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LIVESTOCK PRODUCTION – RECENT TRENDS AND FUTURE PROSPECTS

Abstracts

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OUR FOOD OF TOMORROW IN THE CONTEXT OF SUSTAINABLE FOOD SYSTEMS AND CLIMATE CHANGE: ROLE OF LIVESTOCK

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Providing enough, safe and affordable food is a vital societal goal. Climate change, immigration, the European conflict, and extensive inflation are some of the most pressing contemporary crises directly affecting the food chain's ability to achieve its established goal. Indeed, the food production is increasingly challenged by climate change with the associated weather extremes such as lengthy periods of drought, heat and frequent floods, as well as increasing atmospheric CO₂ concentrations directly affecting the production of feed and food. Drought and heat can not only reduce yields, but rising temperatures can increase susceptibility to disease and the exposure of plants to dangers, e.g., fungal toxins [1]. In respect of climate change, research results already suggest – even if not always without doubt – that the nutrient concentrations in plants could be reduced, while yields should increase [2]. On the other hand, our current food systems harm the environment (i.e., animals are commonly held responsible for about 19–29% of anthropogenic greenhouse gas emissions [3]); all this raises a question whether we can produce enough food and how we must adapt our food systems and nutrition in the future to ensure enough, fair and more sustainable food for all. For this, during the last years, several new criteria have emerged for food production evaluation (i.e., the EU Green Deal). Certain quality labels have emerged for locally, organically and environment-friendly / sustainably-produced food. For farm animals, animal health and welfare issues have received greater importance recently. Ever since, the agricultural production utilises all available national agricultural resources to generate food for a dynamically changing population. Based on the availability of agricultural resources, tradition and other technological opportunities, countries have established and refined their food producing systems, aiming to make use of the best of their agricultural resources, and so supplying their population with nutritious, safe and affordable food, both of plant and of animal origin. The plant-based food systems use the cropland, mainly cereals, legumes, vegetables, oilseeds, and fruits. The animal-based food systems use both resources, croplands and grasslands. Animal products are a guarantor for the supply of the population with healthy and nutrient-rich food. Ruminant production, milk and meat (ruminants) are produced primarily through the use of grassland (regional), without the use of external fertilisers, pesticides, fossil energy and without any competition with cropland. A number of regionally available co-products from different industries as well as food waste can also be easily integrated into the feeding of ruminants. As a result, ruminants play an important role in converting such biomass into high-quality food in a sustainable way. An increased intake of milk and dairy products could improve the population's supply of micronutrients such as calcium, iodine or a number of B vitamins, which are often inadequately consumed. This study is an overview of production of animal food, with a focus on milk and meat. The use of alternatives to animal food, which are intended to replace typical animal protein food, is also shortly discussed. Advantages and disadvantages of different food production systems and forms of nutrition are discussed, the need for changes in consumer behaviour is addressed, and finally the need for research for crisis-resistant food production is outlined.

Keywords: food production, cattle feeding, nutrition, animal health, sustainability.

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AQUAPORINS IN REPRODUCTIVE SYSTEM: NEW MARKERS FOR MALE (IN)FERTILITY IN CATTLE?

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Improving reproductive methods and searching for new solutions is one of the challenges of modern animal breeding. Among the new potential indicators that may allow precise determination of male reproductive potential and elimination of low-fertility males are aquaporins located in the reproductive system (AQPs) [1, 2]. In mammals, of the 13 known AQPs (AQP0–AQP12), as many as 11 were found in the male reproductive organs. AQPs located in sperm are of particular interest in the context of assessing semen quality parameters. According to some authors, their expression may play an important role in the “journey” of sperm in the female reproductive tract and effective fertilisation. Despite the wide range of possibilities related to the measurement of AQPs, little is still known in this respect in farm animals, including cattle. Hence, research was undertaken, the main goals of which were to identify and analyse changes in the expression of all potentially occurring AQPs in the reproductive tract with the growth and development of animals, as well as to identify AQPs in sperm and search for the relationship between their expression and selected semen quality parameters. As a result of the conducted research, the bull will be the first male in which a comprehensive assessment of the occurrence of all known AQPs in the male reproductive system was made. To date, as many as 12 aquaporins, apart from AQP2, have been found in cattle. The age-related changes in the expression and/or distribution patterns of particular AQPs indicate the involvement of these proteins in the normal development and course of male reproductive processes in cattle. The presence of individual AQPs was observed throughout the entire male reproductive system, starting from gonocytes in the youngest animals, through Leydig and Sertoli cells, germ cells in seminiferous tubules, basal and principal cells of epididymis, and ending with vas deferens. Assessment of selected sperm parameters of cryopreserved semen and changes in the expression of individual AQPs indicates that they may be related to its quality.

Keywords: water channel, gonocyte, germ cells, Sertoli and Leydig cells, spermatozoa.

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ADVANCEMENTS IN SURVEILLANCE OF ANTIMICROBIAL SUBSTANCE USAGE IN LIVESTOCK PRODUCTION

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Antimicrobial substances (AB) are extensively used in livestock production to prevent and treat infections, promote growth, and maintain overall animal health. Monitoring AB usage is essential to mitigate antimicrobial resistance (AMR) development, safeguard public health, and support sustainable agricultural practices. However, effective laboratory testing of veterinary drug residues presents significant challenges, requiring advanced analytical equipment to quantify these substances at extremely low levels [1]. Our scientific group within the Institute “BIOR” (Latvia) plays an important role in continuously monitoring and analysing AMR and AB residues in various environmental and biological matrices. Over the past five years, “BIOR” has developed and optimised advanced methods for detecting AB in meat and water samples and contributed significantly to assessing AMR prevalence in bacterial isolates from farms. BIOR chemistry laboratory published several papers dedicated to refining detection techniques, allowing for identifying up to 90 antimicrobial compounds in meat samples with high sensitivity [2, 3]. Research at “BIOR” has consistently demonstrated that antimicrobial residues in meat typically remain within established regulatory limits, reducing concerns about antibiotic exposure from Latvian meat products. However, significant AMR spread has been identified, particularly in zoonotic bacteria like *E. coli* and *Campylobacter*, which has led to recommendations for more stringent monitoring and targeted sampling. “BIOR” has further broadened its research to include the analysis of farm wastewater (WW), developing techniques to detect antibacterial agents at extremely low concentrations, thereby identifying potential contamination risks associated with farm WW. By developing advanced methods with detection limits ranging from 1 to 250 ng/L, “BIOR” identified antibacterial agents in the waters of 8 out of 15 farms in 2022, with enrofloxacin concentrations reaching up to 343 ng/L. The study found significant AMR in *E. coli* and *Enterococcus* isolates from WW samples, with *E. coli* displaying multi-resistance, indicating the potential for farm wastewater to contaminate surrounding environmental waters. In 2023, residues of 19 different antibiotics were detected in various water samples, with concentrations ranging from 1.1 ng/L to 28 µg/L. Tylosin was the most frequently detected antibiotic. Additionally, AMR was prevalent in *E. coli* and *Campylobacter* from pig farms, with several isolates showing multi-drug resistance. Particularly alarming was the detection of carbapenem-resistant bacteria, which raised concerns due to carbapenems being reserved for specific human infections and their prohibition in productive animals.

Keywords: mass spectrometry, antimicrobial substances, antimicrobial resistance, meat.

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HIGH QUALITY OOCYTES AND EMBRYOS – THE KEY TO USE MODERN AND MOST EFFECTIVE BIOTECHNOLOGICAL METHODS FOR ASSISTED REPRODUCTION IN DOMESTIC ANIMALS

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Nowadays, the main, modern assisted reproduction method utilised in cattle breeding industry for genetic selection, is embryo transfer (ET). In the United States of America as well as in the most developed European countries in terms of dairy production, the introduction of genetic selection of bulls and cows naturally coincides with rapid development of ET in cattle. However, in Eastern Europe, this situation is not as obvious. The lack of broad utilisation of the modern and most effective biotechnological methods for assisted reproduction in domestic animals is probably caused by the unstable effectiveness of the results of ET itself. This situation results from variable quality of the oocytes and embryos used for embryo production and then transfer, induced in turn by the lack of objective methods for oocyte and embryo quality assessment. Therefore, the proper assessment of the developmental potential (quality) of oocytes and embryos is not only of pivotal importance to successful reproduction but also gives the possibility to develop most effective biotechnological methods for assisted reproduction in domestic animals. A meta-analysis of the bovine ET data resulted in the pregnancy rate directly influenced by the embryo quality dropping rapidly from over 60% for the grade 1 to just 12% for the grade 4 embryos. Oocyte and embryo quality is a complex feature affected by a broad range of agents. There is also a panel of distinct parameters attributed to *in vivo* and *in vitro* produced embryos. Although the protocol for *in vitro* production of bovine embryos has been described as efficient (blastocyst yield of 30–40%), the quality of IVP embryos is still significantly reduced when compared with their *in vivo* counterparts. It is obvious that suboptimal *in vitro* conditions alter some cellular processes; thus, the embryo has to adapt to culture conditions. The quality of oocytes and embryos results from a great number of factors within the developmental environment, such as donor related age, health, nutrition, distribution of selected organelles in the cells like mitochondria, as well as the morphology, compaction, cavitation, apoptosis or even transcript content in the embryo itself. Moreover, oocyte and embryo quality has also been of special importance for cattle breeders due to commercial implementation of the *ovum pick up* (OPU) method. The numbers of cumulus oocyte complexes (COCs) and blastocysts per one OPU session are rather low in Holsteins and vary in a wide range from 8 to 12 approximately. The lower quality and quantity of the OPU/IVP bovine embryos result from various obstacles connected with the method itself. However, scientists and practitioners have been working continuously to devise the most optimal stimulation protocols to overcome these obstacles and to acquire the best quality COCs.

Keywords: cow, oocyte, embryo, embryo transfer.

GUT MICROBIOTA IMPLICATIONS FOR HEALTH AND PRODUCTION QUALITY IN FARM ANIMALS

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Microbiome is a full collection of microorganisms, their genes, and their microenvironment (habitat) in a specific area. During the evolution, microorganisms and vertebrate animals have established symbiotic relationships [1]. A plethora of species of microorganisms can be detected in the gut of domestic animals, including birds [2, 3]. Microbiota implications for health and production quality in farm animals were discussed at the 4th International Scientific Conference “Livestock production: Recent Trends and Future Prospects” held at the Lithuanian University of Health Sciences on 26–27 September 2024. Comparison of animal and human microbial communities, functions of normal microbiota, and the influence on health and animal immunity were reviewed demonstrating examples of different pathologies associated with the disbalance of microorganisms in the body. The function of probiotics and possible modification of microbiota by adding different feed compounds for different species of domestic animals were discussed, by providing examples from the recent research about how feed supplements can influence microbial communities and animal welfare. Challenges associated with the investigation of microbiomes were also included in the presentation. As the main identified problems insufficient trials with identification of microorganisms up to the species level, a necessity to find and be able to cultivate new probiotic species were mentioned, as well as insufficient number of experiments using animals for studying animal-targeted and not only human host-microbiome interactions.

Keywords: microbiome, microbiota, probiotics, animal welfare, farm animals, production quality.

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SOURDOUGH MICROBIOTA IN COMBINATION WITH BIOENGINEERING TOOLS TOWARDS PREPARATION OF FUNCTIONAL MOLECULES FROM BOVINE COLOSTRUM FOR AGRI-FOOD, NUTRACEUTICAL, COSMETIC AND PHARMACEUTICAL INDUSTRY

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Bovine colostrum, also known as “first milk” or “first food”, is produced by healthy mammary glands until the first 72 hours *post-partum*, after which it acquires the typical physicochemical, microbiological, nutritional and rheological characteristics of milk. Colostrum is composed of unique bioactive compounds, which are divided into two main classes: immunological (immunoglobulins, antibodies, proline-rich polypeptides, glycomacropolypeptides, glycoproteins, lactalbumins, lactoferrin, lactoperoxidase, cytokines, lysozymes) and growth factors [epidermal (EgF), insulin like (IgF-1 and IgF-2), transforming (TgF-A and TgF-B) and plaquette-derived growth factors, vitamins and minerals]. The use of colostrum to treat illnesses and to contribute to the well-being dates from thousands of years ago. The prophylactic and therapeutical use of immune milk presented satisfactory success in the prevention and treatment of bacterial infections produced by *Escherichia coli* strains, in gastroenteritis treatments in children originated by rotavirus, in the treatment of cryptosporidiosis (or cryptococcal disease, a disease caused by one of two species of fungi belonging to the genus of *Cryptococcus*) and diarrhoea in patients with acquired immune deficiency syndrome (AIDS) and other immunological illnesses, and in the prevention of dental caries, among many other examples. In all these cases, colostrum was collected from cows previously hyper-immunised with specific pathogens. Thanks to the biotechnological advances obtained in the last decades, it has been demonstrated that the immunological and growth factors present in colostrum composition are transferable along all the food chain between mammal species and are not species-specific. Hence, a major opportunity to expand the renewed market niche of colostrum has emerged rapidly and its reintroduction in the market depends in biotechnological breakthroughs. In this context, this presentation is focused in research studies that have been undertaken aiming at the implementation of a novel biological technology for the production of functional molecules from bovine colostrum through bioengineering tools based on the ultrasonication in combination with sourdough LAB fermentation and dehydration, resulting in a safe material with constant quality and a very high-added value for the development of innovative products in the pharmaceutical, cosmetic, nutraceutical and agri-food industry. This new technological approach to process and use the bovine colostrum represents a breakthrough that worth exploring.

Keywords: bovine colostrum, lactic acids bacteria (LAB), bioactive compounds, immunological compounds, growth factors, prophylactic, therapeutic.

AGRI-FOOD WASTE AS A VALUABLE SUBSTRATE FOR OBTAINING FEED PROTEIN

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The growing human population and economic development are increasing the demand for meat as an ingredient in human food. Farmed animals are still the primary source of meat [1–3] as are the environmental impacts of agricultural expansion. Here, we project global demand for crop production in 2050 and evaluate the environmental impacts of alternative ways that this demand might be met. We find that per capita demand for crops, when measured as caloric or protein content of all crops combined, has been a similarly increasing function of per capita real income since 1960. This relationship forecasts a 100–110% increase in global crop demand from 2005 to 2050. Quantitative assessments show that the environmental impacts of meeting this demand depend on how global agriculture expands. If current trends of greater agricultural intensification in richer nations and greater land clearing (extensification) continue, the primary raw materials for animal feed are plants, e.g., soya, maize, rye, wheat, potatoes, sugar beet and grasses. In addition to adequate calories, animal feed should be highly digestible, have a high protein content, and have balanced amounts of sugars, starch, vitamins, fats and dietary fibre to ensure rapid growth and maintain livestock welfare [4, 5]. Single cell protein can be a source of high-value feed protein.

The chemical composition of yeast depends on the medium in which it is grown. The protein content can range from 35% to over 55% of the dry matter (DM). The biomass also contains some fats (0.5–8%), carbohydrates (18–43%) and minerals (4–10% of DM). Yeast-based diets are rich in B vitamins and elements such as P, Na, Cu, Mn, Zn and Fe [6]. Yeasts are used as feed for pigs and poultry and less commonly for cattle. When consumed by pigs and poultry, its energy value is about 12 MJ ME/kg and 11 MJ ME/kg, respectively [7].

The cost of SCP must be close to the price of soya protein. To this purpose, yeast culture methods based on agri-food waste are constantly improving. In recent years, our studies have included the cultivation of fodder yeasts in hydrolysates obtained from potato waste pulp (PWP) and sugar beet leaves (SBL).

Our study processed the raw material through several steps of physico-chemical-enzymatic treatment aiming to release simple sugars. We used *Meyerozyma guilliermondii* ATCC 6260, *Scheffersomyces stipitis* LOCK P0047, and *Trichosporon cutaneum* LOCK P0254 as microorganisms.

Three enzyme preparations were used. Termamyl, containing alpha-amylase; SAN Extra, containing glucoamylase and acid alpha-amylase; and Cellic CTec2, a commercial mixture of cellulases, beta-glucosidases and hemicellulose. Hydrolysates of PWP and SBL were enriched in N, Mg and P salts. The cultures were grown using the batch method for 48 hours at $30 \pm 1^\circ\text{C}$ in glass flasks using a laboratory shaker to aerate the medium.

Among all the tested treatment options, raw materials and strains, the highest biomass concentration was observed for the *T. cutaneum* strain (16.25 g DM/L of SBL hydrolysate), and the lowest for the *S. stipitis* yeast cultured in PWP hydrolysate (8.11 g DM/L).

Our results confirm that excess sugar beet leaves and potato waste pulp can be raw materials for yeast protein culture.

Keywords: guilliermondii, cutaneum, stipitis, scp, potato pulp, sugar beet leaves.

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LAURACEAE AND CHLORELLACEAE IN RABBIT NUTRITION: EVALUATION OF GROWTH PERFORMANCE

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After the official ban on the use of growth promoters, feed companies and researchers shift their focus to natural products such as extracts, herbs and spices that can be used as supplements in animal diets to sustain growth performances. The effects of dietary supplementation in rabbit with *Laurus Nobilis* (0.1%), *Chlorella Vulgaris* (0.1%) and their mixtures on growth performances and carcass traits were evaluated. The trial lasted 60 days and was performed on 100 weaned New White Zealand rabbits randomly divided in four groups (CON, LAU, CHLO, MIX). The average daily weight gain, average daily feed intake and the feed conversion ratio were calculated. At the end of the trial, 10 animals per group were selected and slaughtered to calculate carcass weight and yield. The LAU group showed a higher ($P < 0.05$) live weight than the CON and CHLO groups. The ADG values in the last period of the trial (45–60 days) were higher ($P < 0.05$) in the CHLO, MIX and LAU groups than in the CON group. Considering the whole experimental period (0–60 days), no differences ($P > 0.05$) were observed in the ADG values among groups. The feed intake of the rabbits was affected by dietary treatment; in fact, the LAU and MIX groups showed a higher feed consumption ($P < 0.05$) than the CHLO and CON groups. The FCR values were also improved in the LAU and CHLO groups and were significantly lower ($P < 0.05$) compared with CON animals. Hot carcass weight was higher ($P < 0.05$) in the CHLO and CON groups than in the other two groups. The value of carcass yield percentage was higher ($P < 0.05$) in the CHLO group than in the LAU groups and no differences were observed in comparison with the CON and MIX groups. Our data show that *Laurus nobilis*, *Chlorella vulgaris* and their mixture, at the current dosage, can improve growth performance and slaughter indices in growing rabbits. Considering the high polyphenolic content of the two supplements, further studies are needed to highlight their potential effects on the antioxidant status and meat quality parameters of rabbits. More in-depth studies should be carried out to confirm the potential positive effect of these dietary integrations to promote sustainable livestock production.

Keywords: animal feeding, nutraceuticals, growing rabbits, productive traits.

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SYNERGISTIC EFFECT OF BUTYRATE, PLANT EXTRACTS, AND ESSENTIAL OILS ON BROILER CHICKEN GROWTH PERFORMANCE, PHYSIOLOGICAL FUNCTIONS, AND MEAT QUALITY

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Interest in alternative animal feed has risen due to the European Union's ban on antibiotics for subtherapeutic use. Research focuses on altering feed composition with plant, animal, bacterial, and fungal materials, alongside organic acids, essential oils, and bacteriocins, to inhibit pathogenic bacteria. Broiler chicken growth hinges on digestive tract health, including immunity, microflora, efficiency, and nutrient absorption. While studies have explored butyrate, plant extracts, and essential oils separately, there's a gap in understanding their synergistic effects. So, this study aimed to investigate the synergistic effect of butyrate, plant extracts, and essential oils on broiler chicken productivity, physiological processes, and meat quality. The feeding trial was performed with 1-day-old old Ross 308-line combination broiler chickens that were divided into two groups ($n = 103\ 000$ broiler chickens/group): the control group was fed with a standard compound diet, and the experimental group was fed a standard compound diet supplemented with a mixture of butyrate, plant extracts, and essential oils at 1 kg/t. During the feeding trial (1–41 days old), growth performance was recorded, and at the end of the trial, blood samples were taken, as were slaughter performance and sample collection post-mortem. The results showed that the mixture of butyric acids, plant extracts, and essential oils in the experimental group increased the feed conversion ratio during the entire trial period ($P < 0.05$). The used mixture significantly increased total protein, ALT, and AST levels in the blood of broiler chickens on the last day of the feeding trial and the ratio of villus height to crypt depth in the *ileum*. Compared with the control group, feed additives used in the experimental group increased carcass yield, the mass of a fully slaughtered carcass without internal organs, total breast muscle mass without skin, breast muscle width, and the intensity of breast muscle yellowish colour ($P < 0.05$). In conclusion, it can be stated that the synergistic effect of butyrate, plant extracts, and essential oils did not have a positive impact on the growth performance of broiler chickens but had a positive effect on various physiological and morphological parameters.

Keywords: butyrate, essential oils, plant extracts, broiler chickens, performance, physiology, meat quality.

TECHNOLOGICAL POTENTIAL TO ENHANCE THE NUTRITIONAL AND FUNCTIONAL VALUE OF LENTILS AS A SOURCE OF VALUABLE ANIMAL FEED

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The EU protein strategy encourages domestic protein-rich crop production, such as lentils, as the strategy for combating the problems associated with dependence on importing plant-based protein products [1]. While the nutrient absorption of lentils is mitigated due to existing antinutritional factors, the effect can be reduced using various food processing methods which include fermentation [2]. The aim of the study was to evaluate differences in the composition of various biomolecules (free amino acids (FAA), biogenic amines (BA), volatile organic compounds (VOC) and fatty acid composition (FA)) of non-treated and fermented (via submerged (SMF) and solid state (SSF) fermentation, 1:5 and 1:1 lentil/water weight ratio, respectively, at $30 \pm 2^\circ\text{C}$ for 24 h and 48 h with *Pediococcus acidilactici* strain) lentil (variety 'Danaja', *Lens culinaris* Medik.) samples, grown using different technologies. FAA were analysed according to Hua-Lin Cai et al. [3] with modifications. BAs were analysed using the methods described in Ben-Gigirey [4]. FA and VOCs were analysed using the methods, described in detail by Mockus et al. [5]. Fermented samples were found to contain significantly higher amounts of FAA concentrations (except arginine, asparagine, and glutamine), in comparison with the respective control samples. Type of the fermentation showed to be an important factor for all FAA concentrations. Moreover, lentil growing conditions * type of fermentation * fermentation duration interaction was significant for arginine, glutamine, GABA, and tyrosine concentrations in lentils. The BAs detected in all samples were putrescine, spermidine and spermine. Growing conditions * type of fermentation interaction was significant for all detected BAs. The fermentation duration was not significant for C18:0, C18:1 and C18:2 FAs, while an interaction of all the analysed factors was significant for all FAs in lentils. SSF lentils contained a more complex VOC profile (28–39 VOCs in SSF samples) in comparison with respective SMF samples (12–27 VOCs in SMF samples). Finally, it can be stated that the increase of FAAs can result in improved nutrient absorption, while the increase of BAs should be taken into consideration.

Keywords: lentils, amino acids, fatty acid composition, fermentation, volatolomics.

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ASSESSMENT OF ALTERNATIVE PLANT-BASED COCCIDIOSTATS ON THE GROWTH PERFORMANCE, HEALTH, AND PHYSIOLOGICAL STATUS OF BROILER CHICKENS

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The poultry sector in Lithuania is one of the leading branches of livestock farming. The expansion of poultry farming has spurred the quest for enhanced zootechnical parameters. This necessity has led to the exploration of supplements in poultry nutrition aimed at managing pathogens, predominantly *Eimeria spp.*, *Salmonella spp.*, *Clostridium spp.*, and *Escherichia coli*, while also serving as growth enhancers [1]. This study aimed to investigate the impact of alternative plant-based coccidiostats on the performance, health, and physiological functions of broiler chickens, considering the poultry industry's growing interest in natural substitutes for conventional additives. A total of 600 Ross 308-line 1-day-old broiler chickens were divided into two groups (n = 300 broilers/group). The control group received a standard compound feed supplemented with typical coccidiostats, while the experimental group received the standard compound feed added with 500 g/t of alternative plant-based coccidiostats. At the end of the trial (35 days old), 10 chickens from each group were randomly selected and euthanised, and then blood serum, the development of intestines and internal organs, the pH of different intestinal contents, and histomorphometric measurements of the ileum were analysed. The results showed that plant-based coccidiostats had a negative impact on the body weight of broiler chickens but had a positive impact on the feed conversion ratio ($P > 0.05$). Compared with the control group, the experimental group had varying effects on the health of broiler chickens: on day 10, it decreased iron content and increased GGT levels; by day 35, it decreased AST levels and increased iron content ($P < 0.05$). The use of plant-based coccidiostats increased the weight of the intestines with contents and the heart and liver mass but decreased the morphometric measurements of the ileum (villus height and the ratio of villus height to crypt depth) compared with the control group ($P < 0.05$). Although the specific mechanism of action for the alternative plant-based coccidiostats was not revealed in our study, their inclusion in poultry feed can positively impact certain health indicators and physiological functions in broiler chickens.

Keywords: anticoccidial activity, broiler chicken, productivity, physiological functions.

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EFFECTS OF A PHYTOBIOTIC EXTRACT FROM *MACLEAYA CORDATA* ON PIG GROWTH PERFORMANCE AND PORK QUALITY TRAITS

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In animal husbandry, researchers are continually searching for additives that can meet animal growth needs while also improving their health status and growth performance. There is a growing expectation to achieve these results using plant-based additives known as phytobiotics. As natural additives, phytobiotics are safe for animals' health and do not leave any toxic residues in final products such as meat and eggs [1]. Moreover, the main concern for researchers, producers, and consumers is to obtain not only the highest possible quantity of meat but also a healthier and more nutritious product [2]. In this case, the phytobiotic preparation derived from the plant *Macleaya cordata* (*M. cordata*) is a pure and natural additive that could improve feed absorption and reduce protein degradation in the digestive tract. While many studies have examined the use of phytobiotics in poultry production, there is limited data on their effects on the performance of fattening pigs and the characteristics of the resulting meat. Thus, the aim of this study was to investigate the effects of a phytobiotic extract from *M. cordata* on pig growth performance and pork quality. The feeding trial included fifty-two 45-day-old fattening pigs (Landrace x Yorkshire (mother) and Pietrain x Duroc (father)) randomly assigned to two treatment groups (n = 26 pigs/group): BD, receiving a basal diet; MC, receiving BD supplemented with *M. cordata* via water at a rate of 50 g/m³. During the feeding trial, growth performance traits were recorded. At the end of the trial (153 days old), pork quality traits were measured using the Piglog 105 equipment, and the animals were slaughtered. Post-mortem sample collection was then conducted for further chemical analysis. A recent study showed that the body weight of the pigs in the MC group at the end of the feeding trial was higher by 3%, the weight gain per day was higher by 4%, feed consumption during the entire feeding trial period was lower by 6%, and water consumption was lower by 8% compared with the BD ($P > 0.05$). The MC-treated fattening pigs had 13–15% thinner fat ($P < 0.05$), 4% thicker muscles, and 2.27% higher muscularity than BD-treated pigs ($P > 0.05$). However, no significant impact of phytobiotic preparation on pork chemical traits was noticed ($P > 0.05$). Based on the feeding trial results, it can be concluded that supplementation with *M. cordata* significantly enhances the growth performance of fattening pigs. However, this supplementation also led to a reduction in fat thickness and had no discernible effect on the chemical composition of the pork.

Keywords: fattening pigs, phytobiotics, productivity, meat quality.

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THE EFFECT OF ELIMINATING DIETARY MANGANESE SUPPLEMENTATION OR REPLACING ITS STANDARD INORGANIC FORM WITH NANOPARTICLES ON THE IMMUNE STATUS OF RATS

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The aim of the study was to determine the effect of complete exclusion of Mn from the mineral mixture added to the rat diet and replacing the recommended level of MnCO₃ (65 mg Mn/kg diet) with Mn nanoparticles (MnONPs) in the diet on haematological parameters and selected indicators of the immune status of the blood, small intestine, and brain.

The experiment was conducted for 12 weeks on 4-week-old rats randomly assigned to 3 experimental groups (n = 8). The control group (K) received a diet containing the recommended level of Mn, i.e., 65 mg Mn/kg in the mineral mixture in the standard form of MnCO₃. Group B received a diet in which Mn was completely excluded from the mineral mixture. Group N received a diet containing the recommended level of Mn – 65 mg Mn/kg in the mineral mixture in the form of MnONPs (40–60 nm). At the end of the experiment, blood samples were collected from all rats per group. After that, all rats were euthanised, and samples of small intestine tissue and brain were collected. Haematological parameters were determined in the blood of rats. In addition, selected immune status parameters were measured in blood plasma, small intestine tissue, and brain using enzyme-linked immunosorbent assays.

The exclusion of Mn from the dietary mineral formula resulted in a decreased red blood count (RBC), haemoglobin content (HGB), and haematocrit (HCT) ($P = 0.013$, $P = 0.025$ and $P = 0.019$, respectively), C-reactive protein (CRP) level ($P < 0.001$) and ceruloplasmin (Cp) activity ($P = 0.016$), while increasing interleukin 2 (IL-2), immunoglobulin G (IgG) and tumour necrosis factor α (TNF- α) ($P = 0.002$, $P < 0.001$ and $P = 0.032$, respectively) in the blood of rats. Rats receiving a diet without Mn supplements in the mineral formula also showed increased interleukin 6 (IL-6) level ($P < 0.001$) and Cp activity ($P < 0.001$) as well as decreased CRP and immunoglobulin A (IgA) level ($P < 0.001$ and $P = 0.019$, respectively) in the small intestine tissue. This treatment also increased Cp activity ($P < 0.001$) and decreased IgG levels ($P < 0.001$) in the rat brain. Replacing the standard form of Mn (MnCO₃) with MnONPs in the mineral mixture added to the rat diet decreased CRP levels ($P < 0.001$) in the blood plasma. In the small intestine of rats receiving MnONPs instead of MnCO₃ in the diet, decreased CRP and TNF- α levels ($P < 0.001$, both) and increased IL-6 level ($P < 0.001$) were noted. In the brains of rats subjected to this experimental procedure, an increase in TNF- α levels ($P < 0.001$) and Cp activity ($P < 0.001$) was demonstrated, with a simultaneous decrease in IgG levels ($P < 0.001$).

The obtained research results indicate that the presence of manganese is essential for the proper functioning of the immune system, because the exclusion of this micronutrient from the mineral mixture added to the rats' diet worsened the immune status of the organism and resulted in the development of local and systemic inflammation. This procedure also contributed to the development of anaemia in the tested animals. In turn, replacing the recommended level of MnCO₃ with MnONPs in the rats' diet had an adverse effect on the brain, causing local inflammation and worsening the immune response. However, the observed disorders did not translate into a deterioration of the immune status at the systemic level. The obtained research results suggest that the organism, in response to local inflammation and deterioration of the brain's immune response caused by MnONPs, activates adaptive mechanisms that can protect it from their harmful, immuno-toxic systemic effects.

Keywords: manganese, nanoparticles, rat, immune status, inflammation, brain.

VALORIZED FOOD INDUSTRY BY-PRODUCTS – VALUABLE FEED MATERIALS FOR NEWBORN PIGLET NUTRITION

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Facing the challenges of climate change and agricultural sustainability issues, and the still insufficiently effective technological solutions for the valorization of food industry by-products, the search for new technologies in this field becomes especially relevant. Another significant challenge in increasing the sustainability of the livestock sector is the heavy reliance on imported feed raw materials. This not only causes difficulties due to market price fluctuations but also reduces the sustainability of this sector due to the dominance of long supply chains. Therefore, this sector needs to be reorganized as soon as possible by integrating new technologies for efficient processing of local raw materials. Two largest by-products generating sectors in the food industry are the grain and dairy processing industries. Our previous research has shown that the valorization of by-products from the grain (wheat) processing industry, using a composition of thermo-mechanical and biological treatment methods, is a suitable way to produce value-added feed materials for piglets. The valorized wheat by-products demonstrated desirable antimicrobial and antifungal properties. A 21-day experiment with new-born Large White/Norwegian Landrace piglets showed that the created feed raw materials increased the number of lactobacilli in piglet faeces and reduced the feed conversion ratio. Another experiment of ours showed that milk permeate, which contains a high amount of lactose, can be successfully valorized into feed raw materials containing a large amount of galactooligosaccharides. These compounds are desirable in both the food and feed industries due to their prebiotic properties. We found that newborn piglets fed valorized milk permeate from the first day of birth gained weight significantly faster than animals in the control group. In the next stages of this experiment, we plan to evaluate the changes in the microbiota of the piglets, as it is likely that these differences may be related to the better digestion of nutrients and significantly faster weight gain in the piglets. Finally, the tested local feed raw materials, produced by valorizing food industry by-products, could be proposed for piglet nutrition, more sustainable livestock farming and more efficient circular economy development.

Keywords: feed material, food industry by-products, valorization technologies, piglets.

PATHOLOGICAL LESIONS IN PIGS DETECTED DURING POST-MORTEM EXAMINATIONS AND THEIR IMPACT ON PUBLIC HEALTH

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Food safety control is a key factor not only for the public needs but also for their health. Therefore, when assessing pig carcasses during ante-mortem as well as post-mortem examination, it is important to highlight aspects such as the technological and sanitary quality of pig carcasses, and to consider the sensory, physico-chemical and microbiological parameters of pig meat to assess the safety of the product. In addition, the situation regarding the diagnosis of non-infectious animal's diseases in slaughterhouses is unclear. The identification of this situation is of particular importance, since any disorder of the health of an animal not only causes damage to certain organs, but also directly affects the health status and quality of the carcasses of slaughtered animals. This is a crucial part of the food control chain, ensuring food safety and, consequently, public health. The aim of the study was to determine the incidence of pneumonia in slaughter pigs during post-mortem examination and to assess the health status of slaughter pigs. To assess the pathologies of slaughtered cattle at the time of slaughter, a post-mortem analysis was carried out and a detailed analysis of the lung diseases found in pigs was performed. Samples of the diaphragm of pigs slaughtered during post-mortem analysis were collected. A 3.0 x 1.0 x 1.0 cm piece of the diaphragm was placed in a sterile plastic tube and frozen at +20o C until the test was performed. One day before the study, the meat juice samples were transferred to a refrigerator at +4o C and stored for 24 hours. *Salmonella* antibodies in pig meat juice were determined by a screening immuno-enzyme analysis method using the IDEXX HerdChek Swine Salmonella Test Kit (IDEXX Laboratories, Switzerland). The samples were evaluated according to the optical density ratio of the test sample and the positive control sample. During this study, it was found that among the changes attributed to non-infectious diseases, lung pathologies predominated, i.e., 70.4% of clinically healthy slaughtered pigs were affected, followed by liver pathologies (32.5%). Kidney pathologies accounted for 8.9%, heart pathologies for 6.2%, gastrointestinal pathologies for 5.4%, reproductive organ pathologies for 1.2%, and carcass pathologies for 19.84%. The study observed a trend that allows the conclusion that changes in the characteristics of non-infectious diseases are consistently diagnosed and increasing, while infectious diseases are less frequently diagnosed in this case.

Salmonellosis can cause not only economic losses but also a significant harm to human health, necessitating very rapid, accurate, and sensitive methods for detecting *Salmonella*. *Salmonella* is the most common pathogenic microorganism found throughout the environment and a common source of bacterial foodborne-related illness. A practical tool for detecting *Salmonella* infection in slaughtered pigs is the serological examination of meat juice. *Salmonella* was determined in meat juices from healthy and pneumonia diseased pigs. The study found that among pigs with moderate lung damage, 22.90% of meat juice samples were serologically positive for *Salmonella*, while in pigs with severe lung damage, the percentage was 28.70%. In control pigs, serologically positive meat juice samples for *Salmonella* accounted for 0.39%.

Evaluating the results of the conducted studies, various organ and carcass damages of differing severity are identified during post-mortem examination of pigs, which may pose a threat to public health. Therefore, continuous monitoring and studies of this nature are conducted to ensure the delivery of safe food to the market.

Keywords: post-mortem meat examination, pigs, pathological lesions, lungs, *Salmonella*, meat juice.

INVESTIGATION ON MAJOR GENES INFLUENCING BIRTH WEIGHT IN HAIR GOATS

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The effective application of major gene analyses in animal breeding programmes allows for the production of animals with desirable genetic traits in a more efficient and healthy manner. Therefore, the use of major gene analyses is considered an important strategy in animal breeding. This study aims to detect the presence of major genes in the body weight phenotype of hair goats using Bayesian segregation analysis. In this study, 6108 body weight records from 2589 animals were utilised. The mixed inheritance model assumes that genetic variance can be explained by both a major gene and polygenic factors. The segregation analysis model allows for the investigation of a major gene through Bayesian segregation analysis in the presence of pedigree data [1]. This study examined whether a major gene with two alleles (A1 and A2) and frequencies of (1-q) and q, respectively, is associated with body weight in the population. Major locus genotypes were considered as A1A1, A1A2, and A2A2. Due to Hardy-Weinberg equilibrium, the alleles were assumed to combine independently with frequencies (1-q)², 2q(1-q), and q². The polygenic model and the relevant inference can be derived from the interaction of “genotype means * design matrix * matrix containing each individual’s genotype.” Segregation analysis was performed using the IBAY computer programme [2]. The presence of a major gene in the relevant phenotype was determined by examining whether the highest posterior density regions (HPDR) contained 0 and by assessing Mendelian transmission probabilities. Accordingly, for the birth weight trait, the posterior means (\pm SD) of the heritability were estimated as $h^2 = 0.51 (\pm 0.56)$ using the polygenic model and $h^2 = 0.81 (\pm 0.91)$ using the major gene model. It was observed that the heritability estimate for the major gene model was higher than that for the polygenic model.

Keywords: segregation, hair goat, major gene, birth weight.

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IN-SITU AND EX-SITU CONSERVATION OF NATIVE BREEDS IN LITHUANIA

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Within livestock species, the genetic diversity is most obvious as the spectrum and number of breeds. Breeds are defined as populations within a species, the members of which can be identified by a set of characteristics particular to the breed [1]. In ancient times, the names of animal breeds in Lithuania were derived depending on the external features of certain animals: colour, horns, type of wool or geographical region. These breeds, particularly adapted to extreme environments, can be unique sources of income for rural communities. Definition “genetic resources” in this study applies to farm animals and genetic material of breeds ($n = 14$) that are included into the National Conservation Programme (Programme). Farm animal genetic resources are sources of genetic variation of fundamental importance to ensure future genetic improvement, satisfy possible future changes in the markets and in the production environment, and safeguard against disasters that give an acute loss of genetic resources [2]. The financial funds available for conservation of farm animal biodiversity are limited, preventing conservation of all endangered breeds. The choice of breeds that should be included in a well-designed and efficient conservation plan is of fundamental importance. At the moment, the applied rules for selection of breeds for conservation rely on a combination of a few criteria, which are related to the risk status of a breed. Risk status is deduced from the number of breeding males and females, the effective population size and population dynamics like increasing or decreasing population size. In the last 20 years, the decreasing number of conserved animals was stabilised by the Programme and scientific contributions. Effective population size (N_e) for two pig breeds and four cattle breeds under the conservation programme is still below 50 (risk status is endangered [3]); therefore, the survival of the population is uncertain. A critically endangered situation exists in Lithuanian indigenous wattle, Lithuanian White pig and Lithuanian Red cattle (old genotype) populations. There is only one selection of a nucleus per each breed maintained at the Animal Science Institute of Lithuanian University of Health Sciences, where special circular mating schemes [4] are used. Two populations, Lithuanian Blackface sheep and Lithuanian Heavy Drought horse, have $N_e > 200$, but populations are maintained by the *in-situ* conservation programme, and without support, changes in a seemingly non-problematic population can occur very quickly. The combination of live conservation schemes (*in-situ*) and cryo-conservation (*ex-situ*) in Lithuania has two different aims depending on the species: *ex-situ* is actively used to increase the N_e of cattle breeds and reduce genetic drift; *ex-situ* serves as a backup in case the live population runs into genetic problems (loss of breed structure, genetic characteristics, inbreeding and etc.)

Keywords: effective population size, animal genetic resources, conservation programme.

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THE IMPACT OF THE RATIONS CONTAINING PEA MEALS AND HERBAL PREPARATION ON BROILER CHICKENS MEAT QUALITY

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The European Union Member States are dealing with a major issue related to a significant deficit of protein feeds, most of which is covered by imported, genetically modified soybean meal. Developing legume crops, including peas, seems a convenient way to increase self-sufficiency in feed protein. New cultivars of legumes require continuous monitoring of their nutritional value and efficiency in slaughter poultry feeding [1]. The study aimed to evaluate the meat quality of broiler chickens that had received rations containing peas and a herbal preparation. It involved 96 Ross 308 broiler chickens divided into three feeding groups (1, 2, and 3). The birds received loose Starter feed rations ad libitum until day 21 of life, and Grower rations based on wheat, soybean extrudate, soybean oil and mineral and vitamin additives from day 22 to day 42. In rations fed to birds from group 2, the soybean extrudate was partly replaced by pea seed meal (20% – Starter and 25% – Grower), while feed rations in group 3, in addition to pea meal (20% – Starter, 25% – Grower) contained 0.1 g·kg⁻¹ of HERB preparation. On day 42 of life, 8 birds (4 cocks and 4 cockerels) with a body weight representative of a specific group and sex were selected from each subgroup and slaughtered. During dissection, their muscles were sampled for physicochemical and sensory evaluation of meat. We found that the type of feed rations had no material impact on the total protein content and crude fat and crude ash levels either in breast or leg muscles. The combination of peas and a herbal preparation used in feed rations affected the fatty acid profile of lipids in the analysis muscles. Breast muscles of chickens from groups 2 and 3 contained significantly ($P \leq 0.01$) more saturated fatty acids (SFA) and less unsaturated fatty acids (UFA) compared with the muscles of birds from group 1. The initial pH15 of breast muscles testifies that the meat of chickens fed rations with peas (groups 2 and 3) and without peas (group 1) was classified as normal, defect-free meat. After 24 hours of cooling, the acidity of the muscles in all birds was similar. However, glycogenolysis was more intensive in breast muscles than in thigh muscles. As for tastiness, the best was the breast and thigh meat of chickens fed rations containing 20/25% pea seed meal and herbal preparation.

To sum up, the results of the evaluation of breast and leg muscle quality form the basis for recommending the incorporation of pea seed meal in Starter (20%) and Grower (25%) rations, along with 0.1 g·kg⁻¹ of HERB preparation.

Keywords: broiler chickens, rations, pea seeds, herbs, meat quality.

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THE INFLUENCE OF A BOAR FEEDING SYSTEM ON THE PHYSICAL CHARACTERISTICS OF EJACULATES

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The level of reproductive characteristics has a significant influence on the efficiency of animal production. The reproductive capacity of insemination boars depends not only on the genetic potential of the male, but also on environmental conditions. In determining the reproductive performance of boars, the assessment of semen quality is crucial. An incorrectly balanced feed ration affects the deterioration of semen quality and lower libido. The aim of the study was to assess the quality of ejaculates collected from boars used in insemination after the use of two feeding systems. The study was conducted on insemination boars of the following breeds: Landrace, Large White, Duroc and Duroc x Pietrain. The animals were kept in individual pens, in accordance with the requirements for this group of animals, while maintaining appropriate welfare conditions. All boars were fed a complete mixture intended for the nutrition of insemination boars. Ejaculates from the studied boars were collected manually into sterile thermoses with a disposable foil insert lined with sterile gauze, enabling separation of the gelatinous fraction. Immediately after collection, each ejaculate was assessed by determining the following physical characteristics: ejaculate volume, sperm concentration, percentage of sperm showing progressive motility, total sperm count in the ejaculate and number of insemination doses. A total of 4863 ejaculates were assessed. The studied boars were divided into two groups: Group 1 – boars fed with a dry system, Group 2 – boars fed with a wet system. Based on the conducted studies, it was found that the boar feeding system affects the physical characteristics of ejaculates. The obtained means for all boars showed that boars fed a dry system produced ejaculates with a volume smaller by almost 25 mL, but with a sperm concentration higher by 43 thousand/mm³ than boars fed a wet system ($P \leq 0.05$). Ejaculates of these boars contained more sperm, and two more insemination doses were prepared from them than from ejaculates of boars fed a wet feed. Differences in the physical characteristics of ejaculates in boars depending on breed and feeding system were also shown. Landrace boars fed a wet feed produced larger volume ejaculates than boars of the same breed fed a dry system. Ejaculates of a larger volume were obtained from boars of other breed groups using a dry feed. In the ejaculates of Large White boars fed a wet system, higher sperm motility was observed than in the ejaculates of boars of this breed fed a dry system. In the ejaculates of the remaining breed groups, sperm motility occurred at a similar level regardless of the boar feeding system. More insemination doses were prepared from the ejaculates of boars of all the studied breeds fed a dry system than from the ejaculates of boars fed a wet system.

Keywords: boar, ejaculate, nutrition, semen quality.

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CAN CANNABIDIOL INDUCE OR REDUCE STRESS IN BROILER CHICKENS?

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The importance of bioactive nutrients in promoting and modulating gastrointestinal (GIT) function indicates the potential for the development of new and effective applications in disease prevention in birds. One of the most common problems in chickens is necrotic enteritis (NE), caused by the anaerobic bacterium *Clostridium perfringens* (*C. perfringens*), and colibacteriosis caused by *Escherichia coli* (*E. coli*). Cannabidiol is one of the main pharmacologically active phytocannabinoids of the *Cannabis sativa* L. plant, which has no psychoactive effects, but has many demonstrated beneficial properties, including anti-inflammatory, antimicrobial and antioxidant properties [1]. In addition to all these positive biological effects, the question arises as to whether the addition of cannabidiol could have negative consequences on chickens. Heat shock protein 70 (HSP70) is induced in response to cell stresses, protects cells from injury and promotes refolding of denatured proteins. Cortisol concentration is a commonly used indicator to assess acute stress [2]. 204 Ross 308 broilers were divided into 6 groups of 32 each: Group 1 – negative control; Group 2 – control with 30 g/kg CBD; Group 3 – positive control infected *C. perfringens*; Group 4 – positive control infected LPS; Group 5 – with 30 g/kg CBD and *C. perfringens*; Group 6 – with 30 g/kg CBD and LPS. On days 21 and 22 of rearing, chickens in Groups 3, 4, 5, and 6 were infected per os 1 mL with *C. perfringens* or LPS, while other groups were given a 0.9% NaCl solution. At 35 days, 8 birds from each group were euthanised, and a section from the middle part of the jejunum was taken for the mRNA expression of HSP70 gene by real-time PCR and blood to determine a cortisol level by ELISA. In the case of HSP70, which promotes the protection of cells from the lethal effects of oxidative stress [3], we found a significant reduction in its expression levels in *C. perfringens* challenged birds given CBD compared with challenged-only birds ($P = 0.006$) while no differences were observed for LPS challenged (LPS-challenged vs. LPS-challenged and CBD supplemented). It can be concluded that CBD reduced the oxidative stress associated with *C. perfringens* infection because the expression level of HSP70 was the same in the challenged chickens as in the control group. In contrast, the fact that these levels did not differ from the CBD group indicates that the activity of CBD in regulating oxidative stress is induced by a specific stress factor. The concentration of cortisol did not differ among the dietary treatment groups ($P > 0.05$). In conclusions, CBD supplementation to avian diets did not cause a stress-inducing effect resulting in an increase in blood cortisol. Also, by the level of HSP70, CBD exhibited a supportive effect on mechanisms to protect intestinal cells from lethal effects, and its addition alone did not cause a stress-inducing effect by the levels of genes involved in the oxidative stress.

Keywords: cannabidiol, *C. perfringens*, LPS *E. coli*, gastrointestinal tract, heat shock protein 70, cortisol.

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CONNECTIONS BETWEEN MEASURED HOCK BONE PROPORTIONS WITH PERFORMANCE, CONFORMATION AND OSTEOCHONDROSIS DISSECANS STATUS ON DIFFERENT STAGES OF BREEDING PERFORMANCE TRAINING TEST FOR POLISH WARBLOOD HORSE

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The connection between horse osteochondrosis dissecans (OCD) and performance is controversial [1, 2], so detailed study of bone structure seems essential. The study aims to compare the measured hock bone structure proportion indexes with performance and OCD status. The data consists of hock structure proportion indexes (x-ray image measurements), conformation evaluation (3 objective measurements, 4 subjective traits), performance results (6 subjective traits), and OCD evaluation conducted during official performance tests for young Polish Warmblood breeding horses organised by Polish Horse Breeders Association. The four proportion indexes (157–164 observations) were based on bone structure measurements of hock x-ray images of 69 horses (50 mares; 19 stallions), tested twice (before and after) during a basic training test (60–100 days) for young breeding horses (age 1249 days \pm 114.6). The analysis of correlations (SAS programme) showed that conformation traits were connected on the medium level (-0.4 – 0.6 ; $P < 0.05$) with hock structure proportions indexes. The performance traits correlated with proportion indexes mainly for the overall trait – “usability” and walk evaluation (0.3 – 0.4 ; $P < 0.05$). The health OCD status was not connected with any of the measured proportion indexes. The obtained correlations were not the same in both training stages. Results seem informative for the evaluation of hock images and horse performance prediction.

Keywords: horse health, osteochondrosis dissecans status, performance, conformation, hock joint.

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ENVIRONMENTAL AND GENETIC EFFECTS ON MEASURED HOCK BONE PROPORTIONS IN YOUNG POLISH WARBLOOD HORSES UNDERGOING PERFORMANCE TEST TRAINING FOR BREEDING HORSES – AN OBSERVATIONAL STUDY

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Orthopaedic diseases are the most common and problematic health issues for horses in training. A better understanding of the horse's bone structure and its changes related to everyday training may help to predict and avoid injury [1–3]. The objective of the present study was to estimate the influence of basic training effects on hock bone proportions in young horses enrolled in training for Polish Warmbloods officially conducted by Polish Horse Breeders Association. The hock bone proportions in young Polish Warmblood horses ($n = 69$) were studied based on x-ray image measurements done during official breeding horses performance testing. Six measurements were used to identify 4 width and length proportions indexes. The radiological examinations were done before and after horse training, which consists of work with or without the rider at walk, trot, and canter (40–50 min) with some light class (< 100 cm) jumping (< 12 jumps) 6 days a week. The training effect was investigated using analysis of variance (Mixed SAS). The statistical model included a random horse effect and fixed effects: training (1st, 2nd investigation), training centre (1st, 2nd), sex (19 stallion, 50 mares), limb (left, right), and regression on age in days. Pearson correlations between proportions were calculated. Training influenced two proportions. Tibial 3rd metatarsal epiphyses width proportion (AM; $P < 0.0001$) and hock multi-structural lengths proportion (DN; $P = 0.0288$) were greater after training. Distal epiphysis of the tibia (A) seems greater in training than the proximal epiphysis of the 3rd metatarsal epiphysis (M). The width proportions were revealed to be most affected by training, compared with the length proportions. Pearson correlations between measurements changed in training with the overall values from -0.2 to 0.2 . Repeatability of measured indexes was mostly high (> 0.7).

Keywords: horse, hock bone size, training influence.

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EQUINE WELFARE: THE CRUCIAL THEMATIC AREA WITHIN THE EUNETHORSE EUROPEAN PROJECT

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EUnetHorse project [1, 2], funded by Horizon Europe and involving nine countries, is an EU network for knowledge exchange and peer-to-peer learning between actors and stakeholders of the horse sector to improve the resilience of equine farms. The goal of the project is to facilitate connections among various sectors such as sports/leisure, racing, agriculture, and food production. Socio-economic performance, environmental sustainability of the sector and equine welfare and health are three thematic areas (TA) of the project.

This project consists of identifying the needs of equine farmers in each involved country to improve the resilience of their farms by the exchange of existing solutions / good practices. Solutions and good practices will be identified from questions asked to equine farmers. Additionally, some other solutions will be identified from Operational Groups, Focus Groups, EU/national/regional projects and literature reviews. Then, need-solution matching will be done during the European Workshop and solutions will be prioritised accordingly. Each country will acquire a clear knowledge of their situation and priorities, and these will constitute its knowledge agenda for future training and demonstration activities. Potential solutions and best practices will be evaluated using knowledge evaluation methodology and cost-benefit analysis. Each set of solutions and best practices will follow a bottom-up – top-down cycle (ping-pong) between the European and national levels to ensure a good adaptation to each national context and acceptance by farmers. This process will allow the acceptance of solutions and best practices during the exchange activities such as workshops, demonstration and training days and cross-visits.

Up to now, more than 40 interviews have been carried out in each country to help identify and prioritise stakeholders' needs. While working on data collected in each country, nine priority needs were identified at the European level. Within equine welfare and health TA, the project aims to respond to two questions: what practices can be implemented to promote biosecurity measures and prevent emerging diseases and how can horse welfare conditions be improved?

More than 200 solutions relative to equine welfare have been identified so far. Based on existing scientific knowledge on 3F: Food, Friends and Locomotory Freedom, the implementation of best practices in welfare is an ongoing process in multiple farms in Europe, while some equine sectors still appear resistant in answering the basic needs of the horses.

Keywords: horse, welfare, stakeholders, EUnetHorse.

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PROTEOMIC CHANGES IN THE CECAL MUCOSA OF CHERRY VALLEY DUCKS FOLLOWING ZEOLITE SUPPLEMENTATION

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In poultry production, aluminosilicates such as zeolite are used as feed additives primarily due to their high ability to absorb harmful gases, especially ammonia. Additionally, the unique microporous structure of zeolite allows it to effectively bind and neutralise mycotoxins. In ducks, the caecum is characterised by a lower pH and a higher concentration of easily fermentable compounds, with its microbiome playing a significant role in nutrient digestion, absorption, and metabolism [1]. A previous study [2] has shown a significant reduction in the number of pathogenic microorganisms from the Enterobacteriaceae family in the cecum of broiler chickens in response to the addition of zeolite to the feed. Considering the above, the present study aimed to evaluate the impact of a 1% zeolite addition to the feed on the proteomic changes in the caecal mucosa of Cherry Valley broiler ducks. The study involved 24 Cherry Valley ducks, divided into four groups (n = 6). The control groups included males (CM) and females (CF) fed a standard diet, while the experimental groups, males (ZM) and females (ZF), were given a diet supplemented with 1% zeolite. Immediately after slaughter on day 42, caecum fragments were collected. The proteins of the caecal mucosa were separated using two-dimensional electrophoresis (2-DE), and the 2-D images were analysed using PDQuest Analysis software version 8.0.1. Bioinformatic analyses identified 107 spots present on all gels, each with an average coefficient of variation of less than 40%. Statistical analysis of these spots revealed significant changes in 35 of them. Among these, only 3 spots showed increased expression, while 7 exhibited decreased expression in both sexes. For the remaining 25 spots, the direction of changes in response to zeolite supplementation differed between males and females. Supplementing duck diets with 1% zeolite leads to significant changes in the expression of protein spots in the caecal mucosa. The results also reveal gender-specific differences in response to this dietary supplement. Proteins that are statistically significant will be further identified using MALDI-TOF mass spectrometry in subsequent analyses.

Keywords: ducks, zeolite, cecum, proteome.

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HEALTH AND ECONOMIC ASPECTS OF THE USE OF PHYTOBIOTICS IN THE FEEDING OF BROILER CHICKENS

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Since ancient times, plants, especially herbs, have been used to prevent and treat diseases occurring in humans and livestock [1]. Herbs are used, among other things, as a nutritional supplement in the rearing of broiler chickens, improve palatability by stimulating appetite, regulate the gastrointestinal tract and metabolism, and have antidiarrheal, antibacterial and anti-inflammatory effects [2, 3]. The factors that positively affect chicken broiler organisms are the biologically active substances contained in herbs – phytobiotics. Phytobiotics are a broad group of bioactive compounds of plant origin. Plants synthesise them to protect against various pathogens such as bacteria, viruses, and fungi and protect plants' genetic material and photosynthetic apparatus from oxidative damage caused by ultraviolet radiation [4–6]. Phytobiotic raw materials are those parts of plants in which the accumulation of active substances is most significant, most commonly the leaves, rhizomes, roots, flowers, bark, fruits and seeds. To fully exploit the potential of phytobiotics, they should be applied throughout the rearing period (42 days) due to their preventive nature. Economic analyses show that the use of a phytobiotics supplement in the feeding of broiler chickens yields a profit of between 0.074 and 0.087 euro/pc, depending on the production volume.

Keywords: phytobiotics, natural source, herbs, health-promoting effects, economic and production performance.

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THE USE OF MICROALGAE SPIRULINA FOR QUALITY ENRICHMENT OF READY-TO-COOK MINCED CHICKEN MEAT PRODUCTS

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Spirulina, as a potential source of bioactive compounds, is widely used in the production of various dietary supplements, nutraceuticals, and pharmaceuticals. However, these forms are not acceptable to all consumer groups. Therefore, the use of this valuable raw material to enrich traditional products would be very promising. Also, considering the growing problem of malnutrition, especially among children and adolescents, it would be appropriate to enrich ready-to-cook minced chicken meat products (RCMP) with Spirulina, not only to provide them with a greater added value but also to impart acceptable unique sensory properties. The aim of this study was to evaluate the influence of microalgae Spirulina addition on the quality parameters (overall acceptability, colour coordinates, dry matter, pH, cooking loss, intramuscular fat, protein and ash content, amino acids (AA), gamma-aminobutyric acid (GABA), biogenic amine (BA) contents, fatty acid (FA) profile, and malondialdehyde concentration) of RCMP. Lyophilised Spirulina powder (content per 100 g: sodium 1.1 g, total carbohydrates 30.3 g, proteins 60.6 g, calcium 151.5 mg, potassium 1.7 mg, iron 48.5 mg) was provided by Now Foods Company (Illinois, USA). Fresh chicken meat breast was obtained from JSC "Vilniaus paukštynas" (Kaišiadorys, Lithuania). Meat was minced and mixed (separately) with 2% of salt and different quantities of Spirulina powder (1%, 2%, 3%, 4%, 5%, and 6%). Control samples were prepared using minced meat without the addition of Spirulina powder. The RCMP weight was 25 g each. Non-treated and thermally treated (cooked in 100°C water for 10 min) RCMP samples were subjected to analyses. It was established that RCMP prepared with 1%, 2%, 3%, 4%, and 5% of Spirulina showed similar overall acceptability as control ones, despite the fact that RCMP prepared with Spirulina showed intensive green colour ($-a^*$ (greenness) coordinates ranged from -6.13 to -8.36 National Bureau of Standards units). Most of the RCMP with Spirulina showed lower cooking loss; however, other technological parameters were similar to those of control samples. The analysis of free non-essential and essential AA concentrations in RCMP showed that, by increasing Spirulina content in RCMP, in most cases, free AA concentration in thermally treated RCMP was found higher, in comparison with control samples. However, significant differences between GABA concentration in RCMP were not established. Thermally treated RCMP enriched with 4%, 5%, and 6% of Spirulina showed significantly lower spermine concentration, in comparison with control ones. Also, thermally treated RCMP with Spirulina showed a significantly lower malondialdehyde concentration; however, differences between FA profiles were not established. Finally, it can be stated, that 1%, 2%, 3%, 4%, and 5% of Spirulina can be recommended for RCMP enrichment.

Keywords: chicken meat, Spirulina, amino acids, overall acceptability.

THE USE OF APPLE PRODUCTION BY-PRODUCTS FOR QUALITY IMPROVEMENT OF READY-TO-COOK MINCED CHICKEN MEAT PRODUCTS

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The apple processing industry has not yet efficiently utilised the by-products of processing (pomace and its dehydrated forms). Although apple pomace is rich in valuable components, its application in the food industry is limited due to the lack of technological solutions. Due to its unique composition (e.g., high pectin content), the by-products of the apple industry could be an excellent raw material for enhancing ready-to-cook minced meat products (RCMP) because the sensory texture characteristics of pectin are similar to those of fat. This particular characteristic could be applied in the production of ready-to-cook minced chicken products (RCMCP), as these are often characterised as 'dry', 'tough', etc. The aim of this study was to evaluate the influence of apple production by-products (APB) on the quality parameters (overall acceptability, colour coordinates, dry matter, pH, cooking loss, amino acids (AA), gamma-aminobutyric acid (GABA), biogenic amine (BA) contents, and malondialdehyde concentration) of RCMCP. Fresh poultry meat breast was obtained from JSC "Vilniaus paukštynas" (Kaisiadorys, Lithuania). Meat was minced with a meat grinder (hole diameter of 5.0 mm) (Meissner AG, Biedenkopf-Wallau, Germany) and mixed (separately) with 2% of salt and different quantities of the APB (5%, 10%, 15%, 20%, 25%, and 30%). Control samples were prepared using minced meat without the addition of APB. The RCMCP weight was 25 g each. RCMCP were covered with plastic film and stored at -18°C until analysis. Non-treated and thermally treated (cooked in 100°C water for 10 min) RCMCP were subjected to analyses. It was established that the addition of 5% and 10% APB was found to have no effect on the overall acceptability of the RCMCP (mean overall acceptability of the control and RCMCP with 5% and 10% APB was 4.11 points, on a 5.0-point Likert scale). The analysis of colour characteristics demonstrated that, in all cases, thermally treated RCMCP showed lower a^* (redness) and b^* (yellowness) coordinates, in comparison with controls. Also, the addition of APB, in most cases, increased hardness and reduced cooking loss of the thermally treated RCMC, as well as, after cooking, lower losses of most of the essential and non-essential AA were observed. However, no significant differences in GABA concentration in RCMCP prepared with and without APB were found. The main BAs in RCMCP were spermidine and spermine. Thermally treated RCMCP prepared with APB showed a lower concentration of malondialdehyde, in comparison with controls. Finally, it can be stated that 5% and 10% APB can be recommended for RCMCP preparation, because no negative effect on the overall acceptability of RCMCP was found as well as the addition of APB reduced losses of most of the essential and non-essential AA in thermally treated RCMCP and reduced malondialdehyde concentration.

Keywords: poultry meat, convenience foods, apple powder.

CHARACTERISTICS OF THE COOKED TYPE SAUSAGES ENRICHED WITH APPLE POWDER

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The aim of this study was to evaluate the influence of apple powder on the quality parameters (overall acceptability, colour coordinates, dry matter, pH, fat, protein and ash content, free amino acids (AA), gamma-aminobutyric acid (GABA), biogenic amine (BA) concentrations, fatty acid (FA) profile, and malondialdehyde concentration) of a cooked type of sausages (CS). Experimental batches of sausages were produced under laboratory conditions at the Research Center of Meat Processing of Almaty Technological University (Almaty, Republic of Kazakhstan). The recipe of cooked sausages consisted of 35 kg of top-grade fatty beef, 40 kg of chicken meat, 20 kg of raw fat of chicken, 3 chicken eggs, 2 kg of whole dried cow's milk, 2.3 kg of salt, 7.5 kg of sodium nitrite, 0.200 kg of refined sugar and glucose, and 0.040 kg of grounded nutmeg and cardamon. Three recipes were tested: with 0 kg, 3.0 kg and 5.0 kg of apple powder, additionally added to the basic recipe. No significant differences in sausage smell and taste acceptability were found. Sausages also showed similar colour parameters. However, the addition of apple powder increased dry matter content and texture hardness of sausages. Besides, sausages prepared with apple powder showed higher protein content, in comparison with control samples. Most of free non-essential and essential AA concentrations in sausages were similar; however, sausages prepared with apple powder showed, on average by 2 times, a higher GABA concentration. The main BA in sausages was spermine, and its content in the samples was, on average, 53.5 mg/kg. There were no significant differences in sausage FA profile as well as in malondialdehyde concentration. Finally, it can be stated that apple powder can be utilised as a valuable material for cooked type sausage preparation, without negatively affecting their quality.

Keywords: apple by-products, sausages, quality, overall acceptability, technological parameters.

TOXOPLASMA GONDII ANTIBODY DETECTION IN SLAUGHTERED CATTLE

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Toxoplasmosis is one of the most common human zoonoses caused by undercooked meat. *Toxoplasma gondii*, a foodborne pathogen, poses significant concerns for public health [1]. The disease is estimated to affect around one-third of the world's human population and can affect almost all warm-blooded animals [2]. Moreover, toxoplasmosis causes economic losses to livestock farms as a result of reduced milk production, reproduction, infertility, abortions and poor animal health [3, 4]. The aim of the study was to analyse the possible prevalence of *Toxoplasma gondii* in cattle housed differently in Lithuania, and to assess the contamination of beef. Beef diaphragm muscle samples were collected from three slaughterhouses during the period from January to April 2024. The cattle were classified into closed and open environments based on their housing systems. Each sample weighed approximately 150 grams and was stored in individually sealed bags at -20°C to preserve integrity until laboratory analysis. The detection of *T. gondii* antibodies in meat juices was conducted using an indirect ELISA method (ID Screen[®] Toxoplasmosis Indirect Multi-species, ID.vet, France).

A total of 47 beef diaphragm samples were analysed. The findings revealed that 15 samples tested seropositive, indicating a prevalence rate of 31.91%. Additionally, 7 samples were classified as equivocal (14.89%), while 25 samples were confirmed negative (53.20%). The average age of the sampled cattle was found to be 36.46 months. Notably, 12.77% of the cattle were housed in closed systems, whereas 87.23% were in open systems. Importantly, all positive samples originated from cattle in open systems, with a mean age of 39.2 months. Furthermore, no infections were identified in cattle younger than 12 months. Notably, after conducting the Mann-Whitney U Test to analyse the infection data from both groups, we found significant results indicating that cattle held in open systems were indeed infected more frequently with *Toxoplasma gondii* than those in closed systems ($P > 0.05$).

The results show that cattle are exposed to *T. gondii* with high frequency, especially when kept in open systems. It is particularly concerning that meat is not tested for this parasitic protozoan, neither from animals grown in Lithuania nor for imported meat. In order to ensure food safety and quality throughout the food chain, research needs to be carried out to determine the exact prevalence of the pathogen and to look for measures to minimise the potential spread of the parasite along the epizootic chain while minimising the possible risk factors for infection.

Keywords: sero-prevalence, *Toxoplasma gondii*, serology, cattle.

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THE RELATIONSHIP BETWEEN BLOOD B-HYDROXYBUTYRIC ACID CONCENTRATION WITH MILK YIELD, HEALTH AND REPRODUCTION IN HOLSTEIN COWS

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Analysis of beta-hydroxybutyrate (BHB) concentration in dairy cows may allow predicting the properties of cows and help better manage farms [1]. The monitoring of cow metabolism is very important, in the period immediately after calving, when the cows face the consequences of negative energy balance, which result in lower milk production, impaired fertility, and increased frequency of other diseases (mastitis, sub-clinical ketosis, etc.) [2]. Our main objective of the research was to estimate the relationships of BHB concentration with production, reproduction traits and health of Holstein dairy cows.

The research was carried out in Lithuania on a dairy cattle farm of 1187 dairy cows kept in a loose housing system, from which samples were taken: first lactation – 175, second lactation – 157, third – 125, and fourth and higher lactation – 133 cows. In total, 590 Holstein fresh dairy cows were selected for this research. The data were collected from Dairy Plan C21, Version 5.3 management programme (Germany), including lactation number, production, reproduction results, and veterinary records (mastitis, metritis, subclinical ketosis incidence, hoof lesions). Blood BHB concentration was measured using capillary blood samples collected after morning milking. SPSS 25.0 (IBM SPSS Statistics for Windows, Armonk, NY, USA) was used for statistical data analysis. Using descriptive statistics, the results were produced as the mean and standard error of mean ($M \pm SEM$). Mean differences (assessed using the Tukey HSD—Honestly Significant Difference) were considered significant when the P value was < 0.05 . The Pearson correlation coefficient was calculated to define the linear relationship between BHB concentration and investigated indicators.

Analysis revealed a weak positive statistically significant correlation coefficient between blood BHB concentration and first insemination day ($r = 0.184$, $P < 0.001$), insemination rate ($r = 0.158$, $P < 0.001$), milk yield ($r = 0.104$, $P < 0.05$). The effect of BHB was evaluated according to lactation, milk yield per lactation (305 DIM) and health status: healthy, mastitis, metritis, hoof lesions, and sub-clinical ketosis. Dairy cows with BHB concentration reached during subclinical ketosis had a higher insemination rate 17.54% ($P < 0.01$) and approximately a 7-day longer first insemination day and lower 4.45% milk yield ($P < 0.05$) compared with the healthy cows. The investigation of the relationship between BHB concentration and investigated traits (milk yield, lactation, insemination rate, first insemination day) did not show any statistically significant relationship with BHB concentration in healthy cows, $P > 0.05$. While in diseased cows a weak but statistically significant effect was detected: in the mastitis group, BHB concentration with the insemination rate ($r = 0.238$, $P < 0.01$); in the sub-clinical ketosis group: BHB concentration with the insemination rate ($r = 0.242$, $P < 0.05$); in the hoof lesions group: BHB concentration with lactation ($r = 0.321$, $P < 0.05$). The results of this research indicate that the analysis of BHB concentration has a potential for identifying problematic cows in dairy herds and can be a suitable parameter for routine monitoring.

Keywords: BHB, lactation, milk yield, mastitis, metritis, ketosis, hoof.

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ANALYSIS OF EFFECTIVE POPULATION SIZE IN LITHUANIAN DAIRY CATTLE POPULATIONS

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The effective population size (N_e) is a key parameter in population genetics with significant applications in evolutionary biology, conservation genetics, and animal breeding. It provides insights into the rates of genetic drift and inbreeding, which in turn affect the efficacy of evolutionary forces such as mutation, selection, and migration [1–2]. The aim of this study was to investigate the effective population sizes between two open (commercial) populations, i.e., Lithuanian Red and Red-and-White (LRWP) and Lithuanian Black-and-White (LBWP), and two old genotype populations, i.e., Lithuanian Black-and-White (LBW) and Lithuanian Red cattle (LR), which currently are under conservation. The effective population size (N_e) is one of the most commonly used indicators of genetic diversity, and for animal breed conservation purposes, it can be defined in various ways [3–5]. Therefore, in our study the effective population size was calculated using four methods: based on the rate of inbreeding; based on the number of parents; based on the co-ancestry; and based on the regression on equivalent generations. The data on the Lithuanian dairy cattle populations were provided by the Centre for Agricultural Information and Rural Business. The input data consisted of the unique identification of all animals: animal ID, sire ID, dam ID, birthdate and sex. The research showed that the lowest effective population size (N_e) concerning the rate of inbreeding was observed in LBW (23) and LRWP (68), with the highest N_e identified in the LBWP population (462). When considering N_e based on the number of parents, LR displayed the lowest N_e (107), while the highest N_e was found in LBWP (4449). At the end of 2021, the effective population size, based on the co-ancestry, was calculated for LRWP, LBWP, LR and LBW and was found to be 121, 58, 29 and 28, respectively. In 2017–2021, the effective population size based on the regression on equivalent generations decreased in all the breeds. The highest decrease (63%) was found in LR and the lowest (20%) in LBWP. This study demonstrates that current breeding strategies are leading to a significant reduction in genetic diversity in both commercial (open) and old genotype populations of Lithuanian dairy cattle. The effective population size (N_e), calculated using four different methods, revealed critical trends in genetic drift and inbreeding. The ongoing decline in N_e , especially in smaller, conserved populations, poses a serious threat to the sustainability of these breeds. Lower N_e values are closely linked to increased rates of inbreeding and reduced genetic adaptability, making populations more vulnerable to environmental changes and production pressures.

Keywords: open populations, old genotype populations, effective population size.

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PROTEIN FRACTIONS OF ENSILED LUCERNE WITH VARYING LEVELS OF DRY ICE

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The ensiling of lucerne (*Medicago sativa* L.) involves proteolytic transformations, as a result of which some of the true protein (TP) converts into forms of higher solubility. Therefore, the pool of non-protein nitrogen (NPN) compounds increases. The degree and extent of proteolysis in lucerne can be limited by wilting, silage additives, or shortening the aerobic phase of fermentation [1]. The aim of this study was to investigate the characteristics of protein fractions in lucerne silage treated with different levels of dry ice.

Lucerne was harvested during the first cut, at the beginning of the bud formation stage, at a height of 5 cm, using a disc mower (between 12:00 and 1:00 p.m.). Then, it was cut into 25 mm particles. The first part of herbage was ensiled without wilting, while the other was allowed to wilt for 12 and 24 hours. Lucerne was ensiled in micro-silos (1 dm³) with the addition of 0 g, 0.5 g, 1 g, or 2 g of dry ice (DI). After 50% of the micro-silo volume was filled, DI granules (16 mm in diameter) were added, and densifying was continued. It was ensiled in triplicates for 90 days. The protein fractions were analysed according to Licitra et al. [2]. The results were statistically processed by performing an analysis of variance (ANOVA) at a significance level of 0.05, using the XLSTAT package.

There was an effect of DI addition on TP content ($P \leq 0.01$), and the highest concentration of the fraction was obtained in silage after 24 hours of wilting ($P \leq 0.001$), ranging from 33.55% to 42.14% of crude protein (CP). In each group of silage, regardless of the degree of wilting, the addition of 1 g and 2 g of DI significantly increased TP concentration relative to samples with 0 g and 0.5 g of DI. The 0-hour lucerne silage had the lowest TP contents (24.01% to 32.55% CP). As the dry matter (DM) and the amount of DI addition increased, the TP concentration in the experimental silage increased, while the content of the soluble crude protein (SCP) fraction decreased ($P \leq 0.001$). The addition of DI did not affect the content of the neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble crude protein (ADICP) fractions during ensiling ($P > 0.05$). For both NDICP ($P \leq 0.01$) and ADICP ($P \leq 0.001$) fractions, an increasing trend was observed with increasing DM of silage. Silage in the 24-hour group had the smallest increase in ADICP during fermentation.

Ensiling lucerne with the addition of DI at the levels of 1 g and/or 2 g resulted in a more favourable CP composition and a higher forage quality. The results obtained and the analysis conducted suggest that the use of DI as a source of CO₂ to modify the atmosphere of ensiled lucerne could effectively reduce cellular respiration and cell lysis during ensiling, which helps reduce proteolysis.

Keywords: lucerne silage, proteolysis, dry ice.

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CANNABIDIOL AND NANO FORM OF SELENIUM MEDIATE CHANGES IN THE FATTY ACID COMPOSITION OF BREAST MEAT OF CHICKENS APPLIED TO CHALLENGE

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There is a significant correlation between dietary delivered fatty acids (FAs) and those deposited in tissue lipids of poultry. Generally, deposition of saturated fatty acids (SFA) at the cost of polyunsaturated fatty acid (PUFAs) in the tissue lipids deteriorates its healthy properties. On the other hand, FAs, which are deposited as phospholipids, are one of the major elements of cell membranes which play important immunological functions. Arachidonic acid (AA; C20:4n-6), eicosapentaenoic acid (EPA; C20:5n-3), and docosahexaenoic acid (DHA; C22:6n-3) are the precursors of lipid mediators of the inflammation response. Therefore, affecting FA composition is an interesting way of supporting the immune function in birds. Recently, a great attention has been paid to using bioactive agents in the diet of chickens to support their immune function. Substances that have been shown to stimulate immunity include cannabidiol (CBD) and nano selenium (Nano-Se). However, little is known about the host response regarding FA metabolism in chickens as a result of CBD and Nano-Se, in particular in the challenge conditions. Therefore, in the present study, we investigated a potential link between changing the FA composition in the breast meat of chickens as a response to CBD or Nano-Se supplementation in the ordinary conditions or induced stress conditions (challenge with *C. perfringens* bacteria). Birds in the control group and in the positive control group (Con) received a basal diet throughout the experiment. Groups Con + CBD + *C. perfringens* and C + nano-Se + *C. perfringens* were challenged and supplemented CBD or Nano-Se. Birds from Con + CBD + Nano-Se group and C + CBD + nano-Se + *C. perfringens* were fed a Con diet supplemented with both additives, but challenged with *C. perfringens*. The analysis of the FA concentration of the chicken breast meat revealed that the lowest changes in the FA composition were found in the Con group. When the *C. perfringens* challenge was applied, the FA composition of the breast meat did not change markedly. However, supplementation of the birds' diet either with CBD or with Nano-Se resulted in a significant shift in the FA profile in breast meat including changes in SFA, PUFA or PUFA_{n-6/n-3} ratio. The most spectacular ratio was recorded in terms of a negative correlation in the SFA deposition in breast meat as a result of bioactive compound supplementation.

The data indicate that either CBD or Nano-Se have a potential activity in changing FA metabolism in birds applied to different conditions.

Keywords: cannabidiol, nano selenium, fatty acids, immune, broiler chickens.

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THE INFLUENCE OF THE HEALTH CONDITION OF DAIRY HEIFERS ON THE EFFECTS OF THEIR REARING

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Biological and technological progress in animal production has led to an increase in the milk yield of cows. However, obtaining healthy and high-yielding cows depends on the proper rearing of calves. The aim of the study was to determine the impact of health on the course of rearing dairy heifers.

The research material consisted of 147 heifers born between May 2022 and October 2023. During the weaning period, the heifers were observed for the occurrence of symptoms of diseases associated with the rearing period, such as diseases of the digestive and respiratory systems. Body weight was measured twice: on the day of birth and after the end of weaning with a milk replacer. In addition, 45 heifers were qualified for studies on the effectiveness of insemination. In order to achieve the aim of the study, the effect of disease factors on the duration of rearing, the body weight of the heifers, their daily gains in body weight and on the effectiveness of insemination were analysed.

Among the diseases of the calving period, respiratory diseases were the most common especially in the age range from 15 to 90 days of age. Significantly ($P \leq 0.01$) higher daily body weight gains were achieved by healthy heifers compared with those that fell ill at least once during the rearing period. This difference was 64.6 grams. Among 45 heifers subjected to insemination, those that did not show symptoms of disease and had daily body weight gains of ≥ 700 g/day had significantly better effectiveness of the procedure compared with heifers with lower body weight gains (72.7% vs. 47.8%). It was found that the occurrence of diseases during the rearing period affects the reduction of daily body weight gains and final body weight, as well as the extension of the rearing period. Increased disease incidence during the rearing period and, consequently, lower growth and development results also contributed to reduced insemination efficiency in heifers.

Keywords: heifers, health of calves, rearing effects.

POLYMORPHISM OF GROWTH HORMONE GENE (GH) (2141C>G) AND ITS INFLUENCE ON THE CARCASS QUALITY IN BEEF CATTLE

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Growth hormone (GH) gene acts and mediates the growth of bones and muscles. It is known that GH is the main regulator of postnatal somatic growth, stimulating anabolic processes, and skeletal growth [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 (2141C>G) polymorphism and to determine its influence on the carcass quality in beef cattle. Samples of cattle hair follicles were collected from 85 bulls of Angus (41), Limousin (19), Galloway (19) and Simmental (6) cattle. The bulls were raised under the same conditions in Šilutė control bulls feeding station. The hair samples and slaughter data (the ratio of muscle development distributed according to the EUROP system) were obtained from private slaughterhouses, where animals were slaughtered. Bovine genomic DNA was extracted from hair follicles using the Chelex DNA extraction method. Polymorphism of the GH locus was identified using a PCR-RFLP method (*AluI* restriction endonuclease). Investigation of polymorphism 2141C>G of the GH gene showed that the C allele of the GH gene was the most frequent compared with the G allele in the general population of beef cattle. The CG genotype of the GH gene was the most frequent in the studied population (43%) followed by the CC genotype (40%) while the GG genotype had the lowest frequency (17%). After evaluation of the observed and expected heterozygosity across the investigated group of animals, the observed heterozygosity was found lower than expected, indicating the lack of genetic diversity of the studied loci, although the difference was not statistically significant. According to the EUROP carcass classification system, most of the GG genotypes bovine carcasses were classified as the O (fair) carcass conformation class and the 3rd (average) fat coverage class; most of the CG genotype bovine carcasses were classified as the R (good) carcass conformation class and the 3rd (average) fat coverage class; and most of the CC genotype bovine carcasses were classified as U (very good) and R (good) carcass conformation classes and the 3rd (average) fat coverage class. The results were statistically significant (P value – 0.010, $P < 0.05$). In conclusion, the results showed that the GH gene polymorphism (2141C>G) affected carcass conformation and fat coverage in beef cattle.

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

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BLOOD SERUM ELECTROLYTES OF HERITAGE TURKEY HENS AND TOMS ADMINISTERED AQUEOUS *MORINGA OLERIFERA* LEAVES AND SEEDS EXTRACTS

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The serum electrolyte levels are essential in the regulation of nerve and muscle function, hydrating the body, balancing blood acidity and pressure as well as helping build damaged tissues. Hence the need to evaluate the impact of aqueous *Moringa olerifera* leaves and seed extracts on heritage turkey toms. The study was carried out with 36 heritage turkeys of about 8 to 9 weeks old. There were nine turkeys per treatment group: T1 (control); T2 (1% seed w/v); T3 (0.5% leaf and 0.5% seed w/v); and T4 (1% leaf w/v). Each group was replicated three times with three turkeys per replicate. The study lasted for 154 days with all routine management practices duly observed. The data were collected for serum electrolyte analysis through the wing vein using sterile needles and syringes and were analysed within 2 hours of collection. Three (3 ml) of blood samples were drawn from three turkeys in each treatment. This was done on the last day of the trial. The blood serum electrolytes, which are very essential in the maintenance of the body homeostatic function, differed significantly ($P < 0.05$) among the treatment evaluated for the toms (potassium: $3.70 \pm 0.10a$ in T1; $3.1 \pm 0.07c$ in T2; $3.7 \pm 0.13ab$ in T3; and $4.13 \pm 0.00a$ in T4) with the exception of calcium. The calcium level, though not significant, was higher among the *Moringa* administered treatments compared with the control. Potassium levels differed significantly ($P < 0.05$) among the treatment, with T4 (4.13 ± 0.00) being the highest and T2 (3.19 ± 0.01) lowest, which suggests that *Moringa* seed extract may have reduced the potassium levels in the turkey toms. The heritage turkey hens' serum electrolyte levels differed significantly ($P < 0.05$) only in the calcium levels ($8.40 \pm 0.16a$ in T1; $8.86 \pm 0.88a$ in T2; $7.78 \pm 0.23b$ in T3; and $7.89 \pm 0.03b$ in T4). The sodium and potassium levels did not differ significantly ($P < 0.05$) among the female turkeys. Cases of high potassium leading to paralysis and heart problems or low potassium resulting or leading to dehydration and excessive sweating were not witnessed in this study. The sodium level among the male turkeys differed significantly ($P < 0.05$) among the treatments. The observed levels were lowest in T2 (119.043 ± 0.234) thus suggesting that *Moringa* seed extracts may have the potential to balance the body electrolyte level and reduce the risk of high blood pressure as envisaged in this study. This was lower than the results from leaf extracts. The findings confirm the anti-hypertensive qualities of *Moringa*. It may be that the *Moringa* seed has these thiazide or loop diuretics properties that enable the male turkeys on T2 to have reduced levels of sodium and potassium while laying affected the calcium levels in the hens.

Keywords: serum, turkey and extracts.

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MUSIC IN THE PIGSTY – WHAT DO PIGS LISTEN TO?

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The increasing expectations regarding the quality of animal products and farming conditions motivate farmers to seek methods to improve animal welfare [1]. Pigs, as intelligent and curious animals, eagerly explore their environment, which can be enriched with materials such as straw or toys [2, 3]. A relatively new and not yet widespread method of improving welfare is music. Pigs have a hearing range from 42 Hz to 40.5 kHz, with the greatest sensitivity between 250 Hz and 16 kHz, and they use specific vocalisations that last for certain periods and utilise specific frequencies for given situations [4, 5]. Researchers mention the stress-reducing properties of music and its positive effects on production outcomes in other animal species [6–8]. In pig production, attempts have been made to enrich their environment with auditory elements. Classical music, in particular, has been highlighted for its importance – compositions from this genre lower cortisol levels, improve pigs' behaviour, and affect their immune system [9, 10]. Pigs may exhibit preferences regarding the tempo of music and the sound of instruments – piglets prefer slow-tempo string music and fast-tempo wind music [11]. On the other hand, playing rock and roll music to pigs has been found to be detrimental. This genre caused a decrease in the daily growth rate and feed utilisation rate in fattening pigs, which may indicate stress induced by strong sounds [12]. Music can be considered a beneficial environmental enrichment for pigs, improving their welfare by reducing stress and strengthening the immune system. However, particular attention should be paid to the genre of music played in the pigsty.

Keywords: pigs, music, welfare, livestock production.

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EFFECT OF SHOW DIET ON FUTURE PRODUCTIVITY OF HOLSTEIN-FRIESIAN HEIFERS

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Cattle presented at livestock shows serve as the showcase for their owners. The use of specific feeding, referred to as “show feeding”, aims to highlight the best features of the animal, enhance conformation traits, and underline traits correlated with milk production. The objective of the conducted study was to analyse the impact of show feeding of Polish Holstein-Friesian heifers on their future performance and the chemical composition of their milk. The research material consisted of 16 Polish Holstein-Friesian heifers, divided by the analogy method into two feeding groups (8 animals each). The animals were kept in groups in two separate pens measuring 5 m x 9 m, equipped with stalls lined with rubber mats and bedded with wood shavings. The experimental period lasted from the 13th to the 15th month of the heifers’ life (8 weeks). The heifers were fed using the PMR system: Control group (1) – wheat straw, corn silage, haylage, concentrate mixture, dried beet pulp, mineral lick; Experimental group (2) – wheat straw *ad libitum*, concentrate mixture, dried beet pulp. Changes in milk yield and composition were determined up to 100 days after calving and during the 305-day lactation period, based on secondary data obtained from performance evaluation reports conducted within the herd. The results were statistically processed (Statistica 13.0, StatSoft). Heifers from Group 2 achieved a higher milk yield during the first 100 days of lactation (an average of 161 kg more milk), as well as a higher yield during the 305-day lactation (an average of 462 kg more milk) ($P \leq 0.05$). No differences in the chemical composition and quality of milk were found between the feeding groups during the first 100 days of lactation. The analysis of the average chemical composition of milk from cows in both feeding groups during the 305-day lactation period confirmed statistically significant differences in the somatic cell count (SCC): Group 1 – $159.88 \times 10^3/\text{mL}$, and Group 2 – $135.63 \times 10^3/\text{mL}$ ($P \leq 0.05$). The SCC in the milk of the studied heifers ranged from 126 to $159 \times 10^3/\text{mL}$, indicating very good udder health and proper milking processes. The use of increased amounts of dry roughage in the feeding of breeding heifers may positively affect their preparation for consuming larger amounts of feed in subsequent stages of life, positively influencing their future productivity.

Keywords: cattle, heifers, milk yield, show diet.

INFLUENCE OF LYOPHILIZED DRONE BROOD ON BIOCHEMICAL PARAMETERS IN PIG BLOOD

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Bee products are naturally occurring pharmaceutical products with a multidimensional effect on living organisms, including humans. The aim of this study was to evaluate the effects of drone brood in feed on the biochemical parameters of pig serum. Many commonly monitored values serve as indicators of the current metabolic state, as well as cellular integrity disorders caused by pathological processes. In a 21-day experiment, 24 hybrid pigs aged 2 months were used. They were divided into three groups of eight animals each, including a control group and two experimental groups. The pigs were fed a basal diet supplemented with brood homogenate at doses of 100 and 200 mg per 1 kg of body weight. Serum samples were collected from venous blood at the beginning and end of the drone brood administration, and their biochemical parameters were analysed. The administration affected the metabolism of proteins, triglycerides, and cholesterol, but mainly resulted in changes in the activity of liver enzymes such as aspartate aminotransferase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) in the serum. In some cases, there was a significant change in enzyme activity by 2–16%, with AST being the most significantly affected, exceeding more than half of the activity compared with the control group. Despite the fact that the aim and design of the experiment were not intended to monitor feeding parameters, it can be stated that the inclusion of drone brood did not negatively affect the palatability and feed intake, and improved average daily gains by 7 to 9%. The improvement was directly proportional to the concentration of drone brood added to the feed mixture and resulted in a 4% improvement in the conversion rate.

Keywords: drone brood homogenate, biochemical parameters in blood, liver enzymes, pig.

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COMPARISON OF GAITS AND JUMP MOVEMENTS BETWEEN DIFFERENT GENOTYPES IN TRAKEHNER HORSES

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Sports horse breeding is currently most oriented towards the high-performance abilities. This becomes a challenge for small and closed populations. The aim of our study was to determine the performance characteristics of different genotypes present in the Lithuanian Trakehner horse population. Trakehner Horse Breeders Association and Agricultural Data Center provided the data of Trakehner horse evaluations since 1999. The data of 435 protocols of Trakehner horse evaluations at the age of 3 and more years old were analysed. In the protocol walk, trot, canter and free-jumping movements were rated from 1 to 10. Mean and standard deviation values of the scores were calculated for mares and stallions within separate horse genotypes. The genotypes were formed on the basis of Trakehner horse lines described by Kamzolov [1]. All horses were sorted according to pedigree and assigned to the related horse group in accordance with the founders of the stallion lines. The stallion lines were divided within genealogical clusters on the basis of the founder's breed. When describing the lines of old Trakehnen stud, the stallions of Pilger through Einhard line stood out with higher ratings of trot movements (7.0 ± 0.5) and jumping quality (7.4 ± 0.8). The mares of Pilger through Port Arthur were rated at canter movements for 7.0 ± 0.47 and at jumping for 7.5 ± 0.6 . Stallions of the Dampfross through Hyperion line were scored the highest for the quality of trot and canter movements, 7.2 ± 0.6 and 7.1 ± 0.6 , respectively, while ratings of mares jumping quality reached 7.5 ± 0.6 . The stallions of Dampfross through Pythagoras line were also highly rated for jumping quality (7.3 ± 0.5). In the related groups, whose lines were introduced by thoroughbred stallions, the progeny of Saint Cloud group stood out for particularly well-rated walk and trot movements (from 7.1 to 7.7), but they were poorly rated for jumping quality (stallions 6.1 ± 0.6 ; mares 6.3 ± 0.3). Stallions of the Bay Ronald through Gainsborough line were distinguished by the highest jumping rating, which was 7.5 ± 0.6 . The stallions and mares of Prince Rose line showed high ratings for the quality of walk movements (7.3 ± 0.7 and 7.5 ± 0.6 , respectively), and the mares were distinguished in the evaluation of canter movements and jumping (7.3 ± 0.6 and 7.4 ± 0.5 , respectively). The stallions of Langraf line were rated higher in canter movement and jumping (7.1 ± 0.6 and 7.2 ± 0.6 , respectively), while the mares of Nasrullah line stood out with the highest rating of jumping (7.8 ± 0.8). In conclusion, the progeny of all stallion lines originating from old Trakehnen Stud were versatile and rated well for jumping, walk and trot movements. Meanwhile, the progeny of some thoroughbred stallion lines was better rated for walk and trot and some thoroughbred stallion lines were exceptional due to highest rates of jumping. In order to improve the performance of Trakehner horses, it is appropriate to use stallions of Dampfross (especially through Hyperion), Pilger through Einhard, Bay Ronald through Gainsborough and Landgraf lines in mating with typical Trakehner mares.

Keywords: sport horses, gait, jumping, Trakehner.

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THE SEASONAL INFLUENCE ON THE MILK PRODUCTIVITY, QUALITY AND UDDER HEALTH OF DAIRY COWS

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The quality of the milk that arrives at the dairy is mainly evaluated by the SCC, an important health indicator of the mammary gland [1]. During milk production, the concentrations of milk components vary according to the season across the year [2]. The objective of our study was to analyse the effect of the season on milk productivity, quality and udder health of Dairy Cows. The study was carried out on 294 Holstein lactating dairy cows, in accordance with the Law on the Care, Keeping and Use of Animals of the Republic of Lithuania. The herd are kept in loose housing all year round. Milk analysis was performed at the accredited central milk testing laboratory of the Joint Stock Company Pieno Tyrimai, Lithuania. According to the seasons, the cows were divided into 4 groups: spring (n = 85); summer (n = 72); autumn (n = 67); winter (n = 70). After dividing the cows into groups based on seasonal differences, we compared the results from each season to determine the impact of seasonal variations on milk yield, somatic cell count, and udder health. Arithmetic means, their errors and statistical reliability of the data were calculated for each evaluated trait. Statistically reliable data were considered when $P < 0.05$. The highest count of somatic cells was recorded in summer at 424.48 ± 51.35 thousand cells/mL, with a 24% decrease observed in winter; this difference was statistically significant ($P < 0.05$). Subsequent seasons showed no significant variation in somatic cell counts. The highest milk productivity was observed in summer at $4977.17 \text{ kg} \pm 96.94 \text{ kg}$, while the lowest was recorded in spring, with an 18% decrease that was statistically significant ($P < 0.05$). The highest prevalence of udder health issues was observed in cows during the summer season. Across all seasons, subclinical mastitis was more common, occurring 53% more often than clinical mastitis, with results showing statistical significance ($P < 0.05$). In conclusion, our study reveals that seasonality significantly affects milk productivity, quality and udder health in dairy cows.

Keywords: cow, season, somatic cells count, milk productivity, udder health.

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BEE POLLEN QUALITY: INFLUENCE OF THE PREPARATION METHOD AND STORAGE TIME ON THE QUALITATIVE AND QUANTITATIVE COMPOSITION OF ITS PHENOLIC COMPOUNDS

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Bee pollen (BP), made up of flower pollen grains, nectar and bee saliva secretions, contains a wide range of nutrients including proteins, carbohydrates, lipids, dietary fibre, as well as phenolic compounds, various vitamins, minerals, etc. [1]. As the need to consume high quality pollen increases, it is very important to study the amount of biologically active compounds and their variation depending on the preparation method and storage conditions. The aim of this study was to evaluate the changes in phenolic compound composition depending on the storage conditions and method of BP preparation. BP were collected from an apiary located in Pasvalys district (55°96' N, 24°33' E). Dried pollen (at +28°C on the first day and +35°C on the second day) and fresh frozen (–20°C and –80°C) BP samples were prepared. The amount of total phenolic compounds (TPC) was determined by spectrophotometric Folin–Ciocâlțeu method [2]. The amount of total flavonoid compounds was detected using reaction with AlCl₃ [3]. Qualitative and quantitative analysis of the profiles of phenolic compounds in BP was performed by UPLC–MS analysis [4]. The TPC at the starting point was in the range of 23.8 ± 1.0 mg GAE/g to 24.5 ± 1.8 mg GAE/g and after 3 months of storage it was in the range of 21.8 ± 0.4 mg GAE/g DW to 23.8 ± 1.0 mg GAE/g DW. The amount of TPC was not affected up to 3 months of storage in frozen (–20°C and –80°C) pollen samples; however, it was slightly decreased (by 6%) in dried bee pollen ($P < 0.05$). The total amount of flavonoids was in the range of 14.3 ± 0.1 mg RE/g and 15.5 ± 0.3 mg RE/g and remained unchanged after 3 months of storage in either dried or frozen bee pollen samples. After UPLC–MS analysis, 4 phenolic acids and 15 flavonoids were identified in both the dried and frozen bee pollen. P-coumaric acid and chlorogenic acid were observed to be the most abundant phenolic acids in BP. The major flavonoids rutin, quercetin, isorhamnetin-3-glucoside, luteolin-3,7-diglucoside and phloridzin demonstrated the highest amounts and practically did not decrease after 3 months of storage. BP dominated from the following plants: the genus of *Salix* L. and *Brassica* L., *Aesculus hippocastanum* L., *Taraxacum officinale* F. H. Wigg., *Malus domestica* L. and *Prunus cerasus* L. In conclusion, the total phenolic content and the total flavonoid content do not differ at the initial stage in frozen (–20°C and at –80°C) compared with dried bee pollen. The total flavonoid content was not affected by storage of either dried or frozen bee pollen for up to 3 months, but the total phenolic content was slightly decreased in dried bee pollen, while it remained the same in frozen bee pollen. The quantitative composition of individual flavonoids and phenolic acids varied slightly after 3 months of storage.

Keywords: bee pollen frozen and dried, total phenolic content, flavonoids, storage time

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SEMEN QUALITY OF BUCKFAST BEE DRONES

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Honeybee colonies have been declining worldwide for several years, and one of the main factors contributing to this problem is the widespread use of insecticides [1]. Although there has been some research into this issue over the last decade, very little is known about the effects of most chemicals used in agriculture on honeybee reproduction [2]. Even less is known about the reproductive abilities and sperm quality of drones. For this study, 22 semen samples were collected from sexually mature Buckfast bee drones during the month of June. Half of the samples were collected from hives located in urban areas (group A) and the other half from hives located in more rural areas (group B). Sperm analysis consisted of sperm motility, concentration, sperm viability (HOS test), morphology and morphometry. Sperm motility was found to be on average 8.18% higher in group A than in group B ($P < 0.05$). There was no significant difference in sperm concentration between the groups, and the mean value for both groups was 7.69 mln/ μ L (standard deviation (SD) = 2.33). There were no significant differences between the groups in sperm viability, with the mean being 3% higher in group A. Morphological analysis showed a significant difference between the groups ($P < 0.001$). Group B had on average 9% more pathologies in the samples compared with group A. Most abnormalities were detected in the flagellum. After measuring the average length of the spermatozoa in both groups, it was found that the spermatozoa in group A were 10.93 μ m longer ($P \leq 0.05$). During the research, it was found that all evaluated parameters of the drone spermatozoa were well above the average found in the literature [3]. Almost all parameters were higher in group A (urban drones) compared with group B (rural drones). This could indicate that drones from colonies closer to crop fields are affected by possible insecticides and their sperm quality is reduced. Further research with larger study groups and controlled environments is needed to prove this.

Keywords: honeybee, drone, semen, sperm quality.

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DEFENSIN GENE EXPRESSIONS IN HEALTHY UDDER QUARTERS ADJACENT TO INFECTED WITH COAGULASE-POSITIVE OR COAGULASE-NEGATIVE STAPHYLOCOCCI IN HOLSTEIN-FRIESIAN DAIRY COWS

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Defensins are one of the many antimicrobial peptide (AMPs) families. Until now, many of defensins were identified in plants and animals. The activity of defensins indicates their key role in protection against pathogens (bacteria, viruses and fungi) [1]. The aim of the study was to determine the expression of bovine defensin beta 1 (*DEFB1*), defensin beta 4 (*DEFB4*), defensin beta 5 (*DEFB5*), defensin beta 10 (*DEFB10*) and lingual antimicrobial peptide (*LAP*) in the parenchyma of healthy udder quarters adjacent to infected with coagulase-positive or -negative staphylococci. The material for the study consisted of 50 samples selected from 200 samples of udder quarter parenchyma collected from 50 Holstein-Friesian dairy cows. They were between the first to the fourth lactation, slaughtered at the end of lactation due to subclinical mastitis or reproductive problems. Milk samples for microbiological examination were collected aseptically two days before slaughter. Parenchyma samples were divided into five groups: infected with coagulase-positive staphylococci (CoPS, n = 10) and adjacent to them (AHCops, n = 10), infected with coagulase-negative staphylococci (CoNS, n = 10) and adjacent to them (AHCoNS, n = 10) and from whole healthy udders (control group H, n = 10). The mRNA level of genes was determined by RT-qPCR with *GAPDH* and *HPRT1* as references. Analysis of variance with post-hoc Bonferroni correction, after transforming mRNA levels to the natural logarithm scale, was performed with a model including animal as a random effect and health status and lactation number as fixed effects (MIXED procedure, SAS/STAT package, ver. 9.4). Lower *DEFB4*, *DEFB5* and *LAP* expressions were observed in AHCops than in CoPS. While there was no difference between AHCops and H. Expression of *DEFB5* was higher in AHCoNS comparing to the control and AHCoPS groups. Higher expressions of *DEFB4*, *DEFB5*, and *LAP* were found in CoPS than in H. Moreover, the higher *DEFB5* and *LAP* expressions were stated in CoNS than in H. We also found out the higher expression of *DEFB4* in CoPS than in CoNS. No differences in *DEFB1* and *DEFB10* expressions were observed. Summing up, subclinical inflammation caused by CoPS did not affect the expressions of any studied defensin, while CoNS affected the expression of *DEFB5* in the quarter adjacent to them, respectively. It may mean that there is a leakage between udder quarters. Based on the results of cytokine studies at the mRNA and protein levels, it was shown that CoNS infection increased expressions and concentrations of some cytokines in AHCoNS compared with H. This finding also suggests that the bovine mammary gland quarters are not fully independent glands. However, in order to draw precise unambiguous conclusions, the expression of many genes associated with the immune response during mastitis should be examined at both the mRNA and protein levels.

Keywords: mastitis, defensin, mRNA, gene expression, adjacent quarters.

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ANALYSIS OF MILKING PERFORMANCE OF COWS IN DIFFERENT LACTATIONS AND LACTATION PERIODS BASED ON THE ELECTRICAL CONDUCTIVITY OF MILK

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Milk electrical conductivity (EC) and milk flow rate are potential indicators in selection targeting mastitis resistance [1]. Measurement of milk (EC) is one of the indicators of such diagnosis, because it provides an early warning system by monitoring udder health at each milking [2, 3].

The work aims to determine the influence of the EC of milk on milking indicators of cows at different lactations and lactation periods. During the research, the EC of milk of 375 milking cows, milk yield, duration of milking, average and maximum milk flow were analysed. Cows were divided into three groups according to the EC of milk (< 5.0 mS/cm, 5.0–5.5 mS/cm, > 5.5 mS/cm). Lactation was divided into two groups: 1 lactation, 2 and older lactations. Cows were divided into four groups according to the period of lactation (up to 100 days, from 101 to 200 days, from 201 to 300 days and over 300 days of lactation). Averages, errors of averages, and statistical reliability of data (P) were calculated using Microsoft Excel programme. Differences were considered significant at $P < 0.05$.

We found that cows of the second and older lactation with a milk EC of less than 5.0 mS/cm produced 1.74 kg ($P < 0.05$) less milk than cows with a milk EC of 5.0–5.5 mS/cm, and 1.77 kg ($P < 0.05$) less than cows with a milk EC higher than 5.5 mS/cm. As the EC of milk increased, milking duration also increased with the number of lactations and lactation days. In the period up to 100 days of lactation, cows with a milk EC higher than 5.5 mS/cm had a milking duration that was 34 seconds longer than cows with a milk EC lower than 5.0 mS/cm ($P < 0.05$). Cows of the second or later lactations with a milk EC greater than 5.5 mS/cm had an average milk flow of 0.2 kg/min ($P < 0.05$) lower than cows with a milk EC of 5.0–5.5 mS/cm, and 0.23 kg/min ($P < 0.05$) lower than those with a milk EC lower than 5.0 mS/cm. Cows over 300 days of lactation with a milk EC greater than 5.5 mS/cm had an average milk flow rate and maximum milk flow rate of 0.75 kg/min and 1.29 kg/min lower, respectively, than cows with a milk EC of less than 5.0 mS/cm ($P > 0.05$).

In conclusion, we can state that increased duration of milking and decreased average milk flow can be used as additional indicators for evaluating the udder health of cows.

Keywords: milk, electrical conductivity, milk flow, milking duration.

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EFFECT OF SMALL RUMINANT LENTIVIRUS INFECTION ON ACUTE PHASE PROTEIN GENE EXPRESSIONS IN BLOOD LEUKOCYTES OF YOUNG CASTRATED BUCKS

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We hypothesise that natural small ruminant lentivirus (SRLV) infection of young castrated bucks causes changes in the expression of acute phase proteins (APPs). SRLV causes chronic disease in goats and sheep leading to large economic losses [2] while APPs are important markers of viral infection [1]. In the process of diagnosing goat diseases, there are such APPs as serum amyloid (SAA), haptoglobin (Hp), ceruloplasmin (Cp), fibrinogen (Fb), alpha-1-glycoprotein (AGP), and alpha-lactalbumin (LALBA) [2]. The aim of the study was to analyse the response of APP genes to SRLV infection. The study was conducted on 24 young bucks (n = 12 Polish White Improved, n = 12 Polish Fawn Improved). The animals were divided into two groups: control and naturally infected with SRLV but without clinical symptoms, 6 male kids of each breed in the group. The blood samples were collected five times at the age of day 1, 30, 60, 120, and 180 of their lives. The gene expression has been measured using the RT-qPCR method with cyclophilin A as a reference. To analyse the SRLV infection effect on APP gene expression, the one-way ANOVA method was used (SAS/STAT 9.4, ver. 14.3). The Pearson correlation with PROC CORR of SAS package was used to estimate the associations between studied gene expressions. *Cp* and *Fb* expressions were below the detection level. We did not find any effect of infection on the expressions of *Hp* and *AGP* in the blood leukocytes of young castrated bucks. However, we found a negative regulatory effect of SRLV infection on *SAA* and *LALBA* expressions. So far, no impact of SRLV infection on *LALBA* expression was shown in goat blood leukocytes and milk somatic cells. However, a higher level of *SAA* (mRNA and protein) was observed in the blood of infected animals. On the other hand, no differences in a mRNA level and a lower *SAA* concentration were found in milk somatic cells of SRLV-infected goats [2]. Decreased *SAA* and *LALBA* expressions may be early markers for detecting SRLV infections in goat kids.

Keywords: acute phase proteins, gene expression, bucks, blood leukocytes.

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EFFECT OF ENERGY-VITAMIN-MINERAL SUPPLEMENT WITH PROTECTED PALM FAT ON PRODUCTIVITY OF DAIRY COWS

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The feeding strategy and management of cows during the transition period have a key role in health, productivity, and profitability [1, 5]. A successful transition from late pregnancy is highly linked to delivering a healthy calf with a minimum occurrence of metabolic disorders and infectious diseases in early lactation [2]. Fatty acid (FA) supplements are commonly fed to lactating dairy cows with the goal of increasing energy intake, fertility, or milk and component yields. Feeding saturated FA supplements has little risk of disrupting the rumen function, is typically easy to handle on-farm, has less risk of reducing DMI (dry matter intake) compared with unsaturated FA [3], and increases milk and milk fat and protein yield [4]. This study aimed to analyse the influence of energy-vitamin-mineral supplements with protected palm fat on productivity, composition and quality of milking cows and health.

The trial was carried out with 40 Lithuanian black and white cows (2–5 lactation) were selected by analogy principle for the research. They were divided into two groups (control and experimental), and each group consisted of 20 cows. Cows from the control group were fed a common diet, structured from grass haylage, corn silage, wheat and barley flour, rape and soy and minerals. The cows in the experimental group were fed a similar diet, but an energy-vitamin-mineral supplement replaced their diet with protected palm fat.

The amount of milk was estimated at control milkings during the research period. In milk samples, amount of proteins, fat, lactose, the concentration of urea, and somatic cell count were analysed. Besides that, blood samples were taken and analysed during research, showing the concentrations of creatinine, glucose, common proteins, alkaline phosphatase alanine transaminase, calcium, magnesium, and phosphorus. During the experimental period, from cows with a ration of energy-vitamin-mineral supplement with protected palm fat, the basic milk is primed for a total of 9.02% more compared with the cows in the control group ($P > 0.05$). It was determined that after experiment, milk fat content increased 0.49% compared with the control group ($P > 0.05$).

Increased milk fat content in the experimental cows supplemented with energy-vitamin-mineral supplemented with protected palm fat. The volume of lactose and urea in the test group was not significantly different in the trial period compared with the control group. The concentration of urea in cows' milk remained within the physiological norm during the trial period ($P > 0.05$).

Keywords: vitamin, mineral, fat, dairy cows' productivity.

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