys ratio stimulates the immune response of turkeys vaccinated against *Ornithobacterium rhinotracheale* (ORT). The experiment was performed on 576 female turkeys that were randomly assigned to 32 pens. The experiment had a completely randomized design with four dietary treatment groups, 8 replicate pens per group and 18 birds per pen. Two dietary inclusion levels of Lys were analyzed, low ($L_L = \text{NRC}$) and high ($L_H = \text{NRC} + 10\%$). In diets with the low level of Lys, L-Lysine HCl was added to the basal diet to obtain 1.60, 1.50, 1.30 and 1.00 g of Lys per 100 g of feed in 4 successive feeding periods, according to NRC guidelines (1994). L-Arginine HCl was added to the basal diet to obtain 95% and 105% Arg relative to the content of dietary Lys (low and high, A_L and A_H , respectively). The effects of 4 experimental diets, with 2 levels of Lys and 2 levels of Arg ($L_L A_L$, $L_L A_H$, $L_H A_L$, $L_H A_H$), were compared in the study. The inclusion rate of Met in experimental diets exceeded that recommended by the NRC (1994), and DL-Methionine was added to obtain 0.62, 0.59, 0.51 and 0.39 g of Met per 100 g of feed in 4 successive feeding periods. The dietary treatments had no influence on the growth performance of turkeys. It was found that the Lys content of turkey diets should be 10% higher than that recommended by the NRC (1994). The increased Lys content should be combined with the higher Arg level (105% of Lys content). Although the above Arg:Lys ratio did not improve the growth performance of birds, it stimulated their immune system (in particular the immune response following vaccination) and reduced protein nitration as well as protein and DNA oxidation. The present findings can be used to revise and update the existing nutritional recommendations for the optimal levels and ratios of Arg and Lys in turkey diets.

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The Effect of Different Bedding Materials on Hygiene in Calf Housing

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The type and quality of bedding material significantly influence the health and welfare of farm animals that are kept in buildings. The basic function of bedding in calf housing is to absorb moisture and toxic gases, keep the floor dry and shape a beneficial microclimate (1). One of the most popular bedding materials in many countries is straw. Some research indicates that dust levels can be significantly higher in facilities using straw in comparison with alternative bedding materials, whereas dust is an important carrier of microorganisms. Given that calves' immunity builds for a relatively long time, this group of animals may be especially exposed to diseases caused high microbiological contamination. The choice of high-quality bedding material may improve hygiene in calf housing. For this reason, the aim of our study was to determine the effect of classic straw and 6 alternative bedding materials (light pellets, medium sawdust, peat, chopped straw, flax, hemp) on the levels of microbial contamination of air. The calves were kept in accordance with the Polish standards (Journal of Laws 2010, No. 56, item 344) in identically equipped and separated boxes (8 calves on each type of bedding). Quantitative analyses of mesophilic aerobic bacteria and fungi were conducted every 2 days for a period of 2 weeks. Air samples (5 L) were collected with an air sampler MAS-100 Eco[®] (Merck Corp.) at 5 locations in each box. Bacteria and fungi were cultured on solid media (TSA and Sabouraud) and incubated at 35°C for 24 h (bacteria) or 25°C for 120 h (fungi). Microbial counts were expressed in cfu/m³ of air after correction (Feller's formula). After logarithm transformation, the data were normally distributed (Kolmogorov-Smirnov test) and analyzed by ANOVA (StatSoft). Considering bacterial contamination of air, the best quality ($P < 0.01$) characterized peat, light pellet, and sawdust (4.57, 4.59 and 4.64 log₁₀ cfu/m³, respectively). The most contaminated air was registered using traditional straw ($P < 0.01$; 4.79 log₁₀ cfu/m³). Chopped bedding materials (straw, flax, and hemp) had an intermediate effect. Whereas in the case of fungi, chopped flax and hemp had the best impact on the quality of air ($P < 0.01$; 3.96 and 4.09 log₁₀ cfu/m³). The fungal contamination levels were the highest using light pellet and peat ($P < 0.01$; 4.50 and 4.47 log₁₀ cfu/m³). It could be concluded that bedding material significantly influences microbial air quality and hygiene standards in calf housing.

Key words: calf health and welfare, bedding materials, microorganisms, air quality.

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Dairy Cows Stress Assessment by Using Innovative Biomarkers of Herd Management Systems

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The objectives of these studies were to examine the option of using automatic health tracking systems biomarkers as a form of stress indicator and to determine the relationship between biomarkers, blood cortisol levels, and lactate concentration. Ninety cows within 1–30 days were selected and categorized into 3 groups: group 1 – 1–7 days after parturition (dpp) (n = 30); group 2 – 8–14 dpp (n = 30), and group 3 – 15–30 dpp (n = 30) after calving. The cows were milked using Lely Astronaut® A3 milking robots. The pH and temperature of the contents of cow reticulorumen were measured using specific smaX-tec boluses manufactured for animal care. The blood samples were tested for cortisol, lactate concentration. Data about rumination time (RT), milk yield (MY), milk composition, milk somatic cell count (SCC), milk electrical conductivity (EC), consumption of concentrate (CC), weight (BW) were collected from the Lely T4C management program. The RT increased during all of the exploratory periods (with readings between 1.12%–4.90%). A decrease was also observed in the lactate levels (by 1.10 times) and cortisol levels (by 1.98 times, $P < 0.05$) of cows in group 2 (8–14 dpp) compared with an average of group 1 (1–7dpp) in the previous study period (15–30dpp). However, lactate concentrations increased (by 1.84 times, $P < 0.05$) as well as cortisol levels (by 2.09 times, $P < 0.01$) when compared with group 2 on average. The results obtained indicate that RT increased during all exploratory periods, while a decrease of 1.10 times and 1.98 times was observed in lactate levels and cortisol levels, respectively. RT positively correlated with the lactate concentration levels and negatively correlated with cortisol levels during the entire study period. According to this study, there was a positive correlation with milk lactose (ML) during stress, which tends to increase the risk of mastitis and decrease CC, RT, BW, MY, reticulorumen pH, and fat/ protein ratio F/P.

Keywords: biomarkers, stress, rumination time, cortisol, lactate.

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Evaluation of Genetic Correlation Between Lameness and Productivity of Cows

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The literature suggests that severe lameness is associated with poor general health, a weak cow immune system and decreased milk production (1, 2, 3). The aim of our study was to evaluate the genetic relationship between lameness and milk traits in fresh dairy cows ($n = 4726$). Lameness (during 2018–2020) was diagnosed on the visual locomotion scale (4) in 7.2% fresh dairy cows. Parameters such as milk yield, milk lactose, milk fat, milk protein and somatic cell count (SCC) were registered with the help of Lely Astronaut[®] A3 milking robots. Analysis of somatic milk cells was carried out with the logarithmic expression of this indicator: $SCS = (\log_2 (SCC/100)) + 3$ (5). Genetic correlations (r_g) were calculated using programs: PEST 4.2 (Multivariate Prediction and Estimation, 12 March 1999, Linux 2.0.36. Groeneveld E., Kovac M., Wang T. Department of Animal Sciences, University of Illinois) and VCE 4.2.5 (8 December 1998, Linux 2.0.34 i586, written by E. Groeneveld).

The results of phenotypic studies showed that the milk yield of healthy cows (28.36 ± 0.122 kg) was by 1.24 kg more, milk protein ($3.48 \pm 0.006\%$) was by 0.04 percentage points higher than in the group of lame cows ($P < 0.05$). The average concentration of lactose in milk in the group of healthy cows ($4.67\% \pm 0.004\%$) was by 0.10 percentage points higher than in the group of sick cows ($P < 0.05$), and the quality of milk on the SCS scale was better (1.04 times) in non-lame cows ($SCS = 1.89 \pm 0.007$, $P < 0.05$).

After genetic evaluation of the data, we found a positive genetic correlation between lameness scores and somatic cells in milk ($r_g = 0.220$). These results were found to indicate a higher genetic predisposition for cows with higher levels of SCC in milk to lameness. An unfavorable negative genetic correlation was revealed between the assessment of cows for signs of lameness and their milk yield, as well as the content of milk fat, protein and lactose ($r_g = -0.098$ – 0.300). This research shows the genetic links between cow health and productivity, as well as the potential for genetic herd improvement.

Keywords: dairy cows, health, lameness, productivity, genetic correlation.

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Heritability of Lameness in Dairy Cows

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In the last few decades, greater attention has been focused on improving animal welfare in the European Union and globally. Lameness is an important health and welfare concern in dairy farming and has negative implications on productivity and behavior (1, 2, 3). The aim of our study was to evaluate the heritability of lameness and to compare it with heritability of milk traits in fresh dairy cows. The experiment was carried out on 10 commercial dairy farms. Cows were kept in free housing system farms, milking with Lely Astronaut A3 milking robots. A lameness diagnosis (from calving till 30 days after calving) was performed on the visual locomotion scale (VLS) by trained staff (by the same person) according to the standard procedure described by Sprecher et al. (4): 1 = normal, 2 = presence of a slightly asymmetric gait, 3 = the cow clearly protects one or more limbs (moderately lame), 4 = severely lame, and 5 = extremely lame (non-weight-bearing lame). For the study of heritability (h^2), PEST 4.2 (Multivariate Prediction and Estimation, 12 March 1999, Linux 2.0.36, Groeneveld E., Kovac M., Wang T. Department of Animal Sciences, University of Illinois) and VCE 4.2.5 (8 December 1998, Linux 2.0.34 i586, written by E. Groeneveld) programs were used. The following effects and their statistical interpretations were applied in the model: lactation number (fixed), year season (fixed), animal (additive genetic effect, random) and error (random). We found that the heritability of lameness was 0.23. The analysis showed that only a small part of the phenotypic changes in this indicator in the analyzed population was associated with genetic factors. On the other hand, the heritability of milk production and composition ($h^2 = 0.20-0.25$) was at a similar level, and these indicators are used in the breeding program for genetic improvement of dairy cattle.

Keywords: dairy cows, lameness, productivity, heritability.

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Influence of Seasonality on B-Hydroxybutyrate (Bhb) at Early Postpartum Period and Reproduction Performance in Dairy Cows

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High-yielding dairy cows suffer from negative energy balance (NEB) during the first weeks of lactation (1). The NEB depends on zootechnical factors and seasonality. The effect of seasons on cows is controversial, some authors argue that seasons affect the health and reproductive performance of cows (2). The main sign to determine NEB can be evaluated by β -hydroxybutyrate (BHB) concentration in cows' blood. Therefore, the aim of this study was to determine the influence of seasonality on BHB at the early postpartum period and reproduction performance of dairy cows.

The experiment was carried out on 1200 dairy cows. Blood samples were taken from the ear vein at 7–10 DIM in the mornings after milking, and the level of BHB (mmol/L) was determined in different seasons. The number of cows with higher concentration of blood BHB (> 1.2 mmol/L) had a tendency to increase in cold seasons of the year: 28.57% of all cows had increased blood BHB more often in winter ($\chi^2 = 12.857$, $df = 1$, $P < 0.001$) and 41.43% in autumn ($\chi^2 = 2.057$, $df = 1$, $P > 0.05$). An average blood BHB concentration in cows was 11.14% higher in summer and autumn than in other seasons ($P < 0.05$). The number of inseminations depends on blood BHB concentration. Four times inseminated cows had 22.27% higher blood BHB concentration than single inseminated cows ($P < 0.001$), and 23.77% higher than double inseminated cows ($P < 0.001$). The first insemination time correlated with blood BHB ($r = 0.176$; $P < 0.001$).

In conclusion, the season affects the distribution of cows with elevated and average BHB concentration and has influence on insemination rate and first insemination time.

Keywords: cow, NEB, BHB, season, insemination.

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Relationship of Milk Production and Somatic Cell Count in Purebred and Crossbred Cows of Lithuanian Red and Red and White Population

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Cross-breeding of dairy cattle has been used as an alternative to pure breeding and for improvement of various traits. In many cases, crossbreds of the F1 genotype are better than of other genotypes, but the continuous breeding of animals of F1 genotype and the adaptation to the desired genetic combinations of different breeding environments still remain a major challenge. The cross-breeding method produces a heterosis effect, which increases the productivity of cows. This method is recommended for use in productive dairy farms. When using a cross-breeding method to improve dairy herds, it is very important to consider not only the breeds selected for mating, but also the breed of the mother whose characteristics we want to improve and which breed of the father to choose in order to get the desired result.

The aim of this study was to investigate the relationship of milk production and somatic cell count in purebred and crossbred cows of Lithuanian Red and Red and White population. The research was carried out in 7 farms in 2020 with dairy cows ($n = 363$) of different genotypes of Lithuanian Red cattle population. The conditions for keeping and feeding the cows were similar and they were fed equally balanced rations. The milk yield (MY), milk fat (MF), milk protein (MP), concentration of lactose (L), urea (U) and somatic cells count (SCC) were evaluated. Samples were performed each month of the year during control milking. SCC in milk of cows was divided into groups: (1 – ≤ 200 ; 2 – 200–400; 3 – > 400 thousands/mL). For the effects of season, estimation months were assigned (1 – winter, 2 – spring, 3 – summer and 4 – autumn). The statistical analysis of data was performed using the SPSS 20.0 (SPSS Inc., Chicago, IL, USA) software.

We observed that the highest MY and the lowest SCC were estimated in purebred Lithuanian Red cows; the highest milk protein content was detected in purebred Ayrshire cows; the highest milk fat content and urea concentration was estimated in crossbreds of Lithuanian Red x Danish Red; the lowest and below the norm lactose concentration was detected in crossbreds of Lithuanian Red x Swedish Red.

Analysis of different genotypes of cows according to the groups of SCC showed that with increasing SCC, we did not find a direct effect on milk yield and composition except on concentration of lactose. Lactose content in milk of group 1 of SCC was within the norm, while in group 2 and 3 of SCC, as the number of SCC increased, the lactose content decreased.

By using generalized linear model, we estimated that the dependent variables were linearly related to the factors; of all fixed effects, genotype had the impact on fat and urea content ($P < 0.001$); SCC group on milk yield, lactose content ($P < 0.001$) and urea content ($p < 0.01$), season on milk yield, protein, lactose, urea content ($P < 0.001$) and fat content ($P < 0.01$); interaction of genotype with SCC group had an impact only on protein content ($P < 0.05$).

Keywords: genotype, milk production, somatic cell count, season.

In Search of New Potential Markers for Male Fertility in Farm Animals. Aquaporins in the Reproductive System and Metabolomic Profiling of Semen

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Male fertility disorders are a growing problem for both humans and animals. Examination of male reproductive organs and analysis of sperm quality are currently the basic methods for determining the reproductive potential of a male. However, it does happen in everyday animal husbandry practice that fertilization does not occur or its effectiveness is low despite the high functional value of an individual allowed for breeding (1, 2). Hence, it has long been postulated to seek new indicators enabling the precise determination of male reproductive potential and identification of individuals with reduced fertility (3, 4). Studies are conducted in response to the current needs related to animal breeding aimed at answering the question whether the measurement of AQPs in reproductive system and metabolomic sperm evaluation can become a modern, precise and effective indicator enabling the full determination of the male and female reproductive potential and semen quality assessment in the future. The study is carried out on 2 species of farm animals: (1) cattle, a male of the black and white Polish Holstein-Friesian breed, and (2) sheep of the Wrzosówka and Świniarka breeds. The experiment is carried out on the tissues of the male reproductive tract. Bovine tissue samples are collected from 3 age groups: calves 5 to 7 weeks of age, young cattle between 5 and 6 months of age and 1–3 years old reproductive bulls. Sheep tissue samples are collected from 2 age groups: 1–4 months old lambs and adult rams. The presented research involves AQP expression analysis in individual sections of the male reproductive system along with the assessment of animal growth and development and determination of their potential role in the proper course of reproductive processes. The ultrastructure of individual reproductive organs in adult cattle was analyzed. An analysis of AQPs in bovine sperm and metabolomic profiling of semen is also planned, as well as search for relationships between the studied indicators and the quality of sperm and male reproductive potential.

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

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Effects of Beta-Casein Genetic Variants on Milk Composition in the Milk of Dual-Purpose Crossbreed Cows

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Milk is considered nutritious and healthy food for humans. The study aimed to calculate allelic and genotypic frequencies of beta-casein variants (A1 and A2 allele) in crossbred dual-purpose cows and analyze milk production traits of tested cows dependent on beta-casein genotypes. Altogether, genomic DNA of 116 crossbred dual-purpose Simmental cows was collected to estimate beta-casein using the Sanger sequencing method. In the population included in the study, there were homozygote genotype A1A1 (32 animals), A2A2 (33 animals), and heterozygote genotype A1A2 (51 animals). Allele A2 was observed with a frequency of 0.504, and allele A1 with a frequency of 0.496. The most frequent was heterozygous genotype A1A2 (44%), while the homozygous A1A1 genotype was the rarest (27.6%). A2A2 genotype was associated with lower protein content than A1A2 genotype by 0.1% ($P < 0.05$). A breeding program with crossbred dual-purpose Simmental cows could be achieved relatively quick for milk that only contains the beta-casein variant A2.

Keywords: cattle, CSN2 gene, A2 milk, A1A1, A1A2, A2A2.

Antibiotic Resistance Genes Prevalence in *Apis Mellifera* from Different Lithuania Regions

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The honey bee is a major producer of honey. In addition, honey bees are important plant pollinators (1). Genetic analysis of bee microbiota is one of the possible studies that can be used to assess the prevalence of genes leading to antimicrobial resistance in the environment (2). Samples of 111 bees from 4 different Lithuanian apiaries located in Kupiškis, Panevėžys, Prienai and Alytus districts were collected for the study. Group 5 was composed of free-living (wild) bees. Five gene fragments associated with antimicrobial resistance were examined: aminoglycosides (*aph*), beta-lactams (*blaZ*), tetracycline (*tetM*) and sulphonamides (*sul1* ir *sul2*). Four of them were found in bees raised in Lithuania, except for a fragment of the gene encoding aminoglycosides that was not found. The highest frequency of sulphonamide-resistant gene was found in bees. Besides, 43.2% of the samples were positive in more than one gene. The wild bee microbiota had the lowest number of antibiotic-resistant genes. A comparison of antibiotic resistance genes DNA sequences of a varroasis-treated and untreated bee was also performed. The significant difference was only in bees with the *sul2* gene in the microbiota. Bees treated with prophylactic medicinal products have a higher resistance to this group of antibiotics than untreated bees. Analysis of bee results showed no significant difference between particular breeds. We recommend a rational use of antimicrobials to reduce the transfer of genes responsible for antibacterial resistance in the bee microbiota and their possible transmission to the bees themselves, especially by avoiding sulphonamide preparations.

Keywords: honeybee, *Apis mellifera*, antibiotic-resistant genes, microbiota.

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Comparative Analysis of the Nutritional Value of Rabbit Meat: Differences Between Belgian Giants and Crossbreeds

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Due to rabbits' prolificacy and superior feed conversion efficiency, rabbit breeding is becoming more commercially successful as a source of meat (1). Breeders and meat producers have concentrated their attention on small mammals such as rabbits to match market expectations for healthier and leaner production (2). The differences in production across rabbit species are significant and must be studied scientifically. So, this study was aimed at investigating the nutritional value differences between Belgian giants and crossbred rabbits' meat. A total of 14 rabbits (46–130 days old) were selected by weight and divided into 2 groups: crossbreeds (7 rabbits/group) and Belgian giants (7 rabbits/group). During the experiment, housing and feeding conditions were identical for both groups and complied with the statutory norms. And at the end of the feeding test, the following analyses were performed after the slaughter and collection of muscle samples post-mortem: the morphological composition of the carcass, physicochemical properties, lipid oxidation levels (fresh and stored samples) and biogenic amines profile. Morphological studies of rabbit carcasses revealed that crossbred rabbits had slightly greater morphological indices (higher carcass and general muscles weight ($P < 0.05$); higher lumbar spine yield ($P < 0.05$)) than Belgian giants. When compared with the muscles of crossbred rabbits, the muscles of Belgian giant rabbits exhibited with statistically lower inter-muscle fat content by 1.73% ($P < 0.05$). Other nutritional value markers of rabbit meat, such as colour intensity and physical qualities revealed no statistically significant differences across rabbit breeds ($P > 0.05$). Therefore, lipid oxidation levels in rabbits' thighs (24 hours after the slaughter) were higher in crossbred ($P < 0.05$), compared with Belgian giants. However, after 3 months of storage, lipid oxidation levels in the lumbar spine of rabbits were 49% higher ($P < 0.05$) in Belgian giant muscles than in crossbred. After determining biogenic amine profiles in both breeds' lumbar spine samples, the results showed that Belgian giants had significantly higher cadaverine and tyramine levels ($P < 0.05$) compared with crossbreeds. Therefore, higher total biogenic amine content was found in crossbred samples. However, the observed results were not statistically significant ($P > 0.05$). The overall nutritional value of the two species examined did not differ significantly in many aspects, hence there was no evident trend between crossbreeds and Belgian giants.

Keywords: Belgian giants, crossbreeds, meat quality, nutritional value, comparative analysis.

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Suitability of Layer-Type and Dual-Purpose Male Chicks for Capon Production

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The elimination of day-old cockerels of layer or dual-purpose breeds poses a problem for the poultry industry (1). Capon production could contribute to the rational management of unwanted male chicks (2). The aim of this study was to determine the effect of age and breeds on the growth and carcass quality characteristics of caponized cockerels.

The experiment was conducted on 420 cockerels (3 groups: Rhode Island Red RIR, Green-legged Partridge (GIP) and Leghorn Lh; 140 of each breed, 7 replications per group and 20 birds per replication). The birds were raised to 28 weeks of age and were fed commercial diets *ad libitum*. At week 8 of age, birds were surgically castrated in accordance with Commission Regulation (EC) No. 543/2008. The procedure was approved by the Local Ethics Committee in Olsztyn, Poland. From week 12 of age, at 4-weeks intervals, 21 birds (1 bird per replication) were selected randomly and slaughtered. The statistical analysis involved the determination of arithmetic means and SEM. The data were analyzed by two-way ANOVA. The significance of differences in mean values between age groups was determined by the Duncan test. Significance was set at $P \leq 0.05$.

At 8 weeks of age, the average body weight (BW) of all cockerels was similar (RIR – 599.9 g, Lh – 597.8 g, GIP – 565.8 g). Between week 12 and 28, the BW of RIR, Lh and GIP capons increased respectively from 1218.8 g to 3038.2 g ($P < 0.05$), 1084.4 g to 2323.6g ($P < 0.05$) and 1125.0 g to 2187.9 g ($P < 0.05$). The carcass weight of RIR, Lh and GIP capons increased respectively from 738.7 g to 2041.5 g ($P < 0.05$), 654.2 g to 1438.5 g ($P < 0.05$) and 675.5 g to 1418.5 g ($P < 0.05$). From week 16 of age, the RIR capon's BW was significantly higher compared with the BW of Lh and GIP capons ($P < 0.05$). The statistically confirmed increase in carcass weight was found in GIP and Lh capons up to the age of 24 weeks ($P \leq 0.001$). RIR capons had the highest dressing percentage at 28 weeks of age (67.2%). Lh and GIP capons achieved the highest dressing percentage at 24 weeks of age (66.7% and 64.8%, respectively).

Capons of the analyzed breeds, raised under identical conditions, should be slaughtered at different ages. GIP and Lh capons can be slaughter earlier (week 24) than RIR capons (week 28).

Keywords: capon, growth, body weight, carcass weight.

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***GH* Gene (2291A>C) Polymorphism and its Influence on the Economic Traits in Lithuanian Beef Cattle**

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Traditional trait improvement has centered on quantitative genetics, using statistical analysis of phenotypic data to determine animals with the highest genetic merit. This selection approach is most effectively implemented for highly heritable traits that are easily recorded before reproductive age. Genomic selection refers to the use of genome-wide genetic markers to predict the breeding value of selection candidates (1, 2). The growth hormone (GH) gene is a candidate gene for predicting growth and meat quality traits in animal genetic improvement since it plays a fundamental role in growth regulation and development (3, 4). The aim of this study was to investigate the prevalence of GH gene (2291A>C) polymorphism and to determine its influence on the growth rate in beef cattle. Cattle hair follicles samples were collected from 85 bulls consisting of Angus (41), Limousin (19), Galloway (19) and Simmental (6) cattle. Hair samples and the data on daily weight gain records were obtained from Šilutė control bulls feeding station. Bovine genomic DNA was extracted from hair follicles using Chelex DNA extraction method. Polymorphism of GH locus was identified using a PCR-RFLP method. Investigation of polymorphism 2291A>C of GH gene showed that allele A (frequency – 0.947) and genotype AA (frequency – 0.918) were the most common in the general population of beef cattle. Meanwhile, the homozygous CC genotype was the rarest, with a frequency of 0.024. When calculating the influence of GH gene polymorphism (2291A> C) on cattle productivity traits, it was observed that this polymorphism had a statistically significant ($P < 0.05$) effect on cattle live weight, which was determined before cattle slaughter. Evaluation of genotype influence data showed that cattle of AC genotype weighed more than animals of AA or CC genotype. However, after calculating the statistical reliability criterion P value, only a statistically significant result of two characteristics was determined: cattle overweight, kg (from the end of March to the end of June 2017) and live weight, kg. In conclusion, the results showed that polymorphism of the GH gene influences some of the productivity traits of beef cattle.

Keywords: cattle, *GH* gene, polymorphism, PCR-RFLP.

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Gut Response to High or Low Dietary Arginine, Methionine and Lysine Levels in Young Turkeys Applied to Different Challenge Models

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Dietary levels of arginine (Arg), lysine (Lys) and methionine (Met) may be important for the overall health of turkeys. The aim of the study was to determine the influence of feeding diet of low levels (as recommended by NRC)¹ of Arg, Lys and Met (Low ArgLysMet) or diet of high levels (10% higher than recommended by NRC) of Arg, Lys and Met (High ArgLysMet) on performance and the functional status of the gut in turkeys reared either in the optimal conditions or infected with *C. perfringens* or *E. coli* lipopolysaccharide (LPS). A total of 192-day-old female Hybrid Converter turkeys were allocated to 48 pens with 4 birds per pen and 8 replicates per each of the 6 treatment groups. The treatment groups were as follow: (i) birds fed either Low ArgLysMet or High ArgLysMet diets, (ii) birds fed diets as above but orally challenged at day 25, 26 and 27 of age with *C. perfringens* bacteria, or (iii) birds fed as above but orally challenged in 25, 26 and 27 of age with LPS. At day 28 of age, 8 birds from each treatment were sacrificed for sample collection. Feeding birds diet of High ArgLysMet resulted in significantly increased body weight on day 25 of age (before challenge) and on days post-challenge (days 25–28), as well as increased body weight in overall experimental period (0–28). Birds fed diet of High ArgLysMet had significantly lower concentrations of IgA and 8-OHdG enzyme in the blood as well as increased activity of OGG1 enzyme in the jejunal tissue. In the case of the challenge response (*C. perfringens* or LPS), a higher blood or gut tissue concentrations of, among others, IgA, IgM, IgY, Casp 8, APEX 1, 8-OHdG and IL-6 indicate that infection with *C. perfringens* caused greater immunological pressure on the bird gut than LPS. The gut permeability test indicated that a higher concentration of the intestinal permeability marker (FITC-d) in the blood was found in birds infected with *C. perfringens* compared with the control group; however, irrespective to dietary Arg, Lys and Met levels. Our data indicate that feeding diet of increased Arg, Lys and Met levels was not associated either with excessive stimulation of the immune system nor with the increased level of DNA damage in the gut tissue. At the same time, the increased concentration of DNA-repair enzymes in the gut tissue as a result of the administration of High ArgLysMet diet may indicate its beneficial effect on the functional status of the gut-barrier function. This work was supported by the National Science Centre, Grant No. 2017/27/B/NZ9/01007.

Keywords: *C. perfringens*, *E. coli*, essential amino acid, turkeys.

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Bovine colostrum modulates the intestinal homeostasis and diminishes the adhesion and invasion of enteropathogenic *Escherichia coli*

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Bovine colostrum (BC) plays a critical role in the development of neonatal calves. The exact molecular mechanisms of BC and activated intestinal signaling pathway remains poorly understood. Therefore, we aimed to characterize the effects of BC on the intestinal homeostasis and gut permeability using chimeric calve-mice fecal microbiota transplant mice and neonatal rats *in vivo*. Furthermore, we determined the effects of BC on the *in vitro* adhesion and invasion of enteropathogenic *Escherichia coli* O157.

The pre-incubation of intestinal Caco-2 cells with BC (250 µg/mL) resulted in significantly decreased ($p < 0.05$) burden of adhered *E. coli* O157 (2.1 log₁₀ CFU) in comparison to untreated control (UC) (2.9 log/CFU) or autologous milk control (MC) (3.0 log₁₀ CFU). The Caco-2 treatment with BC ameliorated *E. coli* O157 intracellular invasion (2.1 log₁₀ CFU) in comparison to UC (2.9 log₁₀ CFU) or MC (3.0 log₁₀ CFU). The BC activity on *E. coli* virulence was fraction-dependent and peaked at T3 hour *post-partum*. The heat inactivation of BC resulted in significant loss of anti-virulence activity *in vitro*. The BC treatment (10 ml/Kg) of chimeric calve/mice fecal microbiota resulted significantly lower MC permeability in comparison UC. Neonatal rat feeding with BC resulted in greater layer of intestinal mucins, that was most evident when animals were fed with BC fractions collected at 1, 3 and 4 hours post-partum.

Collectively these results demonstrate host-directed anti-virulence properties of BC. BC decreases the intestinal permeability *in vivo* by upregulating mucus production. Further studies are needed to better understand the composition of BC and molecular signaling pathways induced by feeding with BC.

Key words: Bovine colostrum; intestinal permeability, immunomodulation.

Association of Toll-like Receptor 4 Gene Polymorphisms with Cow Milk Production Traits

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Toll-like receptor 4 (*TLR4*) is located on the cell surface and initiates innate immune responses (1). The *TLR4* gene in cattle about 3739-bp contains an open reading frame of 2526-bp encoded 841 amino acids (2). The bovine *TLR4* gene is highly polymorphic (2) and some SNPs in the *TLR4* gene have been associated with milk traits (1, 3).

The research was aimed to investigate polymorphisms c.9421C>T and c.2021C>T and to evaluate their influence on milk fat, protein and lactose percent in Lithuanian dairy cows. Genotypes were determined using RFLP-PCR and detected by performing 2% agarose gel electrophoresis of PCR-RFIP samples and evaluating fragment sizes according to the molecular marker in UV light. The data concerning milk fat, protein, lactose were analyzed. The analysis was performed using program SPSS 22.0. The influence of gene and statistical significance of differences between different genotypes was evaluated by ANOVA (one-factor dispersion analysis). For the influence of the *TLR4* gene polymorphisms on productivity traits in interaction with other factors was evaluated using the linear model. The following factors were analyzed in the model: gene, farm, lactation, duration of lactation.

Blood samples were collected from 150 Lithuanian dairy cows. Genotype and allele frequencies for the analyzed population of cows were calculated. Three genotypes of cows in both polymorphisms were analyzed: CC, CT and TT. The C and T alleles of the c.9421C>T (382 bp) and c.2021C>T (367 bp) polymorphisms were identified based on the amplification of specific primers, followed by digestion with the restriction enzymes *AluI* and *BsiHKAI*. Genotypic frequencies of the c.9421C>T for the CC and CT genotypes were 0.283 and 0.507, respectively, and the allele frequencies for C and T allele were 0.536 and 0.464; meanwhile, genotypic frequency of the c.2021C>T polymorphism for the TC genotype was 0.954, and for the CC genotype it was 0.03. Allele C frequency was 0.490 and allele T frequency was 0.510. Impact of polymorphisms on milk composition indicators in Lithuanian Holstein cows' milk was determined. Genotype TC of c.9421C>T showed higher fat and protein percentage than the other two genotypes, and the highest lactose percentage was established in TT genotype (4.62%). The results of c.2021C>T showed that cows homozygous for the C allele had the biggest influence on milk fat, protein and lactose percentage. After evaluating influence of the *TLR4* gene polymorphisms on productivity traits in interaction with other factors, it has been determined that c.2021C>T polymorphism with lactation influenced milk fat by 3.9% ($P < 0.05$). Polymorphism c.9421C>T together with farm influenced milk fat by 2.5% ($P < 0.05$) and milk protein by 2.2% ($P < 0.05$). The highest effect on cow fat, protein and lactose percent was made by farm and lactation duration ($P < 0.001$).

Keywords: cow, *TLR4* gene, polymorphism, PCR-RFLP.

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The Prevalence of Microorganisms in Milk Depending on Cow Lactation Number and Lactation Period

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Mastitis is caused by a wide spectrum of pathogens and, epidemiologically categorized into contagious and environmental mastitis (1). Contagious pathogens include *Staphylococcus aureus*, *Streptococcus agalactiae*, *Mycoplasma* spp. and *Corynebacterium bovis* (2), while environmental pathogens include *E. coli*, *Klebsiella* spp., *Strept. dysgalactiae* and *Strept. uberis* and the majority of infections caused by these pathogens are clinical and of short duration. The degree of the inflammatory response depends on the invading pathogen, and host factors such as stage of lactation, age, immune status of the cow, genetics, and nutritional status (3, 4). According to Goli et al. (5), younger cows in their first lactation period are more resistant to contagious causative agents and about 13% of first-lactation cows are already infected with *S. aureus*. Many of them remain infected during the whole lactation, unnoticed, and served as reservoirs for the spread of the infection to other cows in the herd. Research indicates that cows older than 7 years are at risk of udder infection.

The aim of this study was to evaluate the isolation frequency of mastitis causing-microorganisms depending on the cow lactation number and lactation period. The study was conducted in a dairy farm with 428 lactating cows. To identify distribution of main milk microorganisms (CNS, *Enterobacteria*, *Str. agalactiae*, *E. coli*, *S. aureus*), milk samples from cow teats were collected. Agents were identified according to standard operating procedures SDP 5.4.4.B.6 guide “Fundamental mastitis-causing bacteria evaluation in milk” developed by “Laboratory and field handbook on bovine mastitis” within 24 h after sampling.

First lactation cows compared with cows of older lactation produced milk with a lower amount of microorganisms (except *E. coli*). With an increasing cow lactation number, the amount of *Enterobacteriaceae* increased as well ($P < 0.05$), while with an increasing lactation period, a greater count of CNS, *Str. agalactiae* and *E. coli* was detected but the biggest number of *Enterobacteriaceae* was established in the first months of lactation.

After assessing the prevalence of microorganisms isolated at different stages of lactation, it was found that with an increasing lactation period a bigger amount of CNS, *Str. agalactiae* and *E. coli* was detected. The highest amount of *Enterobacteriaceae* was detected during the first months of cow lactation. In conclusion, after evaluating microorganisms established in cows with different lactations and lactation periods, no clearly visible trends were found. *E. coli* was more often detected at the end of the cow lactation period. A significant increase of CNS was observed at the end of the first lactation cows than in the cows with the middle lactation period ($P = 0.008$). At the end of the lactation period of the second and third lactation cows, *Enterobacteriaceae* and *Str. agalactiae* were identified more frequently.

Keywords: cow, lactation, microorganisms, milk.

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Aquaponics: The Symbiosis of Fish and Vegetable Growing Systems

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During 2020–2023, Klaipėda University is implementing the project No. 14PA–KL-19-1-08701-PR001 “The use of innovative, environmentally friendly and pollution reducing aquaponics”, by the measure of program for rural development in Lithuania “Communication of knowledge and outreach activities”, activity field “Support for demonstration projects and outreach activities”. An experiment is performed in 10 farms of different categories. The aim of this project is to introduce and promote development of this progressive method in Lithuania. The aquaponics system is a technology and a process during which fish and plants are grown together. It is symbiotic operation of two systems: plants live and grow owing to the fish, and fish grow partly owing to the plants. According to the experience of other countries, this technological system is beneficial both economically and environmentally (1–3). The aquaponics system guarantees an output of two valuable and high quality food products (certain fish and vegetable) in one space saving place. This system can be proceeded all year around, and does not require cultivation and fertilization of the soil and is in need of significantly less water. In an aquaponics system, water cleared by the roots of plants comes back to the pool of fish with less nitrogen compound, and just to fill up evaporated water is needed. This way, water resources are saved and a major part of nitrogen compound does not get in the environment. Plants do not need to be additionally fertilized; therefore, there is no use of herbicides. Electric power is used in the process of growing fish and plants only. The system can be used not only in rural but also in urban territories. Hence, aquaponics is a sustainable and pollution reducing innovative technological system, which has never been practiced in Lithuania before.

Keywords: technology, water, fish, plants, environmental.

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Mobile Tigecycline Resistance (MTR) in Animals Aimed at Human Consumption

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Tigecycline (TIG) is a synthesized third-generation tetracycline antibiotic, which exhibits powerful in vitro activity against a wide spectrum of bacteria. It is one of the last available treatment options used to fight serious infections caused by multidrug-resistant pathogens (1). The increasing prevalence and widespread dissemination of antimicrobial resistance, especially the emergence of multidrug-resistant (MDR) micro-organisms, cause global concern (2). Infections caused by MDR pathogens are high burdens, because of the increased costs, longer hospital stay, and morbidity and mortality rates. The use of tetracycline both in humans and also in animals can, among other factors such as gene mutations, contribute to high-level tigecycline (TIG) resistance. Mobile TIG resistance (MTR) can be detected in diverse bacteria isolated from food animals; therefore, it is important to explore the potential sources as well as its impact in the livestock sector and the one health implications. Although TIG has not been used in animals, animals destined for human consumption can get colonized by TIG-resistant organisms through environmental transfer, consumption of contaminated feed or drinking water. Very recently, plasmid-mediated transmissible tet(X), tet(X3) and tet(X4) genes conferring high-level tigecycline (TIG) resistance were discovered in isolates from food animals, meat and environment in China. Hence, the clinical usefulness of TIG was being threatened by the mobile TIG resistance (MTR). The presence of MTR in the livestock sector is a threat to global food safety and security (3). Through international or domestic food animals/meat trade and travel, TIG-r organisms can be transported from one location to another, making this a worldwide problem. The MTR impacts globally on different chain links, the one health concept, with high economic implications.

Keywords: mobile tigecycline resistance, antibiotic resistance, one health, livestock sector.

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Possibility to Use Sex-Sorted and Unsorted Sperm for *in Vitro* Fertilization

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The main goal of successful livestock farming is to quickly breed healthy offspring of good genetic breeds. This can be achieved through the application of innovative, advanced technologies. Embryo transplantation has become one of the most effective methods that can rapidly improve the genetic potential of breeding stock and thus rapidly increase the number of elite stock in the herd (1). As usual, the prevalent sex sorting techniques, even though being used commercially, need to be further refined for mass scale use of sexed sperm. Whereas sperm sorting techniques that are being used commercially have 90% accuracy (2). The problem is that the fertility when using sex-sorted sperm is lower than that when the unsorted sperm is used (3). Many methods of sperm separation (selective fractionation (centrifugation) and swim-up) have been developed to improve sperm quality. Changes in viability/motility and capacitation/acrosome reaction of sex-sorted sperm could be the reason for the reduced initial fertilization rates in *in vitro* and *in vivo* studies. The aim of this study was to investigate the changes of motility and acrosomal reaction during capacitation of sex-sorted and unsorted sperm and difference to cleavage of oocytes fertilization.

The ovaries of dairy cows were cut out immediately after slaughter and transported within one hour. The diameter (mm) of various follicles was measured. 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 94 COCs (cumulus cells) were aspirated from 29 ovaries. Only normal COCs were used for maturation. *In vitro* matured COCs were fertilized with frozen-thawed sex-sorted and unsorted sperm. Frozen sperm from a Holsteins bulls was thawed at 37°C for 40 s. The thawed first group sex-sorted sperm (n = 17 samples) and the second group of unsorted sperm (n = 17 samples) were capacitated 60 min by a swim-up method. After incubation Trypan blue stain was used to evaluate viability and the acrosome status of spermatozoa. Stained sperm was counted and divided in three groups: non-viable (the spermatozoa stained all), hyperactivated, and with acrosomal changes (changes of spermatozoa head membrane). After fertilization, the embryonic cleavage was evaluated within 48 h (cleavage rate).

The investigate results show that the sex-sorted sperm shows lower capacitation (10%) and the acrosome reaction (6%) rate than the unsorted sperm. Also, the evaluation of the motility after last centrifugation in the first (sex-sorted) group showed that sperm motility decreased by 15.1%, and in the second (unsorted) group it decreased by 12.5%. After fertilization, a lower cleavage rate was determined in oocytes from the sex-sorted sperm (A class oocytes – 37.63% and B class oocytes – 25.84%) and the unsorted sperm (A class oocytes – 47.36% and B class oocytes – 42.1%). In conclusion, this study shows that the unsorted spermatozoa were most suitable for *in vitro* fertilization of A and B grade oocytes. The comparison of oocytes group results shows that inferior quality oocytes require more motility spermatozoa to fertilize.

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

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Association Between *Mblk-1* Gene Polymorphisms and Resistance to *Varroa Destructor* Mites in Honey Bee

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Varroasis is an invasive disease caused by *Varroa destructor* ticks that infect honey bees (*Apis mellifera*) at any stage of their development. The host–parasite interaction between honey bees and the mite *Varroa destructor* is unusual, as honey bee colonies are relatively poorly defended against this parasite. The interaction has existed since the mid-20th century, when *Varroa* switched host to parasitize *A. mellifera*. Infection with these ticks has reduced the viability and productivity of bees, disrupts normal development, reduces weight and resistance to viral diseases. Without acaricides, honey bee colonies typically die within 3 years of *Varroa* infestation (1). However, it has been observed that some bee colonies are able to defend themselves against these parasites without additional chemical or natural substances. The resistance of honey bees is associated with genetic factors that determine their behavior to protect against parasites. *Mblk-1* was identified from a transcriptomic non-hypothesis-driven approach focusing on the honeybee higher brain centers as a candidate that can be potentially important for the highly social behaviors of the honeybee (2). In this study, we aimed to test the contribution of three *Mblk-1* gene polymorphisms to the resistance to *V. destructor* mites. This case–control study involved 117 DNA samples which were genotyped for three single nucleotide polymorphisms using Real-Time Polymerase Chain Reaction method. The distribution of three polymorphisms located at 7454459 (Asn → Thr), 7454648 (Gln → Arg) and 7454648 (Leu → Pro) positions of the *Mblk-1* gene were analyzed among three groups of honey bees: the domestic honey bees treated against *Varroa destructor* ticks, untreated domestic honey bees and wild bee families. Statistical analysis showed that distribution of mutant allele A of the polymorphism at 7454459 (Asn → Thr) position was significantly more common among untreated and wild bees compared with domestic, which are contagious infected with *Varroa destructor* (15.1% and 13.3% vs. 0.8%, $P = 0.004$). The distribution of genotypes was also statistically significant: the mutant AA genotype was more common among uninfected bees, and this genotype was not detected in families treated for the disease. The distribution of alleles and genotypes of the other *Mblk-1* gene polymorphisms at positions 7454648 and 7454648 that also altered the amino acid sequence of the protein was not statistically significant. However, there is a tendency for the mutant, resistance-causing T and A (respectively) alleles of these polymorphisms to be more common among domestic untreated against *Varroa destructor*, and wild bee families.

Keywords: Honeybee, *Apis mellifera*, *Mblk-1* gene, *Varroa Destructor*.

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Zoohygienic Conditions in a Chinchilla Farm and their Influence on Selected Production Indicators

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Despite the fact that chinchillas have been farmed for a century, there are not many studies concerning their behavior, housing requirements or degree of domestication, all of which are important factors in the assessment of the welfare. Different guidelines for keeping and caring chinchillas are focused mainly on the dimensions of the cages, leaving aside the microclimate issues, whilst individual factors such as temperature, humidity, air velocity, cooling, concentration of harmful gases and room lighting determine the welfare of animals. Chinchillas are animals that are particularly sensitive to environmental factors. Unfortunately, to this day, they remain one of the few species of farm animals for which the standards of microclimatic parameters have not been established.

The aim of the study was to assess the influence of microclimatic conditions in a chinchilla farm on production indicators such as fertility and quality of the coat.

The research was carried out in the chinchilla breeding farm in Myślenice (Poland) in two periods: winter (stage I) and spring (stage II). Measurements were carried out in two breeding rooms, in each on 3 racks and 3 levels (level I – the highest cages, level II – medium cages, level III – the lowest cages), altogether in 54 measurement points (cages). The research included measurements of basic microclimatic parameters such as lighting, humidity, water vapor pressure, air movement, temperature, cooling, concentration of carbon dioxide, hydrogen sulphide and ammonia as well as ozone concentration measurements. The obtained results were compared with the assessment of the quality of the fur coat and the reproductive results of chinchillas.

The obtained results indicate that the average fertility (youngsters per year) of chinchillas at individual cage levels was distributed as follows: 1.83 at the 1st level of cages, 1.66 at the 2nd level and 1.82 at the 3rd level.

Based on the conducted microclimatic tests, it can be concluded that the assessed farm had a high hygienic standard. The best production effects (high fertility, the highest quality of fur coat) were found in chinchillas from cages located at the highest levels. The fertility of chinchillas depends on the illumination of the room (the lowest fertility was found in the least lit cages).

Summing up, it can be stated that it is necessary to elaborate zoohygienic standards for chinchilla farms in order to objectify the assessment of animal welfare and obtain high production effects.

Keywords: chinchilla, farm microclimate, fur quality, fertility, welfare.

Effect of a High-Fat Diet and Chromium on Hormones Level and Cr Retention in Rats

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High-fat diet (HFD), especially taken for a long time, can cause obesity as well as many civilization diseases such as diabetes and cardiovascular diseases. Scientific publications have shown that HFD is the reason for hypertriglyceridemia, hyperinsulinemia, and glucose intolerance. The adverse effect of HFD on the organism can be neutralized by adding chromium to the diet. We hypothesized that the addition of chromium to HFD would reduce the negative effect of that diet on the secretion of hormones regulating carbohydrate metabolism and physiologically important neurotransmitters. It was additionally postulated that chromium in the nanoparticle form will be more easily digestible and better retained in the body of rats than chromium in the organic form, and thus the regulatory effect of this form of Cr on hormonal metabolism will be more efficient. The aim of the study was to determine how the administration of HFD supplemented with various forms of chromium to rats affects the accumulation of this element in the tissues and levels of selected hormones (leptin, ghrelin, insulin, glucagon, serotonin, noradrenaline, and histamine). The experiment was conducted on 56 male Wistar rats, which were divided into 8 experimental groups. The rats received for 8 weeks a standard diet or high-fat diet with the addition of 0.3 mg/kg body weight of chromium(III) picolinate (Cr-Pic), chromium(III)-methioninate (Cr-Met), or chromium nanoparticles (Cr-NP). It was noted that chromium in organic forms was better retained in the organism of rats than Cr-NP. However, Cr-Pic was the only form that increased the insulin level, which indicates its beneficial effect on carbohydrate metabolism. In the blood plasma of rats fed HFD, an increased level of serotonin and a reduced level of noradrenaline were noted. The addition of Cr to the diet, irrespective of its form, also increased the serotonin level, which should be considered a beneficial effect. A high-fat diet was shown to negatively affect the level of hormones regulating carbohydrate metabolism (increasing leptin levels and decreasing levels of ghrelin and insulin). This work was supported by the National Science Centre, Grant No. 2020/39/B/NZ9/00674.

Modern Techniques Related to the Control of Veterinary Drug Residues in Meat Samples

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Modern analytical techniques represent a high potential for the analysis of a wide range of food contaminants, especially such sensitive and selective instrumental methods as high-resolution mass spectrometry (HRMS). This approach not only significantly lowers the limit of detection, but also opens opportunities for retrospective analysis of the data obtained. Due to the high selectivity, it is also possible to detect compounds in the presence of interfering matrix components. This allows reduction of the total analysis time due to both faster sample preparation and shorter chromatographic program, as it is possible to identify and quantify compounds that are not completely chromatographically separated. However, the main advantage is the possibility to significantly increase the range of simultaneously detectable veterinary drugs.

This report describes the application of two analytical methods based on HRMS for the analysis of veterinary drug residues in animal origin products. The first study proposes an analytical method for simultaneous identification, screening and quantification of 164 residues and metabolites of pharmacologically active substances belonging to such therapeutic classes as anti-infectious (antibiotics and chemotherapeutics), anti-inflammatory and antiparasitic agents (against protozoa, endo- and ectoparasites), corticoids and agents acting on the nervous and reproductive systems, substances with hormonal and thyreostatic action, and beta agonists. Different sample preparation procedures were compared and optimised for the detection of selected veterinary drugs in chicken, porcine and bovine meat by UHPLC coupled to Orbitrap HRMS. A total of 130 selected compounds in chicken meat, 127 compounds in bovine meat and 123 compounds in porcine meat samples could be quantified with an accuracy ranging from 70% to 120%. The method was successfully used to detect and quantify veterinary drug residues in real samples.

The main purpose of the second study was to develop a fast and reliable analytical method based on ion cyclotron resonance HRMS for the detection of quinolones in poultry meat. The sample preparation procedure was simplified by reducing the procedure to extraction and freezing out steps and the chromatographic separation step was excluded completely. As a result, the total analysis time was reduced to less than an hour. The validation study revealed that the method is capable of detection and confirmation of ten quinolone compounds in poultry indicating the compliance with MRL values. Analysis of treated chickens revealed that the developed method is suitable for the determination of ciprofloxacin and enrofloxacin. The proposed procedure could be one of the fastest quantitative confirmatory methods for the analysis of quinolones available so far.

Keywords: veterinary drug residues, mass spectrometry

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Comparative Analysis of the Nutritional Composition of Eggs from Two Different Poultry Species from Lithuania

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Eggs are one of nature's most nutritious foods, maintaining a low saturated fat content and a high protein content (1). Eggs are a rich source of energy, minerals, and vitamins, all of which are essential for a healthy diet. However, egg quality can be determined by poultry species and comparative data on this case is essential and can aid technological development (2). So, this study was designed to compare Guinea fowl and laying hen egg nutritional value. All the eggs were purchased at local Lithuanian markets, considering the egg laying time. The following indicators were determined when eggs were fresh and after 28 days of storage: egg, yolk, and albumin heights, Haugh unit, yolk colour intensity, eggshell thickness, the pH of the yolk and albumin, dry matter content of yolk, fat, ashes, and protein contents; a sensory evaluation and an analysis of consumers' acceptability by facial expressions were performed. Guinea fowl eggs were observed to have the greater redness of the yolk colour of fresh eggs by 9.69 points; the weight of the shell with film and its strength by 38%; the weight without film by 46%; the shell thickness at the pointed end by 48%, the middle by 58%, and the blunt end by 77%; the shell-to-egg weight ratio by 60% compared with laying hens ($P < 0.05$). Stored Guinea fowl eggs had higher yolk redness (a^*) by 8.11 points, shell thickness at the pointed end by 40%, and the ratio of shell-to-egg weight by 45%, compared with laying hens ($P < 0.05$). No significant differences were found in the chemical composition of Guinea fowl and laying hen eggs. However, when evaluating egg yolk colour characteristics, the results showed that Guinea fowl yolks brightness (L^*) was lower by 8%, the yellowness (b^*) by 21%, but redness (a^*) was more intense compared with the laying hens' yolks ($P < 0.05$). Sensory evaluation showed that consumers rated Guinea fowl eggs as having 18% greater protein colour uniformity, 59% more intense yolk colour, 38% greater total yolk flavour intensity, 21% more acceptable yolk, and 27% less yolk crumbiness than laying hens ($P < 0.05$). The emotional expressions of the consumers' faces while tasting Guinea fowl eggs and comparing them with laying hens showed that the following emotions were expressed more: happiness, contempt, and less fear ($P < 0.05$). To sum up the obtained results, as an alternative to laying hens' eggs, Guinea fowl eggs can be consumed, considering better albumin and shell properties, storage retention, consumer acceptance and emotions expressed by tasting them.

Keywords: Guinea fowl, laying hens, egg quality, sensory analysis, comparative analysis.

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Complex Evaluation of Arabian Horses Bred on Lithuanian and German Stud Farms

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In terms of animal welfare, the sport horse industries generally control themselves. Therefore, it can vary greatly between different countries. However, there are insufficient standards and rules in place for horse training (1). As a result, differences between countries are frequently evident. So, this study was designed to perform an Arabian horse breed complex evaluation by comparing feeding and keeping conditions in Lithuania X and Germany Y studs. Research was carried out with a total of 14 horses, each stud farm consisting of 7 Arabian breed horses, which were selected by gender, age, and similar weight. The horses were assessed on their conformation, temperament, movements, feeding, and keeping circumstances, as well as their body measurement indices. The conformation of the horses showed that statistically significantly higher measurements were obtained for Arabian horses bred in Germany: correspondingly longer shoulder length by 14%, anterior ankle length by 14%, anterior leg circumference height by 28%, pelvic length by 25%, lower leg length by 4%, hind ankle length by 6%, hind leg height by 29% compared to the measurements of horses bred in Lithuania ($P < 0.05$). Evaluation of horse body size indices (large body format, pelvis, pelvis-knee patella, shoulder-foreleg length from wrist joint to hoof) revealed that lower indices were obtained in Lithuania X stud farms compared with Arabian horses bred in Germany ($P < 0.05$). These statistically higher indices were obtained in Germany's Y stud farm horses: shoulder, hind ankle length, forearm-foreleg length from wrist joint to hoof, thighs ($P < 0.05$). After evaluation of temperament, it became clear that the horses of the Arabian breed bred in both Lithuania and Germany had positive characteristics in their manners: 7 horses bred in Lithuania and 6 bred in Germany were peaceful; after assessing the negative characteristics, there were no angry horses bred in Lithuania, and in Germany there were no cowards who did not trust a person. Therefore, in terms of mobility, the walk and trot of horses bred in Germany were of higher quality compared with horses bred in Lithuania. Regarding the feeding of horses in both countries, the norms were in line with the feeding recommendations for sport horses. After analysing the conditions of keeping Arabian horses in Lithuania and Germany, it became clear that horses are kept in different types of stables, but in individual pens, depending on the requirements. In summary, although the feeding and keeping conditions corresponded to the norms in both Lithuanian and German stables, considering the performed measurements and the calculated indices, the Arabian breed horses bred in German Y studs achieved higher indicators.

Keywords: Arabian breed, horse, Lithuania, Germany, conformation, body measurement indices.

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Influence of Puerperal Metritis on the Recovery of the Estrous Cycle After Calving in Modern Dairy Cows

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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 free stall 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 2 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 22. Data were statistically significant when $P < 0.05$.

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, respectively ($P < 0.002$). The same tendency was observed in the MDO and HDO groups, 9.5 ± 1.3 and 7.0 ± 1.4 days postpartum, respectively ($P < 0.002$). 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%, respectively ($P < 0.027$). Moreover, the HDO group cows ovulated their follicle during the first follicular wave faster compared with the MDO group (11.4 ± 2.7 day and 20 ± 1 day, respectively, $P < 0.01$). Also, HDO group cows had a smaller diameter of the ovulatory follicles compared with the MDO group cows (15.3 ± 1.9 mm and 17.3 ± 1.7 mm, respectively, $P < 0.04$).

The conclusion can be drawn that 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.