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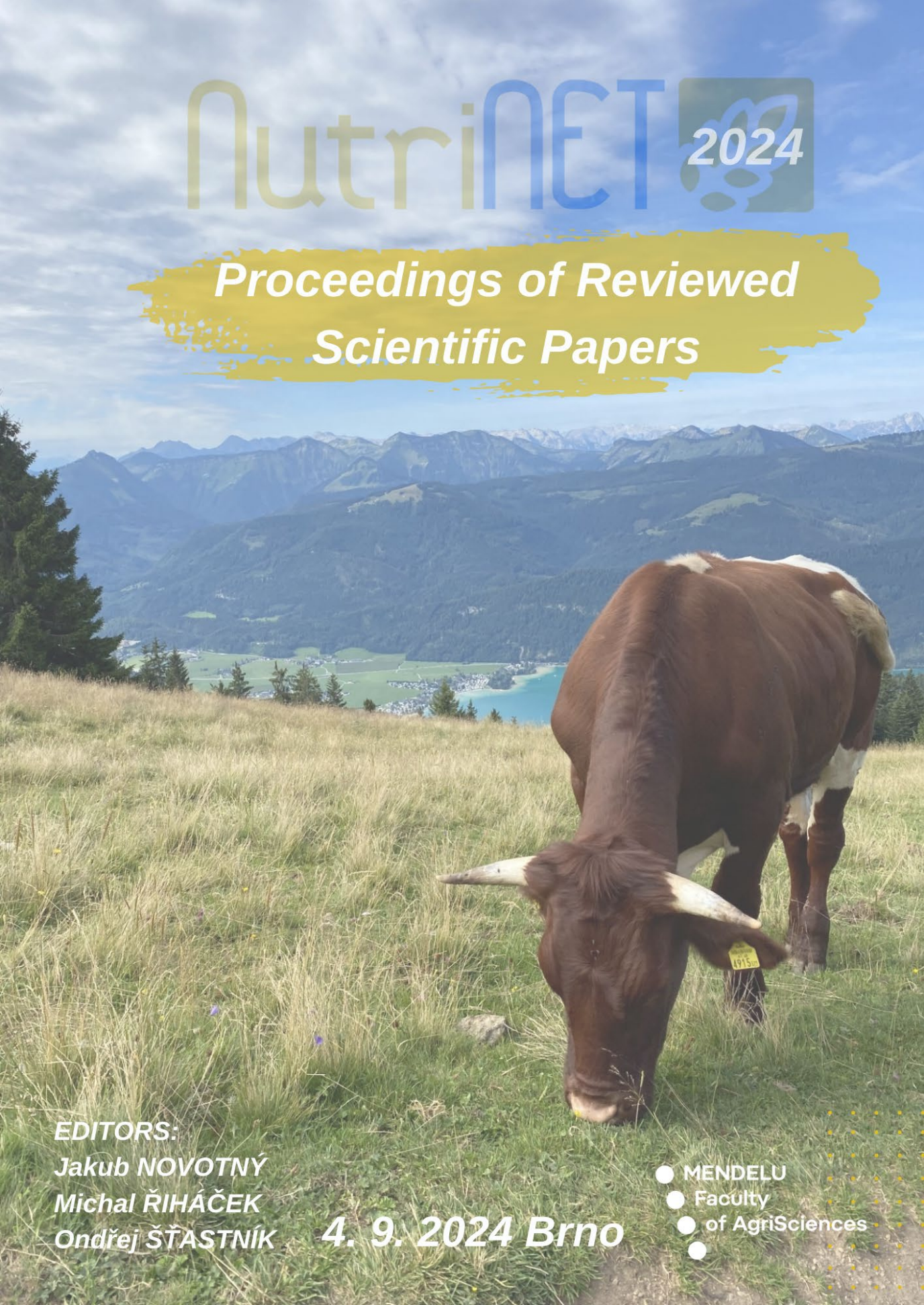

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CONTENT

EFFECT OF CARBON MATERIAL AND BENTONITE ON REDUCTION THE NEGATIVE IMPACTS OF DEOXYNIVALENOL IN RAT ORGANISM

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ABSTRACT

The aim of this study was to investigate the efficacy of mycotoxin adsorbents based on graphene oxide (GO), purified bentonites, and their combination in reducing the negative impact of deoxynivalenol (DON). The experiment involved 48 rats (190 ± 5 g), divided into 8 groups of 6 each. Groups one to seven received a diet containing a high level of DON. Groups one to six had their feed treated with mycotoxin adsorbent. Group one (T1) received a diet with GO (0.25 g/kg), group two (T2) a diet with GO (0.5 g/kg), group three (T3) a diet with purified bentonite (1 g/kg), group four (T4) a diet with purified bentonite (2 g/kg), group five (T5) a diet with GO (0.25 g/kg) and purified bentonite (1.75 g/kg), and group six (T6) a diet with GO

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(0.5 g/kg) and purified bentonite (1.5 g/kg). The control group (C) was fed a diet without DON. The Cmyko group received a diet with mycotoxins without adsorbent. Evaluated parameters included weight and liver enzymes (alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). No statistically significant difference in body weight was observed between groups C and T1–T6 or between groups Cmyko and T1–T6. For liver enzymes, no statistically significant differences were observed in ALP. ALT activity was higher in groups T1, T4, T5, T6, and Cmyko compared to group C. AST activity showed statistically significant differences in all test groups (T1–T6) compared to groups Cmyko and C. In conclusion, a higher dose of GO (0.5 g/kg), purified bentonite, and their combination may be beneficial in mitigating the toxicity of DON.

Keywords: mycotoxins; bentonite; graphene oxide

INTRODUCTION

Mycotoxin contamination in feed is a serious global issue, affecting up to 80% of crop production (Kos et al., 2024). Over 900 mycotoxins have been identified (Habauzit, Lemée, and Fessard, 2024) Their widespread presence in feed, combined with their known adverse effects on livestock health, makes them a significant safety and economic concern (Palumbo et al., 2020).

Adsorption is a surface phenomenon where mycotoxins bind to materials through electrostatic and polar interactions (Freire, Ali, and de Oliveira, 2022). Bentonite, a phyllosilicate clay with a layered crystalline structure, shows variable adsorption efficiency depending on the content of montmorillonite and exchangeable cations (Vila-Donat et

al., 2018). Adsorption mechanisms include chemosorption, electron exchange, hydrogen bonding, ionic interactions, and coordination with carbonyl groups, similar to nutrient adsorption in animal diets (Elliott, Connolly, and Kolawole, 2020). Montmorillonite effectively binds polar mycotoxins such as aflatoxins (AFL), but its efficacy against deoxynivalenol (DON) is limited, despite DON's reactive features such as an epoxide ring, hydroxyl groups, and an α,β-unsaturated carbonyl group (Horky et al., 2021).

Graphene oxide (GO) is a promising material for DON adsorption, however, it was also confirmed that GO has non-specific binding properties for micronutrients (Horky et al., 2020). Its surface, containing oxygen functional groups, contributes to its hydrophilicity (Bytesnikova, Adam, and Richtera, 2021). The unique molecular structure of GO allows it to interact with target molecules through charge transfer, π-π interactions, and hydrogen bonding (Ji and Xie, 2020; Zhou et al., 2023).

The study aimed to assess the impact of purified bentonite and GO on growth performance and liver parameters in rats. We used purified bentonite, which has improved adsorption capacity due to increased binding sites, and GO, known for its effective binding to DON.

MATERIAL AND METHODS

The experiment was conducted at the experimental facility of the Department of Animal Nutrition and Forage Production at Mendel University in Brno, in accordance with the Animal Protection Act No. 246/1992 Coll. The experiment was approved by the Ministry of Education, Youth, and Sports under reference number MSMT-15228/2019-4.

Microclimatic conditions in the laboratory were monitored, primarily limited by temperature, which was measured using a "DATALOGER S 3120 (Voltcraft, Germany)" and maintained within the range of 23 °C ± 1 °C. Additionally, constant air humidity was monitored with the same device and maintained at 60% using an air conditioning unit. The photoperiod was artificially controlled according to a 12-hour light and 12-hour dark cycle with a maximum intensity of 200 lx.

Male Wistar albino rats of the outbred strain were used as the experimental model for this study. The average weight of the rats at the beginning of the experiment was 190 ± 5 grams. The experimental observation lasted for 28 days. The rats were housed in plastic boxes with grates. Throughout the duration of the experiment, the experimental animals had ad libitum access to water and feed. The animals were weighed weekly. Daily monitoring included feed intake and health status. The rats were divided into 8 groups, with each group consisting of 6 males. The first to seventh groups of animals were fed a diet with a high content of DON (4430 µg/kg diet). The first group (T1) of animals had GO added to their feed at a dose of 0.25 g/kg diet. The second group (T2) received GO at a dose of 0.5 g/kg diet. The third group (T3) of rats had purified bentonite mixed into their diet at a dose of 1 g/kg diet. The fourth group (T4) received purified bentonite at a dose of 2 g/kg diet. The fifth group (T5) received a combination of GO at a dose of 0.25 g/kg diet + purified bentonite 1.75 g/kg diet. The sixth group (T6) had a combination of GO at a dose of 0.5 g/kg diet + purified bentonite 1.5 g/kg diet mixed into their diet. The seventh group (Cmyko) was fed a DON diet with defined mycotoxin content without the addition of adsorbents. The eighth control group (C) of rats was fed

a diet with mycotoxin content below the harmful threshold (DON – 238 µg/kg diet).

Table 1. Composition of the rat feed mixture (%)

Component	**(%)**
Maize *	42.9
Wheat *	38.6
Soybean meal extracted (48%)	15.4
Lysine	1.3
Mineral-vitamin premix	1.8

*****_In the experimental groups of rats, corn and wheat were the main sources of DON, while the control group did not have above-limit values of DON in these two ingredients._

The weight of the animals was monitored at regular weekly intervals. After 28 days of the experiment, the rats were euthanized (2 hours after the last feeding). Blood was collected 28 days after anesthesia through cardiac puncture. Blood samples were analyzed for biochemical parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) using the ELISA method as described by Horky et al. (2021).

Data were processed using the statistical software R. The effect of adsorbents on the live weight of rats was tested using one-way analysis of variance. For liver enzyme values, a generalized linear model (GLM) with a gamma distribution was employed. Post hoc analysis compared the treatment groups to the control (C) and Cmyko using Dunnett's test. The null hypothesis was rejected at $p < 0.05$.

RESULTS AND DISCUSSION

Determining the body weight of rats was used to evaluate the performance of animals (Figure 1). Administration of DON at a dose of 4430 µg/kg can negatively impact growth performance in rats, which is also confirmed by the results of Holanda and Kim (2020) at a dose of 3.2 mg/kg DON (Holanda and Kim, 2020). The presence of DON in the feed may lead to feed rejection, consequently decreasing weight gain (Xu et al., 2022). Several studies have demonstrated reduced nutrient digestibility in poultry and pigs in the presence of DON, leading to impaired growth due to histopathological changes in the intestine (Liu et al., 2020; Gallo et al., 2020). Award et al. (2011) found that DON inhibits jejunal villus growth, reducing nutrient absorption (Awad et al., 2011). In our research, no statistically significant differences in the efficacy of the adsorbents among the groups T1–T6 compared to C and Cmyko were observed, however the Cmyko group showed reduced weight gain.

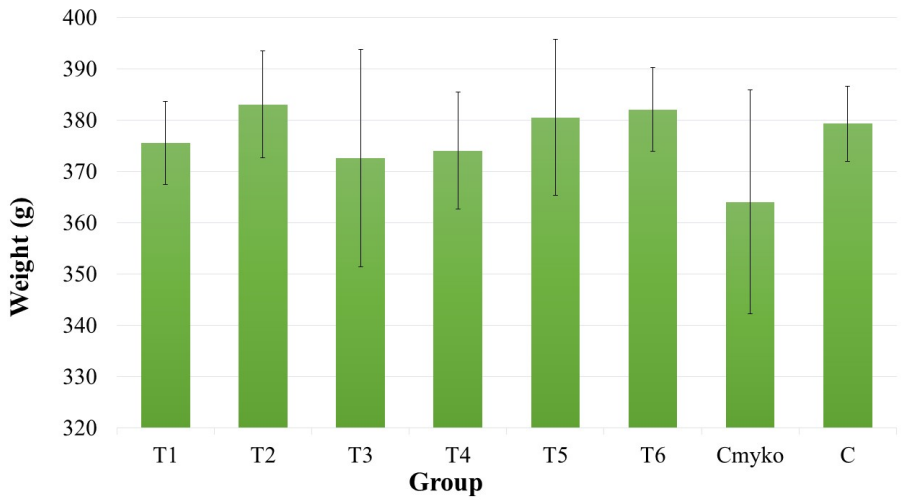

Figure 1: Live weight according to groups

Adsorbents mitigate the harmful effects of mycotoxins by binding to them, reducing their bioavailability and intestinal absorption (Xu et al., 2022). Bentonite's adsorption effects are linked to the physical and chemical properties of mycotoxins (Adegbeye et al., 2020). Horky et al. (2021) reported no significant changes in pig growth with purified bentonites, their study showed improved growth of animals at a dose of 1.5 kg/t (Horky et al., 2021; Horky et al., 2022). Ghazalah et al. (2021) found that bentonite enhanced broiler performance and health. Carbon-based materials vary in their ability to adsorb DON based on type and activation proces (Ghazalah et al., 2021). Carbon-based materials vary in their ability to adsorb DON depending on the type and activation proces (Vila-Donat et al., 2019). Horky et al. (2020) reported that GO adsorbs DON at 1.69 mg/g, with optimal results at 37 °C and pH 5 (Horky et al., 2020). Groups T2, T5, and T6 showed the better growth compared to C and Cmyko, suggesting GO's strong binding capacity for DON. Soybean residue-derived carbon materials achieved up to 98.82% DON adsorption efficiency (Ying et al., 2021) and and Pirouz et al. (2017) demonstrated a 69.6% reduction in DON levels using magnetic graphene oxide (Pirouz et al., 2017).

The liver is essential for metabolism, digestion, detoxification, and excretion. Key liver function tests include ALP, ALT, and AST. Elevated levels of these enzymes can indicate hepatocellular damage, as liver injury causes the release of enzymes from damaged hepatocytes, resulting in increased serum AST and ALT (Martinez et al., 2023; Amer et al., 2022). AST is found in the liver, heart, skeletal muscle, kidneys, brain, pancreas, lungs, leukocytes, and red blood cells, and its elevation can be due to non-hepatic factors. In contrast, ALT is liver-specific, and its increase directly suggests hepatocellular damage

(Kalas et al., 2021). Biochemical assessments should consider the age and sex of rats, as AST, ALT, and ALP levels are higher in males and decrease with age (Patel et al., 2024).

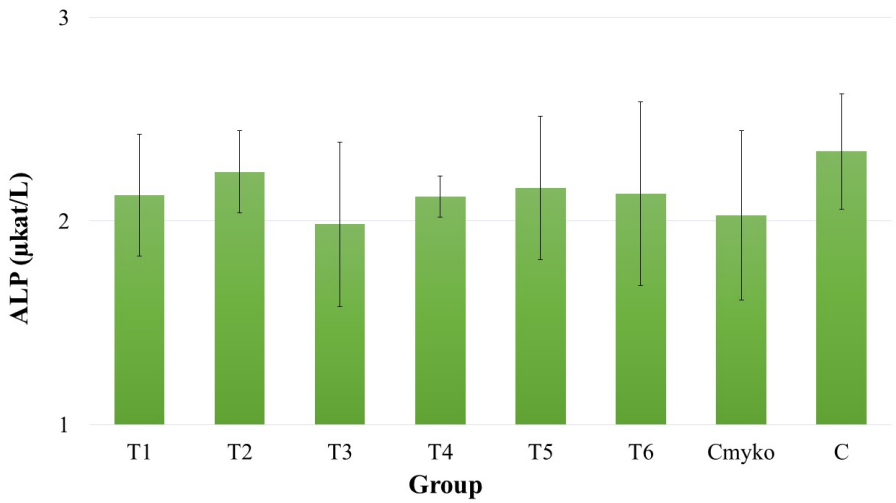

Figure 2: Level of the enzyme ALP (µkat/L)

Enzyme activities in our study were compared across the control group (C), the mycotoxicosis group (Cmyko), and the treated groups (T1–T6) (Figures 2–4). No statistically significant differences were found in ALP values (Figure 2, A).

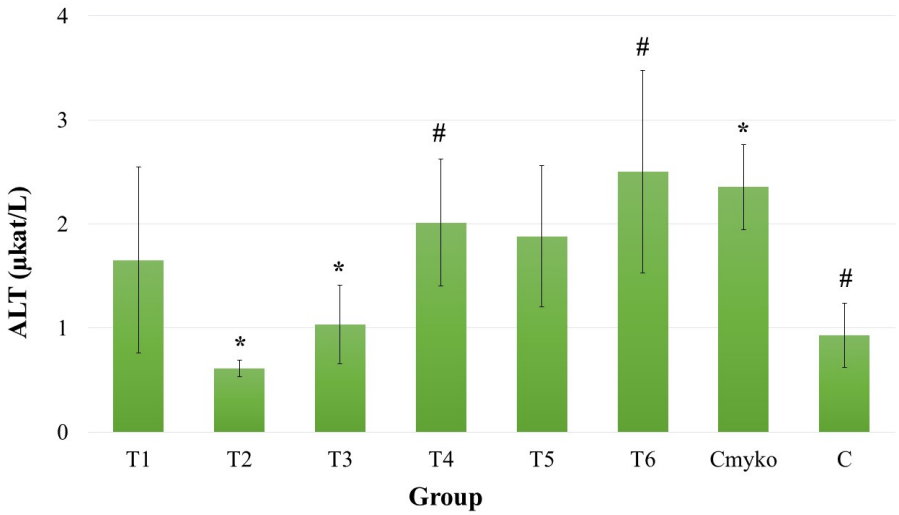

Figure 3: Level of the enzyme ALT (µkat/L)

*****_The group marked with this symbol is statistically significant compared to the group Cmyko; # the group marked with this symbol is statistically significant compared to the group C_

Significant differences in ALT levels were observed in groups T2, T3, and T6 compared to the mycotoxicosis group (Cmyko) and the control group (C) (Figure 2, B). ALT levels were elevated in groups T4 to T6. Patel et al. (2024) report that normal ALT levels in rats range from 0.5 to 1 µkat/L. (Patel et al., 2024). Slight increases in ALT were noted in groups T1 and Cmyko. ALT activity was higher in groups T1, T4, T5, T6, and Cmyko compared to C, suggesting that purified bentonite and the bentonite + GO combination may negatively impact liver function by increasing ALT levels compared to C.

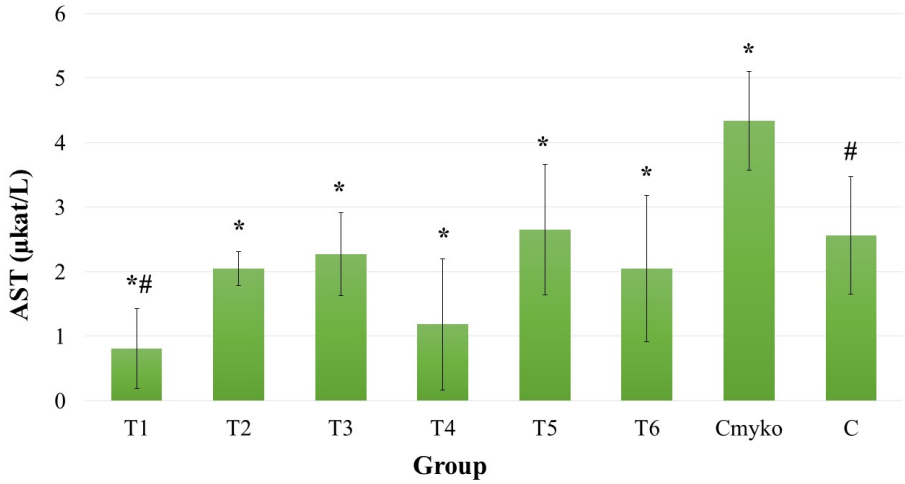

Figure 4: Level of the enzyme AST (µkat/L)

*****_The group marked with this symbol is statistically significant compared to the group Cmyko; # the group marked with this symbol is statistically significant compared to the group C_

Statistically significant differences in AST values were observed in all groups (T1–T6) compared to the Cmyko and the C (Figure 2, C). Significantly lower AST levels were observed in group T1 compared to Cmyko and C. Additionally, significantly lower AST levels were observed in groups T2 through T6 compared to Cmyko. According to Patel et al. (2024), the reference range for AST in rats is 1.4 – 3.78 µkat/L. (Patel et al., 2024). Elevated levels of AST and ALT may indicate hepatic mycotoxicosis (Mgbeahuruike et al., 2018). Increased serum levels of these enzymes were also found in rabbits fed AFL-containing diets (Hassan et al., 2019) and similar results were reported in other mycotoxin studies (Yaman, Yener, and Celik, 2016; Ejiofor et al., 2021). AST levels were lower in groups T1 and T4 compared to the control group (C), while groups T2, T3, T5, and T6 remained within the reference range.

High-dose GO (0.5 g/kg) significantly reduced ALT activity compared to Cmyko, while low-dose GO (0.25 g/kg) significantly reduced AST levels compared to Cmyko and C.These results suggest that higher doses of GO may offer better liver protection against mycotoxins, which is consistent with previous studies (Fu et al., 2015). The T3 group, with a lower dose of purified bentonite, showed similar AST and ALT trends as the higher dose in T2. The T4 group, receiving a higher dose of purified bentonite, exhibited a trend similar to T1. The results indicated that graphene oxide (0.5 g/kg) provided the best protective effect, followed by purified bentonite (1 g/kg), the combination of GO (0.25 g/kg) and purified bentonite (1.75 g/kg), and finally the combination of GO (0.5 g/kg) and purified bentonite (1.5 g/kg).

CONCLUSION

This study aimed to evaluate the impact of two adsorbents at various doses and combinations on rat weight and liver enzymes following exposure to deoxynivalenol (DON). Our research did not reveal significant changes in the growth performance of rats; however, trends were observed in liver enzymes, specifically ALT and AST, compared to the control and mycotoxin-contaminated diet. GO at a dose of 0.5 g/kg demonstrated the most effective protective action against DON-induced mycotoxicosis, followed by purified bentonite at 1 g/kg, and combinations of GO (0.25 g/kg) with purified bentonite (1.75 g/kg) and GO (0.5 g/kg) with purified bentonite (1.5 g/kg). Overall, neither purified bentonite, GO, nor their combinations adversely affected the health status of the rats.

ACKNOWLEDGEMENT

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MELANIN IN BARLEY: FROM ISOLATION TO A POSSIBILITY TO INFLUENCE THE ACTIVITY OF BIOTRANSFORMATION ENZYMES

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ABSTRACT

This study describes the isolation and characterization of melanin from plant matrices using a modified version of the alkaline extraction method originally described by Sava et al. (2001). Melanin was isolated from barley (variety Nudimelanocriton) and purified through a series of organic solvent treatments, acidic hydrolysis, and repeated precipitations, yielding approximately 5 mg of pure melanin from 12 g of barley grain. Isolated melanin exhibited its characteristic properties

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as insolubility in water, acids, and organic solvents, while being soluble in alkaline media and precipitable below pH 3.

Consistent with findings from Caldas et al. (2020) and Glagoleva et Shoeva (2020), melanin samples exhibited in alkaline media a broad-band almost monotonous decrease of UV/VIS absorption from initially 200 nm, with unresolved absorption band at about 270 nm, indicative of complex conjugated structures of aromatic character.

Additionally, effect of melanin on cytochrome P450 1A1/2 enzyme activity was assessed in HepG2 cells using 7-ethoxyresorufin O-dealkylation and high-performance liquid chromatography. Melanin at three concentrations (10 µg/mL, 1 µg/mL and 0.1 µg/mL) did not significantly induce cytochrome P450 1A1/2 enzyme activity (in contrast to CYP1A1/2 potent inducer, 2,3,7,8-tetrachlordibenzodioxin resulting in a nearly sixtyfold increase). These findings contribute to understanding the physicochemical properties of barley-derived melanin and its interaction with hepatic enzymes of xenobiotic biotransformation (as CYP1A1/2).

Keywords: melanin; allomelanin; barley; cytochrome P450; enzyme activity; cell culture; HepG2; UV-visible spectrophotometry

INTRODUCTION

Plant pigments are vital compounds that create the vibrant colours observed throughout the plant kingdom. These pigments not only provide plants with their varied hues but also play crucial roles in important biological processes. While pigments like anthocyanins and carotenoids are well-known, melanins are among the least studied. Though not essential for growth and development, melanin pigments

enhance the survival and competitiveness of species in specific environments (Solano et al., 2014). Melanin, a negatively charged brown or black biomacromolecule found in plants and other living organisms, has shown promising applications in various research fields, including biomedicine, dermocosmetics, nanotechnology, and bioengineering (Hou et al., 2019; Caldas et al., 2020).

In plants, melanin compounds play a crucial role in various functions essential for survival and adaptation. These pigments – in living systems as plants, animals, as well as in humans – primarily protect against environmental stressors such as ultraviolet (UV) radiation, extreme temperatures, microbial attacks, and oxidative damage, as well as chelating metals and, to some extent, involvement in nervous systems. These functions are determined by melanin's chemical and physical properties, including its molecular, supramolecular, and aggregate-level structures (Caldas et al., 2020; Pralea et al., 2019).

The high heterogeneity of melanins makes their analytical characterization quite challenging. Studies on melanin structures has been rare due to difficulties in isolating melanins from natural sources and their poor solubility. Melanins are insoluble in water and common organic solvents like hexane, chloroform, ethyl acetate, ethanol, methanol, or acetone, and can only be dissolved in alkaline solutions (Wang et al., 2006). However, recent advancements have been made in determining the structure of plant melanins using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS). This technique successfully resolved the structure of oat melanin, revealing it as a homopolymer composed of p-coumaric acid, primarily

consisting of low molecular weight oligomers with 3-9 monomer units (Varga et al., 2016).

Melanins can be organized based on their precursor molecules in five categories: eumelanin, pheomelanin, neuromelanin, pyomelanin, and allomelanin (Singla et al., 2021; Peralta et al., 2023). Eumelanin is found in animals, microorganisms, and some fungi. It is derived from tyrosine and is black or brown in colour. Pheomelanin is endemic to higher animals, mammals, or birds. It is also a tyrosine derivative and is red or yellow in colour (Wakamatsu et al., 2002; Bell et Wheeler, 1986). Neuromelanin is predominantly found in the brain, particularly in regions like the substantia nigra and locus coeruleus. It is also derived from tyrosine (Zucca et al., 2014) and its biological function is still under investigation. Plant and fungal allomelanin is often nitrogen-deficient synthesized from phenolic compounds like caffeic, chlorogenic or gallic acid (Guo et al., 2023; Solano et al., 2014).

While the primary structure of melanin, composed of DHI (5,6-dihydroxyindole) and DHICA (5,6-dihydroxyindole-2-carboxylic acid), is widely accepted in the scientific community, its final macromolecular structure remains unclear. Depending on the coupling site of the monomers, melanin can form either a large heteropolymer or, as some researchers suggest, a stacked oligomer. Nonetheless, both structural forms – DHI and DHICA result in melanin particles with identical chemical properties (Caldas et al., 2020).

Given that varieties of products enriched with melanin content are beginning to be developed, it is highly necessary to study its possible influence on the human organism, specifically on the metabolism of foreign substances mediated by cytochromes P450 (CYPs). All of the

recent findings may be a key helping to uncover the potential interaction between melanin pigments and the main enzymes of metabolism of xenobiotics incl. drugs. Melanin may modulate the activity of CYPs through physical binding or sequestration, altering their substrate accessibility and catalytic efficiency. Among human drug-metabolizing enzymes, forms of cytochrome P450 labeled as CYP1A1 and 1A2 are important for metabolism of aromatic structures and for their activation in chemical carcinogenesis. These two enzymes are often collectively named as CYP1A1/2 as they share high amino acid sequence similarity and share also most of substrates incl. the 7-ethoxyresorufin, used also here to evaluate their possible induction and hence an increase of enzyme activity (Anzenbacher et Anzenbacherová, 2001). Revelation of potential crosstalk between melanin and CYP may be crucial for evaluation of safety of future melanin-cereal products.

MATERIALS AND METHODS

Chemicals

Dimethyl sulfoxide (DMSO), penicillin, streptomycin, ethanol, chloroform, methanol, acetonitrile, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), bovine serum albumin, sodium pyruvate, insulin, glucagon, NADP+, and Eagle's Minimal Essential Medium (EMEM 4655) were obtained from Merck (Darmstadt, Germany). Minimum Essential Medium (MEM 31095), foetal bovine serum, glutamin, and non-essential amino acids were obtained from Gibco (Billings, MT, USA). 7-ethoxyresorufin (ETRR) was obtained from Lipomed (Arlesheim, Switzerland). All chemicals were of the highest purity available.

Isolation and purification

The pigment was isolated from the plant matrix via alkaline extraction as described by Sava et al. (2001) with modifications. The grounded barley (2 g) was suspended in 0.5M NaOH (24 mL) in a glass flask. The sample was heated on a heater (IKA Labortechnik) at 100 °C for 1 h with mixing. Supernatant was filtered through gauze and acidified with 7M HCl to pH 2. Mixture was stored at room temperature for 24 h.

After centrifugation at 10000 rpm for 10 min, purification was conducted involving acidic hydrolysis 7 M HCl (12 mL) of the residue at 100 °C for 2 h, to remove carbohydrates and proteins. The resulting suspension was centrifuged at 10000 rpm for 10 min and the residue was washed with water until the supernatant was neutral. The brown-black solid material was washed in turn with chloroform, ethyl acetate and ethanol to eliminate lipids and repeatedly precipitated to extract phenolic compounds. The precipitate was filtered off and washed with water.

The extracted pigment material appears as a dark, glossy powder. It is insoluble in water and most organic solvents, partially soluble in concentrated sulfuric and nitric acids, and completely soluble in sodium hydroxide (Glagoleva et al., 2020).

UV-Vis spectrophotometry

Regarding UV and visible absorption spectrum, melanins of different origin evidence a broad-band monotonic absorption in visible and ultraviolet spectrum with the maximum at 196–300 nm (Caldas et al., 2020, Glagoleva et al., 2020). Melanin was dissolved in 20 mM potassium phosphate buffer (KH2PO4/K2HPO4) with different pH at

final concentration of 50 µg/mL with corresponding solution as a reference. The UV–visible absorption spectrum of melanin was scanned in the wavelength range of 600–200 nm with a UV–visible spectrophotometer (UV-2700i, Shimadzu).

Cell culture

HepG2 cells (ATCC) were cultured in Dulbecco's Modified Eagles medium supplemented with 10% foetal bovine serum, 2 mM glutamine, 1 mM sodium pyruvate, 1% non-essential amino acids, 100 units/mL of penicillin, and 0.1 mg/mL of streptomycin. Cultures were maintained in a humidified atmosphere with 5% CO2 at 37 °C and medium was refreshed every three or four days with subculturing.

Preparation of melanin solutions

Melanin was dissolved in pure DMSO. A series of 10-fold dilutions were then made from the stock solution using DMSO. These DMSO solutions were subsequently added to the culture medium, resulting in a final DMSO concentration of 0.1%.

Measurement of induction of CYP1A/2 enzyme activities in HepG2 cells

HepG2 cells were trypsinized, counted and resuspended in culture medium to a final concentration of 200 000 cells/ well for the CYP1A1/2 assay. 1 mL cell suspension was added to a well of a 12 well culture plate (Merc, Darmstadt, Germany). The 12 well plates were incubated for 24 h in a humidified atmosphere at 37 °C under 5% CO2. The enzymatic activity of CYP1A1/2 was measured via the incubation of HepG2 cells with melanin (concentration range: 10 µg/mL–0,1 µg/mL), TCDD as an inductor and vehicle alone (0,1% DMSO) in a 12-well plate for 24 h. Thereafter, ETRR was added to a

final concentration of 2,6 µM in a culture medium and incubated for 2 h. The supernatant was transferred into Eppendorf tubes, diluted with methanol (1:2), and centrifuged at 14000 RPM at 4 °C for 10 min. The activity of CYP 1A1/2 was measured as an amount of ETRR metabolite – resorufin, using HPLC with fluorescent detection (excitation 535 nm, emission 585 nm) according to methods described by Chang et Waxman (2006).

Cell viability was assessed using the MTT assay. This colorimetric test relies on the activity of oxidoreductase enzymes in living cells to convert the tetrazolium dye MTT into formazan crystals. After 24 hours of treatment, the cells were rinsed with PBS and incubated with a 5 mg/mL MTT solution diluted in serum-free medium (1:10) for one hour. The solution was then removed, and the formazan crystals were dissolved in a DMSO/0.1% NH3 solution. Absorbance was measured spectrophotometrically at 540 nm.

RESULTS AND DISCUSSION

Isolation and purification

In this study, we isolated melanin from barley (var. Nudimelanocriton) (Fig. 1). After further purification with organic solvents, acidic hydrolysis and repeated precipitation, approx. mg of pure melanin was obtained from 12 g of barley grain (Fig. 2). It was insoluble in water, acids and organic solvents, soluble in alkaline media and could be precipitated by acidification below pH 3.

Figure 1. Barley (var. *Caesar*) without increased melanin content (left), barley (var. *Nudimelanocriton*) enriched with melanin content (right).

Figure 2. Melanin isolated from barely, var. *Nudimelanocriton*

UV-Vis spectrophotometry

The absorbance in alkaline aqueous media was higher than in neutral or acid ones (Fig. 3), indicating that melanin prepared was relatively stable under the alkaline pH. The absorbance gradually increased with the pH. The maximum absorption of melanin was reached at about 200 nm and decreased towards the visible region, which is a characteristic feature of melanin absorption spectra ascribed to apparently complex conjugated structures of the melanin molecules (Cockell et John, 1999). No distinct absorption peaks at 260 nm and 280 nm were found, indicating that the content of nucleic acid and

protein impurities was low (Hou et al., 2019). However, an unresolved absorption band at about 270 nm is probably indicative of complex conjugated structures of aromatic character (Fig. 3 and 4).

For melanin samples dissolved in potassium buffer with pH values from 8 to 10, no significant difference was observed between individual spectra (Fig. 4). This optical property of melanin samples in alkaline media may help in choosing an optimal wavelength for detection of melanin in HPLC separation.

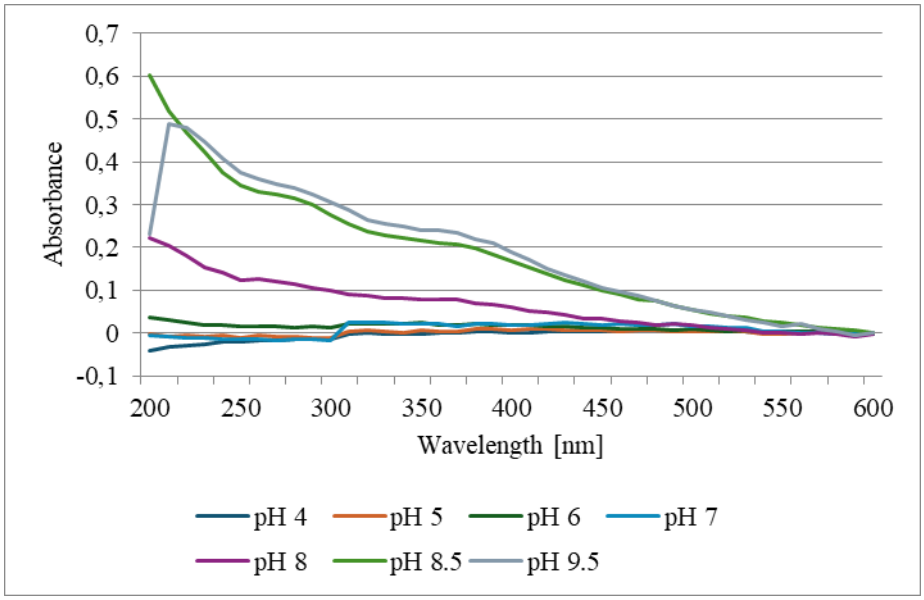

Figure 3. UV–VIS absorbance spectrum of melanin. 50 µg /mL of melanin dissolved in solutions of potassium buffer with different pH.

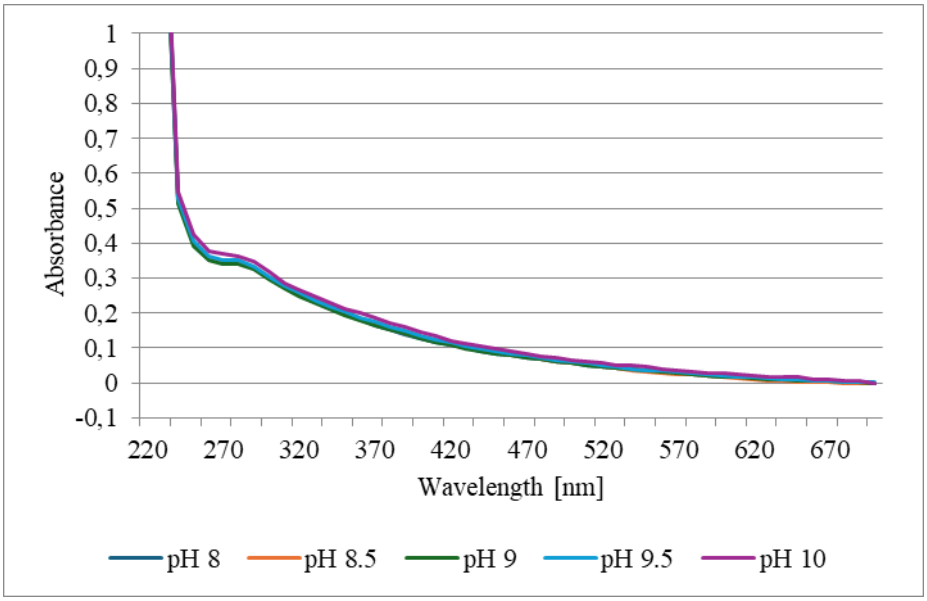

Figure 4. UV–VIS absorbance spectrum of melanin. 50 µg /mL of melanin dissolved in solutions of potassium buffer with different pH.

Induction of CYP1A1/2 in HepG2 cells

The activity of CYP1A1/2 was determined using O-dealkylation of prototypical CYP1A1/2 substrate, 7-ethoxyresorufin, as described by Pearce et al. 1996 and performing high performance liquid chromatography. Melanin tested in three different concentrations showed no significant induction above the basal CYP1A1/2 activity in HepG2 cells. TCDD was used as a control being a potent inducer, with a maximum activity almost sixty times greater than rest of the samples (Fig. 5). Melanin also showed no influence on viability of the HepG2 cell line (Fig. 6).

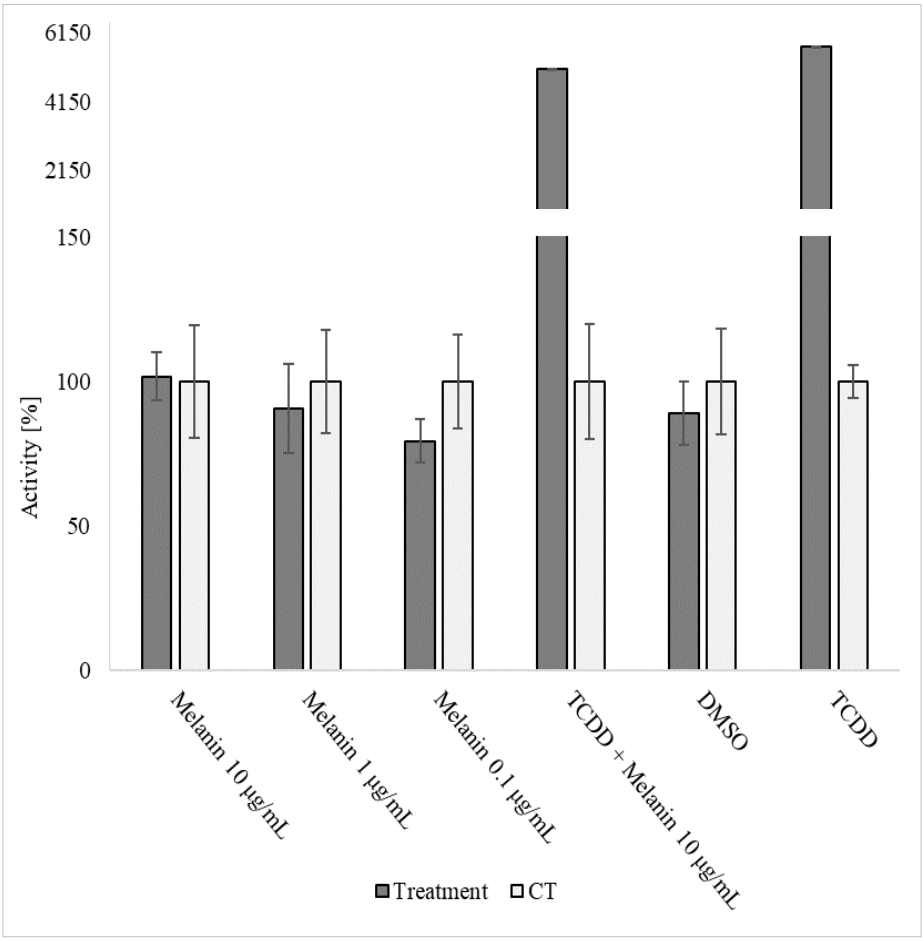

Figure 5. Effect of melanin on the activity of CYP1A1/2 cells after 24 h of treatment. 5 nM TCDD was used as a positive control. All experiments were performed in triplicate, all data were normalized to the control (CT), and each bar represents the mean ± SD of independent experiments.

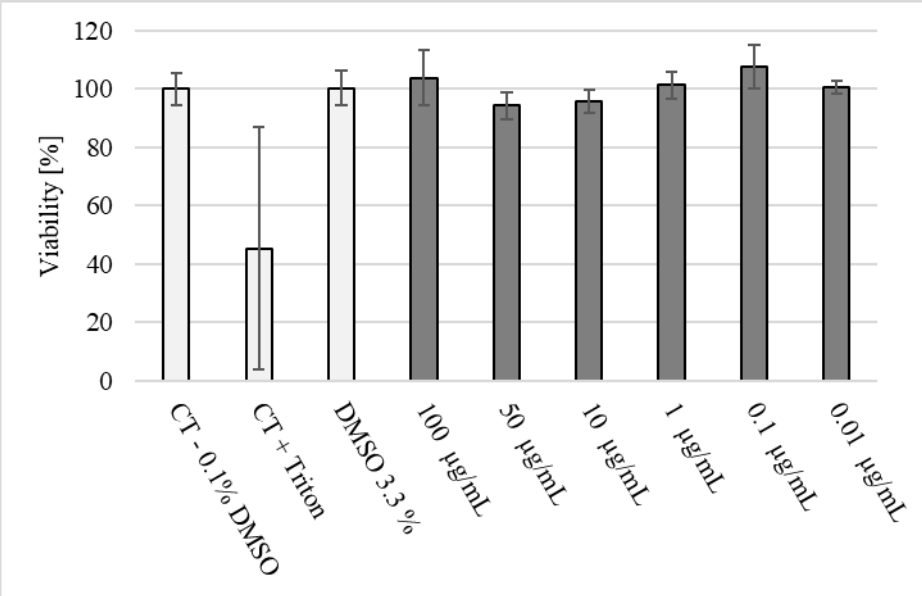

Figure 6: Effect of melanin on the viability of HepG2 cells after 24 h of treatment. All experiments were performed in triplicate, all data were normalized to the control (CT), and each bar represents the mean ± SD of independent experiments.

CONCLUSION

In conclusion, successful extraction and purification of melanin from barley (var. Nudimelanocriton) was realized yielding 5 mg of pure melanin from 12 g of barley grain. Melanin exhibited expected solubility properties, being insoluble in water, acids, and organic solvents, yet soluble in alkaline media and precipitable below pH 3. UV spectral analysis revealed characteristic absorption properties, with maximal absorption beyond 200 nm and stability under alkaline conditions. The absence of peaks at 260 nm and 280 nm confirmed minimal contamination by nucleic acids and proteins, however, an unresolved and broad band at about 270 nm of samples in alkaline

media indicates presence of an absorption due to aromatic structures. These spectral properties may help in HPLC detection of melanin in alkaline matrices. Furthermore, the study showed that barley-derived melanin did not significantly induce CYP1A1/2 activity in HepG2 cells, when compared with the potent inducer TCDD. These findings contribute to the understanding of the properties of barley-derived melanin, offering insights necessary for its potential applications.

ACKNOWLEDGEMENT

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THE USE OF TRITORDEUM IN BROILER CHICKENS DIET AND ITS EFFECT ON PERFORMANCE PARAMETERS

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ABSTRACT

The aim of the experiment was to investigate tritordeum and its potential use in broiler nutrition. The experiment included 90 broilers of the Ross 308 hybrid, divided into 3 groups with 5 replications each. The broilers were fed with 3 feed mixtures (T0, T5, and T40) containing different proportions of tritordeum for 36 days. Feed mixture T0 was mainly composed of corn, wheat, and soybean meal without tritordeum. Feed mixture T5 contained the same components as mixture T0, but it contained 5% of tritordeum. The feed mixture T40 contained 40% tritordeum. Throughout the entire fattening period, feed consumption and live weight gain were recorded, droppings were collected to determine the digestibility of nutrients. At the end of the experiment, the quality of the carcass was also evaluated. The results of the experiment did not show any significant statistical differences between the feed mixtures in terms of feed consumption, feed conversion, live weight gain, or the yield of main meat parts ($P>0.05$). However, significant statistical differences were found when

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comparing the feed mixtures in terms of nitrogen retention ($P < 0.05$). The highest nitrogen retention was observed in feed mixture T5 (67.96%) and the lowest in feed mixture T0 (65.13%). Research has shown the suitability of using tritordeum grain in the nutrition of broilers without any negative impact on production parameters or health. There is an assumption that a certain proportion of tritordeum in the feed mixture may lead to higher nitrogen retention in the organism. As a crop resistant to warmer and drier conditions, tritordeum could potentially replace some traditional cereals in the future. It is important to continue studying new non-traditional feeds in animal nutrition to determine their impact on the organism.

Keywords: alternative feeds; global warming; nitrogen retention, poultry nutrition; Ross 308

INTRODUCTION

In the current era of climate change, with increasing global temperatures and melting glaciers, it is necessary to search for new crops that would be able to adapt to warmer and drier conditions. Tritordeum is a hybrid of barley (*Hordeum chilense*) and durum wheat (*Triticum durum*) (Ávila *et al.*, 2021) and has the potential to adapt to such conditions – it has increased resistance to drought and diseases (De Caro *et al.*, 2024) such as against Septoria leaf blotch, which is one of the most important diseases of wheat (Ávila *et al.*, 2021). The greater drought and heat during the growing season that supports the formation of carotenoids (lutein, zeaxanthin, antheraxanthin or β-carotene). Research by Paznocht *et al.* (2018) showed the highest carotenoid content of 14 selected wheat, barley and tritordeum genotypes in yellow-grained tritordeum HT 439 (12.16 µg/g dry

weight). Lutein, responsible for the yellow color, was the most contained in tritordeum. At the same time, a high content of carotenoids is also achieved thanks to *Hordeum chilense* (Materra *et al.*, 2016). Another positive feature of tritordeum is a higher ability to bind nitrogen from the soil compared to wheat (Martín *et al.*, 1999). To the best of our knowledge, no scientific study has been published to investigate the effect of tritordeum on the production parameters of broiler chickens.

MATERIAL AND METHODS

Animals and experimental conditions

In the experiment, 90 Ross 308 hybrid roosters were used, which were fed for 36 days. The broilers were divided into 3 groups with 5 repetitions each. So, there were 30 individuals in each group. The chicks were housed in balance cages and the stable conditions were set according to the technological instructions of the Aviagen company for Ross 308. During the experiment, the average daily temperature was 24.88°C and the average humidity was 42.48%. Breeding technology corresponded to Decree No. 208/2004 Coll., on minimum standards for the protection of farm animals against cruelty. The chickens had unlimited access to feed and drinking water throughout the experiment. Feed consumption was recorded every day and droppings were collected over 8 days (120 samples in total) to determine nutrient digestibility. The droppings were stored at -20 °C and subsequently lyophilized and subjected to laboratory analyses. The fattened broilers were weighed every day. The experiment was terminated by decapitation of the broilers, followed by the obtained carcasses. These carcasses, i.e. bodies without tarsometatarsus, giblets and necks, were

weighed and the percentage of the carcass from the live weight of the broilers was calculated.

10 chickens from each group were selected, from which the thigh and breast muscles were dissected and the percentage share of thigh and breast muscle from live weight was determined through calculation.

Feed mixtures

The feed mixtures were compiled according to the nutritional specifications for Ross 308 broilers (Aviagen Group, 2019b). Each group of broilers was fed a feed mixture with a different proportion of tritordeum. Tritordeum variety JB1 samples were taken and subjected to chemical analysis. The results of the chemical analysis are shown in Table 1. The first group (T0) did not contain the proportion of tritordeum, wheat was used instead. Group T5 contained 5% tritordeum and group T40 contained 40% tritordeum. The specific composition of the feed mixtures is documented in Table 2. To determine the digestibility of nutrients using indicator methods, 0.3% chromium oxide indicator (Cr_2O_3) was mixed into the feed mixtures. During the experiment, the mixture BR1 was used, which was fed to the chickens until the 10th day of age. BR2 mixure was fed from day 11 to day 36, when the experiment was terminated.

Table 1. Chemical composition of tritordeum (88% dry matter)

CP %	EE %	CF %	ADF %	NDF %	Starch %	Ash %	Carotenoids µg/g
20.28	2.00	1.54	3.53	13.14	50.67	2.47	12.21

CP - crude protein, EE - ether extract, CF - crude fiber, ADF - acid detergent fiber, NDF - neutral detergent fiber

Samples of feed BR1 T0, T5, T40 and BR2 T0, T5, T40 were also taken for chemical analysis. The results of the chemical analysis (calculated on a dry matter basis of 88%) are presented in Table 3.

Table 2. Composition of feed mixtures

	Feed mixture BR1			**Feed mixture BR2**		
Component	**T0**	**T5**	**T40**	**T0**	**T5**	**T40**
Maize (%)	32.82	42.70	20.69	36.29	48.30	27.03
Wheat (%)	13.00	0.00	0.00	15.62	0.00	0.00
Soybean ex. meal (%)	44.21	43.50	30.00	39.50	38.75	25.00
Rapeseed oil (%)	5.00	4.35	4.10	4.10	3.50	3.00
Wheat gluten (%)	0.15	0.10	0.65	0.05	0.00	0.64
Premix (%)	3.00	3.00	3.00	3.00	3.00	3.00
Monocalcium phosphate (%)	0.77	0.60	0.56	0.59	0.60	0.52
Methionine (%)	0.20	0.00	0.00	0.15	0.15	0.15
Ground limestone (%)	0.56	0.45	0.70	0.40	0.40	0.60
Chromium oxide (%)	0.30	0.30	0.30	0.30	0.30	0.30
Tritordeum (%)	0.00	5.00	40.00	0.00	5.00	40.00

Table 3. Chemical composition of feed mixtures (88% dry matter)

Feed mixtures	CP %	EE %	CF %	Ash %
BR1 T0	23.95	6.65	3.74	6.72
BR1 T5	24.00	6.84	4.31	6.76
BR1 T40	22.87	6.24	3.47	6.59
BR2 T0	22.08	5.82	2.48	6.11
BR2 T5	22.02	5.66	2.48	6.14
BR2 T40	22.08	4.80	2.05	5.53

CP - crude protein, EE - ether extract, CF - crude fiber

The data was processed in Microsoft Excel (USA) and StatSoft Statistica version 12.0 (USA) software. One-way analysis of variance (ANOVA) was used. Scheffé's test was used to determine the difference, with a significance level of $P < 0.05$ considered as a statistically significant difference.

RESULTS AND DISCUSSION

The mean live weights of broilers on the day of the start and the day of the end of the experiment (1st and 36th day), average chick gains, feed consumption and feed conversion are shown in Table 4. Only 88 broilers are included in the results, as 2 broilers from the T5 group they died during the experiment. No significant statistical differences ($P > 0.05$) were found in any of the mentioned parameters between the groups fed with different feed mixtures. The average live weight of broilers on the 36th day of fattening was 2,105.51 g, while according to Aviagen's technological instructions, the given broiler roosters should

weigh 2,332 g (Aviagen group, 2019a). According to the technological instructions (Aviagen group, 2019a), the chicken feed consumption should be 3,480 g, while the lowest chicken feed consumption was 3,027.24 g for the T5 group. Furthermore, the technological instructions (Aviagen group, 2019a) state that the 36th day of fattening should have been a feed conversion of 1.492, with the lowest feed conversion in the experiment being 1.43 for the T5 group. According to the results, it can be inferred that adding tritordeum to the feed ration does not reduce the production parameters of broilers and it is possible to use this cereal.

Table 4. Average live weight, average live weight gains, feed consumption and conversion

	In total	**T0**	**T5**	**T40**
n	**88**	**30**	**28**	**30**
	mean ± standard deviation			
ALW of chickens at the beginning of the experiment (g)	43.93 ± 3.63	44.13 ± 3.63	43.86 ± 3.87	43.80 ± 3.52
ALW of chickens on the 36th day of the experiment (g)	2105.51 ± 312.33	2123.60 ± 338.01	2101.07 ± 326.37	2091.57 ± 280.48
AFC per chicken per day (g)	87.40 ± 6.75	88.75 ± 8.56	84.09 ± 2.52	89.37 ± 7.67
FCR per trial period per cage	1.49 ± 0.11	1.50 ± 0.14	1.43 ± 0.05	1.53 ± 0.10

n - number of cases, ALW – average live weight, AWG - average weight gain, AFC - average feed consumption, FCR – feed conversion ratio

Table 5 shows the yields of the main meaty parts of slaughtered chickens. A total of 30 chickens, 10 individuals from each group, were included in this section. The obtained data were subjected to the Scheffé test, which showed no statistically significant differences ($P > 0.05$). Aviagen's technological manual (Aviagen group, 2019a) states that at a live weight of 2,400 g, the carcass yield should be 72.97%, while the highest yield was achieved in the T5 group at 70.03%. Stupka *et al.* (2013) states that the average slaughter yield in chickens is 70 to 76%, which would approximately correspond to the results of the experiment. As for the yield of breast and thigh muscle, according to the technological instructions (Aviagen group, 2019a), it should be 24.03% and 12.88% at a live weight of chickens of 2,400 g. The highest average pectoral muscle yield was measured in the T5 group at 23.22% and the highest average thigh muscle yield was measured in the T40 group at 15.72%. When compared, it is clear that the results of the experiment correspond to the standards for the given hybrid. Another part of this experiment was to clarify the influence of the proportion of tritordeum in the feed ration for broilers on the retention of nitrogenous substances. The data that was collected through faecal sampling and subsequent chemical analysis was evaluated by Scheffé test and the results showed the demonstration of statistically significant differences ($P < 0.05$). In group T5, the highest retention of nitrogenous substances was measured at 67.96%, while the lowest was found in the control group T0, where the retention of nitrogenous substances was measured at 65.13%.

Table 5. Yields of the main meaty parts of slaughtered chickens

	T0	T5	T40
n	**10**	**10**	**10**
	mean ± standard deviation		
Average live weight of chickens (g)	2421.20 ± 239.70	2284.40 ± 348.03	2302.60 ± 287.09
Average carcass yield (g)	1668.48 ± 188.54	1596.80 ± 230.98	1596.41 ± 224.43
Average carcass yield (%)	68.85 ± 1.71	70.03 ± 2.78	69.23 ± 2.09
Average breast meat yield from carcass (%)	33.03 ± 2.83	33.09 ± 2.59	31.73 ± 2.34
Average thigh meat yield from carcass (%)	21.58 ± 1.86	22.33 ± 1.51	22.72 ± 1.78

n – number of cases

Another part of this experiment was to clarify the influence of the proportion of tritordeum in the feed ration for broilers on the retention of nitrogenous substances. The data that was collected through faecal sampling and subsequent chemical analysis was evaluated by Scheffé test and the results showed the demonstration of statistically significant differences ($P < 0.05$). In group T5, the highest retention of nitrogenous substances was measured at 67.96%, while the lowest was found in the control group T0, where the retention of nitrogenous substances was measured at 65.13%. According to the obtained results, it can therefore

be predicted that a certain proportion of tritordeum increases the retention of nitrogenous substances, which is important both from an economic and ecological point of view.

Table 6. Nitrogen retention

	T0	**T5**	**T40**
n	**40**	**40**	**40**
	mean ± standard deviation		
Nitrogen retention (%)	65.13 ± 5.44[a]	67.96 ± 3.73[b]	67.10 ± 0.83[ab]

a,b means statistically significant differences (P < 0.05)); n – number of cases

CONCLUSION

This study did not show a negative effect of tritordeum on the production parameters of broilers or on the quality of the carcass and the main meaty parts. However, a statistically significant difference ($P < 0.05$) was found in the retention of nitrogenous substances between groups T0 and T5. In group T5, which was fed a feed mixture with a proportion of 5% tritordeum, the highest retention of nitrogenous substances was measured at 67.96%. From this, we can conclude that the use of tritordeum in feed mixtures for broiler chickens is feasible as it does not have a negative effect on zootechnical and processing parameters, while a certain proportion of tritordeum can increase the retention of nitrogenous substances. This can reduce costs in the production of compound feed, as the need for these nutrients will be reduced. At the same time, an increase in the retention of nitrogenous substances means less nitrogenous substances excreted in the feces, which has a positive impact on the environment. However, it is important to continue similar research and clarify other effects on the

production and health parameters of farm animals, as well as on the environment.

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INFLUENCE OF SOURCES AND LEVELS OF INORGANIC AND ORGANIC IRON ON EGG QUALITY IN DOMINANT DARKSHELL DS109

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ABSTRACT

With the ever-increasing societal pressure on animal welfare, there is a gradual transition to alternative housing systems. A new development in the field of breeding of laying hens is the breeding of hybrids that can be used in alternative and extensive conditions on the parameters of eggshell colour and uniformity. Different feeding strategies are being tested to maintain the desired colour and uniformity, but these must also be tested in relation to egg quality parameters. The aim of this study was to test the effect of different iron sources and levels on egg quality parameters in the hybrid Dominant Darkshell DS109, which is specifically bred to produce table eggs with dark to chocolate brown eggshell colour. The study included 240 laying hens whose diets were supplemented with two different sources and levels of iron. Our

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results showed a significant effect ($p < 0.001$) of increased organic and inorganic iron levels on the parameters of eggshell thickness (0.403; 0.402 mm vs. 0.395 mm), eggshell weight (5.7 and 5.6 vs. 5.4 g) and eggshell percentage (9.4 and 9.3 vs. 9.1 g). The results also indicate a positive effect of elevated 191 mg/kg Fe-Gly on egg weight (64.1 g vs 62.7 g). There was an effect ($p < 0.05$) of inorganic form of iron on yolk proportion parameter throughout the laying period.

Keywords: laying hens; ferrous sulfate; eggs quality; iron chelate, eggshell quality

INTRODUCTION

In the European Union, with increasing pressure on animal welfare, there is a gradual shift to various non-cage technologies for housing laying hens, which is also reflected in the field of breeding. In addition to the breeding of laying hens for alternative technologies, a new development in the world is the breeding of layers for eggshell colour to increase their attractiveness to both breeders and consumers. The breeding of laying hens for eggshell colour is taking place in hybrids intended for alternative housing systems, which presents the possibility of their increased use in the future. This trend is confirmed by statistics showing that by the end of 2023, almost 61 % of laying hens reared in the European Union (EU Commission, 2024) were housed in non-cage technologies.

One of the main objectives of breeding for eggshell colour is the requirement to maintain uniformity and persistence of eggshell colour throughout the laying period. Various nutritional strategies are used to enhance the production of pigments that cause eggshell colour. The most promising strategy appears to be the use of various macro and

microelements. In addition to testing these feed additives on eggshell colour, it is necessary to test their effect on egg quality parameters. Therefore, this study is devoted to evaluating the effect of supplementation of inorganic and organic forms of iron at different levels on egg quality in the hybrid Dominant Darkshell DS109 intended mainly for alternative housing systems, extensive conditions and backyard production.

MATERIAL AND METHODS

A total of 240 laying hens of the genotype Dominant Darkshell DS109 at the age of 22 weeks were included in the experiment. The laying hens were randomly divided into four groups at this age. Each group had four replicates represented by a total of 60 laying hens. Laying hens were housed in 3-tier battery cages in the experimental facility.

Diet

All experimental groups were fed once a day the same feed mixture, which differed only in the source and amount of iron depending on the premix used, which was added to the feed mixture at 3% concentration. The distribution of laying hens into groups according to the premix used is shown in Table 1. Mixture N1 was fed until the 45th week of laying hens' age at a daily dose of 125 g/head/day with nutrient content of 11.17 MJ of ME and 165.03 g of crude protein per kg/mixture. Mixture N2 was fed until the 72nd week at a dose of 135 g/head/day with nutrient content of 11.7 MJ of ME and 151.51 of crude protein in accordance with the requirements of the breeder of the hybrids. Water were supplied ad libitum. The lighting regime consisted of 14 hours of light and 10 hours of darkness.

Table 2. Schematic division of laying hens into groups according to the premix used

Genotyp of hybrids	Name of group	Type of iron level	N1	N2	Number of repetition	Number of laying hen in repetition
			premix until 45 week	premix from 45 week		
Darkshell DS109	D-K-(K)	Standard	KON	KON	4	60
Darkshell DS109	D-K-O	increased ORG 45t	KON	ORG2	4	60
Darkshell DS109	D-O	increased ORG	ORG2	ORG2	4	60
Darkshell DS109	D-A	increased ANORG	ANORG2	ANORG2	4	60

Layers in the control group (D-K) were supplemented with an inorganic source, specifically ferrous sulphate monohydrate, FeSO4·H2O was used in the premix at a level of 131 mg/kg. The premixes for the organic and inorganic groups of layers contained 45% more iron than the premix for the control group of layers. For the inorganic group (D-A), the same source of iron was used as for the control group, but its level was increased to 191 mg/kg of the feed mixture. Laying hens in the organic group (D-O) were supplemented with an organic source of iron, ferric chelated glycine Fe-Gly, at a level of 191 mg/kg of compound feed. Layers in the D-K-O group were fed the control diet until week 45. From week 45 they were supplemented with the same diet as the organic group.

Egg quality

Egg quality parameters were monitored at regular four-week intervals from 24 - 72 weeks of age of the laying hens. The analysed eggs were

collected on the same day each time to avoid evaluating eggs from the same hen twice within the evaluated week of age.

The egg quality parameters monitored included egg weight, eggshell strength, yolk weight, yolk colour, eggshell weight and eggshell thickness. From the data obtained, the albumen weight, % albumen proportion, % yolk proportion, % eggshell proportion of egg weight were calculated.

Each egg was weighed to determine the egg, yolk and eggshell weight. Each egg was weighed on a KERN KB 1000-2 electronic balance (Germany) to the nearest 0,1 g. The above-mentioned scales were also used to determine yolk weight and eggshell weight. Eggshell strength was determined by the destructive method using an Egg Force Reader (Orka Food Technology, Ltd.). Eggshell thickness was measured on washed and dried eggshells, including the eggshell membranes, at three points - the equator, the sharp and the blunt end of the egg. Measurements were made using a micrometre. Yolk colour was assessed subjectively using a 15-grade Yolk Colour Fan (Hoffman La Roche).

Statistical evaluation

The individual egg quality characteristics were described using the mean. Two-factor analysis of variance was used to evaluate the effect of genotype, age and their interaction on each of the parameters studied. Scheffe's test was used to subsequently test the significance of differences between means for the group factor. Statistical evaluation was performed using Unistat 5.1 software (Unistat Ltd., ENGLAND).

RESULTS AND DISCUSSION

There was a significant effect ($p < 0.001$) of source and level of iron on eggshell weight, eggshell thickness and eggshell proportion there was a significant effect ($p < 0.001$) of source and level of iron. Significant effect of source and level of iron supplemented in the feed was also found for the parameters egg weight, albumen weight, colour and yolk proportion. The effect of age on all egg quality parameters was also demonstrated. The parameters eggshell thickness and yolk colour showed an effect ($p < 0.001$) of the interaction of group and age.

Table 1 shows the effect of the level and source of iron in each experimental group on egg quality parameters over the entire laying period. Over the period of interest, the heavier eggs (61.2 g vs. 60.6 g and 60.2 g) were conclusively ($p < 0.01$) produced by the D-O group layers, which is also confirmed by Xie et al. (2019) for Hy-Line White hybrids when supplementing the diet with an organic iron source, specifically Fe-Gly, which was also used in our experiment. Also Sharlak et al. (2021) found a significantly ($p < 0.05$) higher egg weight in the iron supplemented compared to the non-supplemented experimental group (60.5 g vs 55.3 g). However, no significant difference was found between the iron supplemented groups (Sharlak et al., 2021). The findings of Sharlak et al. (2021) are in agreement with those of Park et al. (2004).

Table 2. Egg quality parameters for the whole laying period

Parameter	Units	Group			Factor		
		D-K	D-A	D-O	Group	Age	Group x Age
Egg weight	g	60,2[b]	60,6[ab]	61,2[a]	<0.01	<0.001	NS
Eggshell strength	N	36,5	38,0	38,0	NS	<0.001	NS
Yolk weight	g	16,4	16,6	16,5	NS	<0.001	NS
Albumen weight	g	38,3[b]	38,4[b]	39,0[a]	<0.01	<0.001	NS
Eggshell weight	g	5,4[b]	5,6[a]	5,7[a]	<0.001	<0.001	NS
Eggshell thickness	mm	0,395[b]	0,402[a]	0,403[a]	<0.001	<0.001	<0.001
Yolk colour	-	11,6[a]	11,9[b]	11,7[ab]	<0.05	<0.001	<0.001
Yolk proportion	%	27,3[a]	27,3[a]	26,9[b]	<0.05	<0.001	<0.05
Albumen proportion	%	63,7	63,4	63,8	NS	<0.001	NS
Eggshell proportion	%	9,1[b]	9,3[a]	9,4[a]	<0.001	<0.001	NS

NS means no statistically significant differences (p>0.05); a,b,c means different letters indicate statistical significance ((p < 0.05)

Egg weight in the D-O group was affected by the conclusively ($p < 0.01$) higher egg albumen weight also in this group (39 g vs. 38.4 g and 38.3 g). The weight, thickness and proportion of eggshell was significantly higher ($p < 0.001$) in the groups whose diet contained elevated levels of iron. Similar conclusions were reached by Sharlak et al. (2021). For the parameter of yolk colour, there was a significant difference in the D-K group compared to the other groups. This finding is in contrast to the study of Seo et al. (2010), who found no conclusive difference ($p > 0.05$) between groups of Hy-Line Brown laying hens given iron in the form of iron-soy proteinate. Also, Xie et al. (2019)

showed no effect of iron source and level on yolk colour in eggs from HY-Line White-produced layers, although the same iron source was used in the Fe-Gly group as in our D-O group.

In the parameter yolk proportion, a significantly higher ($p < 0.001$) proportion of yolk was found in groups D-K and D-A. For the characteristics of eggshell strength, protein percentage, there was no significance ($p > 0.05$) between the tested groups. Our findings correspond in the case of eggshell strength parameter with the results of Xie et al. (2019) and Cao et al. (2023). In contrast, authors Park et al. (2004) found that the effect of FeSO4·7H2O at 200 ppm increased eggshell strength in ISA Brown, which is inconsistent with our results. Also the study of Tu, Zou and Tang (2004) confirmed the beneficial effect of FeSO4·7H2O and Fe-Gly on eggshell strength as well as weight and thickness in Roman hens.

Effect of elevated and organic iron levels on egg quality after 45. week of laying hens' age

Table 2 shows the comparison of the control group with the group in which the inorganic to organic iron source and iron levels were changed in the second phase of the laying period, i.e. after 45 weeks of age. Table 2 shows that the increased amount of iron in the organic form from 45 weeks of age onwards had a conclusive effect on egg weight, albumen, eggshell, thickness eggshell thickness and yolk proportion.

Table 3. Egg quality parameters after 45 weeks of age after changing the source and increasing the iron level

Parameter	Units	Group		Factor		
		D-K-K	D-K-O	Group	Age	Group x Age
Egg weight	g	62,7[b]	64,1[a]	<0.01	<0.001	NS
Eggshell strength	N	35,4	33,8	NS	<0.05	NS
Yolk weight	g	17,8	17,9	NS	<0.001	<0.05
Albumen weight	g	39,2[b]	40,4[b]	<0.01	NS	NS
Eggshell weight	g	5,6[b]	5,8[a]	<0.01	<0.001	NS
Eggshell thickness	mm	0,403[b]	0,413[a]	<0.01	<0.001	<0.001
Yolk colour	-	11,8	11,9	NS	<0.001	<0.01
Yolk proportion	%	28,5[a]	28,0[b]	<0.05	<0.001	<0.01
Albumen proportion	%	62,5	62,9	NS	<0.001	<0.05
Eggshell proportion	%	9,0	9,1	NS	<0.01	NS

NS means no statistically significant differences ($p > 0.05$); a,b means different letters indicate statistical significance (($p < 0.05$)

Characteristics such as egg, yolk and eggshell weight, eggshell thickness, yolk colour, yolk and eggshell proportion were significantly influenced by the age of the laying hens ($p < 0.001$). The characteristics of yolk proportion were also statistically significantly influenced by the age of the laying hens eggshell ($p < 0.01$) and eggshell strength ($p < 0.05$). Kraus et al. (2020) also conducted an experiment with Lohmann Brown Classic and Hisex Brown laying hens enrolled in the experiment at 46 weeks of age and found a conclusive effect of age on egg quality parameters.

For the eggshell thickness parameter, a statistically significant ($p < 0.001$) effect was found interaction of group and age on this parameter of egg quality. Significant effect of the interaction of group and age of laying hens was also confirmed for yolk weight and egg albumen proportion ($p < 0.05$) and also for the parameters colour and yolk proportion ($p < 0.01$).

For the egg weight characteristic, a significant difference ($p < 0.01$) was found, with the D-K-O group laying heavier eggs (64.1 g) than the D-K-K group (62.7 g), which was influenced by the higher eggshell weight (5.8 g vs. 5.6 g) of the D-K-O group compared to eggs produced by D-K-K group hens. Similarly, these eggs also had a thicker eggshell thickness (0.413 mm vs. 0.403 mm) compared to eggs from D-K-K group layers. On the other hand, D-K-K hens produced eggs with a significantly ($p < 0.05$) higher yolk weight (28.5 g vs. 28.0 g) than those from the D-K-O group.

CONCLUSION

In our experiments, a significant effect ($p < 0.001$) of increased supplementation with inorganic and organic iron sources on eggshell quality parameters was found, namely eggshell thickness (0.403; 0.402 mm vs. 0.395 mm), eggshell weight (5.7 and 5.6 vs. 5.4 g) and eggshell percentage (9.4 and 9.3 vs. 9.1 g). It was also found that significantly ($p < 0.01$) heavier eggs (61.2 g vs. 60.6 and 60.2 g) were produced by the D-O group layers due to the significantly higher egg white weight (39 g vs. 38.4 and 38.3 g). The effect ($p < 0.05$) of iron source and level on the qualitative parameters of egg colour and yolk proportion was also found. Increase in organic form of iron after 45 weeks of age had a significant effect ($p < 0.01$) on egg weight (64.1 vs.

62.7 g), eggshell thickness (0.413 vs. 0.403 mm), eggshell weight (5.8 vs. 5.6 g).

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EFFECT OF BACTERIAL INOCULANT ON MYCOTOXIN CONTAMINATION OF ALFALFA SILAGE

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ABSTRACT

The study aimed to investigate the hygienic quality of alfalfa silages, with a focus on the observed concentrations of mycotoxins. It also examined the effect of biological additives, based on homo- and heterofermentative strains of lactic acid bacteria, on the mycotoxin contamination of alfalfa silages. The study monitored several mycotoxins, including total ochratoxins (OTA), total aflatoxins (AFL), total fumonisins (FUM), deoxynivalenol (DON), zearalenone (ZEA), and T-2 toxin (T-2). Mycotoxin concentrations were determined by spectrophotometric immunoenzymatic method (Elisa Reader, Noack SR; Veratox assays, Neogen Ltd., USA) at a wavelength of 650 nm, in alfalfa silage samples that were ensiled into silage units in a volume of 3.5 dm^3. The samples were ensiled in control variant C, without the

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addition of additive, and in experimental variant A with the addition of biological additive (Lactobacillus plantarum, Lactobacillus buchneri) 1.3 x 10^{11} KTJ.g^{-1} at a dose of 10 ml per ton. Both control and experimental treatments were fed in 3 replications (n=3). Analysis of mycotoxic contamination of alfalfa silages revealed statistically significantly ($p < 0.05$) lower fumonisin content in the experimental variant (47.08 µg.kg^{-1}) compared to the control (70.77 µg.kg^{-1}), with a difference of 33.48%. Also, a lower aflatoxin content (3.69 µg.kg^{-1}) was found in the experimental variant compared to the control (4.03 µg.kg^{-1}), which was 8.44%, but these differences were not statistically significant. The contents of DON, ZEA, T-2 and OTA were higher in the experimental variant compared to the control. The mean mycotoxin content of the samples studied showed that alfalfa silages were the most contaminated with ZEA (360.00 µg.kg^{-1}), followed by DON (209.30 µg.kg^{-1}), T-2 toxin (80.38 µg.kg^{-1}), FUM (58.92 µg.kg^{-1}), OTA (53.37 µg.kg^{-1}) and were the least contaminated with AFL (3.81 µg.kg^{-1}). The concentrations of the monitored mycotoxins in alfalfa silages did not exceed the limit values applicable in the EU, which is a prerequisite for ensuring efficient and safe production of animal products.

Keywords: alfalfa silage; mycotoxins; hygienic quality; bacterial inoculant

INTRODUCTION

Silage is the main feed in ruminant nutrition. Silage is a method of preserving forage which allows forage to be stored for a longer period, preserving the nutritional value of the preserved forage. The essence of ensiling is an anaerobic fermentation process in which the pH is reduced by the production of organic acids (mainly lactic acid) by

microorganisms, mainly lactic acid bacteria (LAB; Doležal et al., 2012; Mitrík, 2018; Bíro et al., 2020). However, the growth of microscopic filamentous fungi and potential mycotoxin formation can be triggered by factors such as poor compression, inappropriate dry matter content, insufficient hermetic closure, or rainwater infiltration (Rodríguez - Blanco et al., 2021). The growth of microscopic filamentous fungi also occurs after the opening of the silo when the anaerobic stable environment is disturbed, when the pH is increased and thus the preservative effect of organic acids that suppressed their growth is reduced (Kung et al., 2018). Additives based on homo- and heterofermentative lactic acid bacteria (LAB) strains are often used to enhance the ensiling process and improve silage quality. In addition to improving and accelerating the fermentation process, they also improve the aerobic stability of the silage produced. Fermentation acids are fungistatic, and some LAB strains have the potential to reduce mycotoxin content in silages (Kung et al., 2018; Muck et al., 2018; Gallo et al., 2022). For example, Ma et al. (2017) reported that inoculating *L. plantarum or L. buchneri* linearly reduced aflatoxin B_1 (AFB_1) content in silages, but it is not confirmed whether their antifungal and antimycotoxigenic effects can persist up to the silage feeding stage. The growth of microscopic filamentous fungi and their ability to produce mycotoxins in forages is influenced by a complex of biotic and abiotic factors such as species, its aggressiveness. Furthermore, there are environmental factors such as temperature and humidity, or agrotechnical practice (Alonso et al., 2013). The presence of microscopic filamentous fungi in silage does not automatically imply mycotoxic contamination, and vice versa, the absence of filamentous fungi does not guarantee the absence of mycotoxins (Zain, 2011).

There are more than 400 known species of mycotoxins that are commonly found, but only a few are intensively monitored (Fromme et al., 2016). Ensiled forages can be contaminated by microscopic filamentous fungi and their mycotoxins during the pre-harvest phase (genera *Fusarium, Aspergillus, Alternaria*) and/or post-harvest contamination (species of *Aspergillus* and *Penicillium;* Gallo et al., 2015; Alonso et al., 2013). Mycotoxins are secondary metabolites of microscopic filamentous fungi with low molecular weight. The main mycotoxins that contaminate silages are alflatoxins (AFL), fumonisins (FUM), deoxynivalenol (DON), zearalenone (ZEA) and ochratoxin (OTA; Alonso et al., 2013). By their presence in silages, they can harm animal health, reduce feed intake, reduce performance, and damage the liver. Some are also carcinogenic, teratogenic, immunosuppressive and cause mortality. All these lead to significant economic losses (Ogunade et al., 2018). AFB_1 can be considered as the most toxic, it is a potent carcinogen, mutagen and teratogen that is produced by the genus *Aspergillus* (Bakirdere et al., 2012). After ingestion of contaminated silage with AFB_1, it is metabolized to alflatoxin M_1 (AFM_1) in the body of dairy cows and can be excreted from the animals through all body fluids, including milk. Therefore, its concentration is monitored in milk with a limit of 0.05 $\mu g.kg^{-1}$ (JECFA, 2001; Commission Regulation (EU) 2023/915). DON, ZEA, FUM are fusarium mycotoxins. DON inhibits proteosynthesis, has immunotoxic and cytotoxic effects. It negatively affects feed intake and consequently production. ZEA is estrogenic, which can cause reproductive disorders in both males and females. FUM are hepatotoxic and immunotoxic. They are also considered as potential carcinogens (Rodrigues, 2014). The most common situation in silages is contamination with several types of

mycotoxins simultaneously, which may potentiate their effect on each other or may act synergistically (Cheli et al., 2013). Ruminants are considered to be quite resistant to mycotoxins, due to their rumen microbiota, which is able to partially degrade them into less toxic components. However, ingestion of silage and other feed contaminated with mycotoxins poses a health risk. At the same time, mycotoxins also pose a risk to human health, due to the potential transmission of these toxins through animal products such as milk and meat (Bennett and Klich, 2003; Fink-Gremmels, 2008).

MATERIAL AND METHODS

In cooperation with the university farm SPU s.r.o. Kolíňany farm Oponice, alfalfa (Medicago sativa) from the first mowing at the stage of buttonization was ensilaged. The mass was wilted to a dry matter content of 37% and cut to a theoretical slice length of 20 mm using a self-propelled cutter. The alfalfa was ensiled in two different ways: C (control) and A (experimental variant). In the control variant C, the wilted mass was ensiled without the addition of additives. In experimental variant A, a liquid biological additive based on homo- and heterofermentative lactic acid bacteria (*Lactobacillus plantarum, Lactobacillus buchneri*) 1.3 x 10^{11} KTJ.g^{-1} at a dose of 10 ml per ton was applied to the mass. Alfalfa silage was preserved in three replications (n=3) in the control variant C as well as in the experimental variant A in silage units with a volume of 3.5 dm3. After hermetic sealing, the silage units were stored in the air-conditioned Laboratory of Forage Conservation at the Institute of Nutrition and Genomics at 22 °C. After 8 weeks of storage, the silage units were averaged and sampled. The mycotoxin content of the average samples was

determined. Prior to the determination of mycotoxin concentrations, laboratory samples of alfalfa silage were extracted in 70% methanol (for total aflatoxins, total fumonisins, T-2 toxin and zearalenone), in 50% methanol (for total ochratoxins) and in distilled water for deoxynivalenol. A spectrophotometric immunoenzymatic method at a wavelength of 650 nm (Elisa Reader, Noack SR; Veratox assays, Neogen Ltd., USA) was used to determine the mycotoxin content of the samples. Total ochratoxins (OTA), total aflatoxins (AFL), total fumonisins (FUM), deoxynivalenol (DON), zearalenone (ZEA), T-2 toxin (T-2) were determined.

The results were statistically evaluated using SPSS 26.0 (IBM) statistical program using one-way ANOVA (mean, standard deviation, minimal and maximal values). Tukey's test and Independent samples T-test at the $p < 0.05$ level were used to evaluate the statistical significance of differences.

RESULTS AND DISCUSSION

Several species of mycotoxins were determined in alfalfa silage samples with and without addition of the biological additive, the concentrations of which are shown in Table 1.

The deoxynivalenol (DON) content was 87.51% ($p < 0.05$) higher in the sample with biological additive than in the control. Fan et al. (2021) observed a positive effect of bacterial inoculant on reducing the occurrence of DON in alfalfa silage samples. Increased DON content in corn silage samples with the inoculant compared to the control was also observed by Kalúzová (2023). The average DON content in the analyzed samples was 209.30 $\mu g.kg^{-1}$. Ogunade et al. (2018) reported

an average DON content of 2150.00 µg.kg^{-1} in alfalfa silages. Hodulíková et al. (2016) reported DON content in alfalfa silages from 114.41 to 120.96 µg.kg^{-1} and Juráček et al. (2012) from 365.00 to 379.20 µg.kg^{-1}.

Statistically significant lower content of total fumonisins (FUM) in samples with biological additive compared to C with a difference of 33.48% ($p < 0.05$) was observed. When inoculant was used in maize silage, increased FUM concentrations were observed (Kalúzová 2023; Bakri, 2021; Gallo et al., 2018). The average FUM content in the analyzed samples was 58.92 µg.kg^{-1}. Huerta-Treviño et al. (2016) found an average FUM content of 91.00 µg.kg-1 (fresh alfalfa), Juráček et al. (2014) only 5.40 to 6.27 µg.kg^{-1} (alfalfa silage).

The zearalenone (ZEA) content was lower in the control variant compared to the variant with the addition of the biological additive. The difference between these variants was 11.51 µg.kg^{-1} which was 3.25%. These differences were not statistically significant. Teller et al. (2012) observed lower ZEA concentration in corn silage samples with addition of biological additive compared to control. Among the mycotoxins studied, ZEA occurred at the highest concentration. Its average content in the tested samples was 360.00 µg.kg^{-1}. Huerta-Treviño et al. (2016) reported an average ZEA content of 199.56 µg.kg^{-1} in alfalfa and Ogunade et al. (2018) 533.80 µg.kg^{-1} in alfalfa silages. Adácsi et al. (2022) reported ZEA concentrations lower than 100.00 µg.kg^{-1} in alfalfa silage samples, and Rodríguez-Blanco et al. (2021) did not observe the occurrence of ZEA in alfalfa silage samples.

The T-2 toxin (T-2) content was also lower in the control than in the experimental variant. The difference was 32.33%. Kalúzová et al.

(2022), Wang et al. (2018) and Latorre et al. (2015) confirmed the change in T-2 toxin concentration by using microbial inoculants in maize silages. The average concentration of T-2 toxin in the samples studied was 80.38 $\mu g.kg^{-1}$. Huerta-Treviño et al. (2016) reported a comparable mean T-2/HT-2 concentration in alfalfa silages of 93.71 $\mu g.kg^{-1}$ and Juráček et al. (2014) ranged from 73.30 to 143.50 $\mu g.kg^{-1}$.

The difference in total aflatoxin (AFL) content between the control and experimental treatments was 8.44% and was statistically non-significant. The experimental variant with the addition of inoculant had a lower AFL concentration. Fan et al. (2021) reported a decrease in AFB1 concentration when bacterial inoculants were used in alfalfa silage. Kalúzová et al. (2022) also noted a decrease in AFL concentration following the addition of an inoculant. The average AFL concentration in the analyzed samples was 3.81 $\mu g.kg^{-1}$. A similar mean AFL content in alfalfa silages of 2.77 $\mu g.kg^{-1}$ was also reported by Huerta-Treviño et al. (2016). Rodríguez-Blanco et al. (2021) found an average AFG1 and AFG2 concentration of 2.21 $\mu g.kg^{-1}$ and 0.91 $\mu g.kg^{-1}$, respectively.

Total ochratoxins (OTA) had a lower concentration in the control sample. The difference ($p < 0.05$) between control and experimental samples was 16.56%. Similarly, Kalúzová et al. (2022) reported higher concentration of OTAs after using inoculant in maize silage. The average OTA content in the samples studied was 53.37 $\mu g.kg^{-1}$. Adácsi et al. (2022) reported OTA concentrations in alfalfa silage samples ranging from < 0.50 to 27.57 $\mu g.kg^{-1}$, Juráček et al. (2014) from 13.30 to 13.80 $\mu g.kg^{-1}$, and Huerta-Treviño et al. (2016) reported an average OTA content of 32.74 $\mu g.kg^{-1}$.

When evaluating the mycotoxin content of wilted alfalfa silage, it can be concluded that the biological additive had a positive effect on reducing the content of total fumonisins ($p < 0.05$). A similar trend was observed for total aflatoxins, but the differences were not statistically significant. For the remaining mycotoxins tested, their content was higher in the variant with the addition of the biological additive (DON, T-2, OTA, ZEA). ZEA (360.00 $\mu g.kg^{-1}$) was the most abundant mycotoxin in the alfalfa silage samples, followed by DON (209.30 $\mu g.kg^{-1}$) and T-2 toxin (80.38 $\mu g.kg^{-1}$). The monitored samples did not exceed the permitted acceptable and recommended limits (Directive 2002/32/EC; Commission Recommendation 2006/576/EC; EFSA, 2014).

Table 1. Average concentrations of mycotoxins in alfalfa silage

µg.kg-1 of DM	**DON**	**FUM**	**ZEA**	**T-2**	**AFL**	**OTA**
Control (C)	145.59[a]	70.77[a]	354.24	69.19[a]	4.03	49.29[a]
Additive (A)	273.00[a]	47.08[a]	365.75	91.56[a]	3.69	57.45[a]
Average	209.30	58.92	360.00	80.38	3.81	53.37

DON: deoxynivalenol, FUM: total fumonisins, ZEA: zearalenone, T-2: T-2 toxin, AFL: total aflatoxins, OTA: total ochratoxins. Values with identical indexes in a column are statistically significant ($p < 0.05$).

Previous studies have already indicated that some LAB strains can degrade or inhibit mycotoxins during the fermentation process (Wambacq et al., 2018; Ferrero et al., 2019). Antifungal compounds such as organic acids, carboxylic acids and phenolic compounds produced by LAB can reduce mycotoxins produced by filamentous

microscopic fungi (Peles et al., 2019). Piotrowska (2014) confirmed that *Lactobacillus brevis* and *Lactobacillus plantarum* reduced the concentration of OTA in vitro. Franco et al. (2011) in a similar study found that *Lactobacillus brevis* and *Lactobacillus paracas*ei reduced the concentration of DON in vitro. Ma et al. (2017) revealed the capacity of LAB to bind AFB_1 in vitro, but also in corn silage samples artificially contaminated with AFB_1, when the concentration of AFB_1 in the samples decreased. It can be concluded that microbial additives can be used to prevent the growth of filamentous microscopic fungi and to reduce the mycotoxin content.

CONCLUSION

The results confirmed that a biological additive based on homo- and heterofermentative strains of lactic acid bacteria had a positive effect on the demonstrable reduction of total fumonisins. There was also a reduction in the concentration of total aflatoxins, but the differences were not statistically significant. However, an increase in the content of deoxynivalenol (DON), T-2, ochratoxins (OTA), and zearalenone (ZEA) was observed. These findings suggest that further experiments are needed to verify the effect of additives on mycotoxin concentrations in alfalfa silages. Alfalfa silages were the most contaminated with zearalenone, followed by deoxynivalenol and T-2 toxin. The monitored concentrations of all mycotoxins in lucerne silages did not exceed the maximum permitted limits for mycotoxins in ruminant feed, a prerequisite for ensuring the sustainable and safe production of animal products.

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THE EFFECT OF ENERGY BALANCE AND NON-NUTRITIONAL FACTORS ON PREGNANCY SUCCESS AFTER THE 1ST INSEMINATION IN DAIRY COWS

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ABSTRACT

This study aimed to evaluate energy balance and climatic conditions on pregnancy success after 1st insemination in Holstein cows. Successful conception rate, including the hypothalamic–pituitary–ovarian axis is most sensitive to energy balance and the availability of metabolic fuels. The analyzed negative energy balance of cows with successful insemination was confirmed at a lower level of -14.4 MJ/d, while in 74% of dairy cows with unsuccessful insemination NEB was at the level of -19.4 MJ/day. The level of NEB at the level - 47 MJ/day resp. -53 MJ/d corresponds to a weight loss of 2.3 kg resp. 2.7 kg in the 1st month after parturition. The pregnancy success after artificial insemination in summer is reduced and affected by reduced dry matter intake, the function of the endocrine system's level of hormones and the level of negative energy.

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Keywords: temperature, energy balance, milk, reproduction

INTRODUCTION

The milk yield and reproductive performance are the standard economic barometers of dairy production, which are affected by nutritional factors (composition of feed ration, dry matter intake) and non-nutritional factors. The most important non-nutritional factors include age, breed, lactation order and, last but not least, the temperature of the external environment (M'hamdi et al. 2012). The high temperature of the environment, together with a change in humidity and airflow, causes heat stress with a direct effect on the thermoregulation of animals (Laporta et al. 2017). High production of metabolic heat created during digestion in the rumen (Mader, Davis 2004), along with high temperature, directly proportionally increases the body temperature and frequency of breathing, with a negative effect on milk production and reproductive performance in dairy cows (Domingues et al. 2005).

Up to 65% of the proportion to reduced productions with the impact on the fertility of dairy cows is related to metabolic changes and overall load of metabolism (disruption of digestion in the rumen, change acid-base balance as a result of the heat stress of dairy cows) Up to 65% of the proportion to reduced productions with the impact on the fertility of dairy cows is related to metabolic conversion and overall burden of metabolism (disruption of digestion in the rumen, change acid-base balance as a result of the heat stress of dairy cows) (Vujanac et al. 2012; De Rensis et al. 2017; Liu et al. 2018). A significant impact on the changes in reproductive response during heat stress has a reduction

in dry matter intake, leading to deepening of the negative energy balance (NEB) in the peripartal period.

When significant metabolic changes occur directly, a state of negative energy balance (NEB) with high energy loss affects the recovery of the estrous cycle and the success of further inseminations. NEB leads to lipomobilisation and thus burdens the liver. Recovery of the estrous cycle after calving and subsequent successful insemination depends on several factors associated with the function of organs that undergo a significant load in the peripartum period (Wathes et al., 2007). Important metabolic processes take place in the liver that affects liver function (gluconeogenesis, oxidation of fatty acids, production of insulin-like growth factor (IGF-I) (Butler 2005).

Problems associated with NEB include, for example, delayed first ovulation, prolonged reproductive cycles compared to physiological standard (persistent corpus luteum), long intervals between other luteal phases, when dairy cows do not come into ovulation and others. Various stress factors, diseases (uterine infections), and reduced feed intake before the expected ovulation after luteolysis (Wathes 2007) may also affect cycles without ovulation.

Changing the temperature of the environment mainly elevated temperature during the summer months, as well as deficient intake of nutrients have a direct effect on the endocrine dairy system (Ronchi et al. 2001), causing a reduction concentration of IGF-1 and glucose (O'Callaghan, Boland 1999), and effects the development of follicles and glucose as the main source of energy for the ovaries (Rabiee et al. 1997) with the impact on the manifestation of the heat (estrus) and the conception rate in dairy cows. During the summer months, the effect of increasing temperature on the hypothalamus-pituitary-ovarian axis,

which affects the secretion of hormones, with the manifestation of reduced GnRH secretion, reduced ovarian activity and lower dominance of follicles, is described.

The goals of the work are based on the hypothesis of a published negative effect of energy balance and climatic conditions on milk synthesis and reproduction performance in dairy cows. In this intention the aim of the work was to verify the influence of milk production, the energy balance and heat stress on success pregnancy after the 1st insemination in multiparous dairy cows in breeding conditions on a farm with milk production over 10,000 kg.

MATERIAL AND METHODS

The evaluations were carried out on the selected farm with a controlled nutritional level system and an average annual production over 10 000 kg per cow. Cows were housed in a freestall barn in a separate group and fed a TMR that was formulated to meet NRC (2001) recommendations for 1st phase of lactation of multiparous dairy cows. Multiparous dairy cows (n = 359) were evaluated for the efficiency of utilizing N and energy balance in relation to pregnancy success at first insemination. Samples of prepared TMR in the monitored farm were taken from the feed manger on the control day and there were analysed for dry matter (DM), crude protein (CP), acid and neutral detergent fibre (ADF, NDF), starch and ether extract (EE) contents according to conventional methods according to the Commission Regulation (EC) no. 691/2013. NEL and non-fibrous carbohydrate (NFC) values were calculated using regression equations (NRC 2001).

All cows were enrolled in a Presynch Ovsynch protocol. Controlled reproduction was applied from the 70th day after parturition. The

success of reproduction was evaluated in dairy cows by transrectal ultrasonography of the ovaries and uterus.

Analysis of production parameters on the control day on individually collected milk samples was evaluated for milk production levels in dairy cows, milk components and milk urea. Milk samples were analysed the total protein content, fat, lactose, and urea concentration by near infrared spectrophotometric assay using MilkoScan FT^{+} and BENTLEY FTS at the Central Analytical Laboratory of Milk with accreditation under registration number 096/5878/2015/2.

The energy balance of the nutrients in dairy cows was evaluated by the balance of the amount of actually received energy in TMR in relation to the excreted energy in the nutrients produced by milk, as a positive or negative balance of NEL MJ per day, and determined by calculation according to Daniel et al. (2017).

Efficiency of Nitrogen Utilization (EUN) for group of dairy cows was estimated according to the analysed content of MUN and the amount of milk produced by using regression equation by Huhtanen et al (2015) from meta-analytical assessments of the balance experiments.

The influence of climatic conditions by measuring air temperatures during the year seasons was evaluated using daily measurements from a weather station located approximately 13 km from the farm. Average, maximum and minimum daily temperatures were recorded in a climatological booth at a standard height of 2 m above the ground for 24 hours. Maximum daily temperature was generally recorded with a mercury thermometer 2 hours after peak and minimum temperature was recorded with an alcohol thermometer before sunrise. Information on daily temperatures was provided to us by the Slovak Hydrometeorological Institute of the Slovak Republic.

The achieved results were processed by the statistical program GraphPad Prism9. Each parameter was presented as its mean (x), and standard deviation (SD).

RESULTS AND DISCUSSION

1. Evaluation of nutritional composition

The average concentration of nutrients in TMR at the evaluated farm are presented in Tables 1. The feed ration based on corn, clover and grass silage with the addition of concentrated feed was formulated for multiparous dairy cows for milk production 39.3 kg (3.28% protein and 3.6% fat).

Table 1. Nutritional composition of TMR

		Postpartum phase X ± SD	1st Lactation phase X ± SD
CP	g/kg DM	170.1 ± 2.5	157.2 ± 5.8
Fat	g/kg DM	50.6 ± 3.1	43.6 ± 2.3
ADF	g/kg DM	209.2 ± 5.8	215.9 ± 7.8
NDF	g/kg DM	224.4 ± 9.0	348.2 ± 10.4
Starch	g/kg DM	230.1 ± 8.8	272.2 ± 18.4
NFC	g/kg DM	361.7 ± 11.3	385.9 ± 2.8
NEL	MJ/kg DM	6.60 ± 0.2	6.63 ± 0.3
Dry matter intake	kg	19.0 ± 0.6	24.5 ± 0.74
NEL intake	MJ/d	125.4 ± 2.5	162.4 ± 3.0

Composition: *corn silage 19-33 kg, legume silage 6-17 kg, grass (oat) silage 2-3 kg, grass/alfalfa hay 0.7–1 kg, wheat straw 0.5-0.8 kg, DDGS 4 kg, wheat 0.8–3.5 kg, rapeseed meal 2.2 – 3.8 kg, Feed mixture 4.2 kg*

2. Evaluation of milk production, energy balance and N utilization on reproduction

In the annual assessment, the impact of nutrient intake, production and milk components was analyzed in relation to the success of conception of dairy cows after the 1st insemination. The results of the examinations

are summarized in Table 2. From the total number of 359 evaluated dairy cows with an average daily production of 43.5±7.1 kg and a total milk production of 3003.2 kg until insemination, 25.6% of cows successfully became pregnant after the 1st insemination. In dairy cows that did not conceive at the first insemination, a 1.9 kg higher daily milk production and a 4.5 kg higher milk fat production by the date of insemination was confirmed.

The analysis of the energy balance assessed by the energy intake in the ration and the excretion of nutrients in milk (Daniel et al. 2017) confirmed for the period of the first two months of lactation until the 1st insemination a negative energy balance (NEB) on average -18.2 MJ/day with a lower level of -14.4 MJ/d in dairy cows with successful insemination, while in 74% of dairy cows with unsuccessful insemination NEB was at the level of -19.4 MJ/day.

A higher level of NEB was confirmed at the 1st test control, on average 43 days after calving, where NEB was on average - 47 MJ/day in the group with successful insemination or – 53 MJ/day in the group with unsuccessful insemination. Such a level of NEB corresponds to an average weight loss of 2.3 kg or 2.7 kg in the 1st month after birth. The higher level of the NEB was confirmed in the 1st test of control on average until the 43rd day after calving, where the NEB was on average - 47 MJ/day in the group of dairy cows with successful insemination, resp. - 53 MJ/day in the group with unsuccessful insemination. Such a level of NEB corresponds to a weight loss of 2.3 kg resp. 2.7 kg in the 1st month after parturition. The difference in the level of the NEB within the groups at the 1st resp. 2nd day of the testing is 6.2 resp. 5.0 MJ NEL/day, which corresponds to the energy requirements for synthesis 1.8 - 2.0 kg of milk, and correlates with confirmed average

milk production within groups. The studies by authors (Garverick et al., 2013, Patton et al., 2007) showed that the pregnancy rate at the first insemination is higher in proportion to the decrease in negative energy balance in dairy cows in the postpartum period.

Table 2. Production, milk components and level of energy balance in relation to pregnancy success of dairy cows at the 1st insemination

		multiparous cows (n 359) X ± SD	**1st insemination positive (n 92) X ± SD**	**1st insemination negative (n 267) X ± SD**
Pregnancy rate		**25.6 %**	-	-
DIM at insemination		69 ± 19	69 ± 17	69 ± 15
Milk production	kg/d	43.5 ± 7.1	42.1 ± 7.9	44.0 ± 6.8
Total yield	kg	3003.2 ± 14.1	**2906.9 ± 14.4**	**3034.6 ± 13.9**
Milk fat	%	3.50 ± 0.6	3.50 ± 0.6	3.50 ± 0.7
Total yield	kg	105.1	101.7	106.2
Milk protein	%	3.05 ± 0.3	3.11 ± 0.3	3.02 ± 0.3
Fat/Protein		1.15 ± 0.2	1.13 ± 0.2	1.16 ± 0.2
Lactose	%	4.90 ± 0.1	4.92 ± 0.1	4.90 ± 0.2
Energy Balance	MJ/d	**-18.2± 7.2**	**-14,4 ± 7.0**	**-19.4 ± 6.3**
EUN[1]	%	32.3 ± 0.7	32.1 ± 0.2	32.1 ± 0.4
Milk Urea	mg/dl	26.9 ± 5.6	26.3 ± 4.4 (↑ 21 %)	27.1 ± 5.9 **(↑ 29 %)**
DIM in 1st test day		43 ± 25	43 ± 22	43 ± 27
Milk production	kg/d	41.9 ± 8.3	**40.3 ± 8.2**	**42.4 ± 8.3**
Total yield	kg	1800.4 ± 20.2	**1733.3 ± 17.4**	**1823.2 ± 20.3**
Milk fat	%	3.74 ± 0.9	3.73 ± 0.8	3.74 ± 1.0
Total yield	kg	67.3	64.7	68.2
Milk protein	%	3.12 ± 0.4	3.20 ± 0.4	3.10 ± 0.4
Fat/Protein		1.20 ± 0.3	1.17 ± 0.2	1.21 ± 0.3
Energy Balance	MJ NEL/d	**-51.5 ± 7.2**	**-46.9 ± 7.0**	**-53.1 ± 6.3**
EUN[1]	%	32.1 ± 0.7	31.9 ± 0.2	32.1 ± 0.4
Milk Urea	mg/dl	26.2 ± 7.7	25.7 ± 6.7 (↑ 22 %)	26.4 ± 7.9 **(↑ 29 %)**

DIM – days in milk

EUN – Efficiency of nitrogen utilisation (Huhtanen et al.2015)

3. Evaluation of energy balance and climatic condition on reproduction

The analysis of the effect of the seasonal dependence of production and milk components in relation to the conception rate is summarized in Table 3.

Table 3. Seasonal dependence of production, milk components and the level of energy balance in relation to the success of conception of dairy cows at the 1st insemination

	Spring-Summer (IV. – IX.)		**Autumn-Winter (X. – III.)**	
	1st insemination		**1st insemination**	
	positive (n 38) X ± SD	**negative (n 122) X ± SD**	**positive (n 54) X ± SD**	**negative (n 145) X ± SD**
Pregnancy rate	**24%**		**27%**	
DIM at insemination	70 ± 23	70 ± 17	67 ± 12	67 ± 13
Milk production kg/d	**43.3 ± 6.6**	**44.1 ± 7.4**	**41.3 ± 7.5**	**43.9 ± 6.6**
Total yield kg	3029.6±13.2	**3087 ± 10.3**	2767.1±15.1	**2941.3± 9.1**
Milk fat %	3.36 ± 0.9	**3.43 ± 0.6**	3.60 ± 0.6	3.56 ± 0.7
Total yield kg	101.8	**105.9**	99.6	**104.7**
Milk protein %	2.97 ± 0.3	2.89 ± 0.3	3.22 ± 0.2	3.13 ± 0.2
Fat/Protein	1.14 ± 0.3	1.19 ± 0.2	1.12 ± 0.2	1.14 ± 0.2
Lactose %	4.91 ± 0.1	4.89 ± 0.2	4.92 ± 0.1	4.90 ± 0.1
Energy Balance MJ/d	**-19.1± 8.7**	**-22.0 ± 6.3**	**-15.6± 7.7**	**-23.2 ± 6.3**
EUN[1] %	32.1 ± 0.3	32.0 ± 0.3	32.2 ± 0.2	32.5 ± 0.4
Milk Urea mg/dl	28.2±4.1 (↑34%)	29.5±5.3 **(↑43%)**	24.9±4.1 (↑11%)	25.1 ± 5.6 **(↑17 %)**
DIM in 1st test day	41 ± 26	44 ± 36	43 ± 18	42 ± 15
Milk production kg/d	41.4 ± 8.1	**42.1 ± 8.5**	39.6 ± 8.2	**42.6 ± 8.3**
Total yield kg	1697.4±10.2	**1852.4±10.2**	1702.8±10.2	**1789.2±8.2**
Milk fat %	3.60 ± 0.9	**3.55 ± 0.9**	3.82 ± 0.7	**3.91 ± 1.0**
Total yield kg	61.9	**65.8**	65.0	**70.0**
Milk protein %	3.10 ± 0.4	2.98 ± 0.4	3.27 ± 0.3	3.19 ± 0.3
Fat/Protein	1.17 ± 0.3	1.19 ± 0.3	1.17 ± 0.2	1.23 ± 0.3
Energy Balance MJ/d	**-47.0 ± 8.7**	**-47.1± 10.8**	**-46.9 ± 7.7**	**-58.1 ± 8.7**
EUN[1] %	32.0 ± 0.2	31.8 ± 0.3	31.8 ± 0.2	32.4 ± 0.3
Milk Urea mg/dl	26.2±8.2 (↑34%)	28.6±8.2 (↑42%)	25.3±5.6 (↑13%)	24.6±7.3 (↑18%)

DIM – days in milk

EUN – Efficiency of nitrogen utilisation (Huhtanen et al.2015)

The seasonal evaluation of the analyzed relationships confirmed in the colder months of the year (Autumn-Winter X. - III.) a higher proportion of inseminated animals (55.4%) together with a higher rate of conception (27%) with a higher NEB difference in the group of dairy cows with unsuccessful insemination. In the warmer months of the year (Spring-Summer (IV. - IX.)) with a higher average production of milk and milk fat together with a lower proportion of inseminated animals (44.6%), a lower acceleration rate (24%) was confirmed.

The results can be given in relation to the influence of heat stress with a confirmed share of 64 days with daily temperatures above the thermal optimum level (above 25ºC). The effect of summer heat on the level of conception with a significant decrease in the summer months of 27.7 % compared to the winter months of 42.6 % was confirmed by Wolfenson and Roth (2019). The results of climatological information on seasonal dependence are presented in Table 4. The upper critical temperature for Holstein dairy cows is 25 to 26 °C (Yang et al. 2010). Maximum and minimum temperatures in summer were 35.5 and 14.6 °C, respectively. The average temperature exceeded 25 °C on 70% of test days (64 days out of 92 testing days) during this period, indicating that cows were exposed to high temperatures.

Table 4. Climatological information in seasonal dependence

Items	**Period**			
	Spring	**Summer**	**Autumn**	**Winter**
	X ± SD			
Temperature, minimum (°C)	3.2	14.6	-1.7	-14.5
Temperature, maximum (°C)	21.0	35.5	21.0	6.7
Average temperature (°C)	11.0±4.8	20.4±3.0	9.3±5.0	-2.2±4.9
Days temperatures > 25 °C	-	64	-	-

CONCLUSION

The results of the examination confirmed the tendency of a negative dependence between the amount of milk and milk fat production, as well as the level of NEB at the same time on the first test day of production as well as at the time of the first insemination. The obtained dependencies according to the evaluation of production markers are currently experimentally verified by analyzing the rate of lipomobilization, ketogenesis and the functional load of liver metabolism in dairy cows after parturition and in the insemination phase.

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GREEN SYNTHESIS OF SELENIUM NANOPARTICLES VIA MEDICINAL ZAMBIAN PLANTS

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ABSTRACT

The focus of this study was to produce and select promising selenium nano particlessynthetized in combination with medicinal indigenous Zambian plant extracts. The plants under study were *Bobgunnia madagascariensis*, *Moringa oleifera, Aloe barbadensis, Azadirachta indica, Cissus quadrangulari, Kigelia Africana, and Gliricidia sepium*. After synthesis the brick red colour change on each sample indicated the reduction of Se2O3 to Se0 to form green synthesized selenium nano particles. A total antioxidant capacity analysis was conducted on the green synthesized selenium nano particles. *Bobgunia madascariensis, Moringa oleifera* and *Gliricidia sepium* SeNPs were observed to be promising SeNPs which could be employed in animal nutrition as an antioxidant defence enhancer.

Keywords: Total Antioxidant Capacities; Selenium Nano Particles; Plant Extracts.

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INTRODUCTION

The use of nanotechnology in this era has proven to be the most promising and advancing field of study due to their wide application in technology and applied science to synthesize materials to a nano scale level. This technology coupled with the use of materials from biological sources has been an emerging and effective technical tool to produce ecofriendly nanoparticles. Herbal or medicinal plants have been utilized for prevention and cure of diseases in many parts of the world for so many years now. These plants are comprised of substances that possess nutritive, preventative, and curative properties for many diseases and deficiencies. Plants are also known to contain various compounds such as alkaloids, flavonoids, phenol, tannin, and alcohol which have the capability to reduce metallic ions to nanoparticles with very good stability. Production of SeNPs using green approaches has been found to consume less energy and possess both simple and broad applications and utilizes a reducing agent that is easily accessible and biodegradable.

MATERIAL AND METHODS

Moringa oleifera, Azadirachta indica, Gliricidia sepium, Aloe barbadensis, Cissus quadrangularis, Kigelia Africana, and *Bobgunnia madagascariensis plants* were collected and dried in Zambia.

One mL of plant extract was slowly added to 9 mL solution of sodium selenite (10 mM) under continuous stirring on magnetic stirrer. Mixture was covered by parafilm and let react at 22°C, 600 rmp, 24 hr. SeNPs were stored at 4 °C.

For antioxidant capacity, the spectrophotometry ABTS method was used according to standardized protocol.

RESULTS AND DISCUSSION

After incubation the brick red colour change on the right of each sample indicates the reduction of Se_2O_3 to Se_0 to form SeNPs. This can be seen in figure 1, SeNPs formed, their size and shape influenced the interaction with light, causing the absorption of shorter wavelengths and the reflection or transmission of longer wavelengths, particularly in the red part of the spectrum as observed by other research findings on green synthesis of SeNPs[13,14] . This characteristic red colour serves as a visual indicator and is a result of the collective fluctuation of free electrons on the nanoparticle's surface[14].

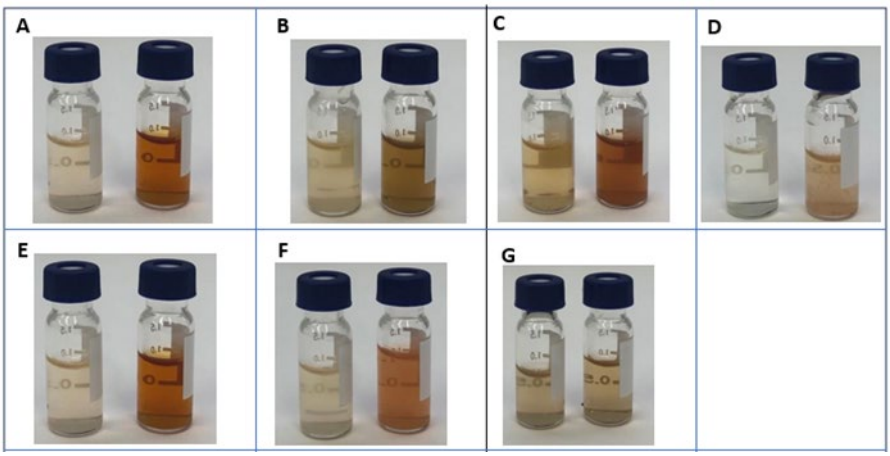

Figure 1: Plant extracts (left) and synthesized SeNPs (right). A) Snake bean, SB (Bobgunnia madagascariensis), B) Moringa, M (Moringa oleifera), C) Aloe, A (Aloe barbadensis), D) Neem, N (Azadirachta indica), E) Veld grape, VG (Cissus quadrangularis), F) Sausage tree, ST (Kigelia Africana) G) Gliricidia, G (Gliricidia sepium).

Due to different chemical composition of plant, efficiency of extraction varied based on selected procedure. For the purpose of the study, extraction methods were evaluated based on antioxidant capacity of the extract.

For extraction of SB, the lowest TAC yield was observed with a 1-hour extraction using 30% and 50% EtOH at 60°C; however, after 24 hours, the TAC yield was comparable to other methods. For extraction of M, both 30% and 50% EtOH resulted in higher TAC yields after 24 hours of incubation compared to other extraction approaches. The highest TAC yield for extraction A was observed using H2O and 30% EtOH at 22°C after just 1 hour. Other extraction methods did not differ significantly in this case. The effect of EtOH concentration and temperature at both time points was observed for extraction N, where higher TAC yields were noted with 30% and 50% EtOH at 60°C compared to H2O at 22°C and 60°C and 30% EtOH at 22°C. Only at 22°C with 50% EtOH was the TAC yield comparable to extraction with water. The impact of extraction time was significant for extraction VG, where 24-hour incubation showed higher TAC compared to 1-hour extraction. For H2O at both tested temperatures and 30% EtOH at 22°C, the TAC yield was lower compared to other extraction methods. For extraction ST, the highest TAC was evident at 60°C after 1-hour extraction in H2O. In EtOH solutions, TAC was lower or comparable to H2O extraction at 22°C. Conversely, extraction with 30% EtOH at 60°C for 24 hours resulted in significantly lower TAC compared to extraction in H2O. Extraction in EtOH solutions at 60°C for 24 hours significantly increased TAC compared to extraction in water or after 1-hour extraction for G. Extraction methods for H were comparable in TAC yield, except for a 1-hour incubation in EtOH solutions at 60°C, where the extraction efficiency was significantly lower compared to other methods.

Overall, the highest TAC yields were observed for SB, M, A and VG while the lowest were for N, ST and G, respectively.

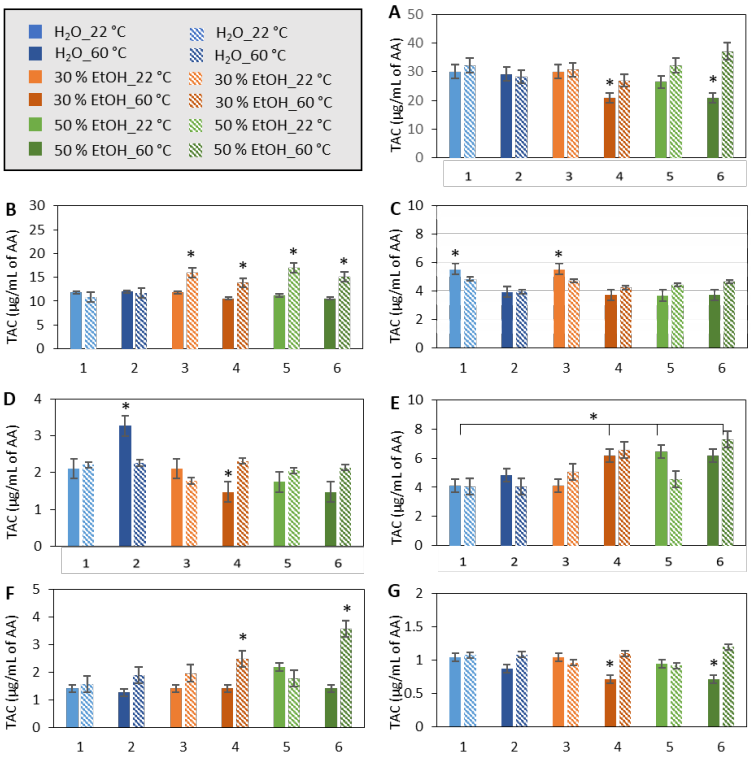

Figure 2: Influence of extraction method on total antioxidant capacity (TAC). TAC is expressed as an ascorbic acid equivalent. A) Snake bean, SB (Bobgunnia madagascariensis), B) Moringa, M (Moringa oleifera), C) Aloe, A (Aloe barbadensis), D) Neem, N (Azadirachta indica), E) Veld grape, VG (Cissus quadrangularis), F) Sausage tree, ST (Kigelia Africana) G) Gliricidia, G (Gliricidia sepium).

Total antioxidant capacity (TAC) of SeNPs is shown on Fig. 3. The highest TAC was observed for SB-SeNPs (1500 µg/mL AA equivalent) and for G-SeNPs (948 µg/mL AA equivalent) and the lowest TAC was measured in ST-SeNPs (193 µg/mL AA equivalent).

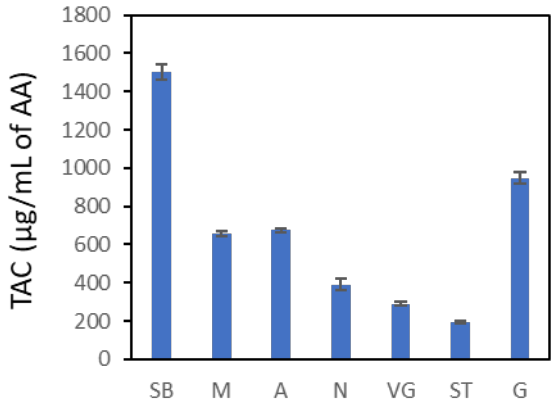

Fig 3: TAC of green synthetized SeNPs

From obtained results is crealy shown that TAC of SeNPs is higher compared to plant extracts. Yields of TAC plant extracts and SeNPs corresponds to each other, however, Girilicia-SeNPs showed higher TAC compared to plant extract. This phenomenon could be explained due to capping agents which are naturally occured in plant extract and SeNPs formation.

CONCLUSION

Based on findings of this research**,** SeNPs are stronger antioxidants compared to plant extracts. SB, M and G SeNPs are promising SeNPs which could be employed in animal nutrition as an antioxidant defence enhancer.

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INFLUENCE OF MODIFICATION THE LENGTH OF STRAW PARTICLES IN TOTAL MIX RATION ON RUMINATION TIME, MILK YIELD, AND MILK COMPOSITION IN THE NUTRITION OF DAIRY COWS

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ABSTRACT

The aim of the experiment was to determine the effect of changing straw length on the behaviour of dairy cows, specifically on activity time (feed intake time, rumination time) or non-activity time (using BouMatic's RealTime Activity program), milk yield (using HerdMetrix program) and milk composition (using True test in 10-day intervals). The 4-week experiment was conducted in a group of high producing Holstein-Friesian dairy cows that were at the peak of their lactation (61-120 days in milk) and were divided into three groups (PLM1;PLM2;PLM3) where they were fed a total mix ration (TMR) with different straw lengths (TMR1;TMR2;TMR3). The group of cows at the peak of their lactation 1 (PLM1) (N=33) were fed TMR1, which

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consisted of straw particles with a length of 3±2 cm. PLM2 (N=29) was fed TMR2 (3±2 cm; 15±2 cm) and the PLM3 group (N=32) was fed TMR3 (15±2 cm). TOMAHAWK Straw mill 404M was used to achieve the desired straw length. The structure of the TMR was also evaluated 3× over the observation period (Penn State Particle Separator method), focusing on the behaviour and selection ability of the dairy cows. TMR structure assessment was performed during the given control periods 4 times/24 h (05:00;11:00;17:00;23:00). The most pronounced selection was observed for PLM3 (TMR3), where the proportion of large particles (+19mm) was as high as 32.92% at 23:00 h., which is 8% higher than the proportion of particles for TMR1 (PLM1) ($p < 0.05$). The results showed that increasing straw length led to an increase in total activity of dairy cows ($p < 0.05$, p-value = 0.000) while non-activity time decreased ($p < 0.05$). There was also a statistically significant increase in milk production (PLM3) with increasing straw length (TMR3), which was an increase of 3.34 kg milk compared to TMR1 (PLM1) ($p < 0.05$, p-value = 0.000). A statistically significant increase with increasing physical structure of TMR was also observed for fat and protein content of milk ($p < 0.05$). Based on the results, it was found that modification of straw length can lead to increased feeding efficiency and consequently higher milk production and quality.

Keywords: dairy cows; sorting; rumination time; milk yield; straw

INTRODUCTION

Nutrition of dairy cows is a key factor influencing the overall performance of dairy cows such as health status, overall activity (feed intake time, rumination time) or non-activity and production ability

(Erickson and Kalscheur, 2020). The performance of dairy cows is not only influenced by the nutritional composition of the total mix ration but also the physical structure of the TMR itself plays a major role (Yang and Beauchemin, 2006a; Yang and Beauchemin, 2006b; Beauchemin, 2018). Yang and Beauchemin (2006b) further describe that the mixing time itself, the type of mixing machine and the cutting machine also influence the total intake or activity and production ability of dairy cows. Miller-Cushon and DeVries (2017) add that forage sorting is also influenced by the frequency of feeding during the day itself, where cows fed twice a day had less selection activity than cows fed once a day. The explanation is that the more a given TMR is available to dairy cows, the more they will select and select for more appetitive particles. Yansari et al. (2004) and Kononoff and Heinrichs (2003) reported that the introduction of so-called physically effective neutral detergent fibre (*pe*NDF) is beneficial for identifying the part of the diet that stimulates the rumination process and is responsible for the formation of the rumen complex (rumen mattress) and for the overall rumen function. Neutral detergent fibre alone only describes the chemical properties of the feed or TMR but not the physical properties (size, density) needed to ensure adequate health status. Yansari et al. (2004) describe that the addition of long particles in TMR provides the desired acetate:propionate ratio, an increase in milk fat concentration and prevention against metabolic disorders (DeVries et al., 2008; Brandstetter et al., 2019; Jurkovich et al., 2019; Shen et al., 2020). Beauchemin and Yang (2005) suggest that to ensure high milk production, it is essential that dairy cows receive energy-rich forages or that sufficient intake of TMR with high energy content is provided. However, an increase in the non-structural carbohydrate content of

TMR, can cause potential problems with decreasing pH values and consequently the development of metabolic disorders (sub-acute ruminal acidosis) which has a negative impact on the microbial representation of fibre-digesting species (cellulolytic bacteria). It is by feeding TMR with sufficient *pe*NDF that the possibility of metabolic disorders will be reduced, as mixing should result in a structure that is sufficiently homogeneous to support minimal selection (Kronqvist et al., 2021). Sova et al. (2013) describe that sorting or selection is an increasing problem in commercial herds. Dairy cows tend to select in favour of smaller particles (pad/starch) and thus take in less fibre (Miller-Cushon and DeVries, 2009; Leonardi et al., 2017). It has been suggested, as Maulfair et al. (2010), that as dairy cows select during the day they ingest different rations, causing fluctuations in the rumen complex (fermentation processes) that can translate in a negative sense into reduced production and poorer health (Miller-Cushon and DeVries, 2009). DeVries et al, (2005) in their research found that the NDF content on the feed table increased during the day, indicating that dairy cows were selecting in favour of finer particles over fibrous components during the day. Also, Leonardi and Armentano (2003) describe that most dairy cows tended to select in favour of finer particles or ingested a higher content of easily fermentable carbohydrates and a lower proportion of fibre than was formulated and supplied. The inclusion of straw in the diets of high producing dairy cows as described by Humphries et al. (2010) may contribute to better activity and consequently productivity of dairy cows. The activity or rumination itself is a mirror result of the effective fibre content in the TMR, which promotes a more stable rumen environment, thus preventing high pH value changes and consequently the development

of metabolic disorders. Yang and Beauchemin (2006b) describe *pe*NDF as the ability of the physical structure of fibre to stimulate the activity of the dairy cow or to activate the process of rumination and then saliva production. Cao et al. (2021) estimate that saliva secreted by the dairy cow can neutralise 30-40% of the volatile fatty acid produced in the rumen environment. In order to maintain proper rumen function, it is necessary to promote salivation, the amount of which depends on the size of the food particles or fibre. The Penn State Particle Separator (PSPS) method is used to determine the *pe*NDF content. It is a tool that provides quantitative determination of feed particle size and total mix ration (Heinrichs and Coleen, 2022). In performing the TMR structure assessment, the focus is not only on the fractions from which the *pe*NDF is determined, but also on the total particle representation on the sieves or on the substrate. The important fact is that in order to make TMR samples objective, the first collection must be secured from the area immediately after TMR establishment or before the cows start to eat and select feed (Heinrichs and Coleen, 2022). Studies by Kononoff and Heinrichs (2003a,b) and Beauchemin and Yang (2005) describe that, based on measurements using the PSPS method, they found that increased *pe*NDF intake was involved in increased rumination time and that it improved overall digestibility. Yang et al. (2001); Kononoff and Heinrichs (2003a) report that the increase in *pe*NDF was also reflected in an increase in milk fat content.

The aim of this experiment was to investigate the effect of changing the length of straw in the total mix ration on the overall activity, production and milk composition of dairy cows. We predicted that different straw lengths would have a significant effect on feed intake time, rumination time and non-activity time, as well as milk production and composition.

MATERIAL AND METHODS

The experiment was realised on the university farm Kolíňany - farm Oponice, where the effect of changing the length of straw in the total mix ration on the overall activity and production ability of high-producing Holstein-Friesian dairy cows was monitored. The experiment was realised in a group of dairy cows that were at the peak of their lactation (61-120 days of lactation). The experiment was carried out for 4 weeks and during these weeks the dairy cows were divided into the following three groups. The group of cows at the peak of their lactation 1 (PLM1) with a head count of 33, where total mixed ration 1 (TMR1) was fed, which consisted of straw cut to an adequate length (3±2 cm). The group of cows at the peak of their lactation 2 (PLM2), with a head count of 29, was fed total mix ration 2 (TMR2), and represented a so-called transitional TMR, meaning that it contained straw particles of 3±2 cm but also straw particles that had a longer cut length (15±2 cm). The third group of dairy cows at the peak of their lactation 3 (PLM3) with a cow number of 32 was fed TMR that contained only particles with a longer chop i.e. 15±2 cm. High producing dairy cows on Oponice farm (PLM1-PLM3) were fed a total mix ration (TMR) which was fed once a day with implementation of feeding every 6 hours. The composition of the TMR together with the content of selected nutrients for the group of cows at the peak of their lactation is presented in Table 1. Straw was cut to the required length using a TOMAHAWK Straw mill 404M. The TMR structure evaluation was realised 3 times during the study period using the Penn State Particle Separator (PSPS) method. Sieves with 19 mm, 8 mm, 4 mm diameter holes and a pad were used. The sample taken from the individual group (PLM1;PLM2;PLM3) was a total of 700±50 grams

from the fifth sampled feeding table locations. The sieving and number of sieving movements represented a total of 80 movements (10 times from each side (5x2). TMR texture assessment was carried out during the given control periods 4x/24 h (05:00;11:00;17:00;23:00). Then, the given data from the PLM1, PLM2 and PLM3 groups were individually averaged into one data for that group. The physically effective neutral-detergent fibre (*pe*NDF) content was obtained from the averaged fractions for the control day (19 mm+ 8 mm=*pef*), which were then multiplied by the analytically determined neutral-detergent fibre content (using the ANKOM 200 Fiber Analyzer). Sampling was always carried out on the day of the structure evaluation, just after TMR establishment (05:00). Daily milk yield data for dairy cows (milked 3 × daily - herringbone type parlour) were recorded and then downloaded using HerdMetrix software. Milk production records included information regarding milking date and time, total milk production (litres), collar number and cow identification number. The True test method (at 10-day intervals) was used to record milk fat and protein content, which allowed milk quality to be monitored accurately and regularly. In this experiment, samples were manually collected by milkers during morning milking into sterile containers, which were then stored at a specific temperature (4°C) and transported to the laboratory for analysis (within 24 hours of collection). The data were used to evaluate the average fat and protein content of the milk of individual cows (subsequently of each group) during the experimental period. The overall activity (feed intake time; rumination time) or non-activity of the dairy cows was also monitored and recorded using BouMatic automated collars (24 h.day-^{1}). Data transmission (between the fitted collar and the receiver installed in the stall) was done at 15-

minute time intervals and then the data were sent at two-hour intervals to the RealTime Activity program. Data downloads were taken every day or every 24 hours throughout the experiment, but in order to increase the accuracy of the results, activity (or non-activity) data from the days closest to the milk sampling date (2 days before; day of sampling; 2 days after; i.e. 5*3=15 days in total/cow) were taken into account. Statistical processing of the results was performed using IBM SPSS ver. 26.0. Descriptive statistics (mean, standard deviation, minimum and maximum values) with one-way ANOVA analysis. Statistical significance of differences between dairy cow groups (PLM1-PLM3) and TMR was expressed using Post Hoc Tukey's test ($p < 0.05$).

Table 1. Composition of the mixed ration and content of selected nutrients

Component	**Total weight of feed (kg)**
Corn silage (%)	47.2
CCM (%)	9.97
Straw (%)	1.23
Rye silage (%)	17.50
Feed Mixture (%)	15.40
Sugar beet strips (%)	8.75
Dry matter intake (kg)	*25.91*
Nitrogenous substances [g]	165.08
Fat [g]	23.37
Fibre [g]	151.41
ADF [g]	189.87
NDF [g]	282.95
Starch [g]	270.86
Total sugars [g]	30.95
NEL [MJ]	7.14

**CCM = Corn Cob Mix, ADF = Aciddetergent fibre, NDF = Neutraldetergent fibre, NEL = Netto energy lactation, kg = Kilograms, g = grams, MJ = Megajoul*

RESULTS AND DISCUSSION

The results showing the selection activity of dairy cows during the period under study are presented in Table 2.

The analysis includes data collected during one month from three groups of peak lactation multiparous 1,2,3 (PLM1;PLM2;PLM3) where different length of straw was fed in each group (TMR1;TMR2;TMR3). Peak lactation multiparous 1 (PLM1) was characterised with TMR1 being fed, with the inclusion of particles or straw that was cut to the desired length (3±2 cm). The second group (PLM2) represented the group where the feed was fed with a transitional ration (TMR2) or fed with a ration that was mixed with short (3±2 cm) and long (15±2 cm) straw particles. The third group (PLM3) was characterised by feeding a diet exclusively with long straw length (15±2 cm). The mean values at 5:00, which represent the average particle content measured immediately after the establishment of the ration, show the variability between the given PLM1-PLM3 groups studied. The feeding ration during the individual measurements during the day (11:00, 17:00 and 23:00) should replicate the values that were measured at 05:00.

Based on the data in Table 2, it can be concluded that in the PLM1 group, where TMR1 was fed (straw length 3±2 cm), dairy cows received TMR more homogenously and selection was less compared to the PLM2 and PLM3 groups ($p < 0.05$). This was particularly indicated by the values measured at the 1st site (19 mm), which varied significantly within the day. In the intermediate ration (TMR2), the addition of longer straw particles increased the selection activity of the cows and the percentage of fractions increased gradually compared to TMR1 ($p < 0.05$, p-value = 0.000).

Table 2. Monitoring intake (/selection) dynamics in peak lactation multiparous groups (PLM1-3)

Evaluated group	Evaluation of structure TMR	Evaluation time	Mean PLM1 (TMR1);N= 12	Mean PLM2 (TMR2);N= 12	Mean PLM3 (TMR3);N= 12	*p*-value
Peak lactation multiparous (PLM)	1st sieve (19 mm)	05:00	12.37[a]	9.01[b]	11.06[c]	0.000
		11:00	14.86[a]	15.89[b]	15.62[c]	
		17:00	21.22[a]	24.45[b]	26.87[c]	
		23:00	24.92[a]	28.51[b]	32.92[c]	
	2nd sieve (8mm)	05:00	48.47[a]	47.97[b]	42.53[c]	
		11:00	48.89[a]	46.37[b]	46.35[c]	
		17:00	48.98[a]	44.42[b]	42.46[c]	
		23:00	51.26[a]	45.74[b]	42.88[c]	
	3rd sieve (4mm)	05:00	12.23[a]	17.84[b]	17.67[c]	
		11:00	12.18[a]	16.41[b]	17.12[c]	
		17:00	10.25[a]	14.31[b]	13.07[c]	
		23:00	9.26[a]	11.44[b]	12.25[c]	
	Pad	05:00	26.93[a]	25.17[b]	28.74[c]	
		11:00	24.07[a]	21.34[b]	20.92[c]	
		17:00	19.55[a]	16.83[b]	17.60[c]	
		23:00	14.20[a]	14.59[b]	11.94[c]	

a-c = Different letters indicate statistical significance ($p<0.05$); TMR1,2,3=total mix ration; PLM1,2,3=peak lactation multiparous groups;N= number of observation.

The most selection was observed at TMR3, where the proportion of large particles (fraction retained on the sieve with a 19 mm hole size) was up to 32.92% at 23:00 h, which is 8% more particles than at TMR1 ($p < 0.05$, p-value = 0.000). The rationale is that after a complete

change in the physical structure of the straw (to longer particles), the dairy cows showed a more pronounced selection in favour of finer particles. A study by DeVries et al. (2005) found that the NDF content gradually increased during the day on the feed table, supporting the fact, as described by Leonardi and Armentano (2003), that dairy cows preferentially select for finer particles, which was also shown in our experiment. A more detailed analysis of the results for sieves with 8 mm and 4 mm diameter holes showed that the percentages of fractions (8 mm - 30-50%; 4 mm - 10-20%) were satisfied for all groups (PLM1-PLM3) ($p < 0.05$, p-value = 0.000). This finding is consistent with that of DeVries et al. (2007), where dairy cows did not show selection towards intermediate particles during the study period ($p = 0.001$). These values indicate that dairy cows mainly selected for particles from 1 sieve (19 mm), which was most affected by the change in straw length ($p < 0.05$). A similar statement is described by DeVries et al. (2007) who describe that in their research they observed dairy cows sorting against long particles or in favour of smaller particles ($p = 0.001$). A large effect of the change in TMR can also be observed on the pad, which had a smaller decrease in fractions during TMR1 feeding compared to TMR2 and TMR3, where the decrease (TMR3) was most evident at 23:00, and the remainder was only 11.94% (PLM3) ($p < 0.05$). These results indicate that the change in total mix ration structure had a significant effect on feed intake by dairy cows ($p < 0.05$). As the total mix ration in the PLM2 group gradually changed (3±2 cm to 15±2 cm), the dairy cows increased their selection, mainly targeting larger particles (+8mm), while decreasing the amount of particles remaining on the pad. This highlights the importance of

proper total mix ration preparation and structure to optimize nutrient intake, efficient feed utilisation and ensure adequate health status.

Table 3. Effect of straw particle size on activity and production ability of dairy cows (mean ±SD)

Parameter	**Group/TMR**			***p*-value**
	PLM1/TMR1 (N^1=495;N^2=99)	**PLM2/TMR2 (N^1=435;N^2=87)**	**PLM3/TMR3 (N^1=480;N^2=96)**	
Total eating time (h.)	4:34:54^a (±1:25:38)	4:44:25^a (±1:22:40)	5:06:09^b (±1:26.52)	
Total rumination time (h.)	7:32:16^a (±1:39:46)	8:08:18^b (±1:39:08)	8:52:11^c (±1:18:41)	
Non-active time (h.)	11:41:51^a (±2:27:19)	11:01:33^b (±2:26:03)	9:58:07^c (±2:01:32)	0.000
Milk yield (kg.day.cow^{-1})	34.35^a (±5.58)	34.86^a (±5.81)	37.69^b (±7.68)	
Fat (%)	3.71^a (±0.61)	3.97^b (±0.81)	3.94^b (±0.63)	
Protein (%)	3.16^a (±0.26)	3.23^b (±0.30)	3.41^c (±0.32)	

a-c = Different letters indicate statistical significance (p<0.05);N1= number of observation;)N2= number of observation for fat anf proteins; TMR1,2,3=total mix ration;PLM1,2,3=peak lactation multiparous; h=hours per day.

In Table 3, we have recorded the average values of total activity and non-activity, and the production ability of dairy cows in the peak group during the study period. Based on these data, a Post Hoc Tukey Test was then used to verify that statistically significant differences existed between the groups. Specifically, the groups (PLM1 and PLM3) were significantly different in selected parameters ($p < 0.05$). The food intake time for TMR1 feeding was 4:34:54 h. which was 0:31:15 h. less

compared to TMR3 (5:06:09 h.) ($p < 0.05$, p-value = 0.000). This difference can be justified by the use of longer particles (15±2 cm) of straw, which caused the dairy cows to select more (see Table 2) and to select smaller and more palatable components from TMR3. Conversely, TMR1 with shorter straw slices (at 3±2 cm) was more readily available, more palatable, which limited the selection process and thus reduced feed intake time. A statistically significant difference in food intake time between PLM2-PLM3 was also identified ($p < 0.05$). Dairy cows fed TMR3 (PLM3) had a significantly longer period of time compared to dairy cows (PLM2) fed TMR2. Analysis of feed intake time between PLM1 and PLM2 indicated a higher p-value (0.136) indicating no statistically significant difference ($p > 0.05$) between the two groups. The reason for this is that since it was an intermediate TMR (TMR2), it contained particles with both smaller section (3±2 cm) and longer section (15±2 cm) and selection was not as necessary as for TMR3 (Table 2). Changes in TMR also had a direct effect on cow behaviour in terms of rumination time ($p < 0.05$, p-value = 0.000). We observed an increase in rumination time when moving from TMR1 (which contained straw particles exclusively with a length of 3±2 cm) to TMR3 (containing longer straw stalks i.e. 15±2 cm). A similar argument is made by Zebeli et al. (2007), where they describe that dairy cows at higher *pe*NDF representation progressively increase rumination time. Rumination time increased progressively for each group based on longer physical structure ($p < 0.05$). Comparison of TMR1 (7:32:16 h.) with TMR3 (8:52:11 h.) showed a difference in rumination time of up to 1:19:54 h. ($p < 0.05$, p-value = 0.000). Changes in total mix ration in terms of increasing rumination time may contribute positively to rumen health and overall digestion in dairy

cows. Increasing the physical structure concentration in the study by Kahyani et al. (2013) increased rumination time, with a 3.7% increase in *pe*NDF content (from 5.7% to 9.4%). We observed a decreasing inactivity time ($p < 0.05$) after introducing longer straw into the TMR, which was related to changes in selection and affected the total time spent ingesting and rumination. Straw of greater length tended to increase the above times due to cows being forced to select more thoroughly and subsequently ruminate longer stalks, as shown in Table 3. The above reductions were observed over the course of each treatment group, with the mean non-active time decreasing by 1:43:43 h from TMR1 (11:41:51±2:27:19) to TMR3 (9:58:07±2:01:32) ($p < 0.05$, p-value = 0.000). There was also a significant increase in milk production between PLM1 and PLM3 groups ($p < 0.05$). In the PLM1 group where TMR1 was fed, the mean milk production was 34.35 kg, where as in PLM3 when TMR3 was fed it went up to 37.69 kg, an increase of 3.34 kg of milk ($p < 0.05$, p-value = 0.000). The increase in milk production suggests that the change in straw length has had a positive effect, but the likely true reason for the higher milk production is indicated in Table 2, where a selection in favour of finer particles (starch) found on the pad can be noted. Starch, being easily digestible, provides a quick source of energy for the cow and the increased intake of these particles improves the overall energy balance of the cow, which is reflected in the higher milk production. The finding that selection against long particles was associated with higher milk production is described by DeVries et al. (2011) ($p = 0.016$). Also, when milk production and fat content were analysed, there was a significant change associated with changes in ration. During TMR1 (PLM1) feeding, milk fat content ranged from 3.71% and in PLM2, fat

content increased to 3.97% during TMR2 feeding, which represented a statistically significant increase ($p < 0.05$). In the PLM3 group, when fed TMR3 the fat content increased mildly (3.94%) but was not statistically significant with PLM2 ($p > 0.05$), indicating a subsequent plateauing and thus that the main increase was between the PLM1 and PLM2 groups ($p < 0.05$, p-value = 0.000). Studies by DeVries et al. (2011); Fish and DeVries (2012) reported that each rejection of long particles (10%) reduced fat content by 0.15% points. Miller-Cushon and DeVries (2017) found a similar association with our results, where milk fat content increased by 0.1% points with the inclusion and benefit of selection for longer particles. The increase in milk constituent content was also evident for protein content and in each group (PLM1-PLM3) ($p < 0.05$, p-value = 0.000). In the TMR1 fed group the protein content of milk was 3.16%, in the intermediate - TMR2 (PLM2) fed group the content increased to 3.23% and in the TMR3 fed group the protein content of milk increased to 3.41% ($p < 0.05$).

CONCLUSION

This study analysed the effect of different straw lengths on the overall activity (feed intake time, rumination time) or non-activity and production ability of dairy cows. The structure of the TMR was also evaluated, where the selection or intake of the different fractions was monitored. The experiment was realised on high producing Holstein-Friesian dairy cows in a peak group, divided into three groups (PLM1;PLM2;PLM3), where each group received TMR with different straw lengths (TMR1;TMR2;TMR3). Food intake time and rumination time increased progressively with increasing straw length and physical structure concentration. PLM3 dairy cows had overall higher activity

compared to PLM1. Statistically significant differences were also observed for milk production and individual milk components, where the greatest increase in production was observed between PLM1 and PLM3 (3.34 kg). Milk fat content increased by 3.97% when fed TMR2 and an increase was also observed in protein content, where it was 3.16% in PLM1 and increased to 3.41% in PLM3 group. When analysing the TMR structure, it was found that as straw length increased, dairy cows selected in favour of finer particles, resulting in a greater percentage of the first sieve. The results suggest that straw length in TMR has a significant effect on the overall behaviour or activity of dairy cows, their milk production ability and milk composition. The PLM3 group of cows showed the most pronounced increase in feed intake time and rumination time, which decreased inactivity time. This increase in activity was related to an improvement in digestive processes and metabolism, which was reflected in increased production and higher fat content in milk. These results are important for the optimisation of TMR in dairy farming because they suggest that adjusting straw length can lead to increased feeding efficiency and subsequently to higher milk production and quality.

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EFFECTS OF FATTY ACID ESTERS AND PHYTOGENIC FEED ADDITIVES ON PERFORMANCE AND HEALTH STATUS OF GROWING PIGS EXPOSED TO HEAT STRESS

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ABSTRACT

The objective of this experiment was to evaluate the impact of phytoadditives and fatty acid esters on heat-stressed pigs, focusing on final weight, average daily feed intake, average daily weight gain and health biomarkers. A mixture of short- and medium-chain fatty acid esters was used in the study (butyric, caproic, capric, lauric acid monoacylglycerol ester) with a concentration of 51% in combination with steroidal and triterpenoid saponins derived from Glycyrrhiza glabra (licorice), Quillaja saponaria (soapbark tree) and Trigonella foenum-graecum (fenugreek) are referred to as glycyrrhizin, diosgenin and quillaja saponins, respectively.

The experimental group exhibited improvements in fattening parameters compared to the control group: final weight increased by 8.5%, weight gain increased by 10.2%, and feed intake increased by 4.9%. Additionally, the health status of the experimental group was affected, evidenced by a 15.6% increase in cortisol activity, a 44.5%

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decrease in haptoglobin levels and a 12.7% increase in heat shock protein-70 levels compared to the control group. These findings suggest that feed supplements comprising fatty acid esters and phytogenic substances can mitigate the adverse effects of heat stress, enhancing performance and health status of the organism.

Keywords: gut health; DanBred; saponins; performance; blood analysis

INTRODUCTION

Heat stress (HS) markedly elevates respiration rates and body temperatures, significantly reduces feed intake, impairs gut health, and increases both mortality and morbidity. These losses are related to the adverse effects of HS on gut permeability and systemic inflammation. HS leads to intestinal hypoxia and reduced blood flow in the intestinal wall, resulting in oxidative stress, apoptosis of intestinal mucosal cells, and increased production of pro-inflammatory cytokines. Another negative effect of HS is the decreased expression of tight-junction proteins, which increases intestinal permeability. This allows pathogens, endotoxins, and metabolites to penetrate from the gastrointestinal tract into the systemic bloodstream (Yan et al., 2006; Montilla et al., 2014; Akbarian et al., 2016; Lauridsen, 2019, Pearce et al., 2012; 2013a; 2013b).

Heat activates the hypothalamic–pituitary–adrenal and the sympathetic–adrenal–medullary axes to regulate the response to the stressors and consequently, elicit changes in the immune response. The release of cortisol during periods of acute stress acts as a stimulus for the immune system; however during chronic stress cortisol secretion can cause immune suppression (Cantet et al., 2021). Cellular exposure

to thermal stress induces a number of anomalies in the functioning of cells which alters the biological molecules, disturbs cell functions, modulates metabolic reactions, induces oxidative cell damage and activates both apoptosis and necrosis pathways, ultimately leading to cell survival, acclimation or cell death depending on the time and success of these alterations (Belhadj et al., 2016). Heat shock proteins-70 (HSP70) has been suggested to function as an indicator of thermotolerance in cells provide protection against hyperthermia, circulatory shock, and visceral ischemia during heat stroke which signifies the central role of HSP in cytoprotection. The HSPs have chaperonic activity ensuring the folding, unfolding and refolding of stress-denatured proteins.

The cellular response is one of the primary pathway by which livestock tries to cope up to the heat stress challenges. This is the pathway that helps the animal to survive the stress condition. The mechanism of action of fatty acid esters in the digestive tract is not detailed in scientific literature. Until now, only short- and medium-chain fatty acids have been described for antimicrobial and gastrointestinal supportive purposes (Rooks and Garrett, 2016; Macpherson et al., 2017). The mechanism of action is currently under development, however, it appears to be an effective solution for digestive issues both post-weaning and in fattening pigs. The function of selected fatty acid esters lies not only in modulating the intestinal microbiome, utilizing their antimicrobial effect independent of pH (throughout the gastrointestinal tract), with activity againts G+, especially *Clostridium perfringens* and G- (*E. coli, Salmonella sp., Campylobacter sp.*) and supporting beneficial microflora (*Lactobacillus sp., Bifidobacterium sp.*), but also their influence on the development, regeneration, and

integrity of the intestinal mucosa. Esters provide an energy source for enterocytes, aid in the formation of longer villi and shallower crypts, thereby increasing the intestinal surface area for nutrient absorption and optimizing the digestion process and immune system function. Esters contribute to the increased synthesis of tight junction proteins and overall improvement of intestinal morphology and function (Batovska et al., 2009, Churchward et al., 2018, Kovanda et al., 2019).

Triterpenoid saponins are found in the roots of Glycyrrhiza glabra (licorice). It is known for its sweet taste and has various pharmacological properties, including anti-inflammatory and antiviral effects. Quillaja saponins are a group of triterpenoid saponins extracted from the bark of Quillaja saponaria (soapbark tree). They are commonly used as emulsifiers and adjuvants in vaccines and have immunostimulatory properties. Diosgenin is a steroidal sapogenin found in Trigonella foenum-graecum (fenugreek). Diosgenin is a precursor for the synthesis of various steroidal drugs and has been studied for its potential health benefits, including anti-inflammatory and anticancer effects. Known for their digestive, anti-inflammatory and antimicrobial properties.

This is a pilot study on the use of a combination of phytogenic adaptogenic substances and fatty acid esters. Similar findings have not been reported in the literature for growing pigs.

MATERIAL AND METHODS

The experiment was carried out at the Czech University of Life Sciences in Prague in an accredited experimental stable and the study was conducted according to the guidelines of the Declaration of Helsinki. 36 DanBred male pigs were divided into two groups, each

consisting of 18 growing pigs. The experimental group received a diet supplemented with a combination of short- and medium-chain fatty acid esters (butyric, caproic, capric, lauric acid monoacylglycerol ester) and steroidal and triterpenoid saponins derived from Glycyrrhiza glabra (licorice), Quillaja saponaria (soapbark tree) and Trigonella foenum-graecum (fenugreek) are referred to as glycyrrhizin, diosgenin and quillaja saponins, respectively. A dose 1 kg/t was used in the experimental group.

While the second group served as the control. The experiment started after the beginning of the pre-fattening phase with an initial weight of around 13.5 kg (± 2 kg) and was conducted for 49 days, with the animals subjected to diurnal thermal stress starting after a 14-day acclimatization period (10 continuous hours per day or night at 33 ± 1 °C). The temperature profile mentioned in the attached Figure 1. The composition and nutritional values of the feed ratio are shown in Table 1. The diet was compiled according to the Nutritional Requirements for DanBred Pigs. All experimental animals were provided *ad libitum* access to feed and drinking water. Feed intake and weight gain was recorded weekly and animals were weighed once a week.

Data processing

Data were analyzed using the statistical software R. The effect of adsorbents on pig performance was tested using one-way analysis of variance (ANOVA). For biochemical marker values, a generalized linear model (GLM) with a gamma distribution was employed. Post hoc comparisons of the treatment groups to the control (C) and experimental group were conducted using Dunnett's test. The null hypothesis was rejected at $p < 0.05$.

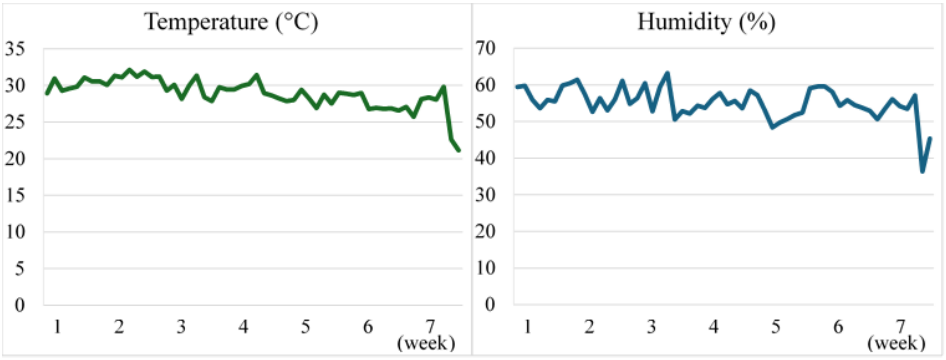

Figure 1. Temperature profile

the ELISA assay kit (CSB-E08317p; Cusabio, USA) following the manufacturer's protocol.

Table 1. Ingredient composition of the experimental diet for pigs

Ingredient	**%**
Barley	40.97
Wheat	34.10
Extracted soybean meal	16.00
Dried sugar beet pulp	3.80
Animal fat	1.20
Calcium carbonate (ground limestone)	1.10
Mineral premix	1.00
L-Lysine HCl 98	0.71
Monocalcium phosphate	0.42
Feeding salt	0.34
L-Tryptophan 20	0.14
L-Threonine 98	0.14
DL Methionine 99	0.08

RESULTS AND DISCUSSION

Specific combination of different esters and saponins showed better fattening parameters compared to the control group: weight gain increased by 10.2%, feed intake increased by 4.9 %, final weight increased by 8.5% (Figure 2. A, B, C). Cervantes et al. (2024) reported a positive effect of adaptogenic substances in their study. According to their results, the average daily weight gain with the use of phytogenic substances was 24% higher than in the control group, and the average daily intake was 18% higher in the experimental group. Similar findings were reported by Kumar et al. (2018) by feeding adaptogenic herbs in broilers during summer time. Body weight gain, feed efficiency and feed intake were affected ($p < 0,05$) *by supplementation of active substances.* This outcome is attributed to the positive influence on the hypothalamus-pituitary-adrenal axis, a reduction in appetite, and an improvement in intestinal permeability. In addition, our measurement of stress hormone cortisol of experimental group showed increased levels by 15.6% compared to the control group (Figure 3. A). Studies indicate that feathers or hair are more suitable biological materials for measuring stress hormones during chronic stress. However, laboratories in the Czech Republic lack established methodologies. Our results suggest that chronic stress is better measured using alternatives to plasma. Previous reports confirmed that corticosterone levels in the feathers of broiler chickens increase during long-term heat stress (Eugen et al., 2019). Consistently, the study by Hossainidoust et al. (2020) indicated that supplementing the diet with astaxanthin during heat stress reduced corticosterone levels in feathers. In our experiment, haptoglobin levels were decreased by 44,5%

(Figure 3. B). Heat-stressed pigs tended to have increased circulating concentrations of haptoglobin, an acute phase marker, and this has previously been reported in heat-stressed pigs (Pearce et al., 2013b, 2014, 2015). In our study, levels of heat shock protein- 70 were increased by 12,7% in the experimental group compared to the control group (Figure 3. C). Various studies have examined the role of HSP70 in preventing the denaturation of proteins by serving as a molecular chaperone and a cell protector. Sheep, buffaloes, cattle, broilers, goats and pigs exhibit elevated HSP70 expression under heat stress, thus improving their adaptation (Flees et al., 2017). However, it remains unclear whether the increased concentration is a positive or negative phenomenon during chronic heat stress.

From the results above, we can conclude that using feed supplements can mitigate the effects of heat stress, enhancing performance while simultaneously improving the organism's health parameters.

The main objective of the study was to demonstrate the effect of fatty acid esters, primarily those with short and medium chains, in combination with plant saponins during heat stress. Due to the innovative nature of these substances, there are no articles comparing similar active compounds under stress conditions. Most publications focus on the non-esterified form, which is pH-dependent, meaning it tends to change its chemical structure (dissociate) as the pH increases in the intestine, thereby losing its antibacterial and other properties. In in vitro tests, esterification of fatty acids demonstrated stability up to pH 7.

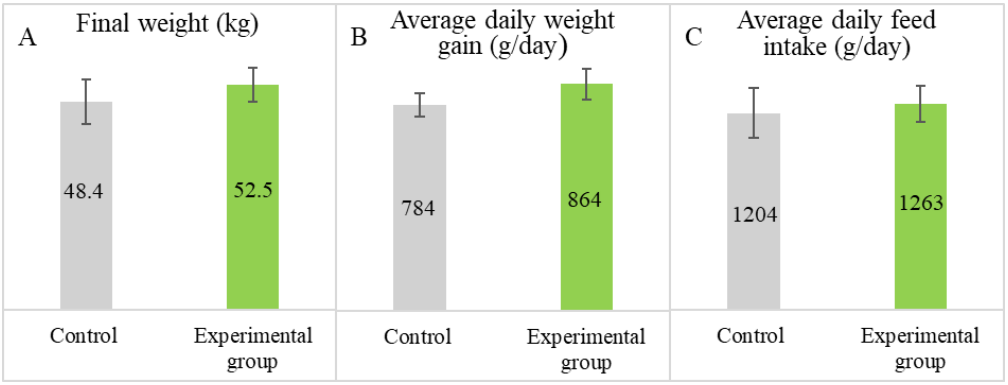

Figure 2. Performance parameters

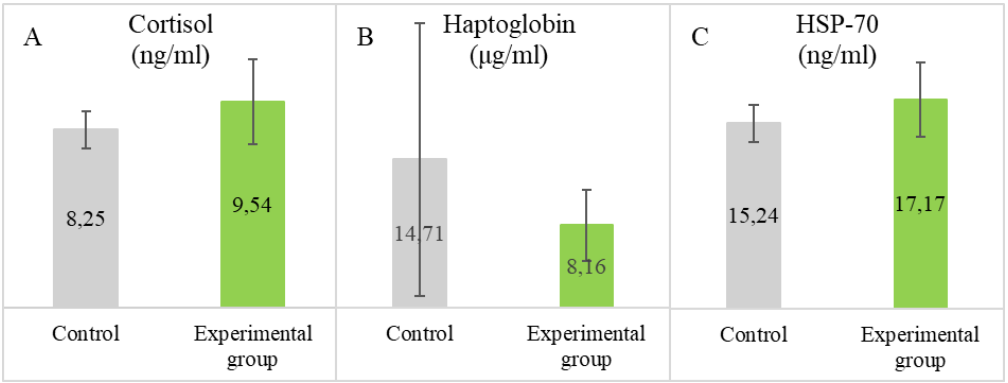

Figure 3. Health biomarkers

CONCLUSION

Supplementation of short- and medium-chain fatty acid esters combined with saponins has a complex effect that supports gut health in pigs. These supplements have both preventive and therapeutic effects, particularly under conditions of increased stress on the animal's body. Future studies will need to be conducted to confirm the physiological role of gut microbiota in HS and develop targeted methods to mitigate adverse HS-related effects in swine.

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THE EFFECT OF INSECT MEALS ON PERFORMANCE PARAMETERS OF JAPANESE QUAILS (*COTURNIX JAPONICA*)

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ABSTRACT

The influence of defatted mealworm meal or defatted black soldier fly larvae meal on performance parameters of Japanese quails was evaluated. Unsexed Japanese quails (n = 300) were divided in to 3 experimental groups: 1) control group without insect meal, 2) group with 10% defatted mealworm meal and 3) group with 10% defatted black soldier fly larvae meal. The experiment lasted for 40 days. The group with defatted mealworm meal had higher carcass yield than control group ($p < 0.05$). Growth performance, other carcass traits and weight of liver, heart and gizzard were not affected by experimental diets.

Keywords: mealworm; black soldier fly; growth; carcass traits; poultry nutrition

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INTRODUCTION

In poultry nutrition, insect can be used as an alternative protein source (Khan, 2018; Elahi *et al*., 2022). The insect is also a source of essential amino acids, fats, monounsaturated and polyunsaturated fatty acids, minerals and vitamins (Rumpold and Schlüter, 2013; Zielińska *et al*., 2015). A black soldier fly (*Hermetia illucens* Linnaeus, 1758), a housefly (*Musca domestica* Linnaeus, 1758), a yellow mealworm (*Tenebrio molitor* Linnaeus, 1758), a lesser mealworm (*Alphitobius diaperinus* Panzer, 1797), a house cricket (*Acheta domesticus* Linnaeus, 1758), a banded cricket (*Gryllodes sigillatus* Walker, 1869), a field cricket (*Gryllus assimilis* Fabricius, 1775) and a silkworm (*Bombyx mori* Linnaeus, 1758) are permitted species of insect as feed for non-ruminant animals in the European Union (Commission Regulation (EU) 2017/893; Commission Regulation (EU) 2021/1925). The Commission Regulation (EU) 2021/1372 allows the inclusion of processed animal protein from insects in poultry diets.

There are not many studies dealing with the inclusion of insect meal in quail diets and the results are quite inconsistent. For example, Silva *et al* (2024) found out the effect of black soldier fly larvae meal on growth performance of Japanese quails, but the relative weight of the digestive organs was not affected by insect meal. Experimental diets with mealworm meal had an effect on final body weight, body weight gain, and feed conversion ratio (FCR), but no effect on feed intake, carcass weight, and carcass yield of Japanese quails (Sabirli and Cufadar, 2019). Zadeh *et al*. (2019) noted the effect of mealworm meal not only on body weight, weight gain and FCR, but also on feed intake, carcass, breast and legs yields. Mealworm meal had no effect on

relative weight of internal organs. On the contrary, Cullere *et al*. (2016) did not notice the effect of the defatted black soldier fly larvae meal on slaughter weight, body weight gain, feed intake, FCR, carcass weight and yield, and breast weight and yield of Japanese quails.

The aim of this study was to evaluate the effect of two insect meals in diets Japanese quails on performance parameters.

MATERIAL AND METHODS

Animals and experimental conditions

A total of 300 seven days old Japanese quails (*Coturnix japonica*) of both sexes were randomly divided in to 3 experimental groups (in total 100 quails per feeding group). The quails were housed in floor system with deep bedding. Microclimate conditions (temperature, humidity) and lighting programme were set according to technological instruction (Hyánková and Hort, 1999). All quails were fed a commercial starter diet for the first week. The quails were fed with experimental starter diets from 7^{th} to 19^{th} day of age and experimental grower diets from 20^{th} to 47^{th} day of age. The quails had unlimited access to the feed and water. The feed consumption was daily recorded, and the quails were weighed every week. At the end of the experiment, the quails were slaughtered.

Experimental diets

In the experiment three diets were used: 1) control diet without insect meal, 2) diet with 10% defatted mealworm meal (TM) and 3) diet with 10% defatted black soldier fly larvae meal (HI). The quails were fed a non-pelleted feed mixture that corresponded to their nutrient

requirements. Compounds and chemical composition of diets are shown in Table 1.

Table 1. Compounds and chemical composition of experimental diets (100% dry matter)

	Starter			Grower		
Compound (%)	**Control**	**TM**	**HI**	**Control**	**TM**	**HI**
Wheat	5.00	14.15	8.40	10.00	20.05	14.60
Maize	34.93	36.50	36.60	47.02	47.25	47.90
Rapeseed oil	4.00	1.69	2.50	3.18	1.06	1.20
Soybean meal	53.00	34.61	39.00	36.50	18.54	22.85
Mealworm meal	-	10.00	-	-	10.00	-
Black soldier fly larvae meal	-	-	10.00	-	-	10.00
Premix*	3.00	3.00	3.00	3.00	3.00	3.00
DL-methionine	0.07	0.05	0.30	0.20	0.10	0.25
L-Lysine	-	-	0.20	0.10	-	0.20
Crude protein	29.73	29.34	29.52	23.20	23.01	23.11
Ether extract	7.27	5.17	6.28	6.35	3.97	4.28
Crude fibre	4.27	4.61	4.63	4.36	4.45	4.62
Crude ash	7.28	7.08	7.40	6.37	6.40	6.38

Legend: TM – 10% defatted mealworm meal; HI – 10% defatted black soldier fly larvae meal. ****Composition of premix for starter*** *(per kg): Lysine 6.7%; Methionine 8.3%; Threonine 3.0%; calcium 21.0%; phosphorus 3.5%; natrium 4.2%; copper 500 mg; iron 2,500 mg; zinc 3,300 mg; manganese 3,300 mg; iodine 34.25 mg; selenium 6 mg; retinol 280,000 IU (international unit); calciferol 166,700 IU; tocopherol 1,500 mg; phylloquinone 50 mg; thiamine 140 mg; riboflavin 280 mg; pyridoxin 200 mg; cobalamin 1,000 µg; biotin 7 mg; niacinamide 1,200 mg; folic acid 60 mg; calcium pantothenate 450 mg; choline chloride 6,000 mg.* ***Composition of premix for grower*** *(per kg): Lysine 7%; Methionine 7.5%; Threonine 3.1%; calcium 19.0%; phosphorus 3.3%; natrium 4.6%; copper 500 mg; iron 2,500 mg; zinc 3,300 mg; manganese 3,300 mg; iodine 34.20 mg; selenium 12 mg; retinol 280,000 IU (international unit); calciferol 165,000 IU; tocopherol 1,500 mg; phylloquinone 44 mg; thiamine 135 mg; riboflavin 280 mg; pyridoxin 200 mg; cobalamin 960 µg; biotin 6 mg; niacinamide 1,200 mg; folic acid 55 mg; calcium pantothenate 445 mg; choline chloride 6,000 mg.*

Sample collection

The 6 individuals from each group were selected for evaluation carcass traits and organ weights. The carcass and organs (liver, heart and gizzard) were weighed. The gizzards were weighed with the cuticle. Carcass yields were calculated as a percentage of live weight. The breast and legs were weighed, and the breast and legs yields were calculated as a percentage of carcass weight. The legs were weighed with bones.

Statistical analysis

The data were processed by Microsoft Excel (USA) and TIBCO Statistica version 14.0 (USA). A one-way analysis of variance (ANOVA) and Scheffé's test were used to determine statistically differences between groups. The value $p < 0.05$ was regarded as a statistically significant difference.

RESULTS AND DISCUSSION

There were no statistically significant differences between feed consumption values in the experiment ($p > 0.05$). In the control group, the average daily feed consumption during the experiment was 24.77 g/bird, in the TM group 25.81 g/bird and in the HI group 25.32 g/bird. Table 2 shows insect meals had no effect ($p > 0.05$) on final weight, average gain and FCR of Japanese quails. The same conclusion was reached by Cullere *et al*. (2016), who fed quails with 10% and 15% defatted black soldier fly larvae meal in diet. Sabirli and Cufadar (2019) found out that mealworm meal influenced final body weight, body weight gain and FCR, where quails fed 2% of mealworm meal had higher final body weight and body weight gain and quails fed

4% and 6% of mealworm meal had worse FCR than other groups. Silva *et al*. (2024) reported the positive effect of black soldier fly larvae meal on body weight, body weight gain and FCR than control group. Zadeh *et al*. (2019) also reported influence of mealworm meal on body weight, daily weight gain, FCR and additionally daily feed consumption. Groups of quails fed 22.5 g and 30 g of mealworm meal had higher body weight and weight gain than other groups. Additionally group with 30 g of mealworm meal in diet had lower feed consumption and better FCR than other experimental groups.

Table 2. Growth performance of Japanese quails

Group	Control	TM	HI
n	100	100	100
		Mean ± SE	
Initial weight (g)	27.16 ± 0.71	27.40 ± 0.56	27.36 ± 0.59
Final weight (g)	252.10 ± 3.08	248.60 ± 3.08	255.94 ± 3.16
Average gain (g)	224.94 ± 3.40	221.20 ± 2.62	228.58 ± 2.17
FCR	4.41 ± 0.08	4.67 ± 0.07	4.43 ± 0.07

No statistically significant differences ($p > 0.05$); TM – 10% defatted mealworm meal; HI – 10% defatted black soldier fly larvae meal; n – number of cases; SE – standard error; FCR – feed conversion ratio

In the experiment the carcass yield was influenced. TM group had higher carcass yield compared to control group ($p < 0.05$). Other carcass traits were without significant differences between experimental groups ($p > 0.05$) (Table 3). Zadeh *et al*. (2019) also found out the effect of mealworm meal on carcass traits, where group of quails fed 30 g mealworm meal in diet had higher carcass yield, breast yield and legs yield compared to other groups. In contrast,

Cullere *et al*. (2016) and Sabirli and Cufadar (2019) did not find out an effect of insect meal on carcass traits.

Table 3. Carcass traits of Japanese quails

Group	Control	TM	HI
n	6	6	6
		Mean ± SE	
Body weight (g)	239.83 ± 5.14	234.50 ± 3.55	246.33 ± 2.62
Carcass weight (g)	162.52 ± 3.50	167.14 ± 2.86	171.86 ± 2.09
Carcass yield (%)	67.79 ± 0.84[a]	71.27 ± 0.53[b]	69.77 ± 0.35[ab]
Breast weight (g)	20.76 ± 0.84	21.79 ± 0.21	22.35 ± 0.78
Breast yield (%)	25.51 ± 0.67	26.09 ± 0.35	26.02 ± 0.91
Legs weight (g)	17.05 ± 0.42	17.31 ± 0.56	18.48 ± 0.52
Legs yield (%)	21.01 ± 0.61	20.74 ± 0.74	21.49 ± 0.42

[a, b] – means statistically significant difference ($p < 0.05$); TM – 10% defatted mealworm meal; HI – 10% defatted black soldier fly larvae meal; n – number of cases; SE – standard error

Table 4. Weight of organs of Japanese quails

Group	Control	TM	HI
n	6	6	6
		Mean ± SE	
Liver (g)	5.11 ± 0.45	4.61 ± 0.18	4.50 ± 0.44
Heart (g)	2.17 ± 0.05	2.20 ± 0.10	2.14 ± 0.10
Gizzard (g)	4.47 ± 0.28	4.43 ± 0.16	4.13 ± 0.29

No statistically significant differences ($p > 0.05$); TM – 10% defatted mealworm meal; HI – 10% defatted black soldier fly larvae meal; n – number of cases; SE – standard error

In Table 4 is shown that the weight of liver, heart and gizzard of Japanese quails were not affected by experimental diets ($p > 0.05$). In studies Zadeh *et al*. (2019) with mealworm meal and Silva *et al*. (2024) with black soldier fly larvae meal in diets of Japanese quails also found out no effect of insect meals on relative weight of gizzard, liver and heart.

CONCLUSION

The influence of 10% defatted mealworm meal or defatted black soldier fly larvae meal on performance parameters of Japanese quails was evaluated. Carcass yield was higher in group with 10% defatted mealworm meal compared to control group. The insect meals did not have any negative effects on other performance parameters like growth, other carcass traits and weight of liver, heart and gizzard.

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EFFECT OF SORGHUM SILAGE IN DIET OF DAIRY COWS ON EIGHTEEN-CARBON FATTY ACIDS IN MILK FAT

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ABSTRACT

The aim of the study was to evaluate the effect of inclusion of sorghum silage into dairy cow diets on the eighteen-carbon fatty acids (FA) in milk fat. The on-farm experiment was carried out on mid-lactating Czech Fleckvieh cows (Agrospol a.d. Knínice, farm Vanovice) and was divided into two consecutive periods of 3 months each. In the first period cows were fed a total mixed ration (TMR) based on maize silage and grass haylage (Control) while in the second period grass haylage was partially replaced by sorghum silage (Sorghum). In each period samples of evening and morning milk were taken from ten cows and were analysed for basic constituents and FA profile. The basic milk components were analysed in an accredited laboratory (LRM Brno-Tuřany). The FA profiles were analysed using gas chromatography with flame ionization detection. The total content of C18 acids was on average 34.6% of all FAs in Sorghum and 29.4% in Control ($P < 0.05$). Similarly, a higher content of stearic (C18:0), oleic (C18:1n9c), linoleic (C18:2n6c) and α-linolenic (C18:3n3) acid was

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found in Sorghum compared to Control ($P < 0.05$). Content of linolelaidic acid (C18:2n6t) was not affected by the treatment ($P > 0.05$). The inclusion of sorghum silage into the ration had a positive effect on the content of polyunsaturated FAs and n-3 FAs ($P < 0.05$) and tended to increase n-6 FAs ($P = 0.064$).

Keywords: α-linolenic acid; linoleic acid; n-3 fatty acids; n-6 fatty acids; saturated fatty acids; unsaturated fatty acids

INTRODUCTION

Milk and dairy products are very important sources of nutrients in the human diet providing energy, high-quality proteins, essential minerals and vitamins (Lock and Bauman, 2004). The primary energy source in milk is fat, which is also a key component contributing to its technological properties. It determines the physical and sensory characteristics and organoleptic properties of dairy products (Lock and Bauman, 2004).

A considerable part of the human population relies on milk as an important source of fat. Milk fat contains various different fatty acids (FAs). From a nutritional standpoint, it is beneficial to reduce the proportion of saturated FAs (SFAs) while increasing the proportion of unsaturated FAs, with a special emphasis on polyunsaturated FAs (PUFAs) in milk (Hanuš et al., 2018). PUFAs, particularly n-3 and n-6 are essential FAs acknowledged to exert pronounced beneficial effects on human health (Angulo et al., 2012). Additionally, monounsaturated FAs (MUFAs) have numerous biological functions that provide health benefits when their intake is increased, particularly when they replace common SFAs in the diet (Calder, 2015). Oleic acid (C18:1n9c) and

α-linolenic acid (C18:3n3) exhibit anti-cancer and anti-atherogenic properties, have positive effect on cholesterol level and improve immune response, linoleic acid (C18:2n6c) and linolelaidic acid (C18:2n6t) improve insulin sensitivity and can therefore help prevent type 2 diabetes (Hanuš et al., 2018).

Dewhurst et al. (2006) recommended that total fat in human diet should contribute 15-30%, SFAs less than 10%, n−6 PUFAs less than 5-8%, n−3 PUFAs less than 1-2% and trans FAs should contribute less than 1% of total energy intake. Samková et al. (2014) outlined that in bovine milk fat, the typical proportions of total FAs are 70-75% SFAs, 20-25% MUFAs, and around 5% PUFAs. The concentration of fat in milk is affected by numerous factors. The most significant are genotype, age, health, stage and number of lactations. Crucially, the concentration of fat in milk and the FA profile of milk can be modified by the dairy cow diet as up to 44% of milk fat can originate from it (Hanuš et al. 2018; Shingfield and Griinari 2007). Despite the extensive metabolism of dietary unsaturated FAs to stearic acid (C18:0), in vitro and in vivo studies have demonstrated that a variety of intermediates, including C18:1, C18:2 and C18:3 are formed during biohydrogenation. The specific profile of these biohydrogenation intermediates produced in the rumen is influenced by the composition of the diet (Shingfield & Griinari, 2007).

Sorghum, a globally significant crop, is, unlike maize, well-adapted to diverse agronomic and environmental conditions, particularly in regions with low rainfall or limited irrigation water. Forage sorghum can produce yields comparable to maize, indicating its potential as a substitute in areas with constrained water supplies. However, there is a trade-off, as maize silage, due to its high grain content, typically offers

superior digestible energy content compared to sorghum forages (Getachew et al., 2016).

Khosravi et al. (2018) observed that substituting maize silage with sorghum silage did not affect milk production, feed efficiency, or the concentrations of milk fat, protein, lactose, and solids-not-fat. However, cows fed the maize silage diet produced higher yields of milk fat, protein, and lactose compared to those fed the sorghum silage diet. Furthermore, cows fed the sorghum silage had a higher percentage of PUFAs in fat compared to the cows fed maize silage.

Research by Cattani et al. (2017) demonstrated that total replacement of maize silage with sorghum silage reduced milk yield, increased the concentration of milk fat and lowered the percentage of PUFAs, but did not negatively influence milk coagulation properties and maintained milk composition. Thus, adding sorghum silage to daily ration can be done without a negative effect on animal performance.

The objective of this research was to assess the concentration of eighteen-carbon FAs and specific FA groups in the milk fat of Czech Fleckvieh cows, in relation to the inclusion of sorghum silage into their diet.

MATERIAL AND METHODS

Design of experiment, animals and feeding

The on-farm feeding experiment was carried out on a group of 120 mid-lactating Czech Fleckvieh cows (Agrospol a.d. Knínice, farm Vanovice) from which ten representative cows were selected for detailed study of milk composition. The trial was divided into two consecutive periods of 3 months each. Each period consisted of an adaptation period (3 weeks) followed by a collection period (60-70

days) in which feed intake and milk yield was monitored and samples of milk were taken three times in monthly interval.

Cows were fed a total mixed ration once daily; during the day the feed was pushed regularly six times per day using an automatic feed pusher. In the first period the TMR diet was based on maize silage, grass haylage and a commercial supplemental feeding mixture (Control) while in the second period grass haylage was partially replaced by sorghum silage (Sorghum). Composition of diets is given in Tables 1 and 2. During the collection period the amount of feed offered as well as the amount of feed refusals was recorded on 3 consecutive days on the same term as milk sampling. Cows were milked twice daily (at 4am and 3pm) and milk yield was recorded.

Table 1. Composition of feed rations (in kg/d, on as fed basis)

Component (kg/d, as fed)	**Control**	**Sorghum**
maize silage	20	21.5
grass haylage	10.5	5
sorghum silage	-	5
sugar beet pulp	7	7
molasses	1.2	1.2
supplemental mixture	9.8	9.7
rapeseed meal	0.85	1
sum of diets	49.35	50.4

Table 2. Composition of supplemental feeding mixture (%)

Component	Content (%)
wheat	29.5
barley	12.5
maize	19
soybean meal	17.5
rapeseed cake	14
SM Production U[1]	3.5
limestone	1.5
salt	0.5
sodium bicarbonate	2

[1]*SM Production U – vitamin and mineral mixture*

Collection and analyses of samples

Samples of feed and feed refusals were taken on 3 consecutive days and were analysed for dry matter content.

Samples of evening and morning milk were taken from ten cows once per month during the regular milk recording. Samples for analysis of basic milk constituents were cooled (6 °C) while samples for FA analysis were kept frozen (-20 °C) until analysis.

The basic milk components (fat, protein, casein, lactose, urea, somatic cell count) were analysed in an accredited laboratory (LRM Brno-Tuřany).

Prior FA analysis evening and morning milk was pooled into one sample relative to milk yield. Milk fat was extracted using a modified Folch et al. (1957) extraction procedure. A mixture of chloroform and methanol was used as a solvent, and to wash out non-lipid components salt solution was used (Eggers and Schwudke, 2016). Transesterification of triglycerides in extracted fat to FA methyl esters (FAMEs) was done by adding hexane and 1.5M methanolic sodium

hydroxide solution in a volumetric ratio of 100:3. FAMEs were analysed by gas chromatography with flame ionization detection using GC Agilent 8860 (Agilent, United States) under optimised conditions (oven program: 40 °C for 1 min to 150 °C @ 25 °C/min to 240 °C @ 2 °C/min, detector: FID @ 250 °C) using the ZB-FAME column (Phenomenex, United States) with dimensions 30m x 0.25mm x 0.20µm. The identification of FAs was carried out using the analytical standards (Restek, United States). In total, 37 FAs were observed, out of which 30 were identified. The FA profile was determined by calculating the ratio of each FA's peak area to the total peak area of all detected FAs.

Statistical evaluation of data

Statistical analysis was performed using Microsoft Excel (Microsoft) and Statistica software (TIBCO Software Inc.). For testing of significance between the groups (Control; Sorghum) T-test for individual FAs and Mann-Whitney U test for specific FA groups were used.

RESULTS AND DISCUSSION

The results presented here are preliminary as the entire experiment has not yet been evaluated and include results from one sampling term in each experimental group (n=10).

The effect of inclusion of sorghum silage into the diet of mid-lactating dairy cows on dry matter intake (DMI) and milk performance is shown in Table 3. The DMI in Sorghum was higher compared to Control ($P < 0.05$). This is in contrast with Cattani et al. (2017) and Khosravi et al. (2018) that found no differences in DMI between cows fed maize

silage- or sorghum silage-based diets. On the other hand, Yang et al. (2019) documented significantly lowered DMI when feeding sorghum silage-based diet. However, it should be noted that in the above-mentioned studies, the sorghum silage completely replaced the maize silage while in our study there was only partial replacement of grass silage. Inclusion of 5 kg of sorghum silage at the expense of grass silage could improve palatability of the diet and positively influence DMI. Further, the contrast between results from literature and our findings can be attributed to the variations in diet composition and use of a different breed.

In our study milk yield and composition was not influenced by the diet ($P > 0.05$) except for the content of urea that was higher in Sorghum compared to Control (Table 3, $P < 0.01$). Some authors (Cattani et al., 2017; Yang et al., 2019), in contrast to our results, reported, that sorghum silage in daily ration, lowered milk yield. However, as mentioned above, in these studies sorghum silage was used as a complete replacement of maize silage. Thus, it can be expected that low amount of sorghum silage in the diet will have a negligible effect on milk yield. In terms of milk components, our study is in agreement with Khosravi et al. (2018), Yang et al. (2019) or Cattani et al. (2017) that also observed no significant differences in the percentage of fat, protein, lactose, somatic cell count or urea concentration when feeding sorghum silage, respectively. Although the content of urea in milk was higher in Sorghum group, still the value was within the physiological range (15 – 30 mg/100 ml, Zadražil, 2002). Except for dietary factors (Roseler et al., 1993) milk urea concentration can be influenced by production and environmental factors such as herd, parity, stage of lactation, individuality of animals, milk yield, time of milking etc. (e.g.

Jílek et al., 2006; Dhali et al., 2005). Thus, the effect of sorghum silage on the milk urea in our study is not unambiguous.

Table 3. Milk yield and composition from cows fed two diets

Item	Control[1]	Sorghum[2]	P
Days in milk	237±69	226±52.5	0.696
Dry matter intake (kg/d)	21.5±0.7	23.98±0.25	0.004
Milk yield (kg/d)	22.8±5.3	23±6.1	0.942
Fat (%)	4.32±0.92	4.2±0.55	0.728
Protein (%)	4.03±0.37	4±0.18	0.822
Casein (%)	3.02±0.25	3.11±0.16	0.354
Lactose (%)	4.62±0.29	4.78±0.18	0.168
Urea (mg/100mL)	15.1±3.66	22.12±3.81	0.001
Somatic cell count (1000/mL)	205.9±136.3	313.1±301.1	0.324

[1] diet based on maize silage and grass haylage; [2] diet based on maize silage and grass haylage that was partially replaced by sorghum silage

Changes in the concentration of observed C18 FAs and specific FA groups are shown in Table 4. Concentrations of C18:0, C18:1n9c, C18:2n6c and C18:3n3 were higher in Sorghum in comparison with Control ($P < 0.05$). Concentration of C18:2n6t did not differ between the two diets ($P > 0.05$). In contrast to this, Khosravi et al. (2018) observed no concentration shifts between cows fed maize diet and cows fed sorghum diet for C18:0 and C18:1n9c. Nevertheless, in accordance with our results, Yang et al. (2019) showed that milk from cows that received sorghum silage contained higher levels of C18:0, C18:1n9c, C18:2n6c and C18:3n3 compared to cows fed maize silage. The same results are reported in research by Cattani et al. (2017) for C18:0 levels.

Table 4. Levels of identified C18 FAs and specific FA groups (% of

total FA) in milk fat of lactating Czech Fleckvieh cows as related to inclusion of sorghum silage into feeding ration

FA	Control[1]		Sorghum[2]		P
	mean	SD	mean	SD	
C18:0	6.55	0.44	8.36	1.34	0.002
C18:1n9c	19.80	1.97	22.71	3.30	0.028
C18:2n6t	0.34	0.05	0.33	0.11	0.870
C18:2n6c	2.33	0.26	2.70	0.37	0.018
C18:3n3	0.41	0.04	0.49	0.08	0.010
SFA	72.65	2.26	69.73	3.59	0.076
MUFA	23.77	2.12	26.20	3.38	0.104
PUFA	3.22	0.33	3.70	0.48	0.021

SD – standard deviation; [1] diet based on maize silage and grass haylage; [2] diet based on maize silage and grass haylage that was partially replaced by sorghum silage

It is important to note that these studies involved different diet compositions and a complete substitution of maize silage with sorghum silage, which contrasts with the methodology employed in our research. Incorporating sorghum silage into the cows' diet can also influence the proportion of SFAs, MUFAs and PUFAs. The concentration of SFAs tended to lower with the inclusion of sorghum silage into the cow's diet ($P = 0.076$). PUFAs concentration has raised for Sorghum in comparison with Control ($P < 0.05$). This can be explained by sorghum affecting rumen fermentation, potentially leading to incomplete biohydrogenation of unsaturated FAs. These changes in concentrations of SFAs and PUFAs are very important and positive. PUFAs, as mentioned previously, play a crucial role in maintaining various bodily functions and have been associated with numerous health benefits,

making them an important component of a healthy diet and a marker of high-quality food products.

Feeding with sorghum silage naturally affected also the total amount of n-3 PUFAs and n-6 PUFAs as showed in Figure 1. The concentration of n-3 PUFAs was higher in Sorghum than in Control representing 17.97% and 17.16% of all PUFAs, respectively ($P<0.05$). The inclusion of sorghum silage into the diet also tended to increase levels of n-6 PUFAs ($P=0.064$). This contrasts with the findings of Cattani et al. (2017) who reported a decline in both, n-6 PUFAs and n-3 PUFAs concentrations in milk after changing from maize silage to sorghum silage. As mentioned earlier, direct comparison with literature is not possible owing to the fact that the majority of research focuses on the total replacement of maize silage by sorghum silage, while our research replaced 5 kg of grass silage with the same amount of sorghum silage. It is noteworthy that the cow breed used in research by Cattani et al. (2017) was Holstein-Friesian dairy cows, which can be another explanation for the differences in the results as cow breed also affects the concentrations of n-3 and n-6 PUFAs (Samková et al., 2014). Moreover, higher levels of n-3 and n-6 PUFAs found in sorghum silage compared to grass haylage (Maggioni et al. 2009) could also partly explain differences between our results and those in literature.

According to Farková et al. (2024) n-6 PUFA intake should be between 5 to 20% of the total energy intake to reduce the risk of chronic diseases, lower blood low-density lipoprotein (LDL)-cholesterol levels and decrease the risk of coronary heart disease. Furthermore, n-3 PUFAs have anti-inflammatory and antioxidant properties (Liput et al., 2021). For these reasons, higher levels of n-3 and n-6 PUFAs after the

partial inclusion of sorghum silage instead of grass silage are perceived positively.

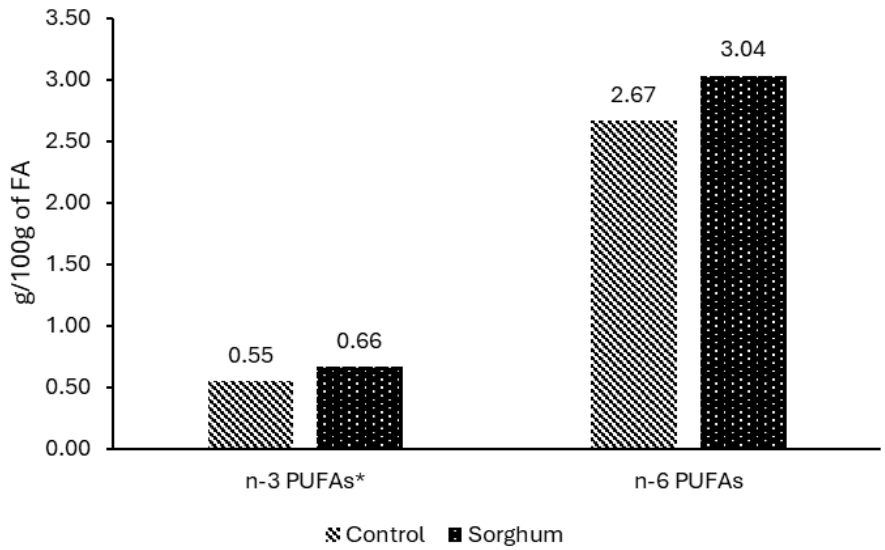

Figure 1. Proportion of n-3 PUFAs[1] and n-6 PUFAs[2] as related to addition of sorghum into feeding ration (Control[3], Sorghum[4]) in milk fat of Czech Fleckvieh cows.

*[1]n-3 PUFAs – sum of C18:3n3 and C20:3n3; [2]n-6 PUFAs – sum of C18:2n6t and C18:2n6c; [3] diet based on maize silage and grass haylage; [4] diet based on maize silage and grass haylage that was partially replaced by sorghum silage; * means statistical significance (P < 0.05)*

The total proportion of all C18 FAs was higher in Sorghum compared to Control (Figure 2, $P < 0.05$). Fat yield was not influenced by the diet ($P > 0.05$). In terms of fat yield, our observations contradict those of Yang et al. (2019) who reported a significant increase of fat yield in milk from cows fed sorghum silage than from those fed maize silage and Khosravi et al. (2018) who observed a decrease in fat yield after changing from maize silage to sorghum silage. The differences in findings can be explained by the interaction of the extent of the change of diet (total vs. partial replacement), the genetic predisposition of

different cow breeds, and other dietary and environmental conditions (Adediran et al., 2010; Hanuš et al., 2018; Sinclair et al., 2015).

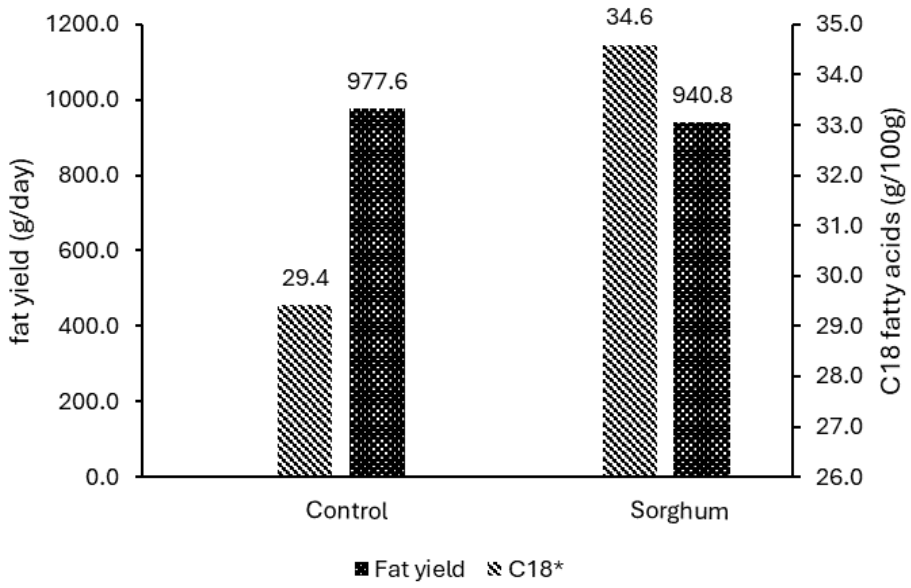

Figure 2. Fat yield[1] (g/day) and total C18[2] FAs content (g/100 g of total FA) comparison related to addition of sorghum silage into feeding ration (Control[3], Sorghum[4])

*[1]Fat yield = fat content (g.kg-1) x milk yield (kg.d^{-1}); [2]C18 – sum of C18:0, C18:1n9c, C18:2n6c, C18:2n6t and C18:3n3; [3] diet based on maize silage and grass haylage; [4] diet based on maize silage and grass haylage that was partially replaced by sorghum silage; * means statistical significance (P < 0.05)*

CONCLUSION

Under the above-described conditions, feeding a sorghum silage as a partial replacement of grass silage in diets of mid-lactating dairy cows positively influenced dry matter intake and maintained milk yield and content of basic milk constituents. This partial substitution of grass silage with sorghum silage resulted in positive changes in C18 fatty acids, namely in increased concentrations of stearic (C18:0), oleic

(C18:1n9c), linoleic (C18:2n6c) and α-linolenic (C18:3n3) acids and a sum of C18 ($P < 0.05$). In addition, content of n-3 fatty acids and polyunsaturated fatty acids was higher ($P < 0.05$), and content of n-6 fatty acids tended to be higher ($P = 0.064$) in Sorghum group compared to Control. These preliminary results suggest that sorghum silages could have a potential as substitute of grass silages in dairy cow diets.

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IN VITRO DIGESTIBILITY OF SILAGE MAIZE HYBRIDS

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ABSTRACT

The aim of this study was to determine in vitro NDF digestibility of different silage maize hybrids using upgraded method in vitro gas production. Digestibility was measured up to 48 hours and the focus of our work was on 30-hour digestibility. Based on our latest work we focused on 2 evaluations: 1. evaluation of NDF digestibility to find correlation between dry matter of silage maize hybrids and NDF digestibility in 30-hour point and 2. Differences of NDF digestibility between silage maize hybrids in 30-hour point. We found highest NDF digestibility 62,80% in dry matter range from 30 – 35% which correlates to our latest work to determine the best silage window. Differences in NDF digestibility of silage maize hybrids in 30-hour point were not significant (P = 0.580) and the range was from 55,16% - 63,82%. In dry matter range 27 – 35% we found 30-hour NDF digestibility from 49,81 – 64,76%.

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Keywords: silage maize hybrid; NDF digestibility; in vitro gas production

INTRODUCTION

Maize silage is very often used as a basis of TMR, and it is important to determine the best time for harvesting and create the best feed from the perspective of nutrients, silage fermentation and digestibility. Neutral detergent fiber (NDF) digestibility is important factor which has impact on the milk production (Krämer-Schmid et al., 2016).

Our latest works showed that FAO groups are not a good prediction model for silage maturity prediction (Mitrík T., Mitrík A., 2022; Mitrík T., Mitrík A., 2023) and to determine silage maturity we need better model as described (Mitrík T., 2023). Based on the results and nutrients we found the best silage maturity level at 30% of dry matter (DM). The aim of this work is to evaluate silage maturity from side of the digestibility. Attempts to predict and describe in vivo digestibility using in vitro digestibility fermentation started in twentieth century but due to poor technique which requires anaerobic environment and inadequate buffers were results using in vitro technique lower than using in vivo method. In 1963 had been developed two-stage Tilley and Terry method which is still widely used today with some modifications. In 1970 Goering and Van Soest developed in vitro NDF digestibility which requires standardization to ensure reproducible results. The newer method developed by Ankom is still used and it requires filter bags with sample and all bags share a common environment with sample weight 0,25 – 0,50 g per sample (William and Hall, 2020). Digestibility is measured after the given incubation time. The latest method is measurement of gas production from in vitro fermentation

which is indirect method to determine digestibility kinetics based on gases production and final sample weight difference before and after the incubation (Tedeschi and Fox, 2020) This method was improved by Pell and Schofiled (1993, 1995), Schofield (2000) and Williams (2000). Based on pitfalls such as particle size, small sample weight, closed bags which floats, small fermentation flasks we developed the new digestibility method.

MATERIAL AND METHODS

An experiment was performed with 7 different maize silage hybrids FAO 200 – 530 and the sample collection was performed in the interval of 34 days at 4 different terms (12.8.2021, 19.8.2021, 2.9.2021, 13.9.2021). Samples (500 – 750 g) were dried at MEMMERT UFE 500 and UFE 700 with < 60 °C 16 - 24 hours. Dried samples were milled by SM-100 (RETCH) to pass a 2 mm sieve and subsequently by TWISTER (RETSCH) with 1 mm sieve. All nutrients were analysed by NIRS Antaris II FT-NIR Analyzer (Thermo Fisher Scientific) on samples with 1 mm grinding using calibrations from FEEDLAB s.r.o. company. Amount of dry matter was evaluated from laboratory dry matter and dry matter analysed by NIRS method. 1,5 g sample after 2 mm mill sieve was taken to the large (8 x 10 cm) open bags handy made from PET mash with mash-opening 36 µm (PET 1500 140/355-31W). Samples for measuring NDF digestibility had chemically isolated NDF ANKOM NDF Method 13 as principally described Van Soest et al. (1991), NRC 2021 and updated by temporarily sealing bag. After NDF determination, bags were placed into ultrasonic water bath to clean detergent from the samples. After that bags were reopened and prepared for isolated NDF digestibility using IN VITRO Ankom Gas Production system. Digestibility was determined as describe Ankom

Gas Production Operator ‘s Manual Appendix C with some modifications. Due to larger bags, we used 1000 ml flasks, and every flask contains 2 sample open bags of isolated NDF with 560 ml of buffer and 140 ml of filtered inoculum. Plastic stick was placed into open bags to kept bag open and preventing to blow the bag. Opening of the bag was set up above the inoculum surface. Data collection was set for every 5 minutes with pressure 1,5 psi. Every sample run included blank flask without sample. After incubation time 48 hours, samples were flushed with hot water, resealed and placed into ultrasonic water cleaner. Cleaned samples were dried at 103 °C and weighed. Final % NDF digestibility was determined as weight difference after incubation using gas production kinetics andcalculated for every hour till 48-hour point using mathematical methods. Statistical evaluation was performer by NCSS 12 (64 bit) – version 12.0.18 – NCSS LLC with ANOVA method.

RESULTS AND DISCUSSION

Table 1, 30 – hour NDF digestibility

sample collection	**DRY MATTER (g/kg)**	**200 - 250**	**250 - 300**	**300 - 350**	**350 - 400**	**400 - 450**	**Total**
1. - 12.8.2021	Count	3	2	2			7
	Mean	**59,80%**	**54,90%**	**61,40%**			**58,80%**
	Min	50,60%	53,80%	61,30%			50,60%
	Max	69,50%	56,00%	61,50%			69,50%
2. - 19.8.2021	Count		5	1	1		7
	Mean		**64,00%**	**66,50%**	**59,90%**		**63,70%**
	Min		59,40%	66,50%	59,90%		59,40%
	Max		67,20%	66,50%	59,90%		67,20%
3. - 2.9.2021	Count		4		1	2	7
	Mean		**54,50%**		**61,50%**	**51,70%**	**54,70%**
	Min		48,40%		61,50%	48,30%	48,30%
	Max		62,30%		61,50%	55,00%	62,30%
4. - 13.9.2021	Count			1	1	3	5
	Mean			**61,80%**	**64,70%**	**57,10%**	**59,90%**
	Min			61,80%	64,70%	55,00%	55,00%
	Max			61,80%	64,70%	59,30%	64,70%
average	Count	3	11	4	3	5	26
	NDF DIGESTIBILITY (%)	**59,80%**	**58,50%**	**62,80%**	**62,00%**	**54,90%**	**59,30%**

Table 2, Average 30-hour NDF digestibility		
HYBRID	**COUNT**	**30 h. IV NDF DIGEST. (%)**
1	4	**58,34%**
2	4	**60,06%**
3	4	**59,03%**
4	4	**60,25%**
5	4	**55,16%**
6	4	**63,82%**
7	4	**58,42%**
average	4	**59,29%**

We found NDF digestibility at 30-hour level with average from 54,90% - 62,80% (Table 1). The highest NDF digestibility was at range 300 – 350 g/kg dry matter (DM) content – 62,80%. We found increasing digestibility till 300 – 350 g/kg DM and decreasing NDF digestibility with rising DM content. The lowest 30 hours NDF digestibility was 48,30% and the highest was 69,50%. Differences in NDF digestibility on the level of hybrids were not significant with P = 0.580 (Table 2 and Chart 1). The average of 30 hours NDF digestibility of all maize silage hybrids was 59,29% with minimum of 55,16% (hybrid 5) and

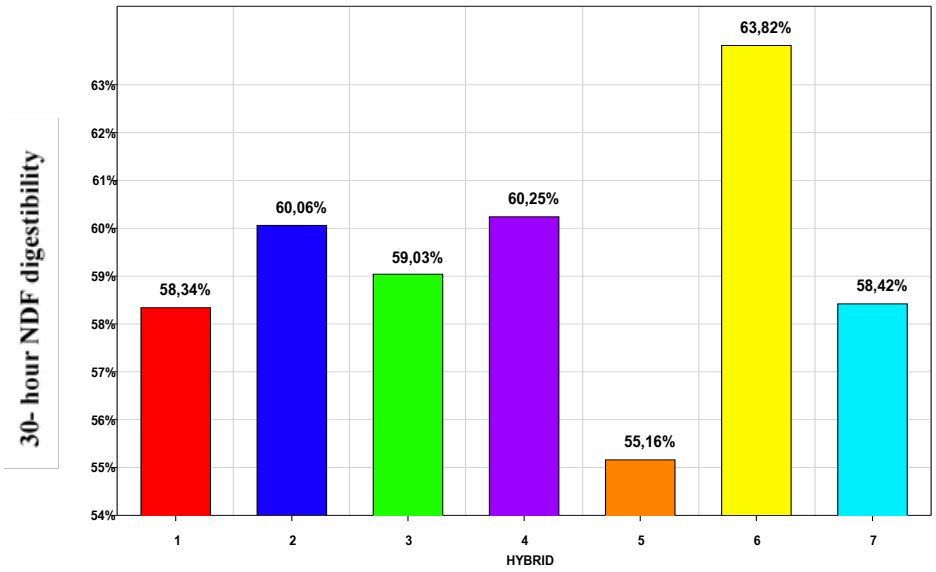

Chart 1, Average 30-hour NDF digestibility from 4 sample collection points

maximum 63,82% (hybrid 6).

These results confirm our hypothesis about silage maturity determination that the best dry matter window for highest NDF digestibility is from 300 – 350 g/kg which correlates with Mitrík T. et al. (2022). NRC 2001 evaluate 30 h. NDF digestibility of maize silage

Chart 2 – Average NDF digestibility from 4 sample collection points with DM 27 – 35%

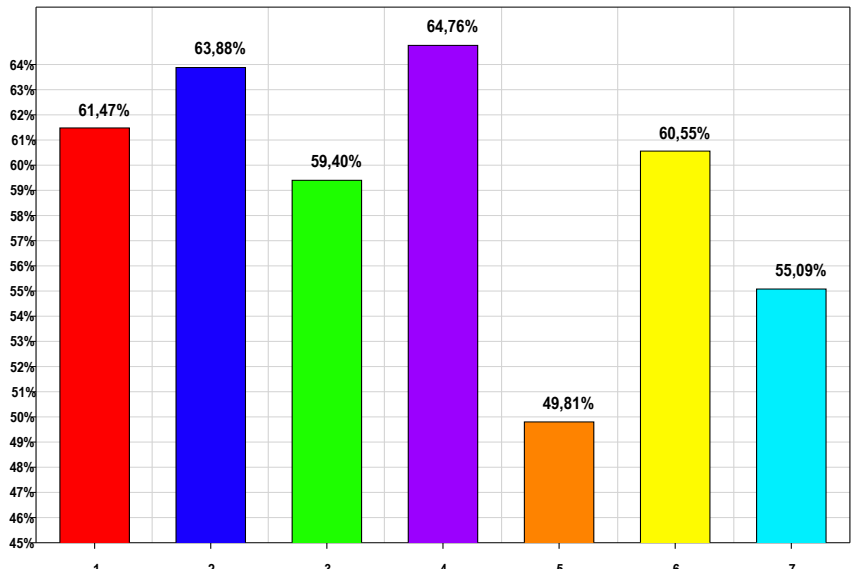

Chart 3 – 30-hour NDF digestibility with DM range 27 – 35%

from 32,5 – 61,2%. Increased maturity of maize is bonded with lowering NDF digestibility (Jensen et al., 2004) and our results support these findings. As describes Mitrík (2023), in this work we choosed silage maturity range from 27% - 35% as range which is the best for ensiling and also with culmination of 30-hour NDF digestibility in that range. In that range we compared average NDF digestibility on the level of hybrids from 0 – 48 hour as show Chart 2. Differences of dynamics of NDF digestibility between hybrids are high.

On the level of hybrid and at 30-hour NDF digestibility point with DM range 27 –35% we found differences with P = 0,476 (Chart 3) In that DM range we can see 30-hour NDF digestibility from 49,81 – 64,76%. These results confirm that differences between hybrids are not strong, but they are present, and they can vary with different maize silage hybrids.

CONCLUSION

The new updated model of determination proved good and reliable results. New model with grinding on 2 mm sieve, higher sample weight, open bigger bags without floating proved repeatability and solved pitfalls described by Weiss et al. (2020) or NRC 2021. On the other hand, this method is more expensive and more difficult for preparation and need more repetitions. Results showed that differences in 30-hour NDF digestibility on the level of hybrid are not statistically significant, but values have wide range from 55,16– 63,82%. On the other hand, we also can see differences of 30-hour NDF digestibility with DM range 27 – 35%. For better evaluation, it is necessary to obtain more data in silage maturity dry matter range 27 – 35%. However, these results also confirm differences between NDF

digestibility of maize silage hybrids and different kinetics of their digestibility.

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EFFECT OF FEEDING HUMIC SUBSTANCES ON THE PRODUCTION PARAMETERS AND PRODUCT QUALITY OF BROILER CHICKENS

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ABSTRACT

Besides the scale of production, the success of livestock farming is also affected by the level of input prices. Feed is one of the more expensive items. The aim is to use the feed components in the feeding process as rationally and efficiently as possible, by increasing their attractiveness in terms of intake, by increasing the digestibility and utilization of nutrients. These effects can also be achieved with the help of additives, which include humic substances. In this work, the influence of humic subtances on the production parameters of broilers and on the quality of poultry products was studied. The experimental group of broiler chickens received a feed mixture with the addition of HumacNatur in a concentration of 0.7 %. At the end of the experiment, the control group of broilers reached an average weight of 2606.4 g and the experimental group 2349.0 g. Feed conversion was 1.63 in the control group and 1.74 in the experimental group. The carcass yield of the control group after dissection was 73.2 % and the experimental group after dissection was 75.1 %. The carcass yield of the pectoral muscle of the control

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group was 27.8 % and of the experimental group was 28.4 %. The carcass yield of the thigh muscle was 28.8 % and the experimental group was 29.1 %. Furthermore, the content of water, dry matter, protein and fat in the pectoral and thigh muscles was determined. The results show that the use of HumacNatur at a concentration of 0.7 % did not have a significant effect on the monitored parameters in broilers.

Keywords: humic compounds; growth rate; poultry nutrition

INTRODUCTION

Humic substances (HS) are natural organic compounds formed through the chemical and biological decomposition of plant and animal residues and the synthetic activity of microorganisms. HS naturally occur in soils, peat, brown coal, and lignin. They are formed through a process called humification, which involves a series of anaerobic enzymatic and biochemical processes (Pivokonský et al., 2010; Trčková et al., 2005). They are classified into three types: humic acids, fulvic acids, and humins (Stevenson, 1994).

The use of HS in poultry nutrition as an alternative feed additive has gained increasing importance, especially after the ban on antibiotics in feed as growth promoters. Humic substances act as growth promoters in the nutrition of both broilers and layers. They improve feed conversion and increase weight gain. Adding HS to drinking water or feed improves most production parameters, such as daily weight gain, in addition to increasing the carcass yield of broilers (Maguey-González, 2022; Karaoglu et al., 2004; Ozturk et al., 2012).

Supplementation of humic acids in the diet of broilers affects the physicochemical and organoleptic properties of meat (Semjon, 2020).

Adding humic substances to broiler feed increased the activity of digestive enzymes (amylase, lipase, and protease), meat protein content, total polyunsaturated fatty acid content, activity of superoxide dismutase and glutathione peroxidase, and serum levels of IgG, IgM, and IgA. It also reduced fat content in meat and malondialdehyde levels compared to broilers that did not have HS in their feed (Mao, 2019).

The aim of this experiment was to study the effect of humic substances on the production parameters and product quality of broiler chickens.

MATERIAL AND METHODS

This study was conducted using 60 one-day-old ROSS 308 broiler chicks. The chicks were randomly divided into two groups: a control group and an experimental group, with 30 birds in each group. The chicks were raised on deep litter under controlled conditions in accordance with the technological guidelines for ROSS 308. They were fed a complete feed mixture, whose main components were wheat meal, corn meal, soybean meal, and a premix of supplements. The feed did not contain antibiotic growth promoters, GMOs, anticoccidials, or meat-and-bone meal. For the experimental group, the feed mixture was supplemented with 0.7% of the natural humic preparation HUMAC® Natur AFM. The control group was fed a standard broiler feed mixture without the additive. The chicks had ad libitum access to water and feed. The composition of the feeds used during the experimental periods is shown in Table 1. The feed was analyzed for dry matter, nitrogenous substances, fat, fiber, starch, and ash according to AOAC (2001).

The weight of each broiler was measured weekly, and feed consumption was recorded daily. Feed conversion was calculated based

on feed intake. At the end of the fattening period (day 42), the animals were weighed, stunned, killed by cervical dislocation, and bled. After bleeding and carcass processing, the broilers were weighed, dissected, and the yield and percentage of breast and thigh muscle, wings, carcass, and abdominal fat were calculated. Meat quality was assessed by determining the content of dry matter, protein, and fat in the breast and thigh muscle.

Data obtained from this experiment were evaluated using GraphPad Prism 3.0 and expressed as mean ± standard deviation (X ± SD). Individual results between groups were statistically compared using a paired t-test, and a P-value < 0.05 was considered a statistically significant difference.

RESULTS AND DISCUSSION

Based on the results of weighing the broilers and the feed consumed, the average weights of the broilers were calculated. The evaluation of these results showed smaller weight gains in the experimental group compared to the control group. The addition of humic substances to the feed throughout the monitored period did not significantly affect or improve the weight gains of the experimental animals. Some researchers have found that supplementation with humic acids has no impact on the live body weight of broilers (Marcinčáková, 2015; Kaya and Tuncer, 2009; Nagaraju et al., 2014). On the contrary, Rath and colleagues (2006) found that treatment with humic acid significantly reduced the body weight of broiler chickens, particularly at higher concentrations. However, many studies have demonstrated that supplementation with humic substances positively affected the live

body weight of broiler chickens (Eren et al., 2000; Karaoglu et al., 2004; Ozturk et al., 2012).

The feed conversion ratio for the entire experimental period was statistically insignificantly higher in the experimental group (control group – 1.63; experimental group – 1.74). The results of this study are consistent with the findings of other researchers who reported a deterioration in feed conversion in the experimental group (Hudák et al., 2020; Rath et al., 2006; Demeterová, 2009). However, Jaďutová et al. (2019) stated that the application of humic substances in amounts of 0.8% and 1.0% in the feed mixture resulted in improved final body weight of broilers and feed conversion ratio.

Table 1. Average weights and average gains of chickens

	Average weight (g)		**Average gain (g)**	
Day	**Control group**	**Experimental group**	**Control group**	**Experimental group**
0.	37.4 ±0.53	37.3 ±0.51	-	-
7.	153.1 ±7.81	150.2 ±5.66	16.5	16.1
14.	403.4 ±40.14	388.9 ±34.24	35.7	34.1
21.	873.9 ±82.98	777.3 ±79.94	41.1	47.3
28.	1328.3 ±145.03	1270.2 ±141.61	76.2	63.4
35.	1952.3 ±198.79	1788.1 ±202.2	76.2	83.2
42.	2606.4 ±289.02	2349.0 ±271.89	85.8	70.3

The eviscerated yield was 73.2% in the control group and 75.1% in the experimental group. The experimental group showed a higher yield of breast and thigh muscle and a lower yield of wings and carcass compared to the control group. The differences are not statistically significant ($P < 0.05$). In thigh muscle, there were minimal differences

between the measured values of dry matter, protein, and fat in the control and experimental groups. All observed parameters did not show any statistically significant differences between the groups ($P > 0.05$). Pistová et al. (2017) and Karaoglu et al. (2004) did not find a positive effect on carcass weight and yield in poultry experiments with the addition of humic substances.

Table 2. Yield of body parts after evisceration (%)

	Control group	**Experimental group**
Eviscerated yield	73.2±1.8	75.1±1.7
Breast muscle yield	27.8±2.0	28.4±1.4
Thigh muscle yield	28.8±1.8	29.1±1.4
Wing yield	10.8±0.8	10.3±0.6
Carcass yield	32.6±1.0	32.0±1.0
Abdominal fat yield	0.5±0.3	0.5±0.2

The breast muscle in the experimental group had a 1.7% higher dry matter content. The protein content was slightly higher in the breast muscle of broilers from the experimental group (by 0.57%). A statistically significant difference ($P < 0.05$) was registered in the fat content. The fat content in the experimental group was 1.48% higher than in the control group.

In the experiment, we observed minimal differences in the content of dry matter, water, protein, and fat in chilled breast and thigh muscle. A significant difference was observed only in the higher fat content of the breast muscle of broilers in the group with added humic substances. Conversely, the fat content in the thigh muscle of the group with the

additive was lower by 1.17%, but not statistically significantly different.

Table 3. Chemical composition of meat (%)

	Breast muscle		Thigh muscle	
	Control group	**Experimental group**	**Control group**	**Experimental group**
Dry matter	25.66	27.36	25.62	25.52
Water	74.34	72.64	74.38	74.48
Proteins	22.01	22.58	20.54	22.05
Fat	1.9	3.38	4.34	3.17

Results from the study conducted by Hudák et al. (2021) indicate that a 0.7% addition of HS in both natural and acidified forms to broiler feed significantly affected the composition and quality of breast meat. The addition reduced the meat's fat content and pH and resulted in a lighter color. The authors also noted a significant impact of adding HS to the feed mixture on meat quality during storage. The oxidative stability and sensory properties of the meat were better compared to the control. When evaluating the natural and acidified forms of HS on the quality of breast muscle meat, they observed a comparable effect. The enhanced effect of the acidified form of HS on growth parameters and meat quality was not confirmed. The addition of 0.7% natural HS preparation shows good potential for significantly improving the quality of produced meat as well as potentially improving the growth parameters of poultry.

CONCLUSION

The results achieved with the 0.7% concentration of humate in the feed mixture indicate that this concentration did not have a significant impact on production parameters and product quality. No negative impact on animal health was observed during the experiment. Further research should focus on testing other concentrations of humic substances, their combinations with other supplements, and the optimal timing for their use in fattening.

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ACTUAL TRENDS IN CALF FEEDING TECHNOLOGY AND THE USE OF PROBIOTIC FEED ADDITIVES IN CALF NUTRITION

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ABSTRACT

The main aim of this study was to evaluate the effect of diverse probiotic strains on the health of the calves during the period of colostrum and milk feeding. The blood samples were also collected to check the level of immunoglobulins in the blood plasma and for the blood count test.

Not only all groups significantly surpassed the normal BRIX value which is 8.4 % BRIX but also all calves that received *Bifidobacterium* supplementation had higher BRIX levels, indicating that *Bifidobacterium* supports effective passive immunity transfer.

In all three weighing there was found no statistical difference observed in average weight gain between groups. However, in the second and third weighing, the BEL combination still showed higher weight gain than any other experimental group.

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As for the frequency of diarrheal diseases, there were no significant differences between individual groups. Nevertheless, the experimental animals receiving single-strain *Lactobacillus* had a higher incidence of diarrhea compared to the control animals, meanwhile the opposite was observed in the *Bifidobacterium* experimental group.

This study shows that supplementation with single-strain or combination probiotics did not significantly affect weight gain, BRIX levels, or the incidence of diarrheal diseases in calves. Despite a numerical increase in weight gain with combination probiotic supplementation, these differences were not statistically significant.

Keywords: calf nutrition, probiotic supplementation, feed supplements

INTRODUCTION

The maternal and fetal blood supplies are separated due to the cow's placenta, preventing the utero transmission of protective immunoglobulins. This makes the intake of colostrum after birth crucial for calves, as it contains immunoglobulins that provide passive immunity until their immune system becomes functional (Godden, 2008). The efficient transfer and absorption of antibodies are influenced by factors such as the method and volume of colostrum intake, immunoglobulin concentration, and timing of colostrum ingestion (Weaver et al., 2008). Low immunoglobulin concentration is associated with high mortality rates due to infections (Blum, 2006).

Newborn calves have poor immune capability, making them vulnerable to infections (Muktar et al., 2015). Calf diarrhea is a common issue that leads to productivity and economic losses for cattle producers. It is a multifactorial disease caused by infectious agents like Cryptosporidium, Clostridium perfringens, Escherichia coli, and

Salmonella, as well as non-infectious factors such as poor sanitation, insufficient colostrum intake, and inappropriate environmental conditions (Cho & Yoon, 2014; Caffarena et al., 2021; Muktar et al., 2015).

The gastrointestinal health of calves plays a crucial role in disease control, with the gastrointestinal microbiota being a key factor. The gastrointestinal tract (GIT) undergoes rapid changes in structure and microbiota composition during early life (Meale et al., 2017). In newborn calves, the GIT is not fully developed, with the abomasum being the main site of digestion. The development of the forestomach, influenced by the intake and quality of solid feed, can take several months after birth (Górka et al., 2018; Guilloteau et al., 2009).

The GIT of calves is believed to be sterile in utero, with colonization occurring during and after birth. This colonization is vital for the maturation of the immune system, influencing the health and performance of the young calves (Klein-Jöbstl et al., 2019; Malmuthuge et al., 2015). In pre-weaned calves, bacterial groups such as Bifidobacterium, Lactobacillus, Fecalibacterium, and Enterococcus are present in fecal samples (Malmuthuge & Guan, 2017). As the forestomach develops, the rumen's microbiome evolves to include bacteria, archaea, protozoa, and fungi, with microbial composition depending on factors such as host age, diet, season, and geographic region (Malmuthuge et al., 2015; Dill-McFarland et al., 2019).

Diarrhea is a leading cause of morbidity and mortality in calves, typically managed with oral antimicrobials. However, associated variable efficacy and rising concerns about antimicrobial resistance makes it an unsuited solution (Smith, 2015). Hence, alternatives like probiotics are being explored. Probiotics are live, nonpathogenic

microorganisms that improve gastrointestinal microbial balance in the GIT (Williams, 2010). Studies show that calves fed probiotic strains such as Lactobacillus plantarum, L. casei, Enterococcus spp., Bacillus spp., Bifidobacterium spp., and Saccharomyces cerevisiae exhibit better health, reduced neonatal diarrhea, and increased growth (Wang et al., 2022; Stefańska et al., 2021).

This study aims to evaluate the effect of selected single and combined probiotic strains on calf growth and prevention of digestive disorders in calves. This research would provide more insight into the potential benefits of probiotics in calf rearing.

MATERIAL AND METHODS

An experiment was performed with a total of 300 calves, randomly assigned to three groups. Each group was further divided into an experimental group and a control group. The first group, L, was fed with probiotics containing the single strain *Lactobacillus sporogenes*, with a total of 83 calves. The second group, B, was also fed with a single strain probiotic containing *Bifidobacterium bifidum* and a total of 70 calves, equally divided into control and experimental groups. The third group, BEL, was given probiotics containing a mixture of *Bifidobacterium bifidum*, *Enterococcus faecalis*, and *Lactobacillus sporogenes*, represented by 147 calves, equally divided into control and experimental groups.

Calves were left with their mother for no longer than two hours after birth. They were then moved to a clean, disinfected outdoor box with straw bedding and weighed. At least two hours after calving, calves received their first drink of frozen colostrum, slowly heated to 39°C with a volume of at least 2.5 liters. The quality of freshly obtained

colostrum was measured using a refractometer. Calves in the experimental group L were given a probiotic pill dissolved in milk from the first feeding with colostrum to the third day of life. Calves in the experimental group B received 3 g of *Bifidobacterium bifidum* before the first feeding with colostrum and subsequently for 21 days, always in the morning feeding. Calves in the experimental group BEL were given a probiotic mixture at the first feeding and subsequently for 5 days, in a dose of 3 g, always before the morning feeding.

Between the third and fifth days, blood was taken from the jugular vein of the calves to obtain samples for immunoglobulin level checks in blood plasma and for laboratory determination of the blood count test. Blood centrifugation was performed at 2000 RPM to obtain plasma for total protein level measurement using a digital refractometer. Blood samples were collected into tubes containing sodium EDTA and sodium fluoride for blood count test and into tubes with Heparin for biochemical analysis. Samples were mixed with anticoagulants immediately after collection, placed in a cooling box, and transported to the laboratory. Biochemical analysis was performed using an Ellipse Dialab device, and a blood count was conducted using an Exigo LABtechnik device.

To monitor weight gain, which was a crucial aspect of the experiment, calves were weighed when moved from the farrowing box to an outdoor individual box at approximately 30 days of age, weighed a second time when moved to group pens at approximately 70 days. Last weighing was at approximately 150 days of age, when calves were at the end of weaning and were completely on a plant-based diet. A two-wheeled cart with built-in tensometric scales was used.

Fresh fecal samples were collected by-hand from the rectum of animals at a depth of 5cm using clean gloves and placed in a sterilized plastic tube. Immediately after collection, samples were stored in a refrigerator at a temperature of −4 °C and transported to the lab.

In describing statistics, we used relative frequencies for categorical variables. Within descriptive statistics, we use valid n, min, median, mean, max, and SD for numerical characteristics. The chi-square goodness-of-fit test was used to detect differences in categorical variables by role in the experiment or by Brix level (≤ 8.4 and ≥ 8.4). To find differences in the numerical characteristics by role in the experiment or according to the Brix level (below 8.4 and 8.4 and over), the method "Compare mean" and t-test were used. All available data were tested; only statistically significant connections are shown in the next part of the presentation. The level of significance for this type of statistic is 0.05 in both cases (< 0.001….; *0.001-0.01….; 0.01-0.05…*).

RESULTS AND DISCUSSION

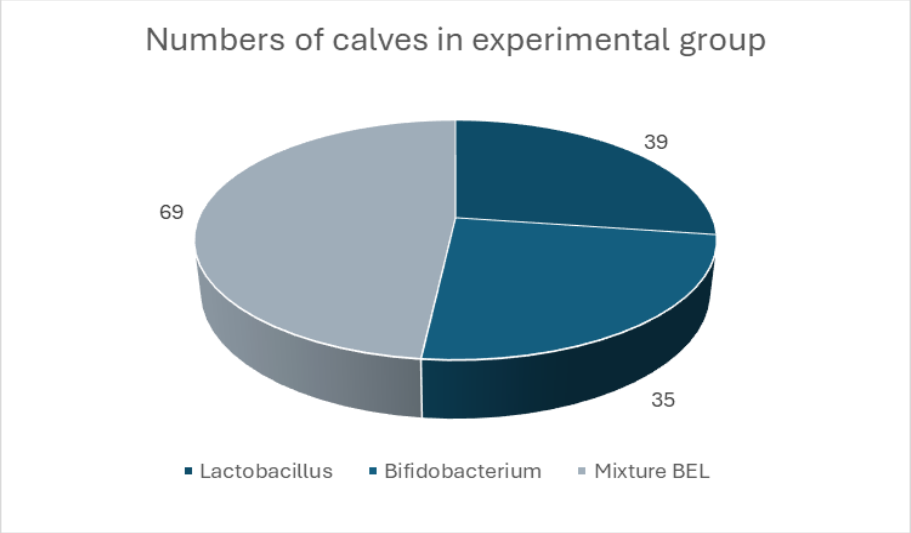

Figure 1. Summary of number of experimental calves in each group

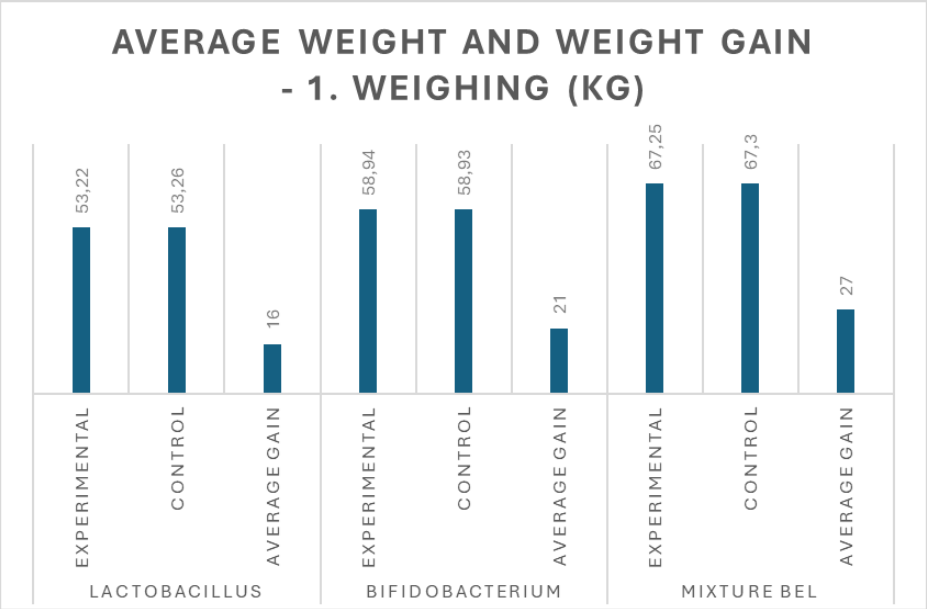

Figure 2. Average weight and weight gain at first weighing

Calves were weighed approximately 30 days at initial weighing. Average weight gain is diameter of all weight gains in each group. We found no statistical difference observed between groups (Figure 2). Both the experimental and the control animals in each group exhibited similar results, showing no significant difference between them.

At second weighing, calves were approximately 70 days of age. We found no statistical difference observed between groups with respect to average weight gain (Figure 3). Again, the experimental and control animals within each group responded similarly with no difference. The BEL combination consistently showed higher weight gain than any other experimental group which might suggest its potential efficacy in promoting growth.

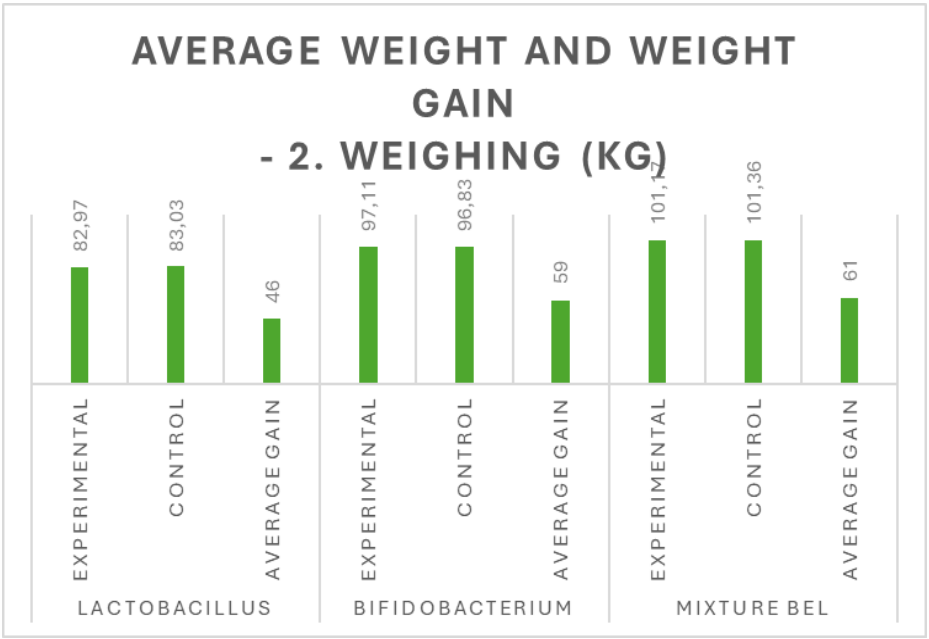

Figure 3. Average weight and weight gain at second weighing

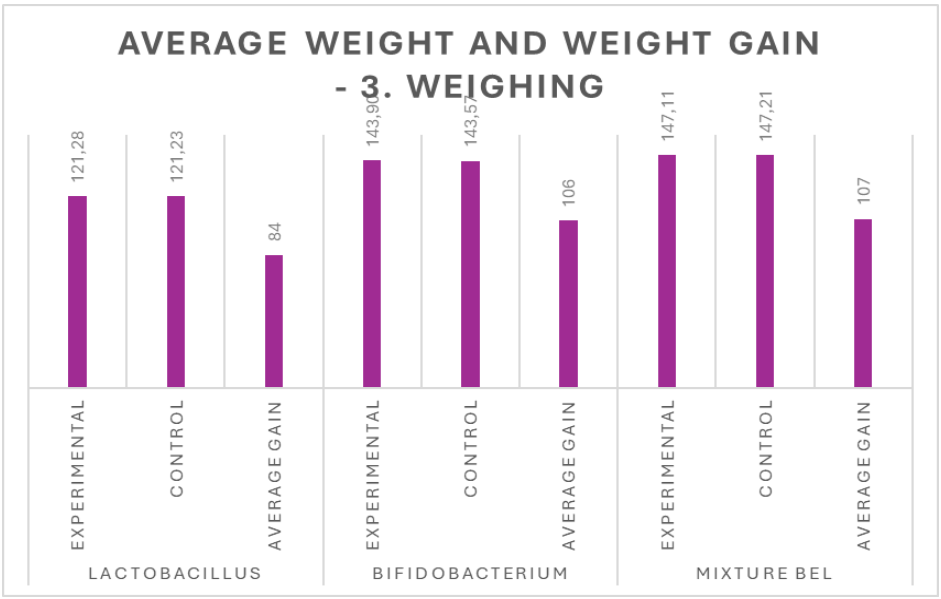

Figure 4. Average weight and weight gain at third weighing

We observed no statistically significant differences in average weight gain between the groups (Figure 4). At the final weighing, both the control and experimental animals in each group were within the same

weight range. Although numerically higher, the weight gain in the experimental animals of BEL combination was not significantly different from that of animals receiving single-strain treatments. This finding is consistent with Guo et al. (2022), who reported no significant differences in body measurement parameters of calves using a multi-strain probiotic over a 30-day period. Additionally, Stefańska et al. (2021) and Fisher et al. (2023) found no significant improvements in growth or live weight gain in their studies on probiotics and multispecies probiotic combinations for dairy calves. Compared to a study carried out by Zábranský et al. (2022) who discovered the positive effect of the combinatiton of *Lactobacillus sporogenes*, *Enterococcus faecium* and *Bifidobacterium bifidum* on live weight and the calves had the highest live gain compared to other groups.

The physiologically normal value for immunoglobulins in blood serum is 8.4% BRIX (Deelen et al., 2014), though it can also be expressed in grams per liter depending on the methodology. The efficient transfer and absorption of antibodies are influenced by factors such as the method and volume of colostrum intake, immunoglobulin concentration, and timing of colostrum ingestion (Weaver et al., 2008). In this study, all calves that received *Bifidobacterium* supplementation had BRIX levels to and above the required threshold, indicating that *Bifidobacterium* supports effective passive immunity transfer. Gaspers et al. (2014) found a correlation between higher birth weights and improved passive transfer of immunoglobulins. On average, all groups significantly surpassed the normal BRIX value, suggesting a positive correlation.

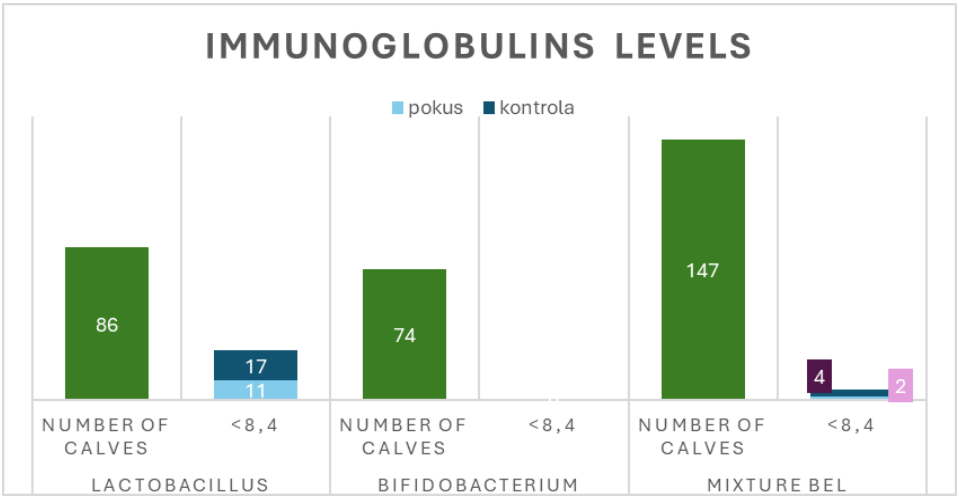

Figure 5. Evaluation of the level of total protein in the blood serum

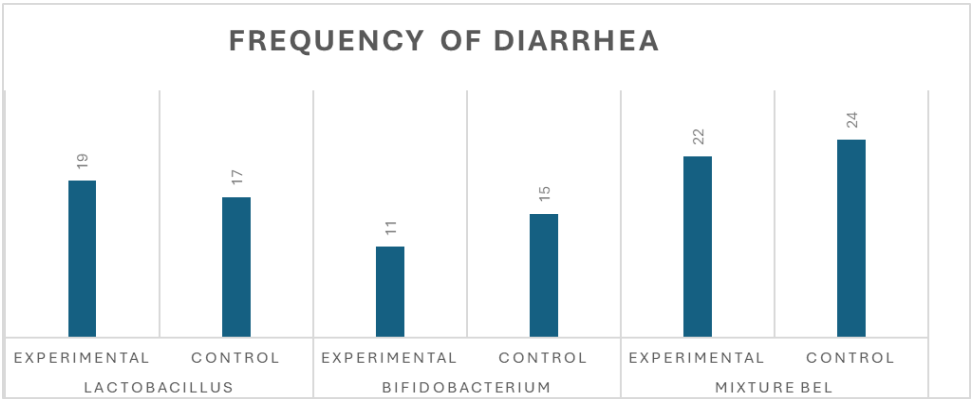

Figure 6. Frequency of diarrhea

The graph below (Figure 6) illustrates the number of diarrheal cases in each treatment group, showing no significant differences. Interestingly, the experimental animals receiving single-strain *Lactobacillus* had a higher incidence of diarrhea compared to the control animals, while the opposite was observed in the *Bifidobacterium* experimental group.

Previous research suggests that multi-strain probiotics are more effective than single strains. *Lactobacillus* inhibits pathogenic bacteria by lowering the pH in the large intestine and competitively attaching to the gut lining (Riddle et al., 2010), which generally supports a healthier gut environment and reduces harmful microbes. *Bifidobacterium*

bifidum disrupts the invasion of host epithelial cells, produces bifidocin to induce cell death in gram-positive bacteria, and synthesizes exopolysaccharides that inhibit pathogen growth (Sarkar and Mandal, 2016). Despite these theoretically beneficial mechanisms, our study found no reduction in diarrhea incidence with the BEL combination.

This report is consistent with the research conducted by Renaud et al. (2019), who investigated the administration of multispecies probiotics and yeast bolus in calves, and Fisher et al. (2023), who assessed the effects of a multispecies probiotic on the health and performance of pre-weaned dairy calves. Both studies found no significant reduction in diarrhea incidence.

CONCLUSION

Our findings show that supplementation with single-strain or combination probiotics did not significantly affect weight gain, BRIX levels, or the incidence of diarrheal diseases in calves. Despite a numerical increase in weight gain with combination probiotic supplementation, these differences were not statistically significant. Specifically, neither individual strains (*Lactobacillus sporogenes*, *Bifidobacterium bifidum*) nor combinations (*Bifidobacterium bifidum*, *Enterococcus faecalis*, and *Lactobacillus sporogenes*) showed a significant impact.

We recommend further investigation into multispecies probiotic combinations in young ruminants, with a focus on selecting strains that may have synergistic effects. Future research should aim to clarify the mechanisms behind the varying results observed in this study compared to previous studies that reported beneficial effects. Such research will enhance the development of effective supplementation strategies.

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EFFECT OF TANNIN TYPE ON *IN VITRO* GAS PRODUCTION

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ABSTRACT

The aim of this study was to determine the effect of different types of tannins on total gas production using in vitro methods using ANKOM Gas Production System. For our study we used 6 products containing tannins commonly available on the market, representing both groups of tannins in the same concentration. Hydrolyzable tannins were presented by the products: FermiTan Harvest Hill, TOP House and Tan Clar. Condensed tannins were presented by the products: Tannin MOX, Tannin FC and VITANIL B. The study using the Ankom Gas Production System was carried out according to Van Soest (1970) with a 5% addition of individual tannins. Incubation was carried out for 48 hours at 39 °C. In our case, there was no evidence of a reduction in the

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amount of gas produced. Both types of tannins increased the amount of gas produced compared to the control sample containing only the standard. Condensed tannins increased gas production more than hydrolyzed tannins.

Keywords: tannins; in vitro; gas production

INTRODUCTION

Tannins belong to the polymeric phenolic compounds present in a number of plant species which is use as feed across agriculture throughout the world. They occur in whole trees, shrubs, but also in by-products of agricultural processing, in leaves and stems of forage crops, cereals and grains, fruits, galls, pods and others (Addisu, 2016). They are also found in food and, as a natural component of food, they affect both desirable and undesirable taste properties of food. In the case of beverages such as fruit juices, beer or wine, tannins are removed by clarification, in order to prevent the formation of turbidity and sediments due to binding to proteins (Food safety, 2024).

Tannins are divided into two groups - group of hydrolyzable tannins and group of condensed tannins. Hydrolyzable tannins are esters of gallic acid or ellagic acid and their derivatives. (Multimediaexpo, 2024). Their occurrence is highest in the tissues of dicotyledonous trees and herbs, and they are stored mostly in leaves (Haslam, 2007). Condensed tannins are non-hydrolyzable oligo- and polymeric proanthocyanidins, which are made up of catechin units and are formed by the condensation of monomeric flavanols (Food safety, 2024). Condensed tannins are not broken down by hydrolysis (Reed, 1995) and may or may not be soluble in water and organic solvents,

depending on their chemical structure and degree of polymerization (Addisu, 2016). Condensed tannins are usually more widespread. Many plants contain both types of tannins. But there are some types of plants that contain only hydrolyzable tannins. Hydrolyzable tannins are thought to be more toxic because they are broken down by microorganisms in the rumen into pyrogallol, which is a hepatotoxin and a nephrotoxin. On the other hand, condensed tannins are not absorbed, but can be incorporated into the mucosa of the gastrointestinal tract and reduce the absorption of other substances there (Lotfi, 2020; Patra – Saxena, 2010; Reed, 1995). Condensed tannins in feed compounds can provide a means to protect feed proteins from degradation in the rumen, thereby increasing the uptake of plant proteins in the small intestine, and this in turn affects animal performance (Piluzza et al., 2013). Polyphenols are the main source of dietary antioxidants and are easily absorbed in the intestine (Kumar – Goel, 2019).

In general, the effect of tannins depends not only on the type of tannin contained, but also on its concentration. In lower concentrations, tannins can have rather beneficial effects, not only on digestion, but on the overall health of ruminants, faster growth of the animal's live mass, higher milk yield, good response of the immune system, reproductive performance, fertility, wool production, resistance to gastrointestinal parasites, prevention bloat and by reducing methane production also on the environment (Aerts et al., 1999; Fonseca et al., 2023; Kelln et al., 2021; Kumar et al., 2014; Mergeduš et al., 2018; Naumann et al., 2017; Tedeshi et al., 2014; Yanza et al., 2024). In higher concentrations, tannins have rather harmful effects on feed intake, rumen microflora, use of nutrients received and overall their production.

MATERIAL AND METHODS

For our study we used 6 products containing tannins, representing both groups of tannins in the same concentration. Hydrolyzable tannins presented by the products: FermiTan Harvest Hill (ellagic tannin from selected wood species), TOP House (fine tannin from ground opal wood from French oak) and Tan Clar (pure hydrolyzed ellagic tannin) (Picture 1).

Picture 1. Selected products containing hydrolyzabled tannins

Condensed tannins presented by: Tannin MOX (condensed tannin of the Quebraco tree), Tannin FC (tannin containing catechin, used to support anthocyanin and polymerize into stable polymers) and VITANIL B (tannin from gall alcohol) (Picture 2).

Picture 2. Selected products containing condensed tannins

The study of apparent digestibility using the ANKOM Gas Production System was carried out according to Van Soest (1970). As inoculum we used a mixture of rumen fluids from 3 cannulated cows. Ruminal fluid was collected 2 hours after morning feeding into pre-tempered thermoses. To each 250 ml glass bottle with septum, was prepared 20 ml of inoculum, 80 ml of final buffer and a weighed 1 g sample of the standard together with a 5% tannin sample. After removing oxygen, the glass bottles were incubated in a thermostat for 48 hours at 39 °C. The temperature and pressure of the gas in the bottles were monitored.

Data has been processed by Microsoft Excel.

RESULTS AND DISCUSSION

From the Table 1 is evident that both types of tannins increased the amount of gas produced compared to the control sample containing only the standard. Condensed tannins increased gas production more than hydrolyzed tannins.

Table 1. Amount of *in vitro* gas production in individual products

		Amount of gas (ml)	Percentage differences
Group 1	Control	1319.42	
	Fermitan	1350.21	increase of 2.33 %
	TOP House	1420.58	increase of 7.67 %
	Tan Clare	1345.81	increase of 2 %
Group 2	Control	1402.99	
	MOX	1464.56	increase of 4.39 %
	FC	1499.75	increase of 6.9 %
	VITANIL B	1627.29	increase of 15.99 %

The results of our study do not agree with the results of studies conducted by other authors. A moderate concentration of tannins in animal food destabilizes protein foams, which makes them safe against flatulence (Kumar et al. 2014, Mergeduš et al. 2019). According to Aerts et al. (1999) it is well documented that bloat occurs when grazing ruminants consume large amounts of legumes (alfalfa or clover for example). Gases produced in the rumen during fermentation cannot be released in the normal way because they are trapped in the persistent foam caused by the rapid release of soluble proteins during chewing and ruminal degradation. However, when these animals graze on legumes containing condensed tannins (such as Onobrychis viciifolia), entrapment does not occur and gases can escape from the digestive tract.

In contrast, Getachew et al. (2008) point out that the addition of tannin had no effect on the amount and rate of gas production but significantly reduced the concentration of ammonia nitrogen. Fagundes et al. (2020)

points to digestibility when, in their study, with the addition of condensed tannins, there was no reduction in nutrient digestibility, nor a deterioration in fermentation parameters (certain types of archaea were suppressed, but overall the number of bacteria in the rumen increased).

For our study, the rumen fluid of dry-resistant crossbred meat breed cows commonly raised in the Czech Republic was used as inoculum. Bueno et al. (2020) in their study showed on the different results of gas production according to the use of different types of inoculum (Holstein dairy cows, Nelore beef cattle, Mediterranean water buffalo, Santa Inês sheep and Saanen goats). Therefore, the results of our study may not be applicable to all ruminants.

CONCLUSION

The study carried out by us opens another field for the study of suitable preparations but also individual concentrations, or combinations of preparations not only to reduce the total volume of gas produced, but also to distinguish individual gases produced and possible reduction of both total and ammonia emissions.

However, for a global solution, it will be necessary to conduct a study not only on cattle as representatives of ruminants, but to conduct a study on several species of ruminants.

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THE INFLUENCE OF HUMATES ON THE CARCAS AND BIOCHEMICAL PARAMETER OF QUAILS

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ABSTRACT

Humic substances (HS) are organic compounds resulting from the decomposition of plant and animal remains. Positive effects of humic substances in the treatment of a whole range of diseases such as inflammation of various organs, gastrointestinal diseases, poisoning, cancer, diabetes, infectious diseases or anemia have been known for centuries. The aim of the work was to determine the influence of humic substances as an organic supplement in a feed mixture on the quality of the carcass and the sensory properties of quail meat. Total 200 Japanese quails were included in the study, divided into four groups (n= 50/group) with two replicates: C (control group, standard diet without additives), group H0.5 (experimental group 1, receiving standard feed + 0.5% HS), group H1.0 (experimental group 2, received standard feed + 1% HS) and group H1.5 (experimental group 3, received standard feed + 1,5% HS). The results of the study show the effect of the addition of

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HS in the H1.0 group of quails, which recorded a lower final weight and carcass yield (P<0.05) after 50 days of fattening. In addition, HS supplementation positively affected lipid parameters, with a lower total fat content in the meat of quails supplemented with 1.0% HS.

Keywords: quail, humic substances, meat, carcass, sensory analyze

INTRODUCTION

Meat plays an important role in the human diet due to the content of proteins, fatty acids, minerals or vitamins. The consumption of poultry meat is becoming more and more popular on a global scale. According to current forecasts, by 2030, poultry will account for 41% of all meat protein intake in the human diet (OECD and FAO, 2021). Modern poultry farming focuses mainly on improving productivity, animal health and the production of healthy meat for consumers (Long et al., 2020). Thanks to intensive farming methods, poultry meat is cheaper and more available, but it brings many problems in farming in the form of increased stress, reduced immunity and infectious diseases. The most frequently reported zoonotic alimentary infections in the EU are mainly caused by agents from the genera Campylobacter and Salmonella, where the primary source of infection is poultry meat and eggs (EFSA, 2023). Humic substances (HS) are considered a suitable alternative to replace antimicrobial substances, due to their number of positive effects on production, immunity and animal health. They have antibacterial, antiviral and antimicrobial effects, thus improving the economy and ecology of animal production (Yasar et al., 2002)

Humic substances are natural organic compounds found in soil, coal, water and other sources, which are formed during the decomposition of plant and animal remains by the action of organisms and abiotic

environmental factors (Marcinčák et al., 2023). They are composed of humic acid, humus, ulmic acid, fulvic acid and minerals (Arif et al., 2019).

Their positive effects in the treatment of a whole range of diseases such as inflammation of various organs, gastrointestinal diseases, poisoning, cancer, diabetes, infectious diseases or anemia have been known for centuries. Modern research mainly focuses on humic acids for their multifaceted positive effects on the organism at the molecular level in both humans and animals (Mudroňová et al., 2020).

HS are characterized by immunostimulating, anti-inflammatory and antiviral properties thanks to the ability to form a protective film on the intestinal mucosa against infections and toxins (Gálik et al., 2023).

Humic substances with a high proportion of humic acids (more than 40%) have been classified by the European Commission as feed materials usable in animal nutrition since 2013 (Marcinčák et al., 2023). Therefore, the aim of study was to determine the influence of HS as an organic supplement in a feed mixture on the quality of the carcass and the sensory properties of quail meat.

MATERIAL AND METHODS

Total 200 quails were used in this study. The quails were housed under the same conditions in cages (150 × 150 × 200 cm), with 50 quails per cage. All quails were fed standard mixture *ad libitum*. They had unlimited access to drinking water. At the beginning of the experiment, the animals were divided into four groups (n= 50/group) with two replicates in each group: C (control group, standard diet without additives), group H0.5 (experimental group 1, received standard feed + 0.5% Humac Natur AFM; Tab. 1), group H1.0 (experimental group 2,

received standard feed + 1% Humac Natur AFM), group H1.5 (experimental group 3, received standard feed + 1,5% Humac Natur AFM).

Table 1. Characteristics of preparations with humic substances

Parameter	**Humic substances***
Particle size	max. 100 µm
pH	5,8
Humidity	max. 15 %
Humic acids	min. 65% in dry matter
Fulvic acids	min. 5 % in dry matter

Note: Humic substances - HUMAC® Natur AFM (Humac s.r.o., Košice, Slovakia)*

In the control group, a crushed basal diet without any additives for growing quails. Feed mixture contain maize, soybean meal extracted, sunflower meal extracted, calcium carbonate, wheat stillage, corn germ, pea hulls, rapeseed meal extracted, NaCl. From nutritional supplements substances vitamin A (min. 10000 IU), vitamin D3 (min. 2500 IU), iron (40 mg), iodine (1.0 mg), copper (15.0 mg), manganese (60 mg), zinc (70 mg), selenium (0.3mg), lysine (2348.2mg). During the fattening period (from the first to the fiftieth day day of age), the second, third and fourth experimental groups were fed basal diet with 0.5%, 1% and 1.5% addition of humic substances (Humac® Natur AFM, Košice, SR; 65% proportion of HS in dry matter).

On the twenty-fifth day of the experiment, part of the quails were slaughtered painlessly in accordance with the applicable legislation. On the fiftieth day another part of the quails were slaughtered. The quails were slaughtered by cutting the *jugular vein* and blood was taken for further examination.

Carcass weight was recorded after removing the head, skin and feathers, viscera and distal parts of the limbs. Carcass yield was determined as the ratio of live weight before slaughtering and carcass weight. The chemical analysis of the basic muscle components was determined from breast muscle samples according to Marcinčák et al. (2023). A muscle sample was taken immediately after slaughtering, wrapped in foil and stored at -80°C until sample analysis. The water content was determined by drying in a dryer at 105°C (Hudák et al., 2021). Proteins were determined on a Kjeltec Auto analyzer, type 1030 (Hanon, Jinan, China). Fats in ground samples with petroleum ether were determined on a Soxhlet apparatus (LTHS 500, Brnenská Druteva, Brno, Czech Republic) according to Semjon et. al. (2020). Muscle pH was analyzed with an InoLab digital pH meter (Wissenschaftlich-Technische Werkstatten, Weilheim, Germany).

The determined values were evaluated by one-factor analysis of variance ANOVA with a significance level of $p < 0.05$. Significance of differences was confirmed using Tukey's multiple comparison test. The results in the tables are presented as mean values (X) and standard deviation (SD).

RESULTS AND DISCUSSION

Table 2 shows the values of live weight, carcass weight and breast muscle weight on 25th day. When evaluating the live weight, we recorded a slightly higher final weight ($P > 0.05$) on day 25 in the experimental group H1.5 compared to the control group. Higher weight in experimental groups H1.5 had a positive effect on higher carcass weight ($P < 0.05$) in comparing with control group.

Table 2. Effect of supplementation on live weight and slaughter parameters of quail on 25th day

Group	Live weight (LW)		Carcass weight (CW)		Carcass yield	Pectoral muscle weight		
	g		g		% LW	G		% CW
	x	sd	x	sd	x	x	sd	x
H0.5	178.5	3.2	107.8	3.5	60.4	29.3	1.6	27.2
H1.0	174.0	12.3	108.8	5.9	62.5	29.3	1.6	26.9
H1.5	185.0	21.8	111.7[a]	10.8	60.4	30.7	3.7	27.5
Control	168.0	13.8	98.0[b]	8.1	58.3	28.7	3.1	29.2

Note: H0.5 – quail group supplemented with 0.5% HS; H1.0 – group of quails supplemented with 1.5% HS; H1.5 - group of quails supplemented with 1.5% HS; a,b - different letters in the column at the level of significance p<0.05.

Table 3 shows the values of live weight, carcass weight and breast muscle weight on 50th day. In the control group H0.5 only a slightly increased value of live weight, carcass weight and carcass yield was recorded. Contrary, supplemented group H1.0 with 1.0% of HS had a lower value of live weight, carcass weight ($P < 0.05$) compared to the control group.

There are many studies focused on the effect of the addition of HS to the feed during the fattening period, but the results are controversial. Some researchers reported a significant effect on the broilers' live weight, weight gain, carcass yield and feed conversion ratio (Karaoglu et al. 2004; Pistová et al., 2016). In contrast, many other studies noted only non-significant effects on the above-mentioned parameters during the fattening period (Yıldız et al., 2013; Nagaraju et al., 2014).

Our finding is similar to the conclusion made by Nagaraju et al. (2014) who reported that broiler chickens fed a diet containing 0.75% and 1%

of HS showed a slightly reduced body weight (P > 0.05) in comparison to the control.

Table 3. Effect of supplementation on live weight and slaughter parameters of quail on 50th day

Group	Live weight (LW)		Carcass weight (CW)		Carcass yield	Pectoral muscle weight		
	g		g		% LW	g		% CW
	x	sd	x	sd	x	x	sd	x
H0.5	299.0	19.6	187.5	12.8	62.7	55.6	5,5	29.7
H1.0	262.0[a]	25.4	162.0[a]	18.2	61.8	49.5	7.6	30.6
H1.5	295.6	25.4	177.0	15.5	59.9	53.5	8.5	30.2
Control	298.6[b]	20.2	184.0[b]	9.7	61.6	56.8	3.7	30.9

Note: H0.5 – quail group supplemented with 0.5% HS; H1.0 – group of quails supplemented with 1.5% HS; H1.5 - group of quails supplemented with 1.5% HS; a,b - different letters in the column at the level of significance p<0.05.

Table 4. Comparison of selected quail meat indicators on 25th day

Parameter	H0,5		H1,0		H1,5		Control	
	x	sd	x	sd	x	sd	x	sd
Water	70.7	0.4	70.5	0.7	71.6	0.5	71.1	0.3
Fat	1.8	0.3	1.3[a]	0.4	1.4	0.3	1.7b	0.2
Ash	1.1	0.2	1.2	0.2	1.6	0.1	1.2	0.2
Protein	24.4	0.3	24.5	0.4	25.4	0.2	24.6	0.4
pH	5.9	0.1	5.9	0.1	5.7	0.1	5.9	0.1

Note: H0.5 – quail group supplemented with 0.5% HS; H1.0 – group of quails supplemented with 1.5% HS; H1.5 - group of quails supplemented with 1.5% HS; a,b - different letters are in a row at the significance level of p<0.05.

The water content in the meat on 25th day was slightly increased (P > 0.05) in the experimental group H1.5 compared to the control group. In experimental groups H0.5 and H1.0, the water content was slightly reduced (P > 0.05) compared to the control group.

The fat content was lower ($P < 0.05$) in H1.0 group compared to the control group. The protein content was not significantly changed in the all supplemented groups compared to the control group (Tab. 4).

Table 5 summarizes the results of selected quail meat indicators on 50th day of experiment. The water content was slightly reduced ($P > 0.05$) in all experimental groups compared to the control group. Significant shifts were occurred in the fat and protein content. In H1.0 and H1.5 groups, the fat content was lower ($P < 0.05$) compared to the control group. In the H1.0 group, there was a difference in lower protein content ($P < 0.05$) compared to the control group.

Table 5. Comparison of selected quail meat indicators on 50th day.

Parameter	H0,5		H1,0		H1,5		Control	
	x	sd	x	sd	x	sd	x	sd
Water	68.0	0.2	67.5	0.3	68.5	0.3	68.7	0.2
Fat	5.5	0.2	3.8a	0.2	4.0^{a}	0.2	5.6^{b}	0.3
Ash	0.6	0.2	0.7	0.2	1.2	0.1	1.1	0.2
Protein	24.5	0.2	23.9^{a}	0.2	25.1	0.3	25.2^{b}	0.2
pH	6.1	0.1	6.0	0.1	5.8	0.1	5.9	0.1

Note: H0.5 – quail group supplemented with 0.5% HS; H1.0 – group of quails supplemented with 1.5% HS; H1.5 - group of quails supplemented with 1.5% HS; a,b - different letters are in a row at the significance level of p<0.05.

CONCLUSION

Based on the effect of the addition of humic substances to the ration of Japanese quails, we can conclude that a lower final weight and carcass yield was recorded in the group of quails H1.0 supplemented with 1.0% addition of HS. In addition, HS supplementation positively affected lipid parameters, with a lower total fat content in the meat of quails supplemented with 1.0% HS. The current results allow us to assume

that the introduction of humic substances into feed mixtures for quail could improve meat quality indicators, especially when reducing the fat content.

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DIETARY EFFECT OF WHEAT VARIETY AND FAT SOURCE ON PERFORMANCE AND EGG QUALITY CHARACTERISTICS OF LAYING HENS

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ABSTRACT

The importance of keeping laying hens lies in egg production. In terms of quality, the most important feature for the simplistic consumer is the colour of the egg yolk. For this reason, either natural or synthetic carotenoids are often added to mixed feed for laying hens. The aim of this experiment was to evaluate the dietary effect of PEXESO wheat with increased carotenoid concentrations in hen diets on performance and egg quality characteristics. Two hundred forty 42-week-old Lohmann Brown hens were divided into 4 treatment groups according to the wheat variety (TERCIE × PEXESO) and fat source (rapeseed oil × pork lard) in the diet. PEXESO wheat in the diet decreased daily feed

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intake ($P < 0.001$). Higher values of the egg yolk colour ($P < 0.001$) and strength of the eggshell ($P < 0.001$) were found in hens that were fed PEXESO wheat diets. The lutein ($P < 0.001$ and $P = 0.001$) and zeaxanthin ($P < 0.001$ and $P = 0.001$) contents in the egg yolks and the oxidative stability of fresh ($P < 0.001$ and $P = 0.008$) and stored ($P = 0.050$ and $P = 0.021$) eggs were positively influenced by PEXESO wheat and pork lard. The diet with PEXESO wheat and rapeseed oil increased the concentration of α-tocopherol ($P = 0.008$) and γ-tocopherol ($P = 0.012$) in the egg yolk. In conclusion, PEXESO wheat increased the retention of biologically active substances, which was subsequently reflected in the performance and quality of the products, i.e. eggs.

Keywords: poultry nutrition; carotenoids; tocopherols; egg quality; yolk colour

INTRODUCTION

Livestock farming is an important part of the agricultural industry, and domestic poultry farming, as a basic animal husbandry industry, plays an important role in promoting economic development, ensuring market supply, and increasing farmers' income (Ruiz-Garcia et al., 2009; Nkukwana, 2018). The use of poultry products (meat and especially eggs) is an effective way for humans to obtain animal protein in daily life (Scholten et al., 2013). The importance of keeping laying hens lies in egg production. Eggs are one of the most nutritious foods. In terms of quality, the most important feature for the simplistic consumer is the colour of the egg yolk (Berkhoff et al., 2020). Preferences for yolk richness vary considerably from country to country according to consumer requirements (Hernandez et al., 2000).

Also, the yolk colour of farm eggs has always been rated higher than that of industrial eggs. This was likely owing to the fact that industrial eggs had a pale-yellow yolk, while the yolk of farm eggs was a more intense yellow with some orange tones (Titcomb et al. 2019). For this reason, either natural or now exclusively synthetic carotenoids are often added to mixed feed for laying hens.

Carotenoids are one of the widespread and ubiquitous lipid-soluble pigments that produce a wide range of colours which are universally found in various plants, micro-algae, bacteria, yeast, mould and fungi (Nabi et al., 2020). They are categorized into two groups according to their chemical structure, in particular the presence or absence of oxygen in the molecule, and according to their functional properties; carotenes, including α-carotene, β-carotene and lycopene and xanthophylls, such as lutein and zeaxanthin (Saini et al., 2015). The animals and birds are incapable of synthesize carotenoids in vivo, so these pigments must be supplemented through diet (O'Byrne & Blaner, 2013). Dietary addition of carotenoid pigments to poultry diet improves the colour of skin, flesh and especially the egg yolk (Carvalho et al., 2009). Grazing poultry obtain xanthophylls from pasture vegetation. In large farms without the access to grazing for the animals, synthetic xanthophylls are added to poultry mixed feed for economic reasons. Xanthophylls of natural origin are gaining popularity among consumers who refuse the use of synthetic substances in livestock nutrition. On this basis, the number of people interested in what have become known as functional foods is expanding.

Recently, carotenoid pigments are of interest to the food and feed industry, researchers, nutritionists and food and feed scientists due to their multiple bioactive and health-promoting benefits including

antioxidant, anti-inflammatory, immunomodulatory and also playing an important role against several diseases (Rao & Rao, 2007; Arain et al., 2018). Dietary supplementation of carotenoids to the poultry birds improves the production performance and health of birds, but also enhance the quality of eggs and meat (Langi et al. 2018). Several studies have suggested that carotenoids reduce the heat and oxidative stress in pre-hatched and post-hatched birds (generally in the host body) through different mechanisms, including quench free radicals, activating antioxidant enzymes and inhibiting the signalling pathways (Sahin et al., 2011; Arain et al., 2018; Langi et al., 2018). Xanthophylls (lutein and zeaxanthin) supplementation in hens' diets has been shown to increase antioxidant capacity and decrease malondialdehyde (MDA) production in the liver and serum (Gao et al., 2013).

Many studies have examined the effects of different sources of lutein and zeaxanthin (e.g. alfalfa concentrate, tomato powder, marigold extract, coloured carrots etc.) on poultry metabolism and the enrichment of eggs and meat with these xanthophylls. Currently tested natural carotenoids are too expensive and therefore are not economically feasible for practical applications. The most commonly used organic source of both xanthophylls is the extract from the flowers of marigold (Tagetes erecta), which is also economically acceptable (Skřivan et al., 2016). Biofortified arable crops can be a special alternative to natural sources of carotenoids. Biofortification is a very cost-effective process that increases the concentration of essential nutrients in crops through agronomic intervention or genetic selection (White & Broadley, 2005). Wheat (Triticum aestivum), as one of the most widely grown cereal crops, accounts for more than 1/4 of field food crop production and nearly 2/3 of the daily energy intake

of the population in several developing countries. Related to this is the fact that the nutritional value of wheat has a significant impact on human health (Wang et al., 2011). Biofortification of wheat with mineral micronutrients has been successful and thus far has the most widespread use. Examples of successful biofortification in terms of carotenoid content are Golden Rice producing β-carotene (Paine et al., 2005) or transgenic Multivitamin Corn providing β-carotene, lycopene, lutein, zeaxanthin, ascorbic acid and folate (Naqvi et al., 2009).

In this experiment, the biofortified wheat variety PEXESO with increased concentration of lutein and zeaxanthin was compared with the common wheat variety TERCIE in the presence of two dietary sources of fat (rapeseed oil × pork lard) with contrasting fatty acid content in mixed feed for laying hens. Vegetable oils and animal fats, which are usually less expensive but may adversely affect the fatty acid composition of animal products, are added to compound feed to balance the metabolic energy requirements of poultry. Therefore, two dietary sources of fat and two varieties of wheat were assessed in this experiment to answer questions about their relationships with metabolism, performance and quality of eggs produced.

MATERIAL AND METHODS

Animals, Husbandry, Diets and Performance

The experiment was performed with two hundred forty Lohmann Brown laying hens (Enterprise for egg production, Kosičky, Czech Republic) at the age of 42 weeks that were housed in enriched cages (10 hens per cage). The hens were assigned to 4 treatments with 6 replicates according to the wheat variety (TERCIE × PEXESO) and fat source (rapeseed oil × pork lard) in the mixed feed. All mixed feeds

in the experiment contained 61% of one or other wheat variety and 3% of one or other fat source. The content of lutein and zeaxanthin in wheat variety TERCIE was 0.45 mg/kg and 0.22 mg/kg, respectively. The content of lutein and zeaxanthin in wheat variety PEXESO was 1.14 mg/kg and 0.79 mg/kg, respectively. Both wheat varieties were bred in the Czech Republic by the company Selgen, a.s. (Prague, Czech Republic). In order to achieve the normal metabolizable energy requirement, it is necessary to add fat to poultry mixed feed. In the experiment, two fat sources with contrasting fatty acids proportions were used. Mixed feeds for hens were mixed in the state enterprise International Poultry Testing (Ústrašice, Czech Republic). Feed and fresh water were provided ad libitum. The technological and microclimatic conditions of the housing corresponded to the management guide of the given Lohmann Brown hybrid. The length of the light day was 16 hours (2.30 – 18.30). The number of hens and their health status were checked daily during the experiment. Hen – day egg production was recorded daily, and feed intake was calculated weekly, both on a per-cage basis. The experiment lasted 10 weeks. The eggs for chemical analyses of the yolk (carotenoid ad vitamin contents and oxidative stability of fat) were collected in the 50th week of age of the hens. Three eggs each replicate were homogenized to form one sample (n = 6). The analyses were carried out in the laboratories of Institute of Animal Science (Prague – Uhříněves, Czech Republic).

Physical Analysis of Eggs

A whole day of egg production (50th week of age of the hens) was analysed for the determination of physical parameters. A total of 438 eggs were analysed. The values were averaged per cage (n = 6). The

yolk colour was evaluated visually with a DSM yolk colour fan (DSM Nutritional Products, Basel, Switzerland) from 1 to 15 and further expressed as a* and b* values using the Minolta SpectraMagicTM NX (Konica Minolta Sensing, Inc., Osaka, Japan). The value a* correspond to redness (– 100 = green, 100 = red) and the value b* to yellowness (– 100 = blue, 100 = yellow). The shell breaking strength was evaluated on the vertical axis using an Instron 3360 apparatus (Instron, Norwood, MA, USA).

Chemical Analyses

The egg yolks were lyophilised before the analytical determination of carotenoids and vitamins. The concentrations of carotenoids, lutein and zeaxanthin, were analysed by a high-performance liquid chromatography (HPLC) system (VP series; Shimadzu, Kyoto, Japan) according to a modified method of Froescheis et al. (2000). The HPLC instrument was equipped with a diode array detector. A Kinetex C18 column (100 × 4.6 mm; 2.6 µm) supplied by Phenomenex (Torrace, CA, USA) was used. A gradient system was established, where eluent A was acetonitrile:water:ethyl acetate (88:10:2) and eluent B was acetonitrile:water:ethyl acetate (88:0:15). The carotenoid content was expressed in mg per kg of egg yolk dry matter. The retention of carotenoids in the yolk was calculated using the following formula:

$$R = \frac{A \times B \times C}{D}$$

R = retention (%)

A = egg mass (g/hen/day)

B = proportion of yolk (%)

C = carotenoid content in fresh egg yolk (mg/g)

D = carotenoid intake (mg/hen/day)

The α-tocopherol and γ-tocopherol contents were evaluated in accordance with the European standards EN 12822-1 (2000) and EN 12823-1 (2000), respectively, by a HPLC system (VP series; Shimadzu, Kyoto, Japan) equipped with diode array detector. Samples were subjected to alkaline saponification with 60% potassium hydroxide (KOH) followed by appropriate diethyl ether extraction. The lipid peroxidation levels in the yolk of fresh eggs and eggs stored on paper trays for 28 days at a temperature of 18 °C and relative humidity of 50 – 55 % were measured using the modified method of Czauderna et al. (2011). A Phenomenex C18 column (Synergi 2.5 µm, Hydro-RP, 100 Å, 100 mm × 3 mm, Torrace, CA, USA) was used for chromatographic analysis (Shimadzu HPLC system (VP series; Shimadzu, Kyoto, Japan) equipped with a diode array detector). Solvent A consisted of water-acetonitrile (95:5, v/v), and solvent B consisted of acetonitrile. The lipid oxidative stability was expressed in mg of malondialdehyde (MDA) per kg of eggs.

Statistical Analyses

The obtained experimental results were analysed by two-way analysis of variance (ANOVA) with the general linear model (GLM) procedure using SAS software (SAS v 9.3 Institute, 2003). The main effects were the wheat variety, the fat source and the interaction between these two factors (wheat*fat). The cage was the experimental unit (n = 6). The results in the Table 1 are presented as the mean, standard error of the mean (SEM) and probability (P) value. A statistical significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

The selected results of dietary effect of wheat variety and fat source on performance and egg quality characteristics of laying hens are summarized and presented in Table 1.

There were no significant differences between groups in the hen – day egg production. Hen – day egg production was slightly higher only in the combination of PEXESO wheat with pork lard (94.70 %) as a source of fat in the mixed feed. Significant differences in egg mass production were due to the fat source in the diet ($P < 0.001$). Higher egg mass production ($P = 0.007$) was found in hens mixed feed with PEXESO wheat in combination with rapeseed oil (59.03 g/hen/day) and pork lard (58.56 g/hen/day) compared to mixed feed with TERCIE and rapeseed oil (58.00 g/hen/day). The main result within the performance indicators in favour of PEXESO wheat was lower daily feed intake ($P < 0.001$). Dietary supplementation with carotenoids improves the production performance and health of poultry birds (Nabi et al., 2020). In addition, carotenoids have antioxidant, anti-inflammatory and immunomodulatory effects, which may also affect the performance of birds (Arain et al., 2018). The positive effect of carotenoids on the performance of broiler chickens is also evident, for example, in a study by Fathi et al. (2022). PEXESO wheat positively increased the final egg yolk colour ($P < 0.001$) according to the DSM yolk colour fan (PEXESO*RO 3.48 DSM; PEXESO*PL 3.52 DSM). The parameters of redness (a*) and yellowness (b*) were influenced by wheat variety ($P < 0.001$) and wheat*fat interaction (a* $P = 0.030$; b* $P = 0.007$), but not by the fat source itself. In particular,

PEXESO wheat in hens mixed feed significantly increased the parameter of yellowness (b*), both in combination with rapeseed oil (26.8) and pork lard (28.7) as a source of fat in the diet. The colour of the egg yolk varies considerably in terms of individual preferences across the European Union (Hernandez et al., 2000). Central European consumers prefer colouring between DSM levels 10 and 14 (Galobart et al., 2004), and therefore the inclusion of PEXESO wheat in the wheat-soybean type of mixed feeds, which, among other things, are common in Europe, will not provide optimal colouration of egg yolk according to the requirements of many consumers. In this case, it is necessary to add another source of colour pigments, such as marigold. The concentration of lutein and zeaxanthin in commercial marigold-based products are many times higher (Skřivan et al., 2016) than in the biofortified PEXESO wheat used in this experiment. The ideal source of natural carotenoids is pasture vegetation. In a study by Skřivan & Englmaierová (2014) it was found that the access of hens to natural grazing together with sequential feeding of wheat increased the concentration of carotenoids in the yolk and significantly raised its colour score to 10.3 according to the Roche Yolk Colour Fan (RYCF). PEXESO wheat increased strength of the eggshell ($P < 0.001$), which is the main technological characteristics of the egg, mainly in terms of production economics. Lutein ($P < 0.001$) and zeaxanthin ($P < 0.001$) levels in egg yolk were consistent with their several-fold higher concentrations in the diets with PEXESO wheat. The deposition of the carotenoids in the yolk was also affected by the source of the fat ($P = 0.001$) in the diet. Higher levels of carotenoids were measured in the case of hens fed pork lard. The higher deposition of lutein and zeaxanthin in the egg yolk was due to the higher dietary concentration

of both xanthophylls in PEXESO wheat and was also supported by their higher retention. The retention of carotenoids, calculated according to above formula, was affected by wheat variety ($P < 0.001$) and fat source ($P < 0.001$). Also, the wheat*fat interaction was significant for the retention of lutein ($P < 0.001$) and zeaxanthin ($P = 0.002$). The highest retention of lutein was observed by PEXESO wheat in combination with both rapeseed oil (55.6 %) and pork lard (55.8 %). On the other hand, the retention of zeaxanthin was highest only in PEXESO wheat with pork lard (69.4 %) compared to the other groups. Lipids have a positive effect on the absorption of lipophilic carotenoids. Dietary fats rich in saturated fatty acids lead to a higher availability of lutein and zeaxanthin compared with fats rich in monounsaturated and polyunsaturated fatty acids (Gleize et al., 2013), which is consistent with the findings of this experiment. The hens mixed feed with PEXESO wheat and rapeseed oil increased the concentration of α-tocopherol (174.1 mg/kg DM (dry matter); $P = 0.008$) and γ-tocopherol (22.98 mg/kg DM; $P = 0.012$) in the egg yolk. The significant increase in tocopherol concentrations was most likely due to rapeseed oil, which is a rich source of these vitamins. The availability of lipophilic vitamins may be affected by the unsaturation of dietary lipids. Prévéraud et al. (2015) showed that a diet containing flaxseed oil resulted in better α-tocopherol bioavailability in adult roosters than a diet containing hydrogenated coconut oil. The increased concentration of α-tocopherol and γ-tocopherol may also be related to the antioxidant properties of carotenoids, which protect these lipophilic compounds against oxidative degradation in the upper gastrointestinal tract (Mortensen et al., 2001). PEXESO wheat, used in this experiment, significantly reduced the process of oxidation of yolk lipids. The process of reducing

the oxidation was undoubtedly contributed by lutein and zeaxanthin, because these natural lipophilic pigments are also powerful antioxidants. The better shelf life of the eggs produced is confirmed by the fact that the carotenoids absorbed from the feed are preferentially deposited in the eggs by the hens. The increased oxidative stability of egg yolk due to lutein and zeaxanthin has also been demonstrated by studies (Englmaierová et al., 2013; Skřivan et al., 2016; Kljak et al., 2021). The formation of MDA oxidation products in fresh and stored eggs decreased in hens fed mixed feed containing PEXESO wheat ($P < 0.001$ and $P = 0.050$) and pork lard ($P = 0.008$ and $P = 0.021$). As measured by the decrease in MDA production, the increased effect of egg storability resulting from lard compared to rapeseed oil in the diet was due to higher proportion of saturated fatty acids. In addition, a significant wheat*fat interaction was found for MDA content in fresh eggs ($P = 0.041$). Increased lipid oxidation in fresh egg yolks (0.448 mg MDA/kg eggs) was observed in the TERCIE wheat and rapeseed oil diet group compared to the other groups. This was due to the lower content of natural carotenoids in TERCIE wheat and probably also to the easier oxidation of the more unsaturated rapeseed oil compared to saturated pork lard.

CONCLUSION

Increasing the content of natural carotenoids in mixed feed for laying hens is highly desirable, both in terms of promoting the health of the farmed animals themselves and the production of quality animal products that are beneficial to human health. The wheat variety PEXESO, with an increased content of lutein and zeaxanthin, included in the hen's diet positively influenced the performance and some

quality parameters of the eggs produced. This was probably related to the higher retention of biologically active substances. However, the colouring effect of the wheat variety PEXESO was insufficient, and the resulting colour of the egg yolk would clearly not satisfy consumer requirements. This wheat can be recommended in combination with rapeseed oil as a source of fat in the diet and also with the addition of another source of natural carotenoids (e.g. from the marigold extract or natural pasture), which together with this wheat will provide the consumer with a quality product. Particularly in terms of preference and optimum colouring of the egg yolk.

Table 1. Selected performance characteristics of laying hens, physical characteristics of egg quality, and vitamin, carotenoid and MDA concentrations in egg yolks

Group	I	II	III	IV	SEM	Probability		
Wheat variety	TERCIE		PEXESO					
Fat source	RO	PL	RO	PL		Wheat	Fat	Wheat*fat
Hen – day egg production (%)	93.48	93.93	93.77	94.70	0.268	NS	NS	NS
Egg mass (g/hen/day)	58.00^{b}	58.15^{ab}	59.03^{a}	58.56^{a}	0.184	NS	<0.001	0.007
Feed intake (g/hen/day)	115.7	114.7	111.6	111.2	0.382	<0.001	NS	NS
DSM yolk color fan	1.62	1.61	3.48	3.52	0.051	<0.001	NS	NS
CIE a* (redness)	-1.57^{c}	-1.59^{c}	-0.93^{b}	-0.80^{a}	0.025	<0.001	NS	0.030
CIE b* (yellowness)	21.9^{c}	21.2^{c}	26.8^{b}	28.7^{a}	0.29	<0.001	NS	0.007
Shell breaking strength (g/cm^2)	4504	4439	4757	4659	33.1	<0.001	NS	NS
Lutein in yolk (mg/kg DM)	1.69	1.91	5.49	6.04	0.368	<0.001	0.001	NS
Lutein retention in yolk (%)	28.5^{c}	39.5^{b}	55.6^{a}	55.8^{a}	2.48	<0.001	<0.001	<0.001
Zeaxanthin in yolk (mg/kg DM)	0.94	1.53	3.98	4.25	0.2677	<0.001	0.001	NS
Zeaxanthin retention in yolk (%)	28.2^{d}	49.6^{c}	63.1^{b}	69.4^{a}	3.45	<0.001	<0.001	0.002
α-tocopherol (mg/kg DM)	156.3^{b}	136.0^{c}	174.1^{a}	141.2^{c}	3.28	<0.001	<0.001	0.008
γ-tocopherol (mg/kg DM)	17.54^{b}	10.13^{c}	22.98^{a}	8.31^{c}	1.104	<0.001	<0.001	0.012
MDA 0 D (mg/kg)	0.448^{a}	0.375^{b}	0.347^{b}	0.337^{b}	0.0109	<0.001	0.008	0.041
MDA 28 D (mg/kg)	0.458	0.416	0.425	0.363	0.0098	0.050	0.021	NS

RO = rapeseed oil; PL = pork lard; SEM = standard error of the mean; ^{a,b,c,d} means in the same row with different superscripts differ significantly; NS = not significant; CIE = International Commission on Illumination; DM = dry matter; MDA = malondialdehyde; D = day

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IMPORTANCE OF PREBIOTIC, PROBIOTIC AND PHYTOBIOTIC FEED SUPPLEMENTS IN CALF NUTRITION

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ABSTRACT

This study is aimed at evaluating the effects of feed additives on health status and live weight gain in calves.

In the experiment, calves were divided into two groups. The first group, the experimental group, was administered RumiForm Digest and RumiForm Digest Yeast+. The second group, the control group was not exposed to any feed additives.

The birth weight of calves in both groups was at the same average values 38 kg. The average weight of the calves at 7 days of age was 46 kg in the control group and the average weight of the experimental group was measured to be 48 kg. A more significant difference in average weights was observed at 90 days after birth. In the control group, the average weight was 137 kg, while in the experimental group the average weight was 152 kg.

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Diarrhoea occurred in both the control and experimental groups. The highest incidence of diarrhoea was recorded in the control group. Diarrhoea disease was recorded in 11 % of the 202 calves. Mortality occurred in both the experimental and control groups. The overall mortality rate was 3.5 %.

In this study, a beneficial effect of probiotic feed supplements on growth was demonstrated. At 7 days after birth and on the 90th day after birth, the p-value < 0.01. The effect of probiotic feed supplements on health status was not demonstrated in this experiment. The p-value was > 0.05 for the incidence of diarrhoea and for the comparison of mortality.

From the data obtained, it can be confirmed that the administration of probiotic feed supplements has a positive effect on weight gain. But a positive effect on health status could not be demonstrated.

Keywords: calves; nutrition; probiotics; prebiotics; phytobiotics; health status

INTRODUCTION

Nutrition is a complex process of interrelated activities (Gaislerová et al., 2019). Adherence to the principles of this complex process is crucial to ensure healthy and resilient individuals who are able to withstand the pathogenic microorganisms that constantly surround them (Rincker et al., 2011; Gaislerová et al., 2019).

In calf rearing, diarrheal diseases are the most common and serious health problem (Cho et al., 2014; Klein-Jobstl et al., 2014). These diseases are the leading cause of mortality in newborn calves (Maier et al., 2022). It is a multifactorial disease caused by a combination of infectious and non-infectious factors (Sung-Hwan

et al., 2019). Diarrhoeal diseases are caused by a variety of factors. From dietary and husbandry to infections caused by different types of enteric pathogens (Illek, 2018a). Diarrhoeal diseases cause significant economic losses in calf rearing (Cho et al., 2014).

Feed additives are used in livestock nutrition for many different reasons. Some additives supplement the needs of essential nutrients, while others affect feed intake or increase nutrient conversion (Wenk, 2000). For this reason, new, effective and gentle feed additives are constantly being sought to promote healthy development of calves (Gaisler et al., 2019).

Many microorganisms are used in livestock nutrition. These microorganisms are referred to as probiotics and prebiotics (Wenk, 2000). Live microorganisms that have a beneficial effect on improving the intestinal microbial balance of the host are referred to as probiotics (Gibson et al., 1995). The most commonly used probiotics include lactic acid bacteria. In particular, the genera *Lactobacillus*, *Streptoccocus*, *Enterococcus* and *Lactoccocus* (Ouwehand et al., 2002). Other bacteria used are representatives of the genera *Bifidobacterium* or the yeast *Saccharomyces cerevisiae*. Probiotic cultures improve resistance to infectious diseases, increase growth performance and production, improve feed conversion, and promote food digestion and nutrient absorption. Effective probiotics in the gastrointestinal tract stimulate health-promoting microorganisms while suppressing pathogenic microorganisms by competitive exclusion (Dawson et al., 1990).

Non-digestible feed ingredients that promote the growth or activity of a particular bacterium or group of bacteria already resident in the digestive tract and improve the health status of the host

are referred to as prebiotics (Gibson et al, 1995). Prebiotics serve as a specific source of nutrients for health-promoting microorganisms that are commonly found in the gastrointestinal tract (Wenk, 2000). The main advantage of feeding prebiotics in animal nutrition, especially in the diet of young animals, is the beneficial effect on the competitive exclusion of pathogenic microorganisms (Savage et al., 1995).

Preparations that are a mixture of probiotics and prebiotics are called synbiotics (Jonova et al., 2021). This term refers to products whose prebiotic component selectively supports the probiotic component. Due to this synergistic effect, probiotics and prebiotics are administered together to restore gut microbial balance (Trafalska et al., 2004). The mixture of probiotics and prebiotics has a positive effect on feed intake, weight gain, milk digestibility and faecal consistency (Marcondes et al, 2016). Roodposhti et al. (2012) add that feeding synbiotics has a positive effect on reducing the incidence of coliforms and to increase IgG levels in newborn calves. In addition, synbiotics are also produced that can reduce greenhouse gas emissions (Jonova et al., 2020). According to Uyeno et al. (2015), the reason for their positive effect on the organism is to provide energy and carbohydrates for rumen microorganisms, which are essential in the fermentation process. In ruminants, products combining oligosaccharides with bacterial cultures are used (Hamala et al., 2012).

Plant extracts, also known as phytobiotics, are natural bioactive substances naturally occurring in plants. These substances affect feed intake, taste and product quality through their pigmentation, digestion, antimicrobial effect and by reducing unsaturated fatty acids in the digestive tract (Wenk, 2000). Phytobiotics exhibit probiotic

effects (Smulski et al., 2020), positively affect animal performance (Nowak et al., 2017) and in dry cow nutrition have a positive effect on colostrum composition and quality. Higher serum IgG concentrations were measured in calves born to cows fed these herbal supplements. Furthermore, a significant reduction in diarrhoeal disease was observed. Phenolic compounds, terpenes, alkaloids, saponins, essential oils, polysaccharides and many other substances have the effect of increasing the phagocytic activity of macrophages, the number of T and B lymphocytes and stimulating interferon synthesis. These substances are found in plants such as *Echinacea*, garlic (*Allium sativa*), *Aloe vera*, mountain arnica (*Arnica montana*), oregano (*Origanum vulgare*) and nettle (*Urtica*) (Frankič et al., 2009). Thyme (*Thymus vulgaris*), oregano (*Origanum vulgare*) or sage (*Salvia officinalis*) have the strongest microbial activity (Windisch et al., 2008). Herbal extracts of oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*) reduce intestinal pathogenic microorganisms responsible for diarrhoeal diseases in calves (Stefanska et al., 2020). Garlic (*Allium sativa*) can also be added to feed in the treatment of diarrhoeal disease in the form of an extract (Kekana, 2014). Another herb used in the treatment of diarrhoeal disease is chestnut (*Castanea sativa*) (Bonelli et al., 2018). According to Smulski et al. (2020), the use of high concentrations of extracts and essences is required to achieve antibiotic-like therapeutic effects. For this reason, phytotherapy is recommended mainly as a preventive measure.

MATERIAL AND METHODS

The experiment took place on a farm of Holstein-Friesian dairy cattle in the southern Pilsen region. Calves were placed immediately after birth in individual outdoor cubicles, where they remained until one month of age. They were then moved, in groups of 10 to 15, to group housing in a calf pen on feeders. The calves were housed there until about 90 days of age and then weaned in the same groups and transferred to a plant-based diet. Finally, at 6 to 7 months of age, they were transferred to a large-capacity calf house.

The calves were fed twice daily with 4 litres of colostrum for the first 4 days after birth. From day 5 after birth, they were also fed twice daily with 4 to 5 litres of native milk, with access to water and starter ad-libitum. They were fed milk formula (MKS) from day 30 of age with gradual weaning until about 3 months of age using automatic feeders. Also with access to water and ad-libitum starter.

Only heifers were included in the study. Feed additives were administered from day 1 after birth until about 1 year of age. During the experiment, the animals were divided into two groups. Individuals in the first group were fed RumiForm Digest (yeast, sorbitol, sodium chloride) - Calf Feed Supplement twice daily at a dose of 10 ml for the first 30 days after birth. Then, from 3 months of age, they were fed RumiForm Yeast+ (calcium carbonate, inactive yeast) at a dose of 50 g until about 1 year of age. The second group of calves was the control group and no feed supplement was given to these calves during the experiment.

Feed consumption (colostrum, native milk, MKS, starter, water) was monitored during the experiment. All calves were sampled for blood

(from the jugular vein) using a haemoscope (HEMOS H-02 for cattle with needle) between the second and fifth day of age. The collected blood was allowed to precipitate for 24 hours at room temperature and the resulting blood serum was used to assess the total protein level using an optical refractometer. The refractometer was calibrated before each use. Further collections were made around day 60 after birth and the total protein level in the blood serum was also determined.

The course of the experiment involved monitoring and recording the health status and weight gains of all calves. Particular attention was paid to diarrhoeal diseases, for which a detailed overview (duration, course, colour and consistency of faeces) was recorded. The course of treatment and drugs used were also recorded.

Calves with any signs of disease were treated according to the standard farm protocol, as established by the farm veterinarian. The course of treatment was guided by the current health status of the individual.

For diarrhoeal disease, the most commonly used rehydration preparation was Glucosol plv.sol. - preparation for oral administration, containing transport and energy components (glucose, glycine), substitutes for ion losses (sodium, potassium), components promoting stimulation (citrate, acetate) and alkalizing components (citrate, acetate, bicarbonate). In addition, Duphalyte was applied - a rehydration solution with a complex composition, containing vitamins, electrolytes, amino acids and nutritional components. This preparation was applied subcutaneously (s.c.). In justified cases, antimicrobials were applied.

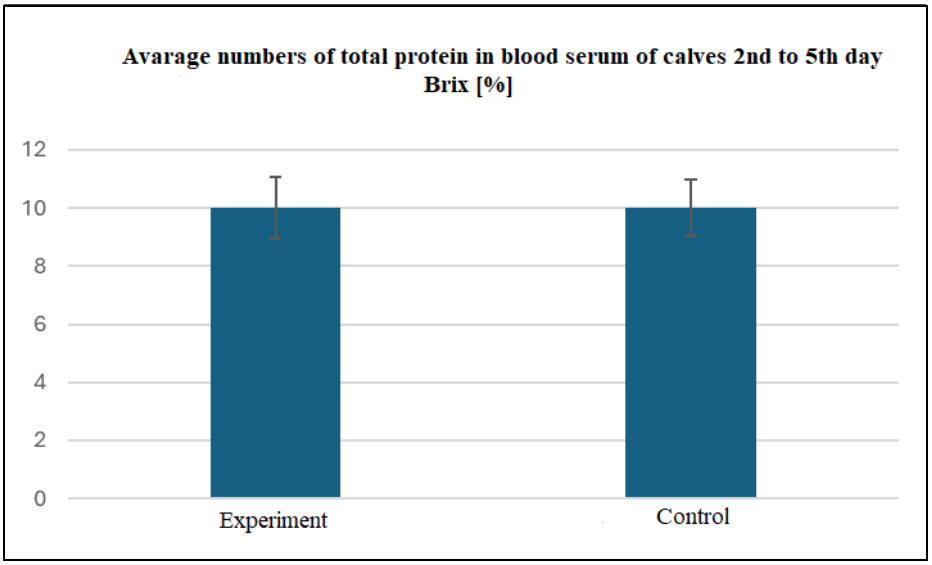
Data were processed using STATISTICA 12 software. Among the tests, chi-square test and t-test were used.

RESULTS

In the study, calves were divided into one experimental group and one control group.

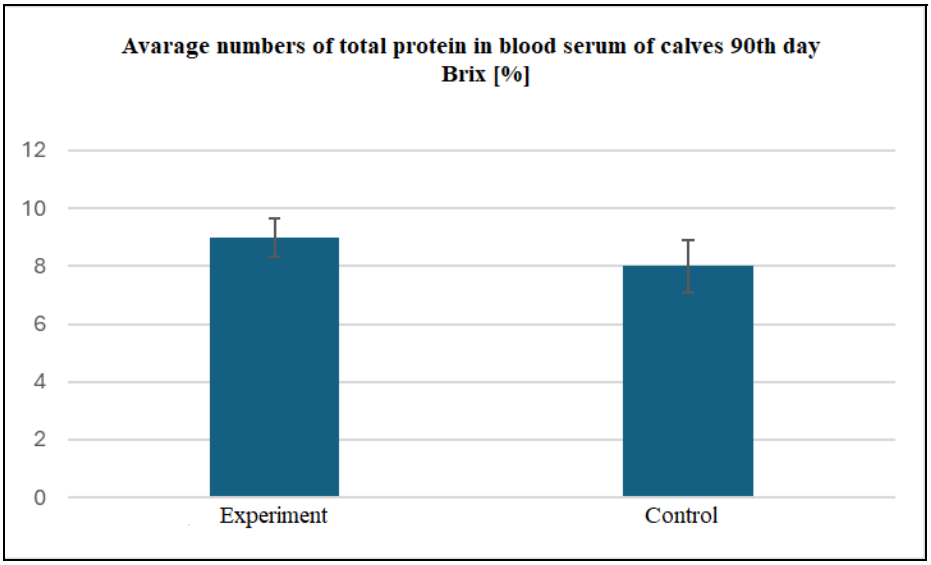
Evaluation of the level of total protein in blood serum

During the study, blood was collected from all animals between days 2 and 5 after birth and then on day 90 after birth. The level of total protein was then determined from the blood serum using an optical refractometer.



Graph 1. Avarage numbers of total protein in blood serum of calves 2nd to 5th day

The mean level of total protein in blood serum (Graph 1) was at the same value of 10.0 Brix [%] between the second and fifth day in both groups. The minimum value found was 5.4 Brix [%], and the maximum value was 12.3 Brix [%]. Based on the parametric Student's t-test, there was no significant difference between the groups (N = 101; t-test = 0.9204; p = 0.1792).

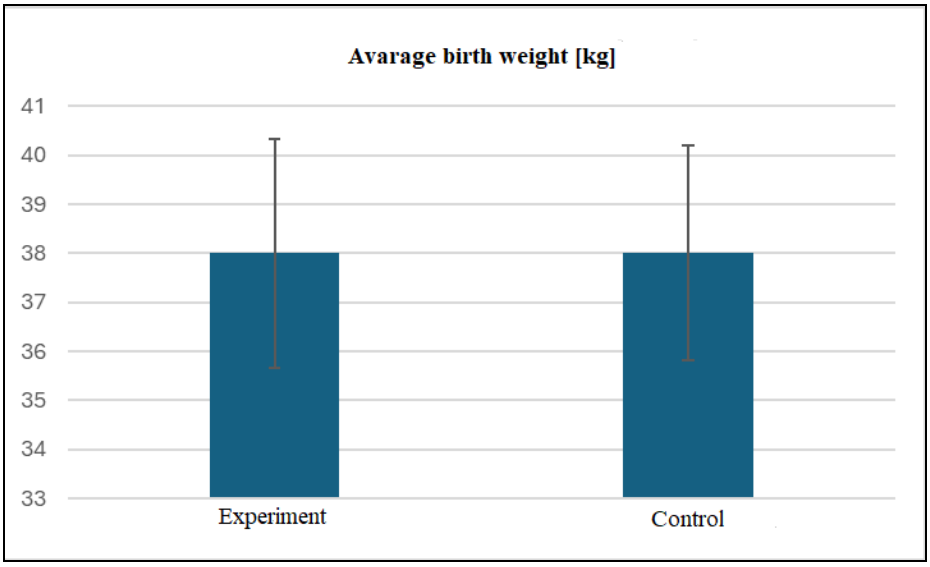


Graph 2. Avarage numbers of total protein in blood serum of calves 90th day

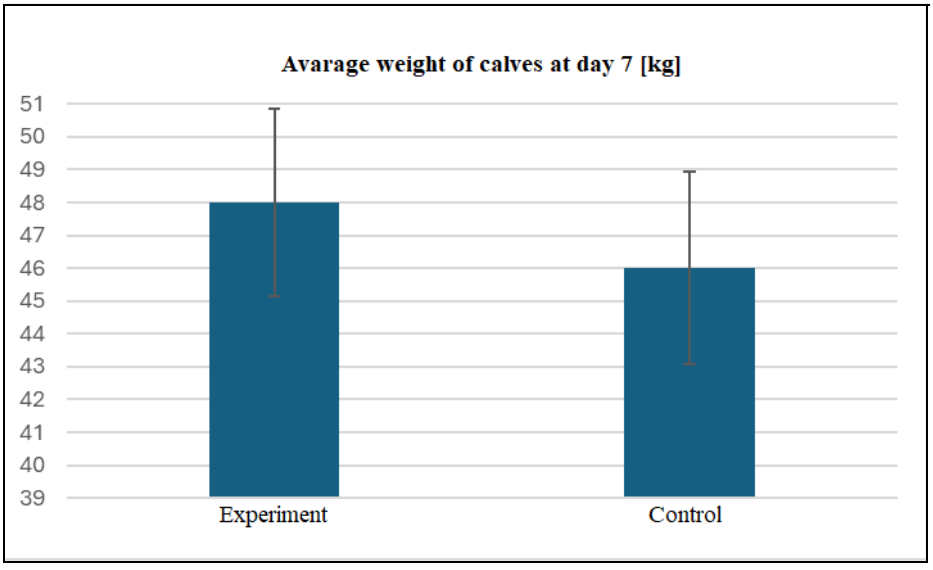
The mean total serum protein counts at 90 days of age (Graph 2) ranged from 8.0 Brix [%] in the control group to 9.0 Brix [%] in the experimental group. The minimum value observed was 7.0 Brix [%], the maximum value measured was 10.7 Brix [%]. Based on the parametric Student's t-test, there was a significant difference between the groups (N1 = 77, N2 = 78; t-test = 2.0692; p = 0.0202).

Growth assessment

Birth weight (Graph 3) was the same in both groups. The mean weight of both experimental and control groups was 38 kg. The lowest weight measured was 30 kg, while the maximum weight recorded was 45 kg. Based on the parametric Student's t-test, it was verified that there was no significant difference between the groups at the beginning of weighing (N = 101; t-test = 0.9927; p = 0.1610).The experiment included weight at birth, at seven days of age and at 90 days of age.



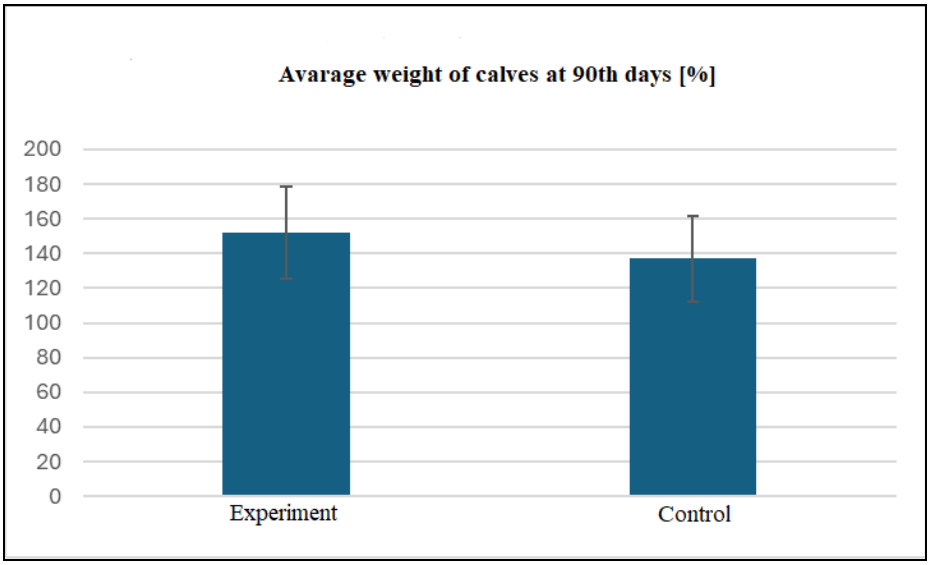
Graph 3. Avarage birth weight of calves



Graph 4. Avarage weight of calves at day 7

The average weight at 7 days of age (Graph 4) was 48 kg in the experimental group, while the average weight of the control group was 46 kg. The lowest recorded value was 36 kg, while the highest was 55 kg. Based on the parametric Student's t-test,

there was a highly significant difference between the groups (N1 = 99, N2 = 101; t-test = 4.8793; p = 0.00000109).

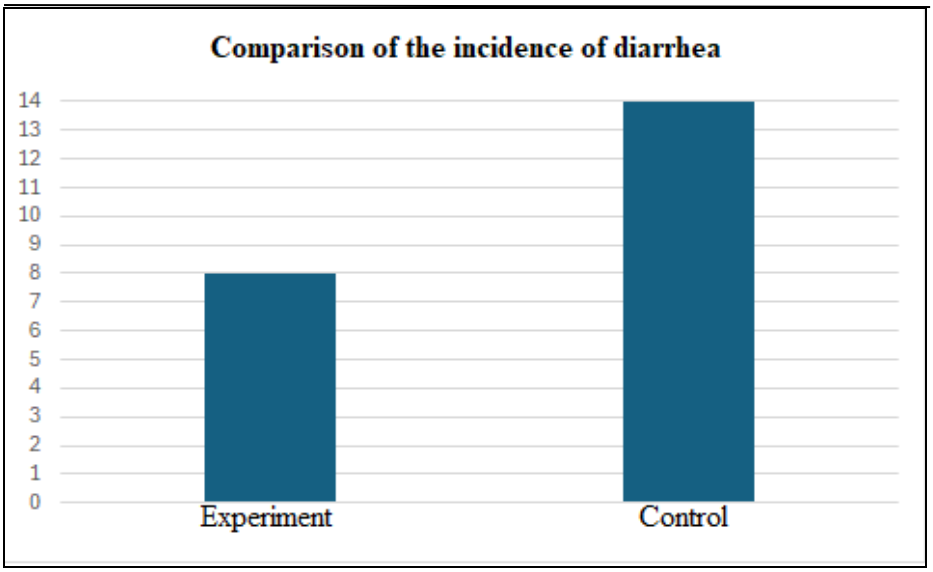


Graph 5. Avarage weight of calves at 90th days

A more significant difference between the groups was observed at 90 days of age (Graph 5), when the average weight of the experimental group was 152 kg, whereas the average weight of the control group was 137 kg. The lowest value recorded was 85 kg and the highest 215 kg. Using a parametric Student's t-test, there was a highly significant difference between the groups (N = 78; t-test = 3.5723; p = 0.0002).

Health assessment

Throughout the experiment, all subjects were monitored and recorded for their health status and treatment progress. In particular, the course and treatment of diarrhoeal diseases were recorded in detail.



Graph 6. Comparison of the incidence of diarrhea between the experimental and control group Diarrhoeal diseases (Graph 6) occurred in both groups. The highest incidence of diarrhoea was recorded in the control group, with 14 individuals suffering from diarrhoea. In the experimental group, diarrhoea occurred in eight individuals. When comparing the experimental group with the control group, no significant difference was found using chi-square test (N = 101; x2 = 1.84; p = 0,1749).

In the experiment, diarrhea in calves (Table 1) was observed from day 6 after birth to calves at 18 days of age. Diarrhoeal disease was most common in animals around day 7 of age. Diarrhoea lasting 1 day was the shortest with the most severe course. Diarrhoea lasted the longest at 10 days. The colour of the faeces ranged from light yellow to brown, with 4 animals showing diarrhoea with blood.

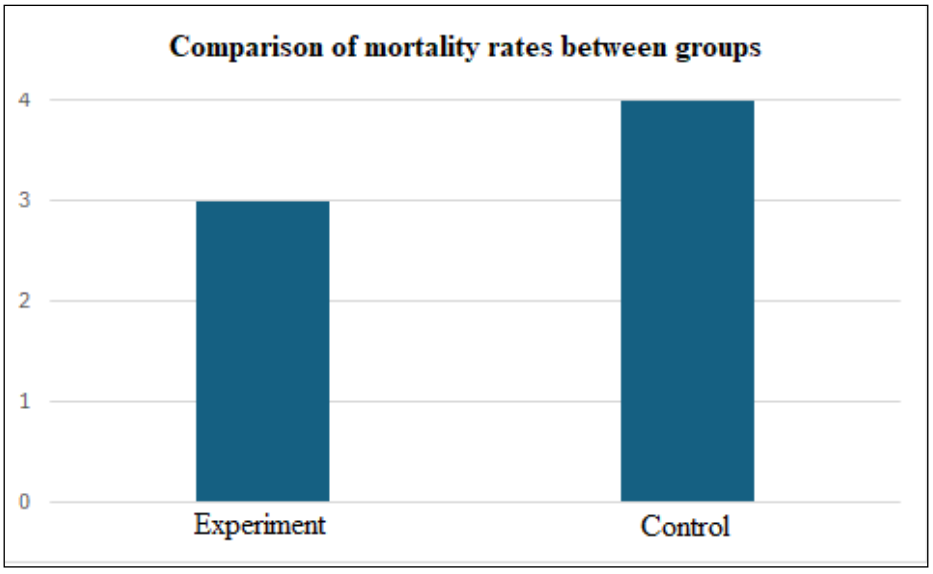
Table 1. Course of diarrhoeal diseases in calves

Group in the experiment	When diarrhea appeared	Number of animals	Duration	Colour, cocsistency
Control	6th day after birth	1	7 days	light brown colour, very watery consistency
	7th day after birth	2	2-5 days	brown colour, with admixture of blood, watery consistency yellow colour, mushy consistency
	8th day after birth	1	10 days	brown colour, with admixture of blood, watery consistency
	9th day after birth	1	5 days	yellow colour, mushy consistency
	10th after birth	3	2-3 days	yellow colour, mushy consistency yellow colour, thin consistency light brown colour, watery consistency
	11th after birth	3	2 days	yellow colour, watery consistency light brown colour, mushy consistency
	13th after birth	1	2 days	light yellow colour, mushy consistency
	18th after birth	2	1-7 days	light yellow colour, thin consistency, with admixture of mucus light yellow colour, very watery consistency, with admixture of blood
Experiment	7th after birth	3	4-8 days	light yellow colour, watery consistency, with admixture of blood yellow colour, mushy consisteny
	8th after birth	1	3 days	light yellow colour, watery consistency
	11th after birth	1	3 days	light brown colour, mushy consistency
	12th after birth	1	9 days	light brown colour, thin consistency
	13th after birth	1	3 days	yellow colour, mushy consistency, with admixture of mucus
	15th after birth	1	6 days	light brown colour, watery consistency, with admixture of mucus

Mortality

The mortalities that occurred during the study were each due to a different cause. In the experimental group, the death of a seven day old heifer occurred with a total serum protein level of 9.1 Brix [%] between days 2 and 5. Mortality occurred unexpectedly without any symptoms. A similar case was the death of a 15 day old calf. In this individual, the serum total protein level between days 2 and 5 was 8.9 Brix [%]. The last mortality within the experimental group was recorded in an individual suffering from diarrhoea.

Within the control group, two deaths were due to injury. Another reason was very severe diarrhoea with a rapid course, which ended in death after the first day. The last mortality occurred in a 53 day old individual with pneumonia.



Graph 7. Comparison of mortality rates between groups

Mortality (Graph 7) was recorded in both experimental and control groups. There were 3 mortalities in the experimental group while 4 mortalities occurred in the control group. Based on statistical analysis chi-square test, there was no significant difference in this case either (N = 101; x2 = 0.07; p = 0.7899).

DISCUSSION

Diarrhoeal diseases are most commonly addressed during the calf rearing period (Cho et al., 2014; Katsoulos et al., 2020). Diarrhoea most commonly occurs around days five and seven of calf age (Urban, 1997). This statement was confirmed in this study. In this experiment, diarrheal disease was the most common problem in calves, and was observed most frequently around day seven of calf age. Diarrhoea was more commonly observed in the control group. A higher number of deaths also occurred in this group.

From the data obtained, there was no evidence of more frequent health problems or deaths in calves with serum total protein levels below 8.6 Brix [%]. This value is considered satisfactory to ensure passive immunity (Bielmann et al., 2010).

From the experiment conducted in this study, a beneficial effect of probiotic feed supplements on weight gain can be demonstrated, but a beneficial effect on calf health cannot be shown. These conclusions are also consistent with the studies by Zábranský et al. (2022) and Zábranský et al. (2023). The study conducted by Zábranský et al. (2022) also found different live weight gains for the different types of probiotics tested. However, this claim is disagreed with by Simon et al. (2001) who claim that the improvements in weight gain and feed conversion are only

sporadic. Authors Uyeno et al. (2015) and Renaud et al. (2019) add that studies focusing on health status are insufficient.

CONCLUSION

The calf rearing period is the most important part of a calf's life. Poor management of rearing (nutrition, housing, zoohygiene, etc.) has a negative impact not only on the viability of the individual but also on its future production. Proper management of rearing will result in a healthy and strong calf and a quality animal that will perform well.

In the study, the average level of total protein in blood serum between day 2 and day 5 was measured to be 10.0 Brix [%]. Total serum protein levels above 8.6 Brix [%] were found in 86% of the animals. Levels below 8.6 Brix [%] were also measured among calves. The minimum value measured was 5.4 Brix [%]. There was insufficient transfer of passive immunity in these calves. The average total serum protein counts at 90 days of age ranged from 8.0 Brix [%] to 9.0 Brix [%]. Total serum protein values above 8.6 Brix [%] were recorded in 75% of calves. The lowest recorded serum total protein level was 7.0 Brix [%] and the maximum was 10.7 Brix [%]. These values are not indicative of the transmission of passive immunity but are indicative of the current quality of the diet and the current health status of the animal. Values below 8.6 Brix [%] indicate a lack of protein in the feed, while values above 10.7 Brix [%] indicate dehydrated calves with overly concentrated blood.

The data obtained in this study showed that the average weight of newborn calves was 38 kg. After seven days, the average weights for both groups remained at approximately the same values. In the experimental group, the average weight was 48 kg and

in the control group, a value of 46 kg was measured. The lowest weight was measured in a calf weighing 36 kg. On the other hand, the highest value was obtained in an individual weighing 55 kg. When weighed at 90 days of age, the experimental group had an average weight of 152 kg and the control group 137 kg.

Diarrhoeal diseases were observed in both experimental and control groups. A higher incidence of diarrhoeal disease was observed in calves in the control group. In this group, 14 calves out of a total of 101 calves developed diarrhoea. Whereas in the experimental group, diarrhoea occurred in eight out of 101 calves. Calves suffered from diarrhoea most frequently around day 7 after birth. The course of treatment was guided by the current health status of the animals. The most severe course of the disease was in the 18-day-old calf. This diarrhoea resulted in the death of the calf.

Mortality occurred within both groups. Among the calves that died were individuals suffering from diarrhea, pneumonia or with trauma. A mortality of 3.5% occurred during the experimental period.

In this study, a positive effect of probiotic feed supplements on weight gain can be statistically demonstrated, but a positive effect on health status cannot be statistically demonstrated.

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IS NARROW-LEAVED LUPINE (*LUPINUS ANGUSTIFOLIUS*) A SUITABLE CRUDE PROTEIN SOURCE FOR RABBIT DIETS? A COMPARISON WITH SOYBEAN MEAL

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ABSTRACT

The present study aimed to evaluate the efficacy of narrow-leaved lupine seeds (NLL) as a protein source in rabbit diets in terms of growth performance, digestive enzyme activity, nutrient digestion, and nitrogen excretion and retention. Different varieties of NLL (Boregine, Jowicz, and Rumba) were used as the replacement of soybean meal (SBM) in the present study. The control diet contained 60 g/kg SBM, and the experimental diets, containing 110 g/kg NLL, differed in the varieties used (Boregine, NLLB diet; Jowicz, NLLJ diet; and Rumba, NLLR diet). For the growth performance trial, 160 rabbits (Hyplus PS

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19 x PS 40; of both sexes; weaned at 32 days of age) were randomly allocated into 4 groups (40 rabbits per group) and fed one of the diets for a period of 42 days. In addition, 40 rabbits weaned at d 32 (Hyplus PS 19 x PS 40; 10 rabbits per diet) were used for the determination of the coefficients of total tract apparent digestibility (CTTAD) of the diets and to determine nitrogen balance and nitrogen retention. The proteolytic activity tended to be lower in rabbits fed the SBM diet than in those of the other rabbits. The CTTAD of the diets were not affected by the dietary treatments. The losses of nitrogen in faeces were not affected by the dietary treatment. There were higher losses of nitrogen in urine (P = 0.006) and also a higher total excretion of nitrogen (by 0.36 g/day; P = 0.013) in rabbits fed the NLLJ diet than in rabbits fed the other diets. Consequently, there was a lower nitrogen retention coefficient in these rabbits (P = 0.008). There was a higher average daily feed intake in rabbits fed the NLLJ diet than in other rabbits (P = 0.035). This corresponded with the worse FCR in rabbits fed the NLLJ diet than in rabbits fed the other diets (P <.0001). The present study revealed that the varieties of narrow-leaved lupine seeds, Boregine and Rumba, represent a suitable dietary CP and can fully replace traditionally used SBM for rabbit diets. Negative results in performance, nitrogen excretion, and nitrogen balance in rabbits fed the diet containing variety Jowicz indicate the importance of choosing a suitable variety of NLL for rabbit diets.

Keywords: rabbit; crude protein; narrow-leaved lupine; feed efficiency; nitrogen retention

INTRODUCTION

The effort toward sustainable agricultural practices has intensified in recent years, with a particular focus on finding alternative crude protein (CP) sources for animal feeds. Within the European Union (EU), the drive towards sustainability is paramount, given the need to reduce dependency on imported feed ingredients, mitigate environmental impacts, and ensure food security (Parisi et al., 2020). Lupines, among legumes, represent a promising alternative with their unique nutrient composition. They are crops well-adapted to the temperate climates of Europe and present a promising alternative to traditional protein sources such as soybean meal (SBM) (Musco et al., 2017; Sońta and Rekiel, 2020). Studies up to date have already shown that white lupine seeds (WLS) are a suitable substitution (partial or full) for SBM as a crude protein source in rabbit nutrition (Volek and Marounek, 2009; Volek et al., 2018). Similarly, narrow-leaved lupine seeds (NLL) could become a suitable CP source and extend the range of domestic protein components. The NLL are rich in nutrients (Lemus-Conejo et al., 2023). Currently, a limited number of studies are available regarding the dietary inclusion of NLL in rabbits. In fact, only Volek et al. (2020) evaluated the dietary inclusion of NLL in comparison with the well-studied dietary inclusion of WLS in rabbits. Concerning SBM, there is no information in the literature regarding the substitution of NLL for SBM in rabbit diets.

Thus, this study aims to evaluate the efficacy of NLL as a protein source in rabbit diets in terms of growth performance, digestion, digestive enzyme activity, and nitrogen retention. Different varieties of NLL were used for the replacement of SBM in the present study.

MATERIAL AND METHODS

Diets

Different CP sources were used for the formulation of the diets (Table 1). The control diet contained 60 g/kg SBM, and the experimental diets, containing 110 g/kg NLL, differed in the varieties used (Boregine, NLLB diet; Jowicz, NLLJ diet; and Rumba, NLLR diet) (Table 2).

The diets met, except for sulphur amino acids (AA) and threonine, the recommendations of De Blas and Mateos (2020) for the nutrient requirements of fattening rabbits (Table 3). The diets were offered *ad libitum* to rabbits as pellets with a diameter of 3 mm and a length of 5 to 10 mm.

Animals and experimental design: performance trial

For the growth performance trial, 160 rabbits (Hyplus PS 19 x PS 40; of both sexes) weaned at day 32 were randomly allocated to one of the four groups (40 rabbits per group), and each group was fed one of the diets for a period of 42 days. Rabbits were housed in wire-net cages (80 x 60 x 45 cm), with 4 animals per cage. Feed intake was recorded daily, and live weight was recorded weekly, both per cage. Average daily weight gain (ADWG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were calculated after the end of the experiment.

Table 1. The chemical composition (% on as fed basis unless otherwise stated) of SBM and selected varieties of NLL used in this study (Boregine, Jowicz, and Rumba)

	SBM	Boregine	Jowicz	Rumba
Dry matter	93.6	87.3	87.3	88.9
Crude protein	42.7	29.4	30.0	27.7
Ether Extract	2.4	5.64	5.14	5.94
Crude fibre	7.61	14.6	12.2	13.7
Neutral detergent fibre	15.1	28.9	27.0	23.4
Acid detergent fibre	10.5	28.4	18.4	17.7
Insoluble dietary fibre	17.32	39.5	40.4	41.5
Soluble dietary fibre	4.10	1.2	0.9	1.8
Ash	7.09	3.28	2.84	3.86
Calcium	2.95	2.34	2.77	3.4
Phosphorus	5.54	4.63	3.42	4.62
Lysine	2.60	1.27	1.36	1.39
Methionine + cysteine	1.28	0.585	0.565	0.619
Threonine	1.70	0.953	0.956	0.945
Total EAA	18.70	10.9	12.3	11.5
α-tocopherol (mg/kg)	2.98	5.61	3.67	4.26
δ-tocopherol (mg/kg)	8.76	1.25	1.59	1.10
γ-tocopherol (mg/kg)	7.29	59.8	54.7	56.2
β-carotene (mg/kg)	0.005	5.79	4.01	5.0
Lutein (mg/kg)	0.242	13.2	9.93	12.6
Zeaxanthin (mg/kg)	0.048	7.20	5.78	8.80

Table 2. Ingredients (% on an as-fed basis) of the experimental rabbit diets based on soybean meal (SBM diet) or NLL varieties: Boregine (NLLB diet), Jowicz (NLLJ diet), and Rumba (NLLR diet).

	Experimental diets			
Component	**SBM**	**NLLB**	**NLLJ**	**NLLR**
Alfalfa meal	28.5	28.5	28.5	28.5
Soybean meal	6	0	0	0
Boregine	0	11	0	0
Jowicz	0	0	11	0
Rumba	0	0	0	11
Wheat bran	33	31	31	31
Sugar beet pulp	9	9	9	9
Oat	15	12	12	12
Barley	6	6	6	6
Vitamin-mineral premix	1	1	1	1
Monocalcium phosphate	0.5	0.5	0.5	0.5
Limestone	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5

Table 3 Chemical composition and nutritive value (% on an as-fed basis unless otherwise stated) of the experimental rabbit diets based on soybean meal (SBM diet) or NLL varieties: Boregine (NLLB diet), Jowicz (NLLJ diet), and Rumba (NLLR diet).

	Experimental diets			
	SBM	**NLLB**	**NLLJ**	**NLLR**
Dry matter	87.8	87.9	87.6	87.8
Crude protein	14.7	15.1	15.0	14.8
Neutral detergent fibre	36.3	35.1	35.6	36.8
Acid detergent fibre	18.2	18.3	18.9	18.1
Acid detergent lignin	4.3	4.3	4.4	4.4
Insoluble dietary fibre	35.3	34.3	35	36.4
Soluble dietary fibre	4.9	4.9	3.2	4.1
Ether extract	2.7	2.8	2.8	2.8
Starch	16.4	15.1	15.3	15.4
Lysine	0.72	0.68	0.68	0.67
Methionine + cysteine	0.51	0.48	0.47	0.48
Threonine	0.60	0.58	0.59	0.59
Total essential amino acids	5.4	5.3	5.3	5.0
Ash	7.3	7.3	6.9	7.0
Particle size distribution (%)				
>1.25 mm	13	15	16	16
>0.630 mm	7	7	7	7
>0.315 mm	8	8	8	8
<0.315 mm	72	70	69	69

Health status, expressed as the sanitary risk index (SRI), was calculated according to the definitions of the European Group on Rabbit Nutrition

(Fernández-Carmona et al., 2005) as a sum of ailing and deceased animals, where the animal was recorded as either ailing or deceased only once. The deceases were recorded daily, and ailments were recorded weekly and monitored by individual observations of clinical signs of digestive disorders (diarrhoea, mucus in faeces, abnormal excretion of caecotrophs faeces) or weight loss.

Digestibility trial, nitrogen balance, and nitrogen retention

The 40 rabbits weaned at d 32 (Hyplus PS 19 x PS 40) were used for the determination of the coefficient of total tract apparent digestibility (CTTAD) of the organic matter (OM), CP (N x 6,25), starch, neutral detergent fibre (NDF), acid detergent fibre (ADF), and gross energy (GE) of the diets in accordance with the European Group on Rabbit Nutrition (Perez et al., 1995) and to determine nitrogen balance and nitrogen retention in accordance with the study of Volek et al. (2020). Rabbits were randomly allocated into four groups (10 rabbits per group) and individually housed in wire-net metabolic cages (50 x 40 x 42.5 cm). Each group was fed ad libitum on one of the four diets, and after an adaptation period that lasted 28 days, the 4-day collection of the whole production of faeces and urine followed afterwards. At the end of the digestibility trial, all rabbits (64 days old) were slaughtered without previous fasting. After laparotomy, the contents of the whole small intestine were immediately frozen (-80 °C) until analysis. The small intestinal contents were used for the analysis of the hydrolytic activities of pancreatic enzymes and the concentration of sialic acid.

Analytical methods

Protein sources, feeds, urine, and faeces were analysed by AOAC (1984, 2000, 2005) methods. Total amylase, protease, and lipase

activities were analysed according to Taubner et al. (2023) with soluble starch, azocasein, and tributyrin as substrates. The analysis of the sialic acid was performed according to Salcedo et al. (2011). For the quantification of the neutral detergent fibre, the methodology of Mertens (2002) was used. Lignin levels were determined by the solubilization of the cellulose in the sulfuric acid (Robertson and Van Soest, 1981). Gross energy was determined by combustion in an adiabatic calorimeter (C5000 control, IKA-Werke, Staufen, Germany). The quantification of ß-carotene, α-tocopherol, δ-tocopherol, and γ-tocopherol in the SBM, lupine seeds, and diets was determined according to the European standards (EN 12822, EN 12823-2) for high-performance liquid chromatography, equipped with a diode-array detector (VP series) (Shimadzu, Kyoto, Japan). The same equipment was used for the quantification of lutein and zeaxanthin in the crude protein sources and diets, following the methodology described by Froescheis et al. (2000).

Statistical analysis

Growth performance, CTTAD of diets, pancreatic enzyme activity, nitrogen balance, and nitrogen retention were examined by one-way ANOVA using the GLM procedure in Statistical Analysis System 9.4 (2012), with type of diet as the fixed effect. For the growth performance assessment, the cage represented the experimental unit. Cages with mortality of 50% and more (i.e., two or more rabbits) were not included in the statistical analysis of growth performance. No corrections concerning the cage feed consumption in cases of morbidity were made. The individual rabbit represented the experimental unit for the CTTAD, nitrogen balance, and nitrogen retention. The data in

tables are presented as least squares means. Health status was assessed using the Fisher's exact test. The individual rabbit was used as the experimental unit. Differences among least squares means with $P \leq 0.05$ were accepted as statistically significant, while $P \leq 0.10$ were considered a trend of significance.

RESULTS AND DISCUSSION

The chemical composition of protein sources and the formulation of the experimental diets used in the present study

The proximate composition of the NLL varieties is within the range reported by the other authors (Bartkiene et al., 2016; Ferchichi et al., 2021). All tested NLL varieties contained lower levels of CP, soluble dietary fibre, and ash and higher levels of EE, NDF, ADF, and insoluble dietary fibre than the SBM. These findings are in accordance with Lagos and Stein (2017).

The diets used in this study were formulated to have similar limiting AA levels. Because of the possible influence of dietary inclusion of synthetic AA on the digestibility of AA/CP in rabbits (Taboada et al., 1994; de Blas et al., 1998), synthetic AA were not added to the diet used. For this reason, the levels of limiting AA in the experimental diets used in this study were slightly below the dietary recommendations for growing-fattening rabbits (de Blas and Mateos, 2020).

Hydrolytic activity of pancreatic enzymes and concentration of sialic acid

The hydrolytic activity of pancreatic enzymes (Table 4) did not differ significantly among dietary treatments, except for the proteolytic

activity, which tended to be lower in rabbits fed the SBM diet than in those of the other rabbits (on average by 193 mg azocasein/g dry matter digesta/h; P = 0.10).

Table 4. Pancreatic enzyme activity and concentration of sialic acid in small intestine of rabbits fed diets based on soybean meal (SBM diet) or NLL varieties: Boregine (NLLB diet), Jowicz (NLLJ diet), and Rumba (NLLR diet).

	Experimental diets				
	SBM	**NLLB**	**NLLJ**	**NLLR**	**P-value**
Rabbits (n)	10	10	10	10	
Live weight[1] (g)	2356	2286	2334	2265	0.714
Amylolytic activity[2]	9.02	6.00	6.11	6.08	0.177
Proteolytic activity[3]	684.7	922.6	883.1	828.3	0.104
Lipolytic activity[4]	8.5	8.6	10.3	10.6	0.174
Sialic acid[5]	2.37	2.43	1.95	3.27	0.291

[1]at d 64 of age; [2]at mg sugar/g dry matter digesta/h; [3]mg azocasein/g dry matter digesta/h; [4]mmolbutyrate/g dry matter digesta/h; [5]µg sialic acid/g dry matter sample.

This finding is more likely related to anti-nutritional factors (ANFs), such as trypsin inhibitors (TI). In this context, Torres et al. (2005) detected no trypsin inhibitors in NLL, whereas the major ANFs in SBM is TI, which may influence the proteolytic activity and subsequently the apparent ileal digestibility of AA and CP, as detected in pigs (Woyengo et al., 2017; Chen et al., 2020). Although the concentration of TI in our study was not determined, a study by Hoffmann et al. (2019) suggest that TI concentration below the threshold (4 mg/g) can negatively affect digestibility of nutrients and growth performance in chickens. In

this context, further experiments should be carried out to evaluate the threshold of TI for rabbits fed diets containing CP sources with TI.

Coefficients of total tract apparent digestibility of the experimental diets, nitrogen balance, and nitrogen retention

Data describing the CTTAD of the diets, nitrogen balance and nitrogen retention are presented in table 5. The CTTAD of OM, CP, EE, NDF, and ADF were not significantly affected by the dietary treatments. The CTTAD of starch was slightly lower in rabbits fed the NLLJ than in those of other rabbits (on average by 0.008; P = 0.048), a finding which is related with a higher ADF/starch ratio in this diet (Gidenne et al., 2000). No significant differences were observed between treatments concerning nitrogen intake. The losses on nitrogen in faeces were not affected by the dietary treatment. There were higher losses of nitrogen in urine (on average by 0.36 g/day; P = 0.006) and also a higher total excretion of nitrogen (by 0.36 g/day) in rabbits fed the NLLJ diet than in rabbits fed the other diets. Consequently, there was a lower nitrogen retention coefficient in these rabbits. These findings may be related to the different AA/CP balances of the diets used in the present study (Volek et al., 2021).

Table 5. Coefficients of total tract apparent digestibility (CTTAD) for the experimental diets, and nitrogen balance a retention in rabbits fed diets based on soybean meal (SBM diet) or NLL varieties: Boregine (NLLB diet), Jowicz (NLLJ diet), and Rumba (NLLR diet).

	Experimental diets				
	SBM	**NLLB**	**NLLJ**	**NLLR**	**P-value**
Rabbits (n)	10	10	10	10	
CTTAD[2]					
Organic matter	0.587	0.600	0.577	0.599	0.653
Crude protein	0.626	0.675	0.672	0.655	0.173
Ether extract	0.915	0.898	0.879	0.898	0.131
Starch	0.952[a]	0.953[a]	0.943[b]	0.948[a]	0.048
Neutral detergent fibre	0.262	0.266	0.281	0.283	0.737
Acid detergent fibre	0.123	0.144	0.125	0.120	0.816
Nitrogen balance[2] (g/day)					
N intake	3.98	3.90	3.98	3.79	0.678
N excretion in faeces	1.49	1.24	1.35	1.31	0.213
N excretion in urine	0.55[b]	0.72[ab]	0.95[a]	0.49[b]	0.006
Total N excretion	2.05[ab]	1.96[ab]	2.30[a]	1.80[b]	0.013
N retention					
Retained N[2] (g/day)	1.94	1.94	1.69	1.99	0.171
Coefficient of N retention[4]	0.49[ab]	0.50[a]	0.42[b]	0.52[a]	0.008

[1] *Determined in rabbits between 60 and 64 days of age.*

[2] *As nitrogen intake – total nitrogen excretion (faeces + urine).*

[4] *As retained nitrogen/nitrogen intake.*

Growth performance of rabbits

The growth performance of rabbits is shown in Table 6. In general, except for the rabbits fed the NLLJ diet, the CP level in the diets used

provided a satisfactory outcome in terms of growth performance, a finding that is in line with Maertens et al. (1997). There were no significant differences in terms of the final live weight of the rabbits at 74 days of age, or ADWG as well. There was a higher ADFI in rabbits fed the NLLJ diet than in other rabbits (on average by 10 g/day; P = 0.035). This corresponded with the worse FCR in rabbits fed the NLLJ diet than in rabbits fed the other diets (on average by 0.34; P <.0001). These findings are apparently related to the lower CTTAD of starch, and the lower coefficient of nitrogen retention detected in rabbits fed the NLLJ diet.

Table 6. Growth performance during the fattening period (32 to 74 days of age) in rabbits[1] fed diets based on soybean meal (SBM diet) or NLL varieties: Boregine (NLLB diet), Jowicz (NLLJ diet), and Rumba (NLLR diet).

	Experimental diets				
	SBM	**NLLB**	**NLLJ**	**NLLR**	**P-value**
Live weight (g)					
At 32 days of age[1]	758	754	746	747	0.871
At 74 days of age	2556	2555	2464	2502	0.554
AWDG (g)					
32-74 days of age	42.8	42.5	40.9	41.6	0.507
ADFI (g)					
32-74 days of age	140[b]	135[b]	146[a]	134[b]	0.035
FCR					
32-74 days of age	3.27[b]	3.17[b]	3.56[a]	3.22[b]	<.0001

[1] *40 rabbits per group at the beginning of experiment*

Table 7. Morbidity, mortality and sanitary risk index (SRI) of rabbits fed diets based on soybean meal (SBM diet) or NLL varieties: Boregine (NLLB diet), Jowicz (NLLJ diet), and Rumba (NLLR diet).

	Experimental diets				
	SBM	**NLLB**	**NLLJ**	**NLLR**	**P-value**
Morbidity					
32-74 days of age	5	3	9	5	0.304
Mortality					
32-74 days of age	1	6	1	3	0.124
SRI					
32-74 days of age	6	9	10	8	0.768

CONCLUSION

The varieties of narrow-leaved lupine seeds, Boregine and Rumba, represent a suitable dietary CP and can fully replace traditionally used SBM for rabbit diets. Negative results in performance, nitrogen excretion, and nitrogen balance in rabbits fed the diet containing variety Jowicz indicate the importance of choosing a suitable variety of NLL for rabbit diets.

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EFFECT OF FEEDING VITAMIN D AND CAROTENOIDS ON PERFORMANCE PARAMETERS OF BROILER BREEDERS

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ABSTRACT

This work was aimed at investigating the effect of the joint effect of vitamin D3 in its hydroxylated form 25-OHD3 and canthaxanthin, on meat type parental flocks performance. These two compounds were added together to the diet, in addition to the technological instructions for the parental flocks. Vitamin D was added at level 69 ppm, i.e. 1 725 IU D3 and canthaxanthin at level 6 ppm. The effect of the administration of these additives on the number of eggs (NE), the number of eggs of hatching quality (HE), and egg weight (EW) was evaluated. Furthermore, the effect of the additives on the mortality and culling of laying females (CH) and the mortality and culling of males (CR) was monitored in the parental flocks, as well as the feed intake of laying hens (FIH) and the feed intake of roosters (FIR). Eggs were evaluated for fertilization and hatchability from individual flocks. Statistical evaluation of the data was evaluated using the Kruskal-

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Wallis method. A beneficial effect of the additives was observed on hatching egg production ($P < 0.05$). Furthermore, the additives had an effect on the reduction of laying hen culling ($P < 0.001$) on one of the three observed farms. The additives also had an effect on the egg hatchability parameter on one of the five farms ($P < 0.05$).

Keywords: parent flock; meat type; vitamin D_3; canthaxanthin; hatchability; hatching egg production

INTRODUCTION

In poultry rearing and breeding, where enormous genetic progress is being made, not only in the meat type of poultry, it is necessary to take special care of the nutrition of these animals. Most companies have established technological guidelines for their hybrids and their parent flocks for optimum production in the context of good breeding economics. But are there ways of taking these already good results even further through nutrition? For parent flocks in particular, nutrition is critically important for the production of hatching eggs and good performing chicks. SAUNDERS-BLADES et al. (2015) reported that most of the nutrients given in the diet are incorporated into the eggs by the laying hens and directly used in embryo development. No new norms have been set recently in mineral and vitamin nutrition for broiler chickens, the most recent NRC standard set for poultry being that in 2012. The question is whether this standard is sufficient for the modern genotypes of both the parent flocks and their offspring which give exceptional growth performance. Here, for example, the recommended dose of vitamin D from that standard can be used as an example, compared to later studies where corrections have already been made.

According to the old NRC (1994), the recommended amount for the lighter white laying type is 300 IU of vitamin D per kilogram of mixed feed per day and therefore 30 IU of vitamin D per day for the normal laying hen. For the brown-hen type, the standard gives a little more, that is 33 IU of vitamin D per day. The higher figure here is due to the higher nutritional status of these hens. In contrast, more recent findings by ATENCIO et al. (2006) set the vitamin D_3 dose at 2,800 IU per animal per day. Here, the research is already focused specifically on meat-type parents. As far as the importance of vitamin D_3 is concerned, it is particularly important for the metabolism and incorporation of Ca and P in the body, where it helps in both the metabolism of absorption and incorporation into the bone itself. It is also of equal importance in the reabsorption of Ca and phosphate by the kidneys (COMBS, 1998). For carotenoids, and here specifically canthaxanthin, their antioxidant properties are of great interest. SURAI et al (2003) describe canthaxanthin as an excellent carotenoid with great antioxidant properties, as well as good potential in preventing lipid peroxidation of many tissues. This is particularly important for developing embryos, which are subjected to extreme oxidative stress during development. The aim of this study was to investigate the effect of these two additives in addition to the amount recommended in the technological guidelines on the parameters of the parental flock and on the parameters of eggs laid by layers of such supplemented flocks, with a focus on fertilization and hatchability of eggs.

The following hypotheses were put forward in this paper:

1. The feed additive based on canthaxanthin and calcifediol has no effect on the number of laid eggs, the number of hatching eggs and their weight, as well as the culling and mortality of laying hens and males in parent flock.

2. The feed additive based on canthaxanthin and calcifediol has no effect on the fertility of hatching eggs and hatchability of chicks.

MATERIAL AND METHODS

Three farms (A, B, C) were evaluated for the data used. For the monitored parameters used (number of laid and hatching eggs, egg weight, mortality and culling of males and layers, and take daily feed intake), data were evaluated once a week throughout the monitoring period. At the same time, different flock ages were taken into consideration and so the results were matched to the same flock age (with and without feeding additives) to eliminate the effect of flock age. For all flocks, feeding and housing conditions were followed according to the technological instructions of the parent flock producer.

Farm A

Parent flocks of ROSS 308 were kept in six halls. There were 18,400 laying females and 2,220 males without additives and 21,600 laying females and 2,580 males with additives in the diet. For egg weight, data for the 28-week observation period were used and for the rest of the parameters for 32 weeks.

Farm B

COBB 500 parents were raised in seven halls. The number of additive supplemented birds was 16,620 females and 1,680 males. The control group without additives had 22,160 laying females and 2,240 males. These flocks were monitored from 27 to 59 weeks of age. Parent flock weight was measured for 31 weeks, egg weight for 19 weeks and all other parameters were evaluated 33 weeks.

Farm C

In this case, ROSS 308 parents were again raised in six halls, as in Farm A. Parameters were evaluated from 26 to 58 weeks of flock age. With supplementation, 18,960 layers and 2,380 males were evaluated, and without supplementation, 18,960 layers and 2,200 males were evaluated. For these flocks, egg weight was evaluated for 32 weeks, males feed intake for only 9 weeks and other parameters for 33 weeks.

Hall equipment and parameters

The same technological equipment was used in all the evaluated halls. The rearing of the parental flock was carried out on deep bedding (straw - mainly cut wheat). Around the laying nests there was a slat system, which normally in parental breeding ensures high hygiene of laid eggs. Feeding for the laying hens was done with a trough feeder system and for the roosters there was the option of both trough feeders and plate feeders, which were placed out of reach of the laying females to prevent the females from eating males feed. The watering system was designed with drip drinkers in all halls. The nests used were automatic emptying nests, followed by a daily collection of eggs laid outside them on deep litter twice a day. Climatic and zootechnical conditions in all halls were ensured by an automatic control unit. This

was particularly important in summer, when the control unit immediately started to adjust the temperature by means of the built-in air conditioning and dampers when the daily temperatures exceeded the optimum.

As far as fertility and hatchability of eggs were concerned, hatching quality eggs suitable for hatching (clean, without cracks) were included. Eggs from farms A and C were evaluated for two production periods and for farm B for only one. Data collection in the hatching facility of the company Trebic s.r.o. (Chropyne) was carried out once a week for both fertility and hatchability and from each flock separately. Only data when the ages of all three flocks could be paired were included in the statistical evaluation, to eliminate the influence of flock age on the results of fertilization and hatchability of eggs.

Farm A

In the first evaluation period (referred to as A1 in the table), which ran for 11 weeks for fertility and 32 for hatchability, 2.5 million eggs were collected as hatching eggs and evaluated in the hatchery from parents not given additives and 2.2 million hatching eggs from parents given additives. In the second production period (A2), evaluated over 23 weeks for hatchability and 7 weeks for fertility, 2.1 million hatching eggs from parents without additives and 1.9 million with additives were evaluated.

Farm B

Here for one production period (B), hatchability was observed for 9 weeks and egg fertilization for 28 weeks. Nearly 3.0 million eggs from parents without additives and 2.2 million eggs from parents with additives were evaluated.

Farm C

Lastly, for the first production period (C1), hatchability was evaluated for 27 weeks, and egg hatchability was evaluated for 14 weeks. The evaluation involved 2.5 million eggs from flocks without additives and 2.3 million eggs with additives added to the parents. The second production period (C2), when for 25 weeks of hatchability and 11 weeks of fertility of hatching eggs were evaluated, more than 2.1 million eggs from swarms without additives and almost 2.0 million eggs from parents with additives were evaluated.

To evaluate these data, the non-parametric Kruskal-Wallis method was used for evaluation throughout the study. To test the given significance, GLM model with LSD test was used to test the data. The program in which the data were evaluated was Unistat® version 10.11.

RESULTS AND DISCUSSION

Effect of additives on parental flock parameters

Neither significant nor non-significant major differences were observed for the feed intake/day parameter, indicating that additives did not have a negative effect. In parental flocks, the aim of the breeder is to ensure that the animals do not take in large amounts of feed and therefore rationing is used. In this way a balanced and healthy flock can be achieved. On farms A, B and C, there were no evident changes in feed intake/day for either males or females. However, in all three cases, intake was slightly increased (in the range of 1-4 g/feed/day). This increase in feed intake was also observed by GARCIA et al. (2002) in their study on the effect of canthaxanthin on egg production related parameters of females (weekly egg production, egg weight and

conversion and feed intake). In this case, they administered canthaxanthin at 60 ppm/kg of feed. This is contradicted by a later study by JOHNSON-DAHL et al. (2017), where, on the contrary, there was a lower weight of supplemented females compared to the control group. However, this phenomenon occurred in older flocks, when, on the contrary, maintaining a lower weight compared to fatter parental flocks is desirable.

Farm A

The results from the number of eggs produced here were the only ones to show a slight increase of 0.3 eggs per hen per week. This increase in egg production was also observed by COTO et al. (2010), when 25OHD3 was given to parental flocks, in parallel with the improvement in parameters such as shell thickness, fertilization and hatchability of eggs. SOUZA et al. (2008) when administered 6 mg of canthaxanthin found improvement in egg fertilization. Another parameter that was significantly improved was the reduction in culling and mortality of females ($P<0.001$), when the same result but not significant in farm C. This could be due, as in the study by KOUTSOS et al. (2003), who found a positive effect on poultry immunity when administering canthaxanthin with lutein (at a dose of 0.8- 38 mg/kg of mixed feed). Furthermore, similar results, but here in terms of vitamin D administration, were observed by KAKHKI et al. (2019) when administering it, a reduction in mortality of parental flocks occured, due to the reduction of fractures and skeletal system diseases.

Table 1. Effect of additives on performance parameters of parent stock on farm A

Parameter	Without aditives	With aditives	P value
Number of eggs per hen per week	4,8	5,1	NS
Number of hatching eggs per hen per week	4,6[b]	4,9[a]	<0,05
Weight of eggs (g)	63,5	63,5	NS
Culling and mortality of laying hens	0,40[a]	0,25[b]	<0,001
Culling and mortality of roosters	0,93	0,87	NS
Daily feed intake laying hens	162	161	NS
Daily feed intake roosters	118	120	NS

On Farm A, the flock was 26-57 weeks old.

Farm B

On this farm, neither a positive nor a negative effect of the addition of vitamin D and carotenoid-based additives was statistically proven in the parental flocks. Higher weekly culling and mortality was also observed in the groups given the additive. Without statistical conclusiveness, however, it is not possible to say with certainty whether this was the effect of the additive or another cause all together.

Table 2. Effect of additives on performance parameters of parent stock on farm B

Parameter	Without aditives	With aditives	P value
Number of eggs per hen per week	4,6	4,5	NS
Number of hatching eggs per hen per week	4,5	4,4	NS
Weight of eggs (g)	63,1	63,4	NS
Culling and mortality of laying hens	0,13	0,14	NS
Culling and mortality of roosters	0,83	1,00	NS
Daily feed intake laying hens	150	151	NS
Daily feed intake roosters	124	125	NS

On Farm B, the flock was 27-59 weeks old.

Farm C

No conclusive result was observed in farm C either. There were identical results for the number of eggs laid and the number of eggs of hatching quality, and for egg weight. However, a reduction was observed with the addition of additives for culling and mortality of the parent flock.

Table 3. Effect of additives on performance parameters of parent stock on farm C

Parameter	Without aditives	With aditives	P value
Number of eggs per hen per week	4,8	4,8	NS
Number of hatching eggs per hen per week	4,6	4,6	NS
Weight of eggs (g)	64,5	64,5	NS
Culling and mortality of laying hens	0,21	0,19	NS
Culling and mortality of roosters	0,64	0,59	NS
Daily feed intake laying hens	174	178	NS
Daily feed intake roosters	130	131	NS

On Farm C, the flock was 26-58 weeks old.

Effect of additives on fertilization and hatchability of eggs

When evaluating the data for the egg fertilization parameter, no statistically positive effect of additives was found. However, an improvement in egg fertilisation can be seen on two of the five farms. ROSA et al. (2012) observed similar results in terms of improvement in parental flock fertility when canthaxanthin was fed at the same level as here (6 mg/kg feed).

A statistically conclusive result can be seen for the egg hatching parameter. Here, on farm A1, there was a significant improvement ($P < 0.05$) of 5% in hatchability with the administration of additives. This agreed with the study by MATILLA et al (2011) where increased supplementation of flocks with 25OHD3 led to higher deposition of

this form of vitamin D in eggs. These eggs then showed better hatchability and chick quality.

Table 4. Effect of additives on fertilisation and hatchability parameters of eggs on individual farms

Farm	Egg fertilisation (%)		P value	Egg hatchability (%)		P value
	Without aditives	With aditives		Without aditives	With aditives	
A1	90,0	93,5	NS	75,4[b]	80,4[a]	<0,05
A2	95,9	96,0	NS	80,6	81,9	NS
B	96,9	96,8	NS	86,3	85,7	NS
C1	94,3	94,1	NS	81,1	81,3	NS
C2	94,5	94,3	NS	83,4	84,1	NS
All farms	94,1	94,7	NS	81,1	82,6	NS

CONCLUSION

When calcifediol and canthaxanthin were administered together and in addition to the technology instructions (i.e. and 100% of the intended need for maintenance and production), several demonstrations of the positive effect of higher doses of these two components were achieved. The following statistically conclusive results can be noted:

Effect of calcifediol and canthaxanthin administration on the parental flock

- When the additives were administered, in one case out of three, there was a 0.15% reduction in weekly mortality and culling of laying hens ($P < 0.001$).
- There was also a significant improvement in hatching egg production ($P < 0.05$) in one case out of three. This was an improvement in this parameter of 0.3 more hatching eggs per week per hen.

Effect of calcifediol and canthaxanthin administration on fertilization and egg hatchability

- In one case out of five, there was a conclusive increase of 5% in hatchability of chicks ($P < 0.05$).

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POSTER SECTION

DIGESTION AND MICROFLORA IN THE INTESTINAL CHYMUS OF CHICKENS AFTER FEED INTAKE OF ESSENTIAL OILS FROM SAGE AND OREGANO

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ABSTRACT

The objective of the study was the observation of the feed intake of essential oils from sage and oregano on enzymatic activities in the chymus of the jejunum and bacterial microflora in the caecum. The broiler chickens ROSS 308 (n=105, age one day) were divided into 3 groups (control, sage, oregano). The diets of experimental groups were supplemented with essential oils isolated from sage (*Salvia officinalis* L.) 2.306 g/kg and from oregano (*Origanum vulgare* L.) 1.179 g/kg. The increased enzymatic activities were observed as follows: a) amylolytic (glucose; μmol/l/min) by 0.09 ($p<0.01$, sage) on days 16 and by 0.05 and 0.04 ($p<0.001$, sage and oregano) on 29, b) cellulolytic (glucose; μmol/l/min) by 0.02 ($p<0.05$, sage) on days 16, by 0.06 ($p<0.001$, sage and oregano) on 29 and by 0.07 and 0.05

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(p<0.01, sage and oregano) on 42. The proteolytic activity (azocasein; µg/ml/min) decreased by 0.29 and 0.41 on day 16 (p<0.01, sage; p<0.05, oregano). The bacterial counts (log CFU/g wet digesta) in the chymus of caecum were increased in experimental groups compared to control on day 42: a) *Lactobacillus* spp. by 0.67 (oregano), b) *Enterococcus* spp. by 0.18 and 0.28 (sage and oregano). Counts (log CFU/g wet digesta) of *Escherichia coli* decreased by 0.66 (oregano) on day 29 and by 0.6 or 0.94 on day 42 (sage and oregano). The faecal digestibility of fibre was increased in all sampling periods on days 16 (p<0.01, exp1; p<0.001, exp2), 29 (p<0.01, sage; p<0.001, oregano) and 42 (p<0.01, sage and oregano). On the contrary, the digestibility of crude protein (CP) decreased on days 16 (p<0.01, oregano), 29 (p<0.05, exp2) and 42 (p<0.05, exp1 and exp2). The application of essential oils from sage and oregano has significantly positive effects on the enzymatic activities (amylolytic, cellulolytic), counts of *Lactobacillus* spp. and *Enterococcus* spp. and the faecal digestibility of fibre of the broiler chickens.

Keywords: poultry nutrition; plant extracts; zootechnical parameters; digestive enzymatic activities; faecal digestibility

INTRODUCTION

The gastrointestinal tract (GIT) of chickens is a complex and dynamic environment where digestion and microbial activities are closely intertwined. The composition of the gut microbiota significantly influences the health, nutrient absorption, and overall productivity of poultry. Recently, there has been a growing interest in utilizing essential oils (EOs) as natural feed additives to enhance poultry health

and performance (Windisch et al., 2008). Use of phytogenic products as feed additives for swine and poultry.

Essential oils derived from plants such as sage (*Salvia officinalis*) and oregano (*Origanum vulgare*) are particularly promising due to their high content of bioactive compounds like thymol and carvacrol, which possess potent antimicrobial, antioxidant, and anti-inflammatory properties (Zhang, 2021).

Research has shown that dietary supplementation with EOs can positively affect gut health by modulating the gut microbiota and improving digestive efficiency. Essential oils can stimulate the secretion of digestive enzymes and alter the microbial populations in the intestinal chyme, leading to enhanced nutrient absorption and growth performance in chickens. Specifically, EOs from sage and oregano have been highlighted for their ability to reduce pathogenic bacteria while promoting beneficial microbial communities in the gut (Hashemipour et al., 2013).

The objective of this study was to compare the influence of dietary intake of essential oils isolated from sage and oregano on some digestive enzymatic activities in the content of the jejunum and on bacterial microflora in the content of the caecum in the feeding trial with broiler chickens.

MATERIAL AND METHODS

The broiler chickens ROSS 308 (n=105, age one day) were from a commercial hatchery. They were randomly divided into 3 equal groups (control, exp1, exp2) and used in the feeding trial on a commercial farm for 42 days. The starting, growing and finishing diets were based on wheat, maize, extracted soybean meal (460 g/kg crude protein - CP),

extracted rapeseed meal (355 g/kg CP), sunflower meal, rapeseed oil and enzymes in commercial preparation Kenzyme W dry (Kemin Industries, Inc.).

The essential oils (100% v/v) isolated from sage leaves (*Salvia officinalis* L., family *Labiateae*) 2.306 g/kg (exp1) and oregano tops (*Origanum vulgare* L., family *Lamiaceae*) 1.179 g/kg (exp2) by steam distillation of plant materials in Calendula Inc. (Nová Ľubovňa, Slovak Republic) were added into the diets of experimental groups. The qualitative and quantitative parameters of both essential oils were determined by gas chromatography (GC) using a Hewlett-Packard 5890 Series II system (injection input split splitless, an HP-5 capillary column, detector FIF, an HP 7673 automatic injector) with nitrogen as carrier gas (Pavlišinová and Danielovič, 2007).

The control group was fed an identical ration without essential oils. Quantitative analyses of dry matter (DM), CP, crude fat (CF), crude fibre, ash, starch, Ca, P and Na were performed in diets (Faithfull, 2002).

The portion of insoluble ash in HCl was determined in feed mixtures as the ash residue after dissolving ash in diluted HCl by weighing (Daněk et al., 2005). The parameters of the diets that were determined are described in Table 1.

The body weights of chickens and the feed consumption were measured and the feed consumption and feed conversion ratio were calculated at 7-day intervals. The samples of chyme from the jejunum were prepared for the quantification of the activities of digestive enzymes as follows.

Table 1. Nutrients in the experimental diets

Ingredients [g/kg]	Experimental diets		
	Starting	Growing	Finishing
Dry matter	772.4	862.3	890.2
Crude protein	268.2	223.1	214.4
Crude fat	12.5	21.6	46.3
Crude ash	66	57.9	55.3
Starch	340.34	385.92	398.02
Ca	9.8	9.1	7.9
P	10.05	8.66	8.26
Na	2.8	2.5	2.3
Methionine	3.92	4.56	4.71
Lysine	10.05	13.39	13.42
Cystine	2.98	2.86	2.9
Insoluble ash	0.143	0.159	0.26
Metabolizable energy (MJ)	11.5	11.8	12

The fresh sample 1.0 g was mixed with 49 ml of sterile TBS buffer (10 mmol/l TRIS-hydroxymethyl aminomethane, 0.5 mol/l HCl, pH 7.0).

The samples were used for the measurement of the nonspecific proteolytic activity using azocasein as the substrate after homogenization. The analysis was performed using the method described by Broderick (1987). The cellulolytic and amylolytic activities were analyzed using methylhydroxyethylcellulose or starch as substrates, following the method by Lever (1977). The total protein concentration was quantified using the method by Bradford (1976).

The samples of chyme for the microbiological studies were prepared according to the method described by Muhl and Liebert (2007).

The identified bacterial groups and species comprised *Lactobacillus* spp., *Enterococcus* spp., and *Escherichia coli*. Total counts of *E. coli* were enumerated on McConkey agar, Enterococcus spp. on Slanetz-Bartley agar, and Lactobacillus spp. on Rogosa agar (Merck Ltd., Germany).

The digestibility checks were conducted on days 16, 29, and 42. The samples of faeces were analyzed for dry matter, crude protein, crude fibre, ash content, and insoluble ash fraction in HCl.

The data presented in this paper are expressed as means ± standard deviation (SD) of a single value (SAS, Version 8.2; SAS Institute Inc., 1999, Cary, NC, USA). The means of the results from treatments were compared using one-way analysis of variance.

RESULTS AND DISCUSSION

We expected that dietary supplementation with essential oils from sage and oregano could improve the growth parameters, digestion of nutrients, enzymatic activities in the jejunum and bacterial flora in the caecum. The results of the presented experimental study were in agreement with the expectations. The percentage ranges of the main components of essential oils used in the trial were as follows: from sage (eucalyptol 8.5%, alpha-thujone 14.8%, beta-thujone 7.2%, camphor 14.9%, borneol 3.7%) and oregano (carvacrol 60%).

The growth data of broiler chickens are summarized in Table 2. The essential oils from oregano significantly increased the average daily gain (ADG) ($p < 0.01$) during the periods of 1–16 days (14.55 ± 3.13 vs. 17.23 ± 4.04), 17–29 days (36.87 ± 13.14 vs. 46.60 ± 10.46), and ($p < 0.05$) 30–42 days of age (69.49 ± 17.99 vs. 80.77 ± 8.38). An increase in the same parameter was observed in the experimental

groups following the intake of essential oils from sage ($p < 0.05$), notably within the periods of 17–29 days (36.87 ± 13.14 vs. 44.89 ± 14.71) or 30–42 days (69.49 ± 17.99 vs. 98.19 ± 12.98) of age.

Table 2. Zootechnical parameters of broiler chickens (n = 105; mean ± SD)

	IBW [g]	FBW [g]	ADG [g/day]			ADFI [g/day]			FCR [g/day]		
day	1	42	1-16	17-29	30-42	1-16	17-29	30-42	1-16	17-29	30-42
Control	38.86±6.71	1746.67 ±340.52	14.55^{a} ±3.13	36.87^{a} ±13.14	69.49^{a} ±17.99	29.39 ±6.33	80.45 ±28.67	160.63±41.58	2.02	2.18	2.31
Sage	40.57±4.75	1947.06 ±274.14	14.49^{a}±3.24	44.89^{b} ±14.71	98.19^{b} ±12.98	28,69 ±6.42	89.52 ±29.33	203.30±26.87	1.98	1.99	2.07
Oregano	39.29±8.31	1840.0 ±231.0	17.23^{c} ±4.04	46.60^{c} ±10.46	80.77^{b} ±8.38	32.80 ±7.69	91.44 ±20.52	160.35±16.64	1.90	1.96	1.99

The enzymatic activities related to digestion (amylolytic, cellulolytic, proteolytic) were assessed in the chyme of the jejunum (Table 3).

The application of both essential oils into feed mixtures significantly increased amylolytic activity ($p < 0.001$) on day 29 of age. The essential oils from sage led to both enhancement ($p < 0.01$) and

reduction ($p < 0.05$) of the mentioned enzymatic activity on days 16 or 42 of age.

Table 3. Enzymatic activities in the chymus of the jejunum of broiler chickens (n=54; mean ± SD)

Age [day]	Group	Amylolytic (glucose) [µmol/l/min]	Cellulolytic (glucose) [µmol/l/min]	Proteolytic (azocasein) [µg/ml/min]
16	Control	$0.17^{a} \pm 0.011$	$0.11^{a} \pm 0.025$	$0.77^{a} \pm 0.063$
	Sage	$0.26^{c} \pm 0.038$	$0.13^{b} \pm 0.023$	$0.48^{c} \pm 0.062$
	Oregano	$0.17^{a} \pm 0.062$	$0.12^{a} \pm 0.017$	$0.36^{b} \pm 0.038$
29	Control	$0.1^{a} \pm 0.013$	$0.07^{a} \pm 0.015$	$0.41^{a} \pm 0.048$
	Sage	$0.15^{d} \pm 0.032$	$0.13^{d} \pm 0.038$	$0.41^{a} \pm 0.035$
	Oregano	$0.14^{d} \pm 0.013$	$0.13^{d} \pm 0.03$	$0.40^{a} \pm 0.055$
42	Control	$0.14^{a} \pm 0.025$	$0.16^{a} \pm 0.025$	$0.60^{a} \pm 0.066$
	Sage	$0.12^{b} \pm 0.015$	$0.23^{c} \pm 0.018$	$0.55^{a} \pm 0.07$
	Oregano	$0.13^{a} \pm 0.021$	$0.21^{c} \pm 0.025$	$0.56^{a} \pm 0.085$

Means with various letters differ significantly: (a,b) $p<0.05$, (a,c) $p<0.01$, (a,d) $p<0.001$

In spite of the fact that the mixture of enzymes was applied to all feed mixtures, the significant increase of amylolytic and cellulolytic activities in the chymus of jejunum was observed only in both experimental groups. The favourable effect of essential oils on amylolytic activities was in agreement with the conclusions of Jang et

al. (2007). Their observation was that activities of pancreatic alpha-amylase and intestinal maltase were elevated in the gastrointestinal apparatus of broiler chickens after the application of a blend of essential oils extracted from herbs. Recent research further supports these conclusions, demonstrating significant enhancements in enzymatic function upon treatment with essential oils (Marcin and Nad', 2017).

The cellulolytic activity significantly increased following the application of both essential oils ($p < 0.001$) on day 29 and ($p < 0.01$) on day 42. The sage essential oils significantly enhanced cellulolysis ($p < 0.05$) on day 16. Because the cellulolytic activity is not endogenous, the significant increase in the chyme of jejunum was caused by the stimulation of a limited population of bacteria with this enzymatic activity with the used essential oils.

A significant decrease in proteolytic activity was observed in both experimental groups ($p < 0.001$, $p < 0.01$) on day 16 of age.

The bacterial counts in the cecal chyme of broiler chickens are summarized in Table 4.

The dietary addition of a blend of essential oils extracted from herbs decreased the population of *E. coli* in ileocaecal digesta (Jang et al., 2007).

Several studies provided evidence for the antimicrobial activities of thymol and carvacrol *in vitro* (de Sousa et al., 2023).

Table 4. Bacterial microflora in the wet digesta in the caecum of broiler chickens (n=54)

Age [day]	Group	*Lactobacillus* spp. [log CFU/g wet digesta]	*Enterococcus* spp. [log CFU/g wet digesta]	*E. coli* [log CFU/g wet digesta]
16	Control	8	5.6	7.04
	Sage	8.18	6.36	6.85
	Oregano	8.2	6.32	6.78
29	Control	8.32	6.2	7.15
	Sage	8.64	6.6	7.11
	Oregano	8.85	6.88	6.49
42	Control	8.48	6.9	7.3
	Sage	8.94	7.08	6.7
	Oregano	9.15	7.18	6.36

On the contrary Cross et al. (2007) observed that dietary treatment with herbs, such as thyme, oregano, marjoram, rosemary and varrow had not any effect on the population of intestinal microflora, apparent metabolisable energy or the calculated coefficients of digestibility. Results obtained in the experiments *in vitro* (Marcin et al., 2006) with the agar spot test and agar diffusion paper disc test demonstrated higher antimicrobial activity of essential oils from oregano than from sage against tested bacterial strains: a/ *E. coli* – pig isolates, haemolytic,

K antigen positive, b/ *Salmonella enterica* var. *enteritidis* – pig isolate, c/ *Enterococcus faecium* M-74 – probiotic strain. The populations of *Lactobacillus* spp., *Enterococcus* spp. and *E. coli* were not significantly influenced by the dietary treatments.

Table 5. Faecal digestibility of broiler chickens (n=72; mean ± SD)

Age [day]	Group	CP (dc)	Fibre (dc)	Ash (dc)
16	Control	$64.12^{a} \pm 0.705$	$28.48^{a} \pm 0.712$	$39.04^{a} \pm 2.467$
	Sage	$61.94^{a} \pm 2.329$	$43.36^{c} \pm 0.997$	$44.64^{a} \pm 3.162$
	Oregano	$58.06^{c} \pm 0.749$	$46.32^{d} \pm 0.787$	$40.94^{a} \pm 1.556$
29	Control	$66.51^{a} \pm 2.501$	$33.99^{a} \pm 0.51$	$55.80^{a} \pm 3.471$
	Sage	$65.26^{a} \pm 2.088$	$43.51^{c} \pm 1.65$	$52.94^{a} \pm 1.853$
	Oregano	$57.61^{b} \pm 3.60$	$50.58^{d} \pm 1.518$	$48.82^{a} \pm 2.289$
42	Control	$64.18^{a} \pm 1.091$	$33.60^{a} \pm 0.874$	$46.18^{a} \pm 3.048$
	Sage	$57.41^{b} \pm 0.861$	$46.07^{c} \pm 0.848$	$49.84^{a} \pm 3.489$
	Oregano	$57.25^{b} \pm 1.202$	$47.18^{c} \pm 1.180$	$45.84^{a} \pm 3.897$

Means with various letters differ significantly: (a,b) $p<0.05$, (a,c) $p<0.01$, (a,d) $p<0.001$; dc – digestibility coefficient

The direct transfer of the results with the essential oils from the *in vitro* experiments to *in vivo* conditions of the digestive tract is very difficult.

The data on faecal digestibility are summarized in Table 5.

Significant differences were observed across all sampling periods for fibre. The digestibility coefficients of crude protein were notably lower with the feed mixture containing oregano in all samples, and with the feed mixture containing sage specifically on day 42 of age.

The fibre digestibility showed a significant increase in both experimental groups during the sampling intervals on days 16, 29, and 42.

The observed increase in fibre digestibility in both experimental groups on days 16, 29, and 42 suggests that oregano and sage positively influence the breakdown and absorption of fibre. This could be attributed to the bioactive compounds present in these herbs, which have been shown to enhance digestive processes. For instance, Castanon (2007) reported that essential oils and plant extracts could improve gut health by stimulating the growth of beneficial microbiota, which aids in fibre degradation. Similarly, Giannenas et al. (2003) found that the inclusion of oregano oil in animal diets enhanced fibre digestibility by increasing the activity of fiber-degrading enzymes.

In the case of the feed mixture containing sage, a notable decrease in crude protein digestibility was observed on day 42. This time-specific effect may indicate a delayed response or adaptation process in the gut microbiota or enzymatic activity in reaction to sage. Kamel (2001) suggested that the effects of herbal supplements on digestive processes might vary over time as the gut environment adapts to the new diet.

CONCLUSION

The addition of essential oils from sage and oregano to the diet of broiler chickens led to positive changes in amylolytic and cellulolytic activities in the chyme of jejunum, as well as in the digestibility

coefficient of fibre and the average daily weight gains of the broiler chickens.

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DIETARY ARTEMISIA ABSINTHIUM EFFECT ON GROWTH RATE AND FEED CONVERSION OF CHICKENS

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ABSTRACT

The aim of our study was to assess the dietary effect of *Artemisia absinthium* L. supplementation on the growth intensity and feed conversion of fattened chickens. A total of 120 female Ross 308 chickens at the age of 21 days were divided into 4 dietary groups. Chickens of the control group were fed a basal diet. Chickens of experimental groups were fed diets supplemented with meal of *Artemisia absinthium*; specifically, the A1, A5 and A10 groups were given diets with the addition of 1, 5 and 10 % *Artemisia absinthium* to the basal diet, resp. The supplementation of *Artemisia absinthium* to diets for fattened chickens led to the alteration of chicken growth rate in the assessed periods ($P < 0.01$), however, the final BW of chickens didn't vary among respective dietary groups ($P > 0.05$). Within the entire experimental period, the FCR value increased with the elevating proportion of *Artemisia absinthium* in a diet.

Keywords: diet; wormwood; meat-type chicken; production performance

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INTRODUCTION

Options to control avian coccidiosis include anticoccidal chemicals, vaccines and natural products. Anticoccidial chemicals, coccidiostats, coccidioides, and ionophores have long been used as a major tool to control coccidiosis in poultry production. While this is an effective and economically acceptable approach, the threat of resistance to these substances and public demands for residue-free meat has encouraged development of alternative control strategies (Chapman et al., 2010).

Vaccinations made up of one or more strains of wild-type or attenuated Eimeria species are among the other option for controlling coccidiosis in poultry farms (Muthamilselvan et al., 2016).

The use of phytogenic products as an alternative to anticoccidal agents can overcome the risks posed by chemical drugs. Moreover, poultry fattened with herbal additives are generally well accepted among current consumers (Srinivasu et al., 2020). The genus *Artemesia* is of interest due to its wide range of pharmacologic properties, such as antioxidant, anti-inflammatory, immune-boosting and antiparasitic effects. The dried plant of *Artemisia absinthium* L. (AA) is, among other specifications, characterised by high levels of proteins, which can play an effective role in fighting against various diseases (Amin et al., 2022).

The aim of our study was to assess the dietary effect of *Artemisia absinthium* L. supplementation on the growth intensity and feed conversion of fattened chickens.

MATERIAL AND METHODS

A total of 120 female Ross 308 chickens at the age of 21 days were used in the experiment. The chickens were divided into 4 dietary

groups of 30 individuals in each. Dividing of chickens in dietary groups was done with the aim of achieving an even distribution of body weight (BW) values and no different average BW among the dietary groups assessed. Chickens were housed in floor pens of the accredited experimental stable of the Department of Animal Breeding, Animal Nutrition and Biochemistry, University of Veterinary Sciences Brno under controlled housing conditions that respected standards used for husbandry of Ross 308 chickens (Aviagen, 2018).

The 2-phase feeding program was used; when the grower diet was fed from 21 to 35 days of age and the finisher diet was fed from 36 to 42 days of age (experiment finishing). The ingredient and nutrient composition of the basal diet is stated in Table 1; the basal diet was offered to chickens in the control (C) group. Chickens of experimental groups were fed diets supplemented with dry meal from whole aerial parts of *Artemisia absinthium* L. In particular, the A1, A5 and A10 groups were given the diets with the addition of 1, 5 and 10 % AA meal to the basal diet, respectively. Feed and water were supplied ad libitum. At 21, 28, 35, and 42 days of age, chickens were weighed and feed intake (FI) was recorded. The average weight gain and FI adjusted for mortality were used to find the feed conversion ratio (FCR; feed/gain).

The arithmetic mean was determined for BW and average daily gain (ADG) in particular dietary groups. To test normality of data distribution in these variables, the Shapiro-Wilk test was used. The normality was found in the both variables. A one-way ANOVA was used to analyse the diet effect on BW and ADG of chickens in their respective ages. Differences among groups were tested by the HSD post-hoc test. The significance was considered at the $P < 0.05$ level. All

statistical analysis were carried on by STATISTICA CZ, v. 10 software.

Table 1. Composition of a basal diet as fed basis

	Grower	Finisher
Ingredient composition (%)		
Wheat	9.38	9.68
Maize	49.5	49.5
Soybean meal	30.5	30.5
Canola oil	4.8	3.5
Monocalcium phosphate	0.7	0.7
Limestone	1.25	1.25
NaCl	0.24	0.24
$NaHCO_3$	0.20	0.20
Lysine	0.14	0.14
Methionine	0.23	0.23
Threonine	0.60	0.60
Maize sprouts	2.0	3.0
Mineral and vitamin premix	0.5	0.5
Mastercube®	0.5	0.5
Nutrient composition (%)		
Crude protein	19.2	19.3
Crude fibre	2.29	2.26
Crude fat	10.0	7.2
Ash	5.36	5.44
Metabolizable energy (MJ/kg)	14.4	14.1

RESULTS AND DISCUSSION

The AA herb meal supplemented to the chicken diet contained (on dry matter basis) following nutrients: 11.4 % of crude protein, 33.5 % of crude fibre, 2.2 % of fat, 44.9 % of nitrogen-free extractives and 8.1 % of ash. The dietary addition of AA to the grower at levels 5 and 10 % in our study led to a decrease in growth intensity ($P < 0.05$, $P < 0.01$, resp.) in the period between 21 and 27 days of age in chickens

compared to the group C, which was also associated with significantly ($P < 0.01$) lower BW of chickens at 28 days of age in the group A10 (Table 2). On the contrary, subsequently in period between 28 to 34 days of age, chickens in the group A10 showed the highest growth intensity ($P < 0.01$).

Table 2. Average body weight and daily weight gain of chickens in relation to *Artemisia absinthium* supplementation to diet

Age	Diet				*P*
	C	**A1**	**A5**	**A10**	
Body weight (g)					
At 21 days	766.6	742.9	735.4	758.4	*0.276*
At 28 days	1312.0^{A}	1265.6	1233.8	1187.3^{B}	*0.001*
At 35 days	1905.3	1882.6	1832.9	1928.6	*0.231*
At 42 days	2571.9	2485.3	2471.6	2589.6	*0.099*
Average daily gain (g)					
21 to 27 days	$77.9^{A,a}$	$74.7^{A,a,b}$	$71.2^{A,b}$	61.3^{B}	*<0.001*
28 to 34 days	84.8^{B}	88.1^{B}	85.6^{B}	105.9^{A}	*<0.001*
35 to 42 days	$95.2^{A,a,b}$	$86.1^{B,b}$	$91.2^{a,B,a}$	$94.4^{A,a,b}$	*<0.001*
21 to 42 days	86.0	83.0	82.7	87.2	*0.060*

^{a,b}: Means within a row with different superscript letters differ at $P < 0.05$; ^{A,B}: Means within a row with different superscript letters differ at $P < 0.01$.

This compensatory growth of chickens fed a finisher diet with addition of 10 % AA in that period was associated with an increase in their average BW, which was not different to the other dietary groups evaluated ($P > 0.05$), as well as, with the higher feed intake and most favourable feed conversion (1.54 kg/kg; Table 3). At the end of the

fattening period, the intensity of the growth did not differ among the chickens in groups C, A5 and A10 ($P > 0.05$), resulting in same final BW at 42 days among dietary groups evaluated ($P > 0.05$). An increase in feed consumption for groups A5 and A10 during this period led to the worsening of feed conversion than in chickens of the group C.

Table 3. Average feed intake and feed conversion of chickens in relation to *Artemisia absinthium* supplementation to diet

Age	Diet			
	C	**A1**	**A5**	**A10**
Feed intake (g/bird/day)				
21 to 27 day	113.9	109.3	108.9	106.5
28 to 34 day	145.4	140.8	141.6	163.2
35 to 42 day	165.1	164.4	170.8	188.0
21 to 42 day	141.5	138.2	140.4	152.6
FCR (kg/kg)				
21 to 27 day	1.46	1.46	1.53	1.74
28 to 34 day	1.72	1.60	1.65	1.54
35 to 42 day	1.73	1.91	1.87	1.99
21 to 42 day	1.65	1.67	1.70	1.75

FCR: feed conversion ratio.

In the course of the entire evaluated experimental period in our study, the FCR value elevated adequately with the increasing proportion of AA in a diet (Table 3). The initial reduction in growth intensity of chickens fed diets with addition of 5 and 10 % AA meal could be

related to both the intrinsic changeover to experimental diets and the likely slightly different nutritional composition of these diets compared to a basal diet (group C). The consequent significant increase in both growth intensity and feed consumption in chickens fed diets with higher AA inclusion indicates a stimulating effect of AA meal on the physiology of chicken GIT. This was likely related to higher levels of digestion, as well as altered nutrient absorption, especially during the period from 28 to 42 days of age. It would be advisable to conduct other profiled study, where diets with different levels of AA would be formulated as isoenergetic and isonitrogenous in order to better understand intrinsic dietary AA effects on chicken performance.

Recently, Cetin et al. (2019) didn't find differences in BW of chickens, when AA was supplemented at the level of 1.2 to 4.7 %; this is not consistent with our results. In addition, increased chicken BW at 42 days of age was reported by Kostadinović et al. (2015) if chickens were fed diets with supplementation of 15 and 20 % AA; the level of 10 % AA supplementation to the diet didn't alter chicken BW in their study. Similarly to our results, Kostadinović et al. (2015) recorded increased FCR values for chickens fed diets with higher AA levels. It should be added that the content of both basic nutrients and biologically active substances in the AA herb can vary significantly depending in particular on the geographical location of origin, the type of soil and the altitude of cultivation, the harvest time, the length and method of storage and so on (Amin et al., 2022).

CONCLUSION

The inclusion of *Artemisia absinthium* in diets for fattened chickens resulted in an alteration of chicken growth intensity in the assessed

period, with the final BW of chickens before slaughter not differing significantly among evaluated dietary groups. Furthermore, as the proportion of *Artemisia absinthium* in diets increased, the feed conversion was worsening proportionally in fattened chickens. In order to further clarify the dietary *Artemisia absinthium* effects on the chicken organism, further studies would be appropriate.

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INSECTS A NEW SOURCE OF NUTRIENTS IN DOG NUTRITION – A REVIEW

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ABSTRACT

Cynologists try to breed their dogs as best as possible within their economic and time possibilities. The dog needs regular exercise, training, treatment, proper nutrition, which will not cause damage to its health. When formulating an appropriate feeding ration, it is necessary to know the needs of the dog, which vary depending on age, breed, workload and breeding conditions. The market offers a wide range of dog food: dried feed mixture, canned food, and BARF. With complete feeds, the nutrients are balanced according to the needs of the category, size, use, or the health status of the dog. Feeds containing insects have a great potential due to the following attributes: a) insecs have a high protein content, b) high levels of lipids, c) vitamins and minerals. These nutrients vary widely depending on the insect species, stage of development, sex and other factors. Insects are rich in elements such as

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calcium, potassium, manganese, sodium, iron, copper, zinc and phosphorus, probably due to their food sources. Insects for dog consumption have a big potential to increase its importance as source of nutrients and as a sustainable and efficient feed source. Basically, insects are a natural food for dogs. Dogs often eat flies, bugs, wasps or even bees as part of a play or as an expression of their hunting instinct. The aim of this article was to get acquainted with possible insect sources and their nutritional composition in dog nutrition.

Keywords: dog; feed; insects; nutrients

People have fed, domesticated, and kept dogs for work and/or pleasure for thousends of years. A hundred years ago, however, dog breeders paid little attention to dog feeding because dogs' diets were very similar to their owners' diets, consisting of what owners could keep, such as bone joints, food scraps, and crusts bread. Only rich people could effort a high quality meat for dogs. Currently dogs are an integral part of our households, very offten considered as a member of the family, and a lot of owners try to provide a dog nutrition in harmony with latest scientific findings and feed safety requirements.

In order to clarify the correct feeding of a dog, it is necessary to know the physiology of its digestion as well as the history, from which we learn that the dog is not purely a carnivore. The dog was domesticated more than 15,000 years ago and comes from a single ancestor - the wolf. The wolf is a typical representative of a carnivore. As a canine animal, the dog is also classified as a carnivore, but after thousands of years of domestication, we can state that the dog is a partial omnivore. Only protein in the form of meat is not enough for a dog to cover all its nutritional needs, it also needs carbohydrates, fats, minerals and

vitamins. In dogs, we observe anatomical peculiarities in the digestive system that distinguish them from other carnivores. A dog's teeth are typical of carnivore. Includes small incisors, strong canines and large molars. With the help of this tooth, the dog does not chew food, but pulls out and swallows whole pieces of food. The digestive tube is relatively short and food passes through it faster. This increases the demands on the quality and digestibility of feed. However, in the course of domestication, the digestive tube was partially lengthened and the dog was given the opportunity to digest even more solid plant food. The dog's stomach is relatively large and this allows the dog to take in a large amount of food at once. For this reason, it is sufficient to feed the dog once a day. Hydrochloric acid, which is present in the gastric juice (0.5%), destroys the bacteria found in the feed. Thus, the dog is not at risk of bacterial infection from the feed. Another peculiarity in dogs are the anal sacs located near the anus. The cams produce a smelly secretion that helps dogs identify each other. Anal sacs react sensitively to the quality of food, and in case of inappropriate feeding (too hard, salty, spicy food) these glands can become blocked and subsequently inflamed (Kváš, 1998; Laukner, 2006; Suvegová, 1994). Even dogs were fed meat, bones, or small rodents and vegetable scraps from the kitchen until industrially produced feed came onto the market. However, feeding with industrial feed meant a total transformation of the organism into a different type of food. It takes about 24 hours for pelleted food to pass through the digestive system, while it takes about 6-8 hours for pure meat to pass through. The digestive tract is constantly burdened with unnatural feed mixtures and this causes improper fermentation in the intestines (Laukner, 2006; Novosádová, 2011; Zieglerova, 2016). Insects are considered a new

source of protein in dog nutrition. Black fly larvae, mealworms and crickets are a good alternative to both protein and oil sources in feed (Wall, 2022). Lisenko et al. (2023) in research on the digestibility of insect feeds for dogs and their effect on blood parameters, faecal properties, volatile fatty acids and intestinal microflora evaluated the effects of feeding three insect feeds in the diet of dogs. Cinerea cockroach, Madagascar cockroach and superworm feed is a sufficient source of protein included in the diet of adult dogs with the potential to replace conventional feed ingredients. Among insect feeds, differences in chemical composition have led to superworm as a highly digestible ingredient for dogs with a lower negative impact on intestinal fermentation products and microbiota profile. Case (2011) reports that a lack of protein in a dog's feed leads to growth retardation, weight loss and deterioration of condition, muscle loss, reduced immunity, bristly fur, loss of appetite, swelling and, in extreme cases, death.

Bosch et al. (2014) in one of the studies evaluated the quality of proteins in insect species. For research, they used: pupae of house flies, adult house crickets, larvae of mealworms - yellow, mealybug, Morio worm larvae and others. House fly, mainly pupae were high in protein, but less digestible. The protein content of crickets was high – similar to fishmeal, but in vitro digestibility was higher. Including insects in a dog's ration can have this effects: a) increases the source of proteins and oils in the feed (Wall, 2022); b) low probability of allergy to insect proteins (Böhm et al., 2018); c) higher protein and fat digestibility and lower fecal dry matter content (Abd El-Wahab et al., 2021); d) no intolerance and no physiological effects (Freel et al., 2021).

In some countries in Europe, pet products are made with insect-based ingredients. Among the most common species used in mixtures were

mealworms and black fly larvae (Wall, 2022). Regulation (EC) No. 999/2001 in order to allow processed animal proteins obtained from insects to feeding aquaculture animals is likely to open up the possibility of producing processed animal proteins obtained from insects in the Union on a larger scale. While the current microbreeding of insects intended as fodder for companion animals can be adequately regulated by existing national control systems, to ensure that the farming of insects on a larger scale in the EU is safe, appropriate provisions are necessary relating to animal health, public health, plant health or environmental risks accepted at the level of EU. As regards insect species reared in the EU, they should not be pathogenic species or such species should they not have any other adverse effects on the health of plants, animals or people; they should not be known as vectors human, animal or plant pathogens and should not be protected or defined as invasive non-native species. With regard to the mentioned national risk assessments, as well as the EFSA opinion of 8 October 2015 perhaps as those insect species which are currently kept in the EU and which meet the above safety conditions for rearing insects for use as feed, identify the following insects: black soldier fly (Hermetia illucens), housefly (Musca domestica), mealworm (Tenebrio molitor), barn fly (Alphitobius diaperinus), house cricket (Acheta domesticus), short-winged cricket (Gryllodes sigillatus) and Gryllus assimilis cricket (Gryllus assimilis). For the use of dried insects as feed for companion animals apply the provisions listed in Annex XIII to Regulation (EU) no. 142/2011. On the basis of the EU Regulation 2021/1925, eight species were added to the seven insect species, namely the silkworm (Bombyx mori).

Insects as a source of nutrients.

Analyzes show that eggs, larvae, pupae and adults contain 15 to 81% protein in dry matter (Xiaoming et al., 2008). The protein content ranges from 30% in worms to 81% in wasps of the genus *Polybia*. In locusts, the proportion of protein ranges from 52 to 77%. For beetles (*Coleoptera*) 36 to 71%, homoptera (*Homoptera*) 33 to 72%, butterflies (*Lepidoptera*) 34 to 71%, dragonflies (*Diptera*) 35 to 61% and in ants, bees and wasps (*Hymenoptera*) 10% to 81% (Ramos-Elorduy, 1997).

Table 1. Total crude protein content (%) (Xiaoming et al. 2008).

Order of insects	Development phase	CP
Coleoptera	Adults and larvae	23 – 66
Lepidoptera	Pupae and larvae	14 – 68
Hemiptera	Adults and larvae	42 – 74
Homoptera	Adults, larvae and eggs	45 – 74
Hymenoptera	Adults, pupae, larvae and eggs	13 – 77
Odonata	Adult dragonflies and nymphs	46 – 65
Orthoptera	Adult and nymphs	23 – 65

CP – crude protein

Table 2. Crude protein and fat content in selected species of invertebrates (%) according to available literature

Species	CP	Fa	Source
Hermetia illucens	35-57	35	Veldkamp et al., 2012
Tenebrio molitor	44-69	23-47	Veldkamp et al., 2012
Tenebrio molitor	50.7	n.d.	Bednářová, 2013
Tenebrio molitor	62.6	16.7	Adámková a Kouřimská, 2016
Alphitobius diaperinus	59.8	28.8	Adámková a Kouřimská, 2016
Musca domestica	43-68	4-32	Veldkamp et al., 2012
Locusta migratoria	62.2	n.d.	Bednářová, 2013

CP – crude protein; Fa – fat; n.d. – not defined

From the point of view of the development stage, larvae and pupae contain the largest amount of fat, in adults individuals, the fat

content is lower (Xiaoming et al., 2008; Chen et al., 2009). In general, the large amount of fat contains the larvae of beetles, caterpillars and termites (Bukkens, 1997). The average crude protein and fat concentration of the selected insects are shown in Table 2.

The caloric value of insects varies between 1225 and 3185 kJ/100g (Ramos-Elorduy et al., 1997). The amount of metabolizable energy depends on the fat content, so larval stages are usually more energy-rich. On the contrary, they have rather proteinaceous species lower energy content (Finke, 2002).

The content of minerals such as calcium, iron and zinc is also interesting, which is higher than in beef, chicken and pork. Likewise, the calcium content of some types of insects is up to 26 times higher than in chicken, pork or beef (Pavelková et al. 2019).

International Platform of Insects for Food and Feed - IPIFF (2022) provides a list of insects approved for the production of processed animal protein intended as feed for farm animals (other than fur animals) within the framework of EU regulation no. 142/2011. In Table 3 are listed the most common insects that were used in the researches mentioned above. However, not all of the cited articles reported the nutritional composition of all insect species, such as for *Musca domestica*. Table 3 shows a large variation in nutritional values not only between insect species, but also between developmental stages within a species.

Table 3. The nutritional composition of different species of edible crickets (Magara et al., 2024; EFSA, 2021a, 2021b, 2021c; EFSA 2022; Tyshko et al., 2021; Payne et al., 2016)

Species	Stage	CP	Fa	Fi	Ash	C	GE
Acheta domesticus	Nymph	62-71	10-23	10	5-9	n.d.	1904
	Adult	n.d.	19-30	n.d.	n.d.	n.d.	n.d.
	Frozen	15	n.d.	n.d.	n.d.	n.d.	477
	DP	60	n.d.	n.d.	n.d.	n.d.	2230
Tenebrio molitor	LL	n.d.	22		2		n.d.
	GL	42	42	n.d.	4	n.d.	n.d.
	Frozen	15	n.d.	n.d.	n.d.	n.d.	619
	DP	56	n.d.		n.d.		2151
Gryllus assimilis	Adult	56	22	8	6	12	1661
	Nymph	56	12	8	n.d.	8	n.d.
Gryllus sigillatus	Nymph	56	n.d.	n.d.	n.d.	n.d.	n.d.
Locusta migratoria	Frozen	14	n.d.	n.d.	n.d.	n.d.	690
	DP	55					2343
Alphitobius diaperinus	Frozen	189	n.d.	n.d.	n.d.	n.d.	669
	DP	61					2084
Hermetia illucens	FF	28	52	n.d.	7	n.d.	n.d.
	DF	55	10	7	8	n.d.	n.d.
Bombyx mori		18	10	n.d.	n.d.	n.d.	536
Musca domestica		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

CP – crude protein (g/100g dry matter); Fa – fat (g/100g dry matter); Fi – fibre (g/100g dry matter); Ash (g/100g dry matter); C – Carbohydrates (g/100g dry matter); GE – gross energy (kJ/100g dry matter); LL – live larvae; GL – ground larvae;DP – dried powder; FF – full fat flour; DF defated flour; n.d. – not defined-1-1

The mineral concentrations of the insects are given in Table 4. In the articles cited, the mineral content was given for only 3 insect species. Either way, both the mineral content and the overall nutritional value of the insects is substantially influenced by the substrate on which the

insects have been reared. The amino acids concentration is shown in the Table 5.

Table 4. Concentration of minerals of the insects in mg/100g (Udomisil et al., 2019; AJAI et al., 2013).

Species	Stage	Ca	P	K	Mg	Na
Tenebrio molitor	LL DP	17 32	319 700	373 727	88 145	40 81
Acheta domesticus	n.d.	150	899	390	137	101
Locusta migratoria	n.d.	n.d.	n.d.	48	148	29
Tenebrio molitor	LL DP	0.4 0.8	4.2 8.2	3.8 4.1	0.8 1.2	n.d.
Acheta domesticus	n.d.	4.4	20	8.8	4.9	n.d.
Locusta migratoria	n.d.	n.d.	16	57	9.9	0.9

LL – live larvae; DP – dried powder; n.d. – not defined

Gryllodes sigillatus

Order: *Orthoptera*; Family: *Gryllidae*

Tropical house cricket, Indian house cricket or banded cricket, native to Southwest Asia, widespread in tropical regions around the world. Due to its high thermal optimum, this species is considered non-invasive in temperate zones. Adults grow up to 20-22 mm, both male and female have reduced wings, in females only a small scale is visible behind the thorax. Tropical house crickets are light yellow in colour and can be easily distinguished from house crickets by two thick, black bands on the thorax and upper abdomen. *Gryllodes sigillatus* is extremely resistant to environmental conditions, and is very productive in mass culture, tolerating the high population density, as well as is immune to

the cricket paralysis virus. Protein content in larvae and imago varies from 60 to 70% (d.m.), with fat content of 20-25% (d.m.) and generally lower chitin content than average crickets. Incubation period (days from egg-laying to hatching) 12, and time to maturity (days from hatch to max body weight) 33-40 (IPIFF, 2024).

Table 5. Amino acid content in different types of insects g/100g (Bednářová et al., 2013)

Species	Ala	Arg	Asp	Cys	Phe	Glu	Gly	His	Ile
Acheta domesticus	3.0	3.5	5.4	0.5	3.8	7.2	2.9	1.8	2.2
Tenebrio molitor	3.2	4.6	4.0	2.9	2.9	6.9	1.5	2.5	3.2
Gryllus assimilis	4.0	8.6	3.0	0.7	0.7	3.6	2.4	1.3	2.1
Locusta migratoria	3.3	2.9	4.1	1.9	5.6	6.2	3.0	1.7	2.5
Bombyx mori	4.8	3.9	6.5	0.9	2.1	3.6	6.6	1.4	2.1
Acheta domesticus	3.6	3.2	1.4	4.1	2.5	2.4	0.4	2.2	3.2
Tenebrio molitor	6.1	3.6	1.9	0.9	1.3	0.3	0.3	0.9	0.7
Gryllus assimilis	5.0	7.9	0.6	1.3	0.6	3.6	1.0	5.4	4.6
Locusta migratoria	5.0	2.9	2.2	3.2	3.9	2.9	1.8	1.7	3.9
Bombyx mori	2.8	3.9	1.9	2.1	2.2	1.3	0.5	1.5	2.4

Ala – alanine, Arg- arginine, Asp – aspartate, Cys – cysteine, Phe – phenylalanine, Glu – glutamate, Gly – glycine, His – histtidine, , Ile – isoleucine, Leu – leucine, Lys – lysine, Met – methionine, Pro – proline, Ser – serine, Thr – threonine, Trp – tryptophan, Tyr – tyrosine, Val – valine

Gryllus assimilis

Order: *Orthoptera*; Family: *Gryllidae*

Jamaican field cricket, tropical species of cricket native to West Indies and Southern part of North America. Due to its high thermal optimum, this species is considered non-invasive in temperate zones. Adults grow up to 25-28 mm, both sexes are fully winged. Adult females are slightly bigger with prominent ovipositor protruding from the abdomen. Crickets are greyish yellow in colour, more robust than house crickets. *Gryllus assimilis* is relatively resistant to environmental conditions, and is productive in mass culture, however under high population density it shows a tendency towards cannibalism. Protein content in larvae and imagines varies from 50 to 65 % (d.m.), with a fat content of 25-30 % (d.m.). Contains a high level of chitin. Incubation period (days from egg-laying to hatching) 12. Time to maturity (days from hatch to max body weight) 42-49 (IPIFF, 2024).

Acheta domesticus

Order: *Orthoptera*, Family: *Gryllidae*

House cricket, native to Southwest Asia, widespread in tropical and temperate zones. Species are native to most of the European countries. Adults grow up to 20-22 mm, both sexes are fully winged. Adult females are slightly bigger with prominent ovipositor protruding from the abdomen. Crickets are greyish yellow in colour. *Acheta domesticus* is resistant to environmental conditions, and is very productive in mass culture, tolerating high population densities. The species is however very susceptible to the Cricket Paralysis Virus. Protein content in larvae and images varies from 60 to 70% (d.m.), with a fat content of 20-25% (d.m.). Incubation period (days from egg – laying to hatching) 11. Time

to maturity (days from hatch to max. body weight) 32-49 (IPIFF, 2024).

Tenebrio Molitor

Order: *Coleoptera*, Family: *Tenebrionidae*

Known as mealworm, species of the darkling beetles. It has a cosmopolitan distribution, being common in Europe, as a pest of the grain storages. The adult beetles are up to 15-18 mm long. It is shiny black or brown with reddish brown elytra. The eggs are oval, whitish, 1.5 mm long. The larvae resemble larvae of other mealworms, at the final stage measuring is up to 25 mm in lenght. *Tenebrio Molitor* is resistant to environmental conditions, and is very productive in mass culture, tolerating high population densities. Protein content in larvae varies from 50-65% (d.m.), with a fat content of 30-40% (d.m.) highly depending on the feed and rearing conditions. Incubation period (days from egg – laying to hatching) 10-12. Time to maturity (days from hatch to max. body weight) 280-400 (IPIFF, 2024).

Alphitobius diaperinus

Order: *Coleoptera*; Family: *Tenebrionidae*

Known as lesser mealworm or litter beetle, species of the darkling beetles. It has a cosmopolitan distribution, being common in Europe, as a pest of the grain storages and poultry farms. The adult beetles are 6 mm long, oval in shape. It is shiny black or brown with reddish brown elytra. Colour is variable among individuals and subpopulations and changing with age. The antennae are paler at the tips and are covered in tiny, yellowish hairs. The elytra have shallow longitudinal grooves. The eggs are narrow, whitish, about 1.5 mm long. The larvae resemble larvae of other mealworms, at the final stage measuring up to 11 mm in length. *Alphitobius diaperinus* is resistant to environmental conditions

and is very productive in mass culture. Protein content in larvae varies from 50 to 65% (d.m.), with fat content of 30-40 % (d.m.) highly depending on the feed and rearing conditions. Incubation period (days from egg-laying to hatching) 10-12. Time to maturity (days from hatch to adult) 280-400 (IPIFF, 2024).

Hermetia illucens

Order: *Diptera*; Family: *Stratiomyidae*

Black soldier fly, composting fly belonging to the soldier fly family. It is supposedly native to South America but is currently widespread in tropic and temporal zones worldwide. As it requires a high level of UV irradiation and temperatures above 24 °C to mate it must be considered as non invasive species in colder zones. The adult flies reach up to 15-18 mm in length. Adults are black, showing mimicry to wasps. The eggs are round, yellow and about 0.5 mm in diameter. The whitish larvae grow up to 25 mm in length, going through six instars to reach the brown prepupa stage. Prepupae leave the moist compost environment to seek for a dry place to pupate, that enables efficient separation of the larvae from the substrate. Larvae of the black soldier fly are very efficient composters, being able to digest a whole variety of organic products. *Hermetia illucens* grows in a wide range of environmental conditions, and is very efficient in mass culture, tolerating high population densities and being able to complete the life-cycle within 3 weeks. Protein content in larvae varies from 40 to 50% (d.m.), with a fat content of 35-45 % (d.m.) with high lauric acid content. Black soldier fly worms are an important source of animal protein. The dry matter content is 30% of their total original larval weight, of which 54% is crude protein (Hwangbo et. al., 2009). Exact composition highly depends on the feed and rearing conditions (IPIFF,

2024). Live pupae consists of 44% dry matter and can be easily store for longer period (Hale, 1973). Incubation period (days from egg-laying to hatching) 4. Time to maturity (days from hatch to max body weight) 12-60 (IPIFF, 2024).

Musca domestica

Order: *Diptera*; Family: *Muscidae*

House fly, most common fly species, with a cosmopolitan distribution, therefore it is a native species in Europe. The adult flies reach up to 10-12 mn in length. Adults are grey to black with four longitudinal dark lines on the back, and the body covered with hair-like protrusions. Eggs are usually laid on decaying organic matter, yellow in colour and about 0.5 mn in diameter. After few days of incubation, they hatch into legless white maggots which after two to five days of development transform into reddish brown pupae of ca. 8 mm in length. *Musca domestica* resistant to a wide range of environmental conditions, and is very efficient in mass culture, tolerating high population densities. Protein content in larvae varies from 40 to 65 % (d.m.), with a fat content of 20-45 % (d.m.). The amino acid profile composition highly depends on the feed and rearing conditions. Incubation period (days from egg-laying to hatching) 1. Time to maturity (days from hatch to max larval body weight) 2-30 (IPIFF, 2024).

Bombyx mori

Order: *Lepidoptera*; Family: *Bombycidae*

Domestic silk moth (*Bombyx mori*) is the lepidopteran whose caterpillar has been used in silk production for thousands of years. Its closest relative is the wild silk moth (*Bombyx mandarina*). Although native to China, the silkworm has been introduced throughout the world and has undergone complete domestication. An adult silkworm has a

cream-coloured body with dark veined wings of 40 to 50 mm span. The female silkworm lays about 300 to 500 eggs, which their hatching period fluctuates based on the environmental conditions. The voracious larvae are monophagous, exclusively feed on mulberry (*Morus spp*.) leaves, and may grow up to 75 mm in length. Pupation occurs within a cocoon composed of two proteins: soluble sericin and insoluble fibroin. Fibroin is the component of silk fiber and is present in single strands of 900-1000 meters long. After 10-14 days of developing, the silkworm moth will emerge. It lives a very brief life of 5-10 days. The silkworm pupae is the remaining after reeling of silk and can serve as feed material. The dry pupae contain 50–82% (d.m.) crude protein and 23–34% (d.m.) crude lipid. It has a rich and balanced content of essential amino acids such as valine, methionine and phenylalanine and is considered a good dietary source of protein for animal feed (poultry, cattle and all types of fish). The silkworm cocoon is made of fibroin (≈75%) and sericin (≈25%), two nutritive proteins that are a source of essential amino acids. Incubation period (days from egg-laying to hatching) 7-14 days at the temperature 23-29 °C and relative humidity 80%. Time to maturity (days from hatch to max body weight) 24-33 days (IPIFF, 2024).

Locusta Migratoria

Order: *Diptera*, Family: *Stratiomyidae*

The common name is migratory locust and it is considered as a pest in many regions of the world. Through history, large swarms of migratory lucusts have caused reducations in croup and vegetation. Migratory locust (*Locusta Migratoria***)** is the most abundant between the *locust* species and occur throughout Africa, Asia, Australia, New Zeland, and seldom in Europe. Their size ranges between 32 to 80 mm and the

development from egg deposit to adult is approximately 3 months depending on the environmetal conditions. Currently the migratory locust can be mass produced in colonies and the adults can be harvested and cosumed as human food (IPIFF, 2024).

The results of research by Bajuk et al. (2021) suggest that dogs allergic to mites can also clinically show cross-reactivity with mealworm proteins. Other hazards associated with the contamination of insects can be the result of flaweed processing produres realted to anthropogenic factors during breeding, packaging, cooking or feeding. Contaminants include the presence of bacteria, mold fungi, mycotoxins and heavy metals, among others. Importantly also, as an undesirable consequence of insects´readily incorporating nutrients from their diet into their body composition, we must be awarw of heavy metal accumulation such as copper, cadmium and lead.

CONCLUSION

From the above cited researches and studies, we can conclude that insects are rich in nutrients. Some insects are richer in live form, some in dried form. Their incubation period and the achievement of the imago is incomparably shorter compared to livestock serving as pet food and they can cover the nutritional needs of pets. Therefore, insects seem to be a good option as a substitute for some types of feed. Dogs allergic to mites can also clinically show cross-reactivity with mealworm proteins. Other hazards associated with the contamination of insects can be the result of flaweed processing procedures realted to anthropogenic factors during breeding, packaging, cooking or feeding. Contaminants include the presence of bacteria, mold fungi, mycotoxins and heavy metals, among others. Importantly also, as an undesirable

consequence of insects´readily incorporating nutrients from their diet into their body composition, we must be awarw of heavy metal accumulation such as copper, cadmium and lead.

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FEED UTILIZATION OF FOXTAIL MILLET (*SETARIA ITALICA*) GRAIN IN BROILER DIETS

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ABSTRACT

The aim of the study is the determination of the retention of crude protein (CP) and crude fat in the organism of broiler chickens after the addition of Foxtail millet (*Setaria italica (L.) Beauv.*) to the diet. At the same time, the performance parameters (live weight, carcass weight, feed conversion ratio (FCR)) were evaluated. Ross 308 male broiler chickens were included in the 36-day experiment, which were fed ad libitum diet of control (C), 5% of Foxtail millet (FM5) and 40% of Foxtail millet (FM40). In addition, their composition included the chromium oxide indicator (0.3%), which was used to determine the coefficients of apparent digestibility and retention of the already mentioned nutrients. The retention of CP was 67.78 % in the control mixture, 66.19% in the mixture FM5 and 68.62% in the mixture FM40. For crude fat, it was on average 84.95% (control mixture), 86.57% (FM5) and 90.21% (FM40). The CP retention of Foxtail millet was set at an average 73.15% over the observed period. There were no significant differences in the biochemical analysis of the blood

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($p > 0.05$) as well as in the production qualities of broiler chickens (assessed by the Scheffe test). The average carcass content was 69.07% for the control group, 69.05% for the FM5 group and 68.93% for the FM40 group. Feed conversion ratio was average 1.28 for control group, 1.32 for FM5 and 1.34 for FM40. Foxtail millet can therefore be included to the diet without negative impact on the health of the chickens or the nutrient retention, which increased compared to the control diet when 40% of the foxtail millet was added to the diet.

Keywords: nutrient retention; poultry nutrition; alternative crop; performance parameters

INTRODUCTION

Verma, Srivastava, and Tiwari (2015) describe millet as a multipurpose crop. It is suitable for human consumption (grain) and animal feed (grain, forage). For human consumption, the grain must be hulled in mills, as the kernel and hull are fused together. Published studies indicate that it has a higher nutritional value than rice.

Millet is primarily used for silage and hay production (University of Kentucky, ©2017). However, it can also be harvested for green mass production. In such cases, harvesting should occur during the early or full heading stage (Hermuth et al., 1997, as cited in Hermuth, Janovská, and Prohasková, 2015).

Rao et al. (2004) identified foxtail millet as a viable option for completely replacing corn in broiler diets without any negative impact on production. Boroojeni et al. (2011) reported that the inclusion of millet in broiler feed positively affected nutrient digestibility and significantly increased body weight at both 21 and 42 days. The presence of essential amino acids important in poultry nutrition is high

in millet, making it suitable for the production of feed mixtures. This is particularly true for methionine, cysteine, threonine, and lysine (Pack, Hoehler, and Lemme, 2003)

In an experiment focused on other objectives, Tirajoh et al. (2014a) examined the effects on breast muscle quality and blood profile in broilers when foxtail millet was gradually incorporated into feed as a replacement for corn. The experiment involved 250 one-day-old chicks, which were divided into 25 groups of ten birds each, with 5 groups receiving one of 5 different feed mixtures: a control mixture, a mixture with 2.5% corn replaced by millet, 5% corn replaced by millet, 7.5% corn replaced by millet, and 10% corn replaced by millet. Depending on the amount of millet used in the feed, the tenderness of broiler meat improved. However, the color of the meat noticeably lightened with the use of feed containing more than 5% millet. No excessively pale or yellowish breast muscle was observed. The experiment concluded that millet can be used as a 10% replacement for corn, resulting in increased meat tenderness.

Batonon-Alavo et al. (2015) also evaluated the response of broilers to the partial or complete substitution of corn with sorghum and millet. They found that feeds containing millet resulted in performance similar to that of corn, while feeds containing sorghum led to a reduction in growth parameters. No impact on feed intake was observed when corn was replaced with sorghum. The results obtained with sorghum substitution highlight the need to develop technological methods that could enhance the utilization of these feeds in broilers.

MATERIAL AND METHODS

Animals and experimental conditions

In 3 replicates, a total of 54 one day old male broiler chickens of Ross 308 were randomly divided into 3 different experimental groups (in total 18 chickens per feeding group) with 6 birds per cage. The lighting program, temperature and humidity was set according to the technological instruction (Aviagen, 2018). Broilers were fed with experimental starter diets until 12 days of age. Chickens were fed with experimental grower diets from 13th day until 36th day of age. The chickens were fed ad libitum. The feed intake of each group was daily recorded. The body weight was regularly noticed. The experimental lasted 36 days. At the end of the trial, broilers were slaughtered, and the carcass parameters were evaluated.

Diets and experimental design

Starter diets were fed until the 11th day of age, and grower diets were fed from the 12th day of age until the end of fattening. For the experiment, 3 variants of the diets were prepared – control C (without Foxtail millet), FM5 (with 5% representation of Foxtail millet-variety Rucereus) and FM40 (with 40% representation of Foxtail millet). The diets were created as isonitrogenous and isocaloric. The diets were non-pelleted and their composition is shown in the Table 1. The chromium oxide indicator was mixed into the diets to determine the retention of nutrients by the indicator method.

Table 1. Compounds and chemical composition of used diets

	STARTER			GROWER		
Component	**C**	**FM5**	**FM40**	**C**	**FM5**	**FM40**
Maize (g/kg)	173.8	171.45	130.0	190.1	170.0	135.5
Soybean meal (g/kg)	438.0	438.0	438.0	300.0	290.0	290.0
Wheat (g/kg)	383.0	343.4	450.0	373.8	353.4	44
Rapeseed oil (g/kg)	42.1	40.0	32.2	57.0	54.1	50.0
Premix* (g/kg)	30.0	30.0	30.0	30.0	30.0	30.0
Limestone milled (g/kg)	6.1	6.0	5.8	4.4	4.4	4.1
Monocalcium phosphate (g/kg)	8.0	8.1	8.7	7.4	7.4	7.9
DL-Methionine (g/kg)	1.2	1.25	1.25	1.5	1.5	1.5
L-lysine	2.0	2.4	2.95	0.0	0.2	0.7
Wheat gluten (g/kg)	50.1	53.7	50.4	33.4	36.4	33.9
Foxtail Millet	0.0	50.0	400.0	0.0	50.0	400.0
Sodium chloride	0.7	0.7	0.7	0.7	0.7	0.7
Chromium oxide (g/kg)	3.0	3.0	3.0	3.0	3.0	3.0
MEN (MJ/kg)**	12.39	12.41	12.49	12.76	12.75	12.91
Crude protein (g/kg)	264.75	231.24	237.33	224.26	219.05	221.68
Ether extract (g/kg)	60.18	60.59	61.22	774.29	72.62	75.19
Crude fibre (g/kg)	30.50	31.38	44.56	30.76	30.29	47.98
Crude ash (g/kg)	64.74	67.02	69.99	62.63	62.60	63.42

Legend: **Premix for starter contains (per kg): L-lysine 2.34 g; DL-Methionine 2.4 g; Threonine 0.99 g; calcium 5.25 g; phosphorus 1.95 g; sodium 1.44 g; copper 15 mg; iron 84 mg; zinc 99 mg; manganese 99 mg; iodine 0.99 mg; selenium 0.18 mg; retinol 13,500 IU (international units); calciferol 5,001 IU; tocopherol 45 mg; phylloquinone 1.5 mg; thiamine 4.2 mg; ri-boflavin 8.4 mg; pyridoxin 6 mg; cobalamin 30 µg; biotin 0.21 mg; niacinamid 36 mg; folic acid 1.8 mg; calcium pantothenate 13.5 mg; cholin chloride 180 mg.*

Premix for grower contains *(per kg): L-lysine 2.58 g; DL-Methionine 2.52 g; Threonine 1.47 g; calcium 5.04 g; phosphorus 1.65 g; sodium 1.38 g; copper 15 mg; iron 75 mg; zinc 99 mg; manganese 99 mg; iodine 0.9 mg; selenium 0.36 mg; retinol 9,900 IU (international units); calciferol 5,001 IU; tocopherol 45 mg; phylloquinone 1.5 mg; thiamine 4.2 mg; ri-boflavin 8.4 mg; pyridoxin 6 mg; cobalamin 28.8 µg; biotin 0.18 mg; niacinamid 36 mg; folic acid 1.71 mg; calcium pantothenate 13.35 mg; cholin chloride 180 mg. ** Apparent metabolize energy, calculated value.;); C-control group; FM5-5% of Foxtail millet in diet; FM40-40% of Foxtaill millet in diet*

At the end of the experimet at 36 days of age, the chickens were weighed and slaughtered. Subsequently, the weight of the carcass without giblets, and neck was determined and the percentage. In selected 12 individuals from each group, breast and thighs muscles were deboned, and the percentage of breast and thighs muscles in live weight was determined by calculation. During the experiment, excreta was regularly collected to determine CP and fat retention (calculation using the indicator method) (Zeman et al., 2006).

Statistical analysis

Data has been processed by Microsoft Excel (USA) and StatSoft Statistica (USA). It was used one-way analysis of variance (ANOVA). For evaluate statistically differences between groups was used the Sheffé´s test and $p < 0.05$ was regarded a level of statistically significant difference.

RESULTS AND DISCUSSION

Table 2 shows the weights of broilers during the experiment. There were no statistically significant differences between the groups in the individual terms ($p > 0.05$). The final weight at 36 days of age corresponds to the performance parameters of Aviagen (2 332 kg) for the given ROSS 308 hybrid (Aviagen, 2019).

Table2. Live weight of broilers fed diet with foxtail millet during the experiment

Age (days)	C	FM5	FM40
n	18		
	Mean (g) ± SD		
1	46 ± 5.2	45 ± 2.7	44 ± 4.4
8	171 ± 26.4	173 ± 21.4	154 ± 23.7.
12	316 ± 51.5	341 ± 46.7	301 ± 54.3
15	463 ± 65.5	501 ± 67.0	448 ± 87.7
22	980 ± 129.3	1 000 ± 114.2	922 ± 207.2
29	1 648 ± 202.2	1 670 ± 181.4	1 655 ± 241.3
36	2 440 ± 303.8	2 435 ± 261.6	2 411 ± 319.9

n.s. ($p > 0.05$); C-control group; FM5-5% of Foxtail millet in diet; FM40-40% of Foxtaill millet in diet; SD-standard deviation

Also, no statistically significant differences were found between the values of average feed consumption per 1 chicken and feed conversion ($p > 0.05$). The FCR value was 1.23 vs. 1.32 vs. 1.35 (C vs. FM5 vs. FM40, respectively). The FCR for all groups was lower than that reported in AVIAGEN (2019) at 1.45. This may be due to the laboratory conditions and minimal movement of the chickens in the cages.

In the experiment conducted by Bugalia et al. (2009), weight gain and feed conversion increased as the millet content in the feed mixture increased. Similar results were obtained by Shekhawat, Mathur, and Kumar (2013), who used millet as a substitute for corn. They observed that both body weight and weight gain increased with the higher levels of millet replacing corn. Likewise, Reddy and Narahari (1997)

achieved similar results when using 40% millet in the feed mixture, with weight gains increasing as the millet level rose. Boroojeni et al. (2011) also confirmed a linear increase in body weight corresponding to higher millet content in the feed. However, in this experiment, the results showed only minimal differences, and with a higher proportion of millet in the feed, there was a decrease in the chickens' weight.

Tirajoh et al. (2014b) evaluated millet as a potential replacement for corn in broiler feeds. However, due to its high phytic acid content, millet may not be able to fully replace all the corn in the feed.

Table 3. Carcass, breast and thighs yield of broiler chickens fed diet with foxtail millet

	C	**FM5**	**FM40**
n		12	
Yield		Mean (%) ± SD	
Carcass	69.07 ± 2.03	69.05 ± 2.2	68.93 ± 1.48
Breast	21.30 ± 1.37	20.59 ± 2.04	20.17 ± 1.44
Tighs	14.14 ± 0.70	14.06 ± 0.83	14.48 ± 0.89

n.s. ($p > 0.05$); C-control group; FM5-5% of Foxtail millet in diet; FM40-40% of Foxtaill millet in diet; SD-standard deviation

The Table 3 presents the carcass yield values of the chickens. No statistically significant differences were found between the groups ($p > 0.05$). Shekhawat and Kumar (2016) reported similar findings. In their study, they replaced corn with millet and did not observe significant changes in the eviscerated carcass weights.

Table 4. Retention of nutrients of boiler chickens fed diet with foxtail millet

	C	FM5	FM40
n	21		
Retention of:		Mean (%) ± SD	
Crude protein	67.78 ± 3.95	66.19 ± 3.61	68.62 ± 3.59
Crude fat	84.95 ± 11.12	86.57 ± 8.36	90.21 ± 6.07

n.s. ($p > 0.05$); C-control group; FM5-5% of Foxtail millet in diet; FM40-40% of Foxtaill millet in diet; SD-standard deviation

The values in the Table 4 for nitrogen and fat retention were evaluated as statistically insignificant ($p > 0.05$). However, slight changes were observed, with a slight increase in the retention of both nitrogen and fat when 40% millet was included in the feed mixture.

Boroojeni et al. (2011), who studied the effects of replacing corn with millet, concluded that incorporating millet into the feed mixture does not adversely affect the digestibility of crude protein. The apparent ileal digestibility of crude protein was, on average, 73.35% with a 33% replacement of corn by millet, 76.05% with a 66% replacement, and 81.73% with a 100% replacement.

In the case of the initial feed mixtures, where the average coefficients of balance digestibility for the FM5 group were 66.19% for crude protein and 86.57% for fat, and for the FM40 group were 68.62% for crude protein and 90.21% for fat over the observed 36-day period, it can be said that the best nutrient utilization for both crude protein and fat was observed in the FM40 group.

CONCLUSION

From the results of the experiment, it can be claimed that Foxtail millet can be included in the broiler´s diet in low and high (40%) proportions, without having a negative impact on productivity or retention of nutrients (NL and fat). Nutrient retention (CP and fat) compared to the control mixture even increased when 40% foxtail millet was included in diet.

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