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An international scientific journal “**Veterinarija ir Zootechnika**” since 1924 publishes original research and review papers on all aspects of veterinary medicine, food safety and animal sciences. From 1952 to 1994 journal was published under the title: “Acta of Lithuanian Veterinary Academy”. After decision of the Research Council of Lithuania under the auspices of the Government of Republic of Lithuania from year 1995 scientific journal „Veterinarija ir Zootechnika” (Vet Med Zoot) was re-established as the Official Organ of the Veterinary Academy (VA) in collaboration with Veterinary Academy, Veterinary Institute, LVA Animal Science Institute, Lithuanian University of Agriculture and Immunology Institute of Vilnius University.

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Linseed Cake in Rabbit Nutrition: Evaluation of Animal Welfare, Livestock Performances and Meat Quality

Zuzana Formelová¹, Ľubica Chrastinová^{1†}, Mária Poláčiková¹, Matúš Rajský¹, Mária Chrenková¹, Zuzana Mlyneková¹, Anna Kalafová³, Ondřej Bučko³, Stanislav Baxa², Ľubomír Ondruška¹, Rastislav Jurčík¹, Aneta Kišová³, Francesco Vizzarri¹.

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Keywords: rabbit, meat quality, functional product, flaxseed, feed additive.

Abstract. A total of 66 post-weaned rabbits (35th day of age meat line M91 a P91 hybrid) were randomly divided into 3 groups for a fattening experiment and kept in standard metal cages with two animals per cage. Rabbits were fed a commercial diet (control group, CG) or a diet supplemented with 5% of agricultural by-products linseed cake (EG1), or a diet supplemented with 10% linseed cake (EG2). The experiment lasted 42 days, until the animals attained the slaughtering weight ≈ 2.5 kg. Individual blood was sampled from a total of 30 rabbits, and five animals from each group were slaughtered at the end of the experiment, for the evaluation of animal welfare and meat quality. Feeding linseed cake substances to rabbits did not negatively influence zootechnical parameters, growth performance and slaughter traits; it had no negative effect on the rabbit's blood biochemical profile either. Experimental diets with 5% linseed cake improved feed conversion ratios, without significantly impacting the final weight of animals. The dietary supplementation positively influenced the meat fatty acids profile, in particular polyunsaturated fatty acids (linoleic acid and CLA), indicating enhanced nutritional quality of the meat as a functional food. Overall, the use of linseed cake, a sustainable agricultural by-product, is a promising dietary strategy to improve the nutritional value of rabbit meat and support efficient, welfare-conscious rabbit production.

Introduction

Oilseeds and the residues of seed processing in the fat and oil industry have a high energy value and a high protein content, leading to different physiological effects and dietetic properties in relation to the types of compressed and extracted scraps. Two specific properties must be considered when feeding extracted linseed meal and cake, namely the laxative effect and the effect on fat consistency. For constipation in animals, the laxative effect of linseed cake or crushed steamed linseed can be successfully used (Abdel-Magid et al., 2009). Linseed meal does not soften animal fats. Considering the mentioned properties, extracted flax meal and cakes can be used in feed mixtures for all species and categories of animals. Linseed and linseed-derived products have been given to rabbits to enhance their fertility, prolificacy, survival and meat quality (Agradi et al., 2023). Recently, Savietto et al. (2025) have evaluated the nutritional composition of linseed-based ingredients in rabbit nutrition, concluding that novel ingredients may improve the feed quality of growing rabbits when included up to 10% in feed formula. Earlier research (Halle and Schöne, 2013; Abdel-Magid et al., 2009) reported an evaluation of nutritional aspects of linseed dry meal cake containing an average of 89.7% dry matter (DM)

of the total, including crude proteins (32.2%), crude lipid (13.0%), crude ash (5.2%), neutral detergent fibre (19.4%), acid detergent fibre (13.0%), and lignin (5.0%). Linseed cake (*Linum usitatissimum*) contains a high proportion of polyunsaturated fatty acids (PUFA) and other bioactive components. One of the richest plant sources of omega-3 polyunsaturated fatty acids is linseed, containing 9% saturated fatty acids, 18% monounsaturated fatty acids and up to 73% of PUFA. Among the total amount of PUFA, 43–56% are alpha-linolenic acid (ALA) (Ribeiro et al., 2013). Linseed is a rich source of both soluble and insoluble fibre and has the highest content of plant lignans, which act as phytoestrogens and antioxidants, among plants used in human and animal nutrition (Patade et al., 2008). The fibre content of flaxseed is significantly influenced by the variety; the highest total fibre levels (60–70%) are found in Agriol, Astella, CDC Bethune, Libra, and Lola, whereas Agram, Flanders, and Raciol contain moderate amounts (50–60%), and Natural has the lowest content (below 50%). Soluble fibre, primarily in the form of mucilage, is predominant, ranging from 40.5% in Natural to 63.0% in Lola, while insoluble fibre remains minimal, from 1.6% in Natural to 8.3% in Astella (Kučka, 2024). Linseed is a source of vitamins A, B, C, D, E and minerals, such as potassium, phosphorus, magnesium, calcium, sulphur, iron, zinc, and sodium.

Flaxseed continues to be the richest known dietary

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source of lignans, particularly secoisolariciresinol diglucoside (SDG). A comparative assessment of 40 flaxseed varieties revealed a significant variability in SDG content (Wu *et al.*, 2023).

In literature, several studies (Abdel-Magid *et al.*, 2009) reported that the dietary application of linseed meal in rabbit nutrition (replacing 50% of crude protein from soybean meal with linseed meal) serves as a good alternative source of protein, improving all digestion coefficients and nutritive values and, thus, resulting in increased total body weight gain and average daily gain of growing animals. Aggoor *et al.* (2007) recommended the dietary use of linseed meal up to 50% of soybean meal protein replacement in rabbit, without adverse effects on reproductive performance and biochemical constituting blood plasma, also underlining advantages in the nutritional and economical aspects. In similar research experience (Halle and Schöne, 2013) on poultry, the dietary inclusion up to 10% of linseed cake did not negatively influence the laying performance of hens, and it was able to enrich yolk fat with polyunsaturated fatty acids.

The aim of the study was to evaluate the effects of dietary use of linseed cake in growing rabbits on growth performance, animal welfare, and selected chemico-physical, nutritional and quality traits of meat.

Materials and methods

Experimental design

A total of 66 post-weaned rabbits (aged 35 days; meat line M91 a P91; both sexes) were randomly divided into 3 groups (22 animals in each group, divided in control CG, experimental group EG1, and experimental group EG2, respectively) kept in standard metal cages, two animals per cage. The

rabbits were fed a commercial diet (KV; TEKRO Nitra, Ltd. Slovak Republic) pellet of 3.5 mm in diameter ad libitum. Water was also provided ad libitum using nipple drinkers. Experimental group EG1 was fed an enriched commercial diet with 5% of linseed cake, while experimental group EG2 was fed the same control diet with 10% of linseed cake. The diet formulation (complete granulated mixture) for all groups is presented in Table 1.

Experimental diets and animals

No anticoccidials, antibiotics or other medications were included in the diet. The feed additive was included in the mashed diets, and then the diets were pelleted. The chemical compositions of diets and feed additive were analysed in accordance with the methods of the Association of Analytical Chemists (AOAC, 2000), and data are reported in Table 2. A cycle of 16 hours of light and 8 hours of dark was used throughout the experiment. The temperature and humidity in the building were recorded continuously by a digital thermograph positioned at the same level as the cages. The heating and forced ventilation systems allowed the building air temperature to be maintained within $14 \pm 1^\circ\text{C}$ throughout the experiment. Relative humidity was about $60 \pm 5\%$. The study was carried in the National Agricultural and Food Centre, Research Institute for Animal Production Nitra. The body weight of each experimental animal was recorded weekly during the whole study, and the data are reported in Table 3 with the following intervals: initial live weight (day 0), intermediate live weight (day 21), and final live weight (day 42). Feed intake was checked daily, and average daily weight gain and feed conversion ratios as well as mortality were calculated mathematically at the end of the experiment. For this study, institutional and national

Table 1. Ingredients of the control and experimental diets⁺

Feed ingredients in g/kg	Control group	EG1 (5% LC)	EG2 (10% LC)
Alfalfa meal	360	342	342
Sunflower meal	55	52	52
Rape-seed meal	55	52	52
Linseed cake	0	50	100
Wheat bran	90	85	81
Oats	130	124	117
Dry malting sprouts	150	143	134
Maize	50	47	43
Sodium chloride	3	3	3
Minerals and vitamins mixture*	17	17	15
Barley	80	75	52
Limestone	10	10	9

⁺ LC – linseed cake, experimental group EG1, experimental group EG2; *Provided per kg diet: Vit. A 6000 IU; Vit. D₃ 1000 IU; Vit. E 50 mg; Vit. B₁ 1.7 mg; Vit. B₂ 8.0 mg; Vit. B₆ 3.0 mg; Vit. B₁₂ 0.01 mg; Vit. K₃ 0.5 mg; biotin 0.2 mg; folic acid 0.5 mg; nicotinic acid 45 mg; Se 0.2 mg; choline chloride 450 mg.

guidelines for the care and use of animals were followed and all experimental procedures involving animals were approved by the State Veterinary and Food Institute of Slovak Republic (Permission code: SK CH 17 021).

The experiment lasted for 42 days. Five animals from each group were slaughtered at the end of experiment day by cutting the jugular vein and the carotid artery after electroanaesthesia (90 V for 5 sec), according to guidelines established by the European Community (n. 86/609/EEC). Carcasses were prepared according to Blasco and Ouhayoun (1996).

Haemato-biochemical analysis

Individual blood was sampled from ten rabbits per each group on day 42 of experiment (n = 10 per group) from the marginal ear vein (*Vena auricularis*)

into dry non-heparinized Eppendorf tubes for biochemical analyses. Blood serum was produced by centrifugation at $3000 \times g$ for 10 min and stored frozen (at -18°C) in plastic vials until analysis. To test the biochemical parameters (total proteins (TP; g/L), albumins (g/L), urea (mmol/L), glucose (mmol/L), triglycerides (mmol/L), and total cholesterol (mmol/L)), commercial kit Dialab (Czech Republic) and an automated biochemical analyser ELLIPSE (AMS, Italy), based on turbidimetric clinical assay, were used according to the manufacturers' instructions and following the methods of serum parameter determination. The activities of blood gamma glutamyl transferase (GGT; U/L), aspartate aminotransferase (AST; $\mu\text{kat/L}$), and alanine aminotransferase (ALT; $\mu\text{kat/L}$) were determined by the colorimetric method (Spectrophotometer UV-

Table 2. Chemical composition of experimental diets⁺ (expressed on DM) and of linseed cake

Chemical analysis [†] in g/kg	Control group	EG1 (5% LC)	EG2 (10% LC)	Linseed cake
Crude proteins	144.80	147.47	147.04	315.42
Crude fibre	138.45	135.97	129.36	118.35
Fat	22.30	37.46	39.54	225.29
Ash	93.48	73.72	74.36	53.56
Starch	204.57	189.22	183.11	nd
Organic matter	796.62	789.49	763.91	946.44
ADF	168.94	234.37	154.16	161.64
NDF	300.14	342.18	273.34	243.61
Calcium (Ca)	11.74	12.99	11.69	3.48
Phosphorus (P)	5.89	6.53	5.89	6.02
Magnesium (Mg)	3.34	2.84	2.93	4.44
Sodium (Na)	1.09	1.06	1.03	0.74
Potassium (K)	11.10	8.68	9.33	11.10
ME MJ.kg ⁻¹	10.74	11.17	11.06	16.47

⁺ LC – linseed cake, experimental group EG1, experimental group EG2; [†]ADF – acid detergent fibre; NDF – neutral detergent fibre; ME – metabolizable energy; nd – not detected. Analyses were performed in triplicate.

Table 3. Effect of linseed cake on growing performance of rabbits⁺ (n = 22)

Parameter [#]	Control group	EG1 (5% LC)	EG2 (10% LC)	SEM	P value [*]
Initial live weight (0 d) g	1175	1178	1213	41.96	NS
Intermediate live weight (21 d) g	2040	2024	1976	14.80	NS
Final live weight (42 d) g	2654	2761	2751	26.27	NS
Feed Intake 0–42d (g/day)	153	149	150	2.96	NS
AFC 0–42 d (g/g)	4.35 ^b	3.95 ^a	4.37 ^b	0.06	*
Mortality (n)	2	1	0	-	NS
Daily weight gain, (g/d)	35.21	37.69	36.61	0.73	NS
Carcass yield at slaughter (%)	53.17	53.08	54.28	0.30	NS

⁺ LC – linseed cake, experimental group EG1, experimental group EG2; [#] AFC – average feed conversion ratio;

^{*} Different letters in the same row mean statistical difference for $P < 0.05$, NS – not significant.

2550 Shimadzu, Kyoto, Japan) using a commercial kit Randox RS 504 (Randox Laboratories Ltd. Crumlin, UK).

Meat chemical composition and nutritional traits

The samples from *musculus longissimus dorsi* (MLD; 30 g) were analysed for proximate composition, specifically, moisture, protein, intramuscular fat, amino acids and fatty acids using spectrometer Nicolet 6700; the data are expressed in g/100 g. The amino acid composition was analysed by ion-exchange chromatography on AAA (Ingos Prague, Czech Republic) after acid hydrolysis with 6M HCl, while methionine and cystine were determined after oxidation hydrolysis.

The samples of MLD were collected immediately after slaughter and stored at 5°C for 24 hours and then chemical-physical analyses were performed (AOAC, 2000). The samples of MLD were homogenised and analysed for individual nutrients. The pH at 24 hours postmortem was measured by a portable pH-meter (model OP-109, Radelkis) with a combined electrode penetrating 3 mm into the muscle. Colour measurements were taken on MLD surface of the carcass at 24 hours after slaughter and were expressed using the CIE L*a*b system (lightness-L*, 0: black and 100: white; redness and greenness-a*; yellowness and blueness-b*) using a Lab Miniscan (HunterLab, Reston, VA, USA).

The energy value was calculated according to the equation (Strmiska *et al.*, 1988):

$$\text{Energy value (kJ/100 g)} = 16.75 \times \text{protein content} + 37.65 \times \text{fat content}.$$

The water holding capacity was determined by the compression method at constant pressure (Hašek and Palanská, 1976). The analysed samples (0.3 g in weight) were placed on filter papers (Schleicher and Schuell No. 2040B, Dassel, Germany) with tweezers previously weighed. Together with the papers, the samples were wedged between Plexiglass plates and then subjected to a pressure of 5 kg for 5 min. The results were calculated based on the difference in weight between the slips containing the aspirating spot and the pure filter paper.

Statistical analysis

The statistical analysis was performed using the SPSS statistical package (version 18.0, 2009, SPSS Inc., USA). Data on productive performance, slaughter parameters, meat chemical composition, meat values of pH and colour, and fatty acid profile of meat were processed with one-way analysis of variance (ANOVA), with the dietary treatment (CON, EG1, EG2) as the source of variation and with the post-hoc Tukey multiple comparison test. All measurements were made in duplicate and the results in tables are reported as means and pooled SEM. The experimental unit for the livestock performance was the cage, while

for the blood parameter and meat quality traits, the experimental unit was the single animal. Mean values within the same row having different superscripts indicate significant difference for ($P \leq 0.05$).

Results and discussion

The experiment was performed at the National Agricultural and Food Centre (NPPC) – Research Institute for Animal Production Nitra. All animals were in good health throughout the trial.

The experimental diets, prepared with or without the linseed cake, were balanced for organic matter, mineral profile, and energy values. In similar previous experience, increasing flaxseed levels (2.5% to 10%) led to obtaining experimental rations balanced for crude protein and digestible energy (Omer *et al.*, 2013).

Results regarding the growth parameters are shown in Table 3. During the fattening period, differences observed at the end of trial induced by the diet on final live weight and feed consumption were not significant. However, differences among treatments ($P < 0.05$) were detected in the average feed conversion ratio, where the lowest value was reported in the EG1 group and the highest in the EG2 group. The overall mortality at the end of the growing phase was 2 (CG) vs 1 rabbit (EG1) and was not related to the type of diet. The average daily weight gain was higher in the experimental groups compared with the control group (35.21 g CG vs 37.69 g EG1 vs 36.61 g EG2), but those differences did not reach the statistical significance. Contrary to our results, Rizwan Tariq *et al.* (2015) showed that enrichment with 7% flaxseed exerted a highly significant effect on weight gain of rabbits, when compared with the control group. The authors also reported the feed conversion ratio (FCR) of the low flaxseed dose (3.5%) as positively lower compared with the control group, increasing the performance of experimental rabbit's growth. Similarly, Abdel-Magid *et al.* (2009) reported that the replacement of conventional ingredients with linseed meal resulted in a significantly increased values in total body weight gain and average daily weight gain of growing rabbits.

Despite variations in the literature, the recorded biochemical blood values fall within the accepted physiological range for rabbit health and welfare (Table 4). The addition of linseed cake to feed mixtures reduced the value of selected indicators of lipid metabolism (triglycerides and cholesterol) in both experimental rabbit groups when compared with the control group, but those differences did not reach the statistical significance. Similar results were reported by Aggoor *et al.* (2007) with no effect on biochemical constituting blood plasma, after the dietary use of linseed meal up to 50% of soybean meal protein replacement in rabbit. Controversially, Ibrahim *et al.* (2021) found that feeding rabbits diets containing 4%, 6%, and 8% flaxseed significantly ($P < 0.05$) decreased serum triglycerides and total

cholesterol levels compared with a control group. The reductions were attributed to the high soluble mucilage fibre and lignin content of flaxseed, which may attenuate cholesterol levels.

In Table 5, the chemical and physical parameters of rabbit MLD muscle samples are reported. No statistically significant differences were found in basic chemical indicators (moisture, proteins, cholesterol, ash, and energetic value), except for fat content. In both experimental groups (EG1 and EG2), the value of fat was statistically lower ($P < 0.05$) compared with the control group (1.39 EG1 and 1.59 EG2 g/100 g vs 1.73 g/100 g CON).

Similarly, Dalle Zotte (2014) reported MLD moisture content in rabbits was 74.6 g/100 g, protein content was 22.4 g/100 g, and fat content was around 1.8 g/100 g. The intramuscular fat content of MLD was higher in the Californian rabbit breed (1.10 g/100 g) compared with the French Lop breed

(0.92 g/100 g). Differences in the intramuscular fat content were likely affected by the breed and the size of the body weight. Consistent with our findings, Martino et al. (2016) reported a water content of 75.3 g/100 g, a protein content of 22.9 g/100 g but a lower intramuscular fat content of 0.70 g/100 g. The mineral content of MLD in the French Lop breed was 0.99 g/100 g, and in the Californian rabbit breed, it was 1.07 g/100 g. Combes (2004) reported a higher mineral content in the rabbit MLD (1.2 g/100 g).

Physical parameters (pH, colour, water holding capacity) were not affected by the dietary treatment, except for the meat redness (a). In both experimental groups (EG1 and EG2), lower values ($P < 0.05$) were observed in comparison with the control group. The colour of meat is the main attractive factor for the consumer at the time of purchase. Similar to our results, Pogany Simonova et al. (2010) detected lower colour measurement of redness (a*), mainly

Table 4. Biochemical analyses on rabbit⁺ blood serum (n = 10)

Parameter [#]	Control group	EG1 (5% LC)	EG2 (10% LC)	SEM	P value [*]
Total protein (g/L)	62.19	60.20	58.95	1.06	NS
Glucose (mmol/L)	6.67	7.68	6.97	0.24	NS
Triglycerides (mmol/L)	1.55	1.10	0.92	0.25	NS
Urea (mmol/L)	4.33	4.49	3.92	0.34	NS
Cholesterol (mmol/L)	2.28	1.29	1.67	0.22	NS
GGT (μkat/L)	0.24	0.22	0.22	0.01	NS
AST (μkat/L)	0.86	0.84	0.96	0.09	NS
ALT (μkat/L)	0.64	0.69	0.53	0.04	NS

⁺ LC – linseed cake, experimental group EG1, experimental group EG2; [#] GGT – gamma glutamyl transferase; AST – aspartate aminotransferase; ALT – alanine aminotransferase; ^{*} NS – not significant.

Table 5. Chemical and physical parameters of rabbit⁺ MLD muscles

Parameters	Control group	EG1 (5% LC)	EG2 (10% LC)	SEM	P value [*]
Content of water (g/100 g)	74.6	73.61	74.08	0.23	NS
Total proteins (g/100 g)	22.69	22.81	22.54	0.10	NS
Content of fat (g/100 g)	1.728a	1.586	1.394	0.20	*
Cholesterol (g/100 g)	0.38	0.39	0.30	0.01	NS
Ash (g/100 g)	0.64	0.66	0.73	0.04	NS
Energetic value (kJ/100 g)	445.12	441.75	430.10	7.56	NS
pH ₂₄	6.06	5.96	6.09	0.03	NS
Colour ₂₄ L	51.28	52.44	52.28	1.19	NS
Redness ₂₄ (500–700 nm) a	2.02a	1.23	1.08	0.22	*
Yellowness ₂₄ (445–578 nm) b	8.99	8.90	7.46	0.38	NS
Water holding capacity (g/100 g)	25.34	24.88	24.37	1.20	NS

⁺ LC – linseed cake; experimental group EG1; experimental EG2; MLD – *musculus longissimus dorsi*; ^{*} Different letters in the same row mean statistical difference for $P < 0.05$, NS – not significant.

in the meat from the rabbits supplemented with a commercial feed additive mixture (XTRACT). Noia *et al.* (2020) reported that the red colour values were lower in rabbits treated with chitosan; in addition, the authors observed an increase in the yellowing and the luminosity of the meat in comparison with the control group. In contrast, Meng *et al.* (2010) reported that meat colour scores and redness values increased when pigs received a probiotic complex of *Bacillus subtilis* endospore and *Clostridium butyricum* endospore. In line with other here mentioned authors, Pelicano *et al.* (2003) also observed that the redness values in the meat of broilers increased in the groups treated with probiotics compared with the control group.

The effect of diet on a fatty acid profile in intramuscular fat is reported in Table 6. Grouped data indicate 35.3%, 36.8%, and 34.5% for saturated fatty acids (SFA), then 49.6%, 49.2%, and 50.3% for monounsaturated acids (MUFA), and 10.7%, 11.4%, and 11.1% for polyunsaturated acids (PUFA) in the control group, EG1 and EG2 groups, respectively. At the end of the dietary treatment, experimental rabbit meat (EG1 and EG2) presented higher values ($P < 0.05$) of linoleic acid and conjugated linoleic acid (CLA) in comparison with the control group. The increment of CLA in products of animal origin is considered a benefit, since bioactive compounds that play a significant role in promoting human health

Table 6. Fatty acid profile (g/100 g FAME) in the rabbit[#] MLD meat

Parameter ⁺	Control group	EG1 (5% LC)	EG2 (10% LC)	SEM	<i>P</i> value [*]
C12:0 (Lauric acid)	0.117	0.123	0.120	0.002	NS
C14:0 (Myristic acid)	1.354	1.349	1.375	0.012	NS
C16:0 (Palmitic acid)	24.296	24.390	24.480	0.094	NS
C17:0 (Heptadecanoic acid)	0.308	0.332	0.327	0.016	NS
C18:0 (Stearic acid)	10.790	10.760	10.680	0.118	NS
Σ SFA fatty acids	35.298	36.840	34.474	0.623	NS
C18:1n-9c (Oleic acid)	37.596	36.750	35.360	1.965	NS
C18:1 11c/15t (Vaccenic acid)	4.848	4.754	4.878	0.055	NS
C20:1 (Eicosanoic acid)	0.601	0.484	0.550	0.033	NS
Σ MUFA fatty acids	49.602	49.154	50.282	0.429	NS
C18:2n-6 (Linoleic acid)	4.432 ^b	6.254 ^a	5.182 ^b	0.341	*
C18:2 9c/11t (Conjugated linoleic acid CLA)	0.116 ^b	0.142 ^a	0.133 ^{ab}	0.007	*
C18:3n-3 (α-Linolenic acid)	0.204	0.154	0.167	0.009	NS
Σ essential fatty acids	8.644	8.484	8.600	0.433	NS
C20:4n-6 (Arachidonic acid)	1.674	1.733	1.804	0.151	NS
C20:5n-3 (Eicosapentaenoic acid)	0.101	0.114	0.107	0.007	NS
C22:5n-3 (Docosapentaenoic acid)	0.130	0.145	0.139	0.009	NS
C22:6n-3 (Docosahexaenoic acid)	0.033	0.037	0.035	0.002	NS
Σ PUFA fatty acid	10.726	11.388	11.116	0.411	NS
PUFA/ SFA	0.304	0.309	0.322	0.004	NS
MUFA/SFA	1.401	1.334	1.459	0.028	NS
Omega 6 fatty acid	8.488	9.300	8.676	0.191	NS
Omega 3 fatty acid	0.466	0.452	0.432	0.067	NS
Saturated index	1.954	1.874	2.030	0.035	NS
Atherogenic index	0.555	0.494	0.489	0.016	NS

^{*}Different letters in the same row mean statistical difference for $P < 0.05$, NS – not significant; ⁺SFA: saturated fatty acids C_{8:0}, C_{10:0}, C_{12:0}, C_{14:0}, C_{16:0}, C_{18:0}, C_{20:0}, C_{22:0}, C_{24:0}; MUFA: monounsaturated fatty acids C_{16:1 n-7}, C_{18:1 n-9c}, C_{22:1}; PUFA: polyunsaturated fatty acids C_{18:2 n-6}, C_{18:3 n-3}, C_{20:4 n-6}; AI: Atherogenic index = (C12 + 4C14 + C16) / (PUFA + C18:1 + other MUFA) (Ulbricht and Southgate, 1991); [#] experimental group EG1; experimental EG2; MLD – *musculus longissimus dorsi*; ⁺ LC – linseed cake; FAME – fatty acid methyl esters.

Table 7. Content of essential amino acid in rabbits[#] MLD (g/100 g)

Parameter ⁺	Control group	EG1 (5% LC)	EG2 (10% LC)	SEM	P value [*]
Arginine	1.141	1.237	1.104	0.03	NS
Cystine	0.267	0.253	0.244	0.01	NS
Phenylalanine	0.735	0.798	0.701	0.02	NS
Histidine	0.783	0.908	0.727	0.02	NS
Isoleucine	0.698	0.690	0.660	0.03	NS
Leucine	1.426	1.402	1.346	0.04	NS
Lysine	1.524	1.507	1.450	0.05	NS
Methionine	0.575	0.595	0.553	0.02	NS
Threonine	0.834	0.788	0.758	0.02	NS
Valine	0.815	0.794	0.776	0.02	NS
ΣEAA	8.798	8.696	8.319	0.25	NS

[#] Experimental group EG1; experimental EG2; MLD – *musculus longissimus dorsi*; ⁺ LC – linseed cake; ^{*}NS – not significant; ⁺ Σ EAA – total essential amino acids.

are the monounsaturated and polyunsaturated fatty acids. Rabbits, as monogastric animals, are unable to synthesise CLA, but thanks to the phenomenon of caecotrophy, they can deposit this acid in their muscles (Corino et al., 2007). Several biological properties are ascribed to CLA, such as antioxidant, antiatherosclerotic and antidiabetogenic properties (Sudiak and Kowalska, 2023). The average PUFA/SFA ratio ranged from 0.304 to 0.322 between the groups. The omega 6 fatty acid content was from 8.49 g/100 g FAME (CG) to 9.30 g/100 g FAME (EG1), and the omega 3 fatty acid content was between 0.43 g/100 g FAME (EG2) and 0.46 g/100 g FAME (CG).

The amino acid composition in MLD muscles is shown in Table 7. The essential amino acid composition is one of the most important nutritional qualities of proteins. Some of these amino acids can be synthesized by living organisms from other nitrogenous material. Other amino acids essential to the diet cannot be synthesized in vivo but must be ingested as such (Henchion et al., 2017). Hernández and Dalle Zotte (2010) reported higher contents of lysine (2.1 g/100 g), leucine (1.7 g/100 g), valine (1.1 g/100 g) and phenylalanine (1.04 g/100 g) in rabbit MLD compared with our results. Wognin et al. (2018) found lower content of phenylalanine (0.77 g/100 g) and a higher value of threonine (0.89 g/100 g) compared with our results.

Conclusion

Feeding linseed cake substances in rabbits did not negatively affect zootechnical parameters, growth performance and slaughter traits; it had no negative effect on a rabbit's blood biochemical profile either. Experimental diets with 5% linseed cake improved feed

conversion ratios, without significantly affecting the final weight of animals. The dietary supplementation positively influenced the meat fatty acids profile, in particular polyunsaturated fatty acids (linoleic acid and CLA), indicating enhanced nutritional quality of the meat as a functional food.

Overall, the use of linseed cake, a sustainable agricultural by-product, is a promising dietary strategy to improve the nutritional value of rabbit meat and support efficient, welfare-conscious rabbit production.

Ethical statement

The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes. All care and experimental procedures involving animals followed the guidelines stated in the Guide for the Care and Use of Laboratory Animals approved by the State Slovak Veterinary and Food Administration and the Ethics Committees of both institutions (Permission code: SK CH 17 021).

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Economic Value of In-Line Milk Analysers for Early Diagnosis and Prevention of Negative Energy Balance in Dairy Cattle

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Keywords: dairy cattle, negative energy balance, milk analyser, prevention economic effect.

Abstract. This study aimed to evaluate the economic benefits of using the in-line milk analyser “Brolis HerdLine” (Brolis Sensor Technology, Vilnius, Lithuania) for the early detection of negative energy balance (NEB) and to assess the value of preventive treatment in dairy cows at a high risk of NEB. A total of 52 Holstein cows were selected and paired based on lactation number, days in milk, and fat-to-protein ratio. The pairs were randomly allocated into two treatment groups: Control group (CON, n = 26) and Test group (TE, n = 26). Cows in the TE group received a single 32.4 g monensin (Kexxtone[®]) controlled-release capsule, while CON cows received no treatment. Milk composition was monitored using the in-line analyser, and energy-corrected milk (ECM) was used to evaluate performance. Over a 200-day period, the TE group produced 9916 kg more ECM than the CON group, resulting in an additional €3056 profit after subtracting treatment costs. On day 20 post-treatment, TE cows showed significantly higher lactose levels (4.64%) than CON cows (P = 0.03). The successful insemination rate was 8.11% higher in the CON group. These results imply that early intervention through the use of an in-line milk analyser to identify cows at a high risk of NEB improves economic performance. The study emphasises how milk analysers can be used to make decisions in real time when managing dairy herds.

Introduction

For dairy cows, innovative technologies not only improve animal welfare but also boost milk productivity and quality, supporting more economically efficient production (Zarba, 2023). The use of sensors and technology enables the collection of vast amounts of data, which must be analysed using advanced statistical methods to draw meaningful conclusions about animal behaviour, health, and welfare (Džermeikaitė, 2023).

Precision livestock farming using on-farm in-line milk analysers is gaining attention as a method for automating the monitoring of animals. Key milk components, such as fat, protein, and lactose, serve as critical health indicators within animal monitoring systems (Uusitalo, 2021). Using spectroscopic technology, an in-line milk analyser provides detailed insights into milk quality, including parameters such as fat, protein, lactose and temperature, which can indicate issues such as NEB (Antanaitis, 2023).

Energy-corrected milk (ECM) is an essential metric in dairy production, as it adjusts milk yield based on its energy content, offering a more precise evaluation of production relative to energy intake (Cabezas-Garcia, 2021). Studies have identified

various factors that influence ECM in dairy cattle, particularly feeding strategies and overall cow performance. Moreover, a statistical analysis of data from Holstein-Friesian cows revealed a negative correlation between the fat-to-protein ratio (FPR) in milk and energy balance, especially during early lactation. This finding highlights the importance of monitoring ECM as an indicator of cows' nutritional status and energy balance (Gohary, 2016).

The connection between ketosis and NEB in lactating dairy cows is crucial and complex. During the transition period (three weeks before to three weeks after calving), dairy cows often experience NEB, which can lead to the development of both subclinical and clinical ketosis as the energy demands for milk production exceed dietary intake. This situation causes body fat to be mobilised, leading to increased ketone production and the risk of metabolic disorders like ketosis and fatty liver (Zhang, 2020). Ketosis is considered one of the most common metabolic disorders, which reduces milk yield, impairs reproductive performance and has significant economic losses due to its detrimental effects on milk production, reproductive performance, and overall herd health (Uusitalo, 2021). In Canadian dairy herds, the cost of a single subclinical case is estimated at approximately \$203, factoring in increased clinical disease rates, prolonged time to pregnancy, culling,

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early lactation deaths, and milk production losses (Steenefeld, 2020). Another study has shown that in the Netherlands, the combined annual herd-level costs of ketosis (both clinical and subclinical) are at €3613 for an average farm, rising to €7371 for high-risk farms. These expenses stem from milk production losses, treatment costs, and managing associated conditions such as mastitis and metritis. A dynamic stochastic simulation model further highlighted the economic burden, estimating the total cost of subclinical ketosis at €130 per case per year, with variation depending on factors like parity and disease incidence (Ha, 2023). Factors such as calving season, body condition score at parturition, and calf birth weight significantly influence ketosis severity, aiding in risk prediction and the implementation of preventive strategies (Dubuc, 2010).

Monensin (MON) is widely regarded as one of the most effective agents for preventing ketosis and negative energy balance. Moreover, the monensin capsule is frequently chosen due to its convenient mode of administration. A systematic review by Mammi *et al.* (2021) revealed that administering monensin as a controlled-release capsule during the transition period significantly improved cow metabolism and health. The treatment enhanced propionate production in the rumen, which supports energy metabolism, and lowered blood BHBA and non-esterified fatty acid levels, indicating a reduced risk of ketosis – a condition commonly associated with NEB in dairy cows (Antanaitis, 2023). Furthermore, the review found that monensin did not adversely affect milk production or composition, demonstrating its potential to maintain energy balance without compromising productivity (Huralska, 2024). Robinson (2020) suggests that monensin supplementation may reduce milk fat yield without significantly affecting milk protein yield.

Although monensin is a widely used antibiotic, it has been associated with negative effects. Monensin can alter the gut microbiome and promote the selection of resistant microbes. Besides, its extensive usage in livestock has sparked worries about the emergence of antibiotic resistance (Lee, 2015). One of the main causes of the worldwide issue of antibiotic resistance is the excessive and improper use of antibiotics in animal production, including cattle farms. Due to their frequent exposure to high doses of antibiotics, cattle may develop and spread germs that are resistant to these drugs (Kasap, 2020). A multifaceted strategy is required to address the issue of antibiotic resistance in cattle farms, which includes limiting the overuse of antibiotics in livestock production and using them sparingly, as well as enacting laws and policies that limit their use as feed additives (Jacobs, 2012).

Based on previous studies showing the link between milk composition, particularly the fat-to-protein ratio and NEB, the use of in-line milk analysers offers a promising method for early detection of metabolic

imbalances. These technologies can continuously and non-invasively monitor milk constituents, allowing for earlier identification of cows at risk. Our hypothesis was that by using milk composition data to identify cows at risk of NEB early on, timely intervention would be possible, leading to better economic results. The aim of this study was to evaluate the economic value of an in-line milk analyser for the early detection of NEB in dairy cows, by comparing productivity and economic outcomes between animals monitored and treated early versus those monitored only. After NEB was detected, monensin was used as a standard intervention; this study did not examine its effects on its own.

Materials and methods

Housing conditions of study animals

This investigation adhered to the Lithuanian Law on Animal Welfare and Protection, with study approval number PK012858. The study was conducted in Lithuania (55.819156, 23.773541) over the period from 14th February to 1st July 2023. The dairy cows were housed in free-stall barns equipped with ventilation systems and were fed a total mixed ration (TMR) tailored to meet their physiological requirements year-round. Feeding occurred daily at 06:00 and 18:00, with a typical TMR formulation for high-producing, multiparous cows. The diet included 25% corn silage, 5% alfalfa grass hay, 20% grass silage, 15% sugar beet pulp silage, 30% grain concentrate mash, and 5% mineral mixture. This ration was designed to support or exceed the nutritional demands of a 500-kg Holstein cow producing 37 kg of milk per day. The cows were milked twice daily, at 05:00 and 17:00, using a parlour system. The chemical composition of the TMR was as follows (Table 1).

Table 1. Chemical composition of TMR

TMR Component	Value
Dry matter (DM)	48.8%
Neutral detergent fibre (NDF)	28.2% of DM
Acid detergent fibre (ADF)	19.8% of DM
Non-fibre carbohydrates (NFC)	38.7% of DM
Crude protein (CP)	15.8% of DM
Net lactation energy	1.6 Mcal/kg

Experimental design

This experiment utilises A/B testing. This method of testing works by splitting sample data into the control (A) and test (B) groups. Cows were selected in pairs so that both animals were in the same lactation, with similar days in milk (DIM) with fat-to-protein ratio values above 1.5 indicating high NEB, and without a noticeable yield drop before or at the time of selection. In total, 52 cows were included because their fat-to-protein ratio was greater than 1.5, which

indicates a high risk of NEB. Cows were chosen before there was a discernible decline in milk production, and there were no clinical symptoms of illness at the time of selection. The average DIM in the control and test groups was 45 days (± 5 days), and the average lactation number for both groups was 1.57 (32 cows were primiparous and 20 cows were multiparous). For a given pair, one animal was put in the test group and the other in the control group. In total, 52 animals were selected with 26 animals in each group. On day 1 of the study, cows in the test group received a single 32.4 g monensin controlled-release capsule (Kexxtone[®], Elanco GmbH, Cuxhaven, Germany) by using a rumen bolus applicator as treatment while those in the control group were only observed. Monensin was used as a standardised preventive treatment once high-risk cows were identified based on milk composition (specifically elevated fat-to-protein ratio), to model a practical on-farm management response following early NEB detection. The study's objective was to replicate a normal response after analyser-based NEB identification, rather than to assess the precise effects of monensin.

After given time, the two groups were compared to assess the economic benefit of BROLIS sensors. It is worth mentioning that not all animals were selected at the same time; therefore, we measure time not by date but by

days since treatment (DST), which was on average 200 days (± 5 days). In the case of the control group DST refers to the days since the beginning of observation. The various procedures of this group comparison are discussed below.

Measurements

The milk fat and protein measurements were performed with "Brolis HerdLine" in-line milk analysers (Brolis Sensor Technology, Vilnius, Lithuania) installed on each milking point in the farm. The devices are factory calibrated, continuously monitored, and do not require any additional reagents. This creates an environment where animals are not stressed by additional interventions and no milk is lost due to continuous measurements of milk composition. In addition, this method allows for accurate comparison of energy corrected milk among animals.

Each cow had its daily NEB recorded using the "Brolis HerdLine" in-line milk analyser (Brolis Sensor Technology, Vilnius, Lithuania). This advanced system incorporates a GaSb widely tunable external cavity laser-based in-line spectrometer operating within the 2100–2400 nm spectral range. Milk flow was continuously monitored in transmission mode throughout the milking cycle. The molecular absorption spectra obtained were processed to determine the levels of the main milk constituents. The analyser provided continuous milk composition measurements for each cow during every milking

session. This compact mini spectroscope was installed on milking stalls or milking robots directly within the milk line and operated without requiring additional reagents or maintenance. Among the measured parameters, the fat-to-protein ratio (FPR) was specifically monitored, providing important insights into the cows' metabolic status, particularly for early detection of NEB.

Methodology

ECM

To assess individual animal's performance, energy corrected milk (ECM) was used. The formula for ECM is as follows:

$$ECM_{kg} = (1 + (fat_a - fat_b) \times k_1 + (protein_a - protein_b) \times k_2) \times milk\ yield_{kg}$$

Where

- fat_a : "fat animal" (%). This term describes the average fat concentration each day.
- $protein_a$: "protein animal" (%). This term describes the average protein concentration each day.
- fat_b : "fat base" = 3.4%.
- $protein_a$: "protein base" = 3%.
- k_1 : fat multiplier = 0.178.
- k_2 : protein multiplier = 0.267.
- $milk\ yield_{kg}$: total daily milk yield (kg).

The base values and multipliers for both fat and protein are set by the Lithuanian authority UAB "Pieno Tyrimai".

Comparison among animals

To account for differences in lactations and days in milk among animals, the comparison was calculated based on the distance from the corresponding average ECM curves. First, average farm lactation curves were calculated (Fig. 1). Then the average fat and protein values for each DIM were determined (Fig. 2). By using the formula from **part 2.4.1**, average ECM curves for the farm were derived.

The distance from the corresponding curve was determined for each day that the animal was observed. Then the cumulative difference and the average difference were calculated.

$$difference\ each\ day_{ecm} = ECM_{animal} - ECM_{farm}$$

$$cumulative\ difference = \sum_{dim=min}^{dim=max} daily\ difference_{ecm}$$

$$average\ difference = \frac{cumulative\ difference}{number\ of\ days}$$

To calculate results for the test and control groups, the corresponding animals were summed or averaged (depending on the measure in question). We do not reference days in milk as our base for time calculations but rather divide by the number of days since treatment. This allows for comparison of animals that were treated at very different moments in time.

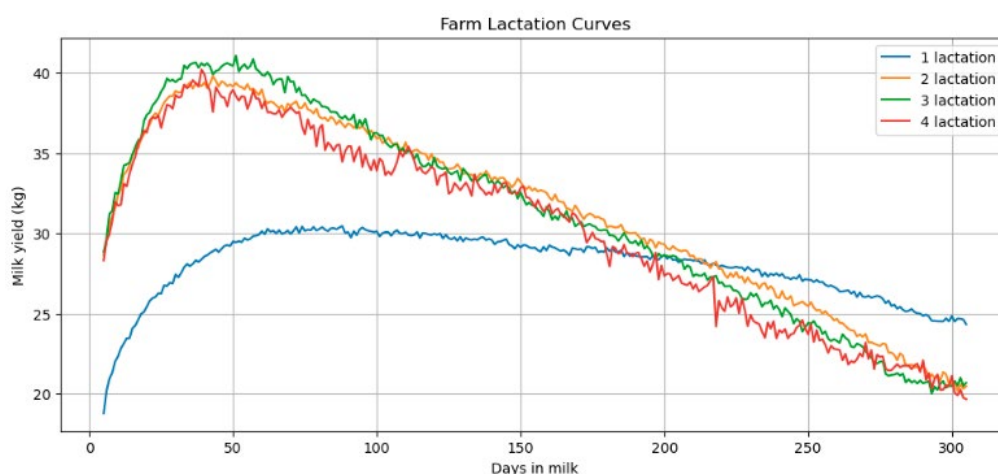


Fig. 1. Farm lactation curves



Fig. 2. Average fat and protein values

Economic evaluation

One of the ways to evaluate benefits of treatments is through calculation of profits. For this, we assumed a price of €0.40 per kg of ECM. The average daily difference in terms of money was calculated by multiplying the average daily difference in ECM by the price. In addition, the price of Kexxtone® was set at €35 per bolus. This allowed for the calculation of the payback period for the farmer.

Observation period

The observation period refers to the duration over which individual cows were monitored following inclusion in the study, specifically to evaluate daily milk composition, FPR, and ECM values. For cows in the test group, this period began immediately after monensin administration; for the control group, it began on the same relative day of selection. The average monitoring duration was approximately 200 days (± 5 days), which was used for the evaluation of ECM production and economic outcomes. Milk composition parameters — including lactose, fat, protein, and FPR — were analysed at selected time points over a 180-day period (days 10, 20, 30, 60, 90,

120, 150, and 180). All 52 cows (26 in each group) were part of the final analysis and stayed in the study until the end of the observation period.

Statistical analysis

The statistical software SPSS 26.0 (SPSS Inc., Chicago, IL, USA) was used to conduct the analysis. Using the Shapiro-Wilk test, the data distribution normality was evaluated. Calculated descriptive statistics were displayed as means \pm standard deviation.

For normally distributed variables, independent samples t-tests were used to compare the control and test groups. In cases where the normalcy assumptions were not fulfilled, the Mann-Whitney U test was utilised. Using two-way repeated measures ANOVA, repeated measurements at various time points were examined.

Differences were considered statistically significant at $P < 0.05$. Treatment costs and group-level ECM differences were used to calculate economic outcomes (such as cumulative profit and payback period), which were then confirmed using descriptive production data.

Results and discussion

By comparing the productivity and profitability results of early-treated cows based on milk composition with untreated controls, this study sought to evaluate the financial impact of using an in-line milk analyser for early detection of NEB in dairy cows.

Monetary

The results suggest that there is a significant difference among the test and control groups in terms of ECM. The summary is available in Table 2.

Table 2. ECM difference between test and control groups

Measure	Difference between test and control group cows
Average daily difference (per cow)	2.35 kg of ECM
	€0.95
Average cumulative difference (per cow) after 200 days	470 kg ECM
	€190

Since not all animals stayed in the experiment for the full 200 days (as mentioned in section 2.4.5), the actual cumulative difference between the two groups is 9916 kg of ECM (or in monetary terms €3966). The farm spent €910 on monensin boluses and thus had €3056 in profit. From an economic perspective, the treatment saved €3056 when comparing the test and control groups. By offering many advantages, especially in the early detection of ketosis, financial benefits, and the resolution of problems associated with negative energy balance in dairy cows, “Brolis Herd line” in-line milk analyser have completely transformed the dairy industry. Continuous health monitoring of individual cows has been made possible, which is essential for early identification of metabolic diseases like ketosis. Real-time monitoring of milk composition and other health indicators is one of in-line milk sensors many noteworthy benefits. For example, the regular analysis of milk components made possible by the use of near-infrared spectroscopic sensors in in-line milk sensors allows for the early detection of ketosis and other health problems without interfering with the cows’ daily routines (Jacobs, 2012). This ability is especially crucial because, if left untreated, ketosis can result in serious health issues and financial losses. Timely interventions made possible by early diagnosis can greatly increase recovery rates and lower the number of severe cases requiring prolonged treatment (John, 2016).

Advanced milk analysers that continuously measure the fat-protein ratio in milk offer many advantages for dairy farms, especially when it comes to improving animal health, milk production, and farm management in general. In order to make well-informed management decisions, real-time analysis of

milk composition is made possible by the integration of technologies like in-line milk analysers. In dairy cattle, the problem of negative energy balance (NEB) is crucial, especially during the transition period. Ketosis and other metabolic diseases are more likely to occur in cows with NEB. In-line milk sensors can help mitigate NEB by allowing for more frequent milking, which can encourage higher feed intake and better energy balance (Bovo, 2021). In-line milk sensors can help cows optimise their feeding habits and enhance their overall energy utilisation by giving them the choice to be milked whenever it is most convenient for them. This will lower the risk of NEB and its related complications (Verde, 2023). Furthermore, herd health management can greatly benefit from the information gathered from in-line sensors about the health and behaviour of individual cows. This information can help farmers further improve productivity and animal welfare if they choose feeding plans and health treatments that are suited to the individual requirements of each cow (Ma, 2016).

Economically, in-line milk sensors can boost milk production and boost dairy farmers’ profits. According to studies, cows milked with in-line milk sensors can yield a lot more milk than those milked the old-fashioned way. For instance, increasing the number of milkings from two to three times a day can increase milk yields by 6% to 25% over the course of full lactations (John, 2016). Since more milk means more sales, dairy producers will directly benefit from this increase in milk yield. Furthermore, in-line milk sensors lower labour expenses related to conventional milking techniques, enabling farmers to more effectively allocate resources (Mecitoglu, 2016).

In this study, cows at a higher risk for NEB were given 32.4 g (Kexxtone[®]) of monensin. The biggest advantage of Kexxtone[®] is that it is a continuous-release drug, which means it does not require a lot of veterinary time per cow. Monensin, an ionophore antibiotic, has been extensively studied for its economic and health benefits in dairy cattle, particularly in enhancing milk yield and improving overall animal health. It has been demonstrated that the veterinary medication monensin significantly improves animal health and farm economics, especially in dairy cattle. Monensin is primarily used to prevent ketosis, a metabolic condition that can negatively affect a cow’s ability to produce milk and general health. According to research, treating cows with monensin can significantly increase their average daily milk production. This rise is explained by the drug’s beneficial effects on the animal’s physiological functions, which raise productivity throughout the cow’s life cycle (Tan, 2020). Monensin modifies the rumen’s microbial population, promoting the development of bacteria that generate propionic acid, a precursor to glucose synthesis that is essential for lactating cows’ energy metabolism (Msellati, 2012).

This has significant ramifications because dairy farmers are more profitable when milk yields are higher, which makes monensin an effective tool for dairy management.

Many scientific studies have been conducted, proving that this medication individually affects the milk yield and health status of cows. In previous studies, we have already found that the administration of monensin significantly increases milk production, with increases ranging from 0.8 kg to as much as 2.8 kg per day (Santos, 2019). Furthermore, another study highlighted that monensin could enhance milk yield by 4.0% compared with control diets, demonstrating its potential to improve economic returns for dairy farmers. The increased milk yield brought on by monensin supplementation has significant economic ramifications. For dairy operations, higher milk production is directly correlated with higher revenue. For instance, a dairy farm with 100 cows might produce an extra 100 kg of milk every day if monensin causes an average increase of 1 kg of milk per cow per day. This financial benefit underscores the importance of monensin as a cost-effective strategy for enhancing dairy farm profitability (Kasap, 2020).

Milk composition difference between test and control groups

Table 3 presents a comparative analysis of key milk composition parameters (lactose, fat, protein, and fat-to-protein ratio) and milk yield between the control group and the test group over a 180-day period. The goal was to evaluate potential differences in milk quality and production efficiency between the two groups.

The only statistically significant difference was in lactose content at 20 days ($P < 0.05$), where the test group showed higher lactose levels (4.29%) compared with the control group. No significant changes were observed in milk yield, fat, protein, or fat-to-protein ratio, indicating that the treatment did not drastically impact these parameters. Monensin is known to reduce the percentage of milk fat and protein, but data on the amount of lactose and its changes in milk are lacking. For example, large-scale studies have reported that monensin has no significant effect on selected components such as lactose, protein, and fat (Kazama, 2010). Overall, it seems that monensin has an indirect impact on the amount of lactose in milk. Studies directly related to changes in lactose are still lacking, so more research is needed to clearly identify and confirm these effects.

This study has a number of advantages: it utilises in-line sensors for milk analysis, offering a non-invasive method to assess dairy cow metabolic status, enhancing animal welfare. It also allows for the early detection of ketosis, which could enhance the management of herd health. Data recorded with in-line sensors allows for the integration of milk composition data with health indicators, helping to create comprehensive health monitoring systems. However, the study also has disadvantages. To confirm these findings, larger cow groups would be beneficial for future studies. The milk fat-to-protein ratio could also be affected by environmental factors, such as heat stress or other environmental factors. Therefore, future studies should consider these actions when evaluating study results.

Table 3. Comparison of milk composition and yield between control and test groups 0, 10, 20, 30, 60, 90, 120, 150, 180 days after Kexxtone administration

Variable	Time Point (days)	Control Mean	Test Mean	T Statistic	P Value
Lactose (%)	10	4.41	4.5	-1.27	0.2
Lactose (%)	20	4.44	4.64	-2.13	0.03*
Lactose (%)	30	4.52	4.52	0.02	0.98
Lactose (%)	60	4.48	4.55	-0.82	0.41
Lactose (%)	90	4.5	4.57	-1.04	0.31
Lactose (%)	120	4.46	4.49	-0.39	0.7
Lactose (%)	150	4.49	4.53	-0.61	0.55
Lactose (%)	180	4.47	4.51	-0.58	0.57
Milk yield (kg/d)	10	27.22	28.93	-0.73	0.46
Milk yield (kg/d)	20	30.94	31.63	-0.26	0.78
Milk yield (kg/d)	30	30.3	30.38	-0.03	0.97
Milk yield (kg/d)	60	31.12	31.55	-0.29	0.77
Milk yield (kg/d)	90	30.74	31.26	-0.36	0.72
Milk yield (kg/d)	120	29.88	30.55	-0.42	0.68
Milk yield (kg/d)	150	29.67	30.34	-0.43	0.67

Table 3 cont.

Variable	Time Point (days)	Control Mean	Test Mean	T Statistic	P Value
Milk yield (kg/d)	180	29.35	30.01	-0.45	0.66
Fat (%)	10	4.66	4.42	0.82	0.41
Fat (%)	20	4.42	4.29	0.59	0.55
Fat (%)	30	4.67	4.24	1.28	0.2
Fat (%)	60	4.6	4.31	1.1	0.27
Fat (%)	90	4.55	4.33	0.94	0.35
Fat (%)	120	4.52	4.35	0.81	0.42
Fat (%)	150	4.47	4.36	0.67	0.51
Fat (%)	180	4.45	4.34	0.63	0.53
Protein (%)	10	3.41	3.29	1.05	0.29
Protein (%)	20	3.33	3.33	0.02	0.98
Protein (%)	30	3.44	3.31	1.09	0.27
Protein (%)	60	3.38	3.3	0.86	0.39
Protein (%)	90	3.36	3.28	0.89	0.38
Protein (%)	120	3.32	3.26	0.65	0.52
Protein (%)	150	3.3	3.25	0.58	0.56
Protein (%)	180	3.28	3.24	0.53	0.6
Fat-to-protein ratio	10	1.39	1.34	0.5	0.61
Fat-to-protein ratio	20	1.33	1.29	0.76	0.45
Fat-to-protein ratio	30	1.36	1.29	0.66	0.5
Fat-to-protein ratio	60	1.36	1.31	0.57	0.57
Fat-to-protein ratio	90	1.35	1.32	0.41	0.68
Fat-to-protein ratio	120	1.36	1.33	0.43	0.67
Fat-to-protein ratio	150	1.35	1.32	0.45	0.66
Fat-to-protein ratio	180	1.36	1.33	0.44	0.66

* – statistically significant

Conclusions

Our findings show distinct advantages in relation to the study's objective, which was to assess the economic worth of an in-line milk analyser for the early identification and avoidance of negative energy balance (NEB) in dairy cattle. In comparison with the control group, cows of the test group produced 9916 kg more milk overall and made €3056 more money after 200 days of treatment. Twenty days following the administration of Kexxtone (monensin 32.4 g), the test group also displayed a higher lactose content (4.29%).

According to these results, cows at risk of NEB can be promptly identified by using an in-line milk

analyser to monitor the fat-to-protein ratio. The standardised monensin intervention used in the analyser-supported management approach enhanced milk performance and financial results. Although monensin is used as a preventive measure, its effects on its own were not assessed. Rather, this study highlights the importance of in-line milk analysers as tools for decision-making in the early detection of metabolic risk in contemporary dairy herd management.

This study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee (the study approval number is PK016965. 6 June 2017).

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Carbon Dioxide Levels in a Cow Milking Parlor

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Abstract. The survey was conducted on a Bulgarian farm with 500 Holstein-friesian dairy cows housed in free-stall dairy operation. Measurements of carbon dioxide (CO₂) levels were taken in 2 x 8 “Herringbone” milking parlor. Monthly within a year, during the morning, midday and evening milkings, measurements were taken at the beginning, half-way through and at the end of the milking of the herd. Carbon dioxide levels were monitored with a Lutron MCH-383SDB (Lutron Electronic Enterprise Co., LTD., Taiwan). At the same time, the temperature-humidity index (THI) levels were recorded using the “Kestrel 5400” Weather Meter (Kestrel Instruments, USA). The highest average values of carbon dioxide in the air of the milking parlor were recorded in the winter season (756.67 ppm), and the lowest in the summer season (435.8 ppm). The highest recorded THI values were in the summer – 72.8, and the lowest in the autumn – 53.5. The highest CO₂ values were found at THI levels around 50, established in autumn and winter, because ventilation in the milking parlor was poor.

Introduction

Ruminant livestock farming is responsible for 18% of greenhouse gas emissions in the EU (EFSA, 2009). As a greenhouse gas carbon dioxide plays an ambivalent role being essential for life on the planet and at the same time having negative effect on climate (Eldesouki et al., 2023). High concentrations of the gas have a negative effect on cows but also on people involved in farming. If animals are exposed to air concentration of carbon dioxide above 1% chronic intoxication, lower productivity and reduced resistance to disease can occur (Vtoryi et al., 2017). CO₂ concentration is affected by three factors, i.e. number of milkings, the type of ventilation system and local climatic conditions (Souza et al., 2024). Microclimate and ventilation are important factors that characterize air quality in farms (Mijid, 2013). Carbon dioxide is one of the key parameters of microclimate. Relative air humidity and concentration of harmful gases like carbon dioxide and ammonia could increase due to poor ventilation. According to Jovović et al. (2015), the carbon dioxide concentration is connected to the design of the barn, ventilation capacity and whether the number of the animals corresponds to their needs of a living space. Improving ventilation is beneficial in terms of animal health, welfare and productivity (Lovarelli, 2024). Carbon emissions in dairy farms are mainly associated with the cultivation and processing of feed, enteric fermentation of cows, the treatment of manure, and the energy consumption in dairy farms (Wang et al., 2024). The creation and provision of a suitable indoor environment especially for the summer season for dairy cows is getting more attention in recent years (Kic, 2022). Animals are milked in different types of milking parlors. Regardless of

the type of the milking parlor, it is located in close proximity to livestock premises. It is necessary to determine the carbon dioxide levels inside a milking parlor, compare them with the outside concentrations and determine effectiveness of building isolation from the influence of temperature and humidity responsible for generation and accumulation of carbon dioxide. All this gave grounds for conducting the present study.

The study aimed to determine the concentration of carbon dioxide in a milking parlor, identify the factors influencing variations of the levels of concentration, and assess the possibility of a risk for the dairy cows' welfare.

Materials and methods

For the purpose of the study, the milking parlor of a dairy cattle farm for 500 Holstein-Friesian cows was selected. The type of milking parlor was 2 x 8 “Herringbone”, no mechanical ventilation. The milking parlor had been installed 10 years prior to the study. Cows were milked three times during the day: at 5:00 a.m., at 12:00 a.m. and at 6:00 p.m. One milking of the herd lasted 2.5 hours.

Triplicate recording of carbon dioxide levels and THI were performed during each milking: at the beginning, half-way through and at the end of the milking. They were carried out during the morning, midday and evening milking at the level where the animals were standing in the milking parlor, from May 2018 to May 2019. Measurements of carbon dioxide and THI levels in the atmosphere were taken 10 meters away from the buildings. Carbon dioxide levels were determined by a “Lutron” MCH-383SDB (Lutron Electronic Enterprise Co., LTD., Taiwan) (Fig. 1).

Weather station “Kestrel 5400 (Kestrel Instruments,



Fig. 1. Lutron MCH-383SDB



Fig. 2. Weather Meter "Kestrel 5400"

USA)" was used for direct measurement of the temperature-humidity index (Fig. 2).

THI values are in 6 classes determined by calculations: class 1 – ≤ 50 ; class 2 – from 50 to 58; class 3 – from 58 to 68; class 4 – from 68 to 72; class 5 – from 72 to 74; and class 6 – ≥ 74 .

A basic statistical processing of data, determining mean values, errors and analysis of variance was performed with MS Excel along with corresponding modules of STATISTICA of StatSoft.

To assess the influence of the factors, the following model was used:

$$Yijklm = \mu + Si + Mj + Pk + THIl + eijklm$$

Where $Yijklm$ is the dependent variable (carbon dioxide); μ is the average for the model; Si is the effect of the reporting season; Mj is the effect of consecutive milking for the day; Pk is the effect of consecutive reporting for the milking; $THIl$ is the effect of THI (class); and $eijklm$ is the effect of uncontrolled factors (error).

The least squares of means by classes of the fixed factors were obtained by ANOVA to fit the model.

Results and discussion

Table 1 presents the average values for the reported CO_2 and THI levels inside the milking parlor and outdoors. Measurements of both indicators in the milking parlor were taken at the beginning of the milking, before production process, animals and people could affect them. The significance of the differences was calculated between the averages for each indicator outside and inside by season.

Average CO_2 values in the outdoor atmosphere were relatively low for all seasons. The lowest average values of CO_2 indoors were for the summer season – 196.3 ppm, and the highest for the winter season – 269.3 ppm.

The anthropogenic factor leads to a steady increase of atmospheric concentration of CO_2 . From 280 ppm concentration of CO_2 in the 1800s, to 320 ppm in 1960, it has now reached 420 ppm, the highest level ever recorded (Oliveira, 2024).

The reported low levels of CO_2 in the area of the farm are due to the fact that it was located in an area with no industrial enterprises, it was less populated and therefore with less traffic. The use of solid fuels for heating in the area was a prerequisite for the reported higher values during cold seasons, especially winter.

The area had a milder climate and high temperatures during the warm months of the year. Seasonal average THI values confirm this. The highest values were recorded during summer – 75.8 and spring season – 70.3.

The values of the temperature-humidity index fall into classes related to different levels of heat stress. Various authors state that heat stress occurs when THI is from 68 to 74 (Herbut, 2018). Armstrong (1994) considers that when the index is below 71 cows are in the comfort zone, values ranging within 72–79 cause mild stress, within 80–89 they cause moderate stress, and when values are above 90, there is severe stress. According to Segnalini et al. (2013), 68 is the threshold value as values of THI between 68 and 72 are considered mild discomfort. Data shows that during summer days inside and outside the milking parlor, THI values that were recorded fall outside the comfort zone for dairy cows. Different studies suggest these values could be classified as moderate stress and uncomfortable conditions or even risky (Segnalini et al., 2013).

The average measured CO_2 values in the milking parlor at the beginning of milking are significantly higher than those outside, i.e., 2 to 3 times for the different seasons. Differences in average values for CO_2 inside and outside the building were significant for all seasons. This was not the case with THI values. The differences inside and outside the parlor were insignificant. Only for the summer season the difference was statistically significant ($P < 0.05$). This indicated a weak isolation of the milking parlor from the influence of external climatic conditions. The

parlor was located next to the animal premise and there was air exchange between them. In addition, the parlor lacked mechanical ventilation and was only ventilated during the warm months when the curtains of semi-open barn were up. All this was a prerequisite for stagnant and low-quality air in the milking parlor at the very beginning of the milking of the animals.

Table 2 presents the results of the analysis of variance for the influence of the factors controlled in this study on CO₂ values in the milking parlor. Consecutive milking (morning, midday and evening) and consecutive reporting for the milking (start, middle and finishing) had no significant effect on CO₂ levels in the parlor. An effect of high statistical significance was reported for the season ($P < 0.001$), followed by THI values (in classes) ($P < 0.05$).

The proximity of the milking parlor to the animals' premise caused continuous air exchange between them. Curtains of the livestock premises were lowered in winter months and on certain days during transition seasons. Humidity of the air in the milking parlor increased, which led to higher values of carbon dioxide in the milking parlor. Jovović et al. (2015) studied 38 dairy cow buildings in the area of 10 municipalities in the region of Bosnia and Herzegovina and reported similar tendencies. Average values of carbon dioxide in their study were 871.57 ppm in winter, varying from 390 to 1690 ppm. During the summer season, ventilation was better because of the opening of windows and doors and, as a result, concentration of

carbon dioxide in the cow premises was lower than in the winter. A study by Vtoryi et al. (2016) showed that the air temperature outside the buildings and the carbon dioxide content in the cowshed were inversely proportional.

Erbez et al. (2015) reported for carbon dioxide levels averaging 627.5 ppm (ranging from 390 to 890 ppm) in loose-housing buildings and 936.7 ppm (ranging from 390 to 1690 ppm) in tie-housing building. Jannat et al. (2025) found even lower carbon dioxide concentration averages of 612.44 ppm, ranging from 643.13 to 536.04 in a milking parlor of a dairy farm. Kaasik et al. (2013) found a clear seasonal variation: in summer, the concentration of CO₂ and relative humidity inside uninsulated loose-housing cattle buildings were lower than in winter, with an overall mean indoor CO₂ concentration of 553 ppm.

Ordinance 44 (MAFWE, 2006) stated that CO₂ concentration could not exceed 0.3% or 3000 ppm. Both values obtained in our study, i.e., the average and the maximum deviations, were below the limit value. The Ordinance concerns livestock premises, and we perceive the milking parlor as such, even though the animals do not spend the whole day there, but the regulation lacks specifics about milking parlors.

Fig. 3 presents the seasonal variation of carbon dioxide values during the different milkings of the day – morning, midday and evening. During the three milkings for the day, the lowest average CO₂ values

Table 1. Carbon dioxide and THI average values inside the milking parlor and outside in the farm area for different seasons

Season	Number n	CO ₂ inside, ppm	CO ₂ outside, ppm	THI inside, value	THI outside, value
		x ± SE	x ± SE	x ± SE	x ± SE
Summer	9	435.8 ± 50.99***	196.3 ± 7.47***	72.8 ± 0.86*	75.8 ± 0.87*
Autumn	6	592.0 ± 104.76**	235.8 ± 18.07**	53.5 ± 1.79-	51.6 ± 2.46-
Winter	3	756.67 ± 23.14***	269.3 ± 12.14***	54.4 ± 0.77-	54.4 ± 0.38-
Spring	11	682.18 ± 53.19***	220.18 ± 10.89***	68.6 ± 2.38-	70.3 ± 2.99-

*** – significance at $P < 0.001$; ** – significance at $P < 0.01$; * – significance at $P < 0.05$; - n.s.

Table 2. Analysis of variance for influence of controlled factors on CO₂ values in the milking parlor

Sources of variation	CO ₂ values inside the milking parlor		
	(n – 1)	MS	F P
Total for the model	12	119 318	3.69***
Reporting season	3	225 681	6.98***
Consecutive milking for the day	2	919	0.03-
Consecutive reporting for the milking	2	68 441	2.12-
THI	5	93 618	2.90*
Error	74	32 318	

*** – significance at $P < 0.001$; ** – significance at $P < 0.01$; * – significance at $P < 0.05$; - n.s.

MS = mean square, F = value of the factor, P = level of significance; “-” lack of significance

were the in the summer season ranging from 432 to 516 ppm, with a maximum deviation of 813 ppm in the evening milking. In winter, the highest average values for CO₂ compared with the other seasons were from 725 to 789 ppm, reaching maximum values above 800 ppm.

Animals coming in the milking parlor result in a rise of carbon dioxide levels. During milking, perspiration and frequency of breathing increase thus leading to indoor air temperature and relative air humidity increase. With each milking session of a technological group of animals, air quality deteriorates (Herbut et al., 2012).

A significant effect of THI values on those of CO₂ in the milking parlor was reported. Fig. 4 presents LS

mean values for CO₂ levels depending on THI values (in classes).

The highest CO₂ values (914.6 ppm) were reported when THI values were lowest – below 50. Medium high values of 591.2 ppm to 605.5 ppm were reported at optimal THI values of 50 to 72. Values of THI above 72 classified as risky were accompanied with an increase in CO₂ values. CO₂ reached 715 ppm when THI was above 74. For both the low and the high THI values, the crucial factor for reducing the level of carbon dioxide in the milking parlor was the ventilation of the premise. During the cold months, the curtains of the barn were down and there was no ventilation at all, resulting in the retention of all harmful gases in the human and animal area.

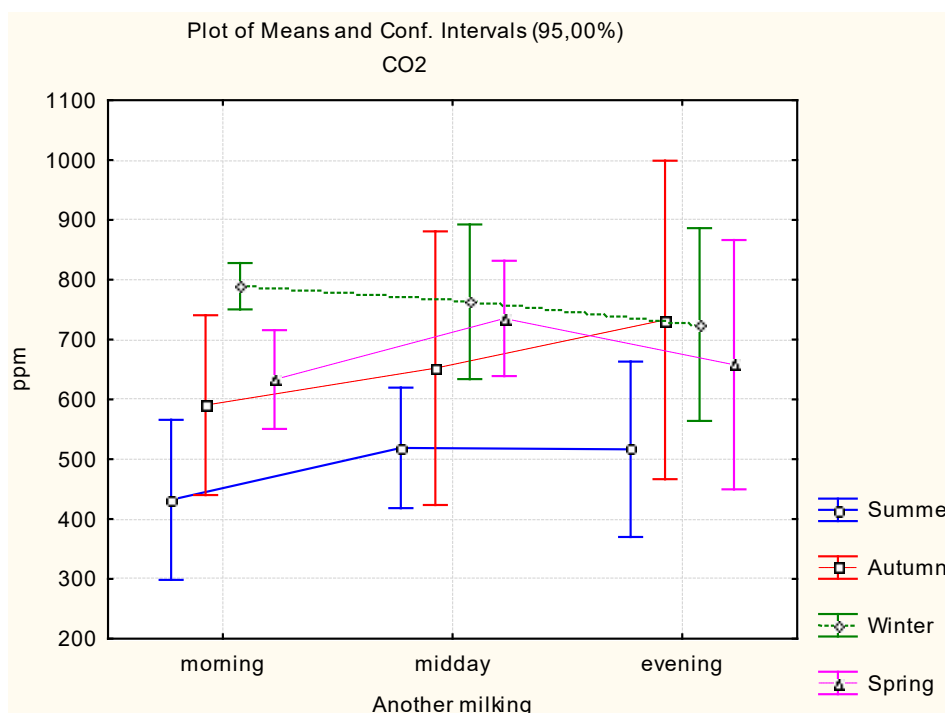


Fig. 3. Seasonal average values and ranging of carbon dioxide during the different milkings for the day

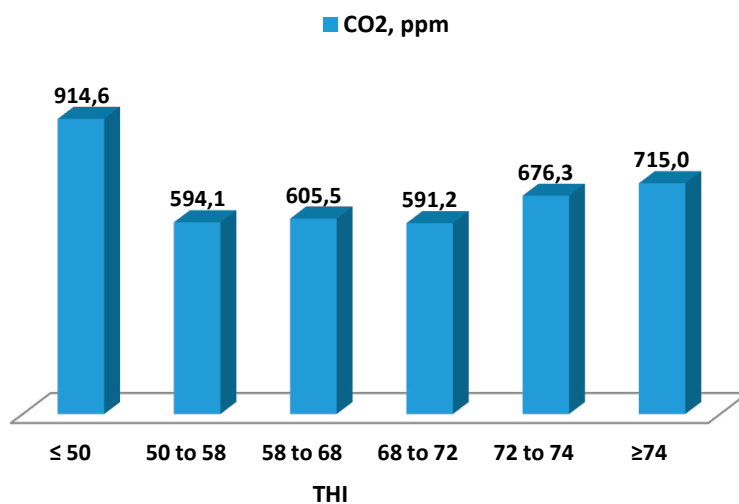


Fig. 4. LS mean values for CO₂ levels depending on THI values (in classes)

During the warm months, although the curtains were raised, outside conditions and inside conditions were the same, which resulted in higher THI values and higher CO₂ levels due to an increase in the respiration rate. Because the livestock facility was not separated from the milking parlor by doors or other barriers, air from the livestock building entered the milking parlor unhindered, which undoubtedly affects CO₂ values.

In a study by Dimov et al. (2019) of three cattle farms over different technological zones in the livestock premises, carbon dioxide values were lowest when the temperature-humidity index was within the range from 58 to 74 and air velocity from 0.6 to 1.0 m/s. High humidity and low temperatures were prerequisites for CO₂ retention in the low air layers of the premise.

Jeelani et al. (2019) suggest little changes for the animal at THI 72, but major physiological changes happen when THI reaches 74. At THI range within 74–79, animals maintain homeostasis, but it is perturbed at THI 80. Overall recalibration of THI is needed to make accurate assessment of heat stress depending on the climatic region. Heat stress in cattle accelerates respiration, pulse rate, and metabolism. Other indicators of heat stress are a decrease in feed intake and an increase in water intake, increased rectal temperature, an increase in water loss through evaporation, and a change in the concentration of hormones in the blood (Koubková et al., 2002). The light increase in carbon dioxide levels in our study was due to the increased respiratory rate corresponding with THI values above 72. Although the cows stay in the milking parlor for a short time, the combination

of high THI and CO₂ values could have a stressful effect on the animals.

Conclusion

The average carbon dioxide air concentration in the milking parlor ranged from 435 to 756 ppm, with the highest values registered in the winter season and the lowest in the summer. The trend for carbon dioxide values by seasons outside the parlor was similar to the reported inside it, but the milking parlor values were 2 to 3 times higher, ranging from 196.3 to 269.3 ppm again lowest in summer and highest in winter. The average values of THI of the air of the milking parlor ranged from 53.5 to 72.8, with the highest values recorded in the summer season and the lowest in the autumn. Outside the milking parlor, the reported THI values ranged from 51.6 to 75.8, again highest in summer and lowest in autumn. Highest values of carbon dioxide were recorded when THI was near 50 or as high as 74 and above. As a preventive measure, a precise regulation of the ventilation system in a milking parlor is necessary, even though the values of the study are in the norm according to the current legislation.

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Comparative Effect of Hormonal and Vitamin Treatments on Reproductive Performance in Rembi Ewes during the Low Sexual Activity Period in Western Algeria

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Keywords: Rembi ewes, hormonal synchronization, vitamin AD₃E, fertility, prolificacy, neonatal mortality.

Abstract. Reproductive efficiency is a key determinant of the profitability and sustainability of sheep farming systems, particularly in arid and semi-arid regions. In Algeria, declining flock productivity, exacerbated by recurrent droughts, forage scarcity, and thermal stress, has raised concerns about the resilience of extensive and semi-intensive sheep production. This study aimed to compare the efficacy of hormonal synchronization, vitamin AD₃E supplementation, and their combination on the reproductive performance of Rembi ewes during seasonal anoestrus. Conducted in spring 2024 on a semi-intensive farm in Aïn Bouchakif (Tiaret, Algeria), the trial involved 60 healthy pluriparous ewes randomly assigned to three groups (n = 20). Group 1 received hormonal treatment (intravaginal progestogen-impregnated sponges followed by PMSG), Group 2 received the same hormonal protocol combined with monthly AD₃E injections, and Group 3 received vitamins only. Fertility, fecundity, prolificacy, birth weight, and neonatal mortality were evaluated. Principal Component Analysis (PCA) was applied to explore multivariate patterns.

The combined treatment (Group 2) yielded the best reproductive outcomes: 100% fertility, 160% fecundity, and 0% neonatal mortality. Group 1 recorded the lowest fertility (80%) and highest neonatal mortality (16.66%), while Group 3 achieved the highest average birth weight (4.25 kg) but lower prolificacy. PCA revealed three distinct reproductive profiles, clearly differentiating the effectiveness of the protocol.

These results underscore the synergistic benefit and practical advantage of combining hormonal synchronization with vitamin supplementation to improve fertility and prolificacy while reducing neonatal losses. This integrative strategy offers a viable and sustainable approach to enhancing reproductive performance in Rembi ewes under the challenging conditions of Western Algeria's steppe regions.

Introduction

The profitability of sheep farming fundamentally relies on the reproductive performance of females, particularly fertility rate, prolificacy, and the number of lambings per year. In Algeria, sheep production is largely dominated by a traditional extensive system accounting for over 90% of all farms (Gani, 2023). This low-input management model often results in modest reproductive outcomes, with fewer than one lamb produced per ewe per year. The underlying causes include the seasonal nature of reproduction, minimal zootechnical monitoring, and high dependence on climatic variability (Zidane et al., 2023).

According to the National Statistics Office (ONS, 2020), the national sheep population was estimated at 30.9 million head, representing about 81% of Algeria's total ruminant livestock. However, this population has shown a worrying decline, with unofficial sources

reporting a drop to around 17 million by 2023 (Le Jeune Indépendant, 2023). This decrease is largely attributed to the cumulative effects of climate change, prolonged drought, rising feed costs, and suboptimal reproductive performance. These stressors are further aggravated by ecological degradation, including the loss of steppe vegetation, reduced forage availability, and worsening female body condition, all of which impair fertility and herd renewal capacity (FAO, 2021).

The Tiaret region, located in the semi-arid steppe zone of northwestern Algeria, illustrates this dynamic. It hosts a significant sheep population, predominantly composed of the indigenous Rembi breed, which is valued for its hardiness, meat conformation, and reproductive potential. Nonetheless, the region has not been spared from the impacts of climatic stress and structural constraints, leading to a marked deterioration in reproductive indicators (Bouacha, 2019).

To overcome the constraints of seasonal reproduction, oestrus synchronization using intravaginal

sponges impregnated with progestagens, followed by equine chorionic gonadotropin (eCG or PMSG) injection at sponge removal, is widely applied in sheep reproductive management. This approach allows induction and synchronization of oestrus during anoestrus periods, facilitating flock planning and enhancing reproductive outcomes (Hameed et al., 2021).

However, the repeated application of this hormonal protocol poses several challenges. Economically, in Algeria, the cost of a full synchronization cycle, including sponge insertion and hormonal injections, can exceed \$17 per ewe, representing a major limitation for smallholder and extensive systems (Meziane et al., 2024). Biologically, studies report that repeated exposure to PMSG may induce antibody formation against the molecule, thereby reducing ovarian responsiveness and compromising fertility (Abecia et al., 2012; Sun et al., 2019). Additionally, intravaginal sponges may provoke local adverse effects such as irritation, metritis, or alterations in vaginal microbiota, especially under suboptimal hygiene or prolonged use (Martemucci and D'Alessandro, 2011; Fashemi et al., 2013). Ethically, PMSG extraction involves repeated blood collection from pregnant mares, raising serious welfare concerns and leading several European countries to ban its use (EFSA, 2011; Veterinary Medical Ethics, 2022).

Given these constraints, vitamin supplementation, especially with the AD₃E complex (vitamins A, D₃, and E), is increasingly considered as an alternative to support reproductive function. These vitamins are essential for ovarian activity, hormonal regulation, embryonic development, and immune function (Bozkurt et al., 2023; Efe et al., 2023). Their administration is cost-effective, easy to implement, and devoid of the immunogenic or ethical concerns associated with hormonal interventions.

While the individual effects of hormones or vitamins have been studied, a direct comparison of these strategies, and especially their synergistic combination, is lacking for indigenous breeds under North African steppe conditions. This study was, therefore, designed to rigorously compare the effects of a conventional hormonal protocol, a vitamin AD₃E supplementation, and their combination on the reproductive performance of Rembi ewes during the seasonal anoestrus. The objective was to identify a viable and affordable strategy to enhance reproductive efficiency and resilience in the face of climatic and nutritional challenges typical of traditional sheep farming systems in Western Algeria.

Materials and methods

Study area and environmental conditions

The trial was conducted on a private farm located in the Sersou plain, between the municipalities of Tiaret and Aïn Bouchakif (Tiaret province), in the northwestern high plateaus of Algeria. The site lies at 964 meters above sea level, with coordinates

35°21'21"N and 1°30'38"E. The region experiences a cold semi-arid Mediterranean climate (BWk according to the Köppen-Geiger classification), characterized by an average annual temperature of 14.9°C, with hot, dry summers (often exceeding 30°C) and cold winters with minimum temperatures frequently below 0°C. The average annual rainfall is approximately 362 mm, mainly concentrated in the autumn and winter months.

The farm follows a semi-intensive production system and is equipped with appropriate infrastructure, including ventilated sheepfolds, covered enclosures, and automatic watering systems. This experimental site was selected for its agro-ecological representativeness of the Algerian high plateaus and the genetic homogeneity of its Rembi ewe flock.

During April, the reproductive activity of Algerian sheep flocks is markedly reduced. This anovulatory state is principally governed by increasing photoperiod, which suppresses gonadotropic activity. The situation is compounded by nutritional stress from declining forage quality and the prevalence of lactational anoestrus. Consequently, inducing oestrus during this period necessitates the application of exogenous hormonal protocols rather than occurring through natural means.

Experimental animals and herd management

A total of 60 clinically healthy pluriparous Rembi ewes, aged between 3 and 5 years and with a postpartum interval of at least 80 days, were selected for the trial. Body condition was evaluated using the Body Condition Scoring (BCS) system, with all animals scoring between 3.0 and 3.5 on a 5-point scale. The ewes were managed under a semi-intensive system involving daily grazing and evening supplementation with 600 g per head of crushed barley and ad libitum access to barley straw and water. A nutritional flushing programme was implemented during March and April 2024, consisting of an additional 230 g per head per day of whole barley.

Experimental design and treatments

The ewes were randomly allocated into three experimental groups of 20 animals each.

In Group 1 (hormonal treatment only), ewes were treated with intravaginal sponges impregnated with 30 mg flugestone acetate (Syncro-part[®], CEVA Santé Animale, Libourne, France) for 14 days. At sponge removal, each ewe received an intramuscular injection of 500 IU of pregnant mare serum gonadotropin (PMSG) (Syncro-part[®], CEVA Santé Animale, Libourne, France). No vitamin supplementation was administered.

In Group 2 (combined hormonal and vitamin treatment), ewes received the same hormonal protocol as in Group 1, along with monthly intramuscular injections of a vitamin AD₃E complex (Adecon[®]

1.9%, FATRO S.p.a, Ozzano Emilia “Bologna”, Italia: 10,000,000 IU vitamin A, 2,500,000 IU vitamin D₃, 10,000 mg vitamin E per 100 mL), administered at a dose of 5 mL per ewe from March to October 2024.

In Group 3 (vitamin treatment only), ewes were exposed to natural mating without hormonal induction but received the same AD₃E supplementation as in Group 2, using identical dosage and timing.

Eight fertile Rembi rams, aged 5 to 7 years and originating from the same flock, were selected as sires. These rams were kept separate from the females until the designated mating period to prevent premature contact.

All animals were individually identified using numbered ear tags. Prior to inclusion in the study, each ewe and ram underwent a comprehensive health check, including general clinical examination, inspection of external genitalia, and assessment of limb integrity. Only animals meeting the inclusion criteria were retained to ensure the internal validity of the experiment.

Oestrus synchronization and mating management

Oestrus synchronization was induced in Groups 1 and 2 through the intravaginal insertion of progestogen-impregnated sponges on April 14 and 18, 2024, respectively (Table 1). Following sponge removal, oestrus detection was conducted at 6-hour intervals for 48 hours by experienced technicians. Oestrus identification was based on standardized behavioural and physiological indicators including restlessness, vulvar hyperaemia and oedema, presence of cervical mucus discharge, and standing reflex when mounted by teaser rams.

The same cohort of eight proven fertile Rembi rams was utilized across all experimental groups. For Groups 1 and 2, controlled mating was implemented immediately after oestrus confirmation using a 1:5 ram-to-ewe ratio during a concentrated 48-hour breeding

period, with rams systematically rotated between these groups. For Group 3 (non-synchronized), natural mating was conducted throughout April 2024 to ensure coverage of at least one complete oestrous cycle (approximately 17 days), thereby accounting for individual variation in cycle onset and maximizing conception opportunities in the absence of hormonal synchronization.

Gestation monitoring and data collection

Gestation status in ewes was monitored using a combined approach of non-return to oestrus records and systematic behavioural assessment. This weekly post-mating surveillance served to confirm initial pregnancy and to diagnose reproductive failures, including early and late embryonic mortality, as well as abortions. Ewes that did not return to oestrus were provisionally classified as pregnant, a status that was subsequently confirmed through documented behavioural indicators such as increased docility, voluntary separation from the flock, and a quantifiable increase in feed intake. All lambing events were systematically recorded. For each birth, the lambing date, litter size, viability status (live or stillborn), and individual birth weights were documented. Neonatal weights were obtained within hours of delivery using calibrated hanging scales to ensure precise measurement.

Reproductive performance indicators

The following indicators were calculated:

- Fertility rate (%) =
$$= \frac{\text{Number of ewes that lambed}}{\text{Number of ewes exposed to mating}} \times 100$$
- Fecundity rate (%) =
$$= \frac{\text{Total number of lambs born (live and stillborn)}}{\text{Number of ewes exposed to mating}} \times 100$$
- Prolificacy rate (%) =
$$= \frac{\text{Total number of lambs born (live and stillborn)}}{\text{Number of ewes that lambed}} \times 100$$

Table 1. Timeline of the experimental protocol applied to the three groups of Rembi ewes

Parameter	Group 1 (Hormonal only)	Group 2 (Hormonal + AD ₃ E)	Group 3 (AD ₃ E only)
Number of ewes	20	20	20
Synchronization status	Synchronized	Synchronized	Non-synchronized
AD ₃ E supplementation	None	5 mL monthly (March–October 2024)	5 mL monthly (March–October 2024)
Sponge insertion date	14 April 2024	18 April 2024	-
Sponge removal date	28 April 2024	02 May 2024	-
PMSG administration	500 IU (28 April 2024)	500 IU (02 May 2024)	-
Sponge retention issues	1 ewe lost sponge	No issues	-
Effective synchronized ewes	19	20	-
Mating period	29 April–01 May 2024	03–05 May 2024	Throughout April 2024
Expected lambing period	30 Sept–06 October 2024	04–07 October 2024	Starting 12 September 2024

- Neonatal mortality rate (%) =
$$= \frac{\text{Number of stillborn lambs}}{\text{Total number of lambs born}} \times 100$$

- Average birth weight (kg): The mean birth weight of lambs was calculated and compared across groups, considering the type of birth (single or multiple).

Statistical analyses

Statistical analyses were conducted using the software PAST version 4.08 (Hammer et al., 2001). Descriptive statistics were calculated for all quantitative variables. Intergroup comparisons were performed using the Mann-Whitney U test, with a significance threshold set at $P < 0.05$. A Principal Component Analysis (PCA) was applied to explore multidimensional associations.

Ethical considerations

The entire experimental protocol was conducted in accordance with international guidelines for the ethical use of animals in research. All procedures adhered to good veterinary practices to minimize stress and ensure animal welfare.

Results

The reproductive performance of Rembi ewes under different treatment protocols is summarized in Table 2, with comprehensive statistical analysis presented in Table 3. The experimental cohort for analysis consisted of 19 ewes in Group 1 (following exclusion of one ewe that expelled the intravaginal sponge), 20 ewes in Group 2, and 20 ewes in Group 3, as detailed in Table 1.

Reproductive efficiency parameters

The combined hormonal-vitamin treatment (Group 2) yielded optimal reproductive outcomes, achieving complete fertility (100%) with absence of neonatal mortality. Vitamin supplementation alone (Group 3) resulted in moderate fertility (85%), while exclusive hormonal treatment (Group 1) showed reduced fertility (80%) accompanied by significant neonatal mortality (16.7%).

Fecundity was significantly enhanced in Group 2 (160%) compared with Group 3 (110%; $P = 0.035$), with Group 1 exhibiting intermediate performance (125%) that did not differ statistically from either group. Prolificacy rates were comparable between

Table 2. Effects of hormonal and vitamin treatments on reproductive parameters in Rembi ewes (Tiaret, Algeria, 2024).

Groups	Number of treated ewes	Ewes that lambed n (%)	Single births n	Twin births n	Triplet births n	Total lambs born n	Neonatal deaths n
Group 1 (Hormonal only)	20	16 (80.0)	8	7	1	25	5
Group 2 (Hormonal + AD ₃ E)	20	20 (100.0)	10	8	2	32	0
Group 3 (AD ₃ E only)	20	17 (85.0)	12	5	0	22	0
Total	60	53 (88.3)	30	20	3	79	5

Table 3. Reproductive performance parameters and statistical comparisons between experimental groups in Rembi ewes (Tiaret, Algeria, 2024).

Parameter	Group 1 (Hormonal only)	Group 2 (Hormonal + AD ₃ E)	Group 3 (AD ₃ E only)	Statistical comparisons (p-value)
Fertility rate (%)	80.0 ± 4.10 ^a	100.0 ± 0.00 ^b	85.0 ± 3.66 ^{ab}	1 vs 2 : 0.040* 1 vs 3 : 0.696 2 vs 3 : 0.080
Fecundity rate (%)	125.0 ± 8.50 ^{ab}	160.0 ± 6.80 ^b	110.0 ± 6.40 ^a	1 vs 2 : 0.214 1 vs 3 : 0.556 2 vs 3 : 0.035*
Prolificacy rate (%)	156.3 ± 6.29	160.0 ± 6.80	129.4 ± 4.69	1 vs 2 : 0.929 1 vs 3 : 0.206 2 vs 3 : 0.164
Neonatal mortality rate (%)	16.7 ± 3.16 ^a	0.0 ± 0.00 ^b	0.0 ± 0.00 ^b	1 vs 2 : 0.021* 1 vs 3 : 0.033* 2 vs 3 : 1.000
Lamb birth weight (kg)	3.02 ± 0.62 ^a	3.48 ± 1.01 ^b	4.25 ± 0.70 ^b	1 vs 2 : 0.026* 1 vs 3 : <0.001*** 2 vs 3 : 0.976

Values are presented as mean ± standard deviation. Different superscript letters (^a, ^b) within a row indicate significant differences ($p < 0.05$); * = Significant ($p < 0.05$); *** = Very highly significant ($p < 0.001$)

hormonally treated groups (Group 1: 156.3%; Group 2: 160.0%), while Group 3 demonstrated lower prolificacy (129.4%), although these differences lacked statistical significance.

Lamb viability and development

Notably, vitamin supplementation eliminated neonatal mortality in both Groups 2 and 3, contrasting significantly with Group 1 ($P = 0.021$ and $P = 0.033$, respectively). Lamb birth weights were maximized in Group 3 (4.25 ± 0.70 kg), significantly exceeding Group 1 (3.02 ± 0.62 kg; $P < 0.001$) while comparable with Group 2 (3.48 ± 1.01 kg; $P = 0.976$).

Multivariate pattern analysis

Principal Component Analysis (PCA) revealed distinct reproductive profiles among treatment groups, with two principal components explaining 100% of total variance (Fig. 1). Group 2 (hormonal + AD₃E) clustered strongly with enhanced fertility (100%) and fecundity (160%) parameters. Group 1 (hormonal only) associated with neonatal mortality (16.7%), while Group 3 (AD₃E only) correlated with improved birth weights (4.25 kg). This clear separation demonstrates the differential efficacy of each treatment strategy and confirms the synergistic effect of combining hormonal synchronization with vitamin supplementation. The multivariate approach provides statistical validation of the hypothesis that combined intervention optimizes reproductive

outcomes beyond single-treatment approaches in Rembi ewes during seasonal anoestrus.

Discussion

Synergistic enhancement of reproductive performance

The present study clearly demonstrates that the integration of hormonal synchronization with vitamin AD₃E supplementation generates a synergistic improvement in reproductive efficiency of Rembi ewes during seasonal anoestrus. Group 2, which received the combined treatment, achieved outstanding performance (100% fertility, 160% fecundity, and complete absence of neonatal mortality). These findings are consistent with previous reports emphasizing the complementary interactions between hormonal induction protocols and nutritional support in optimizing ovine reproductive outcomes (Hameed et al., 2021; Martinez-Ros et al., 2019; Al-Zubaidi et al., 2024; Benia et al., 2022). Importantly, the absence of neonatal losses in vitamin-supplemented groups reinforces the vital role of vitamins AD₃E in sustaining embryonic and foetal development, particularly under the challenging environmental conditions of the Algerian steppe (Gani, 2023; Bouacha, 2019).

Molecular and physiological mechanisms

The enhanced efficacy of the combined protocol likely arises from a convergence of molecular mechanisms through which vitamins potentiate

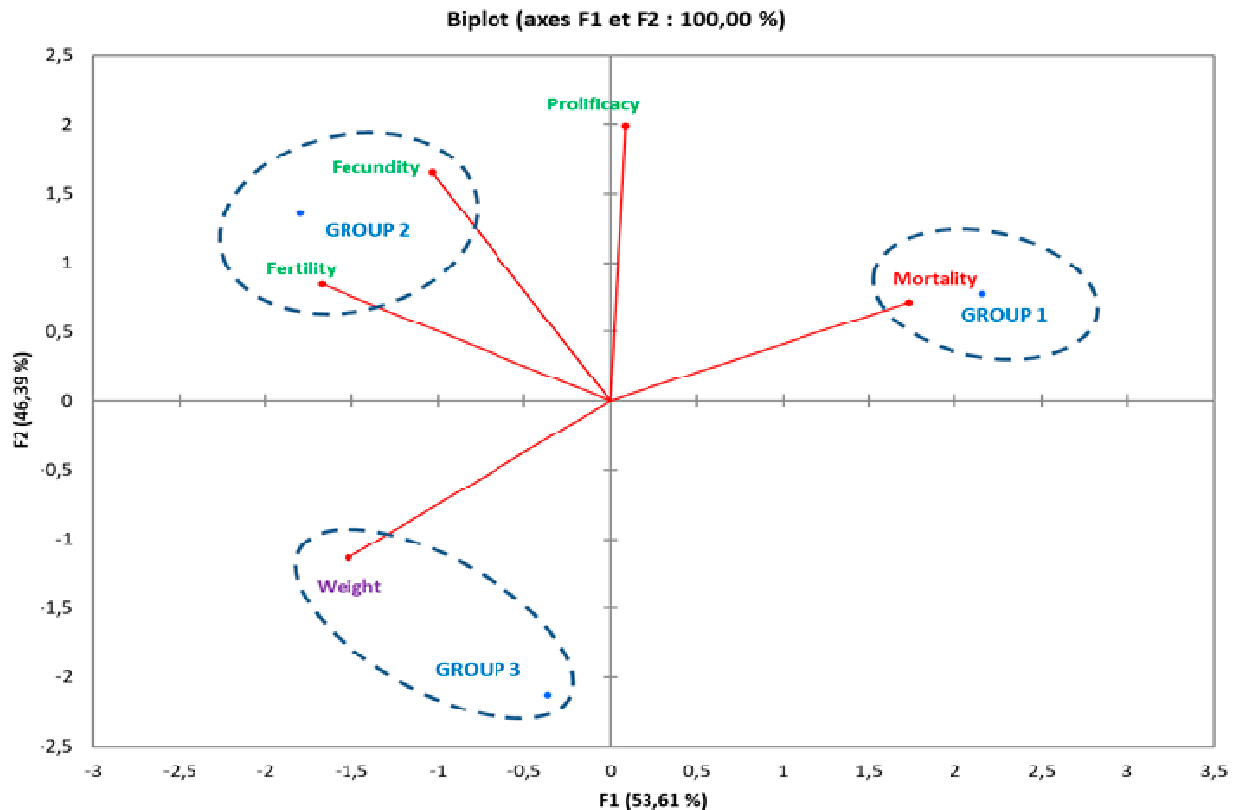


Fig. 1. Projection of three experimental ewe groups on the PCA factorial plane (F1 × F2) based on reproductive and productive performance indicators

hormonal responsiveness. Vitamin D₃ has been shown to upregulate endometrial progesterone receptors, thereby strengthening luteal function and pregnancy maintenance (Meng et al., 2023). Vitamin E exerts protective effects on oocyte integrity by counteracting oxidative stress (Hatami et al., 2022), while vitamin A contributes to follicular growth and maturation (Abdulkareem et al., 2023; Li et al., 2024). Together, these micronutrients create a metabolic and endocrine milieu favourable to the successful action of exogenous hormones, explaining the superior reproductive outcomes observed in the combined treatment group.

Neonatal viability and metabolic efficiency

Vitamin supplementation also had a profound impact on lamb survival and growth. The elimination of neonatal mortality, coupled with improved birth weights, suggests that vitamins AD₃E enhance maternal-offspring metabolic efficiency. Several mechanisms can be proposed: improved colostrum quality and immunoglobulin transfer (Reyes, 2023; Hurlburt, 2025), enhanced antioxidant defence capacity (Borges et al., 2018; Nikolova et al., 2023), and optimized neuromuscular coordination essential for suckling (Stenhouse et al., 2022). These effects are particularly critical in resource-constrained systems, where early neonatal losses remain a major bottleneck to productivity (Dwyer et al., 2016; Samuel and Shortnacy, 2025). The results confirm that beyond fertility enhancement, nutritional support contributes directly to offspring viability and long-term flock sustainability.

Comparative efficacy and economic perspectives

The reduced fertility observed in Group 1 (80%), which relied solely on hormonal synchronization, highlights the limitations of hormonal interventions when nutritional constraints are not addressed. This observation aligns with evidence that metabolic status significantly modulates ovarian responsiveness to exogenous hormones (Larsen, 2021; Martin et al., 2004; Bacha et al., 2017). From an economic standpoint, although the combined protocol produced the most favourable reproductive outcomes, vitamin supplementation alone (Group 3) also yielded substantial improvements at a lower cost. This approach may, therefore, represent a practical and scalable solution for smallholder or resource-limited farmers. The relative cost-benefit of each strategy must be carefully weighed against production objectives, market dynamics, and management capacities.

Practical and ethical implications

In practice, the choice between combined protocols and vitamin supplementation alone depends on the

production system and socio-economic context. While semi-intensive farms with structured breeding programmes may justify the higher investment in combined treatments, extensive systems may prioritize the cost-effective benefits of vitamin supplementation alone (Meziane et al., 2024). Ethical considerations also play a role, particularly regarding the use of PMSG and the necessity of sustainable reproductive management strategies (Veterinary Medical Ethics, 2022). Tailored recommendations for North African semi-arid regions should thus balance biological efficacy, economic feasibility, and ethical acceptability, ensuring that reproductive technologies remain both effective and accessible for livestock keepers.

Conclusion

This study successfully addresses its fundamental research objective by demonstrating that the integration of hormonal synchronization protocols with vitamin AD₃E supplementation significantly enhances reproductive outcomes in Rembi ewes during seasonal anoestrus under semi-arid conditions. The experimental results directly respond to the initial scientific problem of declining reproductive efficiency in North African sheep production systems by providing two evidence-based solutions: a combined protocol achieving optimal reproductive performance (100% fertility, 160% fecundity, 0% neonatal mortality), and a vitamin-only approach offering substantial improvements in lamb viability and birth weights.

The findings establish clear connections between our research aims and outcomes by:

1. validating the synergistic potential of combining reproductive biotechnologies with nutritional management;
2. demonstrating context-appropriate strategies for different production systems;
3. providing scientific justification for targeted nutritional interventions in reproductive management.

These results offer practical solutions to enhance production resilience in semi-arid regions while addressing both economic constraints and animal welfare considerations. Future investigations should focus on elucidating the molecular mechanisms underlying the observed vitamin-hormone interactions and conducting economic analyses to facilitate the implementation of these strategies in traditional farming systems.

This comprehensive approach effectively bridges the gap between experimental research and practical application, providing sustainable solutions to improve reproductive efficiency in challenging agricultural environments while maintaining scientific rigor and practical relevance.

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Guardians of the Forest: European Badgers (*Meles meles*) as Bioindicators of Environmental Health

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Keywords: *Meles meles*, European badger, pollution, bioindicator, sentinel, heavy metal, antibiotic resistance.

Abstract. The European badger (*Meles meles*), a widespread and adaptable mustelid native to Europe and parts of Asia, has emerged as a valuable sentinel species for monitoring environmental health. This review compiles multidisciplinary evidence demonstrating the utility of badgers as bioindicators for environmental contamination, antimicrobial resistance (AMR), and zoonotic diseases. Their omnivorous diet, frequent soil contact, and proximity to human-modified landscapes facilitate exposure to a range of ecological stressors. This review summarizes studies conducted across Europe from 2003 to 2022 that have detected numerous contaminants in badger tissues, including heavy metals (e.g., cadmium, lead, mercury), trace elements, and persistent organic pollutants, highlighting localised environmental pollution. Badgers have also been shown to harbour antibiotic-resistant bacteria such as *Escherichia coli*, *Staphylococcus aureus*, and *Enterococcus* spp., often carrying resistance genes like *SHV-12*, *vanA*, and *tet(B)*, suggesting their role in tracking environmental AMR spread. Additionally, badgers serve as reservoirs or incidental hosts for various zoonotic pathogens, including *Mycobacterium bovis*, *Leptospira interrogans*, *Toxoplasma gondii*, and *Borrelia burgdorferi*. The synthesis of this evidence supports the use of *M. meles* in One Health frameworks as a practical tool for assessing ecological risks and guiding public health and conservation strategies.

Introduction

The European badger (*Meles meles*) is a robust and adaptable mammal belonging to the Mustelidae family, order Carnivora. It possesses a distinctive appearance with a grizzled grey dorsal coat, dark ventral fur, and characteristic black and white facial stripes (Byrne et al., 2012). Badgers are primarily nocturnal, engaging in most of their foraging and social behaviours after dark. It holds a conservation status of “Least Concern” on the IUCN Red List, attributed to its extensive distribution and stable population across Europe and parts of Asia, thriving in varied environments thanks to its generalist diet, behavioural adaptability, and tolerance to human-modified landscapes (da Silva et al., 1993). They construct and inhabit extensive burrow systems known as setts, which can be quite elaborate and are often used communally. On average, they often live in groups of six adults, though associations as large as 23 individuals have been documented (Revilla and Palomares, 2002; Roper, 1992). Their preferred habitats are those offering moderate moisture, rich pastures, and sufficient cover, including tree-, shrub-, or rock-dense areas ideal for concealment and burrow placement. It has an omnivorous and opportunistic diet, with its primary food source including terrestrial

worms, especially earthworms (Goszczyński et al., 2000). They also consume a wide range of animal foods, including insects, molluscs, small mammals (like rabbits), birds, reptiles, amphibians, carrion, and even fish. In addition to animal prey, badgers also feed on plant materials, such as roots, tubers, fruits, seeds, nuts, and fungi (Cleary et al., 2009).

Eurasian badgers interact with human environments in both beneficial and problematic ways. On the positive side, they help control populations of wasps and hedgehogs, and their burrows contribute to biodiversity by providing shelter to other animals (Griffiths and Thomas, 1993) and in all states of Europe west of the border with the former Soviet Union. Within this area it is absent only from the arctic zones, high-altitude areas, and some islands. The Badger is currently a protected species in the UK, the Irish Republic, Spain, Portugal, Italy, Belgium, the Netherlands, Albania, Greece, Estonia, Luxembourg and Hungary, but Luxembourg and Hungary are to reconsider protected status. Elsewhere, the species is either considered as small-game or as a pest, hunting being regulated by closed seasons. At present Finland and Burgenland (Austria). Commercially, badger hair is used in brush-making, and badger hides are occasionally used in textiles. Conversely, they can cause damage to crops, fruit gardens, and property structures through their burrowing activities. They sometimes prey on poultry and are a reservoir for bovine tuberculosis (bTB), a disease that can

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be transmitted to cattle, resulting in significant economic losses for farmers (Cassidy, 2019). Despite their classification as Least Concern by the IUCN, local population densities can vary significantly and may be negatively affected by urban development, road mortality, and disease outbreaks, necessitating regional conservation monitoring (Fig. 1) (Fabrizio et al., 2019; Jenkinson and Wheeler, 1998) and on the number of illegal disturbance events recorded over the study period. The number of, and distance from, public accesses did not influence sett persistence, and the least frequently disturbed setts (i.e. those with an average of one or less disturbance incidents every 2 years).

Because the European badger is widely distributed across various habitats, consumes a broad and varied diet, and often inhabits areas near human activity, it has the potential to serve as a sentinel species (Board on Environmental Studies and Toxicology et al., 1991). A sentinel species is used to detect and monitor the presence and impact of environmental

contaminants within its habitat (Fig. 2) (Ozimec et al., 2015). Through the study of badgers, researchers can identify ecological threats such as infectious agents or other human-induced hazards. This makes the badger a valuable indicator for assessing risks to local wildlife populations, ecosystems, and potentially even human health (Goretti et al., 2018). This work aims to compile the studies that use the *Meles meles* as a sentinel species.

Materials and methods

The initial search on the Web included terms used in combination or isolation, such as “badger”, “European”, “*Meles meles*”, “pesticides”, “pollution”, “contamination”, “antibiotics”, “zoonoses”, yielding articles from digital databases (Web of Science, Scopus, PubMed, SciELO, Research Gate, Google Scholar). The inclusion/exclusion steps were independently reviewed by the two authors of the paper. Only papers in which the species, country, year, and agent were available were included. Another selection criterion



Fig. 1. European badgers (*Meles meles*) were admitted to the wildlife rehabilitation centre for several reasons, such as collisions with vehicles or entrapment

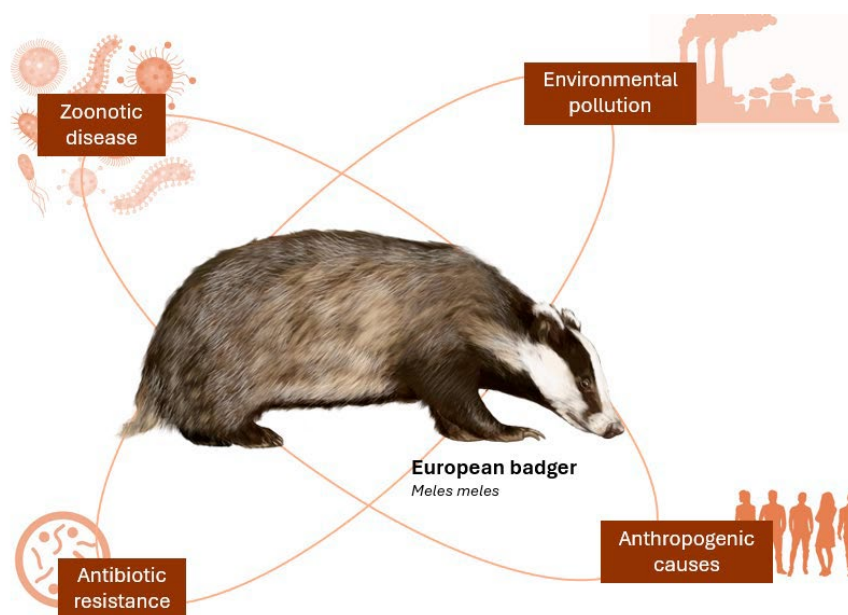


Fig. 2. *Meles meles* as a bioindicator sentinel of environmental ecosystem health (Author: Andreia Garcês)

was language, with only manuscripts in English, Portuguese, Spanish, and French being considered.

European badgers as a sentinel of environmental contamination

European badgers have been increasingly recognised as effective bioindicators of environmental pollution due to their wide distribution, omnivorous diet, and close interaction with the soil (Cleary et al., 2009; Mullineaux et al., 2021). Table 1 presents a review of various studies evaluating environmental contaminants found in European badgers across multiple countries. It summarises key information, including country, years of data collection, sample types analysed, contaminants identified, concentrations measured, and references to the original studies. Data spans from 2003 to 2022, covering nearly two decades of research across various European countries, including Croatia, the Czech Republic, Spain, Poland, Northern Ireland, the United Kingdom, and Italy (Mateo et al., 2012; Ozimec et al., 2015; Squadrone et al., 2022), highlight the accumulation of multiple contaminants in badger tissues such as liver, kidney, muscle, diaphragm, hair, and even tumours (Table 1). The contaminants analysed span a broad spectrum, including heavy metals (e.g., cadmium, lead, mercury), metalloids (arsenic, selenium), trace elements (aluminium, chromium, copper, zinc), and organic pollutants (organochlorine pesticides and polychlorinated biphenyls or PCBs). Concentrations vary significantly by country, tissue type, and pollutant. Notably high

levels of cadmium and zinc were found in the kidneys and livers of Spanish badgers (García-Muñoz et al., 2023), while Italian badger hair revealed high levels of aluminium and iron, suggesting significant environmental exposure (Squadrone et al., 2022). The use of different tissues provides insight into both short- and long-term exposure. Hair, for example, reflects chronic accumulation, while organs like the liver and kidneys indicate more recent or active exposure. Spain reported a wide variety of persistent organic pollutants, including DDE, DDD, Mirex, and several PCB congeners, some of which were found in relatively high concentrations, highlighting localised pollution.

Badger as a sentinel of antimicrobial resistance (AMR)

Table 2 compiles documented findings on antibiotic-resistant bacteria isolated from European badgers, showcasing their potential role as sentinels for environmental antimicrobial resistance (AMR) (O'Hagan et al., 2021). The studies performed from 1997 to 2019 across several European countries, including Ireland, Spain, Poland, Germany, and England, reveal the presence of a wide array of antibiotic-resistant bacteria in badger populations, isolated from faecal, nasal, rectal, and pharyngeal swabs (Osińska et al., 2020). *Escherichia coli* is the most frequently isolated and resistant species, with widespread resistance to beta-lactams (AMP, CZA, CTX), fluoroquinolones (CIP, N), aminoglycosides

Table 1. Review of articles that evaluated environmental contaminants in European badgers (*Meles meles*) regarding the number of animals, substance type, year, sample type analysed, and country

Country	Year	Type of sample	Substance	Concentration	References
Croatia	2009–2010	Muscle, liver, and kidney	As	0.0034 mg/kg, w.w.	(Bilandžić et al., 2012)
			Cd	0.009	
			Cu	2.99	
			Hg	0.005	
			Pb	0.077	
Czech Republic	2011	Liver, kidney, muscle, diaphragm, tumour	As	0.07; 0.02; 0.02; 0.03	(Bukovjan et al., 2014)
			Cd	1.09; 2.06; 0.10; 0.67	
			Cr	0.46; 0.31; 0.12; 0.16	
			Cu	9.47; 5.81; 0.17; 7.74	
			Hg	0.49; 0.67; 0.01; 0.36	
			Pb	2.98; 1.69; 0.26; 0.67	
			Zn	36.20; 28.30; 17.311; 44.54	
Spain	2020–2022	Liver, kidney	Zn	179; 164	(García-Muñoz et al., 2023)
			Hg	0.77; 1.16	
			Cd	4.70; 7.61	
			Pb	0.45	
			As	0.14; 0.08	
Poland	2003–2005	Kidney, liver, pectoral muscle	Hg	0.38; 0.64; 0.25	(Kaliszińska et al., 2009)

Table 1 cont.

Country	Year	Type of sample	Substance	Concentration	References
Spain	2004–2006	Liver	HCB	< 0.01	(Mateo et al., 2012)
			b-HCH	0.48	
			a-Heptachlor epoxide	< 0.01	
			a-Heptachlor epoxide	< 0.01	
			b-Heptachlor epoxide	< 0.01	
			p,p ⁰ -DDE	36.3	
			p,p ⁰ -DDD	5	
			Mirex	84.2	
			PCB 28–31	> 0.01	
			PCB 101	> 0.01	
			PCB 118	1.09	
			PCB 153	12.3	
			PCB 105	> 0.01	
			PCB 138	0.21	
			PCB 126	0.87	
			PCB 128	25.1	
			PCB 156	< 0.01	
PCB 180	< 0.01				
PCB 169	65				
PCB 170	< 0.01				
North Ireland	2017–2018	Liver, muscle, and kidney	Al	6.2; 12.47; 17.41	(Mullineaux et al., 2021)
			As	1.2; 1.2; 1.2	
			Cr	1.3; 2.37; 1.67	
			Cu	77.61; 12.81; 25.83	
			Mo	3.18; 1.4; 1.41	
			Ni	1.39; 1.44; 3.68	
			Pb	1.21; 1.2; 1.47	
			Se	4.22; 1.2; 8.2	
			Sr	0.35; 0.15; 0.54	
			Zn	113.39; 166.16; 83.08	
Croatia	2009–2011	Muscle, kidney, liver	Cd	0.074; 3.046; 0.395	(Ozimec et al., 2015)
			Pb	0.131; 0.190; 0.197	
United Kingdom	2017–2017	Liver	Ag	0.0381	(Sartorius et al., 2023)
			Al	8.37	
			As	0.0906	
			B	1.63	
			Ba	0.487	
			Be	0.0257	
			Ca	286	
			Cd	3.37	
			Co	0.149	
Cr	0.894				

Table 1 cont.

Country	Year	Type of sample	Substance	Concentration	References
United Kingdom	2017–2017	Liver	Cs	0.00707	(Sartorius et al., 2023)
			Cu	32.0	
			Fe	1160	
			K	8140	
			Mg	596	
			Mn	16.0	
			Mo	2.54	
			Na	3930	
			Ni	0.145	
			P	9020	
			Pb	1.38	
			Rb	6.45	
			S	8590	
			Se	3.05	
			Sr	0.328	
			Ti	105	
			Tl	0.0143	
U	0.00235				
V	0.282				
Zn	127				
Italy	2022	Hair	Al	849 ± 51	(Squadrone et al., 2022)
			As	1.1 ± 0.22	
			Cd	0.023 ± 0.001	
			Cr	2.5 ± 0.12	
			Cu	52 ± 0.43	
			Fe	852 ± 62	
			Hg	0.52 ± 0.019	
			Mn	27 ± 1.4	
			Ni	1.7 ± 0.84	
			Pb	0.83 ± 0.054	
			Pd	< LOQ	
			Pt	0.011 ± 0.001	
			Rb	1.0 ± 0.14	
			Sn	0.051 ± 0.001	
V	1.6 ± 0.16				
Zn	131 ± 10				

HCB – Hexachlorobenzene; PCB – Polychlorinated biphenyls; DDE – Dichlorodiphenyldichloroethylene; DDD – Dichlorodiphenyldichloroethane; Cr – Chromium; Cu – Copper; Ni – Nickel; Pb – Lead; Zn – Zinc; Cd – Cadmium; Mn – Magnesium; Hg – Mercury; Al – Aluminum; Ca – Calcium; Fe – Iron; Mg – Magnesium; As – Arsenic; Sr – Strontium; Am – Americium; Cs – Caesium; Pu – Plutonium; Co – Cobalt; V – Vanadium; Rb – Rubidium; Pt – Platinum; Sn – Tin; Tl – Thallium.

Table 2. Antibiotic resistance in European badgers (*Meles meles*) regarding species, country, year, type of sample, bacteria isolated, antibiotic resistance and resistance genes

Specie	Country	Year	Type of sample	Isolated bacteria	Antibiotic resistance*	Resistance genes	Ref.
Badger (<i>Meles meles</i>)	Ireland	2018–2019	Faecal, nasopharyngeal swabs	<i>Salmonella spp.</i> , <i>E. coli</i>	AMP, CZA, CEP, CTX	–	(O’Hagan et al., 2021)
	Spain	2016–2017	Swabs	<i>E. coli</i>	CIP, N, C, S, T	<i>SHV-12</i>	(Darwich et al., 2019)
	Poland	2014–2018	Rectal swabs	<i>E. coli</i>	AMP, S, KAN, C, CIP, S, N, TE	<i>aph(3c)-Ia</i> , <i>strA</i> , <i>aph(3c)-Ia</i> , <i>sul2</i> , <i>tetA</i> , <i>tetB</i> , <i>fbR</i> , <i>cat</i> , <i>sul3</i>	(Osińska et al., 2020)
	Germany	2011	Pharyngeal swab	<i>S.aureus</i>		<i>gapA</i> , <i>katA</i> , <i>CoA</i> , <i>Spa</i> , <i>sbi</i> , <i>nuc1</i> , <i>sarA</i> , <i>saeS</i> , <i>vraS</i> , <i>agrI</i> , <i>hid</i> ,	(Monecke et al., 2013)
	Spain	2015–2015	Faecal	<i>E. coli</i>	AMP, TE	<i>Tet(B)</i>	(Alonso et al., 2017)
	Spain	2012–2015	Nasal and rectal swabs	<i>Staphylococcus spp.</i>	N, P, FOX, FA, CLI		(García et al., 2020)
	England	1997 to 2000	Faecal	<i>Enterococcus spp.</i>	VAN, TEC	<i>vanA</i>	(Mallon et al., 2002)

AMP – ampicillin; E – erythromycin; CD – clindamycin; CEF – ceftiofur; CEP – cephalothin; CPN – cephalixin; CTX – cefotaxime; TE – tetracycline, N – nalidixic acid; CIP – ciprofloxacin; KAN – kanamycin; VAN – vancomycin; FA – fusidic acid; P – penicillin; TEC – tobramycin; FOX – ceftioxin.

(KAN), tetracyclines (TE), and other antibiotic classes (Darwich et al., 2019). Genetic analysis revealed important resistance genes such as *SHV-12* (an extended-spectrum β -lactamase), *tet(B)* (tetracycline resistance), and multiple mobile resistance elements like *aph(3’)-Ia*, *sul2*, *fbR*, and *cat*. Resistance genes such as *SHV-12*, *vanA*, *tet(B)*, and various plasmid-borne genes were detected, indicating potential for horizontal gene transfer. Of particular concern is the detection of *vanA* in *Enterococcus spp.* in England, conferring resistance to vancomycin, a last-resort antibiotic in human medicine (Mallon et al., 2002). Additionally, virulence and regulatory genes in *Staphylococcus aureus* (e.g., *spa*, *sarA*, *vraS*) suggest the presence of potentially pathogenic strains in the badger population (Monecke et al., 2013).

Badgers as a sentinel of zoonotic diseases

Wild animals can act as sentinels for the current health status of the ecosystems they inhabit. The European badger serves as an effective sentinel species for monitoring the presence and circulation of zoonotic pathogens in the environment. As omnivorous mammals with wide-ranging ecological niches – from woodland and farmland to peri-urban areas – badgers are exposed to a variety of infectious agents. Their scavenging behaviour and frequent contact with soil, water sources, and potentially contaminated prey increase their likelihood of acquiring and harbouring zoonotic pathogens. Badgers have been identified as natural reservoirs or incidental hosts for several

zoonoses of public and veterinary health importance, including *Mycobacterium bovis* (the causative agent of bovine tuberculosis), *Leptospira spp.*, *Toxoplasma gondii*, *Echinococcus multilocularis*, and *Trichinella spp.* Due to their relatively stable home ranges and long life span, badgers accumulate exposure to pathogens over time, making them valuable indicators of local and persistent infection risks. Their proximity to livestock and human settlements in many areas also raises concerns about the potential for spillover. Surveillance of zoonotic agents in *Meles meles* populations can thus provide early warning signals for emerging threats, support risk assessments, and contribute to the implementation of One Health strategies aimed at managing diseases at the human–animal–environment interface. While the list of pathogens associated with badgers continues to evolve, Table 3 provides a summary of zoonotic agents identified in the European badger (*Meles meles*).

Discussion

The European badger (*Meles meles*) emerges as a compelling sentinel species for evaluating the health of European ecosystems. Its wide distribution, omnivorous diet, longevity, and close association with soil and human-modified environments place it at the intersection of ecological, public, and veterinary health (da Silva et al., 1993). The present review consolidates a growing body of evidence demonstrating the value of badgers in monitoring environmental contaminants, antimicrobial resistance

(AMR), and zoonotic pathogens – three pillars of One Health surveillance.

Exposure of badgers to heavy metals, metalloids, and persistent organic pollutants (POPs) provides

Table 3. Zoonotic bacteria isolated from European badger (*Meles meles*)

Bacteria		
<i>Mycobacterium bovis</i>	Culture-confirmed infections from lymph/tissue in several countries	(Blanco Vázquez et al., 2021)
<i>Mycobacterium avium subsp. avium & paratuberculosis</i>	Isolated from lymph nodes in Central Italy	(Blanco Vázquez et al., 2021)
<i>Salmonella enterica</i>	Faecal carriage in UK and Italian populations	(Gambi et al., 2022)
<i>Leptospira interrogans</i>	50 % seroprevalence in Andalusia, Spain; antibodies in Slovenia	(Blanco Vázquez et al., 2021)
<i>Anaplasma phagocytophilum</i>	PCR-positive blood/spleen from nine European countries	(Lindhorst et al., 2024)
<i>Ehrlichia</i> spp.	Same continental survey (low prevalence)	(Lindhorst et al., 2024)
<i>Candidatus Neoehrlichia</i> spp.	First detected in badger blood, Hungary	(Hornok et al., 2017)
<i>Borrelia burgdorferi</i>	DNA recorded in badgers/ticks	(Lindhorst et al., 2024)
<i>Rickettsia</i> spp.	Molecular detections in continental survey	(Lindhorst et al., 2024)
<i>Bartonella</i> spp.	Multiple European detections	(Lindhorst et al., 2024)
<i>Coxiella burnetii</i>	Targeted and PCR-positive in some UK studies	(Guardone et al., 2020)
<i>Francisella tularensis</i>	Screened in the UK badgers; considered a risk host	(Guardone et al., 2020)
Virus		
<i>Rabies</i>	Occasional spill-over	(Margalida and Colomer, 2012)
<i>Coronavirus</i>	Discovered in Italian carcasses	(Zamperin et al., 2023)
<i>Caliciviridae</i>	Whole genome from Hungarian badgers	
<i>Badger gammaherpesvirus</i>	Higher viraemia variant reported in 2022	(Tsai et al., 2022)
Protozoaria		
<i>Giardia duodenalis</i>	Zoonotic genotypes in 49% of badgers in Central Italy	(Guardone et al., 2020)
<i>Cryptosporidium</i> spp.	Oocysts in 23% of the same study population	(Maestrini et al., 2022)
<i>Babesia</i> sp. badger types A–C (<i>B. microti</i> group)	Up to 89% prevalence; close relatives infect humans	(Guardone et al., 2020)
<i>Toxoplasma gondii</i>		(M et al., 2018)
<i>Sarcocystis</i> spp.		(K et al., 1994)
<i>Trypanosoma pestanai</i>		(G et al., 2021)
Ectoparasites		
<i>Ixodes ricinus</i> , <i>I. canisuga</i> , <i>I. hexagonus</i> , <i>I. reduvius</i> , <i>I. melicula</i>		(Guardone et al., 2020)
Mange mites (<i>Sarcoptes</i> spp., <i>Demodex</i> spp.)		(Mullineaux and Keeble, 2016)
Helminths and other		
<i>Trichinella britovi</i>	Muscle larvae in the Romanian badger	(Z et al., 2020)
<i>Diroflaria immitis</i> , <i>D. repens</i>	PCR detections across Europe	(Lindhorst et al., 2024)
<i>Thelazia callipaeda</i>	Romanian badger	(Ionică et al., 2019)
<i>Strongyloides stercoralis</i>		(Maestrini et al., 2022)
<i>Eucoleus</i> spp.	Eggs found in faeces; occasional human cases reported	(M et al., 2023)

Table 3 cont.

<i>Fungi</i>		
<i>Trichophyton mentagrophytes</i>	Dermatophytosis confirmed in badgers in Germany, the UK, and the Czech Republic. Lesions observed.	(Segal and Elad, 2021)
<i>Microsporium canis</i>	Occasionally isolated or suspected in badgers via dermatomycoses and contact with domestic animals in Europe.	(Segal and Elad, 2021)

a unique window into localised and long-term environmental contamination (Sartorius et al., 2023). Across Europe, toxic elements such as cadmium, lead, mercury, arsenic, and aluminium have been consistently detected in multiple tissues, with considerable inter-country variability. For instance, Spanish badgers exhibit markedly high concentrations of cadmium and zinc in liver and kidney tissues, while Italian specimens display elevated levels of aluminium and iron in hair, suggesting chronic exposure to industrial pollutants. Notably, the diversity of sampled matrices (liver, kidney, hair, muscle, and even tumours) enhances interpretative depth (Bukovjan et al., 2014), enabling both temporal and tissue-specific insights into pollutant bioaccumulation. Hair sampling, in particular, offers a non-invasive avenue for long-term exposure assessment, applicable to ongoing biomonitoring protocols. Furthermore, the detection of PCBs, DDT metabolites (DDE, DDD), and other organochlorines underscores the vulnerability of badgers to legacy contaminants still circulating in European landscapes despite regulatory bans (Alleva et al., 2006; Florijančić et al., 2013; Kalisińska et al., 2009).

Badgers also serve as reservoirs for antibiotic-resistant bacteria, underscoring their role in tracking environmental AMR dissemination. The presence of multidrug-resistant *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., and *Enterococcus* spp. in badger swabs from countries including Ireland, Spain, and Poland reflects a significant environmental burden of antimicrobial residues and resistant pathogens (Alonso et al., 2017; Gambi et al., 2022; Monecke et al., 2013). Detection of clinically relevant resistance genes such as *SHV-12*, *tet(B)*, and *vanA* – the latter conferring vancomycin resistance – raises concern about horizontal gene transfer in the wild and potential spillover into livestock and human populations (Mallon et al., 2002; Monecke et al., 2013). Furthermore, findings of virulence genes (e.g., *sarA*, *vraS*) in *S. aureus* suggest the presence of pathogenic strains with potential zoonotic implications (Garcia, 2017). These results highlight the necessity of incorporating wildlife into integrated AMR surveillance frameworks, particularly in rural-urban interface zones (Dolejska, 2020).

The extensive catalogue of zoonotic agents isolated from *Meles meles*, ranging from *Mycobacterium bovis* and *Leptospira interrogans* to *Toxoplasma gondii*

and *Trichinella britovi*, reaffirms the ecological role of badgers as a disease reservoir and sentry species (Corner et al., 2011; M et al., 2018; Žele-Vengušt et al., 2021). Several of these pathogens pose a dual threat to livestock productivity and human health, reinforcing the value of badgers in early warning systems for zoonoses. In particular, the consistent association of badgers with bovine tuberculosis across Europe warrants continued monitoring, especially given their overlapping habitats with cattle. The presence of other vector-borne pathogens such as *Anaplasma phagocytophilum*, *Babesia* spp., and *Borrelia burgdorferi* (Lindhorst et al., 2024) further positions badgers within complex vector–host–pathogen networks that require multi-sectoral management approaches. The detection of emerging viruses and protozoa in recent years suggests that badgers may also serve as sentinels for novel or re-emerging infectious agents in the European biome (Hornok et al., 2017).

The integration of badgers into One Health surveillance can support risk assessment and policy development for environmental protection, disease control, and antimicrobial stewardship. Veterinarians, particularly those working in wildlife rehabilitation, public health, or livestock management, are uniquely positioned to contribute to and benefit from badger-based sentinel data. Surveillance findings may inform land use decisions, livestock vaccination strategies, and zoonosis mitigation programmes, especially in regions where badger–livestock–human contact is frequent (Bezerra-Santos et al., 2021; Letková et al., 2006). Nevertheless, challenges remain. Standardisation of sampling protocols, harmonisation of contaminant reporting metrics, and the development of longitudinal studies are necessary to improve cross-regional comparability. Ethical and logistical considerations related to badger handling, particularly in protected areas or disease control contexts (e.g., bTB culling programmes) (Corner et al., 2011; Osińska et al., 2020) must also be addressed with sensitivity to local regulations and public perception (Aranaz et al., 2004).

Despite the growing recognition of *Meles meles* as a valuable sentinel for One Health surveillance, several knowledge gaps limit its full application across Europe. A key constraint is the lack of standardised sampling and analytical protocols. Studies differ in the biological matrices analysed and laboratory techniques

used, hindering cross-country comparisons and the establishment of continental baselines for pollutants, antimicrobial resistance (AMR), and zoonotic pathogens. Developing harmonised methods and centralised data repositories is, therefore, essential to enable consistent spatial and temporal analyses (Aranaz et al., 2004). Most current research provides cross-sectional data, with limited longitudinal monitoring to track temporal trends in contaminant bioaccumulation or pathogen prevalence. Long-term, non-invasive monitoring, using matrices such as hair, faeces, or environmental DNA, would improve understanding of chronic exposures and seasonal variation. Similarly, while AMR has been detected in badgers, the ecological and behavioural pathways driving resistance acquisition and transmission remain poorly understood. Integrating metagenomic and resistome analyses could clarify links between wildlife, livestock, and human environments (Corner et al., 2011; Osińska et al., 2020).

The role of badgers in emerging zoonoses also warrants deeper investigation. Surveillance should be expanded to include novel and re-emerging pathogens using molecular and serological tools, alongside modelling of badger–vector–pathogen interactions under changing climatic and land-use conditions. Additionally, research has been geographically biased toward Western Europe, leaving Eastern and Northern regions underrepresented despite differing

environmental pressures and management practices. Finally, ethical and social challenges surrounding badger research, particularly in tuberculosis control contexts, require attention. Promoting non-lethal sampling and understanding public attitudes toward wildlife surveillance will be key to sustainable monitoring strategies. Addressing these gaps through coordinated, interdisciplinary research will strengthen the integration of badgers into One Health frameworks and enhance their value as indicators of environmental and public health (Bezerra-Santos et al., 2021; Letková et al., 2006).

Conclusion

Eurasian badgers offer a versatile and ecologically relevant model for monitoring environmental health. Their utility as sentinels of pollution, AMR, and zoonotic disease underlines their importance in multidisciplinary One Health strategies. Continued investment in wildlife surveillance infrastructure and intersectoral collaboration will be essential to harness the full potential of *Meles meles* in safeguarding both ecosystem and public health.

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Influence of Maca (*Lepidium meyenii*) on Selected Chemical and Technological Parameters of Rabbit Meat

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Abstract. The aim of this study was to evaluate the effect of dietary supplementation with maca (*Lepidium meyenii*) root powder on selected chemical, nutritional, and technological parameters of rabbit meat, focusing on *Musculus vastus lateralis* (MVL) and *Musculus longissimus dorsi* (MLD). Fifty-four adult male rabbits of the Nitra breed were divided into three groups: a control group without supplementation and two experimental groups receiving 0.3% (E1) and 0.6% (E2) maca root powder in the feed ration for 13 weeks. In the MLD muscle, protein content was significantly higher in group E2 compared with both the control and E1 groups. The content of omega-6 fatty acids was also significantly increased in group E2, while no differences were observed in saturated, monounsaturated, and omega-3 fatty acids. In both muscles, the pH values of meat during storage were significantly lower in E2 compared with the control. Meat colour was also affected, with higher yellowness (b^*) values observed in experimental groups after storage. No significant differences were detected in lipid oxidation parameters among groups. Overall, maca supplementation, particularly at 0.6%, enhanced the nutritional quality of rabbit meat by increasing protein and omega-6 fatty acid content, without compromising oxidative stability.

Introduction

Rabbit meat is a traditional dietary component in European countries, which together account for the second-highest global production of meat rabbits. The majority (83%) of production takes place in Spain (48.5 million rabbits slaughtered), followed by France (29 million) and Italy (24.5 million) (EFSA, 2020). Globally, rabbit meat consumption remains low compared with other types of meat (0.19 kg per capita per year). However, in the European Union (EU), average consumption is around 0.51 kg per capita per year (Spain 1.09 kg, Italy 0.91 kg, and France 0.75 kg) (Szendró et al., 2020). To respond to the needs of the agri-food system and consumers, the scientific community is increasingly focused on developing sustainable feed additives to improve rabbit welfare and meat quality.

Meat quality is influenced by the animal's health before slaughter, the composition of the feed ration, and both microbial and chemical contamination.

Chemical composition significantly affects technological parameters such as colour, texture, water-holding capacity (WHC), and pH (Mínguez et al., 2017; Dalle Zotte, 2002). Age, genetics, diet composition, and processing technology are also fundamental factors influencing meat quality. Rabbit meat is a low-calorie, highly digestible food with low fat and cholesterol content, but rich in unsaturated fatty acids (UFA) (Pavelková et al., 2017). Due to the low content of saturated and high content of UFA compared with other meats, rabbit meat has a favourable nutritional profile (Mínguez et al., 2017). It is considered healthier than other commonly consumed meats due to its high protein and low-fat content (Nistor, 2013). Protein levels in rabbit, chicken, and turkey meat are relatively high (20–21.9%), and the amino acids present are of a high biological value, with a relatively low energy content (427–849 kJ/100 g of fresh meat) compared with other meats (Gašperlin et al., 2010). The fat content of rabbit meat depends largely on feed composition, age, and genetic predisposition to fat storage, typically ranging within 1–12 g/100 g. This is comparable to

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veal (1–7 g/100 g) and chicken (0.9–12 g/100 g) (Combes, 2004). The fatty acid (FA) composition of rabbit meat is influenced by dietary fat sources and, in turn, affects the main physical, chemical, and sensory properties of the meat (Peiretti and Meineri, 2008).

Given these aspects of meat quality, plants and phyto-derivatives have received considerable attention as safe feed additives in animal nutrition. Maca (*Lepidium meyenii*), known as “Peruvian Maca”, originates from South America and has been used as a traditional dietary supplement since the Incan era. Belonging to the *Brassicaceae* family, it was originally cultivated in the central highlands of the Peruvian Andes. Maca is classified into black, purple, and yellow varieties according to hypocotyl colour (Tang *et al.*, 2017). In 1992, the Food and Agriculture Organization (FAO) recommended maca as a safe food. After two decades of development, it has become one of the main products on the global healthcare market (Meissner *et al.*, 2019). Maca roots can reach a circumference of about 20 cm, while plants grow to a height of 10–20 cm. Considerable genetic diversity exists in root morphology, with variations in weight (1–5 kg), shape, and skin and flesh colour (white, cream, yellow, orange, red, and magenta) (Jin *et al.*, 2018). Dried maca hypocotyls are rich in nutrients such as carbohydrates, proteins, lipids, essential amino acids, and free fatty acids, and contain numerous secondary metabolites with strong antioxidant properties (Tafari *et al.*, 2019). Antioxidant levels vary depending on soil composition, harvest time, drying process, and extraction method. Nevertheless, maca consistently contains key antioxidant compounds, including phenols, glucosinolates, alkaloids, and polysaccharides, which perform various metabolic and antioxidant functions (Korkmaz, 2018).

In animal production research, the effects of natural dietary compounds are often assessed by measuring primary and secondary oxidation products, which serve as indicators of oxidative status. These include conjugated dienes, hydroperoxides, and malondialdehyde (Niki *et al.*, 2005; Ross and Smith, 2006). Meat colour, the first quality parameter consumers notice, is influenced by myoglobin (Kirtil *et al.*, 2015). Fat and protein oxidation promote myoglobin oxidation, whereby deoxymyoglobin and oxymyoglobin are converted into metmyoglobin, leading to discolouration (Mancini and Hunt, 2005). Texture, particularly softness and tenderness, is affected by protein and lipid oxidation, which are crucial for consumer sensory preferences. Increased hardness is an undesirable consequence of oxidation and has a negative effect on texture quality. This is particularly relevant for rabbit meat, owing to its higher UFA content, whereas meats with lower UFA content, such as beef, are less affected (Suman and Joseph, 2013). Reduced water holding capacity (WHC) is associated with the formation of protein cross-links, which widen the gaps between muscle

fibres and consequently allow greater water release. Water loss is also influenced by genetic factors and muscle composition (Cayuela *et al.*, 2004).

Therefore, the aim of this study was to evaluate the effect of dietary maca root powder supplementation on rabbit productive performance and selected physical, chemical, and quality parameters of meat.

Materials and methods

Animals and diets

All experiments were conducted in accordance with EU Directive 2010/63/EU and national guidelines for the care and use of animals. All experimental procedures involving animals were approved by the National Agricultural and Food Centre Ethical Committee (Permission code: SK CH 17 021).

Adult male rabbits ($n = 54$; 17.5 ± 0.9 weeks of age; average weight 4.701 ± 0.1425 kg) were divided into three groups, each consisting of 18 animals (three animals per cage). The lighting cycle throughout the trial was 16 hours light and 8 hours dark. Heating and forced ventilation maintained the building temperature at $18 \pm 4^\circ\text{C}$, with relative humidity at $70 \pm 5\%$. The choice of adult rabbits for dietary strategy experiment on meat quality traits is unusual, but it was subordinate to another experiment evaluating the reproductive traits on the same animal population.

The control group (C) received no feed supplement. Experimental group 1 (E1) received 0.3% maca root powder (*Lepidium meyenii*), while experimental group 2 (E2) received 0.6% maca root powder. The maca powder (Bio Maca, GymBeam s.r.o.) was incorporated into the feed components and subsequently pelleted. Water was provided *ad libitum*. The phenolic compound content of the dietary supplement was analysed by HPLC–UV–DAD (Russo *et al.*, 2017) and is reported in Table 1.

The complete feed mixture (Humino Feed, Vetservis s.r.o., Nitra, Slovakia) was used throughout the experiment. Its basic composition was protein 15%, fibre 17%, ash 10%, calcium 0.80%, phosphorus 0.50%, sodium 0.14%, vitamin A 8000 IU/kg, vitamin D₂ 800 IU/kg, and vitamin E 40 mg/kg. According to the manufacturer, the maca powder contained (per 100 g): fat 0.8 g (saturated fatty acids 42.7%, unsaturated fatty acids 53.4%), carbohydrates 76 g, fibre 18 g, protein 12 g, minerals 0.12 mg, vitamin B₁ 0.21 mg, vitamin B₂ 1.95 mg, vitamin B₆ 0.3 mg, calcium 351 mg, potassium 1784.2 mg, magnesium 92 mg, iron 13 mg, sodium 92.1 mg, phosphorus 192 mg, and zinc 4.1 mg.

The experiment was conducted at the Institute of Small Farm Animals, Research Institute of Animal Production – National Agricultural and Food Centre (Lužianky, Slovakia). After 13 weeks of feeding, slaughter took place in slaughterhouse for rabbits at the Research Institute of Animal Production in Nitra. Samples were then subjected to further analyses at

Table 1. Polyphenols content (mg/kg DW) in the natural Maca supplement

Phyto-derivate family name	Compound	Amount in Maca*
Phenolic Acid	Dihydroxybenzoic acid	1.923 ± 0.025
	Vanillic acid	69.430 ± 1.170
Hydroxycinnamic acids	Tans p-coumaric acid	11.727 ± 0.230
	Resveratrol	21.697 ± 0.755
	Ferulic acid	9.371 ± 0.018
Flavonoids	Quercitin	8.238 ± 0.128
	Rutin	61.480 ± 0.459
	Myricetin	9.630 ± 0.111
	Kaempferol	≤ LOD

* Limit of detection; values expressed as means (n = 4) ± standard deviation.

the Institute of Food Sciences, Slovak University of Agriculture in Nitra.

Experimental sampling

Animals were weighed at day 0 and at 30-day intervals throughout the trial. Feed intake was recorded weekly (feed offered and refused). These two parameters were used to monitor and to assess the health conditions of rabbits and the grade of acceptance of diets during the whole experiment.

At the end of the trial, rabbits were weighed and, after a 6-hour fasting period, 10 animals per group were randomly selected for slaughter. Rabbits were stunned electrically (100 V, 50 Hz, 2–3 s) and sacrificed by bleeding according to the guidelines established by the European Community (1099/2009/EC) for the protection of animals during slaughter.

Carcasses were chilled for 24 hours at +4°C and then dissected according to the recommendations of the World Rabbit Science Association (WRSA) (Blasco and Ouhayoun, 1996), with removal of the skin, distal parts of the limbs, genital organs, bladder, and gastrointestinal tract. Carcasses were then weighed, and dressing percentages were calculated. Samples of *Musculus longissimus dorsi* (MLD) and *Musculus vastus lateralis* (MVL) were collected. Physical parameters were measured on freshly cut MLD and MVL muscles. Whole muscles were sampled for chemical analyses, vacuum packed, and stored at –20°C.

Physical and chemical parameters

Physical determinations were carried out immediately after arrival at the laboratory. Within 45 minutes of slaughter, the pH of the MLD and MVL muscles (Hanna HI 99161; Hanna Instruments, Woonsocket, RI, USA) was measured, and the measurement was repeated after 1, 3, and 5 days of storage. Colour measurements were performed on day 0 and after 5 days of refrigerated storage at 4°C,

using a Konica Minolta 2600D spectrophotometer (Konica Minolta, Tokyo, Japan). The instrument was calibrated in the CIE LAB colour space system using a white calibration plate (CR-A43, Minolta Cameras). The colourimeter had an 8 mm measuring area and was illuminated with a pulsed xenon arc lamp at a 0° viewing angle. Reflectance measurements were obtained at 0° with the spectral component included. To allow blooming, trays were opened and data were collected after 30 minutes. Each data point represents the mean of six replicates measured on the chop surface.

Moisture (method 985.41), ash (method 920.153), fat (method 960.39), and crude protein (method 928.08) contents were determined in duplicate on MLD and MVL muscle samples, following the methods of the Association of Official Analytical Chemists (AOAC, 2000).

Cholesterol content was determined according to Du and Ahn (2002) using gas chromatography. Cholesterol was identified based on the retention time of a standard (Sigma Aldrich, St. Louis, MO, USA) and quantified using Chrom Card Data System software (version 1.17). All samples were analysed in triplicate.

Nutritional Traits and Shelf-Life of Meat

Amino acids were determined in fat-free samples by ion-exchange chromatography (free amino acids) and by liquid chromatography (total amino acids) after acid hydrolysis in 6 M HCl. Sulphur amino acids were determined after hydrolysis with hydrogen peroxide and formic acid. Separation was performed using an Amino Acid Analyser AAA 400 (Ingos a.s., Prague, Czech Republic).

The fatty acid composition of intramuscular fat samples was determined after chloroform-methanol extraction according to Folch et al. (1957). Fatty acids were analysed as methyl esters (FAME) (Dal Bosco et al., 2004) using a ThermoQuest TRACE 2000 gas

chromatograph (SACTM-5 column, 30 m × 0.25 mm; Supelco, USA). Fatty acids were identified on the basis of elution times corresponding to the standard (FAME PUFA2, Supelco, Bellefonte, PA, USA).

Health lipid indices were estimated according to Ulbricht and Southgate (1991). From the fatty acid composition, indices of atherogenicity (AI) and thrombogenicity (TI) were calculated using the following formulas:

$$AI = [(4 \times 14:0) + (16:0)] \times [PUFA (n-6 \text{ and } n-3) + MUFA]^{-1}$$

$$TI = [(14:0) + (16:0) + (18:0)] \times [(0.5 \times MUFA) + (0.5 \times n-6) + (3 \times n-3) + (n-3 \times n-6 -1)]^{-1}$$

Lipid oxidation during storage (1, 3, and 5 days at 4°C) was determined using the thiobarbituric acid reactive substances (TBARS) method of Meineri *et al.* (2010), in accordance with ASPA (1996) recommendations. All analyses were performed in duplicate. Absorbance at 532 nm was measured with a spectrophotometer. TBARS values, expressed as mg malondialdehyde (MDA)/kg meat, were obtained using a conversion factor based on a standard curve prepared with MDA (Sigma-Aldrich, Milan, Italy).

Statistical analyses

Statistical analyses were performed using SPSS (version 26.0; IBM, Armonk, NY, USA). The data on growth performance and meat quality parameters (physical and chemical parameters, amino acid profile, and fatty acid profile) were analysed by one-way analysis of variance (ANOVA), with dietary treatment as the main factor. Oxidative stability data were analysed by repeated-measures ANOVA to assess the effects of treatment, time, and their interaction. The experimental unit for growth performance was the cage, while the individual carcass served as the experimental unit for meat quality parameters.

Data are presented as means ± SEM, and statistical significance was accepted at $P < 0.05$.

Results and discussion

Quantification of the polyphenol profile

The qualitative profile of phenolic compounds in maca root powder and the concentrations of individual compounds are shown in Table 1. Rutin and vanillic acid (61.480 and 69.430 µg/kg d.w., respectively) were the main phenolic components, followed by resveratrol, trans-p-coumaric acid, myricetin, ferulic acid, quercetin, and dihydroxybenzoic acid. By contrast, kaempferol was not detected. In accordance with the literature (Jagdale *et al.*, 2021), flavonoids and phenolic acids phyto-derivate families are the most abundant in maca root powder. Considering that the solubility of polyphenols in solvent of different polarity is determined by their structure, different types of extraction solvent and procedures may influence the efficiency of phenolic compounds extraction and their resultant content (Veličković *et al.*, 2014).

Growth performance and carcass characteristics

Throughout the experimental period, the welfare status of the animals was consistently considered good. The present study did not focus on growth performance, as the animals were adults and the number of replicates was relatively low for the evaluation of productive traits. The dietary supplementation with maca root powder did not result in any statistically significant differences in body weight (Fig. 1) and no evident clinical signs were observed in rabbits during the 13-week trial, with an average feed intake of 180 g/day per animal. Similar to our findings, Uchiyama *et al.* (2014) reported no differences in body weight between experimental groups supplemented with

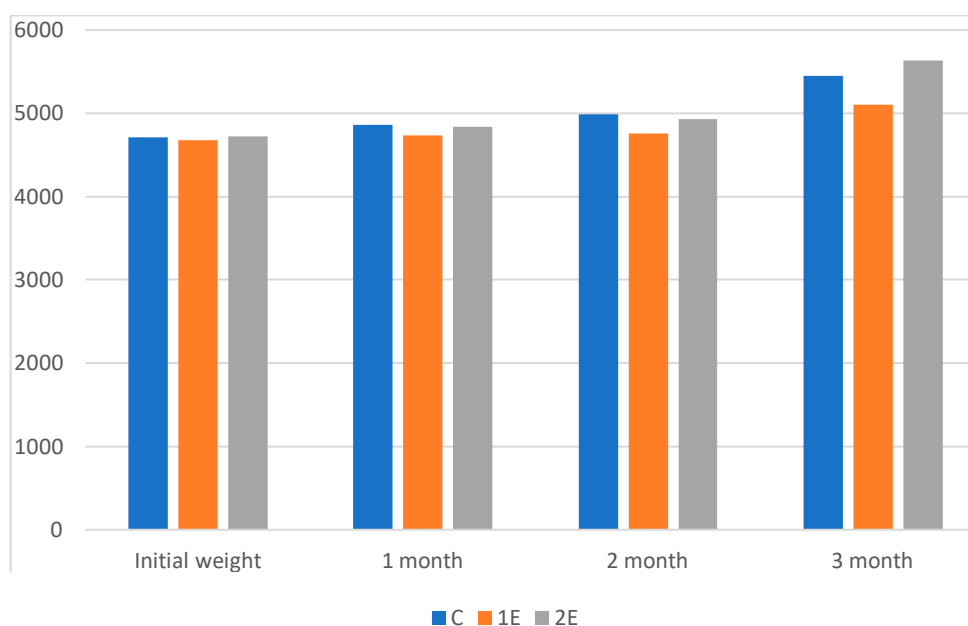


Fig. 1. Comparison of average rabbit live weights (g) during the experiment (months)

maca, whereas Lee et al. (2004) observed faster growth in fish fed with maca.

Chemical composition and nutritional value of meat

In the MLD muscle, protein content in group E2 ($25.550 \pm 0.549\%$) increased significantly ($P \leq 0.05$) compared with both the control ($24.930 \pm 0.493\%$) and group E1 ($24.910 \pm 0.293\%$), suggesting a different nutrient accumulation process in rabbits fed the higher dose of maca. In the MVL muscle, protein content in group E2 did not differ significantly from groups C and E1 (Table 2). All other determinations

regarding meat chemical composition were not affected by dietary treatment. Comparable results were obtained by Semenova et al. (2021), who fed growing rabbits for 45 days with a powdered feed additive titrated at 70% dihydroquercetin. In contrast, other studies (Minardi et al., 2020; Vizzarri et al., 2014) reported no effect on rabbit and brown hare meat traits after supplementation with antioxidant feed additives. Analysis of the protein profile of rabbit meat revealed no significant differences in amino acid content in either of the muscles studied, although some variation among groups was observed (Table 3).

With respect to the lipid fraction (Table 4), the

Table 2. Chemical composition¹ of rabbit meat in the back and thigh rabbit muscles

Item ²	Dietary treatment ³				
	C	E1	E2	SEM	P value ⁴
<i>musculus longissimus dorsi</i>					
Moisture, %	72.94	72.99	73.00	0.010	ns
Crude protein, %	24.93 ^a	24.91 ^a	25.55 ^b	0.115	0.05
Crude fat, %	0.88	0.89	0.78	0.019	ns
Ash, %	1.25	1.21	0.67	0.103	ns
Cholesterol, mg/100g	36.30	35.60	32.60	0.622	ns
<i>musculus vastus lateralis</i>					
Moisture, %	71.86	71.50	71.85	0.065	ns
Crude protein, %	23.80	23.70	23.93	0.036	ns
Crude fat, %	0.76	0.68	0.84	0.025	ns
Ash, %	3.58	4.12	3.38	0.121	ns
Cholesterol, mg/100g	38.80	39.00	39.10	0.048	ns

¹Data are reported as mean values and SEM, n = 10.

²Data expressed as percentage of wet weight.

³Control group (C); experimental group 1 (E1) with the addition of 0.3% of maca; experimental group 2 (E2) with the addition of 0.6% of maca.

⁴Different letters on the same row differed for $P < 0.05$; ns = not significant.

Table 3. Content of selected amino acids in individual experimental groups in the back and thigh rabbit muscles

Parameter ¹ (g/100g)	Experimental groups ²			SEM	P value ³
	C	E1	E2		
<i>musculus longissimus dorsi</i>					
Arginine	1.124	1.144	1.142	0.003	ns
Cysteine	0.270	0.268	0.278	0.002	ns
Phenylalanine	0.724	0.733	0.733	0.002	ns
Histidine	0.729	0.745	0.738	0.003	ns
Isoleucine	0.686	0.701	0.696	0.002	ns
Leucine	1.416	1.436	1.433	0.003	ns
Lysine	1.495	1.522	1.521	0.005	ns
Methionine	0.555	0.566	0.577	0.003	ns
Threonine	0.855	0.861	0.856	0.001	ns
Valine	0.793	0.807	0.796	0.002	ns

Table 3 cont.

Parameter ¹ (g/100g)	Experimental groups ²			SEM	P value ³
	C	E1	E2		
<i>musculus vastus lateralis</i>					
Arginine	1.190	1.227	1.202	0.006	ns
Cysteine	0.250	0.246	0.250	0.001	ns
Phenylalanine	0.759	0.777	0.766	0.003	ns
Histidine	0.769	0.776	0.771	0.001	ns
Isoleucine	0.736	0.760	0.742	0.004	ns
Leucine	1.481	1.520	1.492	0.006	ns
Lysine	1.578	1.625	1.598	0.007	ns
Methionine	0.559	0.566	0.566	0.001	ns
Threonine	0.869	0.880	0.870	0.002	ns
Valine	0.833	0.845	0.844	0.002	ns

¹Data are reported as mean values and SEM, n = 10.

²Control group (C); experimental group 1 (E1) with the addition of 0.3% of maca; experimental group 2 (E2) with the addition of 0.6% of maca.

³ns = not significant.

Table 4. Lipid profile in the back and thigh rabbit muscles

Parameter ¹ (g/100g FAME)	Experimental groups ²			SEM	P value ³
	C	E1	E2		
<i>musculus longissimus dorsi</i>					
MUFA	48.700	49.130	49.200	0.085	ns
PUFA	11.340	11.670	12.160	0.131	ns
SFA	35.090	34.340	35.080	0.136	ns
Omega 3	0.483	0.483	0.492	0.002	ns
Omega 6	9.226 ^a	9.350 ^a	10.020 ^b	0.135	0.05
Essential FA	8.741	8.641	8.646	0.019	ns
<i>musculus vastus lateralis</i>					
MUFA	47.790	47.410	47.930	0.085	ns
PUFA	11.600	12.130	11.840	0.084	ns
SFA	33.230	32.910	33.560	0.103	ns
Omega 3	0.470	0.457	0.456	0.002	ns
Omega 6	8.563	8.613	8.760	0.032	ns
Essential FA	9.289	9.533	9.242	0.049	ns

¹Data are reported as mean values and SEM, n = 10; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

²Control group (C); experimental group 1 (E1) with the addition of 0.3% of maca; experimental group 2 (E2) with the addition of 0.6% of maca.

³Different letters on the same row differed for $P < 0.05$; ns = not significant.

content of omega-6 fatty acids in group E2 (10.020 ± 0.500 g/100 g FAME) was significantly higher ($P \leq 0.05$) than in groups E1 (9.350 ± 0.576 g/100 g FAME) and C (9.226 ± 0.547 g/100 g FAME). These results agree with those of Palazzo *et al.* (2015), who reported an increased proportion of polyunsaturated fatty acids in rabbit meat following supplementation with *Lippia citriodora* extract. Increases in omega-6

fatty acids were also observed by Staerfl *et al.* (2011) in bulls whose diets were enriched with maca and other natural substances. No significant differences were detected in the overall content of saturated, monounsaturated, polyunsaturated, or omega-3 fatty acids. For the selected individual fatty acids analysed, no differences were detected at any level of significance (Table 5).

Table 5. Content of selected fatty acids in the back and thigh rabbit muscles

Parameter (g/100g FAME) ¹	Experimental groups ²			SEM	P value ³
	C	E1	E2		
<i>musculus longissimus dorsi</i>					
Heptadecanoid acid	0.318	0.297	0.327	0.005	ns
Lauric acid	0.104	0.104	0.101	0.001	ns
Myristic acid	1.351	1.373	1.364	0.004	ns
Palmitic acid	24.380	24.410	24.470	0.015	ns
Stearic acid	10.790	10.630	10.700	0.025	ns
Linolenic acid	0.280	0.275	0.260	0.003	ns
Linoleic acid	5.090	5.157	5.547	0.078	ns
Oleic acid	37.860	36.720	38.190	0.244	ns
Vaccenic acid	4.861	4.875	4.834	0.007	ns
CLA	0.128	0.125	0.131	0.001	ns
DHA	0.035	0.033	0.035	0.000	ns
ARA	1.684	1.610	1.730	0.019	ns
EPA	0.106	0.097	0.102	0.001	ns
DPA	0.139	0.137	0.137	0.000	ns
<i>musculus vastus lateralis</i>					
Heptadecanoid acid	0.285	0.288	0.285	0.001	ns
Lauric acid	0.109	0.107	0.103	0.001	ns
Myristic acid	1.412	1.425	1.410	0.003	ns
Palmitic acid	24.530	24.500	24.550	0.008	ns
Stearic acid	10.650	10.490	10.630	0.028	ns
Linolenic acid	0.264	0.261	0.255	0.001	ns
Linoleic acid	4.534	4.466	4.384	0.024	ns
Oleic acid	29.060	26.520	28.340	0.414	ns
Vaccenic acid	4.975	5.011	4.970	0.007	ns
CLA	0.132	0.131	0.127	0.001	ns
DHA	0.029	0.031	0.030	0.000	ns
ARA	1.904	1.899	1.828	0.013	ns
EPA	0.100	0.101	0.095	0.001	ns
DPA	0.129	0.129	0.130	0.000	ns

¹Data are reported as mean values and SEM, n = 10.

²Control group (C); experimental group 1 (E1) with the addition of 0.3% of maca; experimental group 2 (E2) with the addition of 0.6% of maca.

³ns = not significant.

Meat physical parameters

At 45 minutes post-mortem, the lowest pH values of the MLD and MVL muscles were recorded in group E1. The highest pH values were observed in groups C and E2, although these differences were not statistically significant. In the MLD muscle, a significant difference ($P \leq 0.0001$) was recorded on the first day of storage between group E2 (5.154 ± 0.050) and the control group (5.414 ± 0.140). The pH in group E1 (5.239 ± 0.082) was also significantly lower ($P \leq 0.01$) than in the control group. On the third day, the pH of group E2 (5.199 ± 0.063) was

significantly lower ($P \leq 0.0001$) than in the control (5.493 ± 0.149). A significant difference was also observed between groups E2 and E1 (5.356 ± 0.118), with the latter having a higher pH ($P \leq 0.05$). By the fifth day of storage, group E2 again had a significantly lower pH ($P \leq 0.001$) than both group C and group E1.

In the MVL muscle, no differences among groups were observed at 45 minutes post-mortem. However, on the first day of storage, group E2 (5.324 ± 0.068) had a significantly lower pH ($P \leq 0.01$) compared with both group C and group E1. On the third day, the pH of group E2 (5.431 ± 0.090) was again significantly

lower ($P \leq 0.01$) than in the other two groups. By the fifth day, the pH of group E2 (5.190 ± 0.157) remained the lowest of all groups (Table 6).

The pH of meat depends strictly on muscle energy metabolism and plays a key role in maintaining meat quality during storage. Low pH exerts a bacteriostatic effect, thereby regulating microbial growth (Dalle Zotte, 2002). Under our experimental conditions, the lower pH values observed in group E2 after five days of storage in both muscles suggest a biological effect of maca on rabbit meat.

Meat colour is also a critical quality attribute influencing consumer purchasing decisions (Kozioł *et al.*, 2015). Colour measurements (Table 7) showed significantly higher b^* values in MLD muscles of groups E1 and E2 after five days of storage. According to Münch (2004), this may be linked to free radicals produced by lipid oxidation during storage or

handling, which can oxidise haem pigments and cause meat discolouration. Since no differences were detected in the lipid oxidation profile of meat, further investigations are warranted.

Oxidative stability

Groups E1 and E2 showed no significant differences in malondialdehyde content compared with group C throughout the storage period (Table 8). Overall, lipid oxidation increased significantly ($P < 0.001$) with storage time across all groups. Maca supplementation did not alter this trend, with similar increases observed from day one to day five in all groups. In contrast, Staerfl *et al.* (2011) reported improved oxidative stability in bull meat, while Rossi *et al.* (2020) found that feeding growing rabbits brown seaweed and polyphenols enhanced the oxidative stability of meat.

Table 6. pH during storage in rabbit MLD and MVL

Parameter ¹	Experimental groups ²			SEM	P value ³
	C	E1	E2		
<i>musculus longissimus dorsi</i>					
pH					
45 min.	6.691	6.529	6.623	0.026	ns
1 day	5.414 ^a	5.239 ^b	5.154 ^b	0.042	0.01
3 day	5.493 ^a	5.356 ^b	5.199 ^c	0.047	0.01
5 day	5.176 ^a	5.073 ^a	4.949 ^b	0.036	0.001
<i>musculus vastus lateralis</i>					
45 min.	6.704	6.486	6.617	0.035	ns
1 day	5.594 ^a	5.570 ^a	5.324 ^b	0.047	0.01
3 day	5.711 ^a	5.627 ^a	5.431 ^b	0.045	0.01
5 day	5.379 ^a	5.429 ^a	5.190 ^b	0.040	0.001

¹Data are reported as mean values and SEM, n = 10.

²Control group (C); experimental group 1 (E1) with the addition of 0.3% of maca; experimental group 2 (E2) with the addition of 0.6% of maca.

³Letters on the same row differed for $P < 0.01$; ns = not significant.

Table 7. Meat hardness and colour during storage in the back and thigh rabbit muscles

Parameter ¹	Experimental groups ²			SEM	P value ³
	C	E1	E2		
<i>musculus longissimus dorsi</i>					
Colour 24 hour post mortem					
L*	56.930	55.510	56.590	0.741	ns
a*	1.376	0.814	0.885	0.306	ns
b*	7.575	8.109	7.894	0.269	ns
Colour 5 days post mortem					
L*	58.010	58.230	58.730	0.369	ns
a*	1.271	0.939	1.204	0.176	ns
b*	7.923 ^a	9.241 ^b	9.558 ^b	0.867	0.001

Table 7 cont.

Parameter ¹	Experimental groups ²			SEM	P value ³
	C	E1	E2		
<i>musculus vastus lateralis</i>					
Colour 24 hour post mortem					
L*	57.440	55.610	55.560	1.071	ns
a*	0.849	0.806	0.750	0.050	ns
b*	7.869	8.584	8.255	0.358	ns
Color 5 days post mortem					
L*	56.140	54.480	56.100	0.947	ns
a*	1.220	0.835	1.158	0.207	ns
b*	8.391	9.415	8.979	0.514	ns

¹Data are reported as mean values and SEM, n = 10.

²Control group (C); experimental group 1 (E1) with the addition of 0.3% of maca; experimental group 2 (E2) with the addition of 0.6% of maca.

³Letters on the same row differed for $P < 0.001$; ns = not significant.

Table 8. Malondialdehyde content during storage in rabbit MLD and MVL

Malondialdehyde mg/kg*	Experimental groups ⁺			P value [§]			
	C	E1	E2	SEM	D	T	Interaction
<i>musculus longissimus dorsi</i>							
1 day	0.170 ¹	0.167 ¹	0.175 ¹	0.001	ns	ns	ns
3 day	0.185 ²	0.177 ¹	0.190 ²	0.002	ns	0.01	ns
5 day	0.191 ²	0.203 ²	0.212 ³	0.003	ns	0.01	ns
<i>musculus vastus lateralis</i>							
1 day	0.170 ¹	0.167 ¹	0.175 ¹	0.001	ns	ns	ns
3 day	0.185 ²	0.177 ¹	0.190 ²	0.002	ns	0.01	ns
5 day	0.191 ²	0.203 ²	0.212 ³	0.003	ns	0.01	ns

*Data are reported as mean values and SEM, n = 10. ⁺Control group (C); experimental group 1 (E1) with the addition of 0.3% of maca; experimental group 2 (E2) with the addition of 0.6% of maca. [§]Different numbers on the same column differed for $P < 0.01$; ns = not significant.

Conclusion

This study evaluated the effect of *Lepidium meyenii* (maca) as a feed additive in rabbit diets on selected chemical, technological, and quality parameters of rabbit meat. The results showed that dietary maca increased protein and omega-6 fatty acid content in the MLD muscle, thereby improving its nutritional value. The two maca dosages tested had no significant effect on the shelf-life of rabbit meat. Promising findings, such as improved pH values and higher yellowness in meat, support the recommendation of high-dose maca supplementation in rabbit diets as safe and without detrimental effects on rabbit production.

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Ethical Statement

All animal experiments were conducted following the guidelines of EU Directive 2010/63/EU and national guidelines for the care and use of animals. All experimental procedures involving animals were approved by the National Agricultural and Food Centre ethical committee (Permission code: SK CH 17 021).

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Physicochemical Profiling of Traditional Kyrgyz Chobogo

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Keywords: Kyrgyz chobogo, milk residue valorisation, sustainable, zero-waste, Central Asia.

Abstract. This study aims to perform the first comprehensive physicochemical and nutritional characterisation of chobogo, a traditional dairy byproduct with nomadic roots in Kyrgyz food culture. Although it is increasingly popular and commonly consumed in Kyrgyz households, chobogo has remained scientifically undocumented and unregulated in terms of food safety and composition. Validated analytical methods were used to assess its acidity, macronutrient content, and fatty acid and amino acid profiles. The results indicate that chobogo is an energy-rich product with a balanced nutritional composition, containing essential and conditionally essential amino acids, as well as a favourable profile of dietary fats. Its overall characteristics suggest potential health benefits when consumed in moderation, particularly for individuals with high energy needs. These findings provide a scientific foundation for the standardisation and safe use of chobogo in modern food systems. The data also support its potential for industrial production as a culturally significant and functionally valuable dairy ingredient, contributing to the sustainable valorisation of traditional dairy byproducts.

Introduction and objective

Dairy products have long served as a fundamental source of nutrition for many cultures worldwide, offering essential macro- and micronutrients that support human health (Mehwish et al., 2023). In Central Asian countries with hot and dry climates, milk fat was traditionally preserved in the form of clarified butter; in Kyrgyzstan, this product is known as Sary-Mai. It is valued for storage and transport, especially during summer migrations to highland pastures, where herding families spend months without access to industrial foods or refrigeration. In recent years, renewed interest in national cuisine has increased and Sary-Mai has regained popularity as a culturally significant and energy-rich food.

As Sary-Mai production increases, so does the quantity of its byproduct – chobogo. This dense, mildly sweet dairy residue is formed during the melting of cream. Although official data on its production are lacking, household use of chobogo is expanding. Other dairy byproducts, such as buttermilk and whey, are well-recognised for their nutritional value and have been increasingly valorised through their incorporation into food, pharmaceutical, cosmetic, and veterinary applications (Hameed et al., 2023).

chobogo, composed primarily of coagulated milk solids, also holds nutritional potential as a traditional byproduct. Its valorisation aligns with modern sustainability trends in agri-food systems (Khalid et al., 2024; Tahir et al., 2023).

In Kyrgyz nomadic tradition, chobogo is consumed on its own, particularly by children and herders, due to its high energy content. However, despite its growing consumption, no food safety standards currently regulate its production. This study aims to investigate the composition of chobogo to support future standardisation, safety evaluation, and exploration of its potential health benefits for diverse consumer groups.

Materials and methods

Materials

For the study, 100 litres of raw milk supplied to the Alaiku Organics dairy plant in Osh, Kyrgyzstan, were used. The milk from stall-fed cows had the following characteristics: fat content 3.6%, protein content 3.1%, lactose content 4.5%, SNF (solids-not-fat) 8.5%, total solids 12.1%, pH 6.7, and density 1.028 g/cm³. The milk had a yellowish straw hue and a characteristic milky taste without any extraneous odours. The temperature of the milk was 5°C, and it was delivered for processing on February 12, 2024.

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Production of Kyrgyz Chobogo

The raw milk was pre-cleaned of mechanical impurities using a nylon filter (UVMilk, Kyrgyzstan) with a pore size of 50 microns. The filtered raw milk was then pasteurised in a food-grade stainless steel vessel at a temperature of 65–70°C for 30 minutes to eliminate potential pathogenic microorganisms. The pasteurised milk was cooled to 45°C (Chebotarev *et al.*, 2018) and sent to a separator (GEA MSE-500-01-777, Italy) for the extraction of high-fat cream. The freshly separated cream (3.42 kg) with a fat content of 68% was cooled to 5–7°C and left for 12 hours for

cream ripening and fat crystallisation.

The ripened cream (3000 g) was slowly heated to 110–120°C with constant stirring. The subsequent stages of the process are shown in Fig. 1. These include melting the cream, evaporating residual water, coagulating proteins, and caramelising milk sugar. When the milk residue reached a light brown colour, heating was stopped. The melted liquid fat was strained through a fine sieve. The filtered milk fat is called Sary-Mai, and the light brown residue left on the filter is referred to as Kyrgyz chobogo. The final yield was 1820 g of Sary-Mai and 270 g of chobogo.

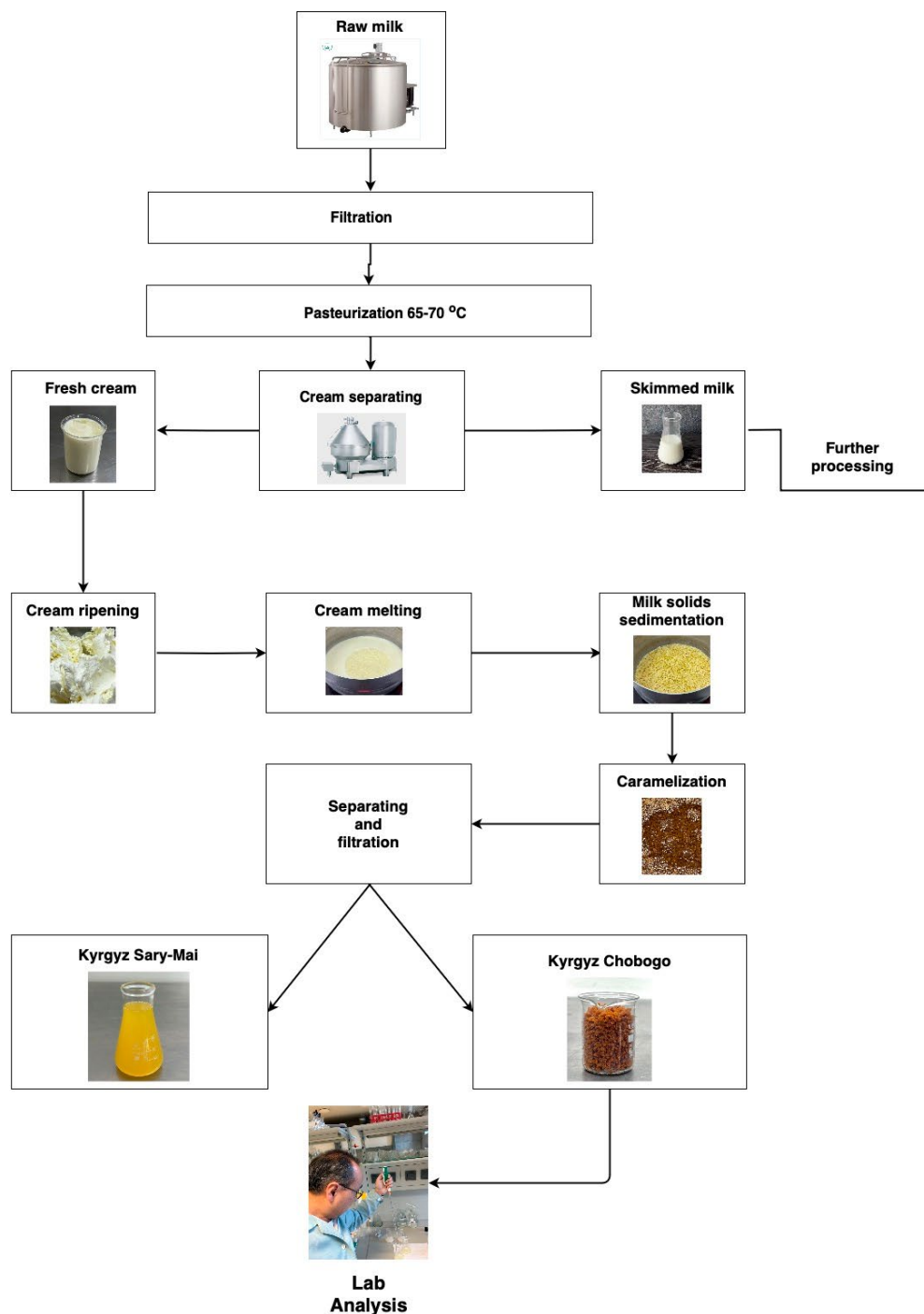


Fig. 1. Production scheme

Determination of physicochemical properties

Chobogo samples were analysed in triplicate for physicochemical and sensory parameters. pH measurements were conducted directly using a pH meter (Sartorius Professional meter for pH Measurement, Germany). Titratable acidity, expressed as a percentage of lactic acid (g/100 g chobogo), was determined according to ISO 11869:2012 standard method (*ISO/TS 11869:2012 – Fermented Milks – Determination of Titratable Acidity – Potentiometric Method*, 2012). Dry matter, moisture, acidity, ash, fat, and protein determinations were performed following prescribed methods: dry matter and moisture ISO 5534:2004c (*ISO 5534:2004 – Cheese and Processed Cheese – Determination of the Total Solids Content (Reference Method)*, 2004), ash AOAC, 2005 (*AOAC (2005) Official Method of Analysis. 18th Edition, Association of Official Analytical Chemists, Washington DC, Method 935.14 and 992.24. – References – Scientific Research Publishing*, 2005), fat ISO 23319:2022 (*ISO 23319:2022 – Cheese and Processed Cheese Products, Caseins and Caseinates – Determination of Fat Content – Gravimetric Method*, 2022), lactose ISO 22662:2024 (*ISO 22662:2024 – Milk and Milk Products – Determination of Lactose Content by High-Performance Liquid Chromatography (Reference Method)*, 2024), and protein ISO 8968-3:2004b (*ISO 8968-3:2004 – Milk – Determination of Nitrogen Content – Part 3: Block-Digestion Method (Semi-Micro Rapid Routine Method)*, 2004).

Colour characteristics were assessed using a CIE Lab* system (1996) with a Chromameter CR-400 (Konica Minolta, Tokyo, Japan), calibrated with a standard white plate. Total colour difference (ΔE) was calculated using the formula (Mileriene et al., 2021):

$$\Delta E = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2},$$

where L_0 , a_0 , and b_0 were values of day 1 and L , a , b were the values measured throughout the storage period. Texture properties were evaluated using a texture analyser CT3 (Brookfield, USA) with TA4/1000 cylinder. The samples were compressed using a TA4/1000 cylindrical probe with a diameter of 38.1 mm (D), length of 20 mm (L), a penetration speed of 1 mm/s, and a penetration depth of 10 mm.

Determination of amino acid profile

The amino acid (AA) compositions of the samples were analysed via ultrafast liquid chromatography (UFLC) with automated o-phthalaldehyde (OPA)/9-fluorenylmethyl chloroformate (FMOC)/Mercaptopropionic Acid (MERC) derivatisation. Standard solutions of the amino acids including alanine, aspartic acid, arginine, cystine, glycine, valine, leucine, isoleucine, threonine, serine, proline, methionine, glutamic acid, phenylalanine, lysine, histidine, tyrosine, asparagine, and tryptophan were used for this analysis (A9781 Sigma-Aldrich, Germany).

To commence the analysis, each sample (approx.

0.4 g) underwent hydrolysis with 25 mL of 6 M HCl for 24 hours at 103°C. The resultant contents were quantitatively transferred into a 250 mL beaker using a 150–200 mL solution of 0.2 mol Na⁺/L, pH 2.20 trisodium citrate dihydrate. The resulting hydrolysate was partially neutralised by the gradual addition of 17 mL of 7.5 N sodium hydroxide solution while stirring continuously, ensuring the temperature remained below 40°C (in a cold-water bath). The pH was adjusted to 2.20 at room temperature using sodium hydroxide solution (7.5 N).

Before injection, all samples were filtered through 0.45- μ m filters. The amino acids were separated using a UHPLC column YMC-Triart C18 (1.9 μ m, YMC co. ltd.) on a UFLC instrument (Shimadzu, Japan), which was equipped with a fluorescence detector RF-20Axs and a pre-treatment function equipped automatic injector SIL-30AC (Shimadzu, Japan). The analytical conditions were as follows: mobile phase consisting of solvent A (20 mmol/L potassium phosphate buffer, pH 6.5) and solvent B (45/40/15 acetonitrile/methanol/water); flow rate set at 0.5 mL/min; column temperature maintained at 45°C; detection wavelengths: RF-20Axs Ex. at 350 nm, Em. at 450 nm to Ex. at 266 nm, Em. at 305 nm (9.0 min). A calibration set comprising five levels was utilised, covering a concentration range of 9.375–150.00 μ mol/L, except for cysteine covering a concentration range of 8.08–75.00 μ mol/L.

Fatty acid profile

The analysis for the identification and quantification of fatty acids (FA) was conducted via gas chromatography utilising a capillary column and flame-ionisation detection. Initially, FA were extracted from a 2 g sample using 15 mL of n-hexane (Chempur, Poland), followed by methylation with anhydrous KOH methanol solution to yield methyl esters, following the protocol outlined in ISO 12966-2:2017 (*ISO 12966-2:2017 – Animal and Vegetable Fats and Oils – Gas Chromatography of Fatty Acid Methyl Esters – Part 2: Preparation of Methyl Esters of Fatty Acids*, 2017).

The analysis of FA methyl esters was carried out using a Shimadzu GC-2010 gas chromatograph (Shimadzu Kyoto, Japan) equipped with a flame ionisation detector (FID) and a 100 m column Restek Rt-2560, with a diameter of 0.25 μ m and thickness of 0.20 μ m, as specified in 12966-4:2015 (*ISO 12966-4:2015 – Animal and Vegetable Fats and Oils – Gas Chromatography of Fatty Acid Methyl Esters – Part 4: Determination by Capillary Gas Chromatography*, 2015). Chromatographic peaks were identified by comparing retention times with a mixture of Supelco 37 Component FAME Mix reagent kit (Supelco Analytical Bellefonte, PA, USA).

The analytical conditions were as follows: a volume of 1 μ L was injected, the column temperature was initially set at 100°C for 4 minutes, then ramped up to 240°C at a rate of 13°C/min and maintained for 63

minutes. The injector temperature was set at 250°C and the detector temperature at 300°C. Nitrogen was employed as the carrier gas.

Each fatty acid was expressed in g/100 g of total fatty acid content. Fatty acids were categorised according to their degree of saturation and the number of double bonds into saturated fatty acids (SFA), unsaturated fatty acids (UFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-3 fatty acids (n3PUFA) and omega-6 fatty acids (n6PUFA). Health lipid indices indicating the quality for pro-atherogenicity, pro-thrombogenicity, and risk of cardiovascular issues were assessed as follows (Pilarczyk *et al.*, 2015):

$$\text{Index of atherogenicity (AI)} = \frac{C12:0+(4*C14:0)+C16:0}{PUFA+MUFA};$$

$$\text{Index of thrombogenicity (TI)} =$$

$$= \frac{C12:0+C16:0+C18:0}{0.5*MUFA+0.5*n6PUFA+3*n3PUFA+\frac{n3PUFA}{n6PUFA}};$$

Hypocholesterolemic and hypercholesterolemic

$$\text{ratio (h/H)} = \frac{C18:1+PUFA}{C14:0+C16:0};$$

$$\text{Desirable fatty acids} = UFA + C18:0.$$

Determination of sensory profile

Sensory analysis was conducted by a trained panel of 7 members, following ISO 8586:2023 guidelines (*ISO 8586:2023 – Sensory Analysis – Selection and Training of Sensory Assessors*, 2023), using a scorecard designed according to the specific attributes of the product being evaluated. The scorecard included criteria such as appearance, aroma, texture, flavour, and overall acceptability, with each attribute rated on a structured scale ranging from 1 (poor) to 10 (excellent) (*ISO 4121:2003(En), Sensory Analysis – Guidelines for the Use of Quantitative Response Scales*, 2003). Panelists were provided with detailed definitions for each sensory attributes to ensure consistency and minimise bias. Evaluations were conducted under controlled conditions, including standardised lighting, temperature, and serving procedures, to ensure reliable and reproducible results. Statistical analysis was performed on the collected data to determine significant differences between samples, employing ANOVA and post-hoc tests as appropriate.

Determination of microstructure by scanning electron microscopy (SEM)

The morphology of the coating and film was analysed using a “Mira3” scanning electron microscope (SEM) manufactured by Tescan Orsay Holding, a.s. (Brno-Kohoutovice, Czech Republic). Samples were manually cut into pieces measuring 0.4 × 0.4 cm with a surgical-grade knife and mounted onto a 51 mm diameter silicon wafer (MicrotoNano, Haarlem, The Netherlands) without the use of

double-sided adhesive carbon discs. Silicon was chosen as the substrate because of its high prevalence of CHON elements in organic samples, which helps minimise potential bias in the results and improves the signal-to-noise ratio. The SEM was operated in high vacuum mode with backscattered electron (BSE) and secondary electron (SE) detectors. Magnification was increased to 1.0 kx to ensure precise dimensional measurements and analysis of element composition, using a 5 kV acceleration voltage.

Statistical analysis

All analytical measurements (physicochemical parameters, composition indicators, amino acid and fatty acid profiles, as well as sensory scores) were performed in triplicate (n = 3). Results are expressed as mean values with standard deviation (mean ± SD). Data processing was carried out using the SPSS statistical package (SPSS Inc., version 24, Chicago, IL, USA).

Since the present study investigates a single production batch of chobogo without comparative treatment groups, statistical analysis was limited to descriptive statistics aimed at evaluating measurement repeatability and within-sample variation. Inferential statistical tests were not applied. A significance level of $\alpha = 0.05$ is reported for reference in future comparative studies.

Results

Physicochemical profile

Titrateable acidity and pH

The acidity and pH of the chobogo sample are presented in Table 1. The titrateable acidity was measured at 0.15 ± 0.01 g/100 g, while the product exhibited a pH of 5.49 ± 0.02, indicating a slightly acidic environment. These acid-base properties are typical for high-fat dairy matrices obtained through heat clarification. A low variation between replicates demonstrates good measurement repeatability.

The relatively low pH, combined with a modest titrateable acidity value, suggests that the product retains a mild acidic profile characteristic of concentrated dairy systems. Such acidity may influence flavour perception, colour formation, and overall shelf stability – aspects relevant for traditional products like chobogo.

Table 1. Physicochemical parameters and colour values

Parameter	Mean ± St. Deviation
Titrateable acidity, g/100 g	0.15 ± 0.01
pH	5.49 ± 0.02
CIELAB colour results:	
L*	36.5 ± 0.8
a*	10.2 ± 0.3
b*	22.4 ± 0.5
c*	24.4 ± 0.6
h*	65.3 ± 1.2

Colour profile

The spectrophotometric CIELAB analysis (Table 1) revealed that chobogo had a moderately dark brown colour typical for heat-treated dairy solids. The L^* value of 36.5 ± 0.8 indicates low lightness, a^* of 10.2 ± 0.3 reflects a reddish tone, and b^* of 22.4 ± 0.5 confirms a strong yellow component. Together with c^* (24.4 ± 0.6) and h^* (65.3 ± 1.2), this profile corresponds to a saturated warm brown hue. Low standard deviations across colour metrics indicate high visual uniformity within the batch.

Microstructure

The SEM micrographs obtained at $1000\times$ magnification (Fig. 2) reveal a heterogeneous microstructure characteristic of sediments formed during Sary-Mai clarification. The surface appears uneven, consisting of alternating depressions and elevations, while the internal morphology demonstrates a porous network with micropores of varying size and shape. Peak-like crystalline formations and dense clusters

are observed, along with occasional microcracks and ruptures, indicating structural heterogeneity.

The presence of pores, aggregated clusters, and fractured regions suggests a complex spatial organisation of proteins, lipids, and mineral residues within the sediment matrix.

Sensory profile

A descriptive sensory evaluation was conducted by trained panelists using standardised methodology to assess the organoleptic properties outlined by (Lawless & Heymann, 2010). The outcomes are summarized in Table 2.

Panelists described chobogo as having a dense, rich texture and oily mouthfeel, with noticeable sour and roasted aroma nuances. The taste was perceived as balanced, combining mild sweetness, sourness, and a lasting aftertaste. Low to moderate standard deviations across parameters indicate consistent perception among assessors and reproducibility of results within this batch.

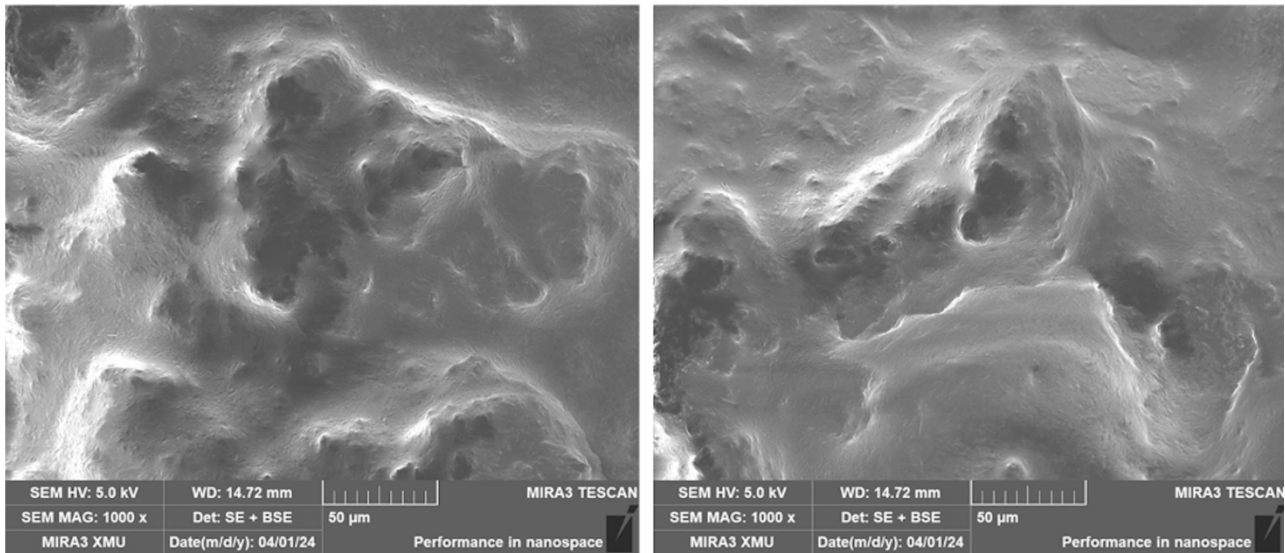


Fig. 2. SEM pictures

Table 2. Sensory profile of chobogo

Descriptive	Mean \pm St. Deviation, units
Colour	7.30 ± 1.72
Texture	7.70 ± 1.41
Overall smell	7.30 ± 1.36
Roasted smell	7.10 ± 1.57
Sour smell	8.20 ± 1.72
Overall taste	6.90 ± 0.75
Consistency	7.86 ± 1.87
Oiliness	8.10 ± 0.84
Sweetness	7.20 ± 1.09
Aftertaste	7.10 ± 1.20
Acceptability	8.50 ± 0.84

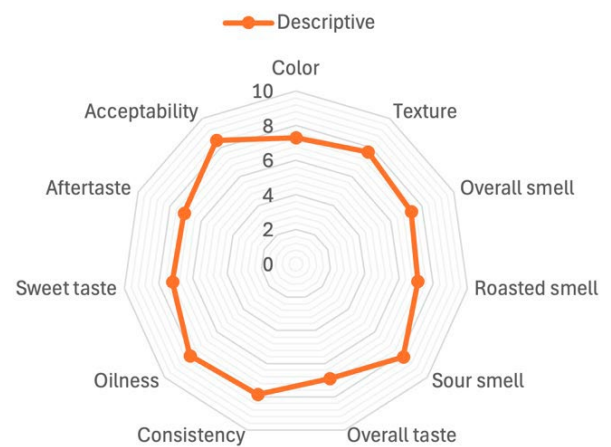


Fig. 3. Sensory profile visualisation of chobogo

Table 3. Composition and energy value of Kyrgyz chobogo

	Dry matter, g/100g	Fats, g/100g	Proteins, g/100g	Ash, g/100g	Carbohydrates, g/100g	Energy value, kCal
Chobogo	90.99 ± 0.10	53.10 ± 0.06	11.93 ± 0.01	2.36 ± 0.05	23.60 ± 0.10	620.0 ± 1.0

Fig. 3 illustrates the distribution of sensory intensities, demonstrating a relatively uniform profile across most evaluated attributes, with high acceptance scores.

Nutritional composition

Composition and energy value

The composition of chobogo is summarised in Table 3. The product contains 90.99 ± 0.10 g/100 g of dry matter, with fats accounting for 53.10 ± 0.06 g/100 g, proteins 11.93 ± 0.01 g/100 g, carbohydrates 23.60 ± 0.10 g/100 g, and ash 2.36 ± 0.05 g/100 g. The resulting energy value is 620.0 ± 1.0 kCal/100 g, indicating a highly concentrated nutritional profile. Low SD values confirm consistency of analytical measurements and stable composition within this batch.

The macronutrient distribution suggests that chobogo is a dense dairy product with high fat and moderate protein content, accompanied by residual carbohydrates and minerals originating from cream.

Amino acid profile

The amino acid composition of chobogo is shown in Table 4. The product contains a broad spectrum of amino acids, including non-essential, conditionally essential, and essential groups.

Among non-essential amino acids, glutamic acid (2.10 ± 0.14 µmol/L) and aspartic acid (0.95 ± 0.07 µmol/L) were predominant, while serine and alanine were present in smaller amounts. Conditionally essential amino acids such as glycine, arginine, tyrosine, cysteine, and proline were detected in moderate concentrations. Chobogo also contained all essential amino acids, with leucine being the most abundant.

Low variation across triplicates indicates reliable quantification and analytical precision.

Fatty acid profile

The fatty acid composition of chobogo is shown in Table 5. Saturated fatty acids (SFAs) were predominant, accounting for 68.85 ± 0.10%, with C16:0 (29.74 ± 0.02%), C18:0 (11.29 ± 0.01%), and C14:0 (11.07 ± 0.01%) being the major contributors. Short-chain SFAs such as C4:0 (3.77 ± 0.11%) and C6:0 (2.24 ± 0.02%) were also present.

Unsaturated fatty acids made up 31.13%, primarily MUFAs (25.50 ± 0.07%), dominated by oleic acid C18:1 (22.39 ± 0.04%), while PUFAs contributed 2.92 ± 0.03%, including C18:2 ω6 (2.23 ± 0.01%) and C18:3 ω3 (0.31 ± 0.00%). Natural trans fatty acids were detected at 2.71 ± 0.01%, mainly C18:1 trans and C18:2 trans.

Health-related lipid indices were calculated as AI = 2.71, TI = 2.86, h/H = 0.35, DFA = 42.41, and the omega-6/omega-3 ratio was 5.94 ± 0.06. Low standard deviations across most fatty acids indicate good analytical repeatability.

Discussion

This study provides the first detailed characterisation of traditional Kyrgyz chobogo, revealing how thermal clarification and concentration processes shape its physicochemical, microstructural, sensory, and nutritional properties.

Physicochemical properties: acidity and pH

The titratable acidity of 0.15 g/100 g (as lactic acid) falls within the expected range for non-fermented high-fat dairy products (typically 0.14–0.18 g/100 g),

Table 4. Amino acid composition in chobogo

	<i>Non-essential</i>				
Amino acids	Aspartic acid	Glutamic acid	Serine	Alanine	
µmol/L	0.95 ± 0.07	2.10 ± 0.14	0.58 ± 0.04	0.35 ± 0.02	
	<i>Cond. Essential</i>				
Amino acids	Glycine	Arginine	Tyrosine	Cysteine	Proline
µmol/L	0.4 ± 0.01	0.38 ± 0.02	0.44 ± 0.03	0.28 ± 0.04	0.22 ± 0.01
	<i>Essential</i>				
Amino acids	Histidine	Threonine	Valine	Methionine	Phenylalanine
µmol/L	0.23 ± 0.02	0.56 ± 0.04	0.48 ± 0.02	0.31 ± 0.05	0.49 ± 0.02
		Isoleucine	Leucine	Lysine	
		0.47 ± 0.01	0.96 ± 0.03	0.39 ± 0.08	

Table 5. Composition of fatty acids

Group	Fatty acid	Content (Mean \pm SD)
Short-chain (SCFA)	C4:0	3.77 \pm 0.11
	C6:0	2.24 \pm 0.02
	C8:0	1.25 \pm 0.01
Medium-chain (MCFA)	C10:0	2.71 \pm 0.01
	C12:0	3.10 \pm 0.01
Long-chain (LCFA)	C14:0	11.07 \pm 0.01
	C15:0	1.30 \pm 0.00
	C16:0	29.74 \pm 0.02
	C17:0	0.86 \pm 0.00
	C18:0	11.29 \pm 0.01
MUFA	C14:1	0.89 \pm 0.00
	C16:1	1.81 \pm 0.01
	C18:1	22.39 \pm 0.04
	Other MUFA	0.14 – 0.26*
PUFA ω 6	C18:2	2.23 \pm 0.01
	Other ω 6	0.01 – 0.15*
PUFA ω 3	C18:3 α	0.31 \pm 0.00
	Other ω 3	0.03 – 0.08*
Trans fatty acids	C18:1 tr.	2.57 \pm 0.01
	C18:2 tr.	0.14 \pm 0.00
	Total TFA	2.71 \pm 0.01
Total groups	SFA	68.85 \pm 0.10
	MUFA	25.50 \pm 0.07
	PUFA	2.92 \pm 0.03
	Omega-6/Omega-3	5.94 \pm 0.06
	AI	2.71 \pm 0.01
	TI	2.86 \pm 0.01
	h/H	0.35 \pm 0.00
	DFA	42.41 \pm 0.12

*Minor fatty acids (< 1%) are grouped and presented as summary values.

indicating the absence of fermentation. The acidity likely reflects the intrinsic properties of the raw cream and minor acid development during heat treatment (Schmidt et al., 1996).

The pH value of 5.49 is lower than that of typical cream (pH 6.4–6.7), which may be attributed to partial disruption of milk buffering systems during heating (Aydogdu et al., 2023), the presence of lactic acid and its salts (Rafiee Tari et al., 2021), and concentration of acidic compounds. Lower pH despite similar acidity suggests redistribution of components during clarification, influencing acid-base balance. These properties correspond to thermal concentration behaviour of dairy systems. However, as only one batch was examined, generalisation requires additional replication.

Colour development during heating

Colour development was consistent with controlled thermal processing. Heating below 130°C induces Maillard reactions and lactose caramelisation, forming a brown hue without burning. This explains the moderate L^* value and the shift toward red-yellow tones (a^* and b^*), similar to thermally-treated dairy residues reported by (Chudy et al., 2020). Concentration of solids during clarification enhances chroma and hue saturation.

Microstructure evolution under heat treatment

The porous morphology observed (SEM) may result from protein denaturation and coagulation during heating (El-Bakry et al., 2018), accompanied by whey-protein release and casein micelle disruption (Kamigaki, 2020). Peak-like aggregates are likely

linked to crystallisation of calcium phosphate and residual fats, while microcracks suggest dehydration and structural breakdown under thermal stress. The combination of smooth and irregular regions reflects redistribution of proteins, fats, and minerals. These microstructural traits align with previously described heat-treated dairy matrices (Rovira *et al.*, 2011) and explain functional behaviour during processing. The observed structure supports potential applications in reprocessing, ingredient development, and culinary uses.

Nutritional composition and energy density

Chobogo presented a highly concentrated energy profile dominated by fat (53.10 g/100 g), resulting in 620 kcal/100 g. Similar concentration-based nutrient enhancement has been reported in processed dairy systems (Rinaldi *et al.*, 2023). While high saturated fat intake requires moderation, such energy-dense foods historically served nomadic communities with high physical demands. Fat also transports vitamins A, D, E, and K, improving micronutrient delivery (Palacios and Gonzalez, 2014). Proteins (11.93 g/100 g) may support metabolic and structural functions, and heat treatment may enhance digestibility (Wada and Lönnerdal, 2014). Residual lactose provides short-chain energy, while minerals present in the ash fraction contribute to dietary mineral intake (Górska-Warsewicz *et al.*, 2019).

Amino acid availability and functional relevance

Chobogo retained a broad spectrum of essential, conditionally essential, and non-essential amino acids, indicating preserved protein value (Asif *et al.*, 2022). Glutamic and aspartic acids dominated the non-essential group, contributing to umami characteristics and nitrogen metabolism (Alencar *et al.*, 2024). Conditionally essential amino acids – including arginine, tyrosine, cysteine, proline – support immune function and tissue repair (Arribas-López *et al.*, 2021). Leucine was the most abundant essential amino acid, relevant for protein synthesis and energy regulation (Rahman *et al.*, 2022). Thermal processes can retain digestibility depending on conditions (Wang *et al.*, 2024), supporting the nutritional potential of Chobogo.

Fatty acid balance and lipid indices

The fatty acid profile was dominated by SFAs (68.85%), notably palmitic, stearic, and myristic acids. Despite associations between high SFA intake and cardiovascular risk, these lipids support membrane structure and energy metabolism (Mensink, 2016). Short-chain fatty acids like butyric and caproic may aid gut health and immunity. MUFAs (25.50%), primarily oleic acid, contribute to cardioprotective effects (Micha *et al.*, 2017), while PUFAs (2.92%) regulate inflammation. The $\omega 6/\omega 3$ ratio (5.94) falls

within recommended limits (Calder and Development, 2020). Natural trans fatty acids (2.71%) align with traditional dairy profiles (Taormina *et al.*, 2024). Lipid indices (AI = 2.71; TI = 2.86; h/H = 0.35; DFA = 42.41) reflect moderate metabolic impact.

Integrated interpretation

Together, the results demonstrate that Chobogo is a high-energy traditional dairy product with preserved amino acid diversity, a structured lipid profile, and a characteristic microstructure developed through thermal treatment. The balance of saturated and unsaturated fatty acids, presence of functional short-chain lipids, and nutrient-dense composition highlight its potential as a culturally relevant food with both historical and modern dietary significance.

Limitations and future perspectives

As analysis was conducted on a single production batch, variation related to seasonality, raw material quality, and processing conditions remains to be evaluated. Future research should incorporate multiple batches, shelf-life evaluation, digestibility studies, and consumer acceptance trials to validate broader applicability.

Conclusion

Chobogo is a culturally significant yet scientifically understudied byproduct of cream clarification. Despite its traditional relevance and increasing consumption, it has lacked regulatory standards, safety documentation, and analytical characterisation. This study provides the first comprehensive dataset describing its physicochemical properties, amino acid and fatty acid composition, sensory attributes, and microstructure, thereby addressing a notable research gap in dairy byproduct valorisation.

Chobogo demonstrated a high dry matter (90.99 g/100 g), elevated energy value (620 kcal/100 g), and substantial fat content (53.10 g/100 g), with proteins (11.93 g/100 g) contributing a complete spectrum of essential and conditionally essential amino acids. The lipid fraction was dominated by saturated and monounsaturated fatty acids, accompanied by a balanced $\omega 6/\omega 3$ ratio (5.94), moderate AI (2.71) and TI (2.86), and low natural trans-fat levels. Sensory evaluation indicated positive acceptability, while SEM analysis revealed a porous crystalline microstructure shaped by thermal concentration.

These findings indicate that chobogo is not only a traditional high-energy food but also a promising candidate for functional and value-added dairy applications, particularly within sustainable and zero-waste processing frameworks. To advance its utilisation, future work should focus on multi-batch variability, shelf-life behaviour, consumer perception across different demographic groups, and nutritional functionality *in vivo*. Establishing standardised processing guidelines and quality parameters could

facilitate its integration into modern dairy production without compromising its cultural authenticity.

Declarations and acknowledgements

Ethics approval and consent to participate

The study was carried out in compliance with the European Union and Lithuanian legal acts regulating scientific research.

This study was approved by the Technological Institute of the Kyrgyz State Technical University Named after I. Razzakov (Approval No. 2024-CCO-EBC-V-017). All participants provided informed consent before participation.

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare no conflict of interest.

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Credit authorship contribution statement

Askarbek Mametjanov: Investigation, Conceptualization, Formal analysis, Data curation, Writing – Original draft. **Mukarama Musulmanova:** Conceptualization, Funding acquisition. **Lina Lauciene:** Formal analysis, Data curation, Writing. **Kristina Kondrotiene:** Formal analysis, Data curation, Writing. **Vitalijs Radenkovs:** Formal analysis, Data curation, Writing. **Sandra Kiseliuviene:** Formal analysis, Data curation, Writing. **Alviya Šalaševičienė:** Formal analysis, Data curation, Writing. **Adele Askarbekova:** Writing – review and editing. **Loreta Serniene:** Investigation, Conceptualization, Formal analysis, Data curation, Writing – review and editing. All authors read and approved the final manuscript.

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Comparative Evaluation of Growth Performance and Carcass Traits in Purebred and Crossbred Pigs under Commercial Production Conditions

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Abstract. The study aimed to evaluate the productive performance of purebred and crossbred gilts under practical production conditions in Slovakia. A total of 164 gilts representing three pure breeds (Large White, Landrace, Duroc) and three hybrid combinations (LW×LA, LA×DU, LW×DU) were assessed for growth intensity, back fat thickness and lean meat content, with all traits standardised to a live weight of 100 kg. Significant differences ($P < 0.001$) were observed between purebred and crossbred groups. Crossbred pigs achieved faster growth (ADG_{100} 635.93 g/day) but showed higher back fat deposition, whereas purebreds exhibited superior carcass leanness (LMC_{100} 62.72%). Within purebreds, Duroc demonstrated the highest lean meat percentage and the lowest fat thickness, while among hybrids the LW×LA combination displayed the most favourable balance of growth and carcass traits. Correlation analysis confirmed strong biological relationships between growth and carcass parameters, particularly the antagonism between backfat and lean meat content. The findings emphasise the influence of a genetic background on production efficiency and highlight the advantages of specific crossbreeding strategies in optimising pig performance.

Introduction

The production performance of pigs is strongly influenced by the genetic background of the population. Numerous studies demonstrate that purebred pig breeds differ significantly in growth rate, carcass characteristics, and overall productivity. Xie et al. (2023) reported that Yorkshire pigs exhibit faster growth and reach a market weight of 100 kg at an earlier age than Duroc pigs. Similar findings were observed by Adebambo (2021), who confirmed higher carcass yields in Large White and Hampshire breeds compared with hybrid pigs, although with increased back fat thickness. In addition, Babicz et al. (2020) highlighted that some native breeds, such as Puławska, may achieve competitive daily gains of approximately 569 g, while offering favourable meat quality traits.

A considerable part of the production efficiency is also associated with heterosis effects. Iversen et al. (2019) reported that heterozygosity has a particularly positive impact on maternal traits such as total number born and litter weight, and it also contributes to improved early growth performance. Therefore, crossbreeding programmes have become an essential component of modern pig production systems. Chen et al. (2018) demonstrated that hybrids such as Duroc

× (Berkshire × Yanan) outperform traditional Chinese breeds in terms of growth rate and carcass traits. Wang et al. (2020) confirmed similar effects in Berkshire × Chenghua hybrids, which achieved higher lean meat content (50.76% vs. 42.58%) while maintaining desirable meat quality characteristics. Moreover, Elbert et al. (2020) showed that different terminal sire lines produce distinct results in growth performance and carcass traits, emphasising the importance of selecting appropriate genetic combinations.

Additional differences between genetic types were described by Christensen et al. (2019), who found genetic correlations between purebred and crossbred performance ranging from 0.75 to 0.96, indicating a strong predictive ability of purebred selection for hybrid performance. Voshchenko (2025) also confirmed that specialised terminal lines, such as PIC-337, can achieve higher live weight gains than traditional purebreds in intensive production systems. At the same time, Li et al. (2020) emphasised the role of environmental factors, showing that enriched housing conditions improve growth rates regardless of the genetic background.

Therefore, comparing the production performance of purebred and crossbred pigs is crucial for optimising growth, reproduction, and the economic efficiency of pig production. Analysing the genetic differences and performance outcomes of these breeding strategies represents an important basis for determining the most

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suitable breeds and genetic combinations for modern and alternative production systems. Accordingly, the aim of this study is to evaluate and compare the productive performance of purebred and hybrid pigs to determine their potential advantages and practical applicability in contemporary pig breeding systems.

Material and methods

Biological material

A total of 164 gilts were evaluated in the experiment, of which 90 were purebred pigs of the Large White (LW), Landrace (LA) and Duroc (DU) breeds, with 30 gilts selected from each breed. The hybrid group consisted of 74 gilts of three genetic combinations: Large White \times Landrace (LW \times LA), Landrace \times Duroc (LA \times DU) and Large White \times Duroc (LW \times DU). For the first two combinations, 30 gilts were included, while the LW \times DU group consisted of 14 gilts. All animals were selected within a live weight range of 80 to 120 kg. The productive performance of gilts was evaluated under practical production conditions on selected farms in Slovakia.

Ethical approval was not required because the data were collected from a commercial pig farm and no direct contact with live animals occurred.

Housing and feeding of pigs

The dataset was collected from commercial pig farms located in various regions of Slovakia. Since the animals originated from multiple production units, exact housing and feeding conditions could not be unified. All farms operated under standard intensive production practices routinely applied in Slovak pig industry. Animals were kept in groups and managed according to common farm protocols. Water was available ad libitum. Feeding was based on complete compound diets intended for growing pigs, with ration formulation determined individually by each farm. Although partial differences in feeding strategy and housing design may have occurred among farms, all pigs were reared under comparable practical production conditions.

Evaluated parameters

During pig performance testing, several production traits were recorded for each pig, including live

weight (to the nearest 1 kg), age in days, average daily gain (g/day), back fat thickness (cm) and lean meat content. The values of average daily gain, back fat thickness and lean meat content obtained on the measurement day were subsequently standardised to a live weight of 100 kg using the corresponding calculation formulas. Back fat thickness and lean meat content were assessed using a Piglog 105 device (SFK Technology A/S, Denmark) (User's Manual Slovakia, 2006). For the measurements, pigs were positioned in a horizontal measuring crate on a solid floor, standing firmly on both front and hind limbs, with the head maintained in a straight horizontal position.

For the determination of back fat thickness, three measurement points (M_1 , M_2 and M_3) were defined and used in conjunction with the Piglog 105 ultrasound device. All points were positioned along a straight longitudinal line on the left side of the animal, at 5–6 cm from the anatomical reference markers A_0 , B_0 and C_0 . Measurement point M_1 was established at the midpoint between the projected locations of A_0 and B_0 . Point M_2 was placed halfway between M_1 and B_0 , whereas M_3 was situated at the midpoint between B_0 and C_0 (Fig. 1). This standardised placement ensured consistent anatomical positioning for all examined animals.

To determine the proportion of muscle tissue, two measurement sites, designated M_1 and M_2M_3 , were utilised. These points were located on a straight line positioned 7 cm laterally from the anatomical reference markers A_0 , B_0 and C_0 on the left dorsal side of the animal. Measurement point M_1 , used for assessing back fat thickness, was situated between the third and the fourth lumbar vertebrae. The combined measurement point M_2M_3 , where both back fat thickness and the depth of the *Musculus longissimus dorsi* were recorded, was positioned between the third and the fourth ribs (Fig. 2).

Average daily gain (ADG), back fat thickness (BFT) and lean meat content (LMC) were recalculated to a standard live weight of 100 kg in accordance with the conversion procedure described by Řeháček et al. (2001). Standardisation was performed to enable objective comparison of animals with different body weights at the time of measurement. The adjusted traits were calculated using the following equations:

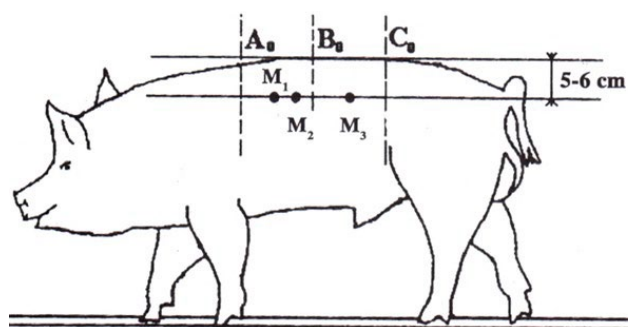


Fig. 1. Backfat thickness measurement points (Řeháček et al., 2001)

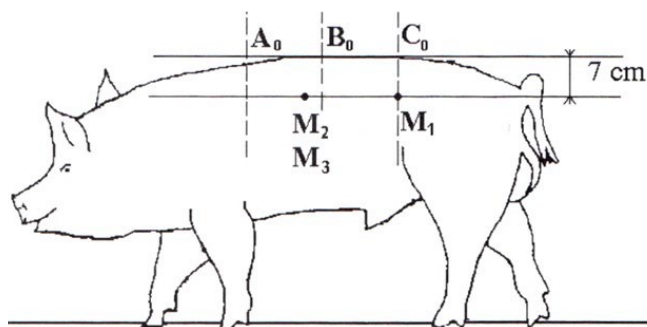


Fig. 2. Measurement points for the lean meat content (Řeháček et al., 2001)

Converted average daily gain on 100 kg live weight (ADG₁₀₀):

$$\text{ADG}_{100} = \text{ADG} - \text{GK} * (\text{LW} - 100)$$

where GK is conversion coefficient for gilts (2.58); LW is live weight of the gilts on the day of measurement; and ADG is the average daily gain on the day of measurement.

Converted back fat thickness on 100 kg live weight (BFT₁₀₀):

$$\text{BFT}_{100} = \text{BFT} - \text{GK} * (\text{LW} - 100)$$

where GK is conversion coefficient for gilts (0.017); LW is live weight of the gilts on the day of measurement; and BFT is the average back fat thickness on the day of measurement.

Converted lean meat content on 100 kg live weight (LMC₁₀₀):

$$\text{LMC}_{100} = \text{LMC} + \text{GK} * (\text{LW} - 100)$$

where GK is conversion coefficient for gilts (0.0859); LMC is lean meat content in % on the day of measurement; and LW is live weight of the gilts on the day of measurement (kg).

Standardisation to a live weight of 100 kg was applied to eliminate the influence of body weight variation among individuals and to ensure objective comparability of growth and body composition traits. Since pigs were measured at different ages and at slightly different body weights, direct comparison of raw values could lead to biased interpretation favouring heavier or older animals. Correcting ADG, BFT and LMC to a common reference weight enables evaluation of performance based on biological efficiency rather than body size, which is particularly important when comparing purebred and crossbred pigs with different growth dynamics. This approach is widely used in live pig evaluation and improves reliability when assessing genetic and performance differences between groups (Řeháček et al., 2001).

Statistical analysis

The results obtained were analysed using IBM SPSS Statistics 20. Comparisons between the groups were performed using one-factor ANOVA analysis of variance, while testing of contrasts was conducted using Scheffe's test at a significance level of $P < 0.05$.

The influence of monitored parameters was tested with the following model equations:

Effect of breed:

$$y_{ij} = \mu + gi + \varepsilon_{ij}$$

where y_{ij} is respected dependent variable, μ is intercept, gi is effect of the breed i ($i = 1$: pure breed, $i = 2$: cross breed), and ε_{ij} is residual error.

$$y_{ij} = \mu + sri + \varepsilon_{ij}$$

where y_{ij} is respected dependent variable, μ is intercept, sri is effect of pure breed and cross breed i ($i = 1$: LW, $i = 2$: LA, $i = 3$: DU, $i = 4$: LW×LA, $i = 5$: LA×DU, $i = 6$: LW×DU), and ε_{ij} is residual error.

Results

Significant differences were found between purebred and crossbred groups in all evaluated traits ($P < 0.001$) (Table 1). Purebred gilts reached a higher final weight (111.29 ± 7.99 kg) and were measured at an older age (172.96 ± 12.14 days) compared with crossbred gilts (102.59 ± 11.51 kg vs. 159.77 ± 11.52 days) ($P < 0.001$). On the other hand, crossbred gilts achieved a significantly higher average daily gain recalculated to 100 kg live weight (635.93 ± 42.82 g/day) than purebreds (615.38 ± 35.54 g/day) ($P < 0.01$). Crossbreeds also showed greater back fat thickness (1.03 ± 0.29 cm vs 0.75 ± 0.20 cm), while purebred gilts had a higher lean meat content ($62.72 \pm 1.67\%$ vs $60.55 \pm 2.40\%$) ($P < 0.001$). The observed differences clearly indicate that hybrid gilts grow faster, but purebreds show better carcass leanness.

Within purebred groups, all evaluated traits also differed significantly ($P < 0.001$). Duroc gilts showed the highest final weight (114.57 ± 5.14 kg) and the greatest lean meat content ($64.36 \pm 0.8\%$) compared with Large White and Landrace (Table 2). However, Duroc had the lowest back fat thickness (0.58 ± 0.06 cm), which supports their well-known carcass quality characteristics. In terms of daily gain, values among purebred breeds were relatively close, with Large White reaching the highest ADG₁₀₀ (619.17 ± 21.71 g/day), followed by Duroc (615.73 ± 50.47 g/day) and Landrace (611.23 ± 28.71 g/day) ($P < 0.001$). Landrace and Large White showed slightly lower meat content than Duroc but had higher back fat thickness

Table 1. Comparison of productive performance between purebred and crossbred gilts

Indicator (unit)	Purebred (X + SD) (n = 90)	Crossbred (X + SD) (n = 76)	P value
Age (days)	172.96 ± 12.14	159.77 ± 11.52	< 0.001
Weight (kg)	111.29 ± 7.99	102.59 ± 11.51	< 0.001
ADG ₁₀₀ (g/day)	615.38 ± 35.54	635.93 ± 42.82	0.01
BFT ₁₀₀ (cm)	0.75 ± 0.20	1.03 ± 0.29	< 0.001
LMC ₁₀₀ (%)	62.72 ± 1.67	60.55 ± 2.40	< 0.001

Legend: ADG₁₀₀: Average daily gain on 100 kg live weight; BFT₁₀₀: Back fat thickness on 100 kg live weight; LMC₁₀₀: Lean meat content on 100 kg live weight; SD: Standard deviation; X: Mean.

Table 2. Productive performance characteristics of Large White, Landrace and Duroc purebred gilts

Indicator (unit)	LW (X + SD) (n = 30)	LA (X + SD) (n = 30)	DU (X + SD) (n = 30)	P value
Age (days)	168.53 ± 10.99 ^a	174.07 ± 7.79 ^b	176.27 ± 15.43 ^b	< 0.001
Weight (kg)	107.83 ± 9.47 ^a	111.47 ± 7.50 ^b	114.57 ± 5.14 ^b	< 0.001
ADG ₁₀₀ (g/day)	619.17 ± 21.71 ^a	611.23 ± 28.71 ^b	615.73 ± 50.47 ^{ab}	< 0.001
BFT ₁₀₀ (cm)	0.85 ± 0.21 ^a	0.82 ± 0.17 ^a	0.58 ± 0.06 ^b	< 0.001
LMC ₁₀₀ (%)	61.98 ± 1.32 ^a	61.81 ± 1.39 ^a	64.36 ± 0.80 ^b	< 0.001

Legend: ADG₁₀₀: Average daily gain on 100 kg live weight; BFT₁₀₀: Back fat thickness on 100 kg live weight; LMC₁₀₀: Lean meat content on 100 kg live weight; SD: Standard deviation; X: Mean; ^{a,b}: different letters in the same row indicate significant differences among the mean values ($P < 0.001$).

($P < 0.001$). These results highlight the variability among pure breeds and confirm superiority of Duroc in carcass lean percentage.

Significant differences were also confirmed between hybrid combinations ($P < 0.001$) (Table 3). The highest average daily gain was found in LW×LA crosses (653.53 ± 33.16 g/day), followed by LA×DU (638.53 ± 45.67 g/day), while LW×DU exhibited the lowest growth intensity (592.64 ± 21.04 g/day) ($P < 0.001$). The LA×DU combination showed the greatest back fat thickness (1.19 ± 0.27 cm), but the lowest lean meat percentage ($59.52 \pm 1.71\%$). In contrast, LW×LA pigs achieved the best balance between growth and carcass traits, with relatively high lean content ($61.62 \pm 2.13\%$). LW×DU crosses had the highest weight at the day of measurement (107.86 ± 4.47 kg) and the oldest age (176.14 ± 2.57

days) ($P < 0.001$). Overall, the results show that crossbreeding significantly influences productive traits and confirms performance advantages of specific hybrid combinations, particularly LW×LA in growth rate.

Correlation analysis (Table 4) revealed significant relationships between age, live weight and production traits recalculated to 100 kg live weight. Age showed a strong positive correlation with live weight ($r = 0.66$; $P < 0.001$), indicating that older gilts were heavier at the time of measurement. At the same time, age was negatively correlated with ADG₁₀₀ ($r = -0.68$; $P < 0.001$), suggesting that younger gilts achieved 100 kg of live weight faster, which supports our earlier findings showing that hybrid pigs reached higher growth rates than purebreds.

Live weight was positively associated with lean

Table 3. Productive performance parameters of hybrid gilts across three genetic combinations

Indicator (unit)	LW×LA (X + SD) (n = 30)	LA×DU (X + SD) (n = 30)	LW×DU (X + SD) (n = 17)	P value
Age (days)	154.80 ± 2.04 ^a	157.10 ± 12.82 ^a	176.14 ± 2.57 ^b	< 0.001
Weight (kg)	101.93 ± 8.47 ^a	100.80 ± 15.35 ^a	107.86 ± 4.47 ^{ab}	< 0.001
ADG ₁₀₀ (g/day)	653.53 ± 33.16 ^a	638.53 ± 45.67 ^{ab}	592.64 ± 21.04 ^c	< 0.001
BFT ₁₀₀ (cm)	0.90 ± 0.26 ^a	1.19 ± 0.27 ^b	0.96 ± 0.27 ^a	< 0.001
LMC ₁₀₀ (%)	61.62 ± 2.13 ^a	59.52 ± 1.71 ^b	60.46 ± 3.24 ^{ab}	< 0.001

Legend: ADG₁₀₀: Average daily gain on 100 kg live weight; BFT₁₀₀: Back fat thickness on 100 kg live weight; LMC₁₀₀: Lean meat content on 100 kg live weight; SD: Standard deviation; X: Mean; ^{a-c}: different letters in the same row indicate significant differences among the mean values ($P < 0.001$).

Table 4. Correlation analysis of growth and live body composition traits in fattening gilts

Indicator	Age	Weight	ADG ₁₀₀	BFT ₁₀₀	LMC ₁₀₀
Age		0.664**	-0.684**	-0.439**	0.375**
Weight			0.085	-0.631**	0.583**
ADG ₁₀₀				-0.032	0.0688
BFT ₁₀₀					-0.844**
LMC ₁₀₀					

Legend: ADG₁₀₀: Average daily gain on 100 kg live weight; BFT₁₀₀: Back fat thickness on 100 kg live weight; LMC₁₀₀: Lean meat content on 100 kg live weight; * Correlation is significant at the 0.05 level (2-tailed). **; Correlation is significant at the 0.01 level (2-tailed).

meat content recalculated to 100 kg ($r = 0.58$; $P < 0.001$), meaning that animals with better growth ability tended to deposit more muscle tissue. On the contrary, a strong negative correlation was observed between weight and BFT_{100} ($r = -0.63$; $P < 0.001$), which confirms that heavier animals within our dataset generally presented a lower standardized back fat thickness.

A highly significant negative correlation was detected between BFT_{100} and LMC_{100} ($r = -0.84$; $P < 0.001$). This result demonstrates a clear antagonistic relationship between fat deposition and lean mass, meaning that individuals with lower back fat have a markedly higher lean content. This supports phenotypic differences previously observed between purebred and crossbred groups, where purebred pigs (especially Duroc) showed superior lean percentage, while hybrids grew faster but accumulated more back fat.

Overall, the correlation results confirm that:

- younger pigs exhibit faster growth (higher ADG_{100});
- higher body weight is associated with leaner carcass composition;
- fat depth and lean meat content are strongly inversely related;
- genotype influences these relationships significantly, as demonstrated by performance differences between purebreds and crossbreds.

Discussion

The results of this study clearly demonstrate statistically significant differences ($P < 0.001$) in production traits between purebred and crossbred pigs, supporting the general assumption that genotype plays a key role in growth performance and carcass quality. Crossbred animals achieved higher average daily gain recalculated to 100 kg live weight compared with purebreds, confirming the positive heterosis effect described by Iversen et al. (2019) and Chen et al. (2018). Faster growth observed in hybrid pigs is likely the result of complementary genetic traits of the parental breeds, which is consistent with previous reports indicating that crossbred combinations often outperform pure lines in growth intensity and efficiency. Similar conclusions were also reported by Elbert et al. (2020), who demonstrated that sire line significantly influences growth performance and carcass yield in hybrid pigs, supporting the observation that hybrid combinations tend to exhibit higher growth dynamics.

In contrast, purebred pigs in the present study achieved higher lean meat content and lower back fat thickness recalculated to 100 kg live weight compared with hybrids. Comparable findings were reported by Adebambo (2021), who observed that purebred pigs may excel in carcass quality traits despite exhibiting lower growth rates. The higher lean meat content observed in purebred gilts may reflect long-term selection pressure focused on carcass composition

within commercial nucleus herds. This interpretation is consistent with Christensen et al. (2019), who reported high genetic correlations between purebred and crossbred performance, particularly for carcass traits. Similar conclusions were drawn by Esfandyari et al. (2020), who highlighted strong genetic correlations between purebred selection response and crossbred performance, indicating that selection in nucleus herds can effectively improve carcass traits expressed in hybrid populations.

These findings reinforce the close interrelationship between growth dynamics and carcass composition. The present results support the concept that crossbreeding strategies enhance growth rate through heterosis, whereas targeted within-breed selection in pure lines contributes to improved carcass leanness. This interpretation aligns well with conclusions drawn by Iversen et al. (2019), Wang et al. (2020) and Chen et al. (2018), who emphasised the complementary roles of hybrid vigour and purebred genetic improvement in modern pig production systems.

When individual purebred lines were compared, the Duroc breed exhibited superior lean meat content and the lowest back fat thickness recalculated to 100 kg live weight, while maintaining daily gain comparable with Large White and Landrace. These results are consistent with earlier reports describing Duroc as a breed characterised by favourable carcass composition and desirable meat quality traits (Xie et al., 2023; Babicz et al., 2020). A similar performance tendency was reported by Kim et al. (2020), who observed lower back fat thickness and improved carcass quality indicators in Duroc pigs and Duroc-based hybrids. The slightly higher body weight at measurement observed in Duroc pigs suggests a greater potential for muscle deposition at comparable ages. In contrast, Large White and Landrace pigs showed slightly higher growth dynamics but accumulated more back fat, which may be related to their maternal-oriented breeding background.

Among the crossbred groups, the LW×LA combination achieved the highest ADG_{100} while maintaining balanced carcass quality parameters, whereas LA×DU pigs exhibited the greatest back fat thickness and the lowest lean meat content recalculated to 100 kg live weight. The superior performance of LW×LA hybrids suggests strong genetic complementarity between the two maternal breeds, potentially enhancing growth metabolism. Conversely, the lower carcass leanness observed in LA×DU crosses, despite the inclusion of the Duroc breed known for lean tissue deposition, may indicate the influence of dominance effects or breed order within the cross. LW×DU gilts reached the highest body weight at measurement but exhibited lower ADG_{100} compared with LW×LA animals, suggesting a more prolonged rather than rapid growth pattern. These findings are consistent with reports by Wang et al. (2020) and Chen et al. (2020), who

demonstrated that crossbreeding outcomes strongly depend on parental breed combinations and terminal sire selection. Similarly, Wang et al. (2021) reported that breed composition affects intramuscular fat content and meat quality, with traditional breeds often exhibiting superior meat quality despite slower growth, which partly reflects the pattern observed in pure Duroc pigs in the present study.

It should be noted that the animals included in this study originated from several commercial farms, where minor differences in housing conditions, feeding strategies and management practices may have occurred. Although all pigs were reared under standard intensive production systems typical for Slovak commercial pig farms, such environmental variation could have partially influenced growth performance and carcass traits. Previous studies have shown that feeding regime, housing conditions and management intensity can modulate carcass trait expression independently of genetic background (Li et al., 2019). Nevertheless, because all evaluated traits were standardised to a live weight of 100 kg, the influence of body weight differences was minimised, enabling a more objective comparison between purebred and crossbred pigs. Therefore, the observed performance differences can be attributed primarily to genetic effects, while the inclusion of animals from different farms reflects realistic commercial production conditions and enhances the practical relevance of the results.

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Overall, the presented data confirm that hybrid pigs exhibit higher growth potential, whereas purebred lines provide advantages in carcass quality. The observed performance differences reflect heterosis effects, breed specialisation and selection orientation, which are consistent with modern crossbreeding strategies aimed at maximising growth efficiency through hybrid vigour while maintaining favourable carcass characteristics through purebred genetic improvement. The integration of the present findings with existing literature suggests that optimal breeding strategies may combine the growth advantages of hybrids with the superior carcass traits of selected pure lines, such as Duroc, particularly within terminal sire systems.

Conclusions

The study confirmed clear production differences between purebred and crossbred pigs. Crossbred gilts grew faster and reached market weight earlier, demonstrating the positive effect of heterosis on growth performance. However, this advantage was accompanied by increased back fat thickness. Purebred pigs, particularly Duroc, showed superior carcass leanness and the highest lean meat content. Among hybrid combinations, LW×LA displayed the most favourable balance between growth rate and carcass traits. Overall, the findings highlight the importance of selecting appropriate breed combinations to optimise growth efficiency and carcass quality in commercial pig production systems.

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ONE HEALTH: CHALLENGES FOR FOOD SAFETY

Abstracts

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DEVELOPING NOVEL FUNCTIONAL FOOD INGREDIENTS FOR MANAGEMENT OF GUT MICROBIOME IN TYPE-1 DIABETES MELLITUS

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A rapid increase in the incidence of Type 1 Diabetes Mellitus (T1DM) in developed countries over recent decades has been observed, with both diet and genetic factors playing a significant role in the onset and progression of the disease. As a result, restoring the natural balance of the gut microbiome has emerged as a promising target for T1DM prevention and management. In this context, the development of functional food ingredients, rich in prebiotic fibers and/or beneficial microorganisms, is expected to contribute to the regulation of microbiota and microbiome balance. In this vein, novel functional food ingredients were developed by immobilizing *Lactocaseibacillus rhamnosus* OLXAL-1 cells on oat flakes, and their effect on gut microbiota, as well as metabolic and inflammatory markers, was examined in a streptozotocin (STZ)-induced T1DM animal model. Administration of the ingredients containing *L. rhamnosus* OLXAL-1, a wild-type strain isolated from Greek olives, resulted in an increase in the relative abundances of lactobacilli and bifidobacteria, along with elevated levels of lactic, acetic, and butyric acids in the feces of the diabetic animals. Additionally, supplementation with oat flakes significantly reduced microbial populations of *E. coli*, *Enterobacteriaceae*, coliforms, staphylococci, and enterococci and lowered IL-1 β levels in the blood plasma of diabetic animals. These findings suggest that probiotic food-based strategies could play a therapeutic role in managing dysbiosis and inflammation associated with T1DM.

Keywords: gut microbiota, prebiotics, probiotics, type-1 diabetes mellitus, cell immobilization, oat flakes.

A CASE STUDY ON THE VALORIZATION OF DAIRY BY-PRODUCTS IN LATVIA

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The main dairy by-products are skimmed milk, buttermilk, and whey. These by-products serve different purposes in the production of (i) low-fat dairy products (skimmed milk, buttermilk), (ii) fermented products (buttermilk, skimmed milk), and (iii) milk concentrates.

The processing of whey remains a challenge for research due to its changeable acidity, perishability, low nutrient concentration, specific equipment and energy consumption requirements for processing.

In Latvia, research on the recycling of by-products includes the development of new products, clinical trials on the effect of buttermilk lipids on the blood lipid profile of menopausal women (Aleksejeva et al., 2025), and studies on the functionality of whey proteins, including analyses aimed at the sustainable utilization of whey permeate.

Permeate consists mainly of water and about 5% of total solids, including lactose, minerals, and acids, and poses recycling and environmental challenges due to its significant chemical oxygen demand and biological oxygen demand. To find the processing solutions for acid whey, research efforts have focused on the production of lactobionic acid production (Šarenkova, 2022), oligosaccharide synthesis (Majore, 2021), and single cell protein production (State Research Programme GreenAgroRes, 2025), which represent the latest research direction at the Food Institute.

This report aims to summarize the results of the research conducted and share experiences on by-product utilization that could help industry optimize the use of existing resources and strategically plan by-product processing into value-added products.

Keywords: dairy by-products, whey, valorization, lactose hydrolysis, single-cell protein.

THE IMPACT OF ALTERNATIVE SOURDOUGH PREPARED FROM EXTRUDED AND FERMENTED WHEAT CEREAL BY-PRODUCTS ON BREAD QUALITY CHARACTERISTICS

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This study aimed to assess the impact of extruded and fermented wheat bran, used as an alternative sourdough, on the quality of wheat bread, with a focus on volatile compounds and their connection to consumer emotional responses induced by bread samples. A comparative analysis of wheat bread prepared with 5%, 10%, and 15% untreated wheat bran and alternative sourdough (wheat bran was extruded at 130°C at a screw speed of 25 rpm and fermented with a *Lactobacillus uvarum* strain) was conducted. The use of untreated wheat bran increased dough hardness and reduced the specific volume of the bread. Adding 5% and 10% of alternative sourdough had a minimal effect on the bread's porosity but resulted in the formation of larger pores. Both untreated wheat bran and alternative sourdough increased mass loss during baking (by 13.38%), with the control sample having the highest values for crust L* (lightness), a* (redness), and b* (yellowness). While untreated wheat bran and alternative sourdough reduced bread firmness during storage, the inclusion of alternative sourdough improved overall acceptability by 26.2%, with a strong positive correlation between overall acceptability and the emotion "happy" (r = 0.8696). The wheat bread prepared with alternative sourdough had higher contents of pyrazine compounds, including methyl-pyrazine, 2-ethyl-pyrazine, 2-ethyl-6-methyl-pyrazine, furfural, 1-(2-furanyl) ethanone, benzaldehyde, and 3-furanmethanol. In conclusion, alternative sourdough not only extends the shelf life of wheat bread but also contributes to a unique volatile compound profile, which is linked to improved consumer acceptability.

Keywords: volatile compounds, porosity, overall acceptability, consumer emotional responses.

ENHANCING PROBIOTIC VIABILITY: EDIBLE COATINGS FROM DAIRY BY-PRODUCTS FOR FUNCTIONAL FOOD APPLICATIONS

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Edible coatings offer an approach for probiotic delivery in food. While some lactic acid bacteria (LAB) survive well *in vitro*, their viability may decline when incorporated into an edible matrix. This study employed a standardized static method (Minekus et al., 2014) to simulate *in vitro* digestion of acid-curd cheese coated with *Lactocaseibacillus paracasei* A11-incorporated films, derived from liquid acid whey protein concentrate and liquid acid whey permeate. A11 counts in the samples were assessed using 16S rRNA gene amplicon sequencing (MiSeq, Illumina). Results demonstrated that LAB counts decreased by 31% post-digestion in uncoated samples, while coated samples with A11 maintained higher stability ($P < 0.05$). In coated samples, pre-digestion A11 counts were 6.40 ± 0.18 log CFU/g (day 1) and 6.38 ± 0.08 log CFU/g (day 10). Post-digestion, A11 counts did not decrease, reaching 6.71 ± 0.17 log CFU/g (day 1) and 6.46 ± 0.12 log CFU/g (day 10). In conclusion, coatings from dairy by-products enhanced A11 stability, achieving 100% survival and preventing a 31% LAB loss post-digestion, highlighting their potential for probiotic delivery in functional foods.

Keywords: probiotics, lactobacilli, edible film-forming solutions, survival.

CHEMICAL FOOD SAFETY RISK IN FERMENTED FISH PRODUCTS OF BALTIC HERRING (*CLUPEA HARENGUS MEMBRAS*)

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Fermentation has been traditionally used worldwide for the preparation and preservation of fish dishes. Many biochemical reactions occurring during fermentation influence the texture and flavor and enhance the nutritional value of the products. Moreover, fermentation decreases the microbiological risks and increases safety of the food. During lactic acid bacteria (LAB) fermentation, organic acids lower the pH, thus inhibiting the growth of spoilage and pathogenic microbes. However, the chemical risk for food safety of Clupeidae family (sardines, herring) fish products may increase due to histamine production during LAB fermentation.

Most of the traditional fermented fish products are produced by spontaneous fermentation. In controlled fermentation, starter cultures containing known specific microorganisms are applied, and it has been rarely used in producing new type of fish products. In our study, we fermented underexploited fish Baltic herring (*Clupea harengus membras*) by controlled fermentation using two commercial starters. The histamine concentration of the fermented samples was analyzed after fermentation and after the storage using an enzymatic method.

The results show that the histamine concentration in our fermented Baltic herring products was low and thus can be regarded chemically safe based on the European Commission legislation Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs. The limits for the accepted histamine levels established for fishery products are between 100–200 mg/kg.

Keywords: fish fermentation, chemical food safety.

THE CHALLENGES RELATED THE PROVISION OF INFORMATION AND THE CONTENT OF SENNA HERBAL TEAS

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Herbal teas used both for food and treatment purposes should be consumed with an understanding of restrictions. The hydroxyanthracenes of senna (*Senna alexandrina* Mill.) are stimulants of bowel movements and modulators of water and electrolyte absorption in the intestinal tract, resulting in a laxative effect. The EFSA has set a daily acceptable intake of 24.83 mg/person per day for an adult (70 kg) for periods not exceeding two weeks. It is important to note that the use of senna teas in children below 12 years of age, during pregnancy or lactation is not recommended. High intakes of hydroxyanthracenes via food products may be subject to restrictions by some authorities. The WHO standardized daily dose is equivalent to 10–30 mg. The aim of this study was to explore the labelling and content of senna teas on the Lithuanian market. The labelling was found to be non-compliant with the recommendations set by the EFSA due to the absence of warnings regarding exemptions for groups of consumers. Furthermore, only two products included restrictions on the duration of use. The content (assayed according to 04/2020:0206 Eur. Ph.; accreditation LA.184-01.) and the daily exposure (from 19.8 to 106.2 mg of hydroxyanthracene glycosides) was found to be sufficiently potent to induce the physiological effects associated with the pharmacological activity of phytochemicals, which could potentially lead to adverse reactions. To ensure the safety of consumers, it is essential to modify the labelling of senna teas, in conjunction with adapting the existing regulatory framework.

Keywords: herbal teas, hydroxyanthracenes, labelling, safety.

PROSPECTS FOR THE TREATMENT OF BEE POLLEN USING BIOTECHNOLOGICAL AND PHYSICAL METHODS: A REVIEW

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Bee pollen is a product produced by bees that contains a variety of nutrients and biologically active compounds (vitamins, phenolic compounds, carotenoids and chlorophylls). Compared with other bee products, bee pollen has a high similarity to bee bread, but its biological value lags behind bee bread, despite the fact that pollen production is more commercialized than bee bread. Bee pollen is criticized by scientists for its limited bioavailability due to its outer wall, called exine. The exine is composed of sporopollenin, which gives plant pollen elasticity, strength and high chemical resistance, so that the pollen can protect itself from external damage. This wall thus protects the substances contained in the pollen, which are necessary for the fertilization of another plant flower. In the case of bee pollen, the exine also protects biologically active substances. In the human organism, the wall covering pollen is not destroyed due to the high chemical resistance of sporopollenin. It is claimed that humans digest only about 10–15% of bee pollen, whereas in bee bread, this wall is weakened by lactic acid bacteria.

Biotechnological methods, such as fermentation or enzymatic hydrolysis, can weaken the resistance of sporopollenin, thereby increasing the release of substances from pollen, and at the same time increasing the bioavailability of bee pollen biologically active compounds along with their nutritional value. Physical processing methods such as ultrasonication or high-pressure processing, or their combination with biotechnological methods, are also promising alternatives for increasing the nutritional value of bee pollen.

Keywords: bee products, sporopollenin, fermentation, enzymatic hydrolysis, biological activity.

IDENTIFICATION OF FISH ALLERGEN IN FOODS USING DROPLET DIGITAL PCR

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Global fish consumption and fish allergy have increased over the years. In allergic individuals, even a small amount of allergens can trigger a potentially lethal reaction, posing a serious concern to human health. Therefore, the food industry bears a great responsibility while indicating the presence of allergens in the label. To verify compliance with labelling requirements and limit the inappropriate use of precautionary allergen labelling, the food sector needs reliable, specific and sensitive methods for the detection of fish in food products. The present study assessed the use of a droplet digital PCR assay for the detection of fish in various processed foods. The method was developed targeting the ribosomal 18S rRNA gene. The specificity, the limit of detection and of quantification, dynamic range and selectivity were assessed. After the optimization and validation of the method, the digital droplet PCR was tested on 37 prepackaged food samples placed on the market. The samples included different types of composite foods either of animal and plant origin in which fish was in the ingredient list and foods in which fish was indicated in traces or not listed. Fish DNA was detected in 88.9% of the samples which included fish in the ingredient list, in 22.2% of the samples declaring traces of fish, while it was never detected in foods in which fish was not declared in the label. No false positive nor false negative results were obtained demonstrating the reliability of the digital droplet PCR for the detection of the fish allergen in commercial foods.

Keywords: droplet digital PCR, human health, allergy, prepacked foods, food labelling.

THE EFFECT OF MOLDS, THEIR VOLATILE ORGANIC COMPOUNDS, AND MYCOTOXINS ON CEREAL GRAIN QUALITY

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Cereal grains are one of the most used raw materials in the food industry. According to statistical data, the highest global production volumes are recorded for maize (1116.34 million tons) and wheat (764.49 million tons). In addition to these major cereal crops, barley, oats, and rye are also in high demand. Therefore, efforts are focused on producing high-quality and safe raw materials for use in the food and feed industries.

This study evaluates the impact of molds, their volatile organic compounds (VOCs), and mycotoxins on the qualitative indicators of wheat, barley, triticale, oat, and rye during storage. Grain samples were collected from a farm in Pasvalys district in September, February, and July. Total mold contamination (CFU/g) was determined, and mycotoxins (AFB₁, ZEA, DON, OTA, T-2) were determined using thin-layer chromatography. Qualitative grain indicators were analyzed using an NIRS 6500 spectrometer, while external and internal mold contamination was assessed by light microscopy. Mold fatty acids and VOCs were examined via gas chromatography-mass spectrometry.

Results showed that mold contamination increased by 14.94% ($P < 0.01$) from the beginning to the end of storage. Mycotoxin levels also increased: AFB₁ by 18.65, OTA by 9.5, and T-2 by 3.5 times ($P < 0.01$). T-2 toxin increased the least, i.e., by 3.5 times ($P < 0.01$). Chemical composition analysis indicated reductions in ash, fiber, and protein levels ($P < 0.01$), with a strong negative correlation between T-2 toxin and protein content. Fat ($P \geq 0.05$), moisture, and starch ($P < 0.01$) levels increased, showing a positive correlation with T-2 toxin and *Rhizopus spp.* spread.

Specific VOCs were associated to mold species, such as 3-ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsilyloxy) trisiloxane (*Alternaria alternata*) and 2,2,4,6,6-pentamethylheptane (*Fusarium sporotrichioides*). *Penicillium viridicatum* exhibited polyunsaturated fatty acids, while *Aspergillus flavus*, dominated by monounsaturated fatty acids, synthesized 2,2,5,5-tetramethylhexane. These biomarkers are valuable for early grain mold contamination detection and prevention.

Keywords: mycotoxins, cereal grains, volatile organic compounds.

ASSESSING BIOGAS OUTPUT FROM FOOD WASTE IN AN EDUCATIONAL SETTING

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In the Food Production Chain Waste course laboratory work, students individually conducted a biogas production experiment. They used self-made demonstration reactors constructed from repurposed plastic bottles, consisting of a main reservoir and a two-part gas collection chamber filled with water, where a floating indicator measured biogas production in milliliters. The main reservoir was filled with 500 mL of goat slurry, 500 mL of water, one boiled sausage (average weight 46 g), and one slice of white bread (average weight 13 g).

The experiment included three groups: one where food was finely chopped, another where food remained unchopped, and a control group with no food added. The ambient temperature was approximately 20°C. The aim was to determine the biogas yield from food waste and whether chopping food increased gas production.

Data were collected from 14 bioreactors installed between 2022 and 2024. Reactors with chopped food produced an average of 3860 mL of gas, whereas those with unchopped food yielded 2388.11 mL. Gas production lasted an average of 12 days (range: 8–18) for chopped food and 15 days (range: 8–28) for unchopped food. Peak production occurred at 2.5 days for chopped food and 4.9 days for unchopped food before declining. Statistically significant differences ($P < 0.05$) were observed on days 2 and 4, with 631.25 mL and 471.25 mL of gas from chopped food reactors versus 155.78 mL and 240.22 mL from unchopped food reactors. These results suggest that finely chopped food waste enhances biogas production efficiency, yielding more gas in a shorter period.

Keywords: biogas production, educational bioreactors, food waste utilization, bioreactor efficiency.

CHANGES IN QUALITY AND CHEMICAL COMPOSITION OF CARROT LEAF PESTO DURING STORAGE

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Carrots are consumed as a root vegetable, and leaves are thrown away. However, they can be a potential source of nutrients. One way of using carrot leaves is to make carrot leaf pesto, but the main quality problem in the pesto production is its short shelf life. The aim of this study was to determine the influence of storage temperature and time on changes in the chemical composition, physicochemical properties and color of the sauce. Carrot leaf pesto sauces were stored at different temperatures: 8°C, 4°C, and –22°C, for different periods of time: 14, 26 and 33 days. Dry matter content, acid value, acidity and peroxide value were determined by the standard methods and the amount of ascorbic acid by titration of samples with 2,6-dichlorophenolindophenol sodium salt solution. The spectrophotometric method was used to determine the amount of chlorophyll *a* and *b* and carotenoids. The antioxidant activity of the carrot leaf pesto was determined using the DPPH radical scavenging activity method, and the color was evaluated using ColorFlex color analyzer (Hunter Lab, USA). Dry matter, vitamin C, total chlorophylls and carotenoids content decreased during storage. The storage temperature had no significant effect on the amount of chlorophyll *a* and antioxidant activity of the sauce. During storage, there was an increase in the acid value, acidity and peroxide value in the sauce except when the pesto was stored at –22°C. The least color change was observed in the sauce stored at –22°C.

Keywords: carrot leaves, byproducts, volarization, pesto, quality, shelf life.

OCCURRENCE OF HISTAMINE IN CANNED FISH SAMPLES (ANCHOVY, MACKEREL, AND TUNA) MARKETED IN SARDINIA (ITALY) BY A COMMERCIAL ELISA FAST-TRACK METHOD

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The biogenic amine histamine is responsible for the so-called “scombroid fish poisoning”. Its formation is due to histidine decarboxylation by bacterial decarboxylases in scombroid fish such as tuna, mackerel and anchovy. Once produced, histamine is not inactivated by common heating used for fish canning, and for this reason, the presence of histamine is generally used as an indicator of the good manufacturing process of canned fishes. The aim of this study was the quantitative determination of histamine in canned fish samples of anchovy (n = 3), mackerel (n = 3) and tuna (n = 3) in brine collected from large retail stores in Sassari, Italy. Histamine concentrations were determined with a commercial ELISA fast-track method. The preparation of the samples was carried out according to the AOAC official method 937.07. Altogether, histamine was detected in all the samples with levels markedly under the food safety criteria indicated by EC Regulation 2073/2005 (< 100 mg/kg). The overall mean histamine concentration was 6.32 ± 2.28 mg/kg in anchovy, 6.17 ± 2.60 mg/kg in tuna and 4.98 ± 5.76 mg/kg in mackerel. A significant difference in histamine concentration was found among the samples of canned anchovy, mackerel and tuna in brine ($P < 0.05$). However, no significant difference in histamine concentration was found between the categories of canned fish. The commercial ELISA test revealed the occurrence of histamine at a very low concentration in some brands of Italian canned fish products. This rapid method offered great benefits and can be routinely applied as a control strategy in the frame of HACCP application.

Keywords: histamine, canning, tuna, mackerel, anchovy, food safety, Elisa.

EFFICIENCY OF OZONATION FOR THE REMOVAL OF AFLATOXIN B₁ AND OCHRATOXIN A IN NUTS

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The aim of this study was to evaluate the efficiency of ozonation in reducing aflatoxin B₁ and ochratoxin A in nuts.

During the study, mold colony-forming units were determined using a dilution method based on ISO 21527-2:2008, and a mycotoxicological analysis was also performed. In total,

22 samples were analyzed: 6 shelled hazelnuts, 8 hazelnuts with shell and 8 peanuts with shell. The study was carried out in two stages. In the first stage, the number of mold CFU before and after ozonation (2.5 ppm, 30 or 60 min) was determined. In the second stage, the concentration of mycotoxins (aflatoxin B₁ and ochratoxin) was determined before and after ozonation treatment.

The results of the study showed that hazelnuts with shell samples were the most infected with mold, from $0.6 \pm 0.1 \log_{10}$ CFU/g to $1.6 \pm 0.1 \log_{10}$ CFU/g. After 30 minutes of ozonation, molds were completely destroyed in all shelled hazelnuts samples ($P > 0.05$). After 60 minutes of ozonation, all molds in nuts were destroyed ($P < 0.05$). Before ozonation, the highest AFB₁ concentrations were detected in shelled hazelnuts samples (up to 5.0 µg/kg), while hazelnuts with shell (75% of all samples) showed the highest levels of contamination with OTA. After 30 minutes of ozonation at 2.5 ppm, AFB₁ concentrations significantly decreased in peanuts and hazelnuts with shell samples ($P < 0.05$). After 30 minutes of ozonation, OTA concentrations were significantly lower in peanuts and hazelnuts with shell samples ($P > 0.05$). Similar results were obtained after 60 minutes of ozonation, although in shelled hazelnut samples, OTA reduction was not statistically significant ($P > 0.05$).

Keywords: ochratoxin A, aflatoxin B₁, molds, ozonation.

THE USE OF APPLE POMACE IN OPTIMIZING SEMI-WHEAT BREAD FORMULATION

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This research investigated the incorporation of freeze-dried and fermented apple pomace into semi-wheat bread formulations to enhance quality without compromising consumer acceptability. Seven bread recipes were examined: a control sample with no pomace and six experimental samples with apple pomace incorporated at 5%, 10%, 15%, 20%, 25%, and 30% levels. The incorporation of apple pomace had a notable influence on the quality parameters. Bread with 20% of apple pomace (BAP20) exhibited the highest specific volume (2.35 mL/g), while bread with 25% of pomace showed the lowest (2.05 mL/g). The 15% pomace formulation demonstrated excellent shape retention, whereas the 20% and 25% variants showed progressively diminished structural integrity. BAP20 represented the optimal balance between nutritional enhancement and sensory characteristics. Crumb porosity analysis revealed a consistent increase in formulations containing 5–20% pomace, with BAP20 achieving maximum porosity (47.82%), followed by a notable decline in the 25% variant. Texture profile analysis indicated that firmness reached optimal levels at 20% pomace incorporation, becoming excessive at 25% concentration due to significant gluten network disruption. Sensory evaluation confirmed that BAP20 received the highest overall acceptability scores, with the 15% formulation achieving acceptable ratings, while the 25% variant scored significantly lower due to pronounced acidity and diminished aroma. Crust characteristics, particularly color and crispness, were most favorable in the BAP20 variant. Finally, the research concludes that 20% of apple pomace represents the optimal concentration for semi-wheat bread formulations.

Keywords: apple pomace, fermentation, semi-wheat bread quality parameters.

CREATING CRACKERS ENRICHED WITH PROTEINS FROM OAT-MILK BY-PRODUCTS AND MILK POWDER

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Crackers are baked goods valued as a snack due to their crunchy, dry texture and long shelf life, usually made from wheat flour, water, salt, and various flavorings. In recent years, there has been an increased demand for healthier, protein-rich snacks. This study assessed high-protein fermented and unfermented composite blends in cracker recipes, incorporating egg-based or flaxseed-based dough, oat milk by-products, and a mixture of milk protein concentrate powder. It examines the impact of these protein combinations on dough characteristics, cracker structure, physicochemical properties, color coordinates, and sensory properties.

Physicochemical analysis showed that the color coordinates of the dough showed noticeable differences, mainly influenced by fermentation and the type of binder used. Texture analysis showed that fermentation affected the dough firmness and elasticity, with egg-based fermented and non-fermented by-product crackers displaying a softer texture compared with the flaxseed-based fermented and non-fermented ones. TTA measurements indicated increased acidity in the fermented samples, which contributed to enhanced preservation potential. Sensory evaluation indicated that the crackers made with egg-based and flaxseed-based fermented by-products were well-accepted for their superior mouthfeel, color, and texture. This research highlights the potential of incorporating milk powder proteins and oat-milk by-products into cracker formulations as a valuable alternative.

Keywords: protein-rich crackers, milk powder, fermented grain by-products, texture analysis, food sustainability.

MOLD AND MYCOTOXIN RISK ASSESSMENT IN BAKERY DUSTS

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Airborne dust and mold spores are considered the primary cause of spoilage in bread products. Airborne contamination is a major source of food contamination because it can carry mold spores. Mold contamination of baked goods also results in economic losses for companies. Dust and the molds and mycotoxins it contains not only affect product quality, but the effects of dust have also been linked to several respiratory diseases in bakery workers.

The aim of this study was to determine the levels of molds and mycotoxins in dust sampled in the bakery environment. Dust samples were collected from a bakery during all seasons of the year. Samples were collected using two different methods: gravimetric and passive. The mold species and mycotoxin concentrations were determined in the dust samples. All data analyses were performed using Statistica software (Microsoft Excel 2016 and SPSS). The amount of dust varied from 0.093 g to 0.433 g and 0.010 g/m³ to 0.051 g/m³ collected by passive and gravimetric methods, respectively. The dominant mold species were *Rhizopus spp.*, *Trichoderma spp.*, *Penicillium spp.*, and *Cladosporium spp.* The mycotoxins OTA, AFL B1 were detected in the dust samples. No positive values were found for the mycotoxins ZEA and DON. Our research data show how crucial it is to identify mold hazards in bakery to ensure the safety of production and health of workers.

Keywords: dust, mold, mycotoxins, bakery.

THE EFFECT OF *LACTOBACILLUS* STRAINS AGAINST *CAMPYLOBACTER JEJUNI* AND *LISTERIA MONOCYTOGENES* IN POULTRY AND SALMON

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Campylobacter jejuni (EFSA's priority foodborne pathogen) and *Listeria monocytogenes* (a resilient seafood-associated threat) pose critical food safety risks. This research evaluates *Lactobacillus helveticus* (*L. helveticus*), *Lacticaseibacillus paracasei* (*L. paracasei*), and acid whey (AW) for enhancing the safety of marinated poultry and salmon fillets. In broiler wings, the combination of *L. helveticus* and AW decreased *C. jejuni* numbers by 1.56 log₁₀ CFU/g while *L. helveticus* alone showed a significant effect (2.55 log₁₀ CFU/g reduction) in broiler fillets by day 5. After inoculation, *L. paracasei* survived well in broiler fillets (8.10 log₁₀ CFU/g, day 5), while AW promoted the growth of *Lactobacillus* in the wings (7.32 log₁₀ CFU/g, day 5), which assisted in the inhibition of *C. Jejuni* counts. The combination of *L. helveticus* and AW had a substantial reduction of aerobic mesophilic bacteria in wings, while AW alone had a similar effect on fillets ($P < 0.05$). In salmon fillets, *L. helveticus* with AW inhibited *L. monocytogenes* by 0.30 log₁₀ CFU/g on day 5 for the samples without skin ($P < 0.05$). This combination also increased lactic acid bacteria in skinless samples by day 5, contributing to *L. monocytogenes* reduction. Additionally, *L. helveticus* and AW decreased aerobic mesophilic bacterial count by 1.11 log₁₀ CFU/g in the samples without skin by day 5 ($P < 0.05$). This study uses a combination of lactic acid bacteria exhibiting probiotic properties and AW to provide a potential way to decrease *C. jejuni* and *L. monocytogenes*, thus improving food safety.

Keywords: *Campylobacter jejuni*, *Listeria monocytogenes*, probiotics, food safety, acid whey.

EVALUATION OF CERTAIN *LACTOCOCCUS LACTIS* STRAINS INTENDED TO BE USED FOR DAIRY FOOD FERMENTATION

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The aim of this study was to evaluate the acidifying and antibacterial activities of ten *Lactococcus lactis* strains isolated from raw and fermented cow and goat milk, and also wheat. Antibacterial activity using agar spot and overlay methods, also in a real food matrix (milk) was evaluated against two foodborne pathogens – *Listeria monocytogenes* and *Staphylococcus aureus* that are significant for the dairy industry. The acidifying activity was assessed by measuring pH changes at 6-hour and 24-hour intervals, with LL16 isolated from raw cow milk emerging as the standout strain, exhibiting the best aroma characterized by a good fermented and slightly creamy profile, along with a solidified gel-like consistency after 24 hours with a pH drop of 2.26 ± 0.01 . *L. lactis* L29 demonstrated the highest antibacterial activity against *Staphylococcus aureus* ATCC 25923 with a 2.35 ± 0.19 cm diameter inhibition zone. Additionally, LL16, LL29, and LL14C showed a strong antibacterial activity against *Listeria monocytogenes* ATCC 7644, each producing an inhibition zone with a diameter of 2.15 ± 0.09 cm. In the milk contaminated with *L. monocytogenes* ATCC 7644 and inoculated with *L. lactis* LL16 strain, the number of *L. monocytogenes* ATCC 7644 after 24 and 48 hours was 1.89 and 4.83 log₁₀ CFU/mL lower, respectively, compared with the control sample. After 72 hours, no *L. monocytogenes* ATCC 7644 were detected at all. *L. lactis* LL16 strain showed potential in controlling *L. monocytogenes* ATCC 7644 and *S. aureus* ATCC 25923 and was selected for further experiments with yoghurt.

Keywords: antibacterial activity, acidifying activity, pH changes.

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VETERINARY TODAY: ADVANCES IN EVIDENCE-BASED VETERINARY MEDICINE IN SMALL ANIMAL CLINICAL PRACTICES

Abstracts

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MANAGEMENT OF STATUS EPILEPTICUS AND CLUSTER SEIZURES IN DOGS AND CATS

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Status epilepticus (SE) and cluster seizures (CS) represent life-threatening neurological emergencies in dogs and cats, requiring rapid recognition and structured intervention. This presentation reviews the ACVIM Consensus Statement and translates it into a practical, stepwise treatment algorithm for clinicians. Definitions and classification of SE and CS are outlined, followed by an evidence-based overview of first-, second-, and third-line antiepileptic therapies, including recommended drug choices, routes of administration, and dosages. Key physiological considerations are discussed, as well as tapering protocols following seizure control. The session emphasizes a flowchart-driven approach that enables veterinarians to make rapid, effective decisions in emergency situations, ultimately improving patient outcomes.

Keywords: status epilepticus, cluster seizures, dog, cat, emergency treatment, antiepileptic drugs, ACVIM consensus.

DIAGNOSTIC VALUE OF CEREBROSPINAL FLUID (CSF) COLLECTION AND ANALYSIS IN NEUROLOGICAL DISORDERS

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Cerebrospinal fluid (CSF) is a clear, colourless liquid that plays a crucial role in central nervous system (CNS) homeostasis. It cushions the brain and spinal cord, delivers nutrients, removes metabolic waste, and helps regulate intracranial pressure. CSF is produced mainly by the choroid plexuses and reabsorbed through arachnoid granulations into the venous circulation. In veterinary medicine, CSF collection is typically performed under general anaesthesia from the cerebellomedullary cistern (*cisterna magna*) or the lumbar subarachnoid space. Analysis of CSF includes measurement of total nucleated cell count, protein concentration, glucose levels, and cytological evaluation to identify bacteria, neoplastic cells, or other abnormalities. These findings are interpreted in conjunction with clinical signs and advanced imaging, such as MRI or CT, to enhance diagnostic accuracy, particularly since CSF abnormalities are often nonspecific [1, 2].

CSF analysis is most valuable for diagnosing inflammatory and infectious diseases, including meningitis, encephalitis, and myelitis, and for detecting neoplastic conditions affecting the CNS (3). Cytology aids in determining the type of inflammation, while culture and molecular techniques can confirm infectious agents. Emerging research is expanding the use of CSF biomarkers for neurodegenerative, traumatic, metabolic, and autoimmune diseases, potentially improving early diagnosis and monitoring of disease progression [4].

Although considered routine, CSF collection carries risks such as haemorrhage, infection, and neurological complications. Proper patient selection, careful technique, and timely sample processing are essential to minimize these risks and to preserve diagnostic accuracy [5]. Overall, CSF analysis remains a cornerstone of veterinary neurology, offering a minimally invasive method to gain insight into CNS pathophysiology and guide targeted therapeutic strategies.

Keywords: cerebrospinal fluid, neurology, meningitis, encephalitis, myelitis, CNS, cytology, protein, spinal tap.

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DETERMINATION OF ANTIMICROBIAL ACTIVITY OF CHLORHEXIDINE-CHITOSAN GEL *IN VITRO*

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Introduction. Skin infections are an increasing concern in both human and veterinary medicine due to factors such as antimicrobial resistance, lifestyle changes, and hygiene habits. The frequent and inappropriate use of systemic antibiotics for superficial skin infections has led to multidrug-resistant pathogens, complicating treatment [1]. This highlights the need for alternative therapies, such as topical antiseptics, to reduce antibiotic use and resistance development.

Objective. This study aimed to evaluate the *in vitro* antimicrobial activity of a chitosan gels containing chlorhexidine against Gram-positive and Gram-negative bacteria.

Methods. The gel's antimicrobial properties were assessed *in vitro* using the agar diffusion method on Mueller-Hinton agar [2]. Tested organisms included reference strains of *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853), along with wild strains of the same species.

Results. The antimicrobial effect of the gels was not dependent on chlorhexidine concentration but was influenced by chitosan content. The 1% chitosan gel showed the strongest inhibitory effect on both reference and wild strains. The results emphasized the importance of testing both strain types, as wild strains were generally more resistant than reference strains. This suggests chitosan boosts chlorhexidine's antimicrobial effect and shows potential for use in skin and wound antiseptics in veterinary medicine.

Conclusions. The study confirmed the synergistic action of chlorhexidine and chitosan, with stronger efficacy against Gram-positive bacteria. Further preclinical trials are planned, and it is expected that chitosan, combined with chlorhexidine, will not only enhance antimicrobial activity but also aid wound healing in damaged tissues.

Keywords: chitosan, chlorhexidine, antiseptic, gel.

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THE ROLE OF MICROSCOPE IN MODERN NEUROSURGERY

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The operating microscope has transformed neurosurgery more than any other innovation. In the early 20th century, modern neurosurgery pioneers such as Harvey Cushing and Dandy Walker carried out brain surgery with limited visualization, often facing high complication rates and restricted access to deeper structures. This changed dramatically in the late 1960s, when Mahmut Gazi Yasargil [1] introduced the surgical microscope which later changed the concept of surgical technique leading to a new term called *microneurosurgery*. For the first time, neurosurgeons could work with high magnification, bright illumination, and refined precision. This not only reduced damage to surrounding brain tissue but also opened the door to procedures that had previously been unimaginable, such as bypassing tiny cerebral arteries. The microscope also stimulated the development of an entirely new set of microsurgical instruments, specifically designed for delicate work in confined spaces. Modern microscopes now offer far more than magnification: they integrate real-time navigation and fluorescence tools such as indocyanine green (ICG) and sodium fluorescein, helping surgeons distinguish tumors, vessels, and functional pathways [2]. By reshaping both the technical and conceptual possibilities of brain surgery, the surgical microscope has become a cornerstone of modern neurosurgical practice.

Keywords: modern neurosurgery, surgical microscope.

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IS ONE PLUS ONE ALWAYS EQUALS TWO?

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Mixed infections of avian haemosporidian parasites (Haemosporida) predominate in wildlife [1]; however, we are still struggling to understand interactions between different parasites and their impact on host fitness. It is conceivable that a host infected with several different parasites may be significantly more affected immunologically than the one infected with only one parasite. We also have no data on whether different vector-borne parasites can be transmitted by a single insect bite. In this work, we aimed to understand if the effect of two haemosporidian parasites on a vertebrate host was equal to their combined effect, and whether mixed infections could be transmitted by one bite of a vector.

We followed the parasitemia, survival and some health parameters (changes in body weight, haemoglobin level, haematocrit value, number of polychromatophils) in domestic canaries experimentally infected with a single infection of *Plasmodium relictum* (genetic lineages pSGS1), *P. relictum* (pGRW11), *Plasmodium elongatum* (pERIRUB01) and their co-infections. In the case of co-infection with *P. elongatum* (pERIRUB01) and *P. relictum* (pSGS1), *P. elongatum* intensity of parasitemia was enhanced by the presence of *P. relictum*, while the parasitemia of *P. relictum* stayed the same [2]. The virulence of this co-infection was similar to that of the more virulent parasite (*P. elongatum*). In the case of co-infection with two lineages of *P. relictum* (pSGS1 and pGRW11), two parasites did not alter overall parasitemia and did not cause higher virulence to the host [3].

We found that two haemosporidian parasites can successfully complete their development in the same mosquito vector at the same time and form the sporozoite stage in mosquito salivary glands. Hence, two vector-borne parasites can be transmitted via one bite of the same infected vector.

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Keywords: co-infection, Culicidae, Haemosporidian parasites, host, *Plasmodium*.

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THE EYES ARE THE WINDOWS TO THE BRAIN

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Vision, menace response, pupillary light reflex, and eye position, together with oculocephalic reflex, are important factors used to evaluate specific brain parenchyma areas [1–3]. However, when dysfunction occurs, a solid understanding of neuroanatomy and neurophysiology is needed to distinguish the correct neuroanatomical localization and/or ophthalmic diseases.

This literature review aims to summarize the current knowledge on the neuro-ophthalmological examination, emphasizing its role in clinical diagnosis and decision-making.

Symptoms such as hypovision, blindness, mydriasis, miosis, nystagmus, strabismus, and anisocoria might indicate involvement of the neuro-ophthalmologic tracts, which are distributed widely through the brain parenchyma. The vision pathway includes the retina, optic nerve, optic chiasm, optic tracts, lateral geniculate nucleus, optic radiations, and visual cortex [1–3]. These structures begin at the fundus of the eye, course ventrocaudally to the base of the skull, and then dorsocaudally until the occipital cortex is reached. Correct eye position and movements are assured by the vestibular system and three cranial nerves: oculomotor, trochlear, and abducens, together with their controlled muscles [1–3]. These structures and their nuclei are mostly located in the mesencephalon, pons, and medulla oblongata. Pupillary light reflex pathway shares its prechiasmatic portion with the visual pathway, but instead it courses to the pretectal nucleus, and parasympathetic nucleus of the oculomotor nerve, to then reach the short ciliary nerves [1–3]. Despite strictly described pathways, quite commonly, eye-related neurological symptoms occur with unusual neurolocalization, such as the thalamus or the cortex of the brain [4, 5]. This proves that correct decisions can only be made by combining neurological and neuro-ophthalmological examinations.

The neuro-ophthalmological examination remains a cornerstone of veterinary neurology and ophthalmology, as most neuroanatomical structures needed for correct vision, eye position, and pupil size are widely distributed through the central nervous system. Early recognition of abnormalities and integration of diagnostic modalities can facilitate timely intervention and prevent further disease progression.

Keywords: veterinary neurology, neuro-ophthalmology, vision, cranial nerves.

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OXIDATIVE STRESS CHANGES DURING LAPAROSCOPIC AND OPEN OVARIECTOMY IN BITCHES WITH THE SAME ANAESTHESIA PROTOCOL

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Laparoscopic ovariectomy (OVE) is increasingly utilised in veterinary practice due to its advantages over open surgery. However, it requires pneumoperitoneum (PNP), typically induced via CO₂ insufflation, to create an operative workspace [1]. The aim of this study was to evaluate the systemic effect of PNP by measuring oxidative stress in blood serum during laparoscopic and open OVE in bitches with the same anaesthesia protocol. A total of 20 client-owned healthy (ASA I class) female dogs were included in this study: laparotomy ovariectomy group (LPTOVE, n = 10) and laparoscopic ovariectomy group (LAPOVE, n = 10). The anaesthesia protocol for dogs of both groups was the following: premedication – methadone hydrochloride 0.5 mg/kg and dexmedetomidine hydrochloride 3 mcg/kg *iv*, induction – propofol 2–4 mg/kg *iv*, anaesthesia maintenance – sevoflurane (vaporiser setting 2%) in O₂ 100%. Postsurgical pain was controlled with buprenorphine hydrochloride 0.015 mg/kg *im* and meloxicam 0.2 mg/kg *sc*. For oxidative stress evaluation, blood samples were collected from *v. jugularis* before premedication (T0), after abdominal incision in group 1 and before PNP in Group 2 (T1), before suturing an abdominal wound in group 1 and before PNP deflation in group 2 (T2) and 2 hours after the surgery (T3). TOS and TAS were determined by using colorimetric assay kits following the manufacturers' instructions. The oxidative stress index (OSI) was calculated as follows: OSI (arbitrary unit) = TOS (mmol H₂O₂ Eq L⁻¹)/TAS × 10 (mmol Trolox Eq L⁻¹) [2]. Statistical analysis was done with SPSS 29.0 software by assessing median, minimum and maximum values. The differences were statistically significant when *P* < 0.05. The median of TOS was as follows: at T0, group 1 – 92.37 [76.40–117.47], group 2 – 86.34 [26.38–97.35] (*P* = 0.199); at T1, group 1 – 96.17 [67.83–102.78], group 2 – 90.83 [43.13–100.74] (*P* = 0.545); at T2, group 1 – 86.31 [0.522–99.23], group 2 – 42.06 [6.34–103.11] (*P* = 0.450); at T3, group 1 – 83.53 [5.06–100.42], group 2 – 87.13 [46.13–125.36] (*P* = 0.151). The median of TAS was as follows: at T0, group 1 – 0.96 [0.05–1.17], group 2 – 0.90 [0.04–1.82] (*P* = 0.821); at T1, group 1 – 0.85 [0.15–1.15], group 2 – 1.09 [0.34–1.99] (*P* = 0.070); at T2, group 1 – 0.71 [0.34–1.12], group 2 – 1.05 [0.05–2.09] (*P* = 0.076); at T3, group 1 – 0.49 [0.04–1.14], group 2 – 0.75 [0.20–1.94] (*P* = 0.450). The median of OSI was as follows: at T0, group 1 – 10.30 [7.08–162.05], group 2 – 8.47 [2.03–235.06] (*P* = 0.406); at T1, group 1 – 11.89 [6.54–56.11], group 2 – 8.07 [4.42–26.75] (*P* = 0.082); at T2, group 1 – 8.53 [0.08–24.60], group 2 – 4.82 [0.96–24.12] (*P* = 0.199); at T3, group 1 – 9.41 [0.76–53.70], group 2 – 11.48 [3.63–45.22] (*P* = 0.821). According to the study data, there was no difference on oxidative stress changes during laparotomy or laparoscopic OVE in bitches with the same anaesthesia protocol.

Keywords: laparoscopic ovariectomy, open ovariectomy, oxidative stress, anaesthesia.

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ADVANCES IN CANINE URINARY BIOCHEMISTRY: FRACTIONAL EXCRETION OF SODIUM AND POTASSIUM

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Currently, the specific gravity or osmolality and the urinary protein-creatinine ratio are the most widely measured biochemical analytes in urine in routine practice. The measurement of new analytes in urine such as the fractional excretions is representing a new field of interest in the companion animal clinics. These fractional excretions can provide interesting information not only about the status of the kidney but also about the general health of the animal, being of particular interest for the evaluation of some aspects such as dehydration or the presence of some endocrine diseases in which the kidney is involved, e.g. hypoadrenocorticism.

In this presentation, we deal with three main topics:

(1) The concept of fractional excretion, which is mainly determined by the need of analyte, the renal function and the hormones involved in the metabolism of the analyte in the kidney.

(2) The fractional excretion of sodium and its application and interpretation, with a special emphasis on its use as a sensitive biomarker of dehydration, overhydration, renal failure or hypoadrenocorticism.

The formula is as follows:

Fractional excretion of sodium = $(\text{Na urine} / \text{Na plasma}) / (\text{creatinine urine} / \text{creatinine plasma}) \times 100$

(3) The transtubular potassium gradient (TTKG) which, in human medicine, is preferred to the fractional excretion of potassium since there is a positive correlation between the TTKG and serum aldosterone levels, and is measured with the formula:

$\text{TTKG} = (\text{K urine} / (\text{Uosm} / \text{Posm})) / \text{K plasma}$.

Uosm is the urine osmolality, and Posm is the plasma osmolality.

This TTKG could help clinicians in diagnosing primary hypoadrenocorticism in hyperkalaemic dogs having a clinical suspicion of this disease. When synthetic ACTH is not available or the result of the ACTH stimulation test is delayed, estimation of the TTKG could help the clinician in initiating mineral and glucocorticoid therapy, especially if life-threatening hyperkalaemia is present [1].

Keywords: urine, sodium, potassium.

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NEUROGENIC BLADDER: NEUROLOGICAL DISORDERS OF MICTURITION AND CHALLENGES FOLLOWING THEIR MANAGEMENT IN DOGS

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Neurogenic bladder (NB) or neurogenic lower urinary tract dysfunction (NLUTD) is a generalised term for the micturition disorders arising from lesions affecting either the central or peripheral nervous systems. It leads to disruption of urinary bladder and urethral function, impairing the process of storage and periodic voiding of urine [1, 2]. If left untreated, micturition disorders can lead to poor life quality or lethal complications; therefore, it is important to understand their pathophysiology and management options.

This report aims to review the literature and scientific publications to provide a brief overview of the aetiology, clinical presentation, and management of NLUTD in canine patients.

Control of micturition involves complex pathways in the central and peripheral nervous systems. Clinical presentation of NB depends on lesion localisation and is classified as lower motor neuron (LMN) bladder or upper motor neuron (UMN) bladder. The UMN bladder derives from pathologies of micturition pathways cranial to sacral spinal cord segments, leading to retention of the urine. The UMN bladder is extremely difficult to manually express. On the contrary, LMN bladder results in constant urine leakage consequent to poor sphincter function, caused by disturbances at the level of sacral spinal cord segments, sacral spinal nerves, pelvic nerves, and sacral plexus, or pudendal nerves [3, 4]. Treatment of NLUTD is highly linked to the primary cause, severity, and duration of urinary incontinence. Management consists of a multimodal approach, such as manual bladder expression or catheterisation, together with a pharmacological treatment with α -adrenergic receptor antagonists, benzodiazepines, parasympathomimetics, or sympathomimetics, depending on the dysfunction type. In chronic cases, surgical resolutions, like placement of a sacral nerve stimulator for bladder emptying in chronically paraplegic dogs, can be considered [3, 5].

Despite high prevalence in neurological clinical practice, NB remains challenging to manage and often raises concerns about owner management and complications affecting patient quality of life.

Keywords: neurogenic bladder, urinary incontinence, urinary retention, dogs.

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FEEDING HOSPITALIZED PATIENTS, MEETING THEIR NUTRITIONAL NEEDS: INTEGRATING PRINCIPLES AND PRACTICE

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Optimal nutritional support is a critical component in the recovery of hospitalised dogs and cats, particularly in the perioperative period. This presentation integrates key principles of clinical nutrition with practical interventions to address the diverse needs of surgical patients. The first part focuses on assessing energy requirements during hospitalisation and identifying nutrients essential for tissue repair, immune function, and inflammation control. Strategies for reintroducing food, maintaining hydration, and managing gastrointestinal tolerance are discussed, including considerations for patients with comorbidities such as obesity.

The second part explores assisted feeding techniques, emphasising the use of feeding tubes in patients unable or unwilling to eat. Indications for tube placement, types of feeding tubes, associated risks, and nutritional calculations are reviewed. The session concludes with recommendations for client education to ensure safe and effective home care, supporting long-term recovery. This integrated approach aims to enhance clinical outcomes through evidence-based nutritional management.

Keywords: clinical nutrition, dogs, cats, feeding tubes.

THE MICROBIOME AND ITS ROLE IN NEUROLOGICAL DISEASES OF SMALL ANIMALS

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The bidirectional communication between the gut and the brain, known as the “gut–brain axis,” is an emerging focus in veterinary neurology. Altered intestinal microbiota can influence central nervous system function through immune modulation, microbial metabolites, neurotransmitter signalling, and barrier integrity [1, 2].

In dogs, dysbiosis has been linked to idiopathic epilepsy, and medium-chain triglyceride supplementation may reduce seizure frequency [1, 3]. Immune-mediated conditions, including meningoencephalomyelitis of unknown origin (MUO), may also be shaped by gut microbial composition, resembling human autoimmune diseases [4, 5]. Age-related microbiome shifts are associated with neuroinflammation and cognitive decline, suggesting a role in canine cognitive dysfunction [2].

Although research is limited, the microbiome shows promise as a biomarker and therapeutic target in neurology. Investigated strategies include diet modification, probiotics, and faecal microbiota transplantation, but further controlled studies are required before clinical application.

This lecture summarizes current evidence on the microbiome–brain axis in dogs, emphasising its relevance for epilepsy, MUO, and cognitive dysfunction.

Keywords: gut–brain axis, microbiome, small animal neurology, epilepsy, MUO, cognitive dysfunction.

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SPATIAL AND RISK FACTOR ANALYSES OF VECTOR-BORNE PATHOGENS AMONG SHELTER DOGS IN LITHUANIA

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Introduction. Vector-borne diseases (VBDs) are a growing health concern in canine populations across Europe, including Lithuania. Shelter dogs, often lacking known health and travel histories, may act as sentinels for local pathogen circulation [1, 2]. This study aims to assess the prevalence and spatial distribution of selected VBDs in shelter dogs in Lithuania and examine associated clinical and hematological findings.

Methods. Blood samples (n = 91) were collected from clinically healthy shelter dogs across various regions of Lithuania. All samples will be screened for *Anaplasma spp.*, *Ehrlichia spp.*, *Borrelia burgdorferi*, and *Dirofilaria immitis* using the IDEXX SNAP 4Dx Plus Test. Each dog will undergo a complete clinical examination and complete blood count (CBC) analysis. Molecular techniques, including polymerase chain reaction (PCR), will confirm and identify pathogens at the species level.

Expected Results. It is expected that both serological and molecular testing will reveal infections with vector-borne pathogens such as *Anaplasma spp.* and *Borrelia burgdorferi* among clinically healthy shelter dogs. This will help identify asymptomatic carriers and assess the potential for disease transmission. The study also aims to outline the current epidemiological situation and highlight potential regional hotspots of infection.

Conclusion. This study combines clinical, molecular, and spatial data to improve the understanding of canine VBD epidemiology in Lithuania. Findings will support the development of targeted surveillance and prevention strategies. Moreover, evaluating environmental and demographic factors will help identify the conditions facilitating pathogen spread, contributing to more effective public and animal health risk assessments.

Keywords: vector-borne diseases, *Anaplasma spp.*, *Ehrlichia spp.*, *Borrelia burgdorferi*.

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MEDIUM-CHAIN TRIGLYCERIDES (MCT) AS A NUTRITIONAL APPROACH IN EPILEPTIC ANIMALS: EVIDENCE AND PERSPECTIVES

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Epilepsy is one of the most prevalent chronic neurological disorders in companion animals, with approximately one-third of affected dogs showing drug resistance despite adequate antiseizure drug (ASD) therapy. The growing interest in dietary interventions has highlighted the potential role of medium-chain triglycerides (MCTs) in seizure management. In a multicentre, prospective, randomised, double-blinded, placebo-controlled crossover trial including 28 dogs with idiopathic epilepsy, MCT supplementation (9% of daily metabolic energy) significantly reduced seizure frequency (median 2.51/month vs 2.67/month; $P = 0.02$) and seizure-day frequency (1.68/month vs 1.99/month; $P = 0.01$) compared with the control oil [1]. Two dogs became seizure-free, three achieved $\geq 50\%$ seizure reduction, while 12 dogs experienced $< 50\%$ reduction and 11 had no improvement or worsened [1]. These results confirm and extend previous findings from a kibble-based MCT diet trial in 21 epileptic dogs, which demonstrated increased serum β -hydroxybutyrate concentrations, reduced seizure frequency, and improved behavioural comorbidities [2]. Beyond seizure control, MCT supplementation was associated with significantly less sedation (baseline $47\% \pm 28\%$ vs MCT $29\% \pm 18\%$; $P = 0.002$), reduced ataxia ($43\% \pm 28\%$ vs $29\% \pm 25\%$; $P = 0.003$), and improved owner-reported quality of life (baseline $38\% \pm 21\%$ vs MCT $10\% \pm 14\%$; $P \leq 0.001$). Owners also reported being less bothered by their dogs' mental status ($P = 0.03$), perceiving a less severely ataxic gait ($P = 0.003$), and better tolerating ASD adverse effects during the MCT period ($P = 0.004$). These findings confirm and extend previous evidence from a kibble-based MCT diet trial in 21 epileptic dogs that demonstrated increased serum β -hydroxybutyrate concentrations, seizure reduction, and behavioural improvements [2]. Overall, clinical trials indicate that MCT supplementation provides both seizure reduction and quality of life benefits in a subset of drug-resistant epileptic dogs, supporting its role as a promising adjunctive management strategy [3].

Keywords: epilepsy, dogs, medium-chain triglycerides, dietary management, seizure control.

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IMMUNOLOGICAL AND DIAGNOSTIC IMAGING FINDINGS IN EVALUATING CLINICAL MANIFESTATION OF FELINE INFECTIOUS PERITONITIS IN CATS

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Feline infectious peritonitis (FIP) is a fatal disease caused by a mutated form of feline coronavirus (FCoV). FCoV exists in two biotypes: the non-mutated feline enteric coronavirus (FECV) and the virulent feline infectious peritonitis virus (FIPV). Transmission occurs via the faecal–oral route, making multi-cat environments (e.g., catteries, shelters) high-risk settings [1, 2]. FIP can present in two main forms: effusive (wet) and non-effusive (dry), though clinical overlap is common [3]. This retrospective study aimed to evaluate immunological markers and ultrasonographic findings in cats with different forms of FIP diagnosed at a veterinary clinic between January 2023 and April 2025. Twelve cats were included (9 males, 3 females) across six breeds: mixed (n = 6), Cornish Rex (n = 2), Burmese (n = 1), Maine Coon (n = 1), Scottish Fold (n = 1), and British Shorthair (n = 1). The median age was 24 months (SD ± 37.38), with the majority (n = 8) over 12 months of age. FCoV antibody titers were higher in cats with mixed-form FIP compared with other forms, but the difference was not statistically significant ($P > 0.05$). Similarly, there was no significant difference in antibody levels between effusive and non-effusive forms ($P > 0.05$). The albumin-to-globulin (A/G) ratio was significantly lower in mixed-form FIP compared with other forms ($P < 0.001$), and lower in wet versus dry FIP ($P < 0.01$). Gamma-globulin levels were significantly elevated in both mixed-form ($P < 0.001$) and effusive FIP ($P < 0.05$) compared to dry FIP. Distinct immunological and ultrasonographic patterns were observed among FIP forms. These findings highlight the diagnostic value of serological markers and imaging findings in differentiating clinical manifestations of FIP and may aid in disease assessment and management.

Keywords: feline coronavirus, feline infectious peritonitis, immunology, ultrasonography.

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SHOULDER INSTABILITY SYNDROME IN DOGS: DIAGNOSIS AND TREATMENT OPTIONS INCLUDING ARTHROSCOPIC LIGAMENT RECONSTRUCTION USING BONE ANCHORS

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Instability of the shoulder joint has been reported to be the most common type of soft tissue pathology in medium-large breed dogs with medial shoulder instability (MSI) being diagnosed in 78% of the cases. MSI in dogs is associated with partial or complete rupture of the medial glenohumeral ligament (MGHL) and/or the subscapularis tendon (SScT). The diagnosis is challenging and is based on orthopaedic examination, abduction angle measurement, radiographic stress views, and arthroscopy, which remains the gold standard for diagnosis and lesion grading. Treatment strategies vary with severity and include radiofrequency-induced capsulorrhaphy, open or modified medial imbrication, extracapsular stabilisation, or arthroscopic ligament reconstruction using bone anchors. In this prospective case series, six dogs (eight shoulders) with grade III–IV MSI underwent arthroscopic reattachment of the MGHL or SScT using knotless bone anchors (PushLock®). Anchors were placed under direct arthroscopic visualisation via a cranio-medial portal. Postoperative evaluation included abduction angle measurement, radiography, and lameness assessment. All dogs showed resolution of lameness within four weeks and returned to full function, including hunting activities. No major complications or relapses were observed during follow-up (2 months–4 years). Advantages of the knotless anchor system included secure and low-profile fixation, minimal tissue manipulation, eliminating arthroscopic knot tying, reduced surgery time and consistent construct strength. Limitations included technical challenges, especially with scapular anchor placement and the small case number. In conclusion, arthroscopic ligament reconstruction with knotless bone anchors is a minimally invasive and effective technique for treating high-grade MSI in dogs, offering reliable joint stabilisation and rapid functional recovery. Further biomechanical and prospective clinical studies are warranted to validate these findings [1–6].

Keywords: medial shoulder instability, dog, arthroscopy, bone anchor, ligament reconstruction.

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SMALL ANIMAL VETERINARY MEDICINE IN LITHUANIA – WHERE ARE WE IN THE CONTEXT OF EUROPEAN VETERINARY SCHOOLS?

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Evidence-based small animal veterinary medicine (EBSAVM) integrates the best available research evidence with clinical expertise and patient-owner values to improve outcomes in companion animals. Across European veterinary schools, EBSAVM has become a unifying scientific and pedagogical approach, shaping curricula, research agendas, and clinical decision-making. Lithuania's veterinary education and research are undergoing rapid development, but their position in the wider European EBSAVM landscape has not been clearly described.

Objectives. The study aims to evaluate how EBSAVM principles are embedded in Lithuanian small animal veterinary education, research, and clinical practice compared with European veterinary schools, and to identify priority areas for strengthening evidence generation and translation into practice.

Methods. We reviewed Lithuanian veterinary curricula, postgraduate training, and research outputs against European standards and initiatives (ESEVT/EAEVE Day One Competences, European Board of Veterinary Specialisation, EBVM Network). A narrative analysis of national research databases, peer-reviewed publications was conducted to map current EBSAVM activities and infrastructures. These data were benchmarked against publicly available information from selected European veterinary schools recognised for leadership in EBSAVM.

Results. Lithuanian veterinary education exhibits strong alignment with core European Day One Competences and growing integration of EBSAVM into undergraduate teaching (critical appraisal, structured clinical questions, and application of systematic reviews). Research in small animal medicine is increasing, particularly in infectious diseases, anaesthesia/analgesia, oxidative stress, but remains fragmented and underrepresented in multicentre European studies and systematic reviews. Stakeholders cited limited access to large, standardised caseloads and databases as a barrier to producing high-level evidence (prospective studies, randomised trials). Continuing professional development in EBSAVM is expanding, yet formal mentorship and residency programmes in evidence-oriented specialties remain scarce.

Conclusions. Lithuania is making substantive progress toward embedding EBSAVM principles in small animal medicine education and practice, but opportunities exist to deepen research capacity, enhance collaborative multicentre studies, and expand postgraduate training aligned with European evidence-based initiatives.

Keywords: evidence-based veterinary medicine, small animal, Lithuania, ESEVT/EAEVE, clinical research, European veterinary schools, benchmarking.

PREVALENCE OF GASTROINTESTINAL AND RESPIRATORY HELMINTHS AMONG CATS IN THE SHELTERS IN LITHUANIA

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Cats are important hosts for different parasites, some of which can be hazardous to humans. The most frequent intestinal parasites of these animals belong to protozoa and helminths: *Giardia*, *Cystoisospora*, *Taenia*, *Dipylidium*, *Echinococcus*, *Toxocara*, *Toxascaris*, *Ancylostoma*, *Uncinaria*, *Eucoleus*, *Trichuris*. During the studies, infection rates among cats in the shelters were 37.4% in Poland [1] and 47.6% in Estonia [2]. Infection with lungworms is also important in veterinary practice, and there was a registered case of *Aelurostrongylus abstrusus* infection in Lithuania [3]. There is a higher risk for parasite infection in outdoor and stray cats. This study aim was to analyse the prevalence of endoparasites in shelter cats in Kaunas, Lithuania.

During this study (September 2024 to August 2025), a total 62 feline faecal samples in two animal shelters, Kaunas, Lithuania were collected. The faecal samples were collected during the first day after adopting from the street (before treatment). Faecal samples were examined using the following methods: sedimentation – flotation (with zinc sulphate flotation solution, 1.45) and Baerman.

Of the total 62 faecal samples, 54.8% were positive for the presence of at least one species of intestinal helminths and 12.9% of respiratory helminths. The highest infection rate was found with *Toxocara cati*, with 45.2 % of cats infected ($P < 0.05$). 17.7% of cats were infected with *Ancylostoma* sp., 14.5% with *Toxascaris leonine* and 6.5% with *Dipylidium caninum*. 11.3% of cats were infected with *A. abstrusus* and 1.6% with *Eucoleus aerophilus*.

The presented study shows that stray cats adopted by a shelter located in the city of Kaunas, Lithuania, were host to a wide range of intestinal parasites, which includes nematodes in gastrointestinal and respiratory tract and cestodes.

Keywords: gastrointestinal parasites, lungworms, parasites, shelter, cats.

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IMPACT OF GENERAL ANAESTHESIA ON TEAR FILM QUALITY IN CLINICALLY HEALTHY MESOCEPHALIC DOGS

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Reduced blink reflex and tear secretion under general anaesthesia (GA) predispose to precorneal tear film (PTF) deficiencies that can be painful and lead to vision loss if untreated [1]. However, limited data exist regarding PTF quality during GA.

This study aimed to evaluate PTF quality in clinically healthy mesocephalic dogs under GA. A prospective, randomised, pre-post treatment study was conducted on 16 client-owned, normal mesocephalic dogs (32 eyes; 10 females, 6 males; age 1–8 years, mean 3.25 ± 2.7 years; mean weight 19.9 ± 10.9 kg) undergoing routine ovariohysterectomy or castration. Tear film parameters – Schirmer Tear Test-1 (STT), tear osmolarity (TO) and tear ferning (TF) – were assessed at five time points: baseline (T0), 10 minutes post-premedication (T10), 5 minutes post-intubation (T5), intraoperatively (TS), and prior to discharge (TD). GA was induced with methadone (0.2 mg/kg) and dexmedetomidine (3 µg/kg), propofol (2–4 mg/kg), and additionally ketamine (1 mg/kg) and fentanyl (3 mcg/kg). Anaesthesia was maintained with isoflurane. Meloxicam (0.2 mg/kg SC) was administered as an anti-inflammatory agent. Statistical analysis was performed using paired t-tests and Pearson correlation.

PTF parameters declined significantly at all intraoperative time points. STT values dropped from a baseline of 21.2 ± 3.3 mm/min to 13.5 ± 5.9 mm/min (T10), 6.4 ± 6.3 mm/min (T5), and 0.8 ± 1.6 mm/min (TS). TO values similarly decreased, while TF scores increased, indicating deteriorated tear quality. A moderate negative correlation was observed between STT and age ($r = -0.47$). Fluorescein staining at TD revealed punctate corneal erosions in 34.4% of eyes. At discharge, PTF parameters remained significantly below baseline.

These findings demonstrate that GA significantly reduces tear production and quality in mesocephalic dogs, with incomplete recovery by discharge. Proactive ocular surface protection during and after anaesthesia is essential to minimise the risk of corneal injury, especially in older dogs.

Keywords: tear osmolarity, tear ferning, dog, general anaesthesia.

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ANALYSIS OF BACTERIAL COLONISATION AND ANTIMICROBIAL RESISTANCE IN ABDOMINAL SWABS FROM DOGS AND CATS

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Septic peritonitis in cats and dogs is a severe condition caused by bacterial infection and inflammation of the peritoneum. It is commonly linked to trauma, ruptured organs, or abdominal surgeries, resulting in the leakage of intestinal or organ contents into the abdominal cavity, causing infection and sepsis. This study aimed to analyse the most commonly isolated bacteria from microbiological abdominal swabs and compare antimicrobial susceptibility testing (AST) results for empirical therapy. Data were collected from dogs and cats that underwent abdominal surgery between January 1, 2023, and April 1, 2025, during which microbiological swabs were taken. Clinical specimens were cultured using standard microbiological techniques, and bacterial identification was performed using MALDI-TOF MS. AST was done using broth microdilution, and results were interpreted according to CLSI guidelines. The study included 18 patients (6 cats and 12 dogs) with a variety of abdominal surgery causes. Bacteria were isolated in 61.11% of the 18 microbiological abdominal swabs, while the remaining 38.89% were negative. Enterotomies and bowel resections were most common (55.56%), followed by traumatic abdominal injuries (27.78%) and isolated cases of cholelithiasis, splenic tumours, and post-urolithiasis complications. All dogs received penicillin-based systemic antibiotic therapy 30 minutes before surgery. Among positive samples, *E. coli* was isolated in 36.36% of cases, *Proteus sp.* and *Enterococcus* in 18.18%, while individual samples contained *Pseudomonas aeruginosa*, *Streptococcus lutetiensis*, *Enterobacter sp.*, and *Klebsiella oxytoca*. Penicillin resistance was found in 66.67% of isolates, likely due to prior use of penicillins. Resistance to aminoglycosides and cephalosporins was seen in 41.67%, while resistance to macrolides (71%), lincosamides (88%), chloramphenicol (50%), and tetracyclines (55%) was also noted.

Keywords: septic peritonitis, antimicrobial susceptibility testing, antimicrobial therapy.

GINGIVAL, SUBMUCOSAL AND PERIODONTAL POCKET INJECTIONS OF PRP (PLATELET-RICH PLASMA) FOR THE TREATMENT OF CANINE PERIODONTAL DISEASE

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Small breed dogs such as Yorkshire Terriers, Toy Terriers, Spitz, Toy Poodles and other breeds have a genetic predisposition to accumulate tartar and plaque faster and suffer from periodontitis [1–3]. As the field of regenerative medicine becomes more popular, more and more attention is being paid to substances that promote tissue regeneration, one of which is platelet-rich plasma (PRP) [4]. The aim of this study was to find out the effectiveness of applying PRP injections in daily small animal veterinary dentistry practice.

A total of 42 adult dogs of various small breeds participated in the study. The PRP group (n = 30) included patients that, after dental hygiene, received PRP injections. All the owners of enrolled dogs voluntarily opted for the PRP treatment option. The control group (n = 12) included patients whose owners did not agree to PRP injections. Under general anaesthesia, plaque and tartar were removed, then the periodontal pockets of all teeth were measured, the degree of periodontitis (PD) and the gingival index (GI) were determined, dental radiographs of the affected teeth were performed, and horizontal bone loss was measured. PRP was injected into the submucosa of affected teeth, under the gums, and into the periodontal pockets in 0.1 mL doses. Repeated assessments and measurements were performed after 30 days. Statistical analysis was assessed using SPSS Statistics 26 software. The data are provided in median and min-max values. The differences were considered significant if the *P* value was ≤ 0.05 . We found that PRP injections statistically significantly reduced the PD compared with days 0 and 30 in the PRP group (day 0 – 3 (2–3), day 30 – 2 (1–2), $P < 0.05$). The GI after PRP injections decreased by 3 times (day 0 – 3 (2–3), day 30 – 1 (0–2), $P < 0.05$) compared with days 0 and 30. The GI after 30 days decreased by 2 times (control – 2 (1–2), PRP – 1 (0–2), $P < 0.05$) compared with the control and PRP groups. The depth of gingival pockets after PRP injections decreased by 2 times (day 0 – 4 (1–8), day 30 – 2 (1–4), $P < 0.05$) compared with days 0 and 30. The depth of gingival pockets after 30 days decreased by 1.5 times (control – 3 (1–5), PRP – 2 (1–4), $P < 0.05$) compared with the control and PRP groups. Horizontal bone loss after PRP injections decreased by slightly more than 2 times (day 0 – 4.20 (1.18–8.54), day 30 – 2.08 (1.03–5.35), $P < 0.05$) compared with days 0 and 30. Horizontal bone loss after 30 days decreased by 1.7 times (control – 3.61 (1.12–5.74), PRP – 2.08 (1.03–5.35), $P < 0.05$) compared with the control and PRP groups. The use of gingival, submucosal and periodontal pocket injections of PRP without any activating substance can be safely and successfully used in the treatment of grade 2–3 periodontitis in small breed dogs in daily small animal veterinary dentistry practice.

Keywords: dog, periodontal disease, platelet-rich plasma, PRP injections.

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TOXOPLASMOSIS IN LITHUANIAN HOUND DOGS: BLOOD TEST EVALUATION, RISK FACTOR ANALYSIS, AND CASE REVIEW

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Toxoplasmosis, a zoonotic disease caused by the parasite *Toxoplasma gondii*, poses a significant threat to both human and animal health. Canines, including hunting dogs, are susceptible to *T. gondii* infection, which can manifest in various clinical forms [1].

This study investigates the seroprevalence of *T. gondii* infection in Lithuanian Hound dogs, categorised into hunting and non-hunting groups, and assesses potential risk factors, including hunting activity, diet, and contact with felines, alongside an evaluation of haematological parameters. A cross-sectional study was conducted on 90 Lithuanian Hound dogs from different regions of Lithuania between March 2023 and December 2024. Blood samples were collected and analysed for antibodies using indirect ELISA and immunochromatographic assays detecting IgG and IgM antibodies. Haematological parameters, including white blood cell count, lymphocytes, eosinophils, and mean platelet volume, were also assessed. Results showed 34.4% of dogs positive for IgG and 18.9% for IgM by rapid test, with ELISA confirming 25.6% positivity. A strong correlation was observed between rapid test and ELISA results ($P < 0.05$). Dogs fed raw wild animal meat had a significantly higher prevalence of infection (28.6%, $P < 0.05$), and hunting dogs were approximately three times more likely to be seropositive (OR = 3.2, 95% CI: 1.5–6.8). No significant associations were found between seropositivity and haematological parameters, gender, age, or contact with felines. A clinical case of a hunting dog from a rural area presenting with lethargy, abdominal effusion, and neutrophilic leucocytosis is described. Serological testing confirmed *T. gondii* infection; despite treatment with clindamycin and, later, sulfonamide-trimethoprim, the dog developed disseminated abdominal pseudotumours and was euthanised. Post-mortem examination confirmed widespread toxoplasmosis.

These results highlight the need for increased awareness and preventive measures regarding toxoplasmosis in Lithuanian Hound dogs, particularly in hunting populations and those consuming raw meat. The findings underscore the importance of regular screening and monitoring for this zoonotic disease, which has implications for public health and canine welfare [2].

Keywords: toxoplasmosis, *Toxoplasma gondii*, Lithuanian Hound, seroprevalence, hunting dogs, ELISA, blood parameters.

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TOXOPLASMA GONDII INFECTION IN COMPANION ANIMALS: CLINICAL AND SEROLOGICAL ASSESSMENT

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Toxoplasma gondii infection is a widespread parasitic disease in dogs and cats, showing varying clinical manifestations from asymptomatic carriage to severe systemic illness [1].

This retrospective study analysed 83 naturally exposed animals (41 dogs, 42 cats) examined between January 2022 and June 2025. Animals were classified as symptomatic or asymptomatic based on clinical signs, including ocular, neurological, gastrointestinal, respiratory symptoms, and fever [2]. ELISA serology showed that cats had significantly higher *T. gondii*-specific IgG antibodies ($P = 0.027$) and globulin levels ($P = 0.017$) than dogs, indicating a stronger chronic humoral response; IgM differences were not statistically significant ($P = 0.13$). Symptomatic animals exhibited elevated IgG ($P = 0.027$), globulin ($P = 0.017$), total protein ($P = 0.037$), and creatinine ($P = 0.029$), suggesting immune activation with renal involvement [3]. Acute phase protein serum amyloid A tended to be raised in symptomatic cases ($P = 0.089$), whereas C-reactive protein levels showed no significant difference. Correlation analysis linked IgM titers with neutrophil percentage ($r = 0.34$), CRP ($r = 0.46$), and SAA ($r = 0.62$), marking acute infection, while IgG correlated positively with globulin ($r = 0.36$), total protein ($r = 0.28$), CRP ($r = 0.49$), and SAA ($r = 0.45$), reflecting chronic exposure and sustained immune system engagement [4].

These findings highlight important species-specific immunological differences, with cats exhibiting more robust IgG and globulin responses compared with dogs. Combining clinical presentation with serological and biochemical markers provides a comprehensive and practical approach for diagnosis, monitoring, and management of toxoplasmosis in companion animals. Furthermore, evidence of renal impairment and systemic inflammation in symptomatic cases underscores the necessity of thorough clinical evaluation and laboratory assessments for optimised therapeutic strategies and improved prognosis [5].

Keywords: *toxoplasma gondii*, companion animals, serology, clinical signs, immunological response, blood parameters.

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STAT GENE EXPRESSION IN CANINE SOFT TISSUE TUMOURS

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Signal transducer and activator of transcription (STAT) proteins play a crucial role in cellular processes such as proliferation, differentiation, apoptosis, and immune response regulation [1]. Aberrant STAT signalling has been extensively studied in human cancers, yet its role in veterinary oncology, particularly in canine soft tissue tumours (STTs), remains underexplored.

This study aimed to investigate the expression profiles of key STAT genes (STAT1, STAT3, and STAT5) in a cohort of canine STTs and assess their potential association with tumour grade, histological subtype, and clinical outcome. Tumour samples were collected from client-owned dogs diagnosed with STTs at veterinary clinics. Gene expression was analysed using quantitative real-time PCR (qRT-PCR), and protein localisation was assessed via immunohistochemistry.

Preliminary results indicate differential expression patterns of STAT genes across tumour subtypes. Notably, STAT3 was significantly upregulated in high-grade tumours compared with low-grade ones ($P < 0.05$), suggesting a role in tumour aggressiveness. STAT1 expression showed variability, potentially reflecting its dual role in tumour suppression and immune modulation. STAT5 expression was relatively consistent but appeared elevated in certain sarcoma variants [1, 2].

Our findings support the hypothesis that dysregulated STAT signalling contributes to the pathogenesis of canine STTs. STAT3, in particular, may serve as a prognostic marker or therapeutic target in aggressive tumours. Further investigation is warranted to explore STAT-mediated pathways and their potential for targeted therapy in canine oncology.

This study provides a foundation for integrating molecular markers into the diagnostic and prognostic evaluation of canine soft tissue tumours, bridging the gap between veterinary and comparative oncology.

Keywords: STAT3, canine tumours, gene expression, soft tissue tumors, veterinary oncology.

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COMPARISON OF THE EFFECTS OF PRP INJECTION AND NSAIDS USAGE ON CANINE QUALITY OF LIFE IN CASES OF ORTHOPAEDIC DISEASES

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Orthopaedic diseases (OD) have a broad impact on canines' health, functions, social relationships and other aspects [1]. OD affects numerous aspects of quality of life (QoL) in dogs [2]. Non-steroidal anti-inflammatory drugs (NSAIDs) are a part of the most common non-surgical treatment options in cases of canine OD [3]. Platelet-rich plasma (PRP) is a beneficial treatment option OD [4–5]. This study is part of an investigation conducted at the Dr. L. Kriaučeliūnas Small Animal Clinic and Biochemistry laboratory of the Neuroscience Institute of the Lithuanian University of Health Sciences between September 2019 and December 2021 [6]. All the dogs matched the inclusion criteria of the research. All the owners of the enrolled dogs voluntarily opted for the non-surgical treatment option; all the animals were blindly assigned to the treatment groups. All the dogs were diagnosed with OD based on the results of the clinical, radiological, and ultrasound examinations. The dogs were divided into two groups for the study. The two groups of dogs were treated for OD with either a PRP intra-articular injection (PRP group, n = 28) or NSAIDs and supplements (NSAIDS group, n = 28). An evaluation of QoL consisted of completing a validated 4 domain questionnaire [7]. The questionnaire was completed 3 times: at day 0, day 14, and day 28. Likert's scale (1 = strongly disagree, 5 = strongly agree) was used for the answers. The results showed significant differences in different treatment effects on 3 domains (happiness, physical functioning, and mental status) at day 28 ($P < 0.05$). The hygiene domain showed significantly different results in different treatment options at day 0 ($P < 0.05$) and at day 14 ($P < 0.05$). ANOVA testing revealed significant changes over time in happiness, physical functioning, and hygiene ($P < 0.01$). The PRP group demonstrated more consistent or improving scores, suggesting that PRP may result in longer-lasting effects, although NSAIDS group showed a trend of worsening over time, especially in the Physical Functioning and Happiness domains.

Keywords: PRP, NSAIDS, quality of life, canine, orthopaedic disease.

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DIAGNOSIS AND TREATMENT OF INFLAMMATORY- INFECTIOUS DISEASES OF THE NERVOUS SYSTEM

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This lecture reviews key inflammatory and infectious disorders of the central nervous system (CNS) in small animals, focusing on discospondylitis, bacterial meningitis, steroid-responsive meningitis-arteritis (SRMA), and meningoencephalomyelitis.

Discospondylitis is an infection of the intervertebral disc and adjacent vertebrae, most commonly bacterial, but occasionally fungal. Diagnosis is based on imaging (radiographs, CT, MRI), and treatment involves prolonged antimicrobial therapy, with surgery reserved for instability or abscessation.

Bacterial meningitis is uncommon and usually results from hematogenous spread or trauma. Diagnosis combines imaging and cerebrospinal fluid (CSF) analysis, and treatment requires targeted antibiotic therapy.

SRMA is an immune-mediated meningitis, typically affecting young-adult dogs. Clinical signs include neck pain, fever, and stiffness. Diagnosis is based on MRI and CSF findings. Corticosteroid monotherapy is the mainstay of treatment, although additional immunosuppressants may be used in refractory or relapsing cases [2], with good long-term outcomes for most patients [1, 3].

Meningomyelitis, often of unknown origin, affects the spinal cord and meninges. Diagnosis also relies on MRI and CSF analysis. Treatment includes immunosuppressive therapy, but prognosis may be guarded, with frequent relapses [4].

Feline CNS inflammatory disorders are also briefly discussed, with feline infectious peritonitis (FIP) being the leading cause of meningoencephalomyelitis. Diagnosis is based on clinical evaluation, imaging, and PCR testing. Antiviral therapies are now available in some regions, improving prognosis.

Early recognition and targeted therapy are crucial to improve outcomes in these conditions.

Keywords: discospondylitis, bacterial meningitis, steroid-responsive meningitis-arteritis, meningomyelitis.

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MOLECULAR AND SEROLOGICAL SCREENING OF FELINE CORONAVIRUS IN CATS FROM A SINGLE SHELTER IN LATVIA

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Feline coronavirus (FCoV) is a highly contagious virus that is a major problem in shelter environments [1–2]. The aim of this study was to determine FCoV prevalence in cats within a single shelter from Latvia, as well as to analyse the relationship between swab and serum sample positivity. Two swab samples (oropharyngeal and rectal) and a blood sample were obtained from 62 cats and subsequently examined for FCoV RNA by RT-PCR and anti-FCoV antibodies by ELISA, respectively. The overall prevalence of FCoV within a shelter was 51.6% (32/62). Of the 62 cats, four tested positive via oropharyngeal swab (6.5%; 95% CI: 1.8–15.7) and 32 via rectal swab (51.6%; 95% CI: 38.6–64.5), showing a low positive correlation ($r = 0.25$) between the swab sample types. Serological analysis detected anti-FCoV antibodies in 31 cats (50.0%; 95% CI: 37.0–63.0%). The overall RT-PCR positivity correlated moderately with ELISA seropositivity ($r = 0.58$), suggesting that seropositive cats are more likely RT-PCR positive. The majority of cats were FCoV positive, and ELISA seropositivity was associated with an increased likelihood of shedding, suggesting that seropositive individuals are more likely to shed the virus.

Keywords: animal shelter, ELISA, feline coronavirus (FCoV), rectal swabs, RT-PCR, serum samples.

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MOLECULAR TECHNIQUES IN SMALL MAMMAL VETERINARY RESEARCH

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Vector-borne diseases pose a significant health threat to small mammals worldwide, often causing severe clinical signs and complicating treatment. Rapid and accurate identification of causative pathogens is essential for effective treatment and prevention. Molecular diagnostic techniques, including polymerase chain reaction (PCR), real-time PCR, and multiplex PCR, have revolutionised pathogen detection by offering high sensitivity, specificity, and rapid results. These methods allow the identification of bacteria, protozoa, and viruses transmitted by vectors such as ticks, fleas, and mosquitoes, even in subclinical infections. Molecular diagnostics also enable the differentiation of strains, detection of co-infections, and monitoring of emerging pathogens, providing essential information for veterinarians and epidemiologists. In this study, we review the molecular tools used for the diagnosis of vector-borne pathogens (including *Babesia canis*, *Anaplasma phagocytophilum*, *Bartonella* spp., *Mycoplasma* spp., and *Dirofilaria* spp.) in dogs, cats, and their ectoparasites in Lithuania [1–6]. We also discuss the prevalence of these pathogens, as well as the advantages and limitations of integrating molecular diagnostics into routine veterinary practice.

Keywords: vector-borne pathogens, cats, dogs, ectoparasites, molecular diagnostics.

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TICK-BORNE ENCEPHALITIS VIRUS IN DOGS: CLINICAL RELEVANCE AND IMPLICATIONS FOR VETERINARY PRACTICE IN ENDEMIC REGIONS

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Tick-borne encephalitis virus (TBEV) is a major zoonotic pathogen in Europe, yet its impact on canine health remains underrecognised in clinical veterinary practice. In a recent large-scale study in Lithuania, one of the most highly endemic countries for TBE, we examined serum samples from 473 dogs collected in two Lithuanian veterinary clinics. Using ELISA for serology and RT-PCR targeting the NCR5 region for viral RNA detection, we found that 21.6% (CI 95% 17.9–25.6) of dogs carried TBEV-specific antibodies, while 18.6% (CI 95% 15.2–22.4) were PCR-positive. Neurological signs were observed in 31.8% of PCR-positive animals, with a case fatality/euthanasia rate of 18.2%. Male dogs were more likely to develop neurological manifestations, and older age, neurological signs, and the presence of antibodies were significantly associated with poor outcomes. Infectious virus was successfully propagated in cell culture from PCR-positive sera, confirming active infection. These findings highlight that TBEV should be considered an important differential diagnosis in dogs presenting with acute neurological disease in endemic areas. Awareness and early diagnostic testing are essential for veterinarians to improve case outcomes and to better understand the epidemiology of this canine pathogen.

Keywords: tick-borne encephalitis virus, dogs, TBEV epidemiology.

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PREVALENCE OF HAEMOTROPIC MYCOPLASMAS IN ASYMPTOMATIC SHELTER DOGS AND POTENTIAL LINK TO SUBCLINICAL ANAEMIA: INSIGHTS FROM GLOBAL DATA AND RELEVANCE FOR LITHUANIA

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Haemotropic mycoplasmas (*Mycoplasma haemocanis*, *Candidatus Mycoplasma haematoparvum*, etc.) are blood-borne, epierthrocytic bacterial pathogens. Although frequently subclinical, they may affect haematologic parameters, particularly in animals under stress or co-infected with other pathogens. While these microorganisms have been studied globally, no data are currently available regarding their prevalence in dogs in Lithuania. Prevalence rates varied widely, from 4.5% in Northern Italy [1] to 56.8% in Iran [2]. Co-infection with both *M. haemocanis* and *C. M. haematoparvum* occurred in up to one-third of infected animals [2, 3]. Older dogs, those with poor body condition, and those infested with fleas were more frequently infected [2, 4]. Although clinical signs were uncommon, some dogs showed mild reductions in haematocrit or haemoglobin, suggesting possible subclinical anaemia [1, 3]. Notably, genetic studies in Spain identified haemoplasma strains in Iberian wolves identical to those found in dogs, supporting potential interspecies transmission [5]. Given the frequent asymptomatic presentation and possible haematological impact, it is essential to establish baseline prevalence in shelter and free-roaming dogs. Such data would support improved disease surveillance, animal welfare strategies, and contribute to One Health perspectives on vector-borne pathogens.

In this study, anaemia was detected in 8.0% (4/50) of shelter dogs, while haemotropic *Mycoplasma* (16S rRNA PCR) was identified in 8.16% (4/49) of tested samples, comprising *M. haemocanis* / *M. haemofelis* and *Candidatus M. haematoparvum* (each 4.08%). No association was found between anaemia and *Mycoplasma* infection ($P = 1.000$), but anaemia was significantly linked to dermatological/hair coat changes ($P = 0.0009$) and *Mycoplasma*-positive dogs were significantly more likely to present with pale mucous membranes ($P = 0.0039$).

Keywords: haemotropic mycoplasma, *Mycoplasma haemocanis*, *Candidatus Mycoplasma haematoparvum*, shelter dogs, subclinical anaemia.

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PARTIAL LAMINECTOMY – A BONE SPARING APPROACH TO CAUDA EQUINA SURGERY

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A *cauda equina* syndrome is a very common neurological disease and a fairly frequent indication for surgical therapy. The scope of this study is a method used on our clinic for nearly five years, which is a period covered by it, e.g. from 1st January 2020 to this day. Within that time frame we have performed spine surgeries on 259 dogs, of which only 14 were diagnosed with cauda equina syndrome, so 6% only. The reason for it is the prevalence of toy, small and medium sized breeds in Croatia. In all dogs, a partial laminectomy of L7-S1 vertebra was performed which was followed by discectomy of L7/S1 intervertebral disc. This approach to lumbosacral joint is still rarely used despite being time saving and tissue sparing. The reason for pursuing this method was our attempt to avoid excessive bone trauma and a need for additional stabilisation. The thinking behind choosing this technique over others was that this way the possibility of epidural fibrosis would be minimised. The lesser the bone trauma, the lesser the scarification. Furthermore, with such an approach, the surgically induced instability is of the smallest degree. Drawback to this technique is slightly lesser visibility, which can be overcome with surgical practice and good positioning of the patient. The outcome of performed surgeries was satisfactory in all patients. Within 2–4 weeks, they all reached significant improvement compared with prior the operation, and none developed a postoperative complication such as epidural fibrosis or instability during approximately six months after the procedure.

Keywords: cauda equina sy., partial laminectomy.

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MOLECULAR DETECTION OF *RICKETTSIA* SPP. IN DOMESTIC CATS AND THEIR ECTOPARASITES IN LITHUANIA

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Vector-borne pathogens are of increasing concern in veterinary and human medicine, as companion animals may act as reservoirs or sentinels for zoonotic pathogens, including *Rickettsia* species. Domestic cats (*Felis catus*), whether living indoors or outdoors, maintain close contact with humans, which may facilitate pathogen transmission [1, 2]. However, knowledge of rickettsial infections in cats across Europe remains limited [3], and no data on the prevalence of *Rickettsia* spp. in cats have previously been reported from Lithuania.

This study aimed to investigate the presence of *Rickettsia* spp. in domestic cats and their associated ectoparasites. Blood samples from cats and specimens of fleas and ticks were collected and analysed using molecular techniques, including PCR amplification and sequencing. The molecular screening revealed the presence of *Rickettsia* spp. DNA in both feline hosts and ectoparasites. Overall, *Rickettsia* spp. was identified in 4.6% (25/543) of cats. Phylogenetic analysis revealed that cats harboured human-pathogenic species, including *R. helvetica*, *R. conorii* subsp. *raoultii*, and *R. felis*. *Rickettsia* prevalence was higher in ticks (25.2%; 81/321) compared with fleas (15%; 23/153). Three species of *Rickettsia* were identified in fleas – *R. helvetica*, *R. conorii* subsp. *raoultii* and *R. felis*, while *R. helvetica* and *R. conorii* subsp. *raoultii* were found in ticks. This study provides the first evidence of *Rickettsia* spp. infection in domestic cats in Lithuania. Sequence analysis demonstrated concordance between feline and ectoparasite isolates, suggesting possible transmission pathways. These findings highlight the importance of continuous surveillance to better understand the epidemiology of rickettsial infections in companion animals and to assess their potential implications for public health.

Keywords: *Rickettsia* spp., domestic cats, fleas, ticks, molecular diagnostics.

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FIRST AUTOCHTHONOUS CASE IN LITHUANIA OF *ANGIOSTRONGYLUS VASORUM* INFECTION IN A DOG

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Angiostrongylus vasorum is an emerging parasite of domestic dogs in many European countries, but until now no cases have been documented in Lithuania. We report the first autochthonous case of *A. vasorum* infection in a 1-year-old male Deutsch Drahthaar (German Wirehaired Pointer) from Molėtai district, Lithuania. The dog, used for hunting, was referred to a referral veterinary clinic on 14 April 2025 due to respiratory signs.

Diagnostic investigations included bronchoscopy, thoracic radiographs, and lung ultrasound, which revealed changes consistent with parasitic bronchopneumonia. Complete blood count showed leukocytosis with eosinophilia, and prolonged prothrombin time (19.0 seconds). Bronchoalveolar lavage cytology and Baermann faecal examination revealed first-stage larvae morphologically consistent with Metastrongylidae. Infection was further supported by a positive Angio Detect™ rapid serological test, which is highly sensitive and specific for *A. vasorum*. Potential cross-reacting pathogens were excluded: the Knott test ruled out *Dirofilaria spp.*, and coprological examinations showed no evidence of other parasitic infections. Samples are currently undergoing molecular confirmation and sequencing at the Department of Veterinary Sciences. Importantly, the dog had never travelled abroad, strongly suggesting local transmission.

The patient was successfully treated with anthelmintics and supportive therapy, with no recurrence of clinical signs reported to date. Based on larval morphology, localisation, clinical findings, and laboratory investigations, *A. vasorum* is considered the most likely causative pathogen.

This case represents the first reported autochthonous infection with *A. vasorum* in Lithuania. Given the clinical significance of this parasite and its potential impact on canine health, this report aims to raise awareness among veterinarians, researchers, and the wider community about the emergence of a new parasitic respiratory disease in the country.

Keywords: *Angiostrongylus vasorum*, canine angiostrongylosis, autochthonous infection, respiratory disease, Baermann technique.

WHAT DO WE KNOW ABOUT CANINE COGNITIVE DYSFUNCTION?

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Canine cognitive dysfunction syndrome (CCDS) is known as a clinical analogue of Alzheimer's disease in human patients [1]. A chronic and progressive neurodegenerative disorder, CCDS, can affect elderly dogs from 8 years old, where the prevalence of the disease, along with worsening clinical signs, increases with age [2].

In an aging brain, reduced antioxidant capacity, amyloid beta accumulation, and the formation of neurofibrillary tangles contribute to neuronal damage, resulting in a progressive decline in learning ability and a loss of basic skills [2–4].

Through clinical examinations, blood tests, and further diagnostic imaging, it is possible to either exclude other potential diseases or diagnose comorbidities that may also be present in elderly dogs. Reduced diameter of the interthalamic adhesion, cortical atrophy, increased size of the lateral ventricles, microhaemorrhages, and white matter hyperintensity are signs of CCDS that can be detected with brain MRI [5]. However, scoring with questionnaires, the behaviour and cognition of dogs is recognised as a practical tool for assessing cognitive decline [6].

Dietary supplementation with antioxidants, docosahexaenoic acid (DHA), L-arginine, B vitamins, and medium-chain triglycerides (MCT) has been shown to improve performance in complex tasks, enhance learning ability, and reduce cognitive dysfunction scores, as well as improve basic skills [7, 8].

Medications such as selegiline, alpha-adrenergic antagonists, and cholinesterase inhibitors showed improvements in the sleep-wake cycle and interactions with family members [9].

Early recognition and diagnosis of CCDS are essential for initiating treatment, which may slow disease progression and preserve both the animal's quality of life and the pet-owner bond.

Keywords: canine, dog, cognitive dysfunction, Alzheimer's disease, DHA, antioxidants.

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PET FOOD IN SCIENTIFIC FOCUS: FROM SOFTWARE-DRIVEN FORMULATION TO VERIFICATION

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The development of nutritionally balanced, legally compliant, and consumer-appealing pet food increasingly relies on science-driven processes supported by advanced digital tools. This study explores the integration of software-based formulation systems with regulatory standards (FEDIAF, AAFCO) and ingredient databases, enabling precise, transparent, and efficient product design for dogs and cats. The formulation process is examined from raw material selection and nutrient balancing to compliance checks for essential nutrients, energy density, and label claims.

Furthermore, the study highlights the critical role of verification methods, as ingredient quality significantly influences pet performance and must be carefully considered during formulation [1]. Methods such as analytical testing, feeding trials, and digestibility studies are essential for validating formulation accuracy and ensuring effectiveness under real-life feeding conditions. Attention is also given to the alignment with pet food legislation, claims substantiation, and traceability throughout the production chain. Analyses have shown that nutrient concentrations in commercial pet foods can significantly differ from declared values, raising concerns about product consistency and regulatory compliance [2].

By connecting digital formulation tools with scientific validation, this study showcases a modern framework for creating pet food products that meet both nutritional integrity and market expectations, ensuring safety, transparency, and animal well-being.

Keywords: pet food formulation, nutritional compliance, FEDIAF standards, aafcoAAFCO standards, software-driven formulation.

Acknowledgments: The authors wish to thank KIKA Group for providing practical insights, industry data, and access to pet food formulation resources, as well as Lithuanian University of Health Sciences (LSMU) for scientific guidance, technical support, and access to laboratory facilities used during this study.

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ANTIMICROBIAL RESISTANCE OF BACTERIA ISOLATED FROM FELINE AND CANINE URINARY TRACTS: IMPORTANCE FOR VETERINARY PRACTICE

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Bacterial infections are the main cause of lower urinary tract infections (UTIs) in dogs and cats. With the increasing resistance of bacteria to antibacterial substances, it is essential to understand the aetiology of cystitis, identify the bacterial species-caused cystitis and determine the susceptibility to antimicrobial substances [1].

The aim of this study was to evaluate incidences of bacterial urinary tract infections in dogs and cats and assess the antimicrobial susceptibility of the isolated pathogens.

A retrospective study was conducted at a small animal veterinary clinic, where the urine culture results from dogs and cats, collected between May 2024 and May 2025, were analysed. Based on positive urine culture, 22 of 47 dogs and 10 of 38 cats were selected for further analysis. In three dogs, infections with two different bacterial species were identified.

The most frequently isolated bacterial species was *Escherichia coli*, found 36% in urine samples of dogs and 30% in cats. *E. coli* isolates from dogs showed a 20% resistance to amoxicillin and amoxicillin/clavulanic and a 12% resistance to trimethoprim-sulfamethoxazole, while bacteria isolates from cats showed a lower resistance to these antimicrobial substances (10% to both amoxicillin and amoxicillin/clavulanic acid). In comparison, *Enterococcus faecalis* and *Pseudomonas aeruginosa* isolated from both dogs and cats were highly multidrug-resistant.

These findings highlight the importance of performing an antimicrobial susceptibility analysis to accurately identify the causative agents of infection and prescribe appropriate therapy, especially in hospitalised patients with urinary tract diseases. Such data will help to reduce the risk of developing and spreading antimicrobial resistance.

Keywords: bacterial cystitis, resistance, antibacterial substances, urine, cat, dog.

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LIGHT IN PROGRESS: A ROLE FOR LASER THERAPY IN ORTHOPAEDIC CARE

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Laser therapy is increasingly recognised as a valuable adjunct in veterinary orthopaedic care. This presentation will examine its current applications, mechanisms of action, and clinical effects in managing musculoskeletal conditions in small animals. From acute injuries and post-operative recovery to chronic degenerative diseases like osteoarthritis, laser therapy offers a versatile treatment option. However, questions remain regarding optimal protocols, dosing parameters, and long-term outcomes, underscoring the need for continued research. Attendees will gain practical insight into the therapeutic potential of laser therapy and how to effectively integrate it into individualized treatment plans.

Keywords: laser therapy, orthopaedic patient, osteoarthritis, dog.

ARE ACUTE PHASE PROTEINS USEFUL IN INFECTIOUS DISEASE MANAGEMENT IN COMPANION ANIMALS?

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Acute phase proteins (APPs) are non-specific biomarkers of the inflammatory and immune system stimulation. According to their behaviour during inflammatory processes, the APPs are categorised into three major categories: positive major, positive moderate and negative APPs. It is highly recommended to include at least one of each when performing biochemistry analyses [1]. Currently, in small animal clinics, the most studied APPs include C-reactive protein (CRP) and serum amyloid A (positive major APPs), haptoglobin and ferritin (positive moderate APPs), and albumin and paraoxonase 1 (negative APPs). Although these markers are highly non-specific and respond to every inflammatory or immune system stimulus, the magnitude of change of their concentration and/or divergent responses of different APPs could help to suspect a possible aetiology. For instance, the highest increases in circulating CRP concentrations, of approximately 100-fold, are detected in sepsis or autoimmune diseases; meanwhile, some infectious diseases, such as leishmania, present small to moderate increases of circulating CRP unless some complication exists [2]. On the other hand, as an example of the added values of performing a profile of APPs, dogs naturally infected with *Babesia canis* present a severe increase in CRP concentrations, while the concentration of haptoglobin remains within the reference range or can be decreased [3]. Whereas, in case of canine pyometra, there is a significant increase in serum CRP and haptoglobin and a decrease in albumin and paraoxonase 1, indicating a severe inflammatory condition. However, serum ferritin, despite being a moderate positive APP, does not show a significant increase [4]. Therefore, including oAPPs in the biochemistry analysis could serve not only for the evaluation of the presence of an inflammatory status and response to treatment monitoring, but also as a valuable diagnostic tool in routine small animal practice.

Keywords: C-reactive protein, haptoglobin, ferritin, leishmaniosis, babesiosis, sepsis.

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COMPUTED TOMOGRAPHIC AND LABORATORY FINDINGS IN DOGS WITH UPPER RESPIRATORY TRACT SYMPTOMS: A RETROSPECTIVE STUDY (2020–2023)

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Upper respiratory tract diseases are common in small animal veterinary practice, often presenting with similar clinical signs regardless of the underlying aetiology. This study aimed to identify the most frequent causes of upper respiratory symptoms in dogs presented to the Veterinary Clinic of the Latvia University of Life Sciences and Technologies between January 2020 and December 2023. A retrospective study was conducted on 105 dogs with clinical signs of upper respiratory tract disease that underwent head computed tomography between 2020 and 2023. The most frequently identified causes were sinonasal neoplasia (32%), nonspecific rhinitis (30%), destructive rhinitis (13%), and dental disease with secondary rhinitis (19%). Neoplastic changes were most common in older dogs (mean age 10 years), with squamous cell carcinoma, osteosarcoma, and adenosquamous carcinoma being the most frequent histopathological diagnoses. CT often revealed bone destruction, with the nasal turbinates, maxilla, and ethmoid bone being most affected. Nonspecific rhinitis was more prevalent in younger dogs (mean age 5.3 years) and associated with lymphoplasmacytic or suppurative inflammation on biopsy. Destructive rhinitis cases frequently involved fungal pathogens (*Aspergillus spp.*) and showed extensive osteolysis. Bacterial cultures identified predominantly opportunistic organisms, including *Staphylococcus spp.*, *Streptococcus spp.*, and *Pasteurella spp.* Dental diseases such as oronasal fistulae were observed mainly in older, small-breed dogs. In conclusion, sinonasal neoplasia and idiopathic rhinitis with secondary infection were the most common findings in dogs with upper respiratory symptoms. The study highlights the importance of advanced imaging and laboratory diagnostics in identifying the diverse aetiologies of upper respiratory disease in dogs and emphasises the need for combined diagnostic approaches to reach a definitive diagnosis.

Keywords: dog, computed tomography, rhinitis, nasal neoplasia, upper respiratory tract, laboratory diagnostics.

PERIOPERATIVE ANALGESIA IN DOGS AND CATS: CURRENT PRACTICES AND ATTITUDES AMONG LATVIAN VETERINARIANS

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Pain induces physiological and neurohumoral responses, increasing stress and discomfort. Although multimodal analgesia, including opioids, is recommended, effective pain management remains challenging, and studies suggest that analgesic use in clinical practice remains suboptimal [1]. Data on Latvian veterinarians' attitudes toward pain management and analgesic use, including opioids, are currently lacking. The objective of this study was to evaluate pain management and opioid use preferences among Latvian veterinarians treating dogs and cats. A prospective online survey was conducted using a structured 23-item questionnaire created in Google Forms. The questionnaire was distributed via the Latvian Veterinary Association at the end of 2023, and data collection concluded on January 1, 2024. Descriptive statistics were used to summarise the responses. A total of 101 practicing veterinarians completed the survey. The data covered pain assessment, analgesic preferences, opioid use, influencing factors, and respondent demographics. Opioids were used daily in 80% of practices, with an increase observed over the past five years. Multimodal analgesia commonly includes opioids combined with NSAIDs (51.1%). Butorphanol (84.7%) and buprenorphine (83.5%) were the available opioids; buprenorphine was most frequently administered (43.2%). Opioid integration was positively evaluated by 96.6% of respondents; 62.4% rated their knowledge as adequate. Opioids were used not only perioperatively but also for therapeutic (47.6%) and oncological (21.4%) pain management. Only 33% of veterinarians reported using formal pain scoring systems; 62.5% relied on clinical experience. This study demonstrates that opioids have been routinely used in Latvian veterinary practices for several years, with a noticeable increase in the past five years. Butorphanol is most frequently used in dogs, while buprenorphine predominates in cats. Opioids are commonly combined with NSAIDs to achieve multimodal analgesia. However, pain assessment remains largely subjective, as most veterinarians rely on clinical experience rather than standardised scoring systems.

Keywords: analgesia, survey, pain management, opioids, cats, dogs.

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SENTINEL LYMPH NODE MAPPING IN CANINE MAST CELL TUMOURS

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Mast cell tumours (MCT) are the most common malignant skin tumours in dogs. MCTs occur in middle-aged dogs with a breed-specific predisposition, e.g. Boxers, Boston Terriers, Weimaraners, Shar-Peis, Golden Retrievers, Labrador Retrievers, Beagles, and Schnauzers. Cutaneous MCT tumours metastasise first to the sentinel lymph nodes and regional lymph nodes and then to distant sites such as the spleen, liver, or bone marrow. The presence of metastases in the lymph nodes (LN), indicating at least stage II disease, is relatively common and a proven negative prognostic indicator [1].

Early detection of LN metastases is crucial for prognosis and better patient care. Accurate detection of metastasised LN prior to surgical excision is difficult. Fine needle aspirates of LN for the detection of MCT metastases have a low sensitivity of 31% [2].

Dogs with low-grade MCTs have a good prognosis after surgical excision of the primary tumour and elective lymphadenectomy of the early metastatic regional LN [3].

Sentinel lymph node mapping is therefore very important. The mapping of sentinel lymph nodes is described: lymphoscintigraphy, colourimetric SLN mapping (using the peritumour injection of blue dye or indocyanine green), radiological lymphography (also known as direct lymphography or radiography), indirect lymphangiography, computed tomography lymphangiography (CTL), near-infrared fluorescence/near-infrared fluorescence-guided lymphography (NIR/NIR-LND) and contrast-enhanced ultrasound (CEUS) [4].

Keywords: mast cell skin tumours (MCT), dogs, sentinel lymph nodes.

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LIVESTOCK PRODUCTION – RECENT TRENDS AND FUTURE PROSPECTS

Abstracts

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THE ZEARALENONE CASCADE: LINKING RUMINANT HEALTH TO HUMAN EXPOSURE

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The contamination of animal feed by mycotoxins, toxic fungal metabolites, is a critical global issue threatening livestock productivity and food safety. Zearalenone (ZEN), an estrogenic mycotoxin from *Fusarium* species, is a ubiquitous contaminant in cereal-based feeds [1]. In ruminants, this threat is amplified when rumen microbes convert ZEN into its more potent metabolite, alpha-zearalenol (α -ZEL). Because α -ZEL has a much higher affinity for estrogen receptors, even low levels of initial feed contamination can lead to significant endocrine disruption. The hormonal imbalance directly interferes with the cow's reproductive cycle, leading to significantly lower conception rates and an increased risk of early embryonic death. Beyond these direct reproductive failures, the overall physiological stress contributes to a notable reduction in milk yield and directly impacts the economic output of dairy farms [2]. Consequently, these potent metabolites can enter the human food chain through milk and meat, posing a direct dietary risk.

This study investigated ZEN concentrations in grain and animal feed and the corresponding presence of its metabolites in livestock urine. Initial monitoring of Latvian grain using high-performance liquid chromatography-mass spectrometry showed a significant 8% non-compliance rate with safety limits implemented in the European Union. A subsequent on-farm investigation involving 120 matched feed and urine samples assessed the extent of the metabolic transformation of ZEN and evaluated risks to animal nutrition and public health. To address the risks identified in this research, proposals for improved on-farm and storage practices were developed to decrease the prevalence of ZEN in animal feed. The study also clarified the potential of the use of urinary zeranone and taleranone as biomarkers for illegal hormone treatment.

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APICULTURE – CHALLENGES, AND PERSPECTIVES

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Apiculture, the science and practice of beekeeping, represents a cornerstone of human interaction with natural ecosystems, agriculture, and biodiversity. Honey bees (*Apis* spp.) provide essential ecosystem services that sustain global food production and ecological balance. Beyond production of honey and other bee products, beekeeping contributes to human livelihoods, medicine, and environmental health [1]. In the 21st century, however, apiculture faces unprecedented challenges – climate change, habitat fragmentation, agricultural intensification, pesticide exposure, spread of pathogens and parasites, and socio-economic pressures have collectively accelerated honey bee colony declines worldwide. Many beekeepers face the challenge of low honey prices in international markets, often driven by oversupply from large-scale producers and unequal competition. Compounding this issue is the widespread problem of honey adulteration [2]. The addition of sugar syrups, rice syrup, or other sweeteners not only undermines consumer trust but also depresses prices for authentic, high-quality honey. Adulterated honey entering global supply chains makes it harder for genuine producers to compete, particularly when testing and enforcement mechanisms are weak. Strengthening quality assurance systems, developing reliable traceability technologies, and enforcing stricter international regulations are, therefore, critical for protecting both beekeepers' incomes and consumer confidence. Future perspectives emphasise the need of data-driven management. Digital beekeeping systems, including smart hives equipped with sensors, enable real-time monitoring of temperature, humidity, hive weight, and bee activity. These systems provide early warnings of colony stress, swarming behaviour, or pest infestations, reducing losses and optimising productivity. In addition to monitoring, selective breeding programmes are being advanced through genomic research. Efforts to develop disease-resistant and climate-resilient bee strains are underway, focusing on traits such as tolerance to *Varroa destructor*, resistance to viral infections, and improved foraging efficiency [3]. From an economic standpoint, apiculture is evolving beyond honey production into a diversified value chain. Hive products such as propolis, royal jelly, pollen, bee bread and beeswax are gaining recognition in nutraceutical, cosmetic, and pharmaceutical industries [4]. Value addition through product differentiation – for example, monofloral honeys, organic certification, or geographic indication labeling – allows producers to capture higher market premiums. In light of these challenges and innovations, the perspectives of the apiculture sector hinge on integrating scientific advancements, regulatory reforms, and market diversification to ensure the resilience of honey bee population and the sustainability of beekeeping.

Keywords: apiculture, sustainability, honey bee health, honey bee products.

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EDIBLE INSECTS AS SUSTAINABLE FOODS: CONSUMER PERSPECTIVE

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Sustainability of food systems has become a major focus in the present century as a result of world overpopulation and a growing need to feed all. According to the United Nations, a significant rise in the population is expected up to 2050. Hence, producing necessary foods in quantity and quality to respond to the expected demand drives agricultural systems and livestock farms to search for alternatives to intensive production techniques, as a way to better preserve the environment and use limited natural resources [1]green (terrestrial plants and insects).

Along with the implementation of innovative and more sustainable agricultural systems and animal rearing techniques, the shift towards alternative, environmentally friendly and more sustainable foods has been encouraged, including by the FAO – Food and Agriculture Organization of the United Nations [2].

Edible insects have been consumed in many and diverse regions of the world since antiquity, being useful and valuable foods for many communities, especially rural populations. While historically insects were collected from the wild for human consumption, the production of insects has been developed as a farming activity or industrial production, following sanitary regulations, thus making them perfectly suitable and safe for human consumption. However, the consumers in regions without a tradition of entomophagy are still facing some reluctance to eating insects, and they show a high degree of discomfort, rejection, and even disgust [3, 4]. Understanding consumer reactions, expectations, motivations and knowledge has been referred to as contributing for a higher acceptability of edible insects and insect-based foods among western countries. The EISuFood project was dedicated to studying the food habits and knowledge about edible insects as sustainable foods in different countries. The countries of the project consortium were Brazil, Cape Verde, Colombia, Croatia, Greece, Latvia, Lebanon, Lithuania, Mexico, Morocco, Nigeria, Poland, Romania, Serbia, Slovenia, Spain, and Turkey. The project has produced important scientific outputs, namely 17 papers already published and some more still being prepared, 2 book chapters, 1 academic thesis, 20 conference presentations, of which one was a key lecture.

Keywords: sustainable food, insect protein, consumer perspective, acceptance.

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AQUAPORINS IN BOVINE SPERM: PROMISING TARGET FOR MALE REPRODUCTIVE STRATEGY

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The search for novel potential biomarkers of cryopreserved semen quality is essential for precise evaluation and reduction of unsuccessful cattle insemination procedures. Literature data indicate that measurement of aquaporins (AQPs) expressed in bovine spermatozoa may be crucial in this regard [1]. The aim of the study was to: (i) analyse the expression of all aquaporins present in bovine frozen-thawed spermatozoa, (ii) investigate the relationships between the identified AQPs and selected parameters of cryopreserved semen, and (iii) determine the potential role of AQPs in successful fertilisation. The study was conducted on cryopreserved semen obtained from local Animal Breeding and Artificial Insemination Centers in the West Pomeranian region of Poland. A detailed analysis was conducted on samples obtained from 29 bulls. Progressive sperm motility measurements were used to categorise semen into two groups with low (L) and high (H) sperm motility. AQP expression analysis was performed using immunofluorescence, immunocytochemistry, polymerase chain reaction, and real-time PCR techniques. Additionally, the concentrations of free amino acids (AA), biogenic amines (BA), and the composition of fatty acids (FA) were determined in both spermatozoa and seminal plasma. The study confirmed the presence of AQP3, AQP7, AQP8, and AQP11 in bovine spermatozoa. In brief, it was demonstrated that the percentage of spermatozoa with intense AQP3 staining in the midpiece and diffuse AQP7 staining in post acrosomal region and midpiece were significantly higher in the H group. Furthermore, AQP3, AQP7 and AQP8 expression showed positive correlations with multiple semen quality parameters and concentrations of selected AA and composition of FA. The results clearly indicate that the analysis of aquaporin distribution in spermatozoa, particularly AQP3 and AQP7, may serve as a useful parameter for assessing the quality of cryopreserved semen in future studies. Detailed analysis of their location and expression allowed for the determination of the potential role of these proteins in the osmoadaptation process to the hypertonic environment of the uterus and fallopian tubes. Our assessment indicates that AQP levels in spermatozoa may serve as a useful marker for predicting fertilisation outcomes. However, further research is required to confirm this observation.

Keywords: water channel, cryopreservation, semen, spermatozoa, bull.

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ANTIMICROBIAL RESISTANCE IN BACTERIA FROM AQUACULTURED FISH

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Aquaculture is a rapidly growing food production sector [1]. It offers potential for food production worldwide, but the rapid growth of aquaculture has raised concerns about the quality and safety of the products, particularly because of antimicrobial resistance and heavy metals [2, 3]. The aim of this study was to investigate the situation of antimicrobial resistance of bacterial isolates from an open fishpond. Bacteria from fish caught in natural surrounding water bodies and their resistance to antibiotics were also examined for comparison. Indicator bacteria and multi-resistant bacteria from two different fish species – Tench (*Tinca tinca*) and Roach (*Rutilus rutilus*) – were investigated. The results showed that the composition of *Aeromonas* and their susceptibility to different antimicrobials were similar in fish from the pond and the lake into which the pond water drains. Meanwhile, the composition of fish bacteria and resistance patterns from the lake into which the pond water does not drain, differ. An analogous situation was found with regard to multi-resistant bacterial isolates. The studies allow us to conclude that in fish cultivated in open ponds and in water receiving inflow from ponds enters, there are multi-resistant bacteria to antibiotics, which may affect the quality of production on the basis of the “One Health” concept.

Keywords: fishponds, fish, bacteria, antimicrobial resistance.

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MILK EJECTION AND OXYTOCIN RELEASE IN A COW-CALF SYSTEM: A REVIEW

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Cow-calf contact (CCC) rearing is gaining increasing scientific and practical attention as an alternative to conventional calf rearing [1]. Its introduction into practice is primarily driven by societal concerns regarding the routine separation of newborn calves from their dams immediately after birth [2]. However, CCC systems raise numerous scientific and management-related questions. Available research shows both positive and negative impacts on cow health, milk production, and welfare [3]. The benefits appear to be more pronounced for calves, especially from the nutritional point of view. CCC systems are established in beef production, where calves are weaned naturally at the age of several months. In contrast, their implementation in dairy systems is more complex: calves typically remain with their dams until the age of two months, while cows are also milked in parlour [3]. The aim of the study was to describe the physiological response of cows to CCC, with the focus on milk ejection. Milk removal from udder is under neuro-hormonal control and requires the release of oxytocin from neurohypophysis into the bloodstream at a threshold level sufficient to reach the myoepithelial cells in the udder [5]. Any emotional stress, especially caused by the separation of the calf during machine milking in the parlour, can centrally inhibit oxytocin release and thus lead to reduced or total inhibition of ejection, resulting in incomplete milk removal from the udder, and, consequently, an increased risk of reduced production during the ongoing lactation [4,6]. Additionally, milking of empty teats may increase the risk of mastitis. Even after permanent separation of calves at weaning, emotional stress can persist for several days disturbing cow behaviour and affecting milk ejection [5]. These findings underline the need to critically evaluate societal expectations regarding calf rearing in dairy farming and to implement CCC systems on the basis of scientific evidence only.

Keywords: cow-calf contact rearing, oxytocin, stress.

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THE INFLUENCE OF BREED AND SEX ON RABBIT PRODUCTIVITY AND MEAT TRAITS

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Rabbit farming is considered a promising branch of animal husbandry, providing high-quality dietary meat and contributing to sustainable livestock production. In addition to nutrition and housing conditions, genetic background and biological factors such as breed and sex are essential determinants of rabbit performance and carcass value. A deeper understanding of how these factors affect productivity and meat quality is important both for optimising production efficiency and for meeting the increasing consumer demand for high-value rabbit meat. The aim of this study was to evaluate the impact of breed and sex on rabbit productivity and meat traits during the fattening period. The research involved rabbits of different breeds and both sexes, monitored from 45 to 105 days of age. Productivity indicators, including average body weight, daily weight gain, feed conversion ratio, and growth rate throughout the trial, were assessed. In addition, carcass morphological composition and the physical and chemical properties of the meat were analysed. The results revealed that males of certain breeds tended to achieve higher body weight and carcass yield, while daily gain varied depending on both breed and sex. Females generally exhibited more favourable meat quality parameters, such as muscle yield, protein and fat content, and water-holding capacity. These findings suggest that both breed and sex exert a measurable influence not only on rabbit growth performance but also on the technological and nutritional properties of the meat. In conclusion, breed and sex are important determinants of rabbit productivity and meat characteristics. Considering these factors in rabbit production may contribute to improved efficiency and product quality, highlighting their relevance for both farmers and the rabbit meat industry.

Keywords: rabbit, breed, sex, productivity, meat parameters.

A REVIEW ON ZEBRAFISH AS A MODEL FOR BEHAVIOURAL ASSESSMENT OF FISH STRESS AND WELFARE

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Aquaculture is the fastest-growing sector in animal food production, with fish health and welfare emerging as a central issue for sustainable growth [1]. Beyond improving nutrition, breeding, and management, the industry is increasingly exploring new compounds and practices to enhance animal stress tolerance and consequent health, particularly during sensitive handling phases such as vaccination, parasite treatments, and transport [2]. In this context, zebrafish (*Danio rerio*) has become an established model in biological research and is now gaining recognition as a relevant species for aquaculture studies due to its low cost, genetic tractability, and the wide range of available experimental tools [3]. Numerous behavioural assays have been developed in zebrafish to evaluate fish welfare in larvae, juveniles and adults, providing measurable indicators of stress or discomfort [4–7]. For instance, in locomotor tracking and startle response assays, hyperactivity, hypoactivity, or abnormal swimming patterns often reflect impaired motor function or acute stress [4, 5]. In the novel tank diving and light–dark preference tests, excessive bottom-dwelling or prolonged avoidance of illuminated areas indicate anxiety-like states [6]. Social preference and shoaling assays highlight welfare alterations through changes in group cohesion, such as abnormal isolation or erratic interactions [7]. Cognitive tests, including the T-maze or conditioned place preference, can reveal deficits in learning and memory, which are often linked to chronic stress or diseases [6]. Together, these assays allow researchers to identify behavioural changes that serve as reliable indicators for stress and welfare. The use of zebrafish behavioural tests in research enables rapid and reproducible screening of dietary compounds, farming practices, and genetic backgrounds, providing valuable insights into fish welfare under different aquaculture scenarios. In conclusion, zebrafish represent a versatile and efficient model to bridge fundamental biology with applied needs in modern aquaculture.

Keywords: aquaculture, zebrafish, fish model, behaviour, welfare.

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THE LEVEL OF SELECTED FATTY ACIDS IN COWS' MILK DEPENDING ON THE BREED AND FEEDING SEASON

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The aim of the study was to determine a level of fatty acid in cows' milk dependent on the breed, feeding system and season. The research material consisted of cows' milk collected from cows from 10 conventional farms in Poland. Cows were fed with haysilage and corn silage (HS, CS) haysilage (HS), or total mixed ration (TMR). Milk for testing was collected in two feeding seasons: summer and winter, from Holstein-Friesian (HF) and Simmental (SIM) dairy cow breeds. In each feeding season, milk was collected two times, at the same time from each farm (at the beginning of the study period and at the end of each feeding season). The highest average concentrations of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were observed in milk from SIM cows, while saturated fatty acids (SFA) were found in milk from HF. In the summer season the PUFA content was 0.12 ± 0.03 g/100 g in the milk from SIM cows, and 0.58 ± 0.56 g/100 g in the milk from HF cows ($P < 0.001$), while the MUFA content was 0.97 ± 0.22 g/100 g and 0.9 ± 0.07 g/100 g, ($P = 0.52$), respectively. In milk from HF cows from the summer season, a significantly higher share of SFA acids was noted compared with the milk from HF cows. The content of these acids was 2.84 ± 0.44 g/100 g in HF cows and 2.74 ± 0.22 g/100 g in SIM cows.

The analysis of the fatty acid profile of milk from the winter season showed that the average content of MUFA and SFA acids was higher in milk from SIM cows. The obtained values were at the level of 0.81 ± 0.17 g/100 g for MUFA ($P = 0.037$) and 2.8 ± 0.46 g/100 g for SFA ($P = 0.043$), while for HF cows, these values were 0.7 ± 0.17 g/100 g for MUFA and 2.33 ± 0.66 g/100 g for SFA. In turn, there were more PUFA in milk from SIM cows (0.12 ± 0.04 g/100 g) compared with milk from HF cows (0.14 ± 0.02 g/100 g; $P = 0.48$).

Feeding cows with haysilage and corn silage (HS, CS) increased the level of MUFA and PUFA, both in the summer season and in the winter season. In turn, feeding in the TMR system increased SFA both in the winter and summer season. The research results indicate that milk from HF dairy cow breeds contained more PUFA and MUFA, and in the case of the feeding season, milk from the summer feeding season had better properties from cows fed with haysilage and corn silage. In the case of SFA, more acids were found when cows were fed TMR.

Keywords: cow milk, fatty acid profile, breed, feeding season, feeding system.

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MICROBIAL DIVERSITY IN BULK TANK MILK SAMPLES FROM DAIRY CATTLE IN LATVIA

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Bulk tank milk (BTM) is the most important source of raw material for dairy products, and its microbial composition and quality are important factors to ensure food safety. Microbial contamination can be impacted by the farm environment, intramammary infections, and milking equipment, showing important insights into farm hygiene and animal health. Knowing the variety and prevalence of bacterial isolates in BTM is essential for managing animal health, milk quality, and monitoring trends in antimicrobial resistance. This study aimed to find out the microbial diversity in BTM from dairy farms across Latvia using commercially available plates such as Columbia Agar, Columbia CNA Agar, Slanetz–Bartley Agar, and Baird–Parker Agar. BTM samples were collected from 18 dairy farms; 18 samples were obtained from the bottom via the tank tap, and 13 samples from the top through the lid. From each plate, up to four morphologically dominant colonies were identified. A total of 91 bacterial isolates were identified with MALDI-TOF mass spectrometry. The most frequently isolated species were *Enterococcus faecalis* (n = 28; 15.4%), *Staphylococcus aureus* (n = 13; 7.1%), and *Escherichia coli* (n = 11; 6.0%). Other clinically relevant bacteria, including *Klebsiella pneumoniae* (n = 6), *Citrobacter freundii* (n = 3), and *Enterobacter cloacae* (n = 2), were detected. In addition, 28 isolates (30.8%) were classified as other environmental bacteria. Comparing sampling sites of the bulk tank revealed that 48 isolates (52.7%) originated from bottom samples and 43 isolates (47.3%) from top samples. *S. aureus* was slightly more common in the top samples (8 vs. 5), while *E. faecalis* was widespread across both positions (15 vs. 13). This study findings underline the importance of taking samples from several areas in bulk tanks to ensure the most precise microbial diversity. The results show that both mastitis pathogens and environmental contamination can give insights into bulk tank milk quality.

Keywords: bulk tank milk, dairy cattle, raw milk microbiota.

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OPTIMIZING PIGLET WEANING DIETS WITH FERMENTED FEED: GROWTH AND HEALTH OUTCOMES

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The gut microbiota plays a vital role in animal health and development. In pigs, the initial microbial colonisation during early life is critical, as it helps shape the long-term gut ecosystem, ultimately impacting growth performance and overall well-being. For this reason, fermented feed materials (FFM) are increasingly recognised for their potential to support piglet health and performance. This study assessed the impact of FFM supplementation in Topigs Norsvin Yorkshire piglets during suckling (days 7–25) and weaning (days 25–69). Piglets were divided into three groups: control (C), receiving a commercial pre-starter and basal diet; Pp, supplemented with *Pediococcus pentosaceus*; and Pa, supplemented with *Pediococcus acidilactici*. During weaning, both Pp and Pa groups received FFM inoculated with selected lactobacilli strains. Results showed that, by day 41, both the Pa and Pp groups had, on average, 13.7% higher body weight than the C group, while feed conversion ratios remained similar. The Pa group exhibited elevated IgG concentration ($P = 0.758$) and both FFM-fed groups showed lower plasma alanine aminotransferase (ALT) activity in treated groups (C – 91 U/L; Pp – 71.8 U/L, Pa – 61.0 U/L). Faecal microbiota analyses indicated greater bacterial diversity and increased lactobacilli counts in FFM-fed piglets, alongside broader volatile compound profiles. Finally, the results of this study indicate that feeding piglets with FFM from suckling through weaning and beyond can positively influence growth and health. By day 69, piglets had higher body weight compared with controls (Pp – 2.15 kg and Pa – 1.65 kg). The control and Pa groups also showed elevated blood plasma IgM levels and lower ALT activity. Faecal samples from FFM-fed piglets displayed firmer texture and increased microbial diversity. Additionally, individual volatile compounds (VCs) in faeces were more variable in the Pa and Pp groups, with significant correlations observed between VCs and faecal microbiological parameters. Overall, the enhanced microbial diversity and variability in VCs in the treated groups may support improved nutrient digestion and absorption, contributing to greater body weight gains.

Keywords: immunoglobulins, volatile compounds, microbiota, blood parameters.

ULTRASONICATION-INDUCED MODIFICATIONS IN THE PROTEOMIC AND LIPIDOMIC PROFILES OF *ACHETA DOMESTICUS* AND *TENEBRIO MOLITOR* BIOMASS

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Insect biomass is increasingly acknowledged as a sustainable source of protein, supplying essential amino acids (AA), fatty acids (FA), and bioactive compounds beneficial for human nutrition. Additionally, insect rearing produces lower greenhouse gas emissions compared with conventional livestock systems [1, 2], particularly when paired with advances in insect genetic resources [3–5]. The aim of this study was to investigate the impact of ultrasonication on the proteomic and lipidomic composition of *Acheta domesticus* and *Tenebrio molitor*. Low-frequency ultrasound (37 kHz, 160 W, 40°C) was applied for 0, 15, 30, 45, or 60 min to assess these effects. Measured parameters included free amino acids (FAA), biogenic amines (BA), fatty acids, pH, colour, total spore-forming aerobic mesophilic bacteria, and volatile compounds (VC), serving as indicators of protein and lipid modifications. Most experimental factors (“sample type,” “ultrasonication,” and “duration”) significantly influenced colour and total bacteria count ($P < 0.001$). Strong correlations were observed between pH and FAA levels, particularly valine ($r = 0.802$), methionine ($r = -0.903$), tryptophan ($r = 0.842$), serine ($r = -0.863$), and tyrosine ($r = 0.831$). In *A. domesticus*, BA concentrations were lowest in whole-insect samples after 60 min, while flour subjected to 60 min of ultrasonication exhibited elevated putrescine, tyramine, spermidine, and total BA (about 2.0–2.3-fold). In *T. molitor*, untreated samples contained the highest BA levels. Ultrasonication induced only minor shifts in FA composition, with linoleic acid (C18:2) predominating in *A. domesticus* and a combination of oleic (C18:1) and linoleic acid (C18:2) in *T. molitor*. Despite the low-frequency treatment, VC profiles and their associations with FAA and FA were altered, highlighting the need for careful monitoring of insect biomass during processing for both food and feed applications. These findings provide novel insights into protein and lipid modifications in insects, supporting the safe and sustainable use of ultrasonication in food and feed processing.

Keywords: amino acid, biogenic amine, fatty acid, volatile compound, insects.

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THE EFFECT OF MANGANESE DEFICIENCY OR DIETARY SUPPLEMENTATION WITH MANGANESE(III) OXIDE NANOPARTICLES ON BONE TURNOVER PROCESSES IN RATS

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The study aimed to determine the effect of manganese deficiency and replacing the recommended level of MnCO_3 (65 mg Mn/kg diet) with Mn_2O_3 nanoparticles ($\text{Mn}_2\text{O}_3\text{NPs}$) in the diet on bone turnover and remodelling in rats. The experiment was conducted for 12 weeks on male Wistar rats randomly assigned to 3 experimental groups ($n = 9$). The first group (control) received a diet containing the recommended level of Mn in the mineral mixture in the standard form of MnCO_3 . The second group received a diet from which Mn was excluded from the mineral mixture. The third group received a diet containing the recommended level of Mn in the mineral mixture in the form of $\text{Mn}_2\text{O}_3\text{NPs}$ instead of MnCO_3 . At the end of the experiment, blood and the right femurs were collected from all rats. Bone-related protein levels were measured in plasma and femur, and additionally, the expression of genes related to osteoblast and osteoclast differentiation was analysed in the femur. Dietary Mn deficiency decreased the levels of bone-specific alkaline phosphatase, tartrate-resistant acid phosphatase 5b, N- and C-terminal propeptides of type I procollagen in both blood plasma and femur of the studied rats. This treatment also resulted in a decrease in the levels of interferon- β , N-terminal telopeptides of type I collagen, RANKL glycoprotein, 1,25-dihydroxyvitamin D_3 , and vitamin K_2 in blood plasma, as well as levels of interferon- γ , C-terminal propeptides of type I procollagen, osteonectin, and calcitonin levels in the femur. In turn, replacing standard MnCO_3 with $\text{Mn}_2\text{O}_3\text{NPs}$ in the rat diet increased IFN- γ levels while decreasing IFN- β and 1,25-(OH) $_2\text{D}_3$ levels in blood plasma. This treatment also decreased BALP and calcitonin levels and the RANKL:OPG ratio in the femur, while increasing *Sp7* and *Ctsk* gene expression in this tissue. All mentioned changes were statistically significant ($P < 0.050$). In summary, both manganese deficiency and replacing its standard form MnCO_3 with $\text{Mn}_2\text{O}_3\text{NPs}$ disrupt bone remodeling processes.

Keywords: manganese carbonate, manganese(III) oxide nanoparticles, bone turnover, femur, rat.

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TWO SEASONAL PROFILES: CONTRASTING PATTERNS OF HONEYBEE VIRUS PREVALENCE ACROSS REGIONS IN LITHUANIA

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Honeybee (*Apis mellifera*) colonies are threatened by multiple viruses, and while seasonal dynamics and environmental conditions strongly influence their prevalence, knowledge of these patterns remains incomplete [1]. Honeybee colonies harbour multiple viruses with diverse transmission routes: some are closely linked to *Varroa destructor* infestation, while others are transmitted primarily via direct contact between bees and through ingestion of virus-contaminated food resources [2]. Understanding the drivers of these patterns is essential for effective disease management. We investigated the seasonal prevalence of four honeybee viruses – acute bee paralysis virus (ABPV), black queen cell virus (BQCV), chronic bee paralysis virus (CBPV), and deformed wing virus (DWV) – in colonies across nine regions of Lithuania during the 2024 beekeeping season. In total, 97 honeybee samples were collected from nine regions of Lithuania (Kaunas, Kupiškis, Panevėžys, Pasvalys, Raseiniai, Šakiai, Šiauliai, Tauragė, and Ukmergė) during spring, summer, and fall of 2024, and screened by PCR for target viruses. Israeli acute paralysis virus (IAPV), sacbrood virus (SBV), and Kashmir bee virus (KBV) were also tested but not detected. Two distinct seasonal profiles emerged. ABPV and BQCV prevalence was highest in spring (41.9% and 87.1%, respectively) and summer (35.5% and 80.6%) and sharply reduced in fall (2.9% and 34.3%), while CBPV and DWV showed the opposite pattern, with low spring levels (6.5% each) and peaks in autumn (31.4% and 77.1%). These findings suggest that different viruses are influenced by distinct epidemiological drivers, with ABPV and BQCV more closely associated with early-season colony conditions, and CBPV and DWV linked to late-season factors, such as *Varroa* dynamics. Understanding these seasonal patterns is critical for improving honeybee health management and mitigating the impact of viral infections on colonies.

Keywords: *Apis mellifera*, viral prevalence, seasonality, Lithuania.

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LACTOFERRIN AS AN EARLY MASTITIS PREDICTOR IN RELATION TO SOMATIC CELL COUNT IN DAIRY COWS' MILK

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Mastitis is the most frequent udder disease in dairy cows. It causes huge economic losses, due to lower milk production, lower milk quality, increasing drug usage and, in most severe cases, early culling; therefore, a healthy herd is a fundamental goal in dairy farming [1]. Lactoferrin (LF) is an iron-binding glycoprotein naturally present in milk and secreted mainly by the mammary epithelial cells or other exocrine secretions which has antimicrobial/antiviral activities, immunomodulatory activity and antioxidant activity [2]. It has been reported that the LF concentration in milk was significantly associated with somatic cell count (SCC); furthermore, it has been demonstrated that the LF concentration changes according to stage of lactation, parity and presence of pathogen, and in fact it is significantly higher in cows with subclinical mastitis compared with healthy ones [3]. Therefore, the aim of this study was to evaluate the amount of antimicrobial protein LF in dairy cows' milk in relation to SCC and validate the theory that LF content rises before somatic cell count rise in milk recording samples. In total, 200 milk samples were collected individually from MR (milk recording) data of 1–7 lactation Holstein breed dairy cows and selected according to the SCC. The measurements were made with high-performance liquid chromatography (HPLC) method. Samples for laboratory evaluation were taken according to the rules for the organisation of performance MRO. Based on the SCC of milk samples, the LF values were distributed within 8 groups. The results of the study showed that the antimicrobial protein LF had a significant correlation with month of lactation and SCC, indicating that both stage of lactation and udder health strongly influence LF concentration levels. All tested models improved significantly with added effects ($P < 2.2 \times 10^{-16}$).

The study suggests that the benefit of this indicator is associated to SCC as an early mastitis predictor, though further methodological advancements are required.

Keywords: lactoferrin, mastitis predictor, mastitis, antimicrobial protein, early mastitis predictor.

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CHEMICAL COMPOSITION OF LITHUANIAN GREEN MACROALGAE *C. GLOMERATA* BIOMASS: POTENTIAL FEEDSTUFF FOR POULTRY ANIMAL MODEL

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From the perspective of the sustainability of animal production, it is necessary to investigate the development of new and innovative feed mixtures in livestock farming that have the potential to improve the overall nutritional value of feeds without negative side effects on animal health, their production and carcass quality. Since algae offer a rich source of beneficial bioactive substances and high protein content, they have become the subject of many studies in animal nutrition in recent years. In our study, we focused on determining the chemical composition of freshwater *Cladophora glomerata* biomass collected from the Jūra River in Lithuania (N55°27'19.58", E22°2'14.72"). After washing and cleaning the biomass, collected samples were dried overnight at 60°C in an oven and were subsequently stored at room temperature for further analysis. The chemical analyses were conducted in accordance with the Official Methods of Analysis of AOAC. Dry matter was represented by organic matter (645.49 g/kg) and ash (354.51 g/kg). Content of crude protein was 264.87 g/kg, crude fibre 170.30 g/kg, nitrogen-free extractive substances 203.97 g/kg, and fat 6.35 g/kg. The analysis of the samples showed a three-fraction fibre composition: the first fraction – neutral detergent fibre (NDF) – was the most abundant component at 237.92 g/kg (of which cellulose had the largest representation 155.81 g/kg, followed by hemicellulose 12.35 g/kg); the second fraction – acid detergent fibre (ADF) – amounted to 225.57 g/kg; and the third fraction was lignin at 69.76 g/kg. Overall results indicate a rich nutritional profile of studied algae and the potential to improve animal health and performance. Obtained data will be useful for a least cost feed formulation methodology in a poultry animal model, to determine the most economical combination of conventional and innovative ingredients that will meet the poultry nutrient requirements and, in terms of precision farming, make the poultry primary production concretely more sustainable and profitable.

Keywords: precision farming, animal nutrition, innovative ingredient, *Cladophora glomerata*.

Acknowledgments: The research was funded by departmental resources of the National Agricultural and Food Centre (Luzianky, Slovakia) and Lithuanian University of Health Sciences (Kaunas, Lithuania).

CANNABIDIOL AND NANOSELENIUM MEDIATES MEAT PROPERTIES IN THE PERIOD OF POST-INFECTION OF *C. PERFRINGENS*-CHALLENGED CHICKENS

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Recently, growing evidence indicates a close relationship between the gut status, the factors determining it, and the processes that condition the physicochemical properties of poultry meat [1]. Bioactive additives, such as cannabidiol (CBD) and nanosized selenium (SeNPs), demonstrate potential in preventing bacterial diseases such as necrotic enteritis in chickens [2]. A total of 432 one-day-old male Ross 308 broilers were divided into 6 experimental groups. The negative control (CON) received a basal diet without supplementation or challenge, while the positive control (CON+) was fed basal diet but challenged with *Clostridium perfringens*. The CBD+ group was challenged with *C. perfringens* and supplemented with 15 g/kg *Cannabis sativa* extract, while the SeNPs+ group was similarly challenged but received 0.3 mg/kg SeNPs. Chicks from CBD + SeNPs and CBD + SeNPs + *C. perfringens* were fed a CON supplemented with both additives, with the latter group also challenged *C. perfringens*. On day 35 of the experiment, breast muscle was sampled for meat quality, fatty acid profile and sarcoplasmic protein evaluation. Supplementation with CBD and/or SeNPs did not negatively affect breast meat quality or the desaturation, thrombogenic and atherogenicity indices. Dietary supplementation with CBD and CBD + SeNPs significantly increased polyunsaturated fatty acid (PUFA) and omega-3 PUFA concentrations of *C. perfringens*-challenged chickens. The PUFA n-6/n-3 ratios in muscle lipids were most significantly associated with groups receiving SeNPs alone or in combination with CBD. The levels of pyruvate kinase/phosphoglucose isomerase in meat exudate from the CBD + SeNPs group were significantly different from those in the CON, CON+ and CBD+ groups, showing the lowest level of this enzyme. THC accumulation in breast muscle was at a concentration of 10 ppm and CBD transfer at 79×10^{-6} . These findings indicate that both CBD and SeNPs can be considered safe and effective feed ingredients in chicken diets under the investigated conditions.

Keywords: cannabidiol, nanoselenium, tetrahydrocannabinol, *C. perfringens*, chicken meat.

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OVARIAN TISSUE ACTIVATION AND THE FUTURE OF FERTILITY TECHNOLOGY

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The preservation of fertility and genetic resources is a growing priority in both human medicine and animal breeding. Ovarian tissue cryopreservation (OTC) aims to preserve the reserve of ovarian follicles, which is essential for preserving genetic material and ensuring fertility in economically important domestic animals. In cattle and other livestock species, the development of breeding systems capable of supporting the growth of immature follicles containing oocytes to the stage when they could mature and be ready for fertilisation would provide a large supply of oocytes for manipulation. The potential embryos derived from those oocytes could in turn be transferred to the recipients in order to shorten the intergeneration interval of genetically selected animals and consequently increase the number of most precious offspring born per animal. Therefore, the activation of primordial follicles, combined with subsequent *in vitro* culture techniques, represent useful tools for the production of domestic animals with improved genetics [1].

In our study, we focused on the intracellular mechanisms controlling ovarian tissue activation in pre-pubertal and pubertal cattle. We hypothesise that mechanical stimulation (fragmentation of ovarian tissue, its vitrification and warming) will modulate intracellular mechanisms in both fresh and cryopreserved samples from pre-pubertal and pubertal animals. Moreover, we speculate that the used method will not induce apoptosis in activated oocytes and surrounding granulosa cells. We observed that ovarian tissues from pre-pubertal and pubertal heifers responded differentially to the applied mechanical stimulation.

Keywords: cow, ovarian tissue activation, vitrification, primordial follicles.

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DIETARY INCLUSION OF *MYTILUS GALLOPROVINCIALIS* MEAL: EFFECTS ON GROWTH PERFORMANCE AND CARCASS TRAITS OF BROILER CHICKENS

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The aim of this study was to evaluate the effects of dietary supplementation with whole *Mytilus galloprovincialis* meal on growth performance and carcass traits of broiler chickens, aiming to improve production efficiency. Three groups of one-day-old Ross 308 broiler chicks (64 birds per group, 32 males and 32 females) were assigned to diets with identical nutritional composition: Group 1 – control with a basal diet; Group 2 – 50% of dietary calcium provided from *Mytilus galloprovincialis* meal and 50% from limestone; Group 3 – 100% of dietary calcium provided from *Mytilus galloprovincialis* meal. The fattening period corresponded to 42 and 49 days of age, during which slaughter analysis was performed on 6 birds of each sex per group. Birds in the control group tended to exhibit higher live body weight and cumulative feed intake, whereas those in Group 3 showed the lowest values. Regarding feed conversion, a trend toward more efficient utilisation was observed in birds from both experimental groups receiving *Mytilus galloprovincialis* meal compared with the control group, with differences of approximately 5.1% and 4.8% at 42 and 49 days of age, respectively. Results from the slaughter analysis indicated a trend towards a higher carcass yield and proportion of valuable meat in birds from the experimental groups compared with the control, observed in both experimental periods (42 and 49 days of age). Economic analysis of feed costs per broiler carcass, per kilogram of broiler meat, and per kilogram of valuable meat showed no significant differences among the groups, although a trend towards more efficient production per unit was observed in birds fed diets supplemented with *Mytilus galloprovincialis* meal. Whole *Mytilus galloprovincialis* meal can partially or fully replace conventional calcium sources in broiler diets, enabling efficient utilisation of mussel by-products.

Keywords: mussel meal, by-products, poultry feeding, feed conversion ratio, chicken meat.

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THE EFFECT OF BREED AND BIRTH SEASON ON LIVE PIG PERFORMANCE

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The aim of the study was to evaluate the effect of breed and birth season on the live gilt's performance. A total of 145 Large White (LW) gilts and 17 Large White x Landrace (LW*La) crossbred gilts at 90 to 100 kg live weight were evaluated. The gilts were divided into groups according to the birth season: spring (24 gilts), summer (49 gilts), autumn (64 gilts) and winter (25 gilts). The parameters monitored were weight (kg), age (days), average daily gain (ADG) in g/day, back fat thickness (BFT) in mm, the *Musculus longissimus dorsi* (MLD) thickness in mm, and lean meat content (LMC) in %. BFT and LMC were measured using an ATLAS ultrasonographic device. LW*La gilts reached a weight of 90–100 kg at 148.82 days of age and LW gilts at 152.00 days of age ($P > 0.05$). ADG was higher in the LW*La crossbreed (634.97 g/day vs LW 624.24 g/day) ($P > 0.05$). The LW gilts had a significantly higher BFT (7.29 mm) than LW*La (6.14 mm) ($P < 0.001$), while differences in MLD thickness were not significant ($P > 0.05$). LW gilts achieved a significantly lower LMC (62.27%) than LW*La (63.05%) ($P < 0.001$). The lowest age at measurement was achieved by gilts born in winter (144.12 days) and the highest in spring (158.25 days) ($P < 0.05$). Similarly, ADG was significantly lowest in gilts born in spring (603.79 g/days) and highest in winter (657.84 g/day) ($P < 0.05$). The season of birth had no statistically significant effect on BFT, MLD thickness, and LMC ($P > 0.05$). The analysis of the results showed a significant interaction between breed and birth period in the indicators age ($P < 0.001$), ADG ($P < 0.05$) and LMC ($P < 0.05$). This suggests that the live gilts performance of individual breeds or crossbreeds can be significantly influenced by changes in housing conditions during the year.

Keywords: pig, breed, birth season, performance.

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POSSIBLE EFFECTS OF PROBIOTIC SUPPLEMENTATION IN FEED AND LITTER ON AMMONIA EMISSION, HEALTH AND PERFORMANCE OF BROILER CHICKENS

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High ammonia concentrations in poultry houses negatively affect broiler health, welfare and productivity. Effective and sustainable strategies to mitigate these effects are increasingly sought [1–3].

The aim of this study was to evaluate the effects of the combined use of *Lactobacillus farciminis* and *Lactobacillus rhamnosus* in feed and litter on ammonia emission and as well as improve the health and productivity of broilers.

In total, 780 unsorted chicks were purchased from a commercial hatchery and randomly divided into two groups: Con (n = 390), which received a basal diet, and ProL (n = 390), which received a basal diet plus a mixture of *L. farciminis* (CNCM I-3699, 7.8×10^6 GU/g) and *L. rhamnosus* (CNCM I-3698, 7.8×10^6 GU/g), at a dose of 4 g / 10 kg feed. The same mixture was applied to the litter at a rate of 10 g/m² in the proL group before the chickens were placed, and then at a rate of 5 g/m² once a week afterwards. Birds were raised for 35 days under identical housing conditions. Growth performance, feed conversion ratio (FCR), and footpad health were monitored. Tracheal samples were collected for histomorphological evaluation.

Probiotic supplementation significantly reduced the concentration of ammonia in the housing environment during the early stages; however, there were no significant differences compared with the control group at the end of the study. Broilers that received probiotics numerically showed a higher body weight gain and improved a feed conversion ratio (FCR), but there were no significant differences in footpad dermatitis between the groups. Histological analysis indicated a better trachea condition, but the results were not significantly better throughout the entire study period.

Combined dietary and litter application of probiotics is a promising approach to reduce ammonia emissions and associated health risks, while enhancing growth performance and welfare in broiler production.

Keywords: Ross 308, *Lactobacillus rhamnosus*, *Lactobacillus farciminis*, poultry, footpad dermatitis.

Acknowledgments: This study was funded by the ESF project 'Transition to the new doctoral funding model at the Latvia University of Life Sciences and Technologies' (No. 8.2.2.0/20/I/001), the Latvia University of Life Sciences and Technologies program 'Realisation of Fundamental research at LLU' project G7 'Changes in postnatal ontogenesis, health status and greenhouse gas emissions of broiler chickens after addition of a new composition of the probiotic mixture to bedding and feed' (No. 3.2.-10/273) and the 'Kekava Food' poultry farm.

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THE ROLE OF LANDSCAPE COMPOSITION ON HONEY PHYSICAL PARAMETERS

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The productivity of honey bee colonies and the quality of honey are closely associated with the characteristics of the surrounding landscape. Urbanisation and agricultural development represent two most prominent and widespread forms of anthropogenic land use; however, their effects on honey bees and bee products remain insufficiently investigated [1, 2]. The objective of this study was to assess the influence of agricultural land, forested areas, and urbanised zones (city center, residential neighborhood, and industrial district) on physical parameters of honey produced by *Apis mellifera carnica* colonies. The parameters analysed included moisture content, electrical conductivity, pH, color intensity, and CIELAB colour coordinates (L^* , a^* , b^*). Honey samples were collected twice, at the end of June and at the end of July. Statistical analyses were performed using SPSS software, and statistical significance was set at $P \leq 0.05$. In the first sampling period, honey from agricultural areas exhibited the lowest moisture content (16.45%) and the lowest pH value (3.72). Honey from forested areas showed electrical conductivity exceeding 0.8 mS/cm and the highest colour intensity (65.47 mm Pfund), indicating the presence of honeydew honey. Analysis of colour coordinates revealed that honey from agricultural areas was the lightest (highest L^*) and most yellow (highest b^*), whereas the highest redness (a^*) was recorded in honey from residential neighborhoods, although this difference was not statistically significant ($P \geq 0.05$). In the second sampling period, the lowest moisture content (18.10%) was recorded in honey from forested areas, while the lowest pH value (3.76) was again found in honey from agricultural areas. Honey from forested areas maintained electrical conductivity above 0.8 mS/cm and exhibited the highest colour intensity. The greatest lightness (L^*) was observed in honey from a city centre, the highest redness (a^*) in honey from agricultural areas, and the most pronounced yellowness (b^*) in honey from residential neighborhoods.

These findings indicate that the structure of the surrounding landscape significantly influences the foraging behaviour of honey bees and, consequently, the physical characteristics of the honey they produce.

Keywords: landscape composition, honey bee, honey, physical honey parameters.

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MICROPLASTIC PRESENCE IN BOAR SEMEN: POTENTIAL RISKS FOR REPRODUCTIVE HEALTH

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Microplastic particles (MPs), defined as plastic fragments measuring less than 5 millimeters, are increasingly detected in the environment due to the extensive production and degradation of plastic materials. Recent studies have demonstrated microplastic accumulation in various biological systems, including reproductive tissues, raising critical questions about their possible effects on human and animal reproductive health [1, 2]. The results of experimental studies have demonstrated that the introduction of MPs during artificial insemination procedures can impair fertility [3]. Therefore, the aim of the present study was to determine the presence of MPs in raw boar semen samples. Fresh, undiluted sperm samples (n = 12) were collected from healthy boars at breeding farms in Lithuania. To minimise secondary contamination, semen samples were collected directly into glass containers with metal lids. All experiments were performed using glassware that had been thoroughly cleaned with ultrapure water (Milli-Q). The detection of MPs was performed by modifying a standard protocol for digestion and filtration, followed by microscopic examination [4]. The presence of MPs was identified in all semen samples that were analysed. The MPs detected in this experiment ranged from less than 50 to 1000 µm in size. The most prevalent polymer types identified were polyethylene (detected in 91.6% of the samples), acrylates (in 75.0% of the samples), and polyester (in 75.0% of the samples), indicating the presence of multiple environmental plastic sources. These findings raise concerns regarding possible biological effects. Previous research indicates that MPs in male reproduction may induce oxidative stress, inflammation, and impaired spermatogenesis, potentially leading to reduced sperm motility, altered morphology, and compromised fertility outcomes [5]. This study provides the initial evidence of microplastic presence in boar semen and highlights the need for further investigations to clarify their impact on boar sperm quality and fertility, as this contamination has not been previously examined.

Keywords: microplastics, boar semen, reproduction, environmental contamination.

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PRELIMINARY RESULTS OF THE FIRST SURVEY ON THE HUNTING SECTOR IN BOSNIA AND HERZEGOVINA

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There are no literature sources on the hunting sector in Bosnia and Herzegovina (BaH). It was our motivation to arrange a screening investigation on site as Short Scientific Mission in the frame of COST Action “SafeGameMeat” about the main principles of organisation of hunting, game meat market and byproduct disposal. Essentially, there are four different hunting associations in the country: Association of Hunting Organisations in Bosnia and Herzegovina in Sarajevo with approx. 20 000 hunters in 48 hunting organisations, mainly in Federation of Bosnia and Herzegovina entity (FBH); The Hunting Association Republic of Srpska (RS) with approx. 20 000 hunters which consists of 105 different member organisations; The Organisation of the Herceg Bosna Hunting Association (based on Croatian majority) in Mostar with approx. 10 000 hunters in 54 organisations across 6 cantons and District Brcko (under the United Nations jurisdiction); The Association of Hunting Societies of the Tuzla Canton, with 7358 hunters in 13 hunting organisations. The organisation of hunting in FBH is divided along four different purposes: economic; sport hunting; economic and sports hunting; and hunting with a special purpose. Similarly, in RS, the hunting sector is organised in four different ways depending on its purpose: special hunting grounds, economic-sports, sports-recreational and economic hunting grounds. Organisation of hunting is uniform throughout the country: every entity gives hunting ground (one per municipality) for rent to the local hunting organisation. And this is the case in the majority of all hunting grounds. Based on surveys in 12 biggest municipal hunting societies, it can be concluded that there is big space for improvement of the hunting sector, i.e. raising awareness about the importance of many topics in game meat hygiene ending with proper treatment of animal byproduct.

Keywords: wildlife management, Balkan countries, questionnaire, game meat.

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ENHANCEMENT OF REPRODUCTIVE FUNCTION IN RABBITS THROUGH ANTIOXIDANT-RICH PHYTOGENIC FEED ADDITIVES: A STUDY OF *CHLORELLA VULGARIS* AND *LAURUS NOBILIS*

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Nutraceuticals, particularly those with antioxidant properties such as phytogetic compounds, have shown significant potential in enhancing male reproductive performance in animals. Supplementation with *Chlorella vulgaris* in male rabbits has been shown to enhance reproductive potential by reducing oxidative stress and upregulating antioxidant enzyme activity in key reproductive organs. Similarly, *Laurus nobilis* supplementation has been reported to improve antioxidant status, lipid metabolism, and immune function in rabbits, which may indirectly support reproductive health. To further investigate these effects, a 3.5 month long feeding trial was conducted at the Research Institute for Animal Production Nitra (Slovak Republic, SK CH 18021). A New Zealand White line of 20 rabbit adult bucks was used for the experiment, divided into two groups. One control group (CON) was fed with commercial pelleted-feed, and one experimental group (EXP) received a natural feed additive mixture (0.1% of dried *Chlorella vulgaris* powder and 0.1% of dried *Laurus nobilis* leaf powder) integrated in pelleted feed. During the experimental period, semen samples were collected and evaluated for two consecutive reproduction cycles. Four quality parameters were analysed using computer-assisted sperm analysis (CASA). The results showed a slightly higher spermatozoa concentration in the experimental group in both collection cycles, but differences were not statistically significant. Ejaculate volume was greater in the experimental group in both cycles, without reaching significance. In the second cycle, spermatozoa motility of the experimental group demonstrated significantly higher percentage (87.83%) compared with the control group (70.63%; $P < 0.01$). Progressive spermatozoa motility followed a similar trend, with improved values in the experimental group during the second cycle, showing a pronounced and statistically significant increase (75.68% vs 50.10%; $P < 0.001$). These findings suggest that dietary supplementation with *Chlorella vulgaris* and *Laurus nobilis* supports reproductive performance in male rabbits by enhancing sperm motility, particularly under prolonged dietary exposure, and serves as effective natural additives in animal fertility management.

Keywords: functional nutrition, reproductive biomarkers, nutraceutical, rabbit semen.

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CONTRIBUTION OF ARTIFICIAL INSEMINATION AS A REPRODUCTIVE METHOD IN THE BULGARIAN SPORT HORSE BREED

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The artificial insemination of mares is a reproductive method that has been well researched for more than a century. Currently, the method is widely used in warmblood horse breeds, but it is still denied or restricted by other breed registers, such as Thoroughbred or some Trotter breeds. In Bulgaria, artificial insemination of mares started to be implemented more widely since 2006, first on mares of various breeds in the national stud farm “Kabiyuk” and, later, on mares of private owners. The Bulgarian sport horse breed has been recently approved in Bulgaria. As with most warmblood horse breeds, the Bulgarian Sport Horse Register has no regulations to restrict or prohibit the application of artificial insemination or any other recent reproductive methods. The Bulgarian Sport Horse Association (BSHA) registers between 30 and 70 foals annually. This study aimed to identify the number of foals produced by artificial insemination registered in the BSHA out of the total number of foals registered from 2006 to 2024. The foals were distributed by year, and the number and breed of stallions used for artificial insemination were traced. The total number of registered foals was 940 during the study period, of which 85 were obtained by artificial insemination. As a percentage, foals conceived by artificial insemination represented 9.04% of all registered new-born foals. This percentage varied from 2.04% in 2010 to 17.46% in 2012. The number of stallions whose frozen semen was used for artificial insemination was 61, of which 57 were stallions with imported frozen semen, and 4 were stallions whose semen was collected, frozen, and stored in Bulgaria. Breeders were most interested in Holsteiner stallions, followed by Oldenburg and KWPN stallions.

Keywords: artificial insemination, sport horse, breeding.

CATTLE BREED DIVERSITY IN TÜRKIYE (1990–2024): AN AI-AUGMENTED, PYTHON-BASED ASSESSMENT OF FAO-DERIVED INDICES

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This study presents an AI-augmented, Python-based evaluation of cattle breed diversity trends in Türkiye (1990–2024) using FAO DAD-IS records. Diversity was assessed through Shannon, Simpson, Pielou's Evenness, Berger–Parker, Menhinick, Hill numbers ($q = 0–2$) and Rényi profiles. Indices peaked in 1990–1994 but collapsed after 2000, with several years (1999, 2004, 2012) showing effective richness reduced to one and Berger–Parker ratios indicating complete dominance. Menhinick values remained low, reflecting homogenisation. Bray–Curtis dissimilarity revealed major structural shifts post-2004, linked to policy and market changes. Population data showed a decline of Anatolian Black from over four million head to near disappearance, contrasted with the stability of Turkish Grey and Eastern Anatolian Red and the persistence of South Anatolian Red and Native Southern Yellow. Coefficient of variation analyses highlighted instability in Anatolian Black and Eastern Anatolian Red, while Holstein and Simmental were more stable. Recent modest recovery reflects FAO and national in situ conservation programmes. All analyses were conducted in Python (NumPy [1], pandas [2], SciPy [3], scikit-learn [4], matplotlib [5], seaborn [6]). AI methods enabled early detection of diversity collapse and asymmetric breed replacement, often overlooked by conventional approaches [7]. This framework underscores the urgency of conserving endangered native cattle breeds in Türkiye.

Keywords: AI-augmented analysis, cattle breed diversity, FAO DAD-IS, in situ conservation, python-based assessment.

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PRACTICAL APPLICATIONS OF 1,3–1,6 B-GLUCANS IN AQUACULTURE: LINKING EXPERIMENTAL MODELS WITH FARMED FISH

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The increasing attention for sustainable aquaculture practices has highlighted the importance of functional feed additives capable of improving animal health, stress tolerance, and resistance to pathogens [1, 2]. Among these bioactive compounds, 1,3–1,6 β -glucans have gained considerable attention due to their immunomodulatory properties [3]. These natural polysaccharides have been shown to modulate both innate and adaptive immune responses in both laboratory and farmed fish species. In practice, β -glucans exert their main biological action by modifying the phagocytic activity of immunocompetent cells, notably macrophages [3, 4]. In addition, they support the immune cells' mitochondrial function, which is deeply involved in orchestrating effective innate and adaptive responses [5]. Recent studies in zebrafish (*Danio rerio*) models have shed light on these mechanisms. In transgenic *Tg(corona:eGFP;lyz:DsRed)* larvae expressing fluorescent macrophages and neutrophils, exposure to 1,3–1,6 β -glucans induced a higher incidence of pro-healing macrophages, suggesting enhanced intestinal immunity [4]. In another transgenic model, the dystrophic *sapje* zebrafish larvae, β -glucan supplementation improved locomotor performance and mitochondrial function, highlighting a link between immunometabolism and muscle physiology [5]. Beyond zebrafish, a study conducted in gilthead seabream (*Sparus aurata*) larvae demonstrated that β -glucan-enriched diets promoted robustness and survival, particularly under conditions of prolonged stress, confirming their potential to enhance resilience in aquaculture species [6]. Furthermore, following caudal fin amputation of adult zebrafish, oral administration of 1,3–1,6 β -glucans decreased mortality and significantly improved both daily and cumulative fin regeneration, suggesting an active role in tissue repair and recovery processes [7]. Overall, these findings confirm that β -glucans act as potent immunomodulators, with effects ranging from macrophage activation to mitochondrial efficiency and tissue regeneration. Their multifaceted action supports their application as functional dietary supplements in aquaculture, promoting improved fish health, stress tolerance, and overall sustainability of farming practices.

Keywords: aquaculture, zebrafish, β -glucans, immunity, welfare.

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GENOMIC RELATIONSHIP MATRICES UNDER LINKAGE DISEQUILIBRIUM: AN EMPIRICAL INVESTIGATION

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Genomic relationship matrices (GRM) are widely applied in estimating genetic relatedness and prediction of additive genetic variance in livestock populations. However, different methods employed in the computation of GRM may yield divergent results, particularly under conditions of linkage disequilibrium (LD). This study systematically evaluated the effects of different LD levels on various genomic relationship matrix (GRM) estimation methods. In this study, 99 individuals (13 males and 86 females) derived from African N'dama × Kenya Boran backcrosses were used, along with 712 123 SNPs [1]. The methodological framework included: (1) a fixed frequency approach (G05); (2) an observed allele frequency approach (GOF); (3) an average minor allele frequency approach (GMF); and (4) a variance-inverse weighted approach (GD). LD levels were defined using the variation inflation factor (VIF) with thresholds of 1, 1.5, 1.8, 2, and 5. For each LD level, correlation coefficients among GRMs were computed, and both the inbreeding coefficient (I) and pairwise relationship coefficient (R) were estimated. Statistical analyses were carried out using R [2]. The results indicated that increasing LD levels were associated with higher correlations among the matrices. In particular, a very strong relationship was observed between the GOF and GD methods ($r \approx 0.99$). Conversely, the correlation between the G05 and GMF matrices was consistently the lowest across all LD levels ($r \approx 0.31$ – 0.33). Regarding inbreeding coefficients, the GMF method produced higher estimates compared with the other approaches, whereas the G05 method yielded lower values. For pairwise relatedness coefficients, the GOF and GD methods provided similar estimates, while the GMF method generated excessively inflated values. These findings indicate that the level of LD considerably influences the estimation of inbreeding and relatedness from genomic relationship matrices. The GOF and GD methods provided more consistent and highly correlated results across different LD levels, while the GMF method tended to overestimate the coefficients. These findings emphasise the necessity of considering LD levels when selecting GRM methods for genomic selection.

Keywords: linkage disequilibrium, genomic relationship matrix, inbreeding coefficient.

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RETROSPECTIVE ASSESSMENT OF THE REPRODUCTIVE EFFICIENCY OF DAIRY COWS RAISED USING AN INTENSIVE FEEDING SYSTEM

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Proper reproduction management on dairy farms is crucial for their profitability, which in turn should result in enough offspring production [1]. Calves, especially heifers, are essential for the future sustainment of the dairy herd. The rearing of calves demands significant resources and time, which influences their future health and development, critical for long-term farm success [2].

The study aimed to compare the reproductive status of experimental females raised using unique intensive feeding system for calves, developed on the family farm, born in 2021–2022 ($n = 41$), with the data from national population averages, obtained from the Association of Polish Cattle Breeders Database. In the intensive calf feeding system, calves obtain colostrum in the first 4 hours of life. Thereafter they are fed with 4 liters of colostrum, twice a day, until day 4. Subsequently, calves are fed with milk substitute with 25.8% protein and 0% fibre, until day 75.4 liters twice a day and then 0.5 liters once a day, until day 85. All the time, calves have ad libitum access to water and dry TMR. After approx. 3 months, calves are grouped together, and forage is introduced from day 125.

We observed daily weight gain of 1.10 kg in the group of experimental calves. 21 days before the planned calving date, the average female weight and height were 645 kg and 148 cm, respectively. The age of the first calving was significantly lower in the studied animals, compared with the age in Polish population ($P < 0.05$). Moreover, the intercalving period was lower in experimental animals (363 days), compared with the general population (418 days) ($P < 0.05$).

The implementation of the unique intensive calf feeding system, developed on the family farm, reduces the time needed to obtain the next generation of dairy cows. This can contribute to improved herd profitability and breeding progress.

Keywords: dairy calves, feeding management, calf welfare, reproductive parameters.

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AN ECONOMIC ANALYSIS OF POULTRY EGG PRODUCTION IN KATSINA METROPOLIS, KATSINA STATE, NIGERIA

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This study aims to bridge knowledge gaps by analysing the economics of poultry egg production in Katsina metropolis, Nigeria, where aspiring farmers face uncertainty due to rising costs and economic instability. It examines socioeconomic characteristics, determines costs and returns, evaluates feed conversion ratio, and estimates feed price ratio to inform decision making and support sustainable poultry egg production. This study collected primary data through structured questionnaires administered to poultry farm managers or their representatives in Katsina metropolis, Nigeria. The questionnaire gathered data on socioeconomic characteristics, production costs, and returns. Data analysis employed descriptive statistics (percentages and frequency distribution) and inferential statistics, including budgetary analysis to determine net farm income. Additional analytical tools used were Feed Conversion Ratio, and Egg Feed Price Ratio. The study revealed that the industry is dominated by young to middle-aged men, with a mean age of 36 years. The majority of farmers are married men with a mean household size of 4 people. The educational level of farmers is relatively high, with 58.1% having tertiary education. Additionally, 48.4% of farmers have 1–5 years of experience, and 42% of farmers engage in farming as their major occupation. Egg sales were found to account for 80.2% of total revenue, while on a per bird basis, the total revenue was approximately (\$5) ₦7720, with a gross margin of (\$2.15) ₦3332. Feed cost is a significant expense, accounting for 74% of total variable cost. The Rate of Return on Variable Cost (RRVC) of 76% and Return per Naira Invested (RRI) of 66% further support the profitability of the business. With an average Feed Conversion Ratio (FCR) of 2.6 and Egg Feed Price Ratio (EFPR) of 1.7, the study concludes that poultry egg production is financially viable in Katsina metropolis, offering opportunities for farmers to improve their income and livelihoods.

Keywords: poultry egg production, economic analysis, feed conversion ratio, egg-feed price ratio, profitability, efficiency.

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EARLY POSTNATAL INDICATORS AS PREDICTORS OF NEONATAL VITALITY AND GROWTH IN IMPROVED VALACHIAN LAMBS

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Monitoring neonatal vitality is essential for ensuring postnatal adaptation and optimising survival and growth in lambs. The Apgar score (AS) is a practical and widely used method for assessing neonatal vigour based on five physiological and behavioural criteria. This study aimed to investigate associations between early postnatal indicators and subsequent growth performance in 38 lambs of the Improved Valachian breed (19 males, 19 females; 24 singletons, 14 twins). Recorded parameters included birth weight (BiW), time to stand (TS), time to first suckling (TFS), AS, and rectal temperature (RT) at 5, 15, 30, and 60 minutes post-partum, and body weight (BW) on days 7, 14, 21, and 28. Male lambs showed significantly higher BiW and greater BW on days 2, 3, and 7 ($P < 0.05$), along with elevated AS at 60 minutes ($P = 0.035$). Singletons had significantly higher BiW and consistently greater BW across all monitored days ($P < 0.001$) and exhibited higher RT at 15 and 30 minutes ($P < 0.05$) compared with twins. TS was strongly correlated with TFS ($r = 0.758$; $P < 0.001$). Both TS and TFS were negatively associated with RT and BW ($P < 0.05$). TS was also inversely related to AS at 15 and 30 minutes ($P < 0.05$). Linear regression identified TS as a strong predictor of TFS ($P < 0.001$). Additionally, BiW and AS at 60 minutes were significant predictors of BW on days 2 and 3 ($P < 0.05$), although the influence of AS declined in later stages. The study demonstrates that behavioural reactivity and physiological condition shortly after birth, as reflected by TS, TFS, RT, AS, and BiW, are closely linked to early adaptation and growth success in lambs.

Keywords: neonatal behaviour, Apgar score, thermoregulation, vigour, lamb growth.

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USEFULNESS OF WHOLE WHEAT GRAIN IN THE FEEDING OF TURKEY HENS

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The use of whole wheat grain in poultry nutrition has a long tradition dating back to the period of extensive rearing. It appears that the use of whole wheat grain in the intensive production of turkeys for slaughter can also bring behavioural, physiological, health-promoting, and economic benefits [1, 2]. The aim of this study was to evaluate the rearing results and post-slaughter parameters of turkeys fed mixtures containing whole wheat grain. The study was conducted on 45 BIG 6 turkey hens, divided into three equal groups (I, II, III), each with five replicates. The birds were kept under standard environmental conditions from 5 to 15 weeks of age. The turkey hens were fed ad libitum with commercial complete feed mixtures, with the addition of whole wheat grain to the mixtures for group II and III birds, at 10% and 20%, respectively. The introduction of whole wheat grain (10% or 20%) to the mixtures had a positive effect on production results, i.e., final body weight and feed consumption per kg of body weight gain. Carcass analysis showed that turkeys fed rations with 20% whole wheat grain had a slightly higher dressing percentage (81.78%) compared with birds from the others. Furthermore, turkeys fed rations with 20% whole wheat grain had better musculature, as evidenced by the percentage of total muscle (52.52%) and breast muscle (31.27%) in the chilled carcass. At the same time, the higher percentage of whole wheat grain in the rations reduced the fatness of turkeys, as the percentage of skin with subcutaneous fat and abdominal fat was 10% lower in the carcasses of these birds, and 25% lower in the percentage of abdominal fat compared with the control group. Based on the results of pH measurements of breast and thigh muscles at 15 minutes and 24 hours post mortem, it was determined that the meat from both the experimental and control turkeys could be classified as normal and free of defects. To sum up, the use of whole wheat grain in the free-choice system alongside pelleted mixtures from 5 to 15 weeks of age provides an opportunity for optimised nutrition, which is the basis for recommending whole wheat grain as a 10% or 20% share in the diet of turkey hens.

Keywords: turkey, wheat, nutrition, rearing performance, slaughter value.

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DYNAMICS OF MILK YIELD AND PROGESTERONE LEVELS DURING THE ESTROUS CYCLE OF DAIRY COWS OF DIFFERENT GENOTYPES

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The milk production and reproduction efficiency are two factors that have the most influence on the profitability of dairy cows (1). The reproduction cycle of cows is directly associated with milk production. Regular calving ensures an optimally short interval between lactations and higher milk yield (2). Milk progesterone level is widely recognised as a valuable parameter for obtaining a comprehensive and direct assessment of a cow's reproduction status (3, 4). The progesterone levels in the milk of high producing cows were higher than in medium and low producing cows (5).

The evaluation of milk yield during milking and milk progesterone levels was carried out using the data of 110 Lithuanian Black and White cows with Holstein genes in their genotype ($n = 43$) and purebred Holstein cows ($n = 67$). The cows were milked 2 to 5 times per day. The parameters were evaluated 10 days before and after insemination and on the day of insemination at each milking. The objective of this study was to focus on the changes in milk yield and progesterone levels during the oestrous cycle of dairy cows of different genotypes.

Statistical analysis of the study results showed that Lithuanian Black and White cows produced more milk than purebred Holstein cows ($P < 0.01$). The dynamics of progesterone levels during the reproduction cycle were similar in both breeds; however, until the day of insemination, Lithuanian Black and White cows had higher progesterone levels, whereas from day one after insemination until the end of the investigation, Holstein cows showed higher levels ($P < 0.05$). Data analysis did not reveal a direct relationship between milk yield and progesterone levels in either breed. Progesterone levels were more dependent on the cows' physiological status during the reproduction cycle, while changes in the physiological status had less impact on the productivity of Lithuanian Black and White cows ($P > 0.05$).

Keywords: dairy cows, milk yield, progesterone, reproduction cycle, insemination day.

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INFLUENCE OF COW BREED AND PARITY ON MILK PRODUCTIVITY AND QUALITY INDICATORS

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Breed and parity influence milk composition and quality (for example, Holstein cows have higher milk fat and protein content, but their milk composition may vary depending on the parity number) [1]. Somatic cell in milk is influenced by many factors, such as animal species, milk production level, lactation stage, and the individual and environmental factors as well as management practices [2]. This study was conducted to assess the influence of cow breed and lactation number on milk yield, as well as compositional parameters (fat, protein, and lactose content), and somatic cell count. The results obtained may be useful for making informed decisions about herd management and for enhancing the efficiency and quality of milk production. The study was conducted on a commercial dairy farm that maintained more than 900 cows of the Lithuanian Black and White (BW), Lithuanian Red (R), and Holstein (HL) breeds. Animals received a complete ration formulated to meet their physiological needs. The farm operates under a loose housing system and features a 2 × 20 herringbone milking parlour. Herd management was facilitated by DelPro software (DeLaval), from which all data used in this study were obtained. For the analysis of the data, statistical indicators were calculated for each evaluated trait (milk content, percentage of milk fat and milk protein, somatic cell count): arithmetic means, mean error and statistical reliability of the data (*P*). Lithuanian Black and White cows exhibited the highest overall milk yield, averaging 9325.8 kg (*P* < 0.05). Lithuanian Red cows produced milk with the highest fat content (4.52 ± 0.10%) and protein content (3.48%; *P* < 0.05). The highest lactose concentration was found in BW cows (4.45%; *P* < 0.05). Milk yield increased with lactation number, peaking in cows during their fourth or later lactations, where the average yield reached 9675.5 kg (*P* < 0.05). Lactose levels decreased with successive lactations, showing the lowest concentration (4.35%) in the oldest group (*P* < 0.05). Peak daily yields were highest during the 3rd lactation at 47.9 ± 0.55 kg, compared to the lowest yield of 35.8 kg in the 1st lactation (*P* < 0.05). The highest total milk yield was recorded in BW cows during their 4th lactation, totalling 10 008.5 kg (*P* < 0.05). In the 1st lactation, Holstein cows achieved the highest single-day yield of 80.0 kg (*P* < 0.05). Lithuanian Red (LR) cows in their 3rd lactation produced the richest milk in terms of fat content (4.87%), while HL in the 4th lactation had the highest protein content (3.57%). Lithuanian Black and White cows in the 1st lactation had the highest lactose concentration (4.58%, *P* < 0.05). This breed also exhibited the lowest average somatic cell count (SCC) across all lactations, averaging 351.7 × 10³/mL, with the lowest value observed in 1st lactation cows (171.7 × 10³/mL). The overall lowest SCC (221.8 × 10³/mL) was recorded in 1st lactation cows (*P* < 0.05). In conclusion, cows tend to reach their highest milk productivity during the 1st and 2nd lactations. However, as cows age, the somatic cell count (SCC) generally increases, while the concentrations of protein and fat in milk typically decline. Observations also indicate that Holstein cows produce the highest milk yield but tend to have a higher SCC.

Keywords: breed, lactation, production, somatic cell number.

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EVALUATION OF THE INFLUENCE OF CALF SEX ON THE QUANTITY AND COMPOSITION OF COWS' MILK

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Scientists are paying increasing attention to factors that may influence milk yield and composition. One such factor is the sex of the calf and its relationship with cow productivity during lactation [1].

Djedović et al. found that cows which gave birth to calves of different sexes or two heifers in their first and second lactations had significantly higher milk yield compared with those that gave birth to two bull calves or a bull calf first. Moreover, the milk fat content in the first and second lactations was observed when Holstein or Holstein crossbreed cows gave birth to a heifer during their first calving [2]. The aim of this work was to evaluate the influence of calf sex on cow milk indicators. The study was carried out on 151 Holstein, Lithuanian Red and Lithuanian Black and White breed cows that had given birth to calves of different sexes. The cows were grouped into 3 groups according to lactation. Group 1 included cows in their second lactation ($n = 51$), group 2 included cows in their third lactation ($n = 50$), and group 3 included cows in their fourth to sixth lactations ($n = 50$). The average milk composition indicators (fat, protein, lactose), somatic cell count, and milk yield of cows were calculated. Differences between the results are considered reliable when $P < 0.05$. The study demonstrated that cows in groups 1 and 2 produced more milk when they gave birth to bull calves (1.52% and 2.40%, respectively), while in group 3, more milk was produced when cows gave birth to heifers (2.67%). Higher protein and fat content in milk was observed in groups 1 and 3 when cows gave birth to heifers, whereas in group 2, milk with higher fat and protein content came from cows that gave birth to bull calves (0.04%). Lactose content was higher in groups 1 and 3 when cows gave birth to heifers (0.1% and 0.02%), while in group 2, lactose levels were similar regardless of calf sex (4.55%). The highest somatic cell count (SCC) in milk was found in groups 1 and 2 when cows gave birth to bull calves (11.55% and 10.48%, respectively), and in group 3 when cows gave birth to heifers (25.31%) ($P > 0.05$). In conclusion, our study shows that calf sex influences milk yield and composition, but the effect varies by lactation group and is not statistically significant.

Keywords: cow, calf, sex, milk yield, composition, lactation

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EVALUATION OF THE QUALITY OF COOLED WARMBLOOD STALLION SEMEN IMPORTED TO LITHUANIA WITHIN 24 HOURS OF COLLECTION

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In Lithuania, the use of artificial insemination in equine reproduction has been steadily increasing its popularity due to the rising levels of equestrian sports. Unlike frozen semen, cooled semen is distributed without an accompanying quality certificate. This lack of documentation limits the capabilities of veterinarians and breeders to assess semen quality. Given the high commercial value of stallion semen, each unsuccessful insemination results in considerable financial loss [1]. This study aimed to evaluate the quality of cooled semen imported into Lithuania within 24 hours of dispatch. Twenty cooled semen samples from stallions of various breeds (aged 4–22 years, representing dressage and show jumping disciplines) were obtained from breeding stations in Belgium (n = 10), the Netherlands (n = 3), and Germany (n = 7). Just after receipt, the semen samples were used in the laboratory to test sperm motility, concentration, viability, and morphology, as well as for mare insemination. Stallion age significantly negatively correlated with motility ($r = -0.56$, $P = 0.031$), while associations with viability ($r = -0.48$, $P = 0.072$) and concentration ($r = -0.50$, $P = 0.057$) showed non-significant negative trends. Breed significantly affected viability ($P = 0.045$) but not motility or concentration. Discipline had no significant effect on any parameter [2]. Morphological assessment showed various abnormalities, but all remained within normal limits. The month of semen collection did not significantly influence motility, viability, or concentration, despite a slightly higher motility detected in April (82.5%) [3]. Similarly, the country of semen origin showed no significant effect. Pregnancy outcomes at 14 days were not associated with semen quality parameters: mares that conceived received semen with marginally higher motility (76% vs. 66%), but viability and concentration did not differ ($P > 0.05$). Quality analysis of cooled stallion semen imported to Lithuania showed that motility, viability and morphological indicators meet international requirements. Nevertheless, conception results varied, so the causes of infertility should be sought in other factors related to the mare or insemination management. As this is the first study addressing cooled semen quality transported to Lithuania, further investigations are needed.

Keywords: stallion, cooled semen, quality, pregnancy rate.

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THE EARLY WARNING SYSTEM AS AN INSTRUMENT SUPPORTING THE IMPLEMENTATION OF THE NATIONAL STRATEGY

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Infectious diseases pose a serious threat to the genetic resources of farm animals. This can lead to a decline in population size, a reduction in breeding value and the loss of valuable traits. Local and endangered breeds are particularly vulnerable due to their small population sizes and insufficient preservation in gene banks, which making recovery after outbreaks more challenging. The FAO has developed the Global Early Warning System for Animal Diseases (GLEWS+) [1], which enables rapid threat detection and supports countries in implementing preventive measures. In Poland, recent threats to animal genetic resources have included African swine fever and highly pathogenic avian influenza, highlighting the need for effective biosecurity, monitoring, and early detection systems. The National Strategy for the Sustainable Use and Conservation of Farm Animal Genetic Resources [2] (Priority 9) emphasises the importance of developing an early warning system for such threats in order to protect farm animals. According to the Polish Act on Breeding [3], the National Research Institute of Animal Production is responsible for informing the District Veterinary Officers about animals under genetic resource conservation programmes. However, there is a lack of mechanisms for rapid feedback and clearly defined procedures in case of threats. Additionally, not all populations are adequately secured in the National Bank of Biological Materials, increasing the risk of irreversible loss. Therefore, improving information exchange, developing response procedures and investing in *ex situ* conservation are essential to safeguarding endangered breeds and ensuring their future restoration.

Keywords: farm animals, endangered breeds, National Strategy, early warning system.

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OESTRUS EXPRESSION AND DETECTION IN ANGUS AND LIMOUSIN CATTLE TO IMPROVE REPRODUCTIVE PERFORMANCE

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In beef cattle herds, maintaining a 12-month calving interval is crucial, necessitating a shortened service period for cows. The window for pregnancy after calving is 65 to 85 days, with approximately 35–40% of cows exhibiting “silent” oestrus, making visual observation challenging [1]. Despite advancements in reproductive physiology knowledge and oestrus detection aids, detecting oestrus remains a major challenge, especially in beef cows with longer non-oestrus periods.

This study investigated differences in the first post-calving oestrus between beef cattle breeds. A total of 50 multiparous Angus and 50 multiparous Limousin cows were monitored using two methods: direct visual observation three times daily for 20 minutes, and digital boluses recording physiological parameters at 10-minute intervals. Reproductive parameters such as oestrus duration, timing of the first oestrus, activity level, body temperature, and oestrus index were evaluated. All cows received SmaXtec boluses at the start of the study, with data continuously collected via SmaXtec messenger[®] software.

Results showed that 76% of cows were identified as in oestrus within 85 days post-calving, aligning with the goal of achieving a 365-day calving interval through insemination within this timeframe. The digital bolus system identified oestrus in 30% of cows between days 22 and 33, 30% between days 34 and 57, and 16% between days 58 and 85. Visual observation, however, missed 70% of the first oestrus events due to low cow activity.

During the research, it was observed that Angus cattle had an average activity index of 19, compared to 17 for Limousin ($P < 0.05$). The system calculated a Heat Index (HI) based on activity and temperature data, reflecting oestrous intensity. The initial post-calving HI was higher for Angus (HI = 84) than for Limousin (HI = 74). Furthermore, Angus cattle had a lower average body temperature over 7 days (39.08°C), which increased by 0.47°C during oestrus, compared with Limousin cattle, which had a higher average (39.33°C) and a 0.41°C increase during oestrus ($P < 0.05$). The average oestrus duration was established at 15 hours, with Angus re-entering oestrus post-calving after 54 days and Limousin after 58 days.

The study revealed more pronounced oestrus signs in Angus cattle and confirmed the applicability of activity and temperature monitoring systems for optimising breeding timing across breeds. These technologies can improve insemination efficiency, reduce labour, and lower costs compared with visual observation. Moreover, earlier detection of initial oestrus signs may shorten the service period and support more efficient reproductive management.

Keywords: beef cattle, estrus, visual observation, bolus.

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ANALYSIS OF FACTORS AFFECTING CALVING DIFFICULTY IN BEEF COWS

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On a selected farm, we analysed 162 calvings of Aubrac, Charolais, and their crossbred cows to identify factors influencing calving difficulty. We evaluated the effects of calf birth weight, month of birth, parity, breed, sex, and bulls on calving difficulty. The average birth weight of calves was 43.7 kg; and they were divided into two groups: up to 44 kg and over 45 kg. The months of birth were categorised as November, December, January, and February, since the farm practices seasonal calving. Parity ranged from heifers up to the 8th lactation. In the analysis, we included 6 Charolais bulls and 8 Aubrac bulls. Calving difficulty was classified into three categories: 1 – unassisted calving, 2 – assistance by one or two people, 3 – use of a calving jack or veterinary intervention. For the analysis, we applied a mixed model (Mixed Model) implemented in the SAS system using the MIXED procedure, with the Restricted Maximum Likelihood (REML) method for parameter estimation. Bulls were included in the model as a random effect. The results showed that parity had a statistically significant effect on calving difficulty ($P = 0.0012$), and the month of calving also proved to be a significant factor ($P = 0.0121$). Other variables studied – breed, sex, birth weight, and bulls – did not show a statistically significant influence. Twin births were excluded from the statistical analysis. These findings suggest that calving management should consider the parity of cows and seasonal calving patterns. The results can contribute to the optimisation of reproductive strategies and reduce the risk of complicated calvings.

Keywords: calving difficulty, beef breeds, parity, seasonal calving, birth weight.

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PHYSICO-CHEMICAL PROPERTIES OF MEAT FROM THE FATTENERS FED MIXTURES CONTAINING PROBIOTIC OR/AND PREBIOTIC

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The withdrawal of antibiotic growth promoters from animal feeding schemes has led to the development of a market for alternative feed additives, including probiotics and prebiotics. Studies [1, 2] have shown that when used together as synbiotics, they have a beneficial effect on biological systems, resulting in improved growth performance; moreover, they affect carcass composition and meat quality. Owing to their inconclusive impact on carcass composition, this study assessed the physico-chemical properties of the *longissimus lumborum* muscle in pigs fed diets supplemented with probiotic, prebiotic, and synbiotic. The study material comprised the *longissimus lumborum* (LL) muscle sampled from PLW×PL fattening pigs assigned to four feeding groups (1, 2, 3, and 4). The fatteners were fed rations based on barley, triticale, non-GMO soybean meal, and mineral-vitamin additives, according to the following scheme: control group (1) – no feed additives; group 2 – 0.3% EM Bokashi; group 3 – 3% inulin; and group 4 – 0.3% EM Bokashi + 3% inulin. The animals were fattened until they reached a body weight of approx. 112kg. The physico-chemical properties of LL muscle as pH, electrical conductivity (EC), natural drip loss, colour (L*a*b* system), water holding capacity (WHC), cooking loss, and shear force were assessed. The results were statistically analysed. The inclusion of evaluated feed additives in the diet of fattening pigs had no significant effect on carcass muscularity or fatness. Supplementation with inulin and EM Bokashi significantly reduced muscle pH at 45 min and 2 h post mortem compared with group 2, and at 144 h post mortem compared with group 1. A significant ($P \leq 0.05$) increase in the EC of muscles was observed in pigs from groups 3 and 4 at 96 h post mortem, and in group 4 at 144 h post mortem, compared with pigs fed rations without additives. No statistically significant effect ($P > 0.05$) of the feeding strategy was observed on the WHC or the LL muscle tenderness. However, the highest drip loss and muscle hardness were recorded in pigs fed diets supplemented with inulin. Regardless of the measurement time, the muscles from pigs in groups 1 and 2 were darker in colour (L*) but less saturated in a* and b* parameters than those from groups 3 and 4 ($P \leq 0.05$). In conclusion, it should be stated that the evaluated feed additives, EM Bokashi and inulin (used separately or in combination), in diets for PLW×PL fattening pigs did not reduce the physico-chemical properties of the LL muscle.

Keywords: pigs, fed additives, nutrition, meat quality.

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PROBIOTIC YEAST IN ANIMAL NUTRITION – FACTS AND PERSPECTIVES

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Intensive animal production requires optimising breed selection, housing, and feeding to ensure welfare and performance. Animal feed must provide not only essential nutrients but also vitamins and trace elements necessary for proper metabolism. The gastrointestinal microflora, whether permanent or transient, has a strong influence on host health, growth, stress tolerance, and disease resistance.

For much of the 20th century, research emphasised the negative impact of pathogenic microflora, which impair health, slow the growth, or cause mortality. The widespread response was routine antibiotic use, often preventive and unjustified. This led to bacterial resistance, environmental contamination, and antibiotic residues in meat and milk, posing risks to both animals and humans. Moreover, antibiotics often devastate gut microbiota, impairing host homeostasis.

Over the past 50 years, research has consistently emphasised the health-promoting properties of beneficial microorganisms. Stable gut microflora supports host well-being, with lactic acid bacteria recognised for their positive homeostatic functions. These bacteria have been widely applied in biopreparations to support livestock production, including cattle, pigs, and smaller animals such as bees.

In the last decade, attention has expanded to probiotic yeasts. Although their probiotic mechanisms resemble those of bacteria, key differences, such as natural antibiotic resistance, enable yeasts to be used alone or in combination with bacterial probiotics. Their application improves gut balance, reduces antibiotic dependence, and enhances production outcomes, including greater weight gain, higher milk yield, and improved disease resistance, particularly in young animals.

Current findings on probiotic yeasts are highly promising, suggesting continued growth in their use within animal husbandry. Ongoing research aims to identify new strains and explore practical applications, reinforcing their potential as sustainable tools for promoting animal health, productivity, and welfare.

Keywords: probiotics, boudardii, lactic acid bacteria.

EFFECT OF MILKING DURATION ON MILK COMPOSITION AND SOMATIC CELL COUNT

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Milk and its composition are key factors influencing profitability of dairy farms [1]. Somatic cell count (SCC) in milk, in addition to other milk components, is crucial for monitoring milk quality and health conditions [2]. Mastitis in dairy cows is a concern worldwide. It is a multifactorial disease influenced by factors at both cow and herd levels, leading to substantial losses throughout the dairy chain [3]. This study aimed to evaluate the relationship between milking duration and key milk quality parameters, including fat and protein content, as well as SCC. The analysis utilised data from 692 cows varying in lactation stage and number, collected during routine test-day milking. Cows were categorised based on milking duration and milk yield per session. Results revealed a negative association between high milk yield and milk component concentrations. Cows yielding ≤ 13.00 kg of milk produced significantly higher fat and protein levels compared with those yielding > 22.01 kg. Second-lactation cows producing 17.01–19.00 kg of milk exhibited higher fat and protein levels than their first-lactation counterparts. Shorter milking durations were generally associated with lower milk yield but higher fat and protein content. Specifically, cows milked for 5 min 31 s – 6 min 30 s had modestly higher fat and protein concentrations than those milked for ≤ 4 min 30 s. However, extended milking durations (6 min 31 s – 7 min 30 s) during early lactation were linked to reduced fat and protein levels. Interestingly, longer milking durations (≥ 6 min 31 s) correlated with increased milk yield but also elevated SCC, indicating a potential compromise between production efficiency and udder health. These findings underscore the importance of optimising milking duration to balance yield, milk quality, and animal health. Further research may help refine milking strategies to enhance both productivity and welfare in dairy herds.

Keywords: milking duration, milk composition, somatic cells.

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APPLICATION OF THE AUBRAC BREED IN SUSTAINABLE CATTLE FARMING SYSTEM IN SLOVAKIA

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Climate change is increasingly affecting agricultural production in Slovakia, including permanent grassland areas. Grazing seasons are marked by more frequent periods of heat and drought, limiting the efficiency of pasture usage emphasising the importance of resilient grazing animals. To adapt livestock production to these challenges and ensure sustainable pasture management, Slovak cattle breeders have turned to diversification through the introduction of robust breeds. The rustic Aubrac breed, known for its resilience to harsh climatic conditions, was imported to Slovakia in 2016 for usage in less intensively cultivable areas. The objective of this study was to evaluate selected performance traits of the Aubrac breed under Slovak conditions (80 heads). Calving performance showed 94.54% unassisted births, while assisted calving and dystocia accounted for 4.36% and 1.09%, respectively. These were significantly associated with high calf birth weights. Similar results have been reported by several other studies [1, 2]. The average birth weight was 41.64 kg, markedly higher than values commonly reported for the breed [3, 4]. The highest average score for calving (1.17 on a four-point scale) was recorded for first-calf heifers. The incidence of assisted calving decreased within increasing parity. The highest culling rate was observed between the second and third calving; however, 11% of cows remained in the herd after the sixth calving, supporting previously published longevity data [3, 4]. ANOVA analysis confirmed a significant effect of age at first calving on total calvings and number of born calves. The optimal age for first calving was found to be between 3 and 3.5 years. The offspring achieved average daily gains of 1.04 kg until weaning, with low variability. The partial results suggest that the Aubrac breed demonstrates promising traits for sustainable beef production under the conditions of Slovakia.

Keywords: Aubrac breed, Slovak conditions, performance traits, sustainable beef production.

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EFFECT OF AGE AT FIRST CALVING AND CALVING INTERVAL ON MILK PRODUCTION IN POPULATION OF THE SLOVAK SPOTTED DAIRY COWS

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Slovak Spotted breed is an important dual-purpose breed with a long farming tradition in Slovak Republic. It is mainly due to its excellent dairy as well as beef production and belongs to the Simmental type of cattle [1, 2]. A moderate age for Simmental dairy cows at first calving (AFC), typically around 24–28 months, is optimal for maximising both first lactation and lifetime milk production [3, 4]. Very young AFC (< 24 months) results in lower peak and total production in the first lactation, while very old AFC (> 28 months) is associated with a longer calving interval (CI) and potentially reducing lifetime productivity due to fewer lactations [5, 6]. The aim of this study was to evaluate the effect of age at first calving and calving interval on milk production in selected population of Slovak Spotted dairy cows during the years 2020–2024. Research database, which contained data collected from 12 298 Slovak Spotted dairy cows (31 925 test-day records), was obtained from the Slovak Animal Breeding Services. Relations among AFC, CI and MY in kg were analysed by Statistical Analysis System (SAS). The results of the basic statistic for AFC are 837.7 days, which represents 27.5 months, CI with 392.1 days and MY with 8108 kg of milk, 3.9% of fat, 3.6% of proteins and 4.9% of lactose. Correlations between AFC and CI with traits of MY were low positive and negative too, mainly with milk content components. In our results, the highest AFC percentage was namely 68.8% in the 24-to-28-month age group. This range typically yields the highest lifetime milk production and productive life, balancing growth with the ability to start milking, with a shorter CI between that better indicates reproductive calving performance and higher lifetime MY, ultimately increasing profitability for dairy farmers.

Keywords: dairy cows, age at first calving, calving interval, milk production, correlation.

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IMPACT OF DIETARY SODIUM BUTYRATE ON MANURE PH IN FATTENING PIGS

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One of the major challenges in pig farming is the emission of ammonia and nitrogen pollution, which negatively affect both animal health within pig production systems and the environment (including water, soil, air and biodiversity). High nitrogen levels in pig diets are a key factor contributing to increased ammonia (NH₃) emissions [1]. The inclusion of dietary additives such as sodium butyrate (SB) aims to reduce protein fermentation in the intestine by acidifying the intestinal chyme and modulating the gut microbiota. The aim of the present study was to evaluate the effect of dietary supplementation with SB on the pH of manure produced by fattening pigs. For this purpose, two groups were established: a control group (fed a basal diet) and an experimental group (fed the basal diet supplemented with 0.1% SB). The trial lasted for the entire fattening period of 70 days, from 90 to 160 days of age. To determine the acidifying effect of SB, pH values of manure were measured from the manure channels. The results indicate that the inclusion of 0.1% sodium butyrate in the compound feed of fattening pigs leads to a significant reduction in manure pH, particularly after the third week of administration ($P < 0.05$). This acidification may be interpreted as an indirect indicator of reduced intestinal pH, which creates more favourable conditions for beneficial microbiota development and limits protein fermentation in the intestinal tract. As a result, a better nitrogen balance and more efficient utilisation of dietary protein by fattening pigs can be assumed.

Keywords: sodium butyrate, pigs, manure pH.

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ROLE OF ESSENTIAL OIL SUPPLEMENTATION DURING THE TRANSITION PERIOD IN MODULATING DAIRY COW HEALTH AND METABOLIC PARAMETERS

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This research assessed the impact of supplementing dairy cows with essential oils (Agolin Ruminant[®]; cinnamaldehyde 60%, eugenol 40%) on productivity, metabolic indicators, and overall health during the transition period. This phase is considered one of the most critical in the production cycle, as it involves calving, uterine recovery, and the initiation of lactation, when the majority of metabolic and infectious diseases typically arise [1]. The trial included 140 multiparous cows, divided into a supplemented group receiving 1 g of Agolin Ruminant[®] daily mixed into the mineral blend, and a control group without additives. Animals were milked with a Lely Astronaut[®] A4 robotic system and fed a total mixed ration twice daily at 07:00 and 19:00. The study period covered 30 days before calving until 90 days postpartum. Cows receiving essential oils produced 4.5–7% more milk between 5 and 90 days in milk compared with controls, together with slight improvements in milk composition, particularly higher fat levels. Supplementation also influenced rumen fermentation by adjusting acetate-to-propionate ratios, which supported better nutrient absorption and improved metabolic efficiency [2]. Blood analyses revealed higher albumin concentrations and a trend toward more consistent regulation of glucose and urea, whereas other parameters, such as AST and minerals, showed little variation between groups, suggesting minimal systemic effects. In terms of milk characteristics, supplemented cows exhibited a modest increase in lactose content and a slight decrease in protein percentage. Somatic cell counts were somewhat elevated in this group, which may signal challenges for udder health. Overall, the findings indicate that essential oils can contribute to improved productivity and feed efficiency during the transition period, with shifts in rumen fermentation likely providing the underlying mechanism.

Keywords: transition period, essential oils, dairy cattle, milk yield.

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DETERMINATION OF THE DOPPLER INDICES IN ULTRASOUND EXAMINATION OF THE COMMON CAROTID, TEMPORAL AND VERTEBRAL ARTERIES IN LAMBS IN THE POSTNATAL PERIOD

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The aim of this study was to determine Doppler indices in the common carotid arteries, temporal arteries, and vertebral arteries in lambs in the first four weeks of life. Peak systolic velocity (PSV), end-diastolic velocity (EDV), PSV/EDV ratio, resistance index (RI), and heart rate index (PI) were determined in the examined arteries. Statistical methods were used to determine the relationships between the studied parameters and the age and sex of the lambs, as well as the location of the arterial vessel. The multivariate analysis of variance showed that the values of Doppler parameters depended on both the age of lambs and the location of the artery ($P < 0.01$). Doppler indices were also shown to be dependent on both the sex of the lambs and the location of the artery ($P < 0.05$). Most Doppler indices in the carotid arteries differed significantly from those in the temporal arteries ($P < 0.01$) and vertebral arteries ($P < 0.01$). In the vertebral arteries, PSV and EDV were significantly lower, and RI and PI were significantly higher, than in the common carotid arteries and temporal arteries ($P < 0.01$). All parameters were positively correlated between those determined in the common carotid arteries and those determined in the temporal arteries. A positive correlation was also found between RI and PI determined in the vertebral arteries and those determined in the common carotid arteries ($P < 0.05$). A positive correlation was also found between PSV and RI in the vertebral arteries and temporal arteries ($P < 0.05$). PSV and RI in the common carotid arteries were higher in ram lambs than in ewe lambs ($P < 0.05$). PSV/EDV in these arteries was also higher in ram lambs than in ewe lambs ($P < 0.01$). Similar differences were observed between PSV, PSV/EDV, and RI in the temporal arteries and vertebral arteries.

This study demonstrated the feasibility of performing Doppler ultrasound and analysing Doppler ultrasound indices in the common carotid arteries, temporal arteries, and vertebral arteries in the early postnatal period.

Keywords: common carotid artery, temporal artery, vertebral artery, Doppler ultrasonography, lamb.

DIFFERENCES BETWEEN INFLUENCE OF SPECIFIC FACTORS IN ACCORDANCE WITH SCALE USE – ORIGINAL OR CONVERTED SPORT HORSE DATA

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Linear profiling offers selection [1] with different traits and scaling [2]. The Polish system consists of 21 conformation and 19 movement traits. The traits are restricted in the range of nine letters from “a” to “i” providing information on whether the trait is at an average level or deviates in one direction. Such scores require transformation into quantitative data or finding appropriate methods for nominal data. The study aimed to compare the impacts of the factors on linear profiling using different scaling. Data from 3854 Warmbloods (3739 mares, aged 3–7) from 17 regions between 2014 and 2025 were analysed. Horses (jumping 19%, dressage 28%, all-around 53%) were evaluated in free movements and/or jumping, depending on the sport type. Variance analyses were conducted with original and converted data. The following transformations were carried out in accordance with scales used in different countries: 1–40 (the Netherlands), –20 – +20 (Belgium), and 1–9 (Sweden). The linear model for the converted scale included fixed effects: sex, age, sport type, region, and year as linear regression (GLM, SAS.) The generalised linear model for original, nominal data included already mentioned fixed effects and year as covariates (GLIMMIX, SAS). Our research concluded that the choice of conversion scale does not affect results substantially for most analysed traits. However, some differences were observed. Region influenced all conformation and gait traits regardless of original or converted character (P value < 0.0001). Year affected almost all traits (36 traits with P value: < 0.0001–0.05) and results were comparable for both approaches. Similar results were obtained for the sport type effect – significant for 19 traits in GLM and for 13 traits in GLIMMIX analyses. Greater differences were observed for sex and age effects. Analysis using original data showed a significant age effect on two more traits, in addition to the other five for both approaches (P value: < 0.0001–0.04); converted data analysis indicated another eleven traits (P value < 0.0001–0.05). Sex significant effect was found for more transformed than original traits: 21 vs 10 variables (P value: < 0.0001–0.05).

Keywords: horses, linear scoring, performance value.

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ASSOCIATIONS BETWEEN CONFORMATION, GAIT AND JUMPING TRAITS IN THE LINEAR SCORING EVALUATION OF WARMBLOOD HORSES

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Linear profiling enables assessment of conformation and primary performance traits. Sport horses' specialisation process results in negative correlations between jumping and dressage traits [1]. Therefore, the study aimed to analyse the preliminary, phenotypical associations between traits of linear profiling. Data included evaluation of 3854 Polish Warmblood horses assessed 2014–2025. The description consisted of 40 traits: 21 conformations, 10 gait characteristics, 9 jumping traits. The traits are restricted in the range of nine letters from “a” to “i” providing information on whether the trait is at an average level or deviates in one direction. The data for analysis was converted from nominal scale “a” to “i” to a quantitative scale 1–9. Associations were analysed using partial correlation from multivariate analysis of variance and Spearman correlations. The linear model included fixed effects: sex (female and male), age (3–7 years), sport type (jumping, dressage, all-round), region (17 levels), and linear regression on assessment year. Both kinds of correlations between analysed traits had similar values, and were mostly significant, positive and low (–0.3 to 0.3). There were no medium or high correlations between conformation and movement traits. Negative significant correlations were found between leg substance and gait, as well as jumping characteristics (the highest with canter [–0.14] and jump elasticity [–0.18]), but also for body shape with back technique in jump [–0.14], body direction with canter impulsion [–0.07] and croup shape with walk correctness [–0.04]. Associations between movement traits were significant, mainly positive, and reached an average level, except for a low level of walk characteristics (< 0.36). Correlation parameters close to high strength of dependence were noted between characteristics of trot and canter, as well as between jumping traits. Within jump traits, correlations were significant and at least a medium level, and a high level was found between back technique and jump elasticity [0.71]. The associations found between conformation and movement traits provide guidance in selecting horses for jumping or dressage. Higher correlations between jumping traits make the horse evaluation and selection easier than for the movement. The results should be confirmed on the genetic level for further discussion on the selection process.

Keywords: Warmblood horses, linear scoring, correlation coefficients.

Acknowledgements: This research was funded by the National Science Centre, Poland, no DEC-2024/08/X/NZ9/01675.

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ASSESSMENT OF GENOME STABILITY IN POLISH LANDRACE BOARS USING THE COMET ASSAY

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Genomic integrity and stability constitute important indicators for the assessment of reproductive performance in breeding males. To identify genomic instability, cytogenetic tests such as the comet assay are used. This method enables the identification of chemical and enzymatic DNA modifications that manifest as DNA strand breaks in somatic cells and mature spermatozoa.

The aim of the study was to evaluate genomic stability in Polish Landrace boars, based on spermatozoa and lymphocyte cells, using the comet assay.

The study used peripheral blood and semen samples collected from 30 boars (two age groups: 12–24 months and older than 24 months, $n = 15$ each). The modified SCGE technique according to [1] was used to identify DNA damage. An Olympus BX53 microscope was used for microscopic analysis. CASP 1.2.2 software was used to analyse degraded DNA. Fifty cells were analysed for each animal. DNA integrity was determined on the basis of the percentage content of DNA in the tail of the comet (%Tail DNA).

The average level of DNA integrity in spermatozoa was %T DNA 17.6 ± 16.2 . In Group 1, the %T DNA value was 5.6 ± 4.3 , whereas in Group 2 it reached 29.5 ± 14.6 . The average level of DNA integrity in lymphocytes was %T DNA 12.2 ± 11.2 . In Group 1, the %T DNA value was 4.9 ± 3.5 , while in Group 2 it amounted to 19.4 ± 11.5 .

A higher degree of genetic material damage was observed in older boars. Moreover, a greater extent of DNA damage was detected in spermatozoa than in lymphocytes in both age groups.

Keywords: genome, stability, boar, comet assay

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MEAT QUALITY OF IMMUNOCASTRATED BOARS AND ENTIRE MALE PIGS SLAUGHTERED AT DIFFERENT BODY WEIGHTS

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Fattening of entire male pigs is associated with the occurrence of boar taint in meat and fat. The conventional method to prevent this is surgical castration; however, this practice has raised ethical concerns among consumers, as in some countries it is performed without anaesthesia up to day 7 of a piglet's life, causing pain and discomfort [1–3]. One alternative is immunocastration [4]. The aim of this study was to compare the quality of meat obtained from immunocastrated boars and entire male pigs slaughtered at different body weights.

In the experiment, male pigs (DanBred × Duroc) were divided into 3 groups of 10 animals each. Control group (C) included immunocastrated boars slaughtered at 120 kg body weight; experimental groups were as follows: E1 – entire male pigs slaughtered at 120 kg, and E2 – entire male pigs slaughtered at 105 kg. Pigs in all groups were fed a complete diet in liquid form three times a day. After slaughter, physicochemical parameters of the meat were analysed, and the levels of indole, skatole, androstenol, and androsterone were measured.

No statistically significant differences were found in the physical parameters of meat between the groups. The chemical composition analysis showed no differences in dry matter, fat, and collagen content in meat obtained from boars in all groups. A higher protein content was observed in the meat of boars from group E1 (23.48%) compared with group C (22.87%) and E2 (22.99%) ($P \leq 0.01$). It was observed that meat from group C boars had the highest mean concentration of indole (43.7 ng/g vs 32.7 ng/g in E1 and 33.0 ng/g in E2), skatole (19.1 ng/g vs 9.9 ng/g in E1 and 11.5 ng/g in E2), and the lowest mean concentration of androstenol (59.6 ng/g vs 70.9 ng/g in E1 and 67.0 ng/g in E2) and androsterone (120.7 ng/g vs 141.3 ng/g in E1 and 147.6 ng/g in E2).

In conclusion, meat obtained from immunocastrated boars and entire male pigs slaughtered at different body weights did not differ in physical parameters. However, meat from group E1 had higher protein content. The reduced levels of androstenol and androsterone in meat from the control group confirm the correct application of immunocastration. The considerable variability in the levels of all analyzed compounds may suggest high individual variation.

Keywords: immunocastration, fattening of entire male pigs, meat quality.

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EFFECT OF LOW-FREQUENCY OSCILLATIONS ON BLOOD CORTISOL CONCENTRATION AND MILK QUALITY IN DAIRY COWS

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Previous investigations have evaluated the impact of low-frequency oscillations on udder function, milk composition, and welfare of dairy cows during milking [1]. We hypothesised that the organism of the cow may respond to a low-frequency oscillation device as an external stressor. In cattle, cortisol produced under stress conditions has been associated with reduced reproductive performance, lowered milk production, and suppression of the immune system [2, 3]. Stress also influences milk composition, decreasing fat and protein content while increasing somatic cell count, which reflects poorer milk quality [4, 5]. The objective of this study was to investigate the impact of low-frequency oscillations on cows' stress level and milk composition. In the first trial, healthy cows were assigned into two groups of 20 cows using the principle of analogy. In the experimental group, low-frequency oscillations were applied to the udder during morning milking [1]. Blood samples were collected from the tail vein right after morning milking to determine cortisol concentration (ELISA). In the second trial, cows were divided into two groups of 10 cows and observed for two weeks. In the experimental group, conventional milking was carried out during the first week, while in the second week, udder stimulation with low-frequency oscillations was applied during each milking. The control group underwent conventional milking throughout both weeks. Daily milk yield was recorded, and at the end of each week, milk samples were collected to determine fat, protein, lactose, and urea content, as well as somatic cell count (SCC). For statistical evaluation, *t* test analysis was used to compare cortisol, milk yield, and milk quality parameters. In the first trial, the cortisol concentration in the blood of cows in the experimental group was 2.81 ng/mL lower ($P = 0.2102$) than in the control group, indicating that low-frequency oscillations did not induce stress during milking. In the second trial, application of low-frequency oscillations in the experimental group during the second week resulted in an average daily increase of 0.99 l of milk per cow ($P = 0.1475$) compared with the first week, when no oscillations were applied. In the control group, the increase between weeks was only 0.26 L of milk per cow ($P = 0.9245$). These differences were not statistically significant. Analysis of milk quality parameters showed that in the experimental group, the milk fat content was 0.58% higher in the second week compared to the first week ($P = 0.038$). Other milk quality indicators (protein, lactose, urea, SCC) did not differ significantly between weeks in either group. In conclusion, low-frequency oscillations applied to cows during milking did not induce stress. Moreover, they tended to increase milk yield and improve milk quality indicators.

Keywords: low-frequency oscillations, cortisol, milk quality, cows.

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ASSESSMENT OF CEREAL STALKS AS A POTENTIAL SOURCE OF MYCOTOXINS

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Cereal stalks are by-products commonly used as straw for animal bedding or as a dietary supplement. They can be a potential source of fungal and mycotoxin contamination, especially under humid climatic conditions [1]. The aim of this study was to screen cereal stalks for the presence of common mycotoxins produced by *Fusarium*, *Penicillium*, and *Aspergillus* species prior to harvesting and storage under field conditions.

Stalk samples were collected on the same day the grains were harvested. A total of 5 wheat stalk samples, 5 barley stalk samples, and 5 triticale stalk samples were collected from completely different fields. The primary moisture content was determined by weighing samples of stalks. The total number of yeasts and molds in the collected samples was determined according to the LST ISO 21527-2:2008 standard [2]. Mycotoxin concentrations (AFL B1, ZEN, and DON) were assessed using thin-layer chromatography, following methods provided by Romer Labs [3, 4].

All tested stalks samples of wheat, barley and triticale were found to be contaminated with fungi, *Fusarium* spp. being the predominant genus. A positive correlation was observed between fungal contamination and the primary moisture content of the stalks. The highest average total number of yeasts and molds was found in triticale stalks (6.08 ± 0.15 log CFU/g). The highest average concentration of AFB1 was detected in barley and triticale stalks (0.85 ± 0.85 µg/kg). The highest concentration of ZEN was found in wheat stalks (175 ± 175 µg/kg), while the highest average concentration of DON was detected in triticale stalks (325 ± 175 µg/kg).

It can be concluded that cereal stalks used for straw production can be a potential source of fungal and mycotoxin contamination. Exposure through the digestive or respiratory tract may lead to health disturbances and decreased productivity.

Keywords: cereal stalks, mycotoxins, fungi.

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2. ISO 21527-2:2008 specifies a horizontal method for the enumeration of viable osmophilic yeasts and xerophilic moulds in products intended for human consumption or feeding of animals, having a water activity less than or equal to 0,95 (dry fruits, cakes, jams, dried meat, salted fish, grains, cereals and cereals products, flours, nuts, spices and condiments, etc.), by means of the colony count technique at (25 ± 1) °C.
3. Aflatoxin B1 and Zearalenone concentrations were determined by the Aflatoxin B1 and Zearalenone Quantitative Thin Layer Chromatography (PLCh) method (ROMER™ Labs, Inc., method code: a/z-ti-01-00.2).
4. Deoxynivalenol concentrations were determined by the Trichothecenes A and B type Quantitative Thin Layer Chromatography (PLCh) method with double sample cleanup (ROMER™ Labs, Inc., method code: CAM-000031-1).

EPIDEMIOLOGY OF BOVINE CRYPTOSPORIDIOSIS IN LATVIA: A SEVEN-YEAR STUDY OF REGIONAL AND AGE-RELATED TRENDS

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This study presents a comprehensive investigation into the epidemiology of *Cryptosporidium* spp. infections in dairy cattle, addressing significant gaps in regional and age-related data in Latvia. *Cryptosporidium* spp. are protozoan parasites that pose a substantial threat to animal health and productivity, making epidemiological surveillance crucial for effective control. Over a seven-year period from 2013 to 2020, faecal samples from 2655 dairy cattle were systematically collected and analysed using flotation methods and the Ziehl-Neelsen staining technique to determine infection prevalence and intensity. The overall prevalence of *Cryptosporidium* spp. was 27%. Our findings revealed significant regional variations, with the highest prevalence observed in the Vidzeme region (31%) and the greatest infection intensity (median = 600 oocysts per gram) in the Kurzeme region. Age-related susceptibility was a critical factor; calves aged 0 to 3 months demonstrated the highest infection rates (39.4%) and oocyst counts (median = 800 OPG). Furthermore, diarrhoea was significantly more prevalent in infected calves within this age group (56.6%) compared with older cattle. These results underscore the critical vulnerability of young calves and highlight distinct geographical patterns of infection within Latvia. This comprehensive analysis provides crucial data for the Latvian dairy industry, emphasising the necessity of implementing targeted, age-specific interventions and region-focused control strategies to effectively manage cryptosporidiosis and mitigate its economic impact.

Keywords: *Cryptosporidium* spp., epidemiology, regional variation, age-related.

EFFECTS OF LITTER SIZE ON EARLY PIGLET PERFORMANCE

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Changes in the body weight of weaned piglets represent a key indicator determining both the productivity and the economic efficiency of pig farming. This parameter is closely linked to litter size, which directly affects the growth rate and health status of individual piglets. Research indicates that larger litters are often associated with a lower average birth weight and greater within-litter weight variation, factors that may increase pre-weaning mortality. Furthermore, birth weight variation within a litter has been shown to reduce piglet survival until weaning. Therefore, to improve farm productivity, it is essential to balance litter size and promote greater uniformity in piglet birth weight [1]. The aim of this study was to evaluate the effects of litter size on piglet birth weight, growth performance, litter uniformity, and survival during the pre-weaning period. The study was conducted with 21 sows, with 3 sows from each parity group (ranging from 1 to 7 parities), selected randomly piglets' parents: the sows (mothers) were Yorkshire × Landrace crossbreeds, and the boar (father) was a Duroc. The parameters investigated included the number and body weight of piglets at birth and at weaning, litter uniformity, sex ratio, average daily weight gain, and causes of mortality. The results revealed significant differences in litter size, body weight, and growth dynamics among the parity groups ($P < 0.05$). The highest number of piglets born, the greatest total litter weight on day 1, and the largest overall litter weight gain were recorded in the fifth-parity group, which also produced the highest number of male piglets ($P < 0.05$). In contrast, the largest number of female piglets was found in the second-parity group ($P < 0.05$). On the day of weaning, the largest litter size was observed in the third-parity group ($P < 0.05$). Analysis of growth indicators showed that the most rapid weight gain and the highest average daily gain over a 21-day period occurred in litters from third-parity sows ($P < 0.05$). The study further demonstrated that one of the most frequent causes of piglet mortality was crushing, particularly common during the first week of life; the highest number of such cases was recorded in the seventh-parity group ($P > 0.05$). In conclusion, litter size and sow parity exert a clear influence on piglet performance and survival. Mid-parity sows (third to fifth) were associated with the most favourable growth and productivity indicators, while larger litters showed greater within-litter variation and increased mortality risks. These findings highlight the importance of considering both litter size and sow parity in herd management strategies to enhance piglet survival and farm efficiency.

Keywords: weaned piglets, sex, weight, litter.

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THE EFFECTS OF MICROALGAE SUPPLEMENTATION ON GROWTH, BLOOD BIOCHEMISTRY AND ORGAN DEVELOPMENT IN BROILER CHICKENS

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Microalgae have gained increasing attention as functional feed additives in poultry nutrition. Spirulina and Chlorella are the most extensively studied, primarily for their nutritional value and health promoting properties [1]. The present study aimed to evaluate whether dietary supplementation with relatively low levels (0.5%) of *Spirulina platensis*, *Chlorella vulgaris*, and a novel species of microalgae in poultry production *Tetrademus obliquus* influences body weight gain, certain biochemical parameters, and internal organ weight in broiler chickens.

Materials and methods. A total of 140 one-day-old Ross 308 broiler chickens were randomly allocated into 4 groups (n = 35): control (BK), *Spirulina* (BS), *Chlorella* (BC), and *Tetrademus* (BT). Birds were reared for 43 days following Ross 308 management guidelines. All groups received the same basal diet, with the respective microalgal supplement incorporated at 0.5% in BS, BC, and BT group diets. Blood and organ samples were collected at a certain period and analysed for TBili, ASAT, TG, cholesterol, urea, ALP, amylase, creat, total protein and albumin.

Results. Significant group effects were detected only for bilirubin, with BC and BT increasing concentrations compared with BK ($P < 0.001$). After adjusting for age, no significant differences in weight were observed between the treatment groups of chickens ($P = 0.777$). Similarly, there were no significant differences in most organ weights ($P > 0.05$). However, there were significant differences in heart weight ($P = 0.029$) and width ($P = 0.001$). Specifically, the BC and BS groups had significantly lower heart weights than the control group, with average reductions of 1.4 g (95% CI: 0.3–2.5) and 1.3 g (95% CI: 0.3–2.2), respectively. Dietary supplementation of broilers with 0.5% microalgae did not significantly affect growth but induced selective changes in bilirubin metabolism and heart development. Further research is needed to clarify potential effects on metabolism and the cardiovascular system.

Keywords: broiler chickens, microalgae, blood biochemistry, organ development.

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RELATIONSHIPS BETWEEN MILK LACTOSE CONCENTRATION AND METABOLIC BIOMARKERS IN DAIRY COWS DURING EARLY LACTATION

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The concentration of milk lactose is increasingly recognised as a non-invasive biomarker that reflects the physiological and metabolic state of dairy cows during the early stages of lactation, characterised by heightened energy demands and metabolic challenges [1]. The purpose of this study was to assess the relationships between various milk composition characteristics, blood biochemical parameters, behavioural indicators and milk lactose levels in Holstein cows that were in clinically good health. Using intraruminal boluses and an in-line milk analyser, 28 cows (9–59 days in milk) were continuously observed. Several important correlations were found by statistical analysis. Blood urea levels and milk lactose showed a positive correlation ($r = 0.429$, $P < 0.01$), suggesting a possible connection to protein metabolism. Additionally, there were weak but significant positive correlations found between lactose and water intake, albumin, and alanine aminotransferase ($P < 0.01$), as well as between lactose and milk yield, iron, glucose, and magnesium ($P < 0.05$). These correlations suggest that enhanced glucose availability, mineral balance, and liver function may be reflected in higher lactose concentrations. Conversely, there were negative correlations with the fat-to-protein ratio ($r = -0.381$) and the milk fat percentage ($r = -0.312$, $P < 0.01$), indicating that lower lactose levels might be associated with lipid mobilisation and altered energy balance, which are frequently connected to subclinical ketosis and negative energy balance [2]. The findings highlight the integrative function of lactose as a biomarker linking production and behavioural characteristics to the metabolism of carbohydrates, proteins, and minerals. In-line monitoring of milk lactose may increase precision livestock management by enabling early detection of metabolic disturbances and offering real-time insight into these intricate physiological interactions. These results support earlier research showing that milk lactose is a useful and instructive metric for herd-level health monitoring in contemporary dairy production [3].

Keywords: milk lactose, dairy cows, early lactation, metabolic biomarkers.

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QUALITY OF HUNTED WILD GAME MEAT IN LITHUANIA

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Since antiquity, wild game has provided essential nutrients and remains central to the diets of many indigenous populations [1, 2]. Game meat represents an alternative to conventional farmed animal products, as it avoids many of the undesirable characteristics associated with intensive industrial livestock farming. A varied diet in the natural habitats of wild animals and higher physical activity are just some of the factors that influence the special taste, appearance, texture, and unique nutritional properties of game meat [3, 4]. This investigation assessed the technological, microbiological, and chemical quality of hunted wild game meat (HWGM) in Lithuania. Fresh carcasses of red deer, moose, roe deer, wild boar, and quail were collected and compared with beef from free-grazing Highland cattle. Samples were stored at -23°C and brought to -4°C prior to analysis. Quail meat exhibited the most alkaline pH (6.73 ± 0.02), the highest intramuscular fat ($4.66 \pm 0.03\%$), and a low malondialdehyde concentration ($2.18 \pm 0.14 \mu\text{mol kg}^{-1}$), indicating minimal lipid oxidation. Its limited water-holding capacity and cooking loss ($1.11 \pm 0.07\%$ and $18.05 \pm 0.07\%$, respectively; $P \leq 0.05$) further confirmed oxidative stability. Conversely, cooking losses exceeding 30% were recorded for red deer and wild boar, reflecting reduced thermal resilience. Wild boar presented the highest moisture content ($5.42 \pm 0.11\%$) and the lowest cholesterol load ($44.2 \pm 2.58 \text{ mg } 100 \text{ g}^{-1}$), whereas roe deer contained the greatest cholesterol concentration ($63.1 \pm 0.72 \text{ mg } 100 \text{ g}^{-1}$). Species strongly affected fatty-acid profiles: moose and roe deer tissues were richest in saturated fatty acids ($> 55\%$), while wild boar and red deer provided the highest proportions of polyunsaturated lipids ($> 30\%$). Wild boar also delivered an advantageous nutrient package, combining elevated protein ($25.19 \pm 0.07\%$), reduced cholesterol and a substantial share of polyunsaturated fatty acids ($25.61 \pm 1.14\%$). Overall, HWGM is a nutritionally valuable resource, but interspecific variation is pronounced. Considering protein yield, lipid quality and cholesterol content, wild boar emerges as the most desirable option for health-conscious consumers.

Keywords: Hunted wild game meat, meat quality and chemical composition, cholesterol, fatty acids, malondialdehyde concentration

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HALLOYSITE-INDUCED DIFFERENTIAL PROTEOMIC RESPONSES IN THE CECAL MUCOSA OF DRAKES AND DUCKS

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Dietary supplementation with halloysite, an aluminosilicate clay mineral, has recently been shown in broiler chickens to improve digestive efficiency and nutrient utilisation, thereby enhancing growth performance [1]. The underlying mechanism is primarily associated with the formation of a protective colloidal layer over the intestinal mucosa, which preserves epithelial integrity and facilitates the removal of pathogenic bacteria and toxins through binding and excretion. Moreover, halloysite inclusion has been linked to increased villus height and a higher villus-to-crypt ratio, resulting in an expanded absorptive surface area and improved nutrient digestibility [2]. Although the beneficial effects of halloysite supplementation have been well-documented in broiler chickens, evidence regarding its impact in ducks remains limited. Therefore, this study aimed to evaluate the influence of 1% dietary halloysite supplementation on caecal proteome alterations of Cherry Valley broiler ducks. The experiment involved 24 ducks, divided into four groups (n = 6). The control groups, consisting of males and females, were fed a commercial diet, whereas the experimental groups received a diet supplemented with 1% halloysite throughout the rearing period. Immediately after slaughter on day 42, caecum fragments were collected. The proteins of the caecal mucosa were separated using two-dimensional electrophoresis and the resulting 2-D gel images were analysed using PDQuest Analysis software. Statistically altered proteins were identified using nano LC-MS. Halloysite supplementation caused moderate changes in the expression of 13 protein spots, with effects that were partly overlapping and partly divergent. Among these, 7 were up-regulated and 5 down-regulated in females. In males, 5 protein spots were up-expressed, while the remaining 8 showed down-expression. In both sexes, this dietary supplement increased the expression of mitochondrial HSP60, which may indicate enhanced protein folding and improved stress protection. Females exhibited stronger activation of sulphur and redox metabolism, as indicated by the up-regulation of sulphurtransferase, whereas this pathway was suppressed in males. Additional changes in cytoskeletal, metabolic and proteostasis-related proteins suggest alterations in energy metabolism, antioxidant defense, and epithelial remodelling, collectively supporting a role of halloysite in modulating gut physiology.

Keywords: ducks, halloysite, cecal mucosa, proteome.

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ANIMAL GENETIC RESOURCES CONSERVATION PROGRAMS IN POLAND OVER THE LAST 25 YEARS

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The National Research Institute of Animal Production coordinates and implements activities related to the conservation of animal genetic resources under the *Breeding Act of 10 December 2020* about Organization of Animal Breeding and Reproduction where Article 34 concerns “Conservation” [1].

The conservation of endangered species and native breeds is carried out using both *in situ* and *ex situ* methods, in accordance with the provisions of the *Convention on Biological Diversity* [2] and this is supported by an annually calculated risk status [3].

For 25 years, conservation efforts have been accompanied by scientific research involving protected breeds, aimed at identifying product quality traits and preserving health and adaptability of native breeds.

According to the Institute’s data, the conservation programmes covered approximately 16 000 animals and 317 bee colonies in 2000. By the end of 2024, the number increased to over 122 000 animals and 766 bee colonies. In 2000, 30 breeds, strains, lines and varieties of farm animals were under protection; currently, this number has now increased to 89. Steady population growth shows that conservation programmes are working.

Although native breeds are often less productive and economically viable compared with commercial breeds, they play a crucial role in preserving genetic, cultural, and ecological heritage. Therefore, their conservation is supported through dedicated measures within the CAP’s Strategic Plan (2023–2027).

Effective conservation depends on collaboration, with breeders playing a key role through exhibitions, product promotion, and practical use of native breeds.

Keywords: native breeds, biodiversity, AnGR conservation programs, CDB, risk status.

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THE INFLUENCE OF DIFFERENT COMBINATIONS OF LAYING HEN LINES AND HOLDING SYSTEMS ON EGG QUALITY

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Egg quality is a crucial factor in poultry production, affecting both consumer acceptance and market value. Genetic background and housing systems are known to influence the physical, chemical, and sensory properties of eggs. The aim of this study was to evaluate the influence of different combinations of laying hen lines and holding systems on the quality of fresh and stored eggs. The study was conducted using combinations of Lohmann Brown hens kept in cages and Lohmann Sandy hens kept in a free-range system, all at the age of 50 weeks. Egg quality parameters were assessed for both fresh eggs and eggs stored for 28 days. Egg weight, protein height, Haff unit, and yolk colour intensity was determined by the Egg Multi-Tester EMT-5200 multi-function automatic egg parameter analyser, eggshell strength by the Egg Shell Force Gauge MODEL-II, and shell thickness by the MITUTOYO electronic micrometre. The results showed that eggs from the Lohmann Sandy line combination had significantly larger egg diameters, higher yolk height, more intense yolk colour, and greater albumen height compared with eggs from the Lohmann Brown line combination, in both fresh and stored eggs. Conversely, shell strength was significantly higher ($P < 0.05$) in eggs from the Lohmann Brown line combination. These findings indicate that hen line and housing system combinations have a substantial impact on specific egg quality parameters. The results suggest that selecting appropriate hen lines and housing systems can help producers optimise egg quality according to market demands, balancing internal quality with shell strength. Moreover, these findings emphasise the importance of integrating genetic selection with suitable management practices to achieve both high productivity and enhanced product quality. Overall, the study provides practical insights for poultry producers aiming to improve egg characteristics while maintaining animal welfare and production efficiency.

Keywords: laying hens, hen lines, housing systems, egg quality.

CHALLENGES IN MANAGING HEAT STRESS IN DAIRY FARMS

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Heat stress remains a major challenge in dairy farming, as elevated temperatures compromise feed intake, milk yield, and reproductive performance. Effective management is further complicated by the high variability of climatic conditions, limited cooling infrastructure, and the economic burden of implementing sustainable mitigation strategies.

The first signs of heat stress can appear at temperature humidity index (THI) levels from 68 to 74, and when THI > 75 it can have a significant negative impact on the composition of cow's milk [1]. The milk fat content decreases immediately when the THI value increases by only 0.01 [2].

The research was carried out at a dairy farm in the year 2024–2025. In total, 30 cows were selected and grouped into three groups according to lactation. Cow's body temperature, milk fat content and THI were calculated daily.

The highest body temperature in August (39.09°C) and, as a result, almost lowest milk fat content were found to be in August (3.79%). The highest THI was in July (70.33). In August, THI was (69.99) and very similar to July in terms of THI. In November, the lowest body temperature (38.76°C), THI (48.31) and the highest milk fat content (4.30%) were observed.

The cows' body temperature decreased from August to November (0.33°C), milk fat increased from August to November (0.51%) and temperature humidity index also decreased from August to November (21.68) ($P < 0.05$). The first lactation cow's milk had the highest milk fat content. In July and November, the first lactation cows had the highest milk fat content. The lowest milk fat content in July was in the second lactation cow's group. In November, the lowest milk fat content was observed in the third lactation cow's group ($P < 0.05$).

In August, when THI was almost highest, the cows' body temperature was also highest, but milk fat content was almost lowest. THI negatively affects health and milk production in cows. Thus, based on the results of the research, the higher THI and the higher cow's body temperature affect the fat content of cow's milk, which decreases ($P < 0.05$). During the study, it was assumed that each cow was in its own individual environment with unique humidity, temperature and humidity index conditions. This resulted in large comparison samples. Therefore, all differences between the study groups were statistically significant.

Globally, heat stress is recognised as a major constraint to dairy production, as elevated THI levels impair cow's health, reduce feed intake, and lower both milk yield and quality. The consistent decline in milk fat content and overall productivity under heat stress highlights the urgent need for effective adaptation and mitigation strategies in dairy farming worldwide.

Keywords: heat stress, dairy cattle, temperature humidity index, body temperature.

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COMPARISON OF MORPHOMETRIC AND SELECTION TRAITS OF RABBIT BREEDS IN LITHUANIA

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Rabbit breeding plays an important role in small animal husbandry, providing valuable insights into genetic diversity, animal performance, and compliance with breed standards. Comparative evaluation of morphometric and selection traits is essential not only for preserving breed characteristics but also for ensuring the advancement of rabbit breeding practices in Lithuania. The aim of this study was to compare morphometric and selection traits of two rabbit breeds commonly kept in Lithuania: Burgundy and California. A total of 30 medium-sized rabbits were included in the study, with 15 individuals from each breed. The animals were clinically healthy, of similar age (8–9 months), and originated from different breeders. All rabbits were purebred, with pedigree certificates and ear tattoos confirming their origin. Exterior features, morphometric measurements, and selection traits were evaluated in detail. The results demonstrated that while Burgundy rabbits showed some minor deviations in selective traits, such as sloping hindquarters, cylindrical body shape, presence of dewlap, or slightly drooping ears, these did not exceed breed standards. Both Burgundy and California rabbits met the required criteria for breed conformity. Morphometric analysis revealed statistically significant differences ($P < 0.05$) between breeds and sexes in traits such as height at withers, chest depth, belly girth, ear length, and shoulder width. However, none of the evaluated rabbits achieved the maximum score (100 points) in the selection assessment, and no statistically significant differences ($P > 0.05$) were found between breeds in the overall scoring system. In conclusion, both Burgundy and California rabbits studied in Lithuania met the established breed requirements, with some minor deviations observed in Burgundy rabbits. Morphometric measurements confirmed clear differences between breeds and sexes, highlighting the importance of such evaluations in maintaining breed standards and guiding future selection strategies in rabbit breeding.

Keywords: rabbit breeds, morphometric traits, selection traits, breeding, standards.

PARASITES IN ALPACAS (*VICUGNA PACOS*) – A CHALLENGE IN HUSBANDRY

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In Europe, the predominant alpaca husbandry system is semi-intensive, providing animals with shelter in buildings and access to outdoor paddocks. In Polish farming practice, the floors of livestock buildings are often covered with sand and topped with a layer of straw, which enhances thermal comfort and insulates the animals from moisture. Although this management system supports animal welfare, inadequate bedding replacement can lead to the accumulation of contaminants, including eggs and oocysts of gastrointestinal (GI) parasites, thereby increasing the risk of reinfection within the herd.

The study was conducted on alpacas in a farm located in central-eastern Poland. Faecal samples were collected directly from the rectum of the animals and from sand in the enclosures. Coproscopic examinations were performed using the Willis-Schlaaf flotation method and the McMaster technique [4]. Nematodes were identified according to protocols described by Taylor et al. [4], Bauer et al. [1], Zajac and Conboy [6], and Thienpont et al. [5]. The presence of parasite eggs in sand was assessed using a modified flotation method [3]. Protozoa of the genus *Eimeria* were identified to the species level according to Bauer et al. [1] and Gomez-Puerta et al. [2].

The overall prevalence of GI parasites in alpacas was 72.4%. Among them, the prevalence of GI nematodes was 67.8%, while the prevalence of *Eimeria* spp. was 31.6%. The most frequently detected nematode was *Nematodirus* spp., and the most prevalent *Eimeria* species was *Eimeria punoensis*. Parasite eggs and oocysts were detected in the sand of 13 out of 20 enclosures (65%).

Gastrointestinal parasites are widespread in alpaca herds and represent a significant health concern. The high prevalence of *Nematodirus* spp. and *Eimeria punoensis* highlights the necessity for regular monitoring and implementation of appropriate prophylactic measures. The presence of eggs and oocysts in enclosure sand indicates that the husbandry environment may facilitate reinfection and the dissemination of parasites within the herd.

Keywords: alpacas (*Vicugna pacos*), gastrointestinal parasites, nematodes, *Eimeria*, sandy floor.

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PRELIMINARY RESEARCH ON THE RESTORATION OF THE LOCAL POPULATION OF LIDZBARK HORSES

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Lidzbark horses are a local type of draft horse developed in the Lidzbark Warmiński region after World War 2. These horses originate from the Vilnius region, where their proper breeding began in 1928 in the areas of Oszmiana, Nowogródek, and the Vilnius region. Previously, this type was believed to have disappeared, but observations conducted in the Warmian-Masurian Voivodeship indicate that there are individuals displaying the phenotypic characteristics of these horses [2].

To determine the occurrence of Lidzbark horses in the Warmian-Masurian Voivodeship, individuals displaying the appropriate phenotypic characteristics were sought for comparison with source materials in terms of basic dimensions and colour. Information from Volume I of the Kzp Stud Book (1964), which lists 20 stallions and 13 mares of the Lidzbark type, was taken into account, as well as information from source materials and publications [1–3]. This information was compared with field-collected data on 19 horses.

The information collected during the study was statistically analysed using the Student *t* test. The results indicated that the measurements of Lidzbark mares recorded in Volume I of the Kzp Stud Book and those of horses currently in existence differ significantly in terms of chest circumference and cannon bone. No statistical difference was found for height at the withers. Among the stallions, the results indicated significant differences in height at the withers, chest circumference, and cannon bone circumference. The prevalence of dun coats was 60.6% currently and 72.2% in 1964, respectively.

The research and observations conducted indicate that in the Warmian-Masurian Voivodeship there are horses (mainly mares) whose conformation and colour correspond to the standards of the Lidzbark horse, as characterised in source materials in the 1960s [2]. Although their basic dimensions differ statistically, these horses largely have ancestors listed in Volume I of the Book of the Lidzbark Horse Code, the section devoted to Lidzbark horses, in their pedigrees. They possess characteristic traits (e.g., colour), which provide a basis for further research on this population.

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CONCENTRATIONS OF CHEMICAL ELEMENTS IN TISSUES OF WILD BOARS FROM SELECTED AREA OF SLOVAKIA

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Venison of game animals is a meat low in fat and cholesterol and rich in health-promoting substances such as vitamins, minerals, and unsaturated fatty acids. The aim of this work was to monitor the occurrence of selected chemical elements in the tissues of wild boars from a selected area of Slovakia with regard to the state of the environment in the selected area. Nine chemical elements (Ca, Mg, Cu, Fe, Zn, Cd, Pb, Cr, Ni) were monitored in tissue samples and determined by the ICP-OES method. From the essential elements, the highest concentrations of Ca (233.13 ± 99.42 mg/kg) were recorded in wild boar muscle samples, and the highest concentrations of Fe (428.76 ± 91.64 mg/kg) were recorded in liver samples. From the toxic elements, the highest concentration was found in the samples of kidneys of wild boar in case of Cd (2.68 ± 0.22 mg/kg). When comparing the concentrations of selected elements in wild boar tissues, we found statistically significant differences between all tissue types in the case of Ca, Mg and Cu ($P < 0.05$). Statistically significant differences ($P < 0.05$) in cadmium (Cd) concentrations were found between all monitored tissues, except between the liver and kidney samples. In case of Ni, statistically significant differences were found only between kidney and muscle samples ($P < 0.05$). In conclusion, it can be stated that the game from the given area is of high quality, rich in essential elements, and the occurrence of toxic elements does not pose a risk; however, since this was a pilot study, monitoring in the given area will continue.

Keywords: game animals, venison, chemical elements, environment.

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THE STALLION EFFECT: CONCENTRATION OF BREEDING USE AND ITS IMPACT ON HORSE POPULATIONS

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Unequal use of stallions is a key driver of the genetic structure in the horse population. In many breeds, a small number of highly valued stallions sire a disproportionately large share of foals, causing the so-called “stallion effect”. We investigated this effect across different breeds, including those in conservation programmes and performance-oriented populations. Using pedigree data of foals born in 2000–2024, we analysed the number of foals per sire, the average and median number of foals per sire, the proportion of foals sired by the top 10% of sires, the proportion of sires with one foal and the proportion of sires accounting for 50% of foals across Baltic Warmblood (BW), Lithuanian Heavy Draught (LHD), Lithuanian Warmblood (LW), Trakehner (TRAK), Žemaitukai (ZEM) and Large-type Žemaitukai (LZEM).

The results showed that the top 10% of sires produced 33–40% of foals in ZEM, LZEM and LHD, while the shares were 51.0% in TRAK, 44.5% in BW and 57.1% in LW. In the two warmblood populations with broadly similar breeding goals, half of all foals came from 124 sires (13.0%) in BW but from just 41 sires (6.6%) in LW, showing much stronger concentration in LW. The median number of foals per sire was 1 in BW and LW, 4 in TRAK, 5 in ZEM, 6 in LZEM and 8 in LHD; likewise, the share of sires with only one foal reached ≈56–59% in LW and BW but only ≈16–21% in LHD, LZEM and ZEM. The gap between the average and the median number of foals per sire (e.g., TRAK 12.3 vs 4.0) further indicates a small group of highly used sires alongside many low-use sires.

These indicators confirm a clear stallion effect – a small subset of sires produces a large share of foals – most pronounced in LW and TRAK, which shrinks the effective sire base and risks loss of genetic diversity. In open populations (BW, LW), this concentration can be managed more readily by broadening and rotating sires, whereas the closed TRAK population requires stricter, line-balanced planning and targeted use of underused sires. Monitoring these parameters within breeds can help improve risk detection, maintain safe inbreeding levels, and safeguard genetic diversity for long-term sustainability.

Keywords: stallion effect, breeding, Lithuanian horses.

POLYMORPHISMS IN SELECTED GENES AND THEIR RELATIONSHIP WITH QUALITY INDICATORS OF LIQUID-STORED BOAR SEMEN

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In swine insemination, liquid-stored semen is most commonly used, and sperm motility is the primary parameter for assessing semen quality, which is influenced by genetic and environmental factors [1–4]. The aim of the study was to determine differences in ejaculate volume, sperm concentration and count, as well as motility of sperm preserved in liquid form, using both computer-assisted semen analysis (CASA) and the traditional microscopic method, in relation to polymorphic variants of the *CD9*, *ESR2*, *DAZL*, and *PIWIL4* genes.

The study was conducted on semen collected from 195 boars of various breeds (Duroc, Duroc × Pietrain, Polish Large White [pbz], PIC, Polish Landrace [wbp]), aged 18–36 months, maintained at two animal breeding and insemination stations in Poland. Semen was evaluated on the first day of storage in liquid form (diluted using Vitasem LD and Gedil extenders) at 16°C. The percentage of progressively motile sperm was determined using both the traditional microscopic method with a Blom's table [5] and the CASA system (Sperm Class Analyzer[®], Microptic S.L., Spain). Statistical analysis was performed using Statistica v.13.3 software (StatSoft, Poland).

For ejaculate volume, a significant difference ($P \leq 0.01$) was found between the *ESR2/GG* genotype ($225.1 \pm 81.7 \text{ cm}^3$) and the other forms: *AA* ($291.4 \pm 129.8 \text{ cm}^3$) and *AG* ($271.7 \pm 77.4 \text{ cm}^3$). Regarding sperm concentration, significant differences ($P \leq 0.01$) were observed between *ESR2/AA* ($351.5 \pm 117.6 \times 10^6/\text{mL}$) and *ESR2/GG* ($438.1 \pm 153.3 \times 10^6/\text{mL}$), and between *DAZL/AA* ($442.01 \pm 164.7 \times 10^6/\text{mL}$) and *DAZL/CC* ($363.9 \pm 102.1 \times 10^6/\text{mL}$, $P \leq 0.05$). For the remaining traits, the differences between genotypic variants were statistically insignificant.

In the percentage of progressively motile sperm assessed by CASA, significant differences were found only within the *ESR2* gene. Homozygous *GG* boars ($75.8 \pm 10.8\%$) differed significantly from the *AA* ($82.3 \pm 6.7\%$) and *AG* ($80.0 \pm 12.1\%$) variants. A strong correlation was found between progressive sperm motility determined by the traditional method and the CASA system ($r_s = 0.86$, $P \leq 0.001$). This confirms the full utility of the widely used traditional method in laboratory practice for evaluating semen quality and qualifying ejaculates for further use in insemination. When analysing the influence of gene polymorphisms on semen traits, the breed/genotype of the boars should also be considered as a significant factor.

Keywords: boar, sperm, progressive motility, *CD9*, *ESR2*, *DAZL*, *PIWIL4*.

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ASSOCIATIONS BETWEEN SELECTED GENE POLYMORPHISMS AND MORPHOLOGICAL INTEGRITY OF LIQUID-STORED BOAR SPERMATOZOA

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During semen preservation, sperm cells undergo structural and functional damage, the extent and nature of which affect their fertilising capacity. This capacity is influenced by both genetic and environmental factors. The aim of this study was to determine morphological changes in boar sperm cells on the first day after semen dilution, taking into account the polymorphisms of the CD9, DAZL, ESR2, and PIWIL4 genes.

The study was conducted on semen samples from 195 boars of different breeds (Duroc, Duroc × Pietrain, Polish Large White [pbz], PIC, Polish Landrace [wbp]), maintained at two animal breeding and insemination stations in Poland. The semen was preserved in liquid form using Vitasem LD and Gedil extenders at a temperature of 16°C. Differential staining (eosin-nigrosin) was used to assess sperm with major and minor morphological defects [1], as well as acrosome damage [3]. For the assessment of membrane and acrosome integrity using fluorescence techniques, the fluorochromes SYBR-14, PI, and PNA were applied [2, 4, 5]. After DNA isolation from sperm cells, gene polymorphisms were determined using the PCR-RFLP method. Statistical analysis was performed using Statistica v.13.3 software (StatSoft, Poland).

Overall, the percentage of minor defects ranged from 3.5% to 5.8%, and major defects from 2.0% to 4.8%, falling within the accepted standards for boar semen quality [6]. Significant differences were found between polymorphic variants of the DAZL gene (AA vs. AC) and ESR2 gene (AA vs. GG). The proportion of sperm with intact acrosomes averaged 85%, with significant differences observed only in the ESR2 gene variants (AA – 87.4%, AG – 86.8%, GG – 82.9%). Statistically significant differences among all gene variants were also found in membrane integrity and acrosome damage as assessed by fluorescent methods. The breed of the animals was shown to have a significant impact as well, which prevents drawing definitive conclusions about the role of these genes in determining the analyzed sperm traits, highlighting the need for studies on a larger number of boars.

Keywords: boar, sperm, preservation, abnormalities, CD9, DAZL, ESR2, PIWIL4.

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SOMATIC CELL SUBPOPULATIONS IN GOAT MILK AND THEIR ASSOCIATION WITH MILK COMPOSITION PARAMETERS

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Somatic cell count (SCC) in goat milk is widely used as an indicator of udder health and milk quality. However, the total SCC alone does not fully reflect the nature of cellular changes occurring during different stages of lactation or in response to subclinical inflammation [1]. This study aims to characterise the subpopulations of somatic cells in goat milk and investigate their potential associations with milk composition parameters. Milk samples were collected from dairy goats at different stages of lactation between April and August 2025. Standardised laboratory methods were used to analyse milk composition (fat, protein, lactose, urea, pH), while cytological evaluation was performed using light microscopy. Somatic cells were classified into four main subpopulations: neutrophils, macrophages, lymphocytes, and exfoliated epithelial cells. The results showed that neutrophils predominated, accounting for an average of 78.2% of total somatic cells, followed by lymphocytes (9.1%), macrophages (7.3%), and epithelial cells (5.4%). Milk samples with elevated SCC contained a higher proportion of neutrophils, whereas epithelial cells were more frequent during periods of active milk secretion and tissue regeneration. Average milk urea concentration reached approximately 50 mg/dL, exceeding the normal range for goats (20–35 mg/dL), suggesting a possible dietary protein–energy imbalance. Such nutritional or management-related factors may have contributed to the elevated SCC values and shifts in cellular proportions, even in the absence of clinical inflammation. Fat and protein levels showed more variable patterns without clear associations with SCC. These findings indicate that changes in somatic cell composition may reflect physiological or management-related factors, such as stress or hygiene, rather than exclusively pathological processes. A more detailed evaluation of cellular composition may therefore provide a better understanding of udder health dynamics and improve the interpretation of SCC thresholds in caprine milk composition [2].

Keywords: goat milk, somatic cell count, neutrophils, macrophages, epithelial cells, milk quality.

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BETA-SITOSTEROL DIETARY SUPPLEMENTATION IN DAIRY SHEEP MODIFIES MILK DELTA 9 DESATURASE INDEXES AND COCCIDIA LOAD

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This research investigated the effects of dietary beta-sitosterol supplementation on productive performance, milk quality, and faecal parasitic load in dairy sheep. A total of 16 Lacaune sheeps in the mid-lactation phase were selected and randomly assigned to two homogeneous groups (n = 8) based on parity, body weight, body condition score (BCS), and milk yield. Both groups were fed the same diet, except that the treatment group received feed supplemented with 0.16% beta-sitosterol. The trial lasted 49 days, including a 14-day adaptation period followed by a 35-day experimental phase. Milk samples were collected individually once a week during morning milking, and pooled faecal samples were collected weekly from each group. Animal weight, milk yield, fat, and protein content were not affected by beta-sitosterol supplementation. Regarding milk fatty acid profile, an increase in palmitoleic acid (C16:1) was observed in the treated group (0.81%) versus control (0.72%). Additionally, a significant decrease ($P < 0.05$) in stearic acid (C18:0) content was observed in the treatment group (11.25%) compared with the control group (12.58%).

The indices of the delta-9 desaturase enzyme showed a variation following dietary supplementation with beta-sitosterol, with significantly higher ratios observed in the treated group. Specifically, the C16:1 cis-9/C16:0 ratio was significantly higher ($P < 0.05$) in the treatment group (0.02 vs 0.03); similarly, the C18:1 cis-9/C18:0 ratio also increased, with a highly significant difference ($P < 0.01$) in the treated animals (1.40 vs 1.60). Parasitological analysis revealed a significant reduction ($P < 0.05$) in coccidia load in the treatment group, from 339 in the control group to 189 oocysts per gram (OPG) of treatment group. The results suggest that dietary beta-sitosterol supplementation may play a role in modulation of desaturase indexes in milk and may help in the control of gastrointestinal parasite. Further research is needed to clarify its mechanism of action.

Keywords: beta-sitosterol, dairy sheep feeding, milk quality, coccidia load, delta-9 desaturase enzyme.

A COMPARATIVE ANALYSIS OF BIRTH WEIGHT AND GROWTH PERFORMANCE BETWEEN AUBRAC AND CHAROLAIS CALVES

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Charolais cattle are among the most widespread beef breeds in Lithuania, while Aubrac cattle are less common but increasingly valued for their adaptability to extensive farming systems [1]. A comparative analysis of Charolais and Aubrac cattle breeds regarding calf birth weights and growth performances is essential for optimising breeding strategies. Additionally, such analysis reveals how each breed to Lithuanian environmental conditions. Monitoring birth weight and weight gain also contributes to animal welfare by enabling early detection of health issues, ensuring smoother calving, and supporting calf vitality. Therefore, the aim of this study was to compare birth weight and growth performance between Aubrac and Charolais calves. A total of 48 Charolais and 100 Aubrac calves were included in the study. The calves were categorised by sex (male or female) and season (born in warm or cold season) to assess the influence of these two factors on birth weight and growth performances. Calves were weighed within 24 hours of birth to determine birth weight. Additional weighing was conducted on days 210 and 365 to calculate weight gain rates for the periods from birth to 210 days, from 211 to 365 days, and from birth to 365 days. Charolais calves were born heavier and exhibited greater growth performance compared with Aubrac calves ($P < 0.001$). Both male and female Charolais calves were born heavier than Aubrac calves ($P < 0.001$). Charolais calves tended to be born heavier than Aubrac calves during both cold and warm seasons ($P < 0.001$). The weight gain of both male and female Charolais calves was statistically significantly higher than that of Aubrac calves across different time periods ($P < 0.001$). Charolais calves exhibited higher weight gain than Aubrac calves during both cold and warm seasons ($P < 0.001$). To conclude, the results showed that Charolais calves were born heavier and achieved greater weight gains compared with Aubrac calves and these same trends persisted when calves were analysed by sex and season.

Keywords: Aubrac, Charolais, weight, growth performance.

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ANIMAL ASSISTED THERAPY – IMPACT ON HUMAN HEALTH AND FUTURE PROSPECTS

Abstracts

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APITHERAPY – A COMPLEMENTARY AND ALTERNATIVE HEALTH CARE SERVICE BASED ON SCIENTIFIC AND PRACTICAL RESEARCH

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Apitherapy is the use of products from honeybees (*Apis mellifera*) for the prevention of diseases, as well as overall health and well-being promotion. It is considered a complementary and alternative medicine (CAM) approach. The main bee products used in apitherapy include propolis, bee pollen, bee bread, royal jelly, bee venom, beeswax and honey. Apitherapy has been practiced for thousands of years in cultures such as Egypt, China, and Greece. In ancient times, those receiving treatment at a sanatorium on the island of Kos were recommended to consume the “sacred sweetness-honey” to strengthen their health. Avicenna (980–1037) wrote: “If you want to preserve your health, be sure to eat honey, it strengthens the soul, helps digestion, refreshes memory and mind.” Dr. Filip Terč (1844–1917), a doctor and beekeeper from Maribor (Slovenia) who successfully cured 543 out of his 658 patients suffering from rheumatic diseases, is considered the father of modern apitherapy. For several years, his birthday, 30 March, has been celebrated as World Apitherapy Day. The first knowledge about bees, honey, and wax in Lithuania was described in the 13th century, where the patron of bees was called Bubilus, and the goddess of bee swarming or reproduction was Austėja. In 1971, the then pharmacist Prof. A. Gendrolis began research on bee venom. In 1992, thanks to him, the pharmacopoeia article on propolis raw material was approved and it became possible to create various forms of medicines from this raw material. In 1975, Dr. L. Mackevičius was the first in Lithuania to use bee venom (by stinging) for treatment and created an original apparatus for this purpose. The Lithuanian Apitherapists Association was established in 1991. On 20th April 2012, the International Federation of Apitherapy (IFA) was founded. The founders of IFA were representatives of Germany, Romania, Hungary, Serbia, Portugal, Ecuador, the Canary Islands and Lithuania. On 14th January 2020, the Seimas adopted the Law “On Complementary and Alternative Health Care (Complementary and Alternative Health Care) of the Republic of Lithuania”. Other adopted legal acts regulating apitherapy services are Order of Minister of Health of the Republic of Lithuania “On Approval of the Description of Requirements for the Provision of Complementary and Alternative Health Care Apitherapy Services” (30/06/2022 No. V-1179) and Order of Minister of Health of the Republic of Lithuania “On the Approval of the Lithuanian Medical Standard 187:2025 *Complementary and Alternative Health Care Apitherapy Specialist*” (24/02/2025; No. V-140). Areas of apitherapy activity; application of apitherapy products; bee hive air therapy and apipuncture. In Lithuania, universities organise training for apitherapy specialists, and it is possible to obtain a license for practice and training as an assistant apitherapy specialist (beekeeper). On 9th February 2023, Traditional, Complementary and Integrated Health Care Coalition was founded at the World Health Organization, and Lithuanian Apitherapy Society is one of the organisational founders of this coalition since 30th March 2023. The Lithuanian Apitherapists Association became a member of the Lithuanian Beekeepers Union on 23th November 2024.

Keywords: apitherapy, alternative health care service.

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THE THERAPEUTIC ROLE OF CATS IN SUPPORTING TERMINALLY ILL PATIENTS

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Cats play a vital role in the lives of terminally ill patients by providing comfort, companionship, and emotional support during challenging times. Their presence can significantly alleviate feelings of anxiety and depression, positively impacting the psychological well-being of individuals facing terminal illness [1]. Petting a cat, releases endorphins and serotonin, which promote a sense of happiness and reduce stress levels. These animals have an innate ability to sense human emotions, often gravitating toward patients who need solace. Their independent yet affectionate nature encourages patients to express their feelings and fosters connections with others, enhancing communication and shared experiences. The responsibility of caring for a cat can also instill a sense of purpose in patients, giving them a reason to engage with life even amidst illness [2]. Animal-assisted therapies, especially those involving cats, are gaining recognition in healthcare settings. Studies show that interactions with animals can reduce pain and improve overall quality of life, making patients more willing to confront their situations. The therapeutic bond between cats and terminally ill patients not only provides temporary relief but also supports emotional healing, allowing patients to navigate their final days with dignity [3]. Moreover, the calming presence of a cat contributes to a serene environment, offering a sense of normalcy amid the turmoil of illness. The routine of caring for a cat can provide structure and serve as a distraction from physical pain and emotional suffering [4]. In summary, the relationship between cats and terminally ill patients can be profoundly therapeutic, enriching their quality of life and helping them cope with their circumstances in a more graceful and peaceful manner [5].

Keywords: cats, terminal illness, emotional support, palliative care, animal-assisted therapy, AAI, pet-therapy, cancer patients.

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WELFARE AND SAFETY OF WORKING HORSES IN FILM PRODUCTION

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In today's modern age, the majority of horses are engaged in sports or recreation, with film sets representing the rare occasion where we still see horses at work. This setting is highly specialised and requires careful adaptation to meet equine needs, supported by continuous oversight from trained professionals. Regulations for animal welfare standards are provided by the American Humane Society [1], and their representative is present at all times when horses are working on film. While Horse Master and its team ensure equine safety and comfort, equal consideration must be given to the well-being of human actors and crew working in close proximity. Interactions may include riding, driving, or handling horses, all of which carry unique challenges and responsibilities. The arrival of horses on set significantly alters the environment, underscoring the profound impact of animal presence on both the production process and those involved.

Keywords: equine, film, welfare, well-being.

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HORSES AND HUMAN WELFARE IN EQUINE ASSISTED SERVICES

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Equine Assisted Services (EAS) are gaining increasing recognition worldwide as a valuable interdisciplinary approach in rehabilitation, education, and social inclusion. With this growth comes the crucial responsibility to safeguard the welfare of all participants — equines, clients, practitioners, and staff members. This presentation explores welfare in EAS as a multidimensional concept rooted in ethical practice, sustainability, and public trust. The Five Domains model provides a framework for evaluating equine welfare beyond physical care to encompass behaviour and mental state. Ensuring equine well-being is not only an ethical obligation but also essential to maintaining a Social Licence to Operate. At the same time, client welfare requires respectful, safe, and dignified interventions, while practitioners need clear boundaries, education, and support to prevent stress and burnout. Common challenges include overwork of equines, inconsistent handling, staff undervaluation, and risks to client safety. Evidence shows that when welfare is prioritised, safety and satisfaction increase for all stakeholders, strengthening the therapeutic process. HETI contributes to this dialogue by connecting practitioners and researchers globally, publishing ethical guidelines translated into several languages, and supporting working groups that advance welfare and ethics. In addition, HETI cooperates closely with major international organisations such as IAHAIO and the Horses and Humans Research Foundation (HHRF), building shared initiatives that strengthen global progress. A joint HETI–HHRF campaign will promote equine welfare through the Five Domains with accessible tools such as welfare pledges, webinars, and educational media. Future initiatives include self-assessment instruments and international frameworks to align best practices across countries while respecting cultural contexts. The presentation emphasises practical strategies that centres can implement immediately, from equine welfare assessments and individualised work plans to staff training and welfare audits. In conclusion, equine and human welfare in EAS are inseparable, ensuring both a shared responsibility and the foundation for ethical and sustainable services worldwide.

Keywords: equine assisted services, welfare, ethics, five domains, sustainability.

DIRECT TESTIMONY OF AN AUTISTIC WOMAN WHO USES CANINE BEHAVIOR READING SKILLS AS A TRANSLATOR OF NEUROTYPICAL HUMAN BEHAVIOUR

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The relationship with dogs, especially those she owns, with whom she has a relationship of mutual knowledge and trust, and who have been specially trained and certified for this purpose as working dogs, is structured on dynamics that use one of the peaks of competence determined by autism and present in her pattern of traits. The high competence in reading the communication signals emitted by these animals compensates for the impossibility or the effort to decipher in a functional way the criteria of neurotypical communication and interaction. Without what can be defined literally a “translation” of the intentions of the human interlocutors with whom she is involved, provided by the dogs, the Lady cannot orient herself in a functional way and for less than short periods, and above all not even to protect herself in social environments, or is not in a sufficiently functional way. But the role of this specific human–animal interaction is deeper and involves the entire perceptive process in every phase and moment. She has modeled her entire experience on the basis of the relationship with the dog, becoming an established professional also in this specific sector. It is not technically impossible for her to do without the constant presence and mediation of dogs occasionally and temporarily, that is, not as a constant condition, but to do so she needs an investment in terms of use of resources that exceeds the threshold of the criterion of protection and well-being. The request for impediment of use or limit, both temporary and environmental, of such a resource could be read, to understand its scope, as a request to deprive oneself or limit the use of tools such as lenses or hearing aids for people who need them, thus putting them in a condition of vulnerability and sensory deprivation.

Keywords: autism, schizophrenia, bipolar disorder, borderline disorder, anxiety, panic disorder, eating disorder, dysmorphic disorder, gender dysphoria, depression, obsessive compulsive disorder, epilepsy, working dogs, accessibility, inclusion, education.

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ANIMAL-ASSISTED INTERVENTIONS (DOGS) TO RELIEVE STRESS IN ONCOLOGY PATIENTS IN WAITING AREAS AND CHEMOTHERAPY ROOMS AT BELLARIA HOSPITAL, BOLOGNA

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The ChiaraMilla Association has implemented Animal-Assisted Interventions (AAI) with dogs and cats across Emilia-Romagna for over 18 years. Its multidisciplinary team operates under Italian Ministry of Health regulations, in collaboration with the National Reference Center for AAI. In Ferrara, the association manages a specialised Animal-Assisted Education center with resident animals registered in Digital Pet, including alpacas, llamas, rabbits, dogs, and cats.

This project, promoted by the volunteer association Onconauti and strongly supported by the Director of the Oncology Department, Prof. Antonio Maestri, was designed to support cancer patients and their families within Bellaria Hospital, Bologna.

The initiative began on January 14, 2025, with a training session for healthcare staff. Weekly sessions followed from January 23, 2025 (Thursdays, 9:00–11:00), with a summer break in August. Each session involved at least two trained dogs, managed by professional handlers and supported by the ChiaraMilla team, including trainees and AAA coordinators who collected data, photos, and session diaries.

Activities included ball games, retrieval, interactive problem-solving tasks, basic obedience, bowling games, grooming, and pet care.

Project objectives were to:

1. Humanise oncology care and make diagnostic, consultative, and therapeutic procedures more bearable;
2. Reduce the emotional and psychological burden of treatment;
3. Improve mood, distract from pain, and foster dialogue among patients and staff;
4. Decrease anxiety, stress, fear, and worry;
5. Promote relaxation through tactile interaction with animals;
6. Encourage mobility and enjoyment through play;
7. Enhance self-esteem and caregiving abilities.

Patient, family, and staff feedback was collected through structured interviews in collaboration with the Department of Psychology.

Preliminary results indicate significant benefits:

1. Patients showed immediate interest, requesting to meet and interact with the dogs.
2. Family members expressed strong appreciation.
3. The animals helped distract patients during medical procedures, reducing perceived pain and fear.
4. Reports included reduced nausea during interactions, highlighting the therapeutic value of distraction.
5. Repeated sessions fostered continuity, trust, and increased communication among patients and with staff.
6. Patients requested photos with the animals, shifting self-perception from illness-centred to positive, emotionally meaningful experiences.
7. Healthcare staff also reported relief, joy, and improved interaction with patients thanks to the presence of animals.

The presence of animals created a more welcoming, less sterile, and emotionally supportive hospital environment for both patients and staff. The project is ongoing and has been renewed for 2026, with plans to add a second weekly session and expand to the Oncology Department of Imola Hospital (BO).

THE FIRST SURVEY OF FISH WELFARE STATUS ON SELECTED CYPRINID AND RAINBOW TROUT FARMS IN BOSNIA AND HERZEGOVINA: PRELIMINARY RESULTS

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Animal welfare assessment is a continuous process of improvement, a process that started only recently for fish welfare [1]. According to the functions-based welfare approach, fish must be able to maintain their biological functions and zootechnical performances [2]. Based on the actual official data from the Agency for Statistics of Bosnia and Herzegovina, the total production of cyprinid ponds and rainbow trout (*Oncorhynchus mykiss*) fish farms in 2023 was 123.3 tons and 3.766,6 tons, respectively, based on the facilities of the surface of 2.042 ha and 110.997 m², in particular. There are not many literature sources on aquaculture production in Bosnia and Herzegovina, especially those about fish welfare conditions on fish farms. It was our motivation to arrange a screening investigation about which knowledge and implementation of principles of the function-based approach to fish welfare are present. Our research assessed four cyprinid fishponds in the municipalities of Derventa, Prnjavor, Brod and Bileca as well as seven rainbow trout fish farms in the municipalities of Trebinje, Sipovo (2 farms), Knezevo, Jezero, and Ribnik (2 farms). The study included a survey with fish farm production managers and the use of a questionnaire based on “The MyFishCheck” [1] with more than 20 questions of important parameters, i.e. personnel training/education, daily check, predator protection, plant cleanliness, mortality documentation, biomass documentation, feeding interval and rate, etc. Additionally, it made measurements of six water quality parameters on-site using portable instruments. Based on the results of the research, it can be concluded that in most fish farms with production up to 100 tons per year, knowledge of the basic principles of fish welfare protection is at a relatively low level and that there is considerable space for improvement of the current situation. This primarily involves maintaining continuous education about welfare-related issues and fish health.

Keywords: common carp (*Cyprinus carpio*), salmonidae, aquaculture, Balkan, well-being, questionnaire.

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TRUST AND COMFORT: DOGS IN ANIMAL-ASSISTED THERAPY

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Trust and comfort are key for dogs to work well in animal-assisted therapy, and they are also essential for the dog's welfare. Welfare means more than health; it includes low stress, feeling safe, and positive emotions. Studies show that dogs with good relationships with their handlers have lower stress, higher levels of calming hormones like oxytocin, and are better able to cope with new or challenging situations [1]. Positive training methods, such as reward-based techniques, strengthen trust and communication, while punishment-based methods can harm welfare and the bond [2]. In therapy settings, a strong human–dog bond helps dogs feel confident and comfortable, which improves their ability to support clients. This presentation will explain why trust and comfort are important for welfare and therapy success and offer practical ideas to strengthen the dog–human relationship.

Keywords: dog training, animal-assisted therapy, dog welfare.

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PERFORMANCE AND WELFARE ASSESSMENT OF HORSES FROM DIVERSE STUD FARMS

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The welfare and performance of horses are influenced by both genetic traits and environmental conditions, including housing, feeding, and overall management practices. Comparative studies across stud farms provide valuable insights into how these factors interact to determine horse health, exterior traits, and well-being. The aim of this study was to evaluate the performance and welfare of horses from two Lithuanian stud farms (X and Y), focusing on their morphological and exterior traits as well as management and feeding conditions. The study was conducted using anatomical–morphological assessment methods of horse body parts. Evaluated parameters included body measurements and indices, exterior traits, and housing and feeding conditions. The results revealed that horses from Stud Farm X demonstrated more favourable outcomes in most body measurements and indices, indicating stronger conformational characteristics ($P < 0.05$). In contrast, exterior evaluations of certain traits, including neck, forelimb, and hindlimb conformation, were more favourable in Stud Farm Y ($P < 0.05$). Housing conditions also differed: stalls in Farm X were slightly longer, while those in Farm Y were wider, but the height of feeders and drinkers was higher in Farm X. Bedding practices varied as well, with Farm X using straw mixed with sawdust and Farm Y relying solely on straw. Feed analysis showed differences in nutritional composition: diets in Farm X contained higher crude protein and balanced fibre fractions, whereas the feed in Farm Y was characterised by higher starch content but lower protein levels. In conclusion, both genetic and environmental conditions influenced the performance and welfare outcomes of the studied horses. While Farm X showed advantages in most morphometric indices and feed quality, Farm Y exhibited better exterior evaluations in certain traits. These findings highlight the importance of integrated assessment of management conditions, feeding, and conformational traits in ensuring optimal horse performance and welfare.

Keywords: horse welfare, stud farms, body measurements, feeding conditions, exterior evaluation.

HEAVY METAL LEVELS IN SEARCH AND RESCUE DOGS' HAIR

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Search and rescue (SAR) dogs, due to their close contact with humans and work in various environmental conditions, are a valuable model for monitoring environmental pollution. Hair, as a tissue reflecting long-term exposure, is a non-invasive biomarker of chemical element accumulation. The aim of this study was to determine the levels of selected heavy metals (Cu, Co, Cd, Fe, Zn, Pb, As) and the non-metal selenium (Se) in the hair of SAR dogs and to assess the impact of the working environment (open field vs. disaster), breed, sex, and neuter status on their concentrations. The analysis showed that dogs working in open fields had higher concentrations of most of the analysed elements compared with dogs working in disaster. Breed also had an impact on metal levels, with retrievers showing the lowest concentrations of Cu, Pb, and Se, suggesting possible genetic predispositions affecting elemental accumulation. No significant differences were observed between sexes or neuter status, confirming the dominant role of environmental and breed-related factors. The results demonstrate that environmental exposure associated with working conditions has a strong influence on the heavy metal load in dogs.

Keywords: search and rescue dog, heavy metals, hair.

FIT FOR THERAPY: REQUIREMENTS FOR DOGS IN ANIMAL-ASSISTED THERAPY ACCORDING TO THE POLISH SOCIETY OF KYNOTHERAPY

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Domestic dogs (*Canis lupus familiaris*) are increasingly used in Polish Animal-Assisted Therapy (AAT), but unlike guide dogs, they follow voluntary professional standards rather than specific laws. The Polish Society of Kynotherapy (PTK) defines canine-assisted therapy as an intervention using a “properly selected and trained” dog handled by a qualified therapist [1]. National guidelines emphasise that therapy dogs must be calm, well-socialised, and highly obedient. For example, PTK recommends selecting dogs from registered kennels (stable pedigrees) and disqualifies any that exhibit aggression [2]. In practice, Polish AAT organisations require dogs to pass formal exams and basic obedience tests [2]. PTK specifies that dogs must demonstrate solid obedience and be at least one year old to qualify for certification. Certification often involves temperament assessments and evaluation of the dog’s interaction with unfamiliar individuals [3]. Handlers are also trained: a PTK kynotherapist programme prescribes 128 hours of theory plus 15 hours of supervised practical work [4]. International best-practice guidelines (e.g. IAHAIO) further stress rigorous welfare standards: dogs must be in “immaculate” health and free from behavioural disorders [5]. By contrast, few countries legally mandate therapy-dog certification. In Poland, however, standards are driven by NGOs and European projects rather than law. This work reviews current Polish requirements for therapy dogs, showing that effective AAT relies on strict training and certification protocols set by professional bodies (in line with international AAT frameworks) to ensure patient and animal welfare.

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REPTILES AND AQUARIUM FISH AS UNCONVENTIONAL PARTNERS IN ANIMAL-ASSISTED THERAPY

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While the benefits of traditional pets on human well-being are well-documented, the potential of unconventional species, such as reptiles and aquarium fish, in animal-assisted services (AAS) and animal-assisted therapy (AAT) remains largely unexplored [1]. Keeping reptiles as pets is common, with millions found in households. Reptiles present unique challenges and opportunities for human-animal interactions. Factors such as the kind of species affect people's attitudes, with snakes often perceived as frightening, while turtles are more popular. Establishing meaningful relationships with reptiles can foster fascination, admiration, and empathy. Reptile-assisted support has been shown to improve internalising behaviours (e.g. withdrawal), externalising behaviours (e.g. aggression), and other behaviours such as social interaction, attention, and thought processes in children with emotional disturbances [2]. The use of aquarium fish in AAS and/or AAT has certain limitations due to the environment in which fish live, compared with other animals [3]. For this reason, aquarium fish are mainly used for non-contact service/therapy, focused on watching/observing the movement of fish. Watching fish has a calming and relaxing effect that increases the patient's calmness and reduces stress, anxiety, fear and discomfort, as well as the risk of zoonotic disease transmission [4]. This intervention is often used in outpatient clinics or specialised dementia units to distract patients from medical procedures (dental procedures, catheterisation, vaccinations, etc.) [4–5]. In addition, aquarium fish are also used for contact therapy – ichthyotherapy – used in the treatment of skin diseases such as eczema and psoriasis as well as for cosmetic purposes – cosmetic peeling, pedicure and manicure, reduction of cellulite and wrinkles. There is limited research focused on accurately identifying stress indicators in reptiles and aquarium fish and understanding their capacity to consent to therapeutic roles. Building trust and recognising the welfare needs of these species are essential steps for their safety and ethical integration into educational or therapeutic contexts.

Keywords: reptiles, fish, aquarium, animal-assisted service, animal-assisted therapy, stress, anxiety.

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SERVICE DOGS IN SEARCH TASKS: PERFORMANCE, WELFARE AND FUTURE PERSPECTIVES

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Service dogs are indispensable partners in search tasks due to their exceptional sense of smell and ability to adapt to various environmental conditions. Their performance is often compared to modern technology, but it turns out that dogs are much more versatile. Analysis of the experience of officers, the insights of trainers and practical tests demonstrated that dogs are able to accurately detect people and specific materials in complex areas, where technical means often face limited capabilities. Technologies such as drones or metal detectors effectively complement search work only under certain conditions but cannot completely replace dogs. The study found that the effectiveness of a service dog depends on proper training, the professionalism of the handler and the interaction between the person and the animal based on mutual trust. The findings show that service dogs remain an indispensable aid in searches. Combining them with modern technology can ensure even better results, while drawing attention to the need to constantly care for the welfare of animals. Future prospects are associated with closer integration of animals and technology, aiming to maximise their advantages in various fields of activity.

Keywords: service dogs, search, efficiency, technology, olfaction, interaction.

DOG INCLUSION IN INSTITUTIONS OPERATING IN KLAIPEDA CITY: ANALYSIS OF EMPLOYEE WELL-BEING, CUSTOMER EXPERIENCE AND REPUTATION OF ORGANISATIONS

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The presence of dogs in workplaces and public spaces is becoming an increasingly popular phenomenon. This shows the increasing openness of society to animals and their positive impact on people. Studies show that they help combat burnout, improve employee productivity and motivation [1]. In addition, the presence of dogs in the workplace promotes team spirit and helps employees to connect with each other more easily [2, 3]. The research involved 24 companies in Klaipėda (12 dog-friendly and 12 non-dog-friendly). A total of 355 respondents were surveyed: 24 managers, 168 employees and 163 customers. A quantitative study was carried out using anonymous questionnaires. The data were analysed using descriptive statistics, the chi-square test and the independent samples t-test. Statistical data analysis was performed using IBM SPSS Statistics 29.0 and Microsoft Excel. The results showed that, according to employees, the presence of dogs in the workplace positively affected their emotional state (63.1%) and mental health (58.9%), reduced stress, improved the working atmosphere, motivation, and job satisfaction. Strong correlations were found between the frequency of dog presence and indicators of employee well-being (e.g., stress reduction, Pearson $r = 0.83$). The main advantages reported by clients were a cosier atmosphere (23.1%), better emotional well-being (20.5%) and greater convenience for dog owners (17.9%). Most respondents (77.9%) had visited dog-friendly establishments and 67% evaluated them positively.

Keywords: dogs, dog-friendly policy, employee well-being, customer experience, organisational reputation, social responsibility.

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THE IMPORTANCE OF ANIMALS IN THE NARRATIVE LIVES OF TRAUMATISED CHILDREN AND THEIR PLAY

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Narrative therapy empowers children to re-author their life stories following exposure to trauma, including experiences of family violence. By externalizing problems and reframing them within a socio-cultural context, children gain mastery and understanding of their emotional worlds. This presentation explores the integration of animal-assisted therapy (AAT) and animal-themed play within trauma-informed narrative frameworks to promote resilience, emotional regulation, and empathy development. Drawing on the Anicare Model for Children and principles of polyvagal and attachment-informed care, the approach emphasizes co-creating positive narratives through structured, supervised interactions with therapy animals and animal-themed play interventions [1,2,4]. In one program, two six-session AAT groups were conducted with approximately 15 immigrant students from Russia, Ecuador, Syria, and Mexico. Through narrative storytelling and interactive work with therapy dogs, including giving commands and sharing their own animal experiences from their home countries, participants demonstrated increased comfort, connection, and self-confidence over time. Observations revealed that animal-assisted interactions helped normalize social communication, foster trust, and promote cultural storytelling that supported emotional safety and belonging [3]. The themes noticed during the discussion were nonverbal communication, identity formation, and hopefulness. Clinical applications include helping children identify emotional triggers, discovering identity and construct healing narratives that strengthen empathy and confidence. This abstract highlights practical, evidence-based methods for integrating AAT into narrative play therapy to support healing and growth among diverse youth populations.

Learning objectives were as follows: (1) to describe how animal-assisted and animal-themed play support the narrative re-authoring process; (2) to identify assessment and safety considerations when working with children exposed to or engaging in animal maltreatment; and (3) to apply trauma-informed and polyvagal-informed techniques for integrating animals into play therapy.

Practical takeaways: supervised animal interactions can scaffold emotional regulation and storytelling; humane education fosters accountability and empathy; polyvagal-based grounding exercises support safety and connection during therapy.

Keywords: play therapy, animal assisted therapy, trauma, narrative therapy, polyvagal theory, immigrant youth.

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THE PHYSIOLOGICAL EFFECTS OF HIPPO THERAPY ON THE HUMAN CARDIOVASCULAR SYSTEM

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Therapeutic riding is an effective form of mental and physical relaxation, which has a proven positive effect on the physiological functions of the human body, especially on the cardiovascular system. The aim of this study was to analyse the effects of therapeutic riding on changes in systolic (sBP) and diastolic blood pressure (dBP) in 68 probands of different ages, performance levels and gender. Therapeutic riding was performed in an indoor arena for 6 months, 20 minutes 3 times a week. The probands were divided according to age into three groups (5–15, 16–40, 41–60 years), according to performance level into beginner, lower intermediate and intermediate, and according to resting blood pressure values into low, normal and high systolic and diastolic blood pressure groups. The results of the measurements showed that therapeutic riding had no statistically significant effect on changes in sBP in children (5–15 years), a slight decrease was noted in adults up to 40 years of age, while in the elderly (41–60 years), there was an increase in sBP, but without statistical significance. In terms of gender, a more significant reduction in sBP was observed in females (127.93 mmHg) compared with males (144.75 mmHg), which was also confirmed by statistical significance ($P < 0.01$). Diastolic blood pressure varied only slightly across the entire proband pool. In probands divided according to performance level, we found that the most balanced dBP level (86.00–86.68 mmHg) was in the beginner category. We recorded a statistically significant difference ($P < 0.05$) before and after riding in the lower advanced category, where the diastolic pressure level dropped from 87.30 mmHg to 82.90 mmHg. Conversely, we recorded an increase in dBP levels in the intermediate group from 83.46 to 88.69 mmHg. The effect of therapeutic riding on dBP was minimal in the different age categories, but after dividing the probands according to resting sBP and dBP levels, a beneficial stabilisation of blood pressure was repeatedly confirmed, especially in probands with low and high blood pressure ($P < 0.001$). We recorded a low level of statistical significance only in the category of riders with high systolic blood pressure, where their measured values decreased by 4.91 mmHg after riding. Regular therapeutic riding may represent a safe form of moderate physical activity with beneficial effects on the regulation of the circulatory system in different population groups.

Keywords: therapeutic riding, blood pressure, systolic pressure, diastolic pressure, cardiovascular health.

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INVESTIGATION OF *DRD4* GENE POLYMORPHISM AND ITS ASSOCIATION WITH CANINE BEHAVIOUR

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In recent years, dogs have become an integral part of human life and are among the most common companion animals. However, undesirable behaviour can negatively affect both animal welfare and owner quality of life [1]. Aggression in dogs, while a normal communicative behaviour, is frequent and generally considered undesirable [2]. Understanding its genetic background is, therefore, important for animal welfare and human safety. The aim of this study was to investigate polymorphism of the dopamine receptor D4 gene (*DRD4*) gene, located in the second intron, which has been associated with unpredictable or spontaneous behaviour and aggressive tendencies in dogs of various breeds. The *DRD4* gene encodes the D4 receptor and is expressed in the limbic system regions responsible for cognition and emotion [3]. A variable number tandem repeat (VNTR) polymorphism consisting of a 17 bp insertion/deletion has been identified in the second intron. A 2013 study reported two alleles: P (194 bp) and Q (211 bp), with the P/P genotype linked to aggression [4]. In this study, 94 dogs from 35 breeds were examined. Based on an owner questionnaire, dogs were categorised as aggressive or non-aggressive, hyperactive or non-hyperactive, and exhibiting or not exhibiting spontaneous behaviour. DNA was extracted from oral epithelial cells using a Chelex solution, and genotypes were determined by PCR. Among aggressive dogs (n = 16), 62.5% carried the P/P genotype compared with 57.7% of non-aggressive dogs (n = 78). The Q/Q genotype was found in 12.5% and 19.2% of these groups, respectively. P/P was the most frequent, and Q/Q was the least frequent genotype. Comparison of the genotyping results with the owners' survey data revealed no statistically significant associations between the *DRD4* gene polymorphism and the expression of aggressive or spontaneous behaviour. Similarly, no association was found between the polymorphism and hyperactivity.

Keywords: *DRD4* gene, canine behavior, aggression, VNTR polymorphism.

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THE EFFECTS OF MEDICINAL LEECHES ON THE HUMAN BODY AND THEIR APPLICATION PRINCIPLES IN COMPLEMENTARY AND ALTERNATIVE HEALTHCARE PRACTICES AND VETERINARY MEDICINE

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Throughout human history people have used living creatures to improve health, relying on the healing powers of nature. Leech therapy is one of the more thoroughly studied natural health methods, based on the unity of all living beings on our planet [1]. One of the important indicators of a health method's value is the length of its use in practice and its developmental history. It is known that leeches feeding on the host's blood overcome blood clotting by releasing about 100 biologically active compounds from their salivary gland secretions including anticoagulants [2]. The use of medicinal leeches for health purposes emerged in ancient times across various cultures as a method for balancing "biological humors," which were believed to contribute to poor health [3]. Around 1930, antibiotics were developed and began to evolve, leading to a decline in the popularity of leech therapy, which came to be viewed as a primitive treatment method [4]. Today, leech therapy has regained significance as an auxiliary method in modern medicine, especially after the U.S. Food and Drug Administration (FDA) approved the use of leeches in 2004 to reduce venous congestion during plastic and microsurgical procedures [5]. In recent decades, new diseases have emerged, prompting further research into the applications of leech therapy. These studies have identified many new indications for its use. As a result, a better understanding of its therapeutic mechanisms has expanded the scope of leech therapy to include the reduction of complications from osteoarthritis, autoimmune diseases, cardiovascular diseases, cancer, and diabetes [6]. Scientific studies and clinical reports indicate that medicinal leech therapy has compelling potential and can be successfully used for both human and animal healthcare purposes.

Keywords: leech therapy, leech therapy and veterinary medicine, effects of leech therapy.

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MANES AND MEMORIES: AN EQUINE-ASSISTED PROGRAM BRIDGING NEURODEGENERATIVE CARE AND GRADUATE CLINICAL TRAINING

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The rise in neurodegenerative conditions underscores the need for innovative, practical, and accessible solutions. Neurodegenerative disease patterns can impact language, memory, and behavior, thereby increasing the need for support in completing daily activities [1, 2]. Growing research supports the use of animal-assisted therapy (AAT) for those impacted by neurodegenerative conditions; however, practical implications are still emerging [3]. This presentation will discuss the creation of the Manes and Memories program, an equine-assisted, community-based initiative for individuals impacted by neurodegenerative conditions and their care partners. This program aims to foster communication, emotional connection, and quality of life through guided interactions with horses. The Manes and Memories program is facilitated by graduate student clinicians in the field of communication sciences and disorders, offering the dual benefit of enhancing the student clinicians' clinical readiness and providing rich, multisensory experiences for the participants. Graduate student clinicians are challenged to develop their professional skills, self-confidence, and flexibility as future practicing professionals. One participant noted that this program offered her father, who lives with a neurodegenerative condition, "an opportunity to have some freedom from an often isolating illness." This presentation aims to describe the practical aspects of implementing similar programs, discuss lessons learned, and opportunities for offering equine-assisted learning in higher education settings.

Keywords: neurodegenerative conditions, equine-assisted learning, social engagement, speech-language pathology, graduate clinical training.

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BEYOND WELFARISM: REIMAGINING HUMAN-EQUINE RELATIONSHIPS THROUGH ETHICAL AWARENESS AND SUSTAINABLE PRACTICES

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The Beyond Welfarism community represents a transformative shift from traditional equine welfare frameworks toward a holistic approach centred on ethical awareness, sustainability, and mutual connection.

This research synthesises insights from interdisciplinary working groups to propose a comprehensive model for human-equine relationships that prioritises flourishing over mere absence of suffering. Traditional welfare approaches have focused primarily on physical health and utility, often overlooking horses' complex emotional, social, and environmental needs.

Our analysis of human motivations for equine engagement reveals five primary drivers: adrenaline and skill development, desire for non-judgmental companionship, symbolic power representation, control-seeking behavior, and healing through emotional resonance.

The proposed framework emphasises six core principles:

1. Ethical awareness that prioritises equine agency and behavioral understanding;
2. Sustainable practices incorporating land regeneration and biodiversity enhancement;
3. Mutual connection based on trust and understanding rather than utility; collaborative innovation through creative, voluntary interactions; comprehensive education and advocacy;
4. Critical self-reflection linking human growth to equine wellbeing; implementation strategies include co-created activities in natural settings, rotational grazing systems, interspecies cohabitation, and therapeutic modalities for aging horses.
5. Economic analysis demonstrates that ethological and regenerative practices offer long-term commercial viability while supporting ecosystem restoration.
6. The study addresses critical gaps in geriatric equine care, proposing dignity-centred approaches for retired horses through continued social integration and gentle engagement.

Results indicate that shifting from ego-driven to empathy-based motivations creates more authentic relationships while supporting environmental sustainability.

This paradigm challenges the fundamental concept of “using” horses, instead promoting voluntary participation in mutually beneficial activities. The Beyond Welfarism community offers a roadmap for transforming equine industries through legislative engagement, certification programs, and community activation, ultimately creating environments where horses achieve genuine happiness rather than merely avoiding suffering.

Keywords: equine welfare, ethical awareness, sustainable practices, human-animal relationships, collaborative innovation, environmental regeneration

Acknowledgments. We thank all participants of the Beyond Welfarism community and working group contributors for their insights and collaborative spirit in developing this transformative framework.

ANIMALS IN EDUCATION: FROM ELIGIBILITY CRITERIA TO THE MORAL DILEMMA BETWEEN ANIMAL WELFARE AND PUBLIC EDUCATION

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In modern society, the use of animals in education has become a subject of intense discussion at the intersection of ethics, science, and public awareness. Key questions arise: which animals can be considered suitable for conscious interaction with humans; where should moral boundaries be drawn; and how can we ensure that every individual involved in educational activities experiences minimal stress and the opportunity to live with dignity. These questions are becoming increasingly urgent as societal expectations toward animals shift, and as the cultivation of empathy and responsibility is recognised as a central goal of education. Evaluating the suitability of animals for educational purposes requires careful attention to their biological, physiological, and ethological needs. Not every species or individual is prepared for interaction with humans as this depends on temperament, environmental conditions, and the expertise of the educator. A particularly sensitive issue is whether it is ethical to involve animals in educational activities if they have never before been used in such contexts. At the heart of this debate lies a moral dilemma: on the one hand, the welfare of an individual animal must be respected; on the other, educational engagement can raise knowledge and awareness in society at large. This tension demands a careful balance. Ensuring animal welfare must remain a top priority, requiring continuous monitoring, respect for individual characteristics, and clear limits on when an animal may or may not be involved. Animals should never be treated as tools, but as equal living beings whose needs are as important as educational goals. Ultimately, educational practices should foster not only knowledge, but also mutual respect and recognition of the intrinsic value of life. At its core, the dilemma asks: one animal and thousands of educated people, or thousands of uneducated people and millions of lost animal lives?

Keywords: animal welfare, ethics, education, human-animal interaction.

ANCIENT SOUND HEALING AND ITS INFLUENCE ON EQUINE PSYCHOLOGY

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Sound therapy or sound healing is a therapeutic approach of sound frequencies to the body and mind of an individual to help improve physical and mental health. There is much controversy over whether sound healing is an effective and efficient treatment method; however, the scientific basis for its effectiveness may point to positive results. The aim of this study was to explore how sound therapy can be beneficial for improving horses' psychology by reducing their fear factors. In this pilot study, 4 horses were given sound healing sessions for a week. During each session, the horses' pulse and body language were assessed. Fear tests for horses with social and physical stimulus were performed to evaluate the effectiveness of sound therapy before and after all sessions. After analysing the results, the change of reaction time was assessed and compared. The results revealed that during the sound sessions, the horses' positive behaviour increased, while their heart rates decreased accordingly ($P < 0.05$). Only one participant allowed being touched by the assessor during the pre-therapy testing, while after the treatment three participants permitted physical contact. The total reaction time in both tests decreased when comparing the horses' responses to stimuli before and after therapeutic sessions. However, the horses' responses to social stimulus were 56.4% higher compared with physical. During the pilot test, no statistically significant relationship was found. In conclusion, sound therapy can be an effective tool for reducing stress in horses and improving animal welfare in various aspects. Even so, there is still too little research to accurately understand the impact of sound vibrations on horse psychology. Minimising environmental factors, expanding the cohort, and optimising research methods opens the opportunity to expand and refine the results, and can give a new way to improve animal welfare.

Keywords: sound therapy, animal welfare, horse psychology.

THE RELATIONSHIP BETWEEN PHYSICAL ACTIVITY AND OBESITY IN CANINE HEALTH

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Dogs' health is becoming an increasingly pressing issue, making it crucial to understand the causes of obesity and other health problems in dogs [1]. Lack of physical activity is an important risk factor that can negatively affect a dog's health. A sedentary lifestyle often leads to weight gain. Moreover, physical activity plays a crucial role in a dog's emotional well-being, as regular exercise reduces stress, stimulates brain activity, and strengthens the bond between the animal and its owner [2]. The aim of this work was to determine the relationship between physical activity and obesity in canine health. The dogs ($n = 31$) were selected randomly, regardless of any factors, but of similar age and breed. Based on a 9-point body condition scoring system (WSAVA), the dogs were classified into 9 categories. Additionally, an effort was made to determine how much time each dog spends being active per day, with activity (as low, medium, or high) assessed according to the FEDIAF scale. Statistical analysis revealed that of the 31 dogs studied, 58.1% were of optimal weight (body condition score 5), 9.68% were underweight (scores 2 or 3), and 32.3% were overweight (score 7) or obese (score 9). When examining the dogs' activity levels, it was observed that most dogs that were physically under-exercised (up to 1 hour of physical activity) were overweight or obese ($P < 0.05$). Regular exercise is important for dogs, as studies show that overweight or obese dogs are usually insufficiently active, whereas dogs whose owners engage them in some form of sport or physical activity are generally at an optimal body weight.

Keywords: dog, activity, obesity, health.

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LEARNING WITH THE HERD: DEVELOPING FIRST-YEAR STUDENTS' NONCOGNITIVE SKILLS THROUGH EQUINE-ASSISTED LEARNING

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In higher education, student success is often measured through cognitive metrics such as GPA and standardized test scores. Yet research increasingly has demonstrated that persistence, resilience, and transformation depend equally – if not more – on noncognitive skills such as self-directed learning, grit, and growth mindset [1–4]. Despite this, higher education institutions continue to privilege what is easily quantifiable while marginalizing the relational, emotional, and dispositional dimensions of learning. This presentation explores the Students Together Reaching Individual Developmental and Educational Success (STRIDES) Equine-Assisted Learning course as a case study for reclaiming the noncognitive learners' aspects facilitative of persistence, growth, and success in education. Designed for first-year college students, STRIDES is not a riding or horsemanship program, but rather an experiential, relationship-centered course that integrates classroom learning with barn-based interactions. Horses, as prey animals, are acutely responsive to body language, presence, and consistency. They do not comply with authority but respond to authentic relational engagement. In this sense, they act as mirrors for students' emotional states, offering immediate feedback that fosters meta self-awareness emotional self-regulation and communication skills. Through structured activities, reflection, and scaffolded challenges, students learn to recognize academic “blind spots,” balance autonomy with structure, and cultivate resilience in the face of setbacks. Weekly reflections and a final “Legacy Statement” anchor these experiences, helping students translate noncognitive insights into academic action plans. Findings suggest that equine-assisted learning environments create powerful opportunities for students to reframe education as relational and transformational, rather than transactional. The presentation will conclude by raising broader implications for educators, administrators, and policymakers: What would redesigning learning environments that recognize belonging, agency, and emotional intelligence as foundational- rather than supplemental - mean to student success? Horses remind us that the deepest learning occurs not through control, but through connection.

Keywords: equine-assisted learning, experiential learning, noncognitive skills, self-directed learning, academic growth mindset, grit, student success, relational pedagogy, first-year college students, belonging.

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THE PROFESSIONAL'S PERSPECTIVE: HOW DO WE BENEFIT WORKING WITH HORSES?

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The relationship between humans and horses began over 6000 years ago with the domestication of wild horses. While the benefits of horseback riding have been recognised since antiquity, and the positive effects of human–horse interaction on individuals with disabilities are well documented, the impact on equine professionals remains largely underexplored [1–2]. This presentation investigates how working with horses affects these professionals. Drawing on personal and shared experiences, it proposes that horses facilitate personal – mental, physical, and social – as well as professional development. Working with horses requires technical expertise (horsemanship skills and knowledge), creative problem-solving abilities, and effective risk management. Professionals may experience stress relief, increased confidence, improved behavioural and emotional regulation, as well as greater empathy and connection. Their work often involves intensive physical activity, especially when caring for and riding horses. Working with horses can provide a sense of purpose, foster networks with other professionals, and demand continuous learning. However, because horses are flight animals, risk assessment must be approached with great caution. Working with equines may involve physical risks (such as injuries and chronic conditions) and mental risks (including stress, burnout, and isolation). Moreover, equine-assisted therapy does not always ensure financial stability and may involve legal and liability challenges.

Keywords: equine professionals, horse-human interaction, professional development, personal growth, mental health, equine-assisted learning.

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PRACTITIONERS' PERSPECTIVES OF EQUINE-ASSISTED SERVICES: A QUALITATIVE STUDY FROM THE UK AND IRELAND

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Equine-Assisted Services (EAS), which incorporate horses in a variety of ways to improve human wellbeing, are becoming increasingly commonplace in today's society. While research regarding the efficacy of EAS is ongoing, the perspectives of those who provide such services have been largely underexplored, despite being well placed to offer unique insights. This study aimed to address this gap by conducting semi structured interviews with 15 practitioners across the spectrum of EAS. These interviews examined practitioners' views on the horse-human bond, how horses may affect change for clients, as well as areas of concern for practitioners and the field in general. Transcripts were analysed via reflexive thematic analysis using a critical realist lens. This methodology aims to explore subjective lived experience while critically examining these views to identify possible underlying mechanisms of change. Five themes were identified. Three related to the effects of the horse-human bond on EAS, including its importance for connection, building relationships, and the enriching value of horses. Two themes explored current challenges to the field of EAS. Findings highlighted the complex nature of EAS, including challenges faced by practitioners, such as minimum standards, competencies to practice, training and governance. Practitioners stressed the importance of having a deep knowledge of horses and equine welfare and the impact of this on the service provided. While these findings demonstrate the benefits inherent within EAS, they also suggest that further progress is needed in the field if EAS is to attain optimum efficacy and professionalisation.

Keywords: equine assisted services, human-animal bond, practitioners, reflexive thematic analysis, One Health, horse and human wellbeing, EAS competencies and training.

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INTEGRATING BOVINES INTO ANIMAL-ASSISTED ACTIVITIES: SELECTION AND TRAINING CASE REPORT

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Bovines are herd animals and tend to develop long-lasting social bonds [1]. These traits can be utilised in developing cattle herds or choosing individual bovines that are suitable for animal-assisted activities (AAA). So far, five temperament patterns have been described in cattle: shyness/boldness, exploration/avoidance, activity, aggressiveness and sociability [2]. However, it can be challenging to identify animals with consistently favourable behaviour.

The aim of this research was to qualitatively assess early temperament traits and changes in their development during the first year of two calves. The study involved Limousin crossbred heifer and a Holstein crossbred steer castrated at four months of age. Both calves were the same age and had been interacting with people every day since birth. At the age of four months, the calves were housed together, and intensive desensitisation training started.

Throughout the period up to 12 months of age, both calves were constantly friendly with their handlers, although the heifer remained cautious with persons which could be potential AAA recipients. In contrast, the steer was consistently willing to interact with various groups of people, without distinguishing between adult and child visitors. This trait of the steer's temperament remained unchanged from birth.

Behavioural dynamics showed significant changes at 10 months of age. While the steer became cautious and expressed agitation at unfamiliar stimuli, the heifer became vigorous and started to demonstrate bullying behaviour within the herd. It is worth noting that this was the age at which calves began to behave independently. However, calves demonstrated predictable behaviour during interactions with both handlers and visitors, specifically during physical contact such as brushing or lying down together during rumination.

After reaching the age of 12 months, the calves entered the next stage of development. During this period, their reactivity decreased, they became more involved in interacting with visitors, and the calves' reactions to environmental stimuli returned to the level demonstrated before puberty started (10–12 months).

This study provides monthly observations that can be developed in more detailed research to determine the best selection and training methods for bovines that will be involved in AAA.

Keywords: cow, cuddling, bovine, therapy, training, selection, activities

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BRIDGING HUMAN AND CANINE ONCOLOGY THROUGH A ONE HEALTH CONCEPTION

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The One Health conception emphasises the interconnectedness of human and animal health and provides a strong foundation for transdisciplinary collaboration in cancer research. Comparative oncology has revealed striking genetic, molecular, and pathophysiological similarities between canine and human tumours, especially regarding mechanisms of drug resistance. Spontaneous canine cancer models more faithfully reproduce the oncogenesis and heterogeneous tumour microenvironment of human malignancies than induced laboratory models, making them an invaluable translational tool for understanding disease progression and testing novel therapies [1]. Strengthening cooperation between human and veterinary medicine, therefore, creates opportunities to accelerate discovery and improve therapeutic outcomes for both species. Emerging evidence also suggests that psychological stress may contribute to cancer incidence in dogs, offering an accessible model to explore behavioural and physiological links between stress and oncogenesis. Similar investigations in humans remain difficult due to socioeconomic and genetic confounding factors [2]. In diagnostic research, trained detection dogs have demonstrated remarkable sensitivity in identifying lung cancer by analysing volatile organic compounds in exhaled breath samples, achieving accuracy rates from 71.3% to 97.6% (mean 83.9%) [3]. These results highlight the potential of canine olfaction as a non-invasive early detection tool that could complement modern molecular screening methods. Therapeutically, Animal Assisted Therapy (AAT) has gained attention for its biopsychosocial benefits in oncology care. Clinical studies with pediatric cancer patients show that interaction with therapy dogs can reduce pain, anxiety, and depressive symptoms, while improving mood, motivation, and social engagement during treatment [4]. Altogether, these findings outline three major domains in which cooperation between humans and dogs is both possible and necessary: comparative pathogenesis, diagnostic innovation, and therapeutic intervention. Integrating these approaches under the One Health framework can enhance translational research, enrich holistic cancer care, and ultimately improve survival and quality of life for both human and canine patients.

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WELFARE AND EFFICIENCY OF SERVICE DOGS: THE ROLE OF SEX DIFFERENCES IN TASK PERFORMANCE

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Service dogs are indispensable partners in human activities, and their performance and efficiency are closely linked to proper selection and welfare. Sex-related characteristics have been observed to influence suitability for different tasks. Previous studies have shown that females tend to show greater obedience and constant attention to their handler [1], which is consistent with the results of this study, where females showed better results in scent recognition and tracking tasks, which require close cooperation with the dog handler [2]. These traits make them more effective in tasks requiring consistency and precision, such as tracking or scent detection. Male dogs, in turn, show stronger defensive instincts and are, therefore, more frequently assigned to tasks where physical strength and rapid reaction are essential.

Sterilisation also plays an important role, as it may reduce excitability and improve obedience, while at the same time potentially influencing health status or decreasing working capacity in certain areas. This underlines the need for individual decisions that consider both the specific characteristics of each dog and the requirements of service work.

The results of a comparative study analysing differences in task performance between male and female service dogs showed that recognising gender differences and addressing welfare needs increases the working efficiency of service dogs. This approach ensures high task quality while maintaining better animal welfare and longer working capacity [2].

Keywords: service dogs, welfare, sex differences, obedience, performance.

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MITOQUINOL MODULATES LIVER RESPONSES TO LONG-ACTING METHYLPREDNISOLONE ACETATE IN DOGS

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Depot corticosteroids, such as methylprednisolone acetate, are commonly administered to dogs for managing inflammatory and immune-mediated disorders [1, 2]. Despite their clinical utility, these long-acting formulations may influence hepatic metabolism and structural integrity, yet data in veterinary species remain scarce [3]. Mitoquinol, a mitochondria-targeted antioxidant, has been proposed to counteract oxidative stress and protect against corticosteroid-associated liver injury [4, 5]. This study investigated the temporal profile of hepatic changes induced by a single intramuscular dose of methylprednisolone acetate (2 mg/kg) in healthy beagle dogs and explored the potential modulatory effects of mitoquinol. Seven dogs were observed for 56 days. Serum activities of alanine aminotransferase (ALAT), gamma-glutamyl transferase (GGT), and alkaline phosphatase (AP) were determined at baseline and on days 7, 14, 21, 28, and 56. Ultrasound-guided liver biopsies were obtained at four time points (days 0, 14, 28, and 56) and evaluated by hematoxylin-eosin, PAS staining, and α -smooth muscle actin immunohistochemistry. Dogs receiving methylprednisolone acetate exhibited significant increases in AP and GGT ($P < 0.05$), peaking on day 7 and day 14, respectively. Histological assessment showed hepatocyte swelling, cytoplasmic vacuolization, and periportal collagen deposition, which persisted in several animals on day 56. In contrast, mitoquinol-supplemented dogs demonstrated smaller fluctuations in enzyme levels and less extensive structural remodeling, indicating potential hepatoprotective effects [4, 6, 7]. These findings suggest that mitoquinol may mitigate hepatic consequences of long-acting corticosteroid therapy in dogs. Such approaches could contribute to maintaining long-term liver health, particularly in animals engaged in therapy and assistance activities where sustained wellbeing is essential.

Keywords: mitoquinol, methylprednisolone acetate, liver protection, dogs, antioxidant therapy, hepatic alterations.

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EFFECTS OF CASTRATION MANDATE FOR PSYCHIATRIC SUPPORT DOG CERTIFICATION: ANALYSIS IN THE PSYCHOEMOTIONAL, CLINICAL AND CULTURAL FIELDS, AND IN THE ZOOTECHNICAL FIELD: ANALYSIS OF THE EFFECTS OF A TWENTY-YEAR PROJECT IN ITALY

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The study illustrates the phenomenon of the imposition of castration in many countries that adopt “blind” an element of the management and organisation model of the service and assistance dog system: the obligation *condicio sine qua non* to castration. Even access to flights of some airlines is determined by this specific criterion. This aspect denies the right of access to users whose dogs are entire. In Italy, Cascina Blue and other organisations in collaboration with selected breeding farms are carrying out significant steps in the sector, employing mares and stallions as working dogs. The results, on different levels, in the short, medium and long term, are surprising. Furthermore, mandatory castration falls within the scope of unconsidered choices that can expose both dogs and owners to risks. The rigidity of this criterion appears unbalanced in a context in which all the others remain vague [1]. This happens in many countries. In others, where regulation is more complex, the imposition of the parameter clashes with the ethics of responsibility and guarantee of animal welfare. Furthermore, in many cases in the field of mental health [2], exposure to the risks of unnecessary operation for life and to the nuances of behaviour that amplify the margin of probability of change, turns out to be an element that calls for reflection. Attitudes of refusal or traumatic events have been recorded and will be illustrated in detail. The work illustrates all these dynamics, in order to promote respectful and responsible management, aimed at the evolution of the system and society, for the advantage of dogs and humans [3].

Keywords: autism, schizophrenia, bipolar disorder, borderline disorder, anxiety, panic disorder, eating disorder, dysmorphic disorder, gender dysphoria, depression, obsessive compulsive disorder, epilepsy, working dogs, accessibility, inclusion, education, dog castration, zootechnic.

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INTERVENTION IN A RESIDENTIAL DAY CENTER FOR ADULTS WITH DISABILITIES IN ITALY: STRATEGIES AND RESULTS

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This abstract presents an ambitious three-year project still in progress, carried out in Milan in a Day Center for the support and services to young people and adults with disabilities. The project uses, among other tools, the relationship with a team of trained and certified dogs, constantly monitored [1]. The dogs are all included in a careful selection programme in collaboration with important breeders [1]. The path has numerous peculiarities because, while remaining within the law and in compliance with every regulatory plan, it is structured on atypical choices. The result, in particular thanks to the valuable role of the human resources involved, is achieved because a rule of management of the behaviour of the dogs has been considered and applied that puts the users in an “up” condition [2]. They are not only users but actively shape the training and behaviour of the various subjects involved. The breeds and species involved in the project are rabbits, dwarfs, and lops, domestic rats, long-haired Scottish Shepherd dogs, Pharaoh Hound, West Highland White Terrier, Chinese Crested Dog, Staffordshire Bull Terrier, Flat Coated Retriever, Golden Retriever, Jack Russel Terrier, and European Cat. The project is a model for future developments in the application of animal-assisted interventions, for its flexibility, competence, exportability of learning and care of relationships and the network [3]. Furthermore, it offers a very interesting perspective on the change and evolution of cognitive skills in humans and animals, solicited in particular ways and contexts.

Keywords: autism, schizophrenia, bipolar disorder, borderline disorder, anxiety, panic disorder, eating disorder, dysmorphic disorder, gender dysphoria, depression, obsessive compulsive disorder, epilepsy, working dogs, accessibility, inclusion, education.

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THE EFFECT OF TWO COMMERCIAL PREPARATIONS ON THE OCCURRENCE OF TICKS IN DOGS

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The aim of the study was to compare (over the following three years) two commercial preparations (Neostomosan and Bravecto) for the occurrence of ticks in hunting dogs. The dogs were divided into groups according to body size and coat length. In the group of large, short-haired dog breeds, after the application of Neostomosan, an average of 2.81 crawling ticks per walk and 0.62 attached ticks per walk with the dog were found during the observation period. In the group of long/wire-haired dog breeds, an average of 1.67 crawling ticks and 0.07 attached ticks were found during the entire observation period. The effectiveness of Bravecto in the group of large, short-haired breeds was determined to be an average of 1.45 crawling ticks per walk and 0.11 attached ticks per walk with the dog over the monitored period. In the group of large, long/wire-haired breeds, an average of 2.50 crawling ticks and 0.01 attached ticks per walk with the dog were found after the application of Bravecto. All attached ticks were dead after the use of Bravecto, while those treated with Neostomosan were alive. Comparable results were obtained for both products studied. Compared with the group of dogs that were not treated with any active substance, the incidence of ticks was several times lower in the groups of treated dogs (for both products).

Keywords: bravecto, neostomosan, ectoparasites, ticks.

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ANALYSIS OF THE RELATIONSHIP BETWEEN MICROCLIMATE CONDITIONS AND FLIGHT PARAMETERS IN FALCONRY-CONDITIONED SAKER FALCONS (*FALCO CHERRUG*)

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The Saker Falcon (*Falco cherrug*) is one of the most frequently used raptors in falconry [1–3]. Birds of prey are complex and intelligent species, prone to behavioural changes if kept in poor conditions or trained improperly [4]. Understanding their flight performance is essential for optimising training and welfare [5,6]. This study evaluated flight parameters of a male Saker Falcon to support falconry practice. Research was conducted at the Astur Falconry Centre, Red Rock Castle, Slovakia, over six months (June–November 2023). Flight data were collected using the Marshall Turbo GPS System, and analyses were performed with SPSS software using Pearson's correlation coefficient (r). The falcon's average body weight was 759.36 g (\pm 25.70), with a maximum of 832 g and a minimum of 725 g. It flew an average distance of 4.484 km (\pm 2.516), with a maximum of 16.6 km and a minimum of 1.1 km. The mean maximum speed was 108.25 km/h (\pm 20.096), ranging from 70 to 166 km/h, while the average speed was 41.32 km/h (\pm 3.949). The bird reached an average altitude of 53.65 m (\pm 29.408), with a maximum of 153 m. Average flight duration was 5 min 55 s (\pm 3:16), with the longest flight 21 min 26 s. Significant correlations ($P < 0.05$) were observed between maximum speed and altitude ($r = 0.217$), distance flown ($r = 0.241$), and duration ($r = 0.238$). Environmental factors influenced flight: air humidity affected maximum speed ($r = 0.33$) and duration ($r = -0.331$) during lower temperatures (18–23°C); humidity below 46% extended free flight ($r = 0.72$; $P < 0.01$); humidity between 55–68% affected maximum altitude ($r = 0.373$). In conclusion, flight characteristics of *Falco cherrug* are interconnected and shaped by environmental factors. These findings support improved falconry training and welfare of raptors in managed care [4–6].

Keywords: Saker Falcon, falconry, flight parameters, environment, training birds.

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THE INFLUENCE OF THE OWNER'S AGE ON THE CHOICE OF DOG BREED

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Keeping a dog can provide many psychological and physical benefits for owners [1]; however, these benefits often depend on the quality of the relationship between the dog and the owner [2]. Therefore, choosing a dog that does not match the owner's expectations and lifestyle may have negative consequences for both parties [3]. The aim of this study was to evaluate the breed selection criteria of dog owners of different ages.

During the investigation, 340 respondents were interviewed. The questionnaire included questions regarding respondents' choices of dog breeds based on breed type, size, appearance, temperament, required care, health, popularity, price, and place of acquisition. Descriptive statistics and the χ^2 test were used to analyse the data, in order to assess the relationships between variables and their strength.

Most respondents across all age groups chose dogs based on the following criteria: very small ($\chi^2 = 3.1145$, $P > 0.05$), short-haired ($\chi^2 = 9.5508$, $P < 0.05$), white dogs ($\chi^2 = 5.5495$, $P > 0.05$), dogs with good character and no special training ($\chi^2 = 1.363$, $P > 0.05$), dogs friendly with everyone ($\chi^2 = 6.1101$, $P < 0.05$), very affectionate dogs ($\chi^2 = 1.333$, $P > 0.05$), dogs with the longest life span ($\chi^2 = 2.4849$, $P > 0.05$), being a family pet ($\chi^2 = 2.7715$, $P > 0.05$), breed popularity does not matter ($\chi^2 = 2.5317$, $P > 0.05$), market price matters ($\chi^2 = 4.3199$, $P > 0.05$) and would look for a dog from a reliable breeder. The need for supervision of the dog ($\chi^2 = 5.2392$, $P > 0.05$) and the breed's predisposition to health problems ($\chi^2 = 5.0651$, $P > 0.05$) were very important criteria for respondents of all age groups. Most respondents across all age groups chose dogs of moderate activity ($\chi^2 = 0.9335$, $P > 0.05$), dogs that should be played with for 1–2 hours a day ($\chi^2 = 9.0159$, $P > 0.05$), or dogs that should be walked once a day ($\chi^2 = 32.5967$, $P < 0.001$).

Keywords: dog, owner's age, dog's appearance, dog's character, dog's need for care.

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THE ROLE OF FARM ANIMALS IN MODERN RECREATIONAL ACTIVITIES: BENEFITS AND CHALLENGES

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Animals constitute an essential component of human life and are increasingly recognised for their contribution to leisure, health, and social well-being. In recent years, the integration of farm animals into recreational contexts has gained prominence for its potential to promote human well-being, enhance educational outcomes, and support sustainable tourism [1]. Evidence suggests that interactions with farm animals reduce stress, improve emotional health, and foster stronger connections with nature, particularly within urban populations. Furthermore, such activities contribute to rural development by attracting tourism and raising awareness of agricultural practices and animal welfare.

Horses are central to many recreational activities, particularly equestrian sports, trail riding and therapeutic riding programmes, because their calm behaviour makes them ideal for therapy settings. Horseback riding offers both physical and emotional benefits, especially for individuals with physical or developmental disabilities [2]. Pigs are increasingly popular in mini zoo parks and farm tours due to their intelligence and social behaviour. They engage visitors, especially children, with their trainability and curiosity, fostering educational opportunities about animal behaviour and welfare. Cows are often featured in dairy farm tours and agrotourism experiences, where visitors learn about milk production and sustainable farming practices. In some cases, cows also participate in therapeutic animal programmes because of their gentle interactions with humans. Sheep and goats are staples of petting farms and interactive animal experiences. Both species are often used for hands-on learning in educational settings, where they provide insight into wool production, grazing behaviours and sustainable farming. Poultry species, including chickens, ducks, and turkeys, serve primarily in educational programmes, where visitors learn about egg production, poultry farming and the role of birds in agriculture. Children particularly enjoy engaging with poultry in petting zoos and egg-collecting activities, which help connect them to food sources.

However, incorporating animals into recreation also presents several ethical and logistical challenges. Ensuring animal welfare, managing zoonotic risks, and balancing the educational aspect with commercial interests require careful planning and regulation. By addressing these issues, we aim to enhance the role of farm animals in fostering meaningful human-animal interactions while maintaining high ethical standards.

Keywords: farm animals, recreational activities, agrotourism, welfare.

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EFFECT OF NUTRITION ON HOOF HEALTH IN HORSES – KEY TO A LONG CAREER AS THERAPY HORSE

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Horses' hooves are vital for stability, movement, and protection, acting as shock absorbers that reduce stress on bones and joints. Proper hoof function is essential for performance, therapy participation, and overall well-being [1, 2]. Because training therapy horses takes time, maintaining their health is crucial. Balanced nutrition rich in protein, vitamins, minerals, and fatty acids strengthens hooves, while poor nutrition can cause cracking, brittleness, or laminitis [3]. This study evaluated the impact of nutrition on horse hoof health, focusing on hoof structure, strength, and growth. The study involved 24 adult horses, divided into two groups of 12 horses each. Group 1 received grass, hay, crushed oats, and dietary supplements, while Group 2 received the same diet without supplements. Physical activity of horses was recorded using a three-point scale. Hoof care was regularly maintained, and growth was measured every eight weeks over six months, before and after farrier visits. Supplemented horses (Group 1) showed significantly greater hoof growth than non-supplemented horses (Group 2). During the first two months, Group 1 hooves grew 0.4 cm more ($P = 0.01$). Growth advantages were seen in both pasture-kept (+0.4 cm, $P = 0.001$) and stall-kept horses (+0.6 cm, $P = 0.01$). By activity, hooves of supplemented horses grew more in all categories: +0.4 cm in low-, +0.5 cm in medium- and +0.5 cm in high-activity horses. At 3–4 months, the growth of horses' hooves in Group 1 exceeded the growth of hooves in Group 2 by 0.6–0.8 cm, with significant differences under both pasture ($P = 0.007$) and stall conditions ($P = 0.009$). After 5–6 months, growth of horses' hooves remained higher by 0.5–0.7 cm, especially in pasture ($P = 0.01$) and stall horses ($P = 0.006$). Activity analysis showed significant differences for low- and medium-activity horses (0.5–0.6 cm, $P \leq 0.05$), but not for high-activity horses. Overall, supplementation improved hoof growth across all conditions, with slightly higher hoof growth in stall-kept and low-activity horses. These results confirm that balanced nutrition, particularly dietary supplementation, plays a key role in promoting healthy hoof growth and structure.

Keywords: horses, diet, hoof health.

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THE PRELIMINARY RESULTS OF SURVEY ABOUT FISH WELFARE STATUS ON SELECTED RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) FARMS IN SERBIA

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Human concerns about fish welfare have increased in recent years. Regardless of the rearing purpose, both the characteristics of the artificial environment and management practices affect the biological functions and mental state of the fish [1]. Based on the official data from the Agency for Statistics of Serbia, the total production of rainbow trout (*Oncorhynchus mykiss*) by fish farms in 2024 was 1790 tons, with facilities covering a surface area of 63.744 m². There are not many literature sources on fish welfare conditions in the context of Salmonidae aquaculture production in Serbia. It was our motivation to arrange a screening investigation about the staff's knowledge and implementation of the principles of the function-based approach to fish welfare. Our research assessed five rainbow trout fish farms in the following municipalities: Despotovac, Paracin, Uzice, Bajina Basta and Kraljevo. The study included a survey with fish farm production managers and the use of a questionnaire based on "The MyFishCheck" [2] with more than 20 questions of important parameters, i.e. personnel training or education, daily check, predator protection, plant cleanliness, mortality documentation, biomass documentation, feeding interval and rate, etc. Additionally, it made measurements of six water quality parameters on-site using portable instruments (air temperature; water temperature; concentration of dissolved oxygen DO; % of DO saturation; conductivity; pH). Based on the results of the research, it can be concluded that in the majority of surveyed farms (80%), the basic principles of fish welfare protection are at a relatively low level, and there exists considerable space for improvement of the current situation. This primarily relates to continuous education about welfare-related issues, including knowledge of the specific requirements of fish, considerate handling, detecting abnormalities in fish behaviour and fish health and how they react if problems show up.

Keywords: Salmonidae, fish pond, estimation, animal wellbeing.

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IMPACT OF PET OWNERSHIP ON MENTAL HEALTH AMONG CANCER PATIENTS: A SYSTEMATIC REVIEW

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Cancer diagnosis and treatment are frequently associated with psychological distress, including anxiety, depression, emotional exhaustion, and social isolation. In recent years, pet ownership has been increasingly recognised as a potential psychosocial resource that may support emotional well-being in individuals undergoing cancer treatment. However, empirical evidence regarding its role remains fragmented. This systematic review aims to synthesise existing research on the impact of pet ownership on the mental health of cancer patients and survivors. A comprehensive literature search was conducted using PubMed, Scopus, Web of Science, and Google Scholar for studies published between 2015 and 2025. Studies were included if they examined adult cancer populations and explored psychological or emotional outcomes associated with pet ownership. Eleven studies met the inclusion criteria, encompassing qualitative, cross-sectional, cohort, and mixed-methods designs. Thematic analysis was employed to identify recurring patterns across studies. Findings indicate that pet ownership is generally associated with improved emotional well-being, reduced feelings of loneliness, and enhanced quality of life among cancer patients [2]. Companion animals were found to provide emotional comfort, routine, and a sense of purpose, which contributed to reduced stress and improved coping during treatment and recovery [3]. Additionally, pet ownership encouraged physical activity and social engagement, indirectly supporting psychological resilience [4]. However, several challenges were also identified. These included the physical and financial burden of pet care, concerns related to infection risk in immunocompromised patients, and emotional stress related to caregiving responsibilities [1]. These findings highlight the dual role of pet ownership as both a source of emotional support and potential stress. Future research should employ longitudinal designs and explore culturally diverse populations to better understand the long-term implications of pet ownership in oncology care and to inform the development of supportive, patient-centred interventions [3].

Keywords: pet ownership, mental health, oncology, psychosocial support, human-animal interaction, coping mechanism.

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EXPERIENCES AND BEST PRACTICES OF ANIMAL-ASSISTED EDUCATION AT KARALIUS MINDAUGAS VOCATIONAL TRAINING CENTER

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At Karalius Mindaugas Vocational Training Centre, animal-assisted activities have become an increasingly significant component of the educational process. About one-third of teachers (34%) involve their own pets in classroom activities. While initially this may appear to be a convenient tool for practical tasks, our experience has shown that the presence of animals in the classroom produces a much deeper impact. It enhances student motivation to learn, reduces stress levels, and contributes to a more positive and supportive learning environment.

A notable initiative within our community is “Reading with a Dog”, organised in the school library. This activity is intended for children who face emotional and psychological difficulties. The dog serves as a patient and non-judgmental listener, which allows children to feel safe, alleviates anxiety, and strengthens their self-confidence. Our observations indicate that this initiative contributes not only to the development of reading skills but also to the improvement of emotional well-being. It is noteworthy that a significant proportion of our students choose the animal caretaker profession precisely because of their own positive experiences with this therapeutic interaction. Most students (around two-thirds) reported being motivated to remain close to animals due to these experiences.

Another important tradition is the annual Christmas ornament workshop, during which decorations are created for animal shelters using recycled materials. This activity fosters creativity, ecological awareness, empathy, and a sense of community. The participation of students’ and teachers’ dogs further enriches the atmosphere, creating a warmer environment and strengthening the therapeutic dimension of the event.

Among our most successful initiatives is the “Hike with Animals” project, in which students and teachers take walks with horses or dogs. This provides opportunities for physical activity in nature while simultaneously reinforcing the therapeutic benefits of human–animal interaction. Such activities outdoors promote relaxation, support emotional health, and nurture a stronger bond with animals.

Animal-assisted activities at our institution go beyond serving as supplementary educational tools. They represent a multidimensional process that supports students in overcoming challenges, enhancing motivation, and even shaping future professional choices.

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STRESS REACTIONS IN HEARING AND DEAF CATS DURING FELINE THERAPY SESSIONS

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Animal therapy is a form of animal-assisted therapy. Felinotherapy is one form of animal therapy, i.e. therapy with a cat (*Felis catus*). Animal therapy encompasses several forms of interaction between the patient and the animal. AAT (Animal Assisted Therapy) and AAI (Animal Assisted Interventions) are animal-assisted therapy, AAA (Animals Assisted Activities) are classes involving animals, and AAE (Animal Assisted Education) is education involving animals [1]. Choosing the right cat for feline therapy and raising it has a significant and crucial impact on the quality and success of the sessions. Particular attention should be paid to the cat's temperament and personality [2]. Other characteristics, such as sensory development, are also important. The sense of hearing plays a crucial role in survival, serving to detect threats, acquire and search for food, and communicate with other species. Deaf cats, especially those living outdoors, are exposed to numerous dangers, including cars and various predators [3]. But is hearing essential for their work in animal therapy? This study aimed to assess and compare the reactions of hearing cats and cats with hearing loss in the context of cat therapy. Four cats participated in the study – two with normal hearing and two deaf. The cats were observed during five cat therapy sessions attended by children aged 5 to 11. Several behavioural indicators of stress were analysed, including defensive aggression, loss of appetite, increased alertness, low body posture, urination outside the litter box at home, decreased activity, escape, hiding, avoidance of humans, vocalisation, and panting. Additionally, a survey was conducted among caregivers of hearing and hearing-impaired cats (n = 48) regarding their animals' reactions to potentially stressful stimuli in their everyday environment. The results show that cats with hearing loss exhibit significantly lower stress responses compared with cats with normal hearing. Cats with hearing loss established contact with children more quickly and showed no significant signs of stress. These results suggest that hearing loss may, in some cases, act as a factor in reducing environmental stress, which may be important when selecting cats for cat therapy.

Keywords: cat, feline therapy, welfare, stress, animal therapy, deaf cats.

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ETHICAL ASPECTS AND ANIMAL WELFARE IN THE FIELD OF HUMAN HEALTH PROMOTION: A LITERATURE REVIEW

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The aim of the work was to conduct a literature review of scientific research and scientific articles, to summarise the latest knowledge on ethical aspects and animal welfare in the field of human health promotion, and to critically examine animal selection processes, temperamental characteristics and training methodologies. The main focus was on ethical imperatives that ensure the welfare of animals working in therapy, nutrition, work, rest, play and active leisure, including stress recognition and reduction, promotion of animal autonomy and planning for healthy aging and retirement, all framed by the concept of “One Welfare”. In order to ensure sustainable and ethical animal-assisted interventions, it is necessary to adhere to the “One Welfare” principle, which recognises the close connection between the well-being of people, animals and the environment.

The literature review showed that ethical aspects and animal welfare in the field of human health promotion are an area that has not been studied much by scientists and little has been written about it so far. More attention is paid to the selection and training of suitable animals for human health promotion. Research shortcomings include methodological limitations and standardised assessments of suitability for work in the field of human health promotion, mainly taking into account the benefits received from the animal for humans, and ethical imperatives and animal welfare are still given too little attention. Dogs are the most popular animals working in therapy; therefore, it is necessary to create and implement guidelines that would include the selection of therapy dogs, humane training, work and rest regime and constant monitoring, after all, ensuring that the welfare of therapy dogs is not only an ethical obligation, but also an essential condition for achieving real and long-term benefits for human health [1–6].

Keywords: ethics, animal welfare, therapy dog, human health promotion through dog assistance.

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THE ACCESSIBILITY OF MIGRANTS TO EQUINE-ASSISTED THERAPY FOR TRAUMA TREATMENT ASSOCIATED WITH PTSD

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This master's thesis explores the accessibility of equine-assisted therapy as a trauma treatment for migrants suffering from Post-Traumatic Stress Disorder (PTSD). Migrants, particularly those who have fled conflict zones, often face not only psychological challenges but also barriers related to language, culture, and religion. Equine-assisted therapy presents a unique approach that transcends these barriers. Unlike traditional therapeutic methods, the horse serves as a non-judgmental companion, offering emotional safety and warmth [1, 2]. This non-verbal relationship allows PTSD patients to engage and express emotions in ways that verbal communication may hinder, providing a form of support that can be particularly beneficial for individuals who struggle to articulate their experiences [3, 4].

To examine the effectiveness and accessibility of equine-assisted therapy, a qualitative research design is employed, utilising semi-structured interviews guided by a carefully constructed interview template [5, 6]. This research focuses on the experiences of various stakeholders, including employees at institutions, treatment centres, private practitioners, and patients receiving therapy for PTSD, whether or not they have participated in equine-assisted therapy. Understanding the availability and perceptions of this therapeutic approach is critical, as the onset of therapy can significantly impact a migrant's long-term mental health [7, 8].

The interview guide will be meticulously designed to avoid language barriers that could impede communication, ensuring that participants feel comfortable and able to express themselves fully [9]. This study aims to shed light on the unique needs of migrants who have experienced the trauma of war and displacement and to highlight the potential of horses as effective facilitators of healing [10, 11].

Keywords: Equine-assisted therapy, trauma treatment, migrants, post-traumatic stress disorder

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EQUINE STUDIES AND COMMUNICATION RESEARCH

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Understanding equine communication is increasingly recognised as essential for safeguarding both horse and human welfare, as misinterpretation of behavioural cues can compromise safety, emotional well-being, and the effectiveness of equine-assisted interactions [1].

The objective of the research was to investigate equine body language, behaviour and the interrelationship between horses and humans, aiming to uncover the dynamics of their communication. This study utilised a mixed-methods research design, integrating both quantitative and qualitative approaches to gain a comprehensive understanding of horse–human communication. Quantitative data were collected through a structured survey conducted with horse owners, aiming to explore their relationships with horses and the training methods they applied. Qualitative insights were obtained through direct behavioural observation, focusing on horses' responses to human interaction. In addition, standardised tests assessing sociability temperament, voluntary approach behaviour, and tolerance to physical contact were used to provide further depth to the analysis, offering nuanced insights into equine behavioral responses and communicative tendencies [2–4]. Two evaluators took part in all tests, and the impact of human gender (man, women) on the horses' fears and communication was examined. The statistical analysis of data was conducted using the SPSS 30.0.0.0 software. The chi-square (χ^2) test was used to analyse frequency distributions, allowing for the evaluation of differences in observed frequencies. Spearman's rank correlation coefficient was calculated to assess the relationships between variables. Findings were considered statistically significant at $P < 0.05$.

The results indicated that horses that tolerated physical contact—particularly on the neck and shoulders—demonstrated higher levels of trust and social openness. Behavioural variability was linked to individual temperament and prior experience, with tactile interaction emerging as a key factor in human–horse bonding ($P < 0.01$). Horses exhibited distinct behavioural responses toward familiar versus unfamiliar individuals. While most showed a preference for familiar human, a subset displayed avoidance of stranger, indicating the role of recognition and trust in equine social behaviour ($P < 0.05$).

These findings underscore the importance of tactile communication and individual recognition in fostering trust-based human–horse relationships, with implications for welfare, training, and safety in equine-assisted contexts.

Keywords: equine behavior, body language, communication, horse–human interaction

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ANIMAL ASSISTED THERAPY IN PRISONS

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Detainment marks a disruption in the flow of one's life. Any contact with the outside world is limited according to the sentence, which is meant to aid the reeducation of the convict. However, the lack of social relationships has a negative psychological effect on the people deprived of their freedom, who not only develop behaviour pathologies ("post-incarceration syndrome") and manifest anxiety and depressive disorders, but also can experience a decline in healthy social skills [1].

In this case, it is becoming increasingly more obvious that participation in Assisted Education projects with a dog can restore the convict's chance to experience a healing relationship and to develop empathy, respect and attentiveness [2]. The service perfected by the "Do Re Miao" Association in over ten years of efforts for the detained population in Tuscany records encouraging data in favour of the popularisation of these "good practices".

Keywords: detainment, Animal Assisted Therapy, empathy, social skills, dog.

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