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PROCEEDINGS

THE USE OF LUPIN IN LAYING HEN NUTRITION: A REVIEW OF ITS EFFECTS ON PERFORMANCE, EGG QUALITY AND HEALTH STATUS

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ABSTRACT

The aim of this review was to evaluate the potential of narrow-leafed (*Lupinus angustifolius*), yellow (*Lupinus luteus*) and white lupin (*Lupinus albus*) as alternative protein sources in laying hen nutrition and to summarize their effects on productive performance, egg quality and health status. Relevant studies published during the last 10–15 years were identified through searches of scientific databases using keywords related to lupin, laying hens, poultry nutrition, and legumes.

Available studies show that lupin can partially replace soybean meal in the nutrition of laying hens without a negative effect on performance, when included at appropriate levels. Narrow-leafed lupin is the most extensively studied species, with recommended inclusion levels of 15–20 %. Yellow lupin shows comparable effects on egg quality, although recommended inclusion levels vary among studies. White lupin has been studied less frequently, with dietary concentrations up to 15–20 % generally considered safe.

Across species, the most consistently reported benefits include improved yolk pigmentation and a more favourable fatty acid profile. Several studies have also described positive effects on gut environment and intestinal fermentation. In contrast, excessive lupin inclusion may impair performance and egg quality, likely due to antinutritional factors and reduced nutrient digestibility.

Overall, lupin represents a promising alternative protein source for laying hens; however, its successful use depends on lupin species, inclusion level, feed formulation, and seed processing methods.

Keywords: alternative protein sources; legumes; soybean meal replacement; poultry feeding; productive performance; fatty acid profile; gastrointestinal health



INTRODUCTION

The search for alternative protein sources has become one of the major priorities of European livestock production. Following the ban on meat and bone meal in animal feeding, soybean products have become the dominant protein source in feed formulations for livestock and poultry. However, the increasing cost of imported soybean commodities, together with concerns regarding genetically modified crops, has intensified interest in locally produced alternative protein sources (Abraham et al., 2019).

Among the most promising alternatives are cultivated lupin species (*Lupinus* spp.), particularly their seeds, which contain crude protein concentrations comparable to those of soybean seeds (David et al., 2024; Struti et al., 2020). In addition, modern lupin cultivars are characterized by relatively low concentrations of antinutritional factors compared with other grain legumes and by a favourable profile of fatty acids (Gresta et al., 2023). The use of lupin seeds in poultry diets depends on appropriate dietary formulation and adequate balancing of limiting amino acids (Lanza et al., 2025).

Although earlier studies reported adverse effects on laying hen performance, mainly due to nutritional imbalances and antinutritional compounds, more recent research indicates that properly formulated lupin-based diets can achieve performance comparable to conventional soybean-based diets (Jeroch et al., 2016).

The aim of this review was to summarize current knowledge regarding the use of different lupin species and cultivars in laying hen diets, with particular emphasis on their effects on productive performance, egg quality, health status and gastrointestinal function. In addition, selected technological processing methods used to improve the nutritional value and digestibility of lupin seeds are discussed.

MATERIAL AND METHODS

This review was based on scientific publications focusing on the use of different lupin species in diets for laying hens. Relevant studies were identified through searches of the Web of Science, ScienceDirect and Scopus databases.

The literature search was conducted using the keywords “lupin”, “*Lupinus albus*”, “laying hens”, “nutrition”, “poultry” and “legumes”, as well as their various combinations. Studies published within the last 10–15 years were considered for inclusion. Publications were selected according to their relevance to the topic, with primary emphasis placed on studies evaluating the effects of dietary lupin inclusion on productive performance, egg quality and health-related parameters in laying hens.

RESULTS AND DISCUSSION

Narrow-leafed lupine

Production performance and egg quality

Most studies evaluating narrow-leafed (blue) lupine in laying hen diets compared different inclusion levels with conventional soybean-based diets, while some also assessed seed processing methods and enzyme supplementation.

Studies evaluating narrow-leafed lupine generally indicate that inclusion levels up to 20% do not adversely affect laying performance or egg quality (Dražbo et al., 2014; Kowalska et al., 2020). Improved yolk pigmentation and a more favourable fatty acid profile were consistently reported. Park et al. (2016) even observed increased egg production in hens fed lupine-containing diets, while lower inclusion levels were associated with a higher proportion of oversized eggs. However, the effects on eggshell quality remain inconsistent. Dražbo et al. (2014) reported improvements in eggshell thickness, weight and strength, whereas Kowalska et al. (2020) and Park et al. (2016) found no significant effects. A slight reduction in egg weight was observed only at the highest inclusion level (25 %) evaluated by Kowalska et al. (2020). Lee et al. (2016) evaluated the use of 15% narrow-leafed lupine as a protein source in laying hen diets, comparing whole and dehulled seeds, with or without enzyme supplementation. Similar to previous studies, no adverse effects on laying performance or hen health were observed. Lupine inclusion resulted in redder and lighter yolks, while a tendency toward reduced eggshell thickness was reported, particularly in enzyme-supplemented diets. No negative effects on gastrointestinal health or bacterial load were detected.

Seed processing technologies may improve the nutritional value of lupine and its utilization by laying hens. Kponouglo et al. (2024) demonstrated that fermentation improved the nutritional quality of lupine-based diets and resulted in better egg production, egg quality, and maintenance of body weight, whereas germinated grains were associated with reduced laying performance and body weight loss. In addition, all experimental diets reduced egg cholesterol content.

Similarly, Tiruneh et al. (2025) reported positive effects of ground and roasted narrow-leafed lupine seeds on performance and economic efficiency in dual-purpose chickens. The authors suggested that processed lupine could replace a substantial proportion of soybean meal, although direct comparison with laying hen studies is limited due to differences in production type. Tomczak et al. (2024) found that partial replacement of soybean meal with narrow-leafed lupine did not impair laying performance but significantly affected egg protein composition. Higher lupine inclusion was associated with lower protein content in albumen and higher protein content in yolk. According to the authors, a combination of lupine and reduced soybean meal may represent the most practical compromise between nutritional quality and feeding efficiency.

Health, gastrointestinal and metabolic effects

In addition to production performance, the effects of blue lupine on the gastrointestinal tract, gut microbiota, and metabolic processes in laying hens have also been investigated.

Zdunczyk et al. (2014) reported that blue lupine positively modulated the caecal microbiota and increased its fermentative activity, while Lee et al. (2016) found no adverse effects on gastrointestinal health, bacterial load, or overall hen health status.

Wójcik et al. (2024) showed that short-term supplementation with narrow-leaved lupine had no adverse effects on oxidative balance, whereas long-term inclusion of 20–25% may impair liver function and increase oxidative stress. Inclusion levels of 10–15% appeared to be the most favourable, showing only minor adverse effects together with a slight improvement in antioxidant activity. Similarly, Grabowski et al. (2025) observed changes in hepatic protein expression and increased synthesis of acute-phase proteins, particularly at the 25% inclusion level, suggesting activation of metabolic and immune responses under high lupine supplementation.

Yellow lupine

Production performance and egg quality

Several studies have evaluated yellow lupine in combination with other legumes or oilseed meals as an alternative to soybean meal in laying hen diets.

Kowalska et al. (2021) reported that yellow lupine-based diets improved several egg quality traits, including albumen quality, yolk pigmentation, and fatty acid composition. Similar effects on egg quality were observed by Kuźniacka et al. (2020), who additionally reported no adverse effects on laying performance, despite a reduction in albumen height and an increase in albumen weight. Likewise, Krawczyk et al. (2024) found that replacing soybean meal with legumes, including yellow lupine, did not affect laying performance but altered several egg quality characteristics, including yolk colour, Haugh units, shell porosity, and lysozyme activity. In contrast, Rutkowski et al. (2015) reported reduced laying performance, egg weight, and feed efficiency at higher legume inclusion levels, although improvements in albumen quality and yolk pigmentation were also observed. Based on these findings, the authors recommended limiting legume inclusion to approximately 20% of the diet.

In contrast to mixed diets, several studies have evaluated yellow lupine as the main protein source in laying hen diets. Rutkowski et al. (2017) reported that inclusion levels up to 20% had no adverse effects on performance, whereas 25% lupine reduced laying rate, egg weight, feed intake, and feed efficiency. Increasing lupine inclusion improved yolk pigmentation and slightly increased Haugh unit values, but was also associated with poorer eggshell quality. Conversely, Krawczyk et al. (2015) found no negative effects on laying performance, feed

conversion, eggshell quality, or albumen quality at inclusion levels up to 30%. Similar to previous studies, yolk colour intensity increased with lupine supplementation, while favourable changes in yolk fatty acid composition and a slight reduction in cholesterol content were also observed.

Gastrointestinal function and gut microbiota

Studies investigating yellow lupine have evaluated not only production performance but also its effects on intestinal morphology and microbial fermentation. Zdunczyk et al. (2019) reported no adverse effects of yellow lupine (10–30%) on production performance, nutrient digestibility, or blood parameters, except for a reduction in cholesterol concentration. Lupine inclusion induced changes in intestinal morphology that may reflect improved absorptive capacity and was associated with enhanced caecal fermentation, increased short-chain fatty acid production, lower pH, reduced ammonia concentrations, and higher microbial enzymatic activity. However, the authors noted that higher inclusion levels may increase the content of antinutritional factors, particularly non-starch polysaccharides and raffinose family oligosaccharides.

White lupine

Production performance and egg quality

White lupine has been less extensively studied than narrow-leaved or yellow lupine, but available evidence suggests that it can partially replace soybean meal in laying hen diets without adverse effects on performance or egg quality when included at appropriate levels. Kubis et al. (2018) recommended inclusion levels of 6–12%, whereas Mierlita (2013), Criste et al. (2017), and Laudadio and Tufarelli (2011) reported satisfactory results at approximately 15–20% inclusion. Higher concentrations generally impaired performance, particularly egg production, egg weight, and feed intake (Kubis et al., 2018; Mierlita, 2013; Criste et al., 2017).

In addition to maintaining acceptable performance, white lupine was associated with improvements in egg quality, including enhanced yolk pigmentation and a more favourable fatty acid profile (Criste et al., 2017; Laudadio and Tufarelli, 2011). Similarly, Timová et al. (2020) reported reduced saturated fatty acids and increased mono- and polyunsaturated fatty acids in egg yolks from hens fed white lupine diets, indicating improved nutritional value of eggs.

Blood parameters

Straková et al. (2021) evaluated the effects of replacing soybean meal with dehulled and whole white lupine (cv. Zulika) on blood biochemical parameters in laying hens. Long-term lupine feeding did not adversely affect the health status of hens and had no effect on most blood indicators. However, lupine inclusion was associated with reduced concentrations of total plasma protein, cholesterol, calcium, and triacylglycerols, together with increased alkaline phosphatase activity.

Comparison of lupine species

The interpretation of comparative results is complicated by considerable variability in experimental conditions. Individual studies have used different lupine species and cultivars, varying dietary inclusion levels, and different seed processing methods. Direct comparison of results is therefore limited.

All three lupine species show potential as partial replacements for soybean meal in laying hen diets. Narrow-leafed lupine is the most extensively studied species, with relatively consistent evidence supporting inclusion levels of 15–20%. Yellow lupine produces comparable effects on egg quality, although recommended inclusion levels vary more widely (20–30%) and effects on production performance are less consistent. White lupine has been investigated least frequently, with most studies recommending lower inclusion levels (approximately 5–15%), although successful use at 15–20% has also been reported.

Across species, the most consistent benefits include improved yolk pigmentation and a more favourable fatty acid profile, resulting in enhanced nutritional value of eggs. In contrast, high dietary inclusion levels may reduce laying performance, egg weight, and feed efficiency, likely due to the presence of antinutritional compounds such as non-starch polysaccharides and raffinose family oligosaccharides.

Several studies also suggest beneficial effects of lupine on gut microbial activity and fermentation processes, whereas excessive long-term inclusion may negatively affect hepatic metabolism and oxidative balance. Lupine-containing diets generally do not adversely affect blood biochemical parameters and may contribute to reduced cholesterol concentrations in both blood and eggs.

Overall, when used at recommended inclusion levels, lupine does not adversely affect laying performance, egg quality, or health status and represents a promising alternative protein source for laying hens.

Tab. 1: Comparison of the effects of different lupin species in laying hens

Species	Recommended inclusion (%)	Effects on performance	Effects on egg quality	Health effects
<i>L. angustifolius</i>	15–20	generally no negative effects	Improved yolk and PUFA profile	Positive effects on gut microbiota; adverse liver effects at high levels
<i>L. luteus</i>	20–25	Variable results among studies	Improved yolk colour, variable shell quality	Enhanced gut fermentation and SCFA production
<i>L. albus</i>	6–15	Generally no negative effects	Improved fatty acid profile	Favourable blood biochemical parameters

CONCLUSION

Lupine represents a promising alternative to soybean meal in laying hen nutrition. Available studies indicate that, at appropriate inclusion levels, it does not negatively affect production performance or health status and may improve egg quality, particularly yolk color intensity and fatty acid profile.

Narrow-leafed lupine is the most extensively studied species, with a recommended inclusion levels ranging from 15–20 %. Yellow lupine shows comparable effects; however, greater variability exists among studies regarding optimal inclusion levels. White lupine is the least studied, with suggested inclusion levels typically not exceeding 15 %, or in some cases 20 %.

High dietary inclusion of lupine may impair laying performance, egg weight, and feed conversion, most likely due to antinutritional compounds and reduced nutrient digestibility. Conversely, several studies suggest beneficial effects on gut environment, fermentation processes, and selected metabolic parameters.

Further research is needed to define optimal inclusion levels for individual lupine species and to evaluate processing technologies aimed at improving nutritional value and reducing antinutritional factors.

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EFFECT OF PEA PROTEIN ON BROILER PERFORMANCE

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ABSTRACT

The aim of this experiment was to investigate the effect of incorporating pea protein into broiler diets on growth, feed intake, feed conversion, yield, and the parameters of the main meat cuts. Pea protein was incorporated into the diets as a substitute for soybean meal. The cultivation of soybeans and their import into Europe currently appear problematic from an environmental perspective; therefore, alternative protein sources are being sought to reduce the use of soybeans in animal diets. The experiment included 128 Ross 308 broilers, which were fattened for 36 days. Four groups were formed—a control group (K) and three experimental groups, which were fed a diet containing pea protein that replaced soybean meal at levels of 25 % (H25), 50 % (H50), and 100 % (H100). The highest slaughter weight was recorded in the H50 group, at 2,179 g, while the lowest slaughter weight of 1,715 g was found in the H100 group ($P < 0.05$). Likewise, the highest average weight gain was observed in the H50 group, and the lowest average weight gain in the H100 group ($P < 0.05$). Neither feed intake nor feed conversion was affected by the inclusion of pea protein in the broiler diets ($P > 0.05$). In the H100 group, there was a decrease in yield compared to the other groups ($P < 0.05$) as well as a reduction in breast weight, which was also reflected in the breast percentage of slaughter weight ($P < 0.05$). Conversely, the lowest proportion of thighs in broiler body weight was measured in the H50 group and the highest in the H100 group ($P < 0.05$). The results indicate that incorporating pea protein into broiler feed formulations is feasible and may even have a positive effect on certain production parameters. However, replacing soybean meal with pea protein in the diets may impair growth and broiler carcass quality parameters.

Keywords: alternative protein source; carcass; fattening; animal nutrition; poultry; Ross 308



INTRODUCTION

Sustainability and reducing the negative impact of human activity on the environment are increasingly discussed topics today. The agricultural sector, and specifically livestock production, is closely linked to these issues. Feed is the most costly item in livestock farming. In poultry farming, this cost item accounts for 70–75 % of total costs (Mbukwane *et al.*, 2022), which only underscores its financial burden on the operation. Soy is an irreplaceable protein feed in the diet of non-ruminants. However, from today's perspective, it may seem rather “controversial” precisely from an environmental standpoint. The deforestation of rainforests to expand soybean croplands, coupled with costly imports to Europe that consume significant amounts of fossil fuels, increases the pressure to find alternatives to reduce the consumption of this feed (Te Pas *et al.*, 2021; Pexas *et al.*, 2023). Feed selection should therefore not be solely a matter of cost or nutritional quality; the environmental impact is also coming to the forefront (Thornton *et al.*, 2023). One way to reduce the negative environmental impact is to increase the proportion of locally sourced feed. In 2024, global soybean production totaled 397 million tons, with 86.2 % grown in South America. Europe accounted for only 2.7 % of global production, as 8 million tons of soybeans were grown there. However, soybean cultivation continues to intensify. Over the past 50 years (1974–2024), production has increased nearly eightfold. Insects, seaweed, or legumes are mentioned as alternative protein feeds that could at least partially replace soy (Čumplík *et al.*, 2026). Peas are also one of the possible alternatives. Although dry pea production amounted to 14 million tons in 2024, Europe accounted for less than 54 % of it; specifically, 6 million tons of peas were grown in Europe (Faostat, 2026). The local availability of peas in European conditions may play an important role in the future when selecting protein feeds for diets not only for broilers but for livestock in general.

MATERIAL AND METHODS

A total of 128 male Ross 308 broiler chickens were monitored as part of the experiment; they were fattened for a period of 36 days. The broilers were reared in accredited cage systems in the experimental rearing halls of building M at Mendel University in Brno. The broilers were housed in groups of 8 in 16 cages. Each group consisted of 4 repetitions. Feeding of the broilers and simultaneous data collection took place regularly at the same time to ensure that the results obtained were as objective as possible. Over the course of 36 days, the broilers were fed 3 types of diets (starter diet, grower diet, and finisher diet) to adhere to phase-specific nutrition. The component composition of diets is shown in Tables 1, 2, and 3. The diets contained varying proportions of pea protein. The nutritional composition of the pea protein is shown in Table 4. The control mixture did not contain pea protein (K). In the first experimental mixture, 25 % of the soybean meal was replaced with pea protein (H25); in the second experimental mixture,

50% of the soybean meal was replaced with pea protein (H50); and in the third experimental mixture, 100% of the soybean meal was replaced with pea protein (H100). The diets were analyzed to determine the content of basic nutrients—dry matter, crude protein, crude fat, crude fiber, and crude ash. The results of the chemical analysis of the diets are presented in Table 5. During the experiment, broiler performance—weight gain, feed intake, and feed conversion ratio—was monitored. The health status of the chickens will also be monitored based on the performance data, clinical assessment, and mortality rate. Light, temperature, and humidity conditions will be set according to the technical guidelines for Ross 308 (Aviagen, 2025). On day 36, the chickens were slaughtered by decapitation, and the carcasses were subsequently evaluated. Data obtained during the experiment were statistically analyzed using one-way analysis of variance in Statistica 14. For evaluated differences between groups was used the Sheffé's test and $P < 0.05$ was a significance level determinate statistically significant difference.

Tab. 2: *Feed components of starter diet*

COMPONENTS (%)	TREATMENTS			
	K	H25	H50	H100
Corn	30.00	36.13	37.50	48.50
Wheat	25.99	24.00	26.70	25.00
Soybean meal	35.00	27.00	18.30	X
Rape oil	5.00	4.80	5.32	5.33
Premix	3.00	3.00	3.00	3.00
Lysine	0.16	0.14	0.12	0.05
Methionine	0.22	0.23	0.23	0.25
Pea protein	X	4.00	8.00	17.00

K – control group, H25 – 25 % soybean meal replaced with pea protein, H50 – 50 % soybean meal replaced with pea protein, H100 – 100 % soybean meal replaced with pea protein

Tab. 3: *Feed components of grower diet*

COMPONENTS (%)	TREATMENTS			
	K	H25	H50	H100
Corn	37.77	33.03	42.60	47.40
Wheat	24.50	33.40	26.90	30.00
Soybean meal	29.60	22.30	14.80	X
Rape oil	4.82	4.95	5.40	5.30
Premix	3.00	3.00	3.00	3.00
Lysine	0.13	0.14	0.09	0.05
Methionine	0.18	0.19	0.21	0.25
Pea protein	X	3.00	7.00	14.00

K – control group, H25 – 25 % soybean meal replaced with pea protein, H50 – 50 % soybean meal replaced with pea protein, H100 – 100 % soybean meal replaced with pea protein

Tab. 4: Feed components of finisher diet

COMPONENTS (%)	TREATMENTS			
	K	H25	H50	H100
Corn	39.20	42.64	43.28	42.69
Wheat	29.00	28.55	30.23	37.45
Soybean meal	23.40	17.40	12.90	X
Rape oil	5.00	5.00	5.20	5.50
Premix	3.00	3.00	3.00	3.00
Lysine	0.22	0.21	0.19	0.16
Methionine	0.18	0.20	0.20	0.20
Pea protein	X	3.00	5.00	11.00

K – control group, H25 – 25 % soybean meal replaced with pea protein, H50 – 50 % soybean meal replaced with pea protein, H100 – 100 % soybean meal replaced with pea protein

Tab. 5: Nutritional composition of the pea protein (%)

	Dry matter	Crude ash	Crude protein	Crude fat	Crude fiber
Pea protein	88.00	4.09	70.68	0.31	0.29

Tab. 6: Chemical analysis of the diets at 88 % dry matter (%)

	Crude ash	Crude protein	Crude fat	Crude fiber
Starter diet				
K	6.48	19.83	6.32	2.59
H25	5.95	19.44	6.16	2.45
H50	5.61	19.35	6.83	2.14
H100	4.68	18.65	7.15	1.64
Grower diet				
K	5.65	18.16	6.64	4.04
H25	5.24	17.66	6.90	3.81
H50	4.66	17.15	7.09	2.09
H100	3.90	16.72	6.99	1.79
Finisher diet				
K	5.09	16.28	6.63	4.17
H25	4.80	16.16	6.77	3.91
H50	4.57	16.06	7.11	4.00
H100	3.75	16.23	7.22	3.93

K – control group, H25 – 25 % soybean meal replaced with pea protein, H50 – 50 % soybean meal replaced with pea protein, H100 – 100 % soybean meal replaced with pea protein

RESULTS AND DISCUSSION

By the end of the starter period (1–14 days of age), significant differences in body weight were already observed between the individual groups ($P < 0.05$). Broilers from groups K and H100 had significantly lower body weights than those from groups H25 and H50. The highest live weight was measured in group H25 (399 g), though this difference was not statistically significant compared to group H50 ($P > 0.05$). At the end of the grower period (15–28 days of age), the same trend from the previous period continued. Here as well, lower live weights were measured for chickens from groups K and H100 ($P < 0.05$). However, the highest live weight was found in group H50 (1,300 g), though this was not statistically significant compared to group H25 ($P > 0.05$). At the end of the experiment (day 36 of the broilers' age), the highest slaughter weight was achieved by chickens from group H50 (2,179 g), though this was not statistically significant compared to group H25. As was the case throughout the experiment, the lowest weight was recorded in the H100 group (1,715 g), which also lagged behind the control group in terms of weight during the finisher period. Conversely, compensatory growth was observed in the K group during the final phase of fattening. According to the technical guidelines for the Ross 308 hybrid (Aviagen, 2022), broilers should reach a live weight of 2,552 g by 36 days of age. The body weight of broilers in different fattening periods is shown in Table 6. On the contrary, the study by Kirn *et al.* (2024) states that up to 30 % inclusion of peas in the feed ration of broilers does not lead to lower performance. Similarly, the inclusion of pea hulls in a proportion of 4.5 % or pea protein concentrate in a proportion of 10 % did not negatively affect the slaughter weight of broilers. Laudadio and Tufarelli (2011) also state that up to 40 % inclusion of peas in diets did not worsen growth, feed consumption, or feed conversion. Dotas *et al.* (2014) even state that a proportion of 48 % peas in the diet had no effect on the growth of broilers.

Tab. 7: Body weight of broilers in different fattening periods (g)

PERIOD	TREATMENTS				SEM	P-VALUE
	K	H25	H50	H100		
Starter (14-day)	318 ^b	399 ^a	397 ^a	283 ^b	8.00	0.00
Grower (28-day)	1,118 ^b	1,294 ^a	1,300 ^a	1,011 ^b	21.92	0.00
Finisher (36-day)	1,897 ^{bc}	2,111 ^{ab}	2,179 ^a	1,715 ^c	33.63	0.00

^{a,b,c} means statistically significant differences ($P < 0.05$), SEM – Standard Error Mean, K – control group, H25 – 25 % soybean meal replaced with pea protein, H50 – 50 % soybean meal replaced with pea protein, H100 – 100 % soybean meal replaced with pea protein

The effect of pea protein on feed intake and feed conversion ratio was not evident in the final data for the entire fattening period (1–36 days of age) ($P > 0.05$). In contrast, a statistically significant difference was observed in body weight gain ($P < 0.05$). These results mirror the trend observed for body weight at 36 days of age. Here as well, the highest body weight gain was achieved by chickens in the H50 group (2,141 g) and the lowest by the H100 group (1,676 g). These results confirm that the slaughter weight of the chickens was not influenced by their initial live weight at placement. According to the technical guidelines for the Ross 308 hybrid (Aviagen, 2022), at 36 days of age, feed intake should be 200 g and the feed conversion ratio 1.408. Feed intake, feed conversion ratio, and body weight gain of the broilers are shown in Table 7. According to a study by Kirn *et al.* (2024), the inclusion of pea protein concentrate in the broiler diet at a rate of 10% did not lead to a deterioration in the feed conversion ratio. On the contrary, a deterioration in this parameter occurred when pea hulls were included in the diet at levels of 3% and 4.5%. Dotas *et al.* (2014) report that even a 48% proportion of peas in the diet does not lead to a reduction in feed intake or a deterioration in feed conversion.

Tab. 8: Feed intake, feed conversion ratio, body weight gain

	TREATMENTS				SEM	P-VALUE
	K	H25	H50	H100		
Feed intake per broiler (g)	107	98	103	97	2.43	0.47
Feed conversion ratio	2.1	1.7	1.7	2.1	0.08	0.11
Body weight gain (g)	1,859 ^{bc}	2,072 ^{ab}	2,141 ^a	1,676 ^c	53.84	0.00

^{a,b,c} means statistically significant differences ($P < 0.05$), SEM – Standard Error Mean, K – control group, H25 – 25 % soybean meal replaced with pea protein, H50 – 50 % soybean meal replaced with pea protein, H100 – 100 % soybean meal replaced with pea protein

Replacing 100% of soybean meal with pea protein had a negative effect on carcass quantitative parameters, as shown in Table 8. The H100 group had the lowest carcass weight (1,176 g) and the lowest breast weight of all groups (123 g) ($P < 0.05$). Conversely, the inclusion of pea protein had no effect on thigh weight ($P > 0.05$). Similarly, the proportion of breast weight relative to live weight was lowest in the H100 group (14.04%), with the highest proportion recorded in the H25 group (19.09%) ($P < 0.05$). Conversely, the proportion of thigh weight was highest in the H100 group (14.71%) and lowest in the H50 group (12.92%) ($P < 0.05$). According to the study by Kirn *et al.* (2024), it does not lead to a reduction in the proportion of breast or thigh meat in the carcass. Laudadio and Tufarelli (2011) add that even 40% peas in the broiler diet do not have a negative effect on the proportion of monounsaturated and polyunsaturated fatty acids or on the n-6/n-3 ratio.

Tab. 9: Quantitative parameters of carcass

	TREATMENTS				SEM	P-VALUE
	K	H25	H50	H100		
n	8	8	8	8	-	-
Body weight (g)	2,040 ^a	2,233 ^a	2,248 ^a	1,752 ^b	45.04	0.00
Carcass (g)	1,392 ^a	1,556 ^a	1,561 ^a	1,176 ^b	34.66	0.00
Breast (g)	182.9 ^a	213.1 ^a	197.9 ^a	123.0 ^b	7.25	0.00
Thigh (g)	137.0	150.0	145.1	128.9	2.91	0.05
Breast meat (% from BW)	17.77 ^a	19.09 ^a	17.61 ^a	14.04 ^b	0.40	0.00
Thigs meat (% from BW)	13.38 ^{ab}	13.46 ^{ab}	12.92 ^b	14.71 ^a	0.21	0.01

n – number of samples, ^{a,b} means statistically significant differences ($P < 0.05$), SEM – Standard Error Mean, BW – body weight, K – control group, H25 – 25 % soybean meal replaced with pea protein, H50 – 50 % soybean meal replaced with pea protein, H100 – 100 % soybean meal replaced with pea protein

CONCLUSION

This study has shown that the inclusion of pea protein in diets for broilers is possible. In this way, the use of soybean meal can be reduced by 50 % in diets for Ross 308 broilers. However, it is necessary to add that according to our results, soybean meal cannot be fully replaced by pea protein. Complete replacement leads to a decrease in the intensity of broilers and can also negatively affect the quality of the carcass, especially by reducing the weight of the breast. However, partial replacement (25 and 50 %) of soybean meal with pea protein does not negatively affect the growth of individuals, but according to our results, it can even improve growth. The highest live weight gain was observed in the H50 group. As for the effect of the inclusion of pea protein on the carcass, 25 % and 50 % replacement of soybean meal does not negatively affect the yield, breast weight, thigh weight, or the proportion of breast weight in live weight. However, in the H50 group, a negative effect on the thigh weight parameter from the live weight fraction was observed. Our results indicate that at least 50 % replacement of soybean extracted meal with pea protein does not impair broiler performance.

As soybean is widely used as a protein source in animal nutrition, reducing its inclusion in feed formulations could contribute to a decrease in the intensity of soybean cultivation. Modifying the choice of dietary protein sources may help mitigate the environmental impacts associated with animal production. Furthermore, increased utilization of locally produced feed ingredients represents an additional strategy for reducing the pressure on tropical deforestation and lowering fossil fuel consumption associated with feed transportation. Therefore, the identification and adoption of alternative protein sources constitute important steps toward enhancing the sustainability of agricultural production systems.

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LIVER HEALTH IN THE PERIPARTURIENT PERIOD AND ITS EFFECT ON MILK PRODUCTION AND REPRODUCTION IN DAIRY COWS

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ABSTRACT

The periparturient period is a critical phase in dairy cows because negative energy balance and lipid mobilization may impair liver function, milk production, and reproductive performance. The aim of this study was to evaluate the relationship between energy balance, liver health indicators, milk production, and pregnancy success after the first insemination in multiparous dairy cows from a commercial herd with high milk production. Forty cows were assessed using production records, reproductive data, biochemical blood parameters, and calculated liver indices, including the liver functionality index (LFI) and liver health index (LHI). The results indicate that cows with more favorable liver health indicators showed better metabolic adaptation after calving and more favorable production and reproductive outcomes. The findings support the practical use of LHI as a simple tool for early identification of cows at risk of impaired transition performance.

Keywords: liver, energy balance, milk production, reproduction

INTRODUCTION

The periparturient period, approximately 21 days before and 21 days after calving, represents a phase of substantial physiological and metabolic changes in dairy cows. This metabolic challenge is associated with the high energy requirements for fetal growth in late gestation and preparation of the mammary gland for the subsequent lactation (Haitao et al. 2025). The early postpartum period is particularly critical because feed intake is often insufficient to meet the energy demands associated with tissue recovery and onset of lactation. This results in negative energy balance (NEB) and increased lipomobilization (Wathes et al. 2007; LeBlanc 2012). Reduced feed intake decreases ruminal propionic acid production, an important precursor for glucose synthesis (Weber et al. 2013).



To compensate for the energy deficit, dairy cows mobilize adipose tissue through lipolysis, releasing non-esterified fatty acids (NEFA) into the bloodstream. These fatty acids are subsequently metabolized in the liver (Mao et al. 2026). When circulating NEFA concentrations are high, hepatic metabolic capacity may be exceeded, resulting in lipid accumulation in the liver and contributing to fatty liver development (White 2015; Haitao et al. 2025). Disruption of metabolic processes during the periparturient period increases the risk of metabolic disorders, particularly ketosis, which negatively affects milk production. In addition, secondary reproductive health problems may occur, including retained placenta, bacterial uterine infections, and impaired estrous cyclicity (Abdelli et al. 2017).

The first 21 days after calving are also influenced by inflammatory conditions associated with increased cytokine production as a consequence of lipid mobilization (Contreras, Sordillo 2011). During this phase, cytokines contribute to the redistribution of nutrients among tissues and organs according to current physiological priorities and also affect dry matter intake (DMI) and reproductive performance in dairy cows (Montagner et al. 2016). In the liver, cytokines are involved in inflammatory responses, increasing the concentrations of positive acute-phase proteins and decreasing negative acute-phase proteins, including albumin (Bionaz et al. 2007).

In high-producing dairy cows, proper liver function is essential for maintaining high milk yield and minimizing health disorders. The liver is one of the most important metabolic organs because it is responsible for key processes such as gluconeogenesis, fatty acid metabolism, and protein synthesis, while also playing an important role in immune regulation (Drackley 2025). A healthy and functional liver is therefore a prerequisite for sufficient gluconeogenesis and the synthesis of metabolites required for milk production in the mammary gland. Impaired liver function during the periparturient period is often associated with reduced milk yield and shorter lactation length (Weber et al. 2013).

One approach to evaluating the extent of inflammatory processes and liver functionality is the liver functionality index (LFI), which considers changes in serum concentrations of bilirubin, albumin, and cholesterol in dairy cows on days 3 and 28 after calving (Zhou et al. 2016). LFI can be used to assess the success of adaptation to early lactation. However, a limitation of this indicator is the need for repeated sampling at precisely defined time intervals (Kerwin et al. 2022). LFI enables assessment of the extent of the inflammatory response and prediction of its potential impact on animal health (Bertoni and Trevisi 2013). In intensive systems, cows with low LFI have been shown to have lower dry matter intake, reduced milk production, and a higher incidence of disease (Trevisi et al. 2012). However, it has not been clearly demonstrated whether similar results occur in semi-extensive systems, where environmental factors such as heat stress must also be considered (Montagner et al. 2016).

Another approach to assessing hepatic load is the liver health index (LHI), which is based on a single blood sample collected 3–12 days after calving. This method

may help farmers identify cows at risk of impaired adaptation to lactation at an early stage. LHI uses serum concentrations of bilirubin, cholesterol, and albumin, which are individually compared with the values of other cows in the herd using the mean and standard deviation. Cows with higher LHI values have been reported to have higher milk yield, more days in milk (DIM), and lower NEFA concentrations (Kerwin et al. 2022; Nelson et al. 2024).

The aim of this study was to evaluate the relationship between energy balance, liver function, milk production, and pregnancy success after first insemination in dairy cows under farm conditions with an average milk production above 9,000 kg. The underlying hypothesis was that impaired energy balance and reduced liver function during the periparturient period negatively affect productive and reproductive indicators in dairy cows.

MATERIALS AND METHODS

The evaluation was carried out on a selected dairy farm with a controlled nutritional management system and an average annual milk production of 8,000–10,000 kg per cow. Cows were housed in a freestall barn in a separate group and fed a total mixed ration (TMR) formulated according to NRC (2001) recommendations for different phases of the production cycle in multiparous dairy cows. Multiparous dairy cows ($n = 40$) were evaluated for nitrogen utilization efficiency and energy balance in relation to pregnancy success after first insemination. Samples of the TMR were collected from the feed manger on the control day and analyzed for dry matter (DM), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), starch, and ether extract (EE) using conventional methods according to Commission Regulation (EC) No. 691/2013. Net energy for lactation (NEL) and non-fibrous carbohydrate (NFC) values were calculated using regression equations according to 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 was performed on the control day using individually collected milk samples. Milk yield, milk components, and milk urea were evaluated. Total protein, fat, lactose, and urea concentrations were analyzed by near-infrared spectrophotometry using MilkoScan FT⁺ and BENTLEY FTS at the Central Analytical Laboratory of Milk, accredited under registration number 096/5878/2015/2.

Energy balance was evaluated as the difference between the actual energy intake from the TMR and the energy output in milk nutrients, expressed as positive or negative NEL balance in MJ/day and calculated according to Daniel et al. (2017).

Efficiency of nitrogen utilization (EUN) was estimated for the group of dairy cows based on the analyzed MUN content and milk yield using the regression equation developed by Huhtanen et al. (2015) from meta-analytical assessments of balance experiments.

Statistical analyses were performed using GraphPad Prism 9. Each parameter was presented as its mean (x), and standard deviation (SD).

Blood samples for biochemical analysis were collected at regular intervals: 7 days before the expected calving date, 7 days after calving, 20 days after calving, and 60 days after calving. Samples were collected from the coccygeal vein in the morning after feeding and immediately transported to the laboratory for processing.

Metabolites of intermediary metabolism were determined photometrically using diagnostic kits and an Ellipse biochemical analyzer (Spain). The assessment included markers of energy metabolism (CHOL, cholesterol; TG, triacylglycerol; NEFA, non-esterified fatty acids; BHBA, beta-hydroxybutyric acid), protein metabolism (TP, total protein; ALB, albumin; urea), and liver load (BIL, bilirubin; AST; GGT).

To assess hepatic load, LHI was calculated using samples collected 7–10 days after calving. Serum concentrations of bilirubin, cholesterol, and albumin were determined in the laboratory and subsequently used in the LHI calculation according to Kerwin et al. (2022).

Milk performance and reproductive records were obtained from the software program used on the evaluated farm and from the Breeding Services of the Slovak Republic (PLIS).

RESULTS AND DISCUSSION

Evaluation of nutritional composition

The average nutrient concentrations in the TMR used on the evaluated farm are presented in Table 1. The feed ration, based on corn and ryegrass silages with the addition of concentrate feed, was formulated for multiparous dairy cows with a target milk production of 45 kg/day, 3.3 % milk protein, and 3.6 % milk fat.

Evaluation of milk production, energy balance, nitrogen utilization, and reproduction

The evaluation of milk production, energy balance, and the efficiency of nitrogen utilization according to days in milk is summarized in Table 2. Analysis of energy balance, assessed on the basis of energy intake in the diet and nutrient output in milk according to Daniel et al. (2017), confirmed different levels of negative energy balance in the evaluated cows. The mean energy balance ranged from -43.4 MJ/day shortly after calving to -3.5 MJ/day at peak lactation. Negative energy balance reflects the degree of lipid mobilization and, at the beginning of lactation, corresponds to the energy requirements for the synthesis of approximately 14 kg of milk.

These findings indicate that the extent of postpartum negative energy balance represents an important biological mechanism linking metabolic load, liver function, and subsequent reproductive success in dairy cows.

Tab. 10: *Nutritional composition of the total mixed ration (TMR)*

	Antepartum	Postpartum	Early lactation
	X ± SD	X ± SD	X ± SD
DM g	346.9 ± 65.0	426.3 ± 39.7	412.7 ± 9.3
CP g/kg DM	153.3 ± 12.1	164.9 ± 12.4	164.8 ± 16.6
PDIN	99.4 ± 6.6	113.3 ± 9.5	112.8 ± 12.4
PDIE	87.0 ± 10.6	111.3 ± 9.8	109.3 ± 8.9
NDF g/kg DM	425.8 ± 42.5	327.3 ± 19.6	330.2 ± 10.2
ADF g/kg DM	275.1 ± 32.5	192.8 ± 10.9	195.7 ± 9.3
Starch g/kg DM	172.9 ± 46.3	271.7 ± 24.7	280.9 ± 31.5
EE g/kg DM	30.7 ± 4.0	33.5 ± 5.9	32.3 ± 2.8
Ash g/kg DM	74.0 ± 7.8	66.3 ± 4.9	69.0 ± 3.5
NEL MJ/kg DM	5.89 ± 0.5	6.99 ± 0.3	7.00 ± 0.15
DMI kg	19.3 ± 0.7	20.5 ± 0.8	23.6 ± 0.9
NEL intake MJ/d	113.7	143.3	165.2

Composition: corn silage 26 – 30 kg, ryegrass silage 10 kg, GPS silage 9 kg, wheat straw 0.5 – 0.8 kg, corn cob mix (CCM) 5.0 – 7.5 kg, dried distillers grains (DDG) 3.5 kg, rapeseed meal 4.0 – 4.3 kg, CornGold 2.0 – 2.5 kg, molasses 0.5 kg, and feed mixture 1.8 – 2.2 kg.

DM = dry matter; DMI = dry matter intake.

Tab. 11: *Milk production, energy balance, and efficiency of nitrogen utilization*

	<25 DIM	25 – 60 DIM	60 – 90 DIM
Milk production, kg/day	33.85 ± 6.3	44.60 ± 12.1	43.40 ± 12.3
Milk fat, %	4.75 ± 1.29	3.39 ± 1.65	2.95 ± 1.03
Total yield, kg	1.42 ± 0.6	1.38 ± 0.6	1.24 ± 0.5
Milk protein, %	3.41 ± 0.35	3.18 ± 0.34	3.31 ± 0.18
Total yield, kg	1.05 ± 0.4	1.39 ± 0.3	1.43 ± 0.4
Fat-to-protein ratio	1.39 ± 0.36	1.04 ± 0.39	0.89 ± 0.31
Lactose, %	4.81 ± 0.18	4.79 ± 0.33	4.77 ± 0.25
Energy balance MJ NEL/day	-43.4 ± 38.8	- 31.2 ± 35.4	- 3.5 ± 31.5
EUN %	29.2 ± 4.9	31.7 ± 4.1	31.9 ± 2.43
Milk urea, mg/dL	24.43 ± 10.66	25.17 ± 10.98	21.93 ± 7.02
MUN, mg/dL	11.48 ± 5.04	11.83 ± 5.16	10.31 ± 3.30

In the evaluated cows, the highest mean milk production was recorded during the first phase of lactation, reaching 43.40 ± 12.3 kg/day. Increased milk production may reduce fertility, particularly as a consequence of inadequate transition-period management, different levels of postpartum NEB, or a higher incidence of disease. The extent and duration of NEB are closely related to dry matter intake. NEB accompanied by lipid mobilization affects the physiological mechanisms of the reproductive endocrine cascade. At the beginning of lactation, milk synthesis

is associated with increased growth hormone concentrations, which redistribute nutrients mainly in the liver and adipose tissue (Lucy, 2008). As a consequence of NEB, insulin concentrations remain low, preventing the increase in hepatic growth hormone receptors and IGF-I secretion, thereby negatively affecting reproduction. Insulin and IGF-I are then unable to act synergistically with gonadotropins on ovarian cells, which may prevent ovulation of the dominant follicle (Butler 2005) and delay the resumption of cyclicity (Wathes 2007). Increased milk production is also negatively correlated with the intensity of estrus expression (Madureira et al. 2015) or with reduced locomotor activity (Yániz et al. 2006).

Evaluation of liver health and liver function

Concentrations of intermediary metabolism metabolites in dairy cows during the evaluated period from 7 to 60 days in milk are shown in Table 3. Mean concentrations of cholesterol, bilirubin, and AST increased between sampling points compared with the other evaluated metabolites. The concentrations and trends of intermediary metabolism metabolites corresponded with physiological changes after calving and during early lactation. At the level of intermediary metabolism, the degree of NEB is assessed mainly through the concentration of non-esterified fatty acids (NEFA). In the present evaluation, serum NEFA concentrations did not differ markedly among cows and averaged 0.25–0.28 mmol/L.

Tab. 12: *Metabolites of intermediary metabolism in dairy cows from 7 to 60 days in milk*

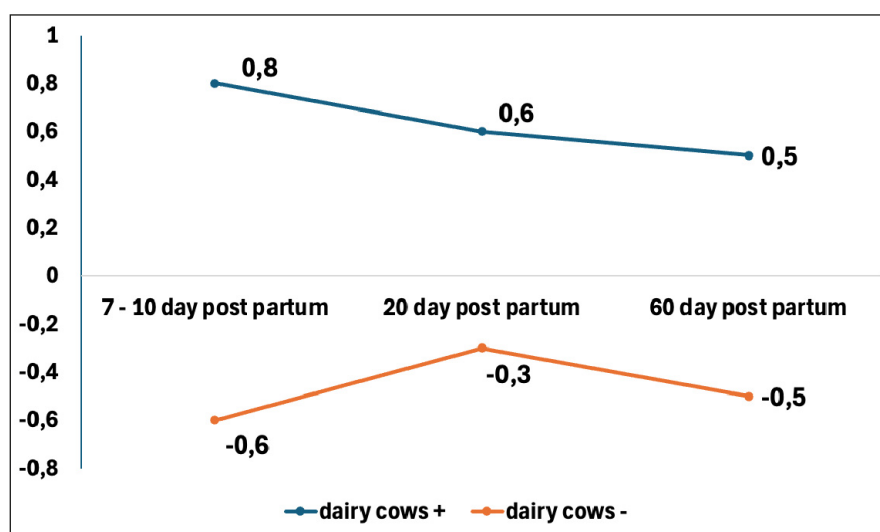
	7–10 days postpartum	20 days postpartum	60 days postpartum
TP g/l	71.91 ± 6.31	78.83 ± 4.88	73.07 ± 6.74
ALB g/l	33.65 ± 3.43	33.65 ± 3.30	33.92 ± 3.15
UREA mmol/l	4.25 ± 0.91	4.20 ± 1.05	4.98 ± 1.24
CHOL mmol/l	2.07 ± 0.66	2.60 ± 0.89	4.44 ± 1.60
TG mmol/l	1.01 ± 0.13	0.98 ± 0.08	1.00 ± 0.08
NEFA mmol/l	0.28 ± 0.17	0.25 ± 0.11	0.26 ± 0.15
BHBA mmol/l	0.76 ± 0.32	0.73 ± 0.32	0.80 ± 0.30
BIL mmol/l	3.17 ± 1.19	4.24 ± 2.85	6.64 ± 5.26
GGT µkat/l	0.45 ± 0.20	0.45 ± 0.13	0.54 ± 0.18
AST µkat/l	1.38 ± 0.29	1.42 ± 0.30	1.54 ± 0.38

Peak lactation is characterized by NEB, higher blood NEFA concentrations, and the most pronounced increase in milk production (Drackley, 1999). Postpartum increases in NEFA concentration and decreases in glucose concentration are negatively correlated with pregnancy success at first insemination in artificial insemination programs (Garverick et al. 2013). BHBA concentrations can be used as a marker of NEB, and increased BHBA values associated with hyperketonemia negatively affect milk production, culling rate, and reproductive performance (McArt et al. 2012). McArt et al. (2012) confirmed that cows with subclinical ketosis

between days 3 and 7 after calving were less likely to become pregnant after first service during the first 30 days postpartum than cows in which ketosis developed on day 8 or later.

According to Ospina et al. (2010) and McArt et al. (2013), elevated serum concentrations of BHBA and NEFA in dairy cows during the periparturient period represent a risk factor for the success of the first postpartum insemination. Under these conditions, both the immune and endocrine systems are involved in adaptation. The resulting metabolic load may impair reproductive performance and reduce milk production, which are key indicators of profitability in dairy farms (Esposito et al. 2014).

The liver functionality index (LFI) was calculated according to Bertoni and Trevisi (2013) from albumin, cholesterol, and bilirubin concentrations measured 7 or 10 days and 20 days after calving. In the positive group of cows (+), the LFI value was -0.55, which was higher than in the negative group of cows (-), where LFI reached -3.54. Mezzetti et al. (2021) reported that cows with the lowest LFI values had the lowest milk yield, the poorest reproductive parameters, and the highest incidence of disease at the beginning of lactation. Therefore, LFI calculation represents a useful tool for monitoring the adaptation of dairy cows to the postpartum period and early lactation.



Graph 1: Liver health index in dairy cow

The liver health index (LHI) was subsequently determined in selected cows and is shown in Graph 1 for both evaluated groups (+ and -). In the positive group of cows (+), LHI ranged from 0.5 to 0.8, whereas in the negative group (-), it reached negative values ranging from -0.6 to -0.3. Kerwin et al. (2022) reported that cows with higher LHI had a lower incidence of reproductive disorders after calving, higher milk yield, and better pregnancy success than cows with lower LHI values. LHI assessment can therefore be considered an economically and practically advantageous monitoring method under farm conditions, as it requires only one blood sample shortly after calving.

Available studies indicate that severe negative energy balance during the periparturient period and an increased incidence of metabolic disorders are associated with impaired liver function. Low LHI values are usually related to increased concentrations of NEFA and BHBA, which negatively affect subsequent reproductive performance and the overall health status of dairy cows in the herd (Ospina et al. 2010; Kerwin et al. 2022).

CONCLUSION

Both LHI and LFI use serum concentrations of bilirubin, albumin, and cholesterol to evaluate the functional status of the liver. LFI assesses the dynamics of these indicators between repeated sampling points after calving, whereas LHI provides a practical assessment of liver health based on a single sample collected during the early postpartum period. From the perspective of routine herd monitoring, LHI is therefore a more suitable tool for the early identification of at-risk cows and for supporting decisions regarding nutritional and veterinary interventions (Zhou et al. 2016; Kerwin et al. 2022).

Assessment of liver health using LHI may help identify at-risk animals shortly after calving without the need for repeated testing later in lactation. Early diagnosis may therefore contribute to more targeted management of negative energy balance, reduction of metabolic risks, and improvement of both productive and reproductive efficiency in the herd.

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EFFECTS OF CORN DISTILLERS DRIED GRAINS AND CORN GLUTEN IN THE DIET OF DAIRY COWS: A COMPARATIVE ANALYSIS OF THEIR IMPACT ON MILK YIELD

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ABSTRACT

The experiment compared the effect of wet distillers' grains with solubles (WDGS) and enriched corn gluten (CG) on the milk production and milk composition of 86 Holstein dairy cows. The experiment group received 4 kg of WDGS per cow in their daily total mixed ration (TMR), while the control group received 4 kg of CG per cow. The results demonstrated that the CG group achieved a significantly higher milk yield at week 7 of the trial (April) compared to the WDGS group (40.63 kg vs. 37.21 kg, $p \leq 0.01$). In contrast, the WDGS group showed a significantly higher milk protein content from week 3 to week 11 of the trial ($p \leq 0.01$) and higher fat content at week 11 (4.21 vs. 3.96%; $p \leq 0.05$). Higher levels of milk urea were also observed in the WDGS group ($p \leq 0.01$) during multiple sampling periods. Both by-products proved to be effective subsites for concentrate mixtures. While CG promoted a higher milk volume, WDGS improve milk quality parameters (protein and fat concentrations). However, the use of WDGS requires more precise balancing of nitrogenous fractions and energy in the rumen due to its higher acidogenity and nutritional variability.

Keywords: nutrition; daily ration; Holstein cattle; milk parameters; milk urea; bioethanol by-products, fatty acids

INTRODUCTION

The production of bioethanol from crops, mostly corn and wheat, has undergone significant technological advancements over the past decades, leading to the large-scale production of nutritionally valuable bioproducts (Malik et al. 2024; Garnsworthy et al. 2021). In the Czech Republic, this development was accelerated



by legislative changes after 2008, which introduced mandatory biofuel additive requirements. Modern distilleries produce corn distillation by-products as distillers' grains with solubles (DGS) in dried (DDGS) or wet (WDGS) form. This is significantly different from water-based, low-nutrient feeds produced in the past.

Current DDGS represent a highly concentrated source of crude protein (more than 300 g/kg dry matter), with roughly 50% of these nitrogenous substances consisting of rumen-undegradable protein (RUP), which is crucial for high-producing dairy cows in the early stage of lactation. Despite the high protein content, a low concentration of essential amino acid lysine remains a limiting factor in corn by-products (Kalscheur 2005; Kudrna et al. 2011). Alongside DGS, corn gluten feed (CGF) is an important by-product of the wet-milling process. It is valued for its high digestibility of its neutral detergent fiber (NDF) and its potential to partially replace energy-dense cereals (Ertl et al. 2015; St-Pierre & Weiss 2015).

Research by Mammi et al. (2022) suggests that incorporating distillery by-products into the feed ration can increase milk production. However, high doses of WDGS carry the risk of increased diet acidogenicity and potential development of subacute ruminal acidosis. Considering that feed costs often represent more than 50–60% of total milk production costs, the use of agricultural by-products could be an economic necessity. Although the properties of WDGS are well documented, comparative studies evaluating the effects of WDGS versus enriched corn gluten on milk production are limited.

This experiment aims to conduct a comparative analysis of the effects of incorporating WDGS into the diet of Holstein dairy cows, compared with the corn gluten diet, on milk production.

MATERIAL AND METHODS

Animals and experimental groups

The experiment was conducted on a commercial herd of Holstein dairy cows. The experiment involved four production sections of dairy cows, which were divided into two treatments, with two sections serving as the experimental group and another two as the control group. From the base population of both treatments, a total of 86 cows were selected using the principle of analogical pairs, with 43 cows from the experimental group and 43 cows from the control group. The cows were selected based on three key parameters: lactation number, days in milk (DIM) and milk yield prior to the start of the experiment (Table 1). The experiment lasted for 16 weeks.

Tab. 13: Dairy cows from experimental ($n = 43$) and control ($n = 43$) groups before the start of the experiment

N	Lactation number	Days in milk	Milk yield (kg)
WDGS group	2.44 ± 1.26	92.56 ± 41.35	39.05 ± 6.81
CG group	2.44 ± 1.26	93.0 ± 39.47	39.01 ± 5.52

n – number of cases, WDGS – wet distillers' grains with solubles, CG – corn gluten

Sample collection

Data on milk yield and milk composition were obtained from the official milk recording scheme conducted by the Czech-Moravian Breeders' Corporation (ČMSCH). The monitored parameters included milk yield, concentration of milk components (fat, protein, lactose), urea, citric acid, ketone bodies (acetone and beta-hydroxybutyrate), and free fatty acids. Samples of WDGS were collected weekly for laboratory nutritional analysis.

Experimental diets

The diet was presented as a total mixed ration (TMR). The experimental group received WDGS at a rate of 4 kg per cow per day on a fresh matter basis. The control group received the same amount (4 kg/cow/day) of enriched corn gluten (CG), a feed supplement consisting of corn gluten, soluble fractions from molasses, and fiber, with an average dry matter (DM) content of 64 %. The diet rations were formulated to ensure that nutrient concentration differed minimally between the two group. The calculated compositions of the rations are shown in Table 2. Due to the introduction of a new alfalfa silage for the final three weeks of the experiment, the ration components were recalculated to maintain a stable nutrient concentration through the entire study.

Tab. 14: Nutritional composition of the ration during the experiment

	Date	March	CG	13. May	CG
	units	WDGS		WDGS	
NEL	MJ/kg DM	6.39	6.29	6.20	6.08
NL	g/kg DM	161.02	159.22	155.66	154.9
NDF	g/kg DM	322.87	320.48	344.99	346.00
ADF	g/kg DM	195.45	201.28	201.89	212.12
Starch	g/kg DM	238.1	226.51	251.21	231.42
NSC	g/kg DM	404.32	390.64	397.12	377.61
DM	kg	27.93	27.98	28.14	28.80

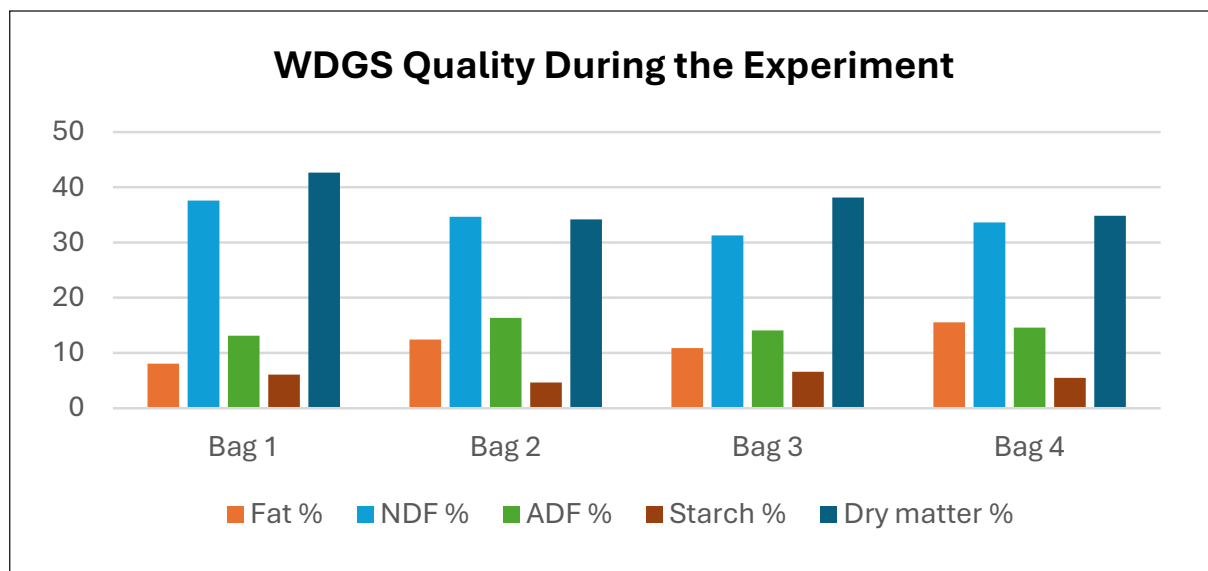
CG – Corn gluten, WDGS – Wet distiller's grains with solubles, NEL – Net Energy for Lactation, NL – Net Energy for Lactation, NDF – Neutral Detergent Fiber, ADF – Acid Detergent Fiber, Starch – Starch, NSC – Non-Structural Carbohydrates, DM – Dry Matter

Statistical analysis

Data were statistically analysed using MS Excel. Mean differences between the two feeding groups were evaluated using an unpaired Student's t-test after verifying the equality of variances with a F-test. Statistical significance was declared at $p \leq 0.05$. Additionally, a one-way analysis of variance (ANOVA) was performed to evaluate differences in nutrient composition among the four separate batches (bags) of WDGS used. When significant differences were detected, post hoc comparison were performed using Tukey's honestly significant difference (HSD) test at significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

We determined that the nutritional composition of the WDGS varied considerably throughout the experiments (Graf 1).



Graf 1: WDGS Quality During the Experiment

Averages of samples taken from a same bag: Bag 1 (13.2. – 12.3); Bag 2 (13.3 – 1.4.), Bag 3 (2.4 – 30.4.); Bag 4 (1.5 – 28.5)

The crude fat content showed quite high variability, ranging from 8% to 16%, with significant differences between individual storage bags; the highest values were found in bag 4 and the lowest in bag 1. Similarly, substantial variability was found in NDF content (31–38%), which was significantly higher in bag 1 compared to the others. In contrast, the percentage of ADF did not show significant differences and its concentration in individual bags ranged from 13 to 16%. Starch content varied from 4.6% to 6.6%. These fluctuations in fat and starch contents indicate that the net energy density differ between individual batches of WDGS. The last evaluated parameter was dry matter content which is crucial for the aerobic stability of wet by-products during storage. Dry matter ranges from 34% to 43% with bag 1 having the highest values, followed by bag 3, while bags 2 and 4 had lower values. These differences in the composition of the WDGS during the experiment could influenced the results, because for high producing dairy cows is important stability of the nutritional composition of the ration. Our findings reflect the natural variability of distillery co-products, where composition varies depending on the production process or aerobic deterioration during storage.

The effect of the dietary treatments on milk production parameters is summarised in Table 3. The results showed that cows fed the corn gluten (CG) had a significantly higher milk yield after week 7 of the trial (40.63 kg vs. 37.21 kg; $p \leq 0.05$). In the other months, the differences were smaller and did not reach statistical significance, though the CG diet maintained a numerically higher trend. Buenavista et al. (2021) and Benchaar et al. (2013) stated that co-products such

as WDGS can partially replace corn grain and protein sources such as soybean meal, without negatively affecting milk production if their inclusion is in the range of 20–30 % of dietary DM. The higher milk production observed in the CG group could be attributed to higher stability of the nutrient content in the ration. The next factor might be differences in fiber digestibility. Corn gluten is valued for its high NDF digestibility, serving as an effective energy substitute for corn grain while also promoting rumen stability (Wickersham et al. 2004; Magalhães Wanderley et al. 2025). The higher milk yield in the CG group may thus relate to the dietary characteristics of corn gluten, whose fermentable fiber helps maintain an optimal pH in the rumen, reduces the risk of subacute ruminal acidosis, and supports total tract organic matter digestibility. This statement is supported by Magalhães Wanderley et al. (2025). By contrast, the diet containing WDGS was more acidogenic, particularly due to the final nutrient balancing of the diet. Mammi et al. (2022) also stated that the addition of WDGS can result in a more acidogenic diet. The WDGS diet was richer in both starch and non-structural carbohydrates (NSC). WDGS used in our experiment contain 4–6 % of starch and in TMR was the total starch also slightly higher (Table 2).

Throughout the experimental period, the WDGS group consistently had a higher milk protein percentage compared to the CG group, with statistically significant differences from the 3 up to the 11 weeks (Table 3). This outcome aligns with Magalhães Wanderley et al. (2025), who reported that partial as well as complete replacement of conventional concentrate increased the milk protein concentration. As shown in Table 2, the WDGS diet maintained a slightly higher crude protein level (161.02 a 155.66 g/kg DM) compared to CG (159.22 and 154.90 g/kg). Although the difference in NL can be explained by the varying content and quality of protein in the two feeds. Literature data indicate that WDGS or DDGS contain 55–65 % of rumen undegradable protein (RUP or by-pass protein) of total crude protein. Compared to this, corn gluten feed contains a bypass protein of only approximately 20–22 %, with the rest being rumen-degradable protein (Darabighane et al. 2020). Therefore, the higher milk protein content in the WDGS group was likely driven by an increased supply of bypass protein absorbed directly in the small intestine and is not dependent on microbial synthesis of protein in the rumen. The specific amino acid profile of this protein, which avoids rumen degradation, is key to formulating feed rations (Maskaľová et al. 2014).

The WDGS group also reached a significantly higher milk fat content in May (4.21 % vs 3.96 %; $p \leq 0.05$), with a similar numerical trend observed during the other sampling period. Milk fat content is primarily influenced by acetate production from ruminal fiber fermentation and the fat content of the diet itself. Although WDGS are generally considered more acidogenic than CG, they did not exert a negative effect on milk fat percentage in this study. We hypothesize that the higher milk fat concentration was supported by the higher fat content within the WDGS itself. According to our analysis, the fat content of the WDGS ranged between 8 % and 16 %. Consequently, the WDGS diet group had a higher NEL

(6.39 and 6.20 MJ/kg DM) than the CG diet (6.29 and 6.08 MJ/kg) (Table 2). This is consistent with the scientific literature stating that distillers' grains (DGS) often have an energy value 10–15% higher than corn gluten meal, due to their higher fat content (Moureau et al. 2010).

Milk ketone levels (acetone and BHB) remained low and well within the physiological range for both groups. These low concentrations confirms that neither diet induced a negative energy balance. Although significant differences in BHB occurred in April and May, no clear trend was established, and the absolute differences were minimal.

Milk urea levels were significantly higher in the WDGS group in March, April, and June (Table 3). Higher concentration of urea is typically result from a lack of insufficient amounts of available saccharides, poor synchronisation between protein degradation and available energy for microbial growth, or an excessive supply of degradable protein. The concentration of crude protein in our diets was around 15% (which complies with the standard recommendations), the WDGS diet was slightly higher in crude protein than the CG diet. Nevertheless, because WDGS have higher by-pass protein than corn gluten, this pronounced increase in milk urea was unexpected and warrants further investigation into ruminal nitrogen efficiency.

To perform a comprehensive evaluation, raw milk yields were converted to fat-corrected milk (FCM) and energy-corrected milk (ECM). As shown in Table 4, no statistically significant differences were observed for FCM and ECM, despite slightly higher numerical values in the CG group. These quite small not statistically significant differences confirm that both feed by-products provide highly comparable production efficiencies. The choice between them should therefore be guided by individual farm objectives (e.g. milk volume vs. enhancing milk component concentration).

Tab. 15: Milk yield and milk composition in group fed wet corn distillers' grains (WDGS, n = 43) and corn gluten (CG, n = 43) during the experiment

Sampling Date (since start of feeding)	4. March		3. April		6. May		3. June	
	CG		CG		CG		CG	
Groups	WDGS	CG	WDGS	CG	WDGS	CG	WDGS	CG
Milk Yield (kg)	38.50±6,05	39.97±6.10	37.21±5.94**	40.63±5.91**	34.67±5.96	35.94±5.43	33.38±6.56	34.59±5.00
Milk Fat (%)	4.18±0,54	4.02±0.44	4.16±0.51	4.00±0.86	4.21±0.48*	3.96±0.49*	3.95±0.65	3.87±0.54
Milk Protein (%)	3.31±0,24*	3.20±0.25*	3.39±0.21*	3.27±0.23*	3.43±0.25**	3.23±0.25**	3.41±0.23	3.38±0.21
Fat/Protein Ratio	1.26±0,16	1.26±0.16	1.23±0.13	1.23±0.28	1.23±0.11	1.23±0.16	1.16±0.17	1.15±0.16
Milk Lactose (%)	4.95±0,09	4.93±0.09	4.98±0.09	4.99±0.08	4.97±0.10	4.96±0.09	4.90±0.10	4.87±0.09
Urea (mg/100 ml)	28.18±4.87**	23.56±6.29**	31.24±3.33*	29.70±3.78*	31.60±3.66	30.96±3.53	31.33±2.84**	27.99±3.81**
Citric Acid (%)	0.15±0.03**	0.17±0.03**	0.13±0.03**	0.17±0.04**	0.14±0.03	0.13±0.02	0.13±0.03**	0.17±0.05**
Acetone (mmol/l)	0.06±0.03	0.04±0.04	0.05±0.03*	0.03±0.03*	0.03±0.03	0.04±0.03	0.06±0.04	0.06±0.06
BHB (mmol/l)	0.04±0.03	0.04±0.03	0.05±0.03**	0.02±0.02**	0.05±0.03**	0.08±0.03**	0.07±0.03	0.06±0.06
FFA (mmol/100 g fat)	0.96±0.66	1.19±0.86	0.89±0.28	0.95±0.35	1.00±0.38*	1.19±0.39**	1.09±0.45	1.12±0.32

WDGS – Wet distiller's grains with solubles, CG – Corn Gluten, BHB – beta-hydroxybutyrate, FFA – Free Fatty Acids, * statistically significant difference between groups ($p \leq 0,05$), ** statistically significant difference between groups ($p \leq 0,01$)

Tab. 16: Fat corrected milk (FCM) and energy corrected milk (ECM) in group fed wet corn distillers' grains (WDGS, $n = 43$) and group fed corn gluten (CG, $n = 43$) during the experiment

	n	FCM	t-test	ECM	t-test
		3,50 %	p-value		p-value
4. March	WDGS	42.65±7.13	0.6742	42.48±6.60	0.7079
3 weeks	CG	43.29±6.94		43.02±6.63	
3. April	WDGS	41.05±6.25	0.0685	41.12±5.99	0.0591
7 weeks	CG	43.83±7.67		43.80±6.95	
6. May	WDGS	38.49±6.19	0.9970	38.63±6.05	0.8665
11 weeks	CG	38.50±5.78		38.42±5.54	
3. June	WDGS	34.78±8.55	0.2638	35.16±8.51	0.2519
16 weeks	CG	36.52±5.51		36.91±5.23	

FCM – Fat corrected milk for 3.5 % of fat, $FCM = (0.4324 \times \text{kg milk yield}) + (16.216 \times \text{kg milk fat})$ Western et al. (2020).

ECM – Energy-Corrected Milk, $ECM = (0.327 \times \text{kg mléka}) + (12.95 \times \text{kg mléčného tuku}) + (7.20 \times \text{kg mléčného proteinu})$ Western et al. (2020).

CONCLUSION

This experiment confirmed that both wet distillers' grains with solubles (WDGS) and enriched corn gluten (CG) serve as highly effective partial replacements for concentrate mixtures in the diets of high-producing dairy cows. The corn gluten (CG) diet supported a significantly higher raw milk yield at week 7 of the trial and numerically was found similar trend also in other samplings. Conversely, wet distillers' grains with solubles (WDGS) proved to be a tool for enhancing milk component quality, resulting in significantly higher milk protein percentages from week 3 to week 11 and an increased milk fat content in week 7.

However, because no statistically significant differences were detected in standardized milk production parameters (FCM and ECM), both by-products demonstrate comparable overall production efficiency. The choice between utilizing WDGS or CG should therefore be determined by specific farm management priorities, whether the primary goal is to maximize total volumetric milk yield (CG) or to enhance the concentration of milk fat and protein components (WDGS). Furthermore, the inherent nutritional variability of wet distillery by-products observed across different batches underscores the necessity of regular laboratory monitoring to prevent diet instability.

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SELECTED BIOMARKERS OF ANTIOXIDANT STATUS IN HORSES

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ABSTRACT

Oxidative stress represents an important factor affecting health status, reproductive performance, physical performance, and adaptive mechanisms in horses. The aim of this study was to evaluate selected biomarkers of antioxidant status in clinically healthy American Quarter Horses and to assess their usefulness in the evaluation of oxidative balance. The study included 13 clinically healthy horses consisting of seven mares and six geldings aged between 4 and 15 years, used for breeding and recreational purposes.

Blood samples were collected from the *vena jugularis*. Serum samples were used for the determination of total antioxidant status and malondialdehyde concentration, whereas glutathione peroxidase activity was determined from heparinized whole blood.

The mean total antioxidant status reached 2.42 ± 0.53 mmol/L, glutathione peroxidase activity 862 ± 265 U/L, and the mean malondialdehyde concentration 0.131 ± 0.022 μ mol/L. The obtained results indicated a favorable antioxidant profile in the evaluated horses.

The results suggest that clinically healthy American Quarter Horses maintained under the conditions of the studied farm in eastern Slovakia do not exhibit signs of increased oxidative stress. The obtained data provide preliminary information on the antioxidant status of this breed and may serve as a basis for further studies focused on the influence of age, reproductive status, nutrition, and physical activity on the oxidative balance of the equine organism.

Keywords: American Quarter Horse; malondialdehyde; glutathione peroxidase; oxidative stress



INTRODUCTION

Oxidative stress represents a state of imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense mechanisms of the organism. Excessive production of free radicals may result in oxidative damage to lipids, proteins, and nucleic acids, thereby affecting cellular homeostasis and physiological functions. In horses, oxidative stress is considered an important factor influencing health status, athletic performance, and adaptive responses of the organism (Shono et al., 2020; Arfuso et al., 2022).

The antioxidant defense system consists of both enzymatic and non-enzymatic mechanisms. Among the most important enzymatic antioxidants is glutathione peroxidase (GPx), which participates in the detoxification of peroxides and protects cellular membranes against oxidative damage. Total antioxidant status (TAS) reflects the overall antioxidant capacity of the organism, whereas malondialdehyde (MDA), a final product of lipid peroxidation, is widely recognized as a reliable biomarker of oxidative damage in horses (Brkljača Bottegaro et al. 2018; Yildirim and Apaydin Yildirim, 2023).

Several studies have demonstrated that physical exercise significantly influences the oxidative balance in horses. Increased oxygen consumption during muscular activity leads to enhanced production of reactive oxygen species and subsequent alterations in oxidative and antioxidant biomarkers. Sport and endurance horses frequently exhibit changes in oxidative stress parameters and antioxidant responses, indicating the importance of adaptive mechanisms in maintaining redox homeostasis during exercise (Brkljača Bottegaro et al., 2018; Arfuso et al., 2022).

Nutrition and trace mineral intake also play an important role in the regulation of antioxidant defense mechanisms. Latham et al. (2021) demonstrated that supplementation with complexed trace minerals influenced antioxidant enzyme activity and improved the physiological response to transportation stress in young horses. Adequate antioxidant capacity may contribute to the maintenance of physiological functions and reduce the risk of oxidative damage to tissues.

The American Quarter Horse is one of the most popular horse breeds worldwide and has become increasingly common in Central Europe, including Slovakia. However, information on the antioxidant status of clinically healthy American Quarter Horses maintained under Slovak breeding conditions remains limited. The present study aimed to evaluate selected biomarkers of antioxidant status, including total antioxidant status, glutathione peroxidase activity, and malondialdehyde concentration, and to provide preliminary reference data for horses maintained under breeding conditions in eastern Slovakia.

MATERIALS AND METHODS

Experimental Animals

A total of 13 clinically healthy American Quarter Horses were included in the study. The American Quarter Horse is a breed originating from the United States, which began to spread more significantly in Central European countries following the social changes that occurred at the turn of the 1980s and 1990s. Despite the increasing popularity of this breed, information regarding the antioxidant status of American Quarter Horses kept under the conditions of the Slovak Republic remains limited.

The experimental group consisted of seven mares and six geldings aged between 4 and 15 years, used for breeding and recreational purposes. The body weight of the horses ranged from 470 to 540 kg. The body condition of all animals was assessed using the 9-point Body Condition Score (BCS) system and corresponded to normal body condition (BCS = 5) (Potter et al., 2024).

The horses originated from a breeding farm located in the Spiš region of eastern Slovakia and were kept under outdoor housing conditions. The study was conducted during the winter period when the animals were not grazing. The diet consisted primarily of meadow hay obtained from a local supplier, which represented the main component of the ration. Throughout the study period, the horses had unrestricted access to drinking water.

All animals were regularly dewormed according to the recommended veterinary program. Prior to inclusion in the study, all horses underwent a clinical examination and showed no signs of disease or other health disorders that could have influenced the evaluated parameters. During the study period, all animals were maintained under comparable management and husbandry conditions.

Blood Sampling and Sample Processing

Blood samples were collected from the *vena jugularis* of all examined horses under standard conditions, with emphasis on minimizing animal stress during sampling. Blood intended for biochemical and antioxidant analyses was collected into serum separator tubes (SST). Additional blood samples for the determination of glutathione peroxidase (GPx) activity were collected into heparinized tubes. Following collection, the samples were transported to the laboratory and processed as soon as possible under standard laboratory conditions. Blood samples collected into SST tubes were subsequently centrifuged at 3500 rpm for 10 minutes to obtain serum. The separated serum was used for the determination of selected antioxidant parameters, whereas heparinized whole blood was used for the determination of GPx activity.



Fig. 1: Laboratory workflow for the determination of antioxidant parameters
Source: Own photographs.

Determination of Total Antioxidant Status (TAS)

Total antioxidant status was determined using the Randox Total Antioxidant Status kit NX2332, Randox Laboratories Ltd., United Kingdom. The method is based on the inhibition of the formation of the ABTS® radical cation. The absorbance was measured spectrophotometrically at 600 nm using a UV-VIS spectrophotometer ONDA V-10 PLUS (Giorgio Bormac S.r.l., Italy). The TAS values were calculated according to the manufacturer's instructions using calibration procedures and expressed in mmol/L.

Determination of Glutathione Peroxidase Activity (GPx)

Glutathione peroxidase activity was determined using the Ransel kit RS504, Randox Laboratories Ltd., United Kingdom. The assay is based on the oxidation of reduced glutathione by cumene hydroperoxide in the presence of glutathione reductase and NADPH.

The decrease in NADPH absorbance was monitored at 340 nm using a UV-VIS spectrophotometer ONDA V-10 PLUS (Giorgio Bormac S.r.l., Italy). The average change in absorbance per minute was calculated according to the following equation:

$$\Delta A/\text{min} = (A1 - A3) / 2$$

GPx activity was calculated using the following equation:

$$\text{GPx (U/L)} = 8412 \times (-\Delta A/\text{min})$$

The results were expressed as U/L of hemolysate.

Determination of Malondialdehyde Concentration (MDA)

Malondialdehyde concentration was determined using the thiobarbituric acid reactive substances method according to Ohkawa et al. (1979). Malondialdehyde reacts with thiobarbituric acid to form a colored MDA-TBA complex, which was measured spectrophotometrically at 532 nm using a UV-VIS spectrophotometer ONDA V-10 PLUS (Giorgio Bormac S.r.l., Italy), (Ohkawa et al., 1979).

MDA concentration was calculated according to the Beer-Lambert law using the following equation:

$$\text{MDA } (\mu\text{mol/L}) = A \times 6.41$$

where A represents the absorbance measured at 532 nm.

Statistical Analysis

The obtained data were statistically evaluated using basic descriptive statistics. Mean values, standard deviations, minimum values, and maximum values were calculated for all evaluated parameters.

RESULTS AND DISCUSSION

The total antioxidant status values obtained in the evaluated horses ranged from 1.81 to 3.85 mmol/L, with a mean value of 2.42 ± 0.53 mmol/L (Table 1). The highest TAS value was observed in horse No. 12, whereas the lowest value was recorded in horse No. 11. The relatively narrow range of TAS values suggests homogeneous antioxidant capacity within the studied group. Higher TAS values indicate

Tab. 17: Individual values of antioxidant parameters in American Quarter Horses

Horse	TAS (mmol/L)	GPX (U/L)	MDA ($\mu\text{mol/L}$)
1	2.31	770	0.115
2	2.12	1392	0.115
3	2.23	1178	0.122
4	2.77	967	0.115
5	2.04	1102	0.109
6	2.69	833	0.141
7	2.46	736	0.122
8	2.73	652	0.109
9	1.92	538	0.135
10	2.23	648	0.128
11	1.81	551	0.160
12	3.85	1106	0.135
13	2.27	736	0.192

Source: Own results

Tab. 18: Summary statistics of selected antioxidant biomarkers

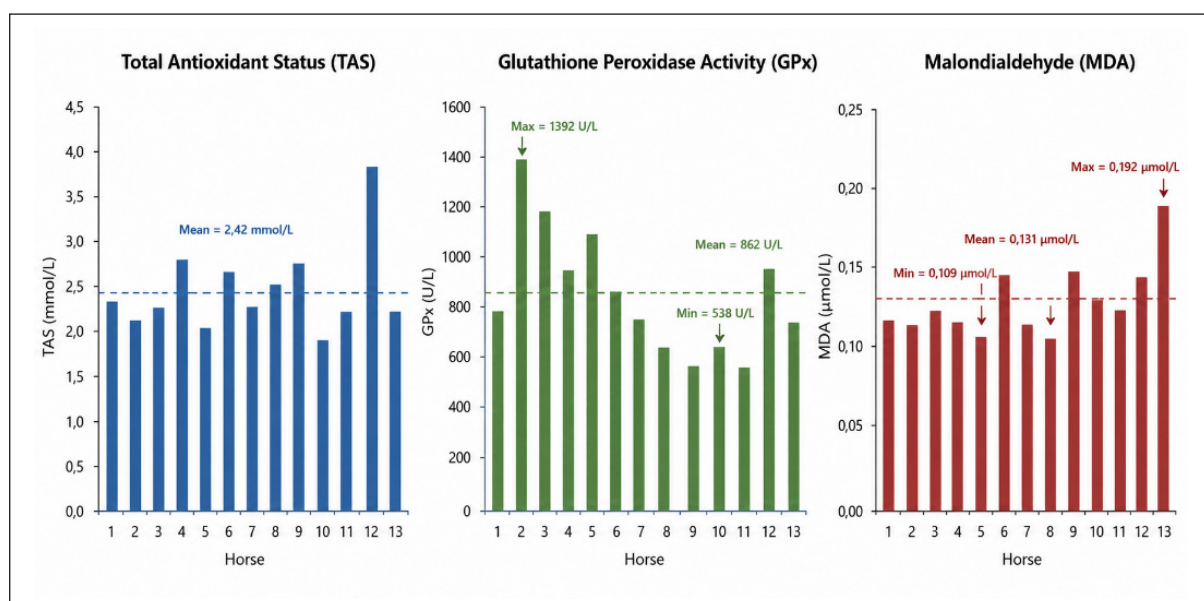
Parameter	Mean $\pm$ SD	Minimum	Maximum
TAS (mmol/L)	2.42 $\pm$ 0.53	1.81	3.85
GPx (U/L)	862 $\pm$ 265	538	1392
MDA (μ mol/L)	0.131 $\pm$ 0.022	0.109	0.192

Source: Own results

greater total antioxidant capacity and a more effective defense against oxidative stress. The obtained results correspond with findings reported by Brkljača Bottegare et al. (2018), who demonstrated that antioxidant status in horses is influenced by metabolic activity and exercise intensity.

Glutathione peroxidase activity ranged from 538 to 1392 U/L, with a mean value of 862 ± 265 U/L. The lowest activity was observed in horse No. 9, while the highest activity was detected in horse No. 2. Higher GPx activity may indicate more efficient enzymatic antioxidant defense mechanisms. The observed variability among individual animals indicates differences in enzymatic antioxidant defense. GPx plays an important role in the detoxification of hydrogen peroxide and lipid hydroperoxides, thereby protecting cellular structures from oxidative damage. Latham et al. (2021) demonstrated that antioxidant enzyme activities in Quarter Horses may be affected by nutritional status and environmental conditions. Similar observations were reported by Niedźwiedź and Jaworski (2014), who identified changes in antioxidant enzymes in horses experiencing oxidative stress.

The mean MDA concentration reached 0.131 ± 0.022 μ mol/L, with values ranging from 0.109 to 0.192 μ mol/L. The highest concentration was observed in horse No. 13, whereas the lowest values were detected in horses No. 5 and No. 8.

**Fig. 2:** Individual values of selected antioxidant biomarkers in American Quarter Horses

Abbreviations: TAS – total antioxidant status; GPx – glutathione peroxidase; MDA – malondialdehyde.

MDA is considered an important marker of lipid peroxidation and oxidative damage. The relatively low concentrations observed in the present study may indicate a low degree of oxidative stress in the examined horses. Similar findings were reported by Yildirim and Apaydin Yildirim (2023), who observed lower MDA concentrations in horses exhibiting a favorable antioxidant status. Increased MDA concentrations have been associated with various pathological conditions and elevated oxidative stress in horses.

Overall, the obtained results suggest a favorable antioxidant profile characterized by relatively high TAS values, adequate GPx activity, and low MDA concentrations, indicating effective antioxidant defense mechanisms in the evaluated horses. The observed values may serve as preliminary reference data for clinically healthy American Quarter Horses maintained under comparable breeding conditions. Low malondialdehyde concentrations suggested a low degree of lipid peroxidation, whereas adequate glutathione peroxidase activity and relatively high total antioxidant status values indicated a well-functioning antioxidant defense system.

CONCLUSION

In clinically healthy American Quarter Horses, the mean TAS value was 2.42 ± 0.53 mmol/L, GPx activity reached 862 ± 265 U/L, and the MDA concentration was 0.131 ± 0.022 μ mol/L. Low MDA concentrations together with adequate GPx activity and higher TAS values indicate preserved antioxidant protection and a low level of oxidative stress. The obtained results may serve as preliminary reference values for the antioxidant status of American Quarter Horses kept under conditions of the Slovak Republic. Considering the increasing popularity of western riding and the growing interest in breeding American Quarter Horses in Slovakia, the present findings contribute to expanding the knowledge of the antioxidant status of this breed, for which data under local conditions remain limited.

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EFFECTS OF DIFFERENT MAIZE CULTIVATION SYSTEMS AND MICROBIAL INOCULATION ON THE AEROBIC STABILITY OF MAIZE SILAGE

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ABSTRACT

Freshly chopped whole-plant maize cultivated under narrow-row spacing (0.375 m) and conventional row spacing (0.75 m) was ensiled in laboratory-scale mini silos with or without a microbial inoculant containing *Lactococcus lactis* and *Lentilactobacillus buchneri*. The aim of the study was to compare the fermentation characteristics and quality parameters of silages produced under the two cultivation systems after six days of aerobic exposure. In addition, the effect of microbial inoculation on the aerobic stability of maize silage was evaluated.

Silages produced from maize grown under narrow-row cultivation had a significantly lower ($p < 0.05$) dry matter content than silages produced under the conventional cultivation system. The untreated narrow-row silage also contained significantly less ($p < 0.05$), crude fibre (205.48 ± 0.59 g/kg DM) than the untreated conventional-row silage (218.46 ± 1.08 g/kg DM). In both inoculated silages, the proportion of lactic acid in total fermentation acids was significantly lower (51.59 % and 52.04 %; $p < 0.05$), whereas the proportion of acetic acid was significantly higher (45.90 % and 45.08 %; $p < 0.05$) than in the untreated controls.

After six days of aerobic exposure, the untreated narrow-row silage exhibited the highest pH (5.82; $p < 0.05$). In contrast, silages treated with the microbial inoculant maintained a stable pH of approximately 4.0 throughout the aerobic exposure period. The untreated narrow-row silage also reached the highest temperature (38 °C), indicating intensive aerobic deterioration. Inoculation with a microbial inoculant containing *L. lactis* and *L. buchneri* significantly improved the aerobic stability of maize silage by reducing both silage heating and pH increase during aerobic exposure.

Keywords: maize silage; microbial inoculants; aerobic stability; row spacing; fermentation



INTRODUCTION

Maize cultivated under narrow-row spacing has attracted increasing attention because of its potential to improve crop performance under changing climatic conditions. Narrow-row cultivation promotes earlier canopy closure, thereby reducing soil water evaporation compared with conventional row spacing (Barbieri et al., 2012). In addition, denser plant stands help suppress weed growth and reduce the risk of soil erosion (Barbieri et al., 2008). Previous studies have shown that row spacing and plant density can influence both the yield and morphological characteristics of maize intended for silage production (Baron et al., 2006). These changes may subsequently affect the chemical composition, fermentation characteristics, and feeding value of maize silage.

Maize silage is one of the most important forage sources for ruminants worldwide owing to its high energy concentration, good palatability, and high dry matter yield (Pasa & Pasa, 2015). Whole-plant maize is generally harvested at a dry matter content of 30–35%, which provides favourable conditions for lactic acid fermentation and ensures efficient preservation of the forage (Kung et al., 2000). Successful ensiling depends on the rapid establishment of anaerobic conditions and the predominance of desirable lactic acid bacteria, resulting in a rapid decline in pH and inhibition of undesirable microorganisms.

Once the silo is opened, however, silage is exposed to oxygen, initiating a series of microbial and biochemical processes collectively referred to as aerobic deterioration. Yeasts are typically the first microorganisms to metabolise residual water-soluble carbohydrates and lactic acid, leading to heat production, carbon dioxide release, alcohol (especially ethanol), nutrient losses, and a gradual increase in silage pH (Muck, 2010). As aerobic deterioration progresses, the elevated pH creates favourable conditions for the growth of moulds and other spoilage microorganisms, resulting in further deterioration of silage quality (Woolford, 1990). Aerobic stability is therefore considered one of the key indicators of silage quality and is commonly defined as the time required for silage temperature to increase by 2 °C above ambient temperature after exposure to air (Kung et al., 2000).

Microbial inoculants are widely used to improve silage fermentation and enhance aerobic stability. Homofermentative bacteria, such as *Lactococcus lactis*, rapidly convert water-soluble carbohydrates into lactic acid, promoting a rapid decline in pH during the initial stage of fermentation (Hindrichsen et al., 2012). In contrast, heterofermentative species, particularly *Lentilactobacillus buchneri*, convert part of the lactic acid into acetic acid, which possesses strong antifungal activity and effectively inhibits the growth of yeasts and moulds during feed-out (Comino et al., 2014). Numerous studies have demonstrated that inoculants containing *L. buchneri*, either alone or in combination with other bacterial species, substantially improve the aerobic stability of maize silage by increasing acetic acid production (Ranjit & Kung, 2000; Tabacco et al., 2011; Silva et al., 2014; Kok et al., 2024).

The present study was conducted within the framework of the NAZV research project QL24010237 entitled *Cultivation of Maize Using Narrow-Row Technology in a Precision Agriculture System (Agriculture 4.0) under Climate Change Conditions – Stabilisation of Forage Production and Sustainable Intensification of Agricultural Production in the Czech Republic*. The objective of this study was to evaluate the effects of two maize cultivation systems and microbial inoculation with *L. lactis* and *L. buchneri* on the fermentation characteristics and aerobic stability of maize silage following six days of aerobic exposure.

MATERIAL AND METHODS

Plant material and silage preparation

Maize (*Zea mays* L.) hybrid KWS Monumento was cultivated at the experimental field station in Žabčice (Czech Republic) using two row-spacing systems: narrow-row cultivation (0.375 m) and conventional cultivation (0.75 m). The crop was harvested at a stubble height of 20–25 cm and chopped to a theoretical length of cut of 10 mm.

Freshly chopped forage from each cultivation system was ensiled in laboratory-scale silos (mini silos) in six replicates. Half of the silos were treated with a microbial inoculant containing *Lactococcus lactis* and *Lentilactobacillus buchneri*, while the remaining silos served as untreated controls. All silos were stored at an ambient temperature of 20–25 °C for five months before being opened for laboratory analyses.

Chemical analyses

After opening, the silages were analysed for dry matter (DM), pH, starch, acidity of the aqueous extract (KVV), lactic acid, volatile fatty acids (VFA), crude protein (CP), crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF), and ash content. Chemical analyses were performed according to Commission Regulation (EC) No. 152/2009, as amended by Commission Implementing Regulation (EU) 2024/771, which specifies official methods for feed sampling and laboratory analysis. All analyses were carried out at the Institute of Animal Nutrition and Forage Science, Mendel University in Brno.

Lactic acid and volatile fatty acids were determined by isotachopheresis (INOSEP 2017-2).

Determination of Ammonia Content

Ammonia concentration was determined using the Conway microdiffusion method. Approximately 5 mL of boric acid solution was placed in the central compartment of each Conway dish, while 10 mL of the aqueous silage extract and 1 mL of potassium carbonate solution were added to the outer compartment. The rim of each dish was sealed with glycerol, and the dishes were immediately closed to prevent ammonia losses.

After gentle mixing of the outer compartment, the dishes were incubated at room temperature for 20 h. Subsequently, Tashiro indicator was added to the boric acid solution, and the absorbed ammonia was quantified by titration with 0.05 N sulphuric acid until a stable pale-pink endpoint was reached.

Ammonia concentration was calculated using the following equation:

$$NH_3(\%) = V_{H_2SO_4} \times 0.0918 \times f_{H_2SO_4}$$

where

- $V_{H_2SO_4}$ = volume of 0.05 N sulphuric acid consumed during titration
- $f_{H_2SO_4}$ = correction factor of the sulphuric acid solution.

Preparation of Extracts

For each silage sample, two subsamples (50 g each) were homogenised with 450 mL of distilled water for 3 min using a blender. The homogenate was subsequently filtered through a 119- μ m (UHELON 59 S, SILK & PROGRESS, spol., s.r.o., Czech Republic) laboratory sieve and then through qualitative filter paper (FILTRAK 388) to obtain a clear aqueous extract for further analyses.

Aerobic stability

Aerobic stability was evaluated by continuously monitoring silage temperature during aerobic exposure. After the initial analyses, 350 g of silage from each treatment was transferred into individual polyethylene containers and exposed to air under laboratory conditions. Temperature probes were inserted into the centre of each sample, and silage temperature was recorded automatically at 1-h intervals.

Following six days of aerobic exposure, the silage samples were analysed again for DM, pH, KVV, ammonia concentration, lactic acid, volatile fatty acids, and basic chemical composition.

Statistical analysis

Data were analysed using a two-way analysis of variance (ANOVA), with cultivation system and microbial inoculation as the main experimental factors. Treatment means were compared using Tukey's honestly significant difference (HSD) test. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

As shown in Table 1, silages produced from maize cultivated under the narrow-row system exhibited lower dry matter (DM) contents (350.0 and 352.6 g/kg) than silages produced under the conventional cultivation system, in which DM contents reached 397.7 and 389.96 g/kg.

The highest crude protein (CP) concentration was observed in the inoculated narrow-row silage (77.31 ± 0.09 g/kg DM; $p < 0.05$), whereas the untreated silage

produced from conventionally grown maize had the lowest CP content (67.55 g/kg DM). Similar increases in crude protein following inoculation with *L. buchneri* have previously been reported by SILVA et al. (2014) and BUMBIERIS Jr. et al. (2017). A comparable trend was observed in the present study, where microbial inoculation increased the crude protein content in both cultivation systems.

The untreated narrow-row silage also contained significantly less crude fibre (205.48 ± 0.59 g/kg DM) than the untreated conventional-row silage (218.46 ± 1.08 g/kg DM; $p < 0.05$).

Results obtained from the experiments, including their statistical evaluation and commentary, should be presented graphically or in table-form and the author should comment on the results and confront them with data published elsewhere.

The fermentation characteristics of the silages after opening are presented in Table 2. The lowest pH value (3.83; $p < 0.05$) was recorded in the untreated narrow-row silage, whereas the highest pH (3.96) was observed in the inoculated silage produced under the conventional cultivation system.

Barry et al. (1980) reported that lactic acid accumulation during fermentation lowers silage pH, thereby suppressing undesirable microbial activity and improving nutrient preservation. In the present study, the acidity of the aqueous extract was significantly higher in inoculated silages (1952.0 ± 193.82 and 2045.7 ± 116.16 mg KOH/100 g) than in the untreated controls ($p < 0.05$), indicating more intensive fermentation.

The concentrations and proportions of fermentation products are summarised in Table 3. According to SHAVER (2003), lactic acid should account for approximately 65–70% of total fermentation acids in well-preserved maize silage. This criterion was fulfilled by both untreated silages, in which lactic acid represented approximately 80% of total fermentation acids. In contrast, the inoculated silages contained significantly lower proportions of lactic acid (51.59% and 52.04%; $p < 0.05$). The reduced proportion of lactic acid resulted from the activity of the heterofermentative bacterium *L. buchneri*, which promoted the conversion of lactic acid into acetic acid. Consequently, the proportion of acetic acid increased significantly, reaching 45.90% and 45.08% in the inoculated silages.

The lactic acid concentration in well-fermented maize silage generally ranges from 2 to 4% of DM. In the present study, lactic acid concentrations ranged from 3.46 to 4.45% DM, which is consistent with values reported for high-quality maize silages

Krooneman et al. (2002) reported that acetic acid concentrations typically range between 1 and 3% DM. In the present experiment, untreated silages contained less than 1% acetic acid, whereas inoculated silages contained significantly higher concentrations (3.19% and 2.99% DM; $p < 0.05$).

These findings agree with those of Kung and Ranjit (2000), who demonstrated that inoculation with *L. buchneri* significantly increased acetic acid production. Similar results were reported by Kok et al. (2024), who observed enhanced acetic

Tab. 19: Chemical composition of maize silage immediately after silo opening

Row spacing (metre)		0.375	0.75	0.375	0.75
Treatment		Control		Lc. Lactis + Len buchneri	
n		6		6	
Variables	units	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DM	g/kg	350.00 ^a ± 0.6390	397.70 ^b ± 0.5623	352.60 ^a ± 0.9997	389.36 ^b ± 0.8084
Ash	g/kg	35.48 ^a ± 0.0934	38.70 ^b ± 0.0688	38.53 ^b ± 0.0752	40.06 ^c ± 0.0611
Crude protein	g/kg	71.61 ^b ± 0.1729	67.55 ^a ± 0.1967	77.31 ^c ± 0.0941	70.26 ^b ± 0.1324
Crude fat	g/kg	26.57 ^a ± 0.1396	26.37 ^a ± 0.1306	29.84 ^b ± 0.0842	29.45 ^b ± 0.0826
Starch	g/kg	304.60 ^a ± 1.4140	325.42 ^{ab} ± 1.0578	322.02 ^{ab} ± 2.1329	332.50 ^b ± 1.4700
Crude fiber	g/kg	205.48 ^a ± 0.5851	218.46 ^b ± 1.0843	213.68 ^{ab} ± 0.6007	214.94 ^{ab} ± 0.7174
ADF	g/kg	247.98 ^a ± 0.8371	273.64 ^d ± 0.5510	260.30 ^{ab} ± 0.7660	288.92 ^c ± 1.0415
aNDF	g/kg	416.36 ^c ± 1.7677	457.23 ^{ab} ± 0.8125	444.50 ^a ± 1.2553	468.38 ^b ± 1.3003

Means of value in a row marked with different letters are significantly different between groups ($p < 0.05$), Tukey's HSD test, n – number of cases, SD, standard deviation; DM, dry matter; aNDF, neutral detergent fiber determined with α -amylase; ADF, acid detergent fib

Tab. 20: Fermentation characteristics of maize silage immediately after silo opening

Row spacing (metre)		0.375	0.75	0.375	0.75
Treatment		Control		Lc. Lactis + Len buchneri	
n		6		6	
Variables	units	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
pH	g	3.83 ^a ± 0.03688	3.89 ^b ± 0.02137	3.89 ^{ab} ± 0.01789	3.96 ^c ± 0.05820
WEA mg KOH/100	g	1383.9 ^a ± 65.93	1382.1 ^a ± 78.16	1952.0 ^b ± 193.82	2045.7 ^b ± 116.16
NH ₃	%	0.0372 ± 0.0142	0.0372 ± 0.0144	0.0389 ± 0.0131	0.0601 ± 0.0408
NH ₃ – N	%	0.0307 ± 0.0117	0.0307 ± 0.0119	0.0321 ± 0.0108	0.0495 ± 0.0336

Means of values in a row marked with different letters are significantly different between groups ($p < 0.05$), Tukey's HSD test, n – number of cases, SD, standard deviation; WEA, water extract acidity; NH₃, ammonium; NH₃-N, ammonia nitrogen.

Tab. 21: Fermentation products of maize silage immediately after silo opening

Row spacing (metre)		0.375	0.75	0.375	0.75
Treatment		Control		Lc. Lactis + Len buchneri	
n		11		12	
Variables	units	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
LA/VFA	% of DM	4.01 ^b ± 0.2238	4.16 ^b ± 0.5497	1.09 ^a ± 0.2243	1.10 ^a ± 0.1659
LA	% of DM	4.45 ^c ± 0.6413	4.27 ^{bc} ± 1.1702	3.58 ^{ab} ± 0.5107	3.46 ^{ab} ± 0.5852
AA	% of DM	0.91 ^a ± 0.1485	0.80 ^a ± 0.2578	3.19 ^b ± 0.5273	2.99 ^b ± 0.5336
PA	% of DM	0.12 ^c ± 0.0180	0.11 ^{ac} ± 0.0261	0.08 ^b ± 0.0168	0.09 ^{ab} ± 0.0276
BA	% of DM	0.08 ± 0.0232	0.14 ± 0.2054	0.09 ± 0.0097	0.10 ± 0.0116
VFA	% of DM	1.11 ^a ± 0.1594	1.05 ^a ± 0.3538	3.37 ^b ± 0.5483	3.19 ^b ± 0.5604
Total organic acids	% of DM	55.56 ^{ac} ± 7.9338	53.21 ^c ± 14.6952	69.51 ^b ± 8.4874	66.42 ^{ab} ± 9.9396
Proportion of LA	%	80.03 ^b ± 0.9271	80.36 ^b ± 2.7786	51.59 ^a ± 4.6586	52.04 ^a ± 4.0002
Proportion of AA	%	16.36 ^a ± 0.5149	14.72 ^a ± 1.3765	45.90 ^b ± 4.5690	45.08 ^b ± 4.0262
Proportion of PA	%	2.14 ^b ± 0.2972	2.20 ^b ± 0.4314	1.20 ^a ± 0.1495	1.32 ^a ± 0.3134
Proportion of BA	%	1.47 ^{bc} ± 0.6509	2.73 ^c ± 3.3040	1.31 ^a ± 0.1031	1.55 ^{ab} ± 0.1151

Means of values in a row marked with different letters are significantly different between groups ($p < 0.05$), Tukey's HSD test, n – number of cases, S – Standard deviation, DM, dry matter; LA, lactic acid; AA, acetic acid; PA, propionic acid; BA, butyric acid; VFA, volatile fatty acids; LA/VFA ratio, ratio of lactic acid to volatile fatty acids. Total organic acids represent the sum of lactic, acetic, propionic and butyric acids

acid production and improved aerobic stability in silages treated with *L. lactis* and *L. buchneri*. As expected, propionic acid was absent or detected only in trace amounts, which is typical of well-fermented maize silages with relatively high dry matter contents.

As shown in Table 4, weight loss after fermentation was significantly higher ($p < 0.05$) in the untreated narrow-row silage (21.08 g). Following six days of aerobic exposure, both narrow-row silages contained significantly higher crude protein concentrations than the corresponding conventional-row silages. The crude protein content increased significantly in the untreated narrow-row silage, from 71.61 to 79.98 g/kg DM. Woolford (1990) reported that exposure of silage to air results in the depletion of readily oxidisable forage components. Consequently, structural carbohydrates, crude protein, and ash represent a greater proportion of the remaining dry matter. Similar findings were reported by Tabacco et al. (2011), who observed increases in crude protein and ADF concentrations with increasing duration of aerobic exposure.

According to Rezende et al. (2011), exposure of silage to aerobic conditions is associated with several characteristic changes, including increases in pH, temperature, the proliferation of aerobic microorganisms, and ammonia nitrogen concentration. Similar trends were observed in the present study.

The fermentation characteristics of the silages after opening are presented in Table 5. Following six days of aerobic exposure, ammonia and ammonia nitrogen concentrations increased in all silages except for the untreated narrow-row silage. Bumbieris Jr. et al. (2017) reported that exposure to air promotes the growth of aerobic microorganisms, particularly yeasts and moulds, which utilize nutrients present in the silage. During this process, protein degradation may also occur as a result of the activity of *Clostridium* spp. and other undesirable microorganisms. Deamination of amino acids releases ammonia, contributing to an increase in ammonia nitrogen concentration.

Microbial inoculation effectively prevented the increase in pH during aerobic exposure. In contrast, the pH of untreated silages increased to values exceeding 5.0. The untreated narrow-row silage exhibited the highest pH value (5.82; $p < 0.05$), indicating the greatest degree of aerobic deterioration. According to Kung and Ranjit (2001), an increase in pH following exposure to air is one of the most reliable indicators of aerobic spoilage. Lactate-assimilating yeasts, including species of *Saccharomyces*, *Candida*, *Cryptococcus*, and *Pichia*, are considered the primary initiators of aerobic deterioration because they metabolise lactic acid, thereby eliminating the principal factor responsible for maintaining a low silage pH (Kung & Ranjit, 2000).

The acidity of the aqueous extract was significantly lower ($p < 0.05$) in the untreated silages from both cultivation systems (402.7 ± 360.36 and 672.6 ± 699.84 mg KOH/100 g), reflecting the depletion of fermentation acids during aerobic deterioration.

Tab. 22: Chemical composition of maize silage after six days of aerobic exposure

Row spacing (metre)	0.375	0.75	0.375	0.75
Treatment	Control	Control	<i>Lc. Lactis + Len buchneri</i>	<i>Lc. Lactis + Len buchneri</i>
n	6	6	6	6
Variables	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
Fermentation weight loss				
DM	21.08 ^b $\pm$ 14.038	10.68 ^{ab} $\pm$ 3.3075	6.38 ^a $\pm$ 0.9071	6.28 ^a $\pm$ 0.6847
Ash	351.71 ^a $\pm$ 1.1026	405.74 ^b $\pm$ 0.7255	361.61 ^a $\pm$ 0.6567	399.19 ^b $\pm$ 0.2690
Crude protein	39.76 $\pm$ 0.3723	40.95 $\pm$ 0.0758	38.50 $\pm$ 0.1066	40.668 $\pm$ 0.0682
Crude fat	79.98 ^b $\pm$ 0.6110	68.87 ^a $\pm$ 0.1420	76.22 ^b $\pm$ 0.1804	69.56 ^a $\pm$ 0.0735
Starch	27.72 ^a $\pm$ 0.1767	26.48 ^a $\pm$ 0.1610	31.82 ^b $\pm$ 0.0870	28.71 ^a $\pm$ 0.1527
Crude fiber	309.04 $\pm$ 1.6887	318.57 $\pm$ 1.3142	323.09 $\pm$ 1.1828	324.45 $\pm$ 1.1330
ADF	214.53 $\pm$ 1.5247	215.95 $\pm$ 0.6430	203.81 $\pm$ 0.6003	212.45 $\pm$ 0.8066
aNDF	285.28 $\pm$ 2.4305	289.80 $\pm$ 0.9642	271.15 $\pm$ 1.0824	278.98 $\pm$ 0.7347
	458.07 $\pm$ 2.6304	457.96 $\pm$ 1.3385	433.66 $\pm$ 1.2989	450.28 $\pm$ 1.6680

Means of values in a row marked with different letters are significantly different between groups ($p < 0.05$), Tukey's HSD test, n – number of cases, SD, standard deviation; DM, dry matter; aNDF, neutral detergent fiber determined with α -amylase; ADF, acid detergent fiber

Tab. 23: Fermentation characteristics of maize silage after six days of aerobic exposure

Row spacing (metre)	0.375	0.75	0.375	0.75
Treatment	Control	Control	<i>Lc. Lactis + Len buchneri</i>	<i>Lc. Lactis + Len buchneri</i>
n	6	6	6	6
Variables	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
pH	5.82 ^b $\pm$ 1.4017	5.07 ^{ab} $\pm$ 1.0821	3.92 ^a $\pm$ 0.0337	3.98 ^a $\pm$ 0.0316
WEA	402.7 ^a $\pm$ 360.36	672.6 ^a $\pm$ 699.84	2123.7 ^b $\pm$ 161.68	2134.3 ^b $\pm$ 113.29
NH₃	0.0371 $\pm$ 0.0129	0.0484 $\pm$ 0.0138	0.0479 $\pm$ 0.0163	0.0484 $\pm$ 0.0150
NH₃ – N	0.0306 $\pm$ 0.0106	0.0398 $\pm$ 0.0113	0.0395 $\pm$ 0.0134	0.0398 $\pm$ 0.0123

Means of values in a row marked with different letters are significantly different between groups ($p < 0.05$), Tukey's HSD test, n – number of cases, SD, standard deviation; WEA, water extract acidity; NH₃, ammonium; NH₃–N, ammonia nitrogen.

According to Velho et al. (2006), the increase in silage temperature during aerobic exposure is caused by the metabolic activity of spoilage microorganisms. As these microorganisms degrade nutrients, energy is released in the form of heat, resulting in progressive heating of the silage mass.

Table 6 shows the aerobic stability of corn silage after six days of aerobic exposure. In the present study, untreated silages reached higher maximum temperatures than inoculated silages (38.35 and 31.83 °C, respectively), indicating greater susceptibility to aerobic deterioration. Consequently, the aerobic stability of the untreated silages was reduced. In contrast, silages treated with *Lactococcus lactis* and *Lentilactobacillus buchneri* exhibited improved aerobic stability under both cultivation systems with aerobic stability increasing to 79.4 h and 63.4 h, respectively. These findings are consistent with those of KOK et al. (2024), who also reported enhanced aerobic stability in maize silage treated with the same bacterial combination.

According to Doležal et al. (2012), aerobic deterioration inevitably results in nutritional losses. Similarly, the untreated narrow-row silage reached a temperature of approximately 38 °C after six days of aerobic exposure. Muck and Pitt (1993) suggested that prolonged exposure to temperatures of this magnitude may lead to gradual protein denaturation, thereby reducing the nutritional value of the silage.

In the present study, inoculation with *L. lactis* and *L. buchneri* increased the aerobic stability of narrow-row silage by approximately 50 h, whereas the corresponding improvement in conventionally grown maize silage was only 11.8 h. Although these results confirm the positive effect of microbial inoculation on aerobic stability, the observed improvement was considerably lower than that reported by KOK et al. (2024), who achieved an extension of aerobic stability of approximately 150 h.

Tab. 24: *Aerobic stability of maize silage after six days of aerobic exposure*

Row spacing (metre)	0.375	0.375	0.75	0.75
Treatment	Control	<i>Lc. Lactis + Len buchneri</i>	Control	<i>Lc. Lactis + Len buchneri</i>
Aerobic stability (hours)	28.4	79.4	51.6	63.4
Average environmental temperature	24.20	24.20	24.20	24.20
Maximum temperature	38.35	26.27	31.83	27.66
Maximum pH	5.82	3.92	5.07	3.98

Maize silage is particularly susceptible to aerobic deterioration after silo opening because of its high concentration of water-soluble carbohydrates. The application of microbial inoculants is therefore an effective strategy for improving aerobic stability during the feed-out period. Inoculants containing a combination of homofermentative and heterofermentative lactic acid bacteria are especially suitable for maize silage.

In the present study, maize cultivated under two different row-spacing systems was ensiled with a microbial inoculant containing *Lactococcus lactis* and *Lentilactobacillus buchneri*. Silage produced from maize grown under narrow-row cultivation had a significantly lower dry matter content (350.0 and 352.6 g/kg) than silage produced under the conventional cultivation system (397.7 and 389.96 g/kg; $p < 0.05$). The untreated conventional-row silage contained significantly more crude fibre (218.46 ± 1.08 g/kg DM) but the lowest crude protein concentration (67.55 g/kg DM). The highest pH after fermentation was recorded in the inoculated conventional-row silage (pH 3.96; $p < 0.05$).

Inoculation with *L. buchneri* significantly reduced the proportion of lactic acid (51.59% and 52.04%) while increasing the proportion of acetic acid (45.90% and 45.08%), reflecting the metabolic activity of this heterofermentative species.

Following six days of aerobic exposure, the untreated narrow-row silage exhibited the greatest fermentation losses, the highest pH (5.82), and the lowest aerobic stability. In contrast, silages treated with the microbial inoculant maintained a stable pH of approximately 4.0 and reached lower maximum temperatures during aerobic exposure. Overall, the results demonstrate that microbial inoculation with *L. lactis* and *L. buchneri* effectively enhanced the aerobic stability of maize silage produced under both cultivation systems.

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EFFECT OF CATTLE BREED ON MUSCLE PROTEIN

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ABSTRACT

The study evaluated differences in muscle protein quality in the *m. longissimus, pars thoracis* of bulls from the Aberdeen Angus and Czech Fleckvieh breeds reared under identical extensive pasture-based conditions. A significantly higher crude protein content ($P \leq 0.05$) was found in Czech Fleckvieh bulls (886.96 ± 87.36 g/kg of dry matter) compared to Aberdeen Angus bulls (812.48 ± 59.31 g/kg of dry matter). The Czech Fleckvieh breed also exhibited greater concentrations of non-essential amino acids (389.79 ± 37.92 vs. 363.91 ± 44.48 g/kg of dry matter; $P \leq 0.05$), essential amino acids (405.95 ± 38.95 vs. 382.25 ± 41.00 g/kg of dry matter; $P \leq 0.05$), and total analysed amino acids (795.74 ± 74.85 vs. 746.16 ± 82.71 g/kg of dry matter; $P \leq 0.05$) than Aberdeen Angus. Breed significantly affected the content of most analysed amino acids ($P \leq 0.05$), whereas no breed differences were detected for tyrosine and phenylalanine ($P > 0.05$). Lysine was the most abundant essential amino acid, with contents of 73.43 ± 8.23 g/kg of dry matter in Aberdeen Angus and 76.57 ± 8.87 g/kg of dry matter in Czech Fleckvieh ($P \leq 0.05$). Among non-essential amino acids, glutamic acid was the most abundant, reaching 108.97 ± 14.35 g/kg of dry matter in Aberdeen Angus and 119.42 ± 12.47 g/kg of dry matter in Czech Fleckvieh ($P \leq 0.05$). These findings suggest that breed influences crude protein concentration and the amino acid profile of muscle tissue.

Keywords: genotype; pasture; beef; nutrients; nutritional quality

INTRODUCTION

Beef is recognised as an important dietary source of protein, with its protein concentration remaining relatively stable (Gunduz and Cayiroglu 2024; Mujic et al. 2025). It is also characterised by a nutritionally favourable amino acid profile, particularly due to the presence of essential amino acids that cannot be synthesised by the human body (Samicho et al. 2013). Glutamic acid and lysine occur at the highest concentrations among amino acids present in beef (Wu et al. 2016). Ruminants obtain a substantial proportion of amino acids through microbial



fermentation in the rumen. Dietary protein is primarily degraded by ruminal microorganisms and transformed into microbial protein, which is digested and utilised in the small intestine. Rumen undegradable protein passes to the small intestine, where it contributes to meeting nutritional requirements that cannot be fully covered by microbial protein alone. For this reason, cattle diets should provide an appropriate balance of both rumen degradable and rumen undegradable protein (Zahradkova 2009).

The nutritional quality of muscle tissue is strongly associated with cattle breed. Compared with dairy and dual-purpose breeds, beef breeds are generally characterised by a higher protein content in muscle tissue. Differences in maturation pattern are also important, as early-maturing breeds tend to deposit fat sooner, whereas late-maturing breeds remain in the muscle growth phase for a longer period before intensive fat deposition occurs (Solarczyk et al. 2020). In addition, variation in protein concentration and amino acid composition may depend on the muscle type (Samicho et al. 2013; Fortova et al. 2018). Reported effects of grazing on muscle protein content remain inconsistent (Hwang and Joo 2017; Fruet et al. 2018; Najjar-Villarreal et al. 2019; Siphambili et al. 2020), likely due to seasonal changes in forage quality, as well as differences in pasture botanical composition and grazing management.

MATERIAL AND METHODS

The study involved 65 bulls of the dual-purpose breed Czech Fleckvieh and 65 bulls of the beef breed Aberdeen Angus. Czech Fleckvieh was the only dual-purpose breed, and Aberdeen Angus represented the largest group of beef breeds grazed in the study area. All animals were raised under identical conditions within an extensive pasture-based system in the Prachatice District, South Bohemia Region, Czech Republic (828 m altitude). Cattle were managed under a free-grazing system with unrestricted movement and minimal human intervention. During the main grazing season (March–November), pasture was the main component of the diet. Pasture consisted mainly of red fescue (*Festuca rubra*), meadow fescue (*Festuca pratensis*), smooth meadow-grass (*Poa pratensis*), and white clover (*Trifolium albi*). In winter, animals were fed haylage, hay, and straw. Water was supplied through tanks. Salt and mineral licks were provided ad libitum, while conserved forage in the form of bale feed was offered when necessary. The stocking density was approximately one animal per 2 ha. Bulls were slaughtered between 16 and 18 months of age over a two-year period depending on slaughterhouse capacity.

Muscle samples were obtained at the Volary slaughterhouse, ZEFA, from 65 Aberdeen Angus and 65 Czech Fleckvieh bulls slaughtered between 16 and 18 months of age. From each carcass a 500 g sample of the *m. longissimus, pars thoracis* was taken. Dry matter (g/kg) was determined gravimetrically after drying the samples at 105 °C. Crude protein content (g/kg) was calculated as nitrogen determined by the Kjeldahl method multiplied by the factor 6.25 and analysed using

a Buchi analyser (Centec automation, company s.r.o., Prague, Czech Republic). Amino acid composition was determined following acid hydrolysis using an automatic amino acid analyser AAA 400 (INGOS, a. s., Prague, Czech Republic) based on the colour reaction of amino acids with ninhydrin. The analysed amino acids included aspartic acid (Asp), threonine (Thre), serine (Ser), glutamic acid (Glu), proline (Pro), glycine (Gly), alanine (Ala), valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu), tyrosine (Tyr), phenylalanine (Phe), histidine (His), lysine (Lys), and arginine (Arg).

Statistical analysis focused on the number of samples (n), mean ($\bar{x}$), and standard deviation (SD). Data normality was evaluated using the Shapiro–Wilk test in Unistat version 6.5 (Unistat Ltd., London, UK), where the data fulfilled the condition of normal distribution ($P > 0.05$). Homogeneity of variances was assessed using the F -test ($P > 0.05$). F -test was followed by the Student's t -test, and differences between mean values were considered statistically significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Table 1. demonstrates breed-related differences in muscle crude protein content, with higher values observed in Czech Fleckvieh (CF) compared to Aberdeen Angus (AA). Crude protein content reached 886.96 ± 87.36 g/kg of dry matter in CF and 812.48 ± 59.31 g/kg of dry matter in AA ($P \leq 0.05$). These findings suggest that cattle breed influences muscle protein content. CF reaches the stage of intensive fat deposition later than AA, while AA breed is characterised by greater intramuscular fat content (Bures and Barton 2018; Papanikolopoulou et al. 2025). Papanikolopoulou et al. (2025) also reported that breed can affect muscle protein content. Compared with Limousine and Holstein bulls, Aberdeen Angus bulls exhibited the lowest protein content and the highest fat content in the *m. longissimus dorsi*. However, no breed-related differences in protein content were reported in the *m. longissimus lumborum* of Aberdeen Angus and Czech Fleckvieh bulls fed the same diet in intensive feeding system and slaughtered at approximately 17 months of age (Bures and Barton 2018).

Table 1 shows differences in the content of non-essential amino acids (NEAA), essential amino acids (EAA), and total analysed amino acids (AAs) in the muscle tissue of the evaluated cattle breeds, with consistently higher values observed in the CF breed across all assessed parameters. The NEAA content reached 363.91 ± 44.48 g/kg of dry matter in AA and 389.79 ± 37.92 g/kg of dry matter in CF ($P \leq 0.05$). Similarly, EAA content was higher in CF, amounting to 405.95 ± 38.95 g/kg of dry matter compared to 382.25 ± 41.00 g/kg of dry matter in AA ($P \leq 0.05$). The AAs content was 746.16 ± 82.71 g/kg of dry matter in AA and 795.74 ± 74.85 g/kg of dry matter in CF ($P \leq 0.05$). Significant breed-related differences were identified for nearly all analysed amino acids ($P \leq 0.05$), except for Tyr and Phe, for which no significant differences were detected ($P > 0.05$). Glu was the predominant amino acid within the NEAA group, with values of 108.97 ± 14.35 g/kg of dry

matter in AA and 119.42 ± 12.47 g/kg of dry matter in CF ($P \leq 0.05$). Among EAA, Lys was the most abundant amino acid, reaching 73.43 ± 8.23 g/kg of dry matter in AA and 76.57 ± 8.87 g/kg of dry matter in CF ($P \leq 0.05$). These findings suggest that breed may significantly influence amino acid composition and affect the nutritional quality of muscle protein. Consistent with our study, Koutsidis et al. (2008), Vopalensky et al. (2017), Chen et al. (2022) and Ge et al. (2023) reported that breed can affect amino acid composition and content of total analysed amino acids. Our study is consistent with the results reported by Vopalensky et al. (2017). In both studies, the Aberdeen Angus breed was raised under identical pasture conditions and feeding management. The main difference between the studies was the number of analysed samples. The results were highly comparable, indicating that breed is likely a primary factor affecting the amino acid composition of the *m. longissimus thoracis*. Consistent with our study, Lys was the most abundant EAA and Glu was most abundant NEAA in different muscles across cattle breeds (Samicho et al. 2013; Vopalensky et al. 2017; Ge et al. 2023; Merayo et al. 2023).

Tab. 25: Amino acid profile and crude protein content (g/kg of dry matter) in the muscle tissue of Aberdeen Angus and Czech Fleckvieh bulls

Nutrients	Aberdeen Angus $\bar{x} \pm SD$	Czech Fleckvieh $\bar{x} \pm SD$	P value
Crude protein	812.48 ± 59.31	886.96 ± 87.36	*
AAs	746.16 ± 82.71	795.74 ± 74.85	*
EAA	382.25 ± 41.00	405.95 ± 38.95	*
Thre	33.67 ± 4.12	36.00 ± 3.64	*
Val	37.41 ± 4.36	40.14 ± 4.20	*
Met	19.06 ± 2.67	21.54 ± 2.57	*
Iso	35.44 ± 3.95	37.86 ± 3.71	*
Leu	61.43 ± 6.47	65.51 ± 6.93	*
Phe	30.66 ± 3.32	31.61 ± 3.76	ns
His	31.89 ± 4.65	34.34 ± 4.63	*
Lys	73.43 ± 8.23	76.57 ± 8.87	*
Arg	59.26 ± 6.56	62.37 ± 7.55	*
NEAA	363.91 ± 44.48	389.79 ± 37.92	*
Asp	70.10 ± 8.06	74.49 ± 7.50	*
Ser	29.19 ± 3.58	31.81 ± 6.20	*
Glu	108.97 ± 14.35	119.42 ± 12.47	*
Pro	38.03 ± 8.16	41.40 ± 5.19	*
Gly	40.73 ± 6.32	43.16 ± 5.73	*
Ala	45.48 ± 5.86	48.55 ± 5.17	*
Tyr	31.42 ± 4.22	30.97 ± 4.01	ns

mean($\bar{x}$) $\pm$ standard deviation(SD), AAs–total analysed amino acids, EAA–essential amino acids, NEAA – non-essential amino acids, ns = not significant, * $P \leq 0.05$

CONCLUSION

The results demonstrated that cattle breed significantly influenced crude protein concentration and the amino acid composition of muscle tissue in bulls reared under identical extensive pasture-based conditions. Czech Fleckvieh bulls exhibited higher crude protein content as well as greater concentrations of essential, non-essential, and total analysed amino acids in the *m. longissimus, pars thoracis* compared with Aberdeen Angus bulls. Breed-related differences were identified for the majority of analysed amino acids, whereas tyrosine and phenylalanine were not affected by breed. These findings suggest that cattle breed contributes to variation in the nutritional quality of beef muscle tissue.

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FROM LARVAE TO MEAL: INSECTS UNDER PROCESSING PRESSURE

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ABSTRACT

The industrial conversion of insects into high-value protein meals is a cornerstone of the modern circular bioeconomy, where minimizing processing losses is critical for economic viability. This study analyzed the processing losses and mass balance of two economically important insect species – yellow mealworm larvae (*Tenebrio molitor*) and adult house crickets (*Acheta domestica*) – during post-harvest technological processing, including freezing, lyophilization (freeze-drying), and homogenization (mechanical milling). For crickets, three dietary regimens were compared using five replicates each: a traditional plant-protein diet (PP), an insect-protein diet (IP), and a combined-protein diet (CP). For mealworms, two dietary groups were evaluated using five replicates each, specifically a control group (CG) and an insect-protein group (IG). The results showed that losses during freezing and milling did not differ significantly among groups CG and IG ($p > 0.05$). In contrast, losses during lyophilization differed significantly among groups CG and IP ($p < 0.05$). Significant within-species variations in lyophilization losses were also observed, both in *Acheta domestica* and *Tenebrio molitor* ($p < 0.05$). These findings indicate that dietary composition does not affect mechanical processing losses but significantly influences moisture retention and subsequent drying efficiency. Understanding these mass balance parameters allows for the optimization of industrial post-harvest processing of insect biomass.

Keywords: *Tenebrio molitor*; *Acheta domestica*; black soldier fly larvae meal; alternative feed; biomass yield; freeze-drying; homogenization



INTRODUCTION

Insect biorefining is a key driver of the global circular bioeconomy, with the yellow mealworm (*Tenebrio molitor*) and house cricket (*Acheta domesticus*) leading EU food and feed applications due to efficient feed conversion and established mass-rearing (Tettamanti et al., 2022; Bordiean et al., 2022; Siddiqui et al., 2024; Conway et al., 2024). While black soldier fly (*Hermetia illucens*, BSF) larvae meal is widely studied as a sustainable replacement for soybean meal in livestock and aquaculture (Tettamanti et al., 2022; Le Boucher et al., 2024), its use as a feed ingredient for rearing other commercial insect species—and its subsequent effect on the technological quality of harvested biomass—remains unexplored.

Diet composition directly dictates insect growth, metabolism, and final biochemical profile, including protein, lipid, and chitin content (Bordiean et al., 2022; Orkusz et al., 2023). Because biochemical composition shapes how biological materials behave during processing, dietary changes likely alter post-harvest characteristics, though this relationship has received minimal scientific focus.

Post-harvest processing ensures microbial safety and product stability (Marzoli et al., 2023). Freezing is standard for ethical extermination and enzyme reduction (Cámara-Ruiz et al., 2023), while freeze-drying preserves nutritional and structural integrity (Krzyżaniak et al., 2022; Yan et al., 2023). The efficiency of freeze-drying depends on matrix properties like moisture distribution and lipid composition, which vary with diet (Krzyżaniak et al., 2022). Finally, milling yields a homogeneous powder (Yan et al., 2023) whose flowability and handling are governed by particle size, residual moisture, and lipids, directly affecting product recovery (Suhag et al., 2024; Weiss et al., 2021).

The aim of the present study was to quantify the biomass losses occurring during successive post-harvest technological processing steps — freezing, lyophilization, and mechanical milling — in two species of edible insects, the house cricket (*Acheta domesticus*) and the yellow mealworm (*Tenebrio molitor*), fed diets differing in protein source (plant-based, insect-based, or a combination of both, depending on species), and to evaluate the mass recovery throughout the entire processing chain.

MATERIAL AND METHODS

Experimental animals and dietary treatments

Two edible insect species were used in this study: the house cricket (*Acheta domesticus*) and the yellow mealworm (*Tenebrio molitor*). Insects were reared under controlled conditions at a temperature of 25 °C for crickets and 27 °C for mealworms, relative humidity of 50 % for crickets and 60 % for mealworms, and a photoperiod of 12 hours light and 12 hours dark for both species, for a period of 45 days.

Crickets were divided into three dietary groups (n = 5 replicates per group), differing in their main protein source. The plant-protein diet (PP) was based on

wheat and soybean meal as the primary protein source, supplemented with rapeseed oil and a standard mineral premix (limestone, salt, monocalcium phosphate, sodium bicarbonate). The insect-protein diet (IP) was formulated identically, with the soybean meal fully replaced by black soldier fly (*Hermetia illucens*) larvae meal as the sole protein source. The combined-protein diet (CP) contained a partial replacement of soybean meal with black soldier fly larvae meal (50:50), resulting in a mixed plant- and insect-based protein source. All three diets were formulated to be isonitrogenous and isoenergetic.

Tab. 26: Composition of experimental diets for *Acheta domesticus* (%).

Ingredient (%)	PP	IP	CP
Grains	48.28	47.70	47.34
Legumes	41.00	–	20.50
Insect protein	–	42.40	21.40
Oilseeds	7.00	5.60	6.80
Minerals	2.72	3.30	2.96
Additives	1	1	1
Total	100.00	100.00	100.00

Mealworms were divided into two dietary groups (n = 5 replicates per group), based on wheat bran as the basal component (78.68%). The control group (CG) received wheat bran combined with a soybean-based protein concentrate (21.32%), identical to the plant-based protein source used in the cricket PP diet. The insect-protein group (IG) received wheat bran combined with black soldier fly (*Hermetia illucens*) larvae meal (21.32%) as the sole protein source, with no soybean-based component included. Mealworms were provided with agar as a water source throughout the experimental period.

Tab. 27: Composition of experimental diets for *Tenebrio molitor* (%).

Ingredient (%)	CG	IG
Grains	78.68	78.68
Legumes	21.32	–
Insect protein	–	21.32
Total	100.00	100.00

Post-harvest processing

At the end of the feeding trial, insects were harvested and subjected to a standardized sequence of post-harvest processing steps: freezing, lyophilization, and mechanical milling. Insects were killed by freezing at -20 °C for 24 hours, which is widely regarded as an ethical killing method that simultaneously reduces enzymatic activity and microbial deterioration prior to further processing. Frozen insect biomass was freeze-dried using a GREGOR Instruments freeze-dryer

(GREGOR instruments, s.r.o., Czech Republic) a shelf temperature of 20 °C to 30 °C, condenser temperature of -55 °C, and chamber pressure of 10 to 50 Pa, for a duration of 72 hours. Freeze-dried samples were mechanically milled using an IKA MultiDrive basic mill (IKA-Werke GmbH & Co. KG, Germany; distributed by VERKON, s.r.o.) to obtain a homogeneous powder suitable for further analysis.

Determination of processing losses

Biomass mass was recorded using an analytical balance (with a readability of 0.001 g) before and after each processing step (freezing, lyophilization, milling). Processing losses (%) at each step were calculated according to the following equation:

$$\text{Loss}(\%) = \left[(m_1 - m_2) / m_1 \right] \times 100$$

where m_1 is the biomass mass before the given processing step and m_2 is the biomass mass after the given processing step. Mass recovery throughout the entire processing chain was calculated as the cumulative product of the recovery rates of the individual steps.

Statistical analysis

Data are expressed as mean and standard deviation (SD). All statistical analyses were performed using IBM SPSS Statistics (version 25.0). Differences among dietary groups were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test. A significance level of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Losses during freezing and milling

Losses during freezing (0.18–2.63 %) and milling (0–1.30 %) did not differ significantly among groups ($p > 0.05$ for both variables, Table 3). This shows that these two steps are relatively unaffected by dietary composition. Their mass losses are also negligible compared with lyophilization. Freezing produced consistently low losses, supporting its use as an ethical and simple killing method. It also inhibits enzymatic activity and microbial spoilage before further processing. Compared with other killing methods, freezing has been shown to better preserve protein quality and limit browning in insect biomass (Leni et al., 2019). Milling losses were similarly low. This is expected, since the material is already largely dehydrated after lyophilization. At this stage, milling mainly fragments the solid matrix rather than causing further loss of mass, moisture, or fine particles (Suhag et al., 2024; Waiss et al., 2021).

Lyophilization losses – overall analysis and species effect

In contrast, losses during lyophilization were highly significant among groups ($p < 0.001$). Tukey's post-hoc test identified three homogeneous subsets: group

CG showed the lowest losses ($62.25 \pm 1.22\%$), group IG an intermediate value ($65.02 \pm 0.40\%$), and groups CP, IP, and PP the highest losses (72.60 – 73.65%). These three groups did not differ significantly from each other (Table 3).

Tab. 28: Biomass losses (% , mean $\pm$ SD) during post-harvest technological processing across all groups.

Group	Freezing (%)	Lyophilization (%)	Milling (%)
CG	0.20 ± 0.06	62.25 ± 1.22	0.08 ± 0.09
IG	2.63 ± 5.57	65.02 ± 0.40	0
PP	0.18 ± 0.14	72.60 ± 0.37	0.79 ± 0.83
IP	0.38 ± 0.40	73.65 ± 0.30	1.30 ± 1.76
CP	0.31 ± 0.21	73.29 ± 0.67	0.94 ± 0.83
p-value	0.484	< 0.001	0.178

This pronounced species-level difference (62 – 65% in *Tenebrio molitor* and 72.6 – 73.65% in *Acheta domesticus*) likely reflects an intrinsic difference between the two species. Mealworm larvae and adult crickets naturally differ in their initial water and lipid content. Larvae of *T. molitor* contain markedly higher lipid levels ($\sim 32\%$ of dry weight) than adult *A. domesticus* ($\sim 15\%$ of dry weight), as shown in a direct comparative analysis of insect lipid profiles (Paul et al., 2017). This difference in lipid content, together with differences in body structure and developmental stage between the two species, may affect how water is bound within the biological matrix, and therefore how available it is for sublimation during lyophilization.

Species-specific differences in the hygroscopic and moisture-related properties of edible insect powders have been reported before, both for house crickets and for black soldier fly larvae (Kamau et al., 2018). Oven-drying has also been shown to affect protein digestibility differently in *T. molitor* and *A. domesticus*, which supports the idea of species-specific responses to dehydration (Hammer et al., 2023). Differences in chitin content between the two species may play a role as well, since chitin content and structure vary considerably between *Coleoptera* and *Orthoptera* (Abenaim & Conti, 2025).

Lyophilization losses – within-species diet effect

In addition to the species effect, a statistically significant effect of diet was also observed within both species (Tables 4 and 5).

This consistent pattern across both species suggests that replacing plant-based protein with BSF larvae meal systematically increases losses during lyophilization. A plausible mechanism relates to the distinct lipid fraction of BSF meal, which is rich in medium-chain saturated fatty acids, particularly lauric acid; larvae are able to synthesise this fatty acid largely independently of the fatty acid profile of their rearing substrate (Ewald et al., 2020). Nevertheless, the overall fat content and fatty acid profile of BSF larvae can still be modulated by dietary fat sources and levels, and larvae have been shown to bioaccumulate dietary fatty acids in their

Tab. 29: Biomass losses (% , mean $\pm$ SD) during post-harvest technological processing in *Acheta domesticus*.

Group	Freezing (%)	Lyophilization (%)	Milling (%)
PP	0.18 $\pm$ 0.14	72.60 $\pm$ 0.37	0.79 $\pm$ 0.83
IP	0.38 $\pm$ 0.40	73.65 $\pm$ 0.30	1.30 $\pm$ 1.76
CP	0.31 $\pm$ 0.21	73.29 $\pm$ 0.67	0.94 $\pm$ 0.83
p-value	0.525	0.012	0.801

Tab. 30: Biomass losses (% , mean $\pm$ SD) during post-harvest technological processing in *Tenebrio molitor*.

Group	Freezing (%)	Lyophilization (%)	Milling (%)
CG	0.20 $\pm$ 0.06	62.25 $\pm$ 1.22	0.08 $\pm$ 0.09
IG	2.63 $\pm$ 5.57	65.02 $\pm$ 0.40	0
p-value	0.357	0.0013	0.059

tissues (Li et al., 2022). Dietary fat originating from the BSF-based diet may therefore accumulate in insect tissues and subsequently alter the physicochemical structure of the biological matrix, the distribution of bound and free water, and consequently the efficiency of sublimation during lyophilization. A higher lipid fraction within a biological matrix has been reported to negatively affect the efficiency of the freeze-drying process and the physical quality of the resulting product (Bhatta et al., 2020). Furthermore, the drying method and associated thermal history have been shown to interact with the fat and protein fraction of BSF-derived material, affecting its structural and nutritional properties (Huang et al., 2019), while processing-related enzymatic reactions (e.g., phenoloxidase activity) have been reported to alter protein solubility in *T. molitor* and *H. illucens* in a matrix-dependent manner (Janssen et al., 2019). Taken together, these mechanisms indicate that a higher lipid content in the biological matrix may, on the one hand, encapsulate water molecules and slow their evaporation, and on the other hand, alter fat tissue structure such that more water is released during the sublimation phase transition – in either case resulting in a change in overall mass loss relative to a plant-based diet.

CONCLUSION

This study demonstrated that losses during freezing and mechanical milling were low and largely unaffected by species or dietary composition, confirming that these two processing steps are robust across different rearing conditions. In contrast, lyophilization was the critical step in the post-harvest processing chain, showing both a pronounced species effect — with *Tenebrio molitor* consistently losing less mass than *Acheta domesticus* — and a consistent, statistically significant diet effect within both species, whereby replacing plant-based protein with black soldier fly (*Hermetia illucens*) larvae meal increased lyophilization losses. These

findings indicate that dietary protein source does not influence the mechanical stages of processing but does meaningfully affect moisture-related behaviour during freeze-drying, likely through diet-driven differences in the lipid fraction of the biological matrix. From a practical standpoint, these results suggest that industrial lyophilization protocols for edible insects should be adjusted according to both species and diet formulation to optimise drying efficiency, energy use, and final product recovery. Future research should further investigate the underlying compositional and structural mechanisms — particularly lipid distribution and water-binding capacity — driving these differences and should extend this assessment to a broader range of insect-based diets and species relevant to the circular bioeconomy.

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INSECT MEAL IN THE BROILER CHICKEN DIETS

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ABSTRACT

Insects are increasingly being investigated as a promising source of protein with high nutritional value. The aim of this study was to evaluate growth performance, carcass characteristics and nitrogen retention coefficient in chickens fed insect meal. Chickens were divided into four groups: Control group (without insect meal), three groups receiving diets containing 5%, 15%, 30% inclusion of black soldier larvae meal. The 30% inclusion of insect meal in diets showed negative effects on all monitored parameters (body weight, feed conversion ratio, carcass yield, thigh yields and nitrogen retention coefficient).

Keywords: poultry nutrition; black soldier fly; growth; carcass; nitrogen retention

INTRODUCTION

The growing demand for animal products has increased interest in alternative protein sources for livestock nutrition (Makkar et al., 2014). Traditionally, soybean meal (SBM) and fish meal (FM) have been among the most commonly used protein feeds in poultry nutrition (Sánchez-Muros et al., 2014). However, these feeds are largely dependent on imports and are associated with economic and environmental impacts (Makkar et al., 2014). For these reasons, measures have been implemented to promote the use of alternative protein sources. The European Union has authorized several insect species for inclusion in poultry and pig diets.

Generally, insects are rich sources of protein and lipids (Makkar et al., 2014). However, insects contain chitin, which may be more difficult to digest for non-ruminants (Sánchez-Muros et al., 2014). Nutrient composition and chitin content vary according to insect species, developmental stage, diet and processing method (van Huis et al., 2013).

Several studies have evaluated insect meal in broiler diets, but results remain inconsistent. Several authors reported maintained or improved growth performance, feed conversion ratio (FCR), and carcass traits following partial replacement



of SBM or FM (Bovera et al., 2015; Dabbou et al., 2018). In contrast, other studies observed negative effects on growth performance, FCR and carcass characteristics (Šťastník et al., 2021; Hartinger et al., 2021).

The aim of this study was evaluated performance parameters and nitrogen retention.

MATERIAL AND METHODS

Animals, experimental conditions and diets

The experiment was conducted at the accredited experimental facilities of Mendel University in Brno. A total of 96 one-day-old male broiler chickens of hybrid combination Ross 308 were divided into four groups, each with four replicates. The trial lasted for 35 days. The lighting program, temperature and humidity was set according to the technological instruction for hybrid combination. Four diets were fed: Control (without insect meal, "IM0"), and three experimental diets receiving inclusion of black soldier fly larvae meal (5% "IM5", 15% "IM15" and 30% "IM30") as a partial substitute of SBM. The experimental diets were prepared by Tekro, spol., s.r.o. and were balanced so that the compared diets corresponded in their basic nutritional composition (particularly in crude protein, crude fat, crude fibre, and energy content). During the first 14 days, the chickens were fed experimental starter diets, followed by grower diets, and finally finisher diets, which were fed during the last week. During the experiment, the chickens had unlimited access to feed and water.

Sample collection

During the experiment, feed consumption was recorded daily and FCR was subsequently calculated. The chickens were weighed every week to determine growth curves. At the end of the experiment, the chickens were weighed and slaughtered.

Slaughter performance parameters were evaluated in selected average individuals (eight individuals from each group). Among the slaughter performance parameters carcass yield was evaluate. Breast and thigh were weighed, and the proportion of the muscle parts was determined.

Nitrogen retention was determined using the indicator method with chromium oxide (Cr_2O_3) added to the experimental diets. Excreta samples were collected at regular intervals throughout the experimental period. A detailed description of the methodology is provided by Šťastník et al. (2025).

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

Figure 1 shows changes in the average body weight of broiler chickens during the experiment. The IM30 group had significantly lower body weight from 14 days of age to the end of the experiment compared with other groups. At the end of the experiment, the IM0, IM5, IM15 groups had weight around 2 kg. Chicken growth could have been limited by the chitin content present in insect meal. Even relatively low inclusion levels (2% and 5%) of mealworm meal reduced the body weight of broilers; however, the final body weight still exceeded 2 kg (Šťastník et al., 2021). Similarly, Facey et al. (2023) observed a decrease in broiler body weight with increasing replacement of SBM by black soldier fly larvae meal. In contrast, Dabbou et al. (2018) reported an opposite trend, with broiler body weight increasing as the inclusion level of black soldier fly larvae meal in the diet increased. Bovera et al. (2015) found out no effect of complete replacement of SBM with mealworm meal on broiler body weight.

In Table 1 is shown the feed intakes and FCR, which were influenced by inclusion of insect meal in diets. In starter, grower and finisher phases and overall, the IM30 group had lower feed intake than other groups. Due to its reduced feed intake, the IM30 group showed a significantly higher FCR in all phases of the fattening period except the finisher phase, during which no significant differences were observed between groups. Facey et al. (2023) also observed lower feed intake and higher FCR in groups fed diets containing black soldier fly larvae meal. Bovera et al. (2015) observed a positive effect of mealworm meal on FCR.

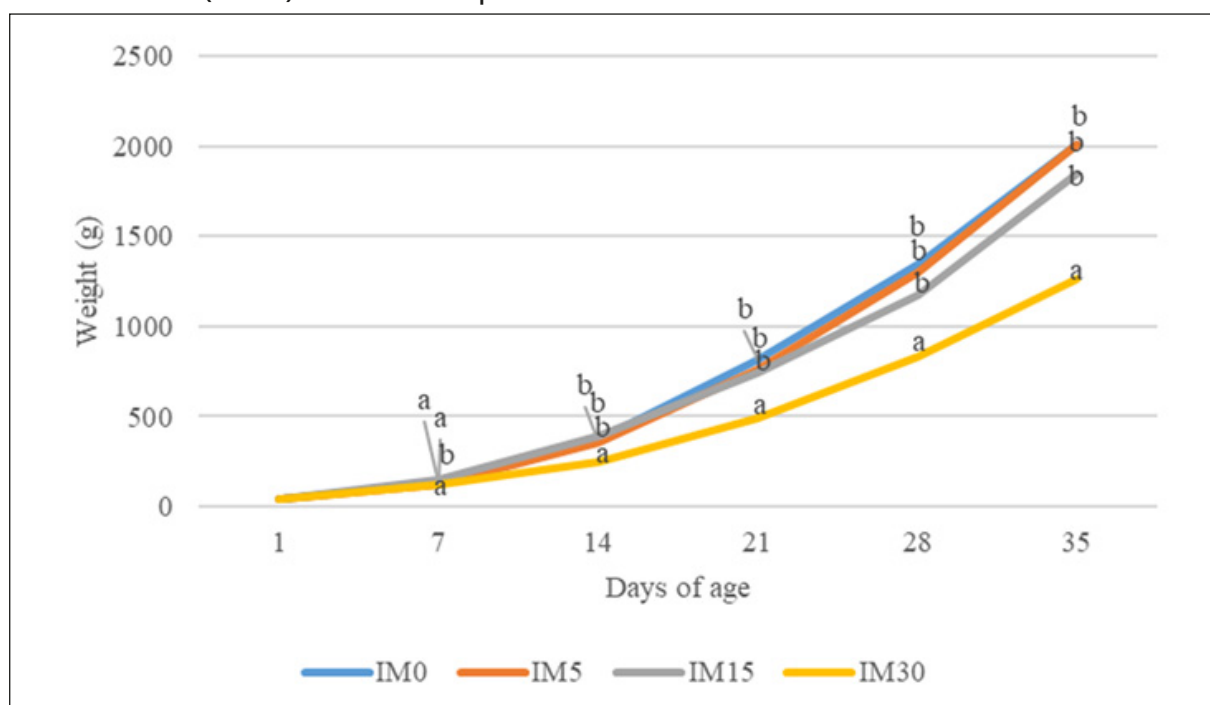


Fig. 1: Changes in the average body weight of chickens during the experiment

a, b – means statistically significant differences ($P < 0.05$); IM0 – Control (without insect meal); IM5 – 5% inclusion of insect meal in diet; IM15 – 15% inclusion of insect meal in the diet; IM30 – 30% inclusion of insect meal in the diet.

Tab. 31: Average feed intake and FCR during experiment

	Phases	IM0	IM5	IM15	IM30
Feed intake (g/bird/period)	Starter	436 ^b	379 ^b	413 ^b	297 ^a
	Grower	1440 ^c	1359 ^{bc}	1296 ^b	1010 ^a
	Finisher	1335 ^b	1300 ^b	1336 ^b	1015 ^a
	Overall	3211 ^b	3037 ^b	3045 ^b	2323 ^a
Feed intake (g/bird/day)	Starter	31 ^b	27 ^b	29 ^b	21 ^a
	Grower	103 ^c	97 ^{bc}	93 ^b	72 ^a
	Finisher	191 ^b	186 ^b	191 ^b	145 ^a
	Overall	92 ^b	87 ^b	87 ^b	66 ^a
FCR	Starter	1.33 ^{ab}	1.20 ^a	1.17 ^a	1.43 ^b
	Grower	1.48 ^a	1.44 ^a	1.66 ^{ab}	1.75 ^b
	Finisher	2.01	1.83	2.05	2.40
	Overall	1.63 ^{ab}	1.54 ^a	1.69 ^{ab}	1.92 ^b

^{a, b, c} – in row means statistically significant differences ($P < 0.05$); FCR – feed conversion ratio; IM0 – Control (without insect meal); IM5 – 5% inclusion of insect meal in the diet; IM15 – 15% inclusion of insect meal in the diet; IM30 – 30% inclusion of insect meal in the diet.

The results presented in Table 2 for the nitrogen retention coefficient ° of finisher diets are consistent with the previously discussed findings. The nitrogen retention coefficient was lowest in the IM30 group compared with other groups. These findings further support the negative impact of the treatment on broiler performance. Chitin may be reason for this negative effect. The negative effect of mealworm meal on the ileal digestibility coefficient of crude protein was reported by Bovera et al. (2016). In contrast, Dalmoro et al. (2021) observed an increasing ileal nitrogen digestibility coefficient in broilers receiving diets containing mealworm meal.

Tab. 32: Nitrogen retention coefficient of finisher diets

	IM0	IM5	IM15	IM30
n	8	8	8	8
Nitrogen retention (%)	56.46 ^b	54.63 ^b	52.11 ^b	36.86 ^a

^{a, b} – in row means statistically significant differences ($P < 0.05$); n – number of cases; IM0 – Control (without insect meal); IM5 – 5% inclusion of insect meal in the diet; IM15 – 15% inclusion of insect meal in the diet; IM30 – 30% inclusion of insect meal in the diet.

Table 3 shows slaughter performance parameters of selected broiler chickens. The differences were found out in carcass yield and thigh yields. In these parameters, the IM30 had lower values than other groups. Only breast yields were not affected by inclusion of insect meal. Hartinger et al. (2021) observed tendency toward lower carcass yield in broilers fed diets containing 100 g/kg of black soldier fly larvae meal. Dörper et al. (2024) observed no effects of insect-derived feed ingredients on slaughter performance parameters.

Tab. 33: Slaughter performance parameters

	IM0	IM5	IM15	IM30
n	8	8	8	8
Body weight (g)	1974 ^b	1909 ^b	1951 ^b	1518 ^a
Carcass yield (%)	67.95 ^b	69.34 ^b	68.58 ^b	64.08 ^a
Breast yield (% of BW)	16.53	18.14	17.45	18.12
Breast yield (% of CW)	24.32	26.15	25.44	28.45
Thigh yield (% of BW)	15.71 ^b	15.92 ^b	15.60 ^b	13.66 ^a
Thigh yield (% of CW)	23.12 ^b	22.96 ^{ab}	22.76 ^{ab}	21.33 ^a

^{a, b} – in row means statistically significant differences ($P < 0.05$); n – number of cases; BW – body weight; CW – carcass weight; IM0 – Control (without insect meal); IM5 – 5% inclusion of insect meal in the diet; IM15 – 15% inclusion of insect meal in the diet; IM30 – 30% inclusion of insect meal in the diet.

CONCLUSION

Insect meal is widely considered a promising alternative protein source that can partially replace conventional protein feeds due to its high crude protein content and the rapid growth cycle of insects. The present results indicate that higher dietary inclusion level of insect meal impair broiler growth performance, increase FCR, and reduce carcass yield. These findings are further supported by decreased nitrogen retention coefficient.

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THE EFFECT OF (QUEBRACHO) TANNINS ON METABOLICAL PROCESSES IN THE RUMEN DURING SIMULATED DIGESTION IN BIOREACTOR (*IN VITRO*)

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ABSTRACT

The present study investigated the effect of dietary tannins on ruminal fermentation parameters using a continuous *in vitro* bioreactor system (Sartorius Biostat A plus Bioreactor System). The experiment was carried out in the form of a cross-over design and compared a control diet with a tannin-enriched diet supplemented with 5 % of quebracho tannins. The incubation lasted 16 days, during which each diet was fed for 8 days, consisting of 5 days of adaptation followed by 3 days of sampling. The incubations were repeated three times. The results demonstrated that tannin supplementation increased pH, altered redox potential, and significantly reduced dry matter digestibility. Furthermore, a marked decrease in volatile fatty acid (VFA) concentrations was observed, although the acetate-to-propionate ratio remained unaffected. These findings indicate that tannins primarily suppress rumen fermentation by limiting substrate degradation and microbial activity, thereby influencing overall fermentation efficiency.

Keywords: *in vitro* rumen fermentation; gas chromatography; pH; redox potential; digestibility; volatile fatty acids.

INTRODUCTION

Rumen fermentation represents a key metabolic process in ruminants, where complex plant materials are converted by a diverse microbial community into VFAs that serve as the principal energy source for the host animal (Ding et al., 2023). *In vitro* fermentation systems are widely utilized to study ruminal processes because they allow precise control of experimental conditions, reduce variability and reduce the need for animal usage compared with *in vivo* experiments



(Scicutella et al., 2025). Tannins are polyphenolic secondary metabolites that are abundant in many plant species and can significantly influence digestive processes through interactions with proteins, carbohydrates, and microbial enzymes. Their effects on ruminal fermentation are complex and concentration-dependent, ranging from inhibition of microbial activity and reduced digestibility to potential benefits such as improved nitrogen utilization or reduced methane emissions (Frutos et al., 2004). Despite extensive research, the overall impact of tannins on rumen metabolism remains incompletely understood due to variability in tannin structure, concentration, and experimental conditions. The aim of this study was therefore to evaluate the effect of tannin supplementation at 5% of dry matter on selected fermentation parameters, including pH, redox potential, dry matter digestibility, and VFA production, using a continuous *in vitro* bioreactor system.

MATERIAL AND METHODS

The experiment was carried out using continuous incubations *in vitro* in a bioreactor (Sartorius Biostat A plus Bioreactor System) that simulated ruminal conditions, including a constant temperature of 39 °C, strict anaerobic environment maintained by continuous CO₂ flow, homogeneity using a metallic stirrer rotating at 20 rpm, and a steady inflow of artificial saliva (McDougall, 1948) dosed into the bioreactor vessel every 15 minutes using a peristaltic pump, averaging a flow of 1038 ml per day. The working volume of incubation media was maintained at 1 L. Each experimental run lasted sixteen days and consisted of two phases of equal duration. In the first phase, the bioreactor was fed using a control diet (Table 1), whereas in the second phase the same diet was supplemented with quebracho tannins using tannin concentrate Tannivin® SR (Erbslöh), at a concentration corresponding to 5 % of dry matter. The bioreactor was fed once daily with 10 g of the respective diet, administered via nylon bags. Each bag was incubated in the bioreactor for 48 h and then replaced with a fresh one. Each phase included a five-day adaptation period followed by a three-day sampling period during which measurements and sample collections were conducted (Table 2). The entire experiment was repeated three times.

Tab. 34: Composition of the feed ration used during fermentation in the bioreactor.

Components	Unit	Composition
Maize silage	%	40,5
Grass haylage 1 st cut	%	21,3
Sugar beet cake	%	14,2
Molasses	%	2,4
Supplemental mixture ¹	%	19,9
Rapeseed meal	%	1,7
Total	%	100

¹ Supplemental mixture - composition (%): wheat (29.5), barley (12.5), corn (19.0), soybean meal (17.5), rapeseed meal (14.0), mineral-vitamin premix SM Production U (3.5), limestone (1.5), salt (0.5), sodium bicarbonate (2.0)

Tab. 35: *Timeline of a single bioreactor run*

Bioreactor run	
16 days	
8 days – control diet	8 days – tannin-enriched diet
5 days – adaptation period	5 days – adaptation period
3 days – sample collection	3 days – sample collection

Rumen fluid formed the basis of the digestive environment and served as the inoculum for cultivation in the bioreactor. It was collected on the first day of each bioreactor run from the rumen of two dairy cows using an esophageal tube (a private farm Lenka Klíčová, Božice, 1st and 3rd run) or from cannulated cows (Agrovýzkum Rapotín Ltd., 2nd run). This change was due to restrictions imposed due to the risk of foot-and-mouth disease transmission. After collection, rumen fluid was transferred to the laboratory in a heat-stable box, homogenized and filtered through four layers of gauze. The feed retained on the gauze was used as an initial nutrient source to support adaptation and initiate fermentation processes.

During the sampling period, 2 ml of liquid inoculum was collected from the bioreactor for subsequent VFA analysis. For the determination of dry matter digestibility, part of the feed was incubated in six F57 filter bags (O.K. SERVIS BioPro, Czech Republic), 0.5 g per bag, plus one blank bag. After 48 hours of incubation, filter bags were removed, rinsed externally, dried, and weighed. pH and redox potential were measured using an InPro3253i/SG/225 electrode (METTLER TOLEDO) with values being recorded every 20 minutes. Buffer consumption and effluent volumes were recorded daily at 8:45. Digestibility was calculated from feed and residue weights using equation below.

$$\text{Dry matter digestibility (\%)} = \left(100 - \left(W3 - (W1 \times C1)\right) \times 100\right) / W2$$

Where:

- W1 = weight of empty bag
- W2 = feed sample weight
- W3 = dried bag weight after incubation
- C1 = blank correction factor

The VFA profile analysis was performed using gas chromatography with an Agilent Technologies 8860 GC System equipped with a DB FATWAX UI column (30 m × 0.32 mm × 0.25 µm) and a flame ionization detector. The profile consisted of the three most abundant VFAs: acetic acid, propionic acid, and butyric acid.

Inoculum samples were first homogenized, and subsequently centrifuged using a Microspin 12 (Biosan) for 10 minutes at a relative centrifugal force of 4000× g. After centrifugation, 1000 µl of supernatant was collected and mixed with 200 µl of 25% metaphosphoric acid. The samples were then incubated at 4 °C for 30 minutes and centrifuged again for 10 minutes at 12,000× g. For gas chromatographic analysis the resulting solution was diluted 1:9.

Gas chromatography parameters:

- Injection split: 20:1; 250 °C
- Carrier gas: Helium
- Column flow rate: 1.5 ml/min
- Oven temperature: 60 °C with a temperature gradient of 20 °C/min to a final temperature of 180 °C
- Detector temperature: 250 °C
- Fuel gas: Hydrogen flow of 30 ml/min
- Oxidizing gas: Compressed air flow of 400 ml/min
- Injection volume: 1 µl

Data was analyzed using software Statistica. A general linear model was used with treatment (control, tannin-enriched diet) as a fixed effect and experimental run (Run 1, 2, 3) as a random effect. Graphs were created in Microsoft Excel. Outliers in box plots were defined using the interquartile range (IQR), with values outside $1.5 \times \text{IQR}$ considered as outliers.

RESULTS

Chart 1 shows pH values from three measurement days for the control group and the tannin-enriched group across all three bioreactor runs. Chart 2 presents redox potential values in the same manner.

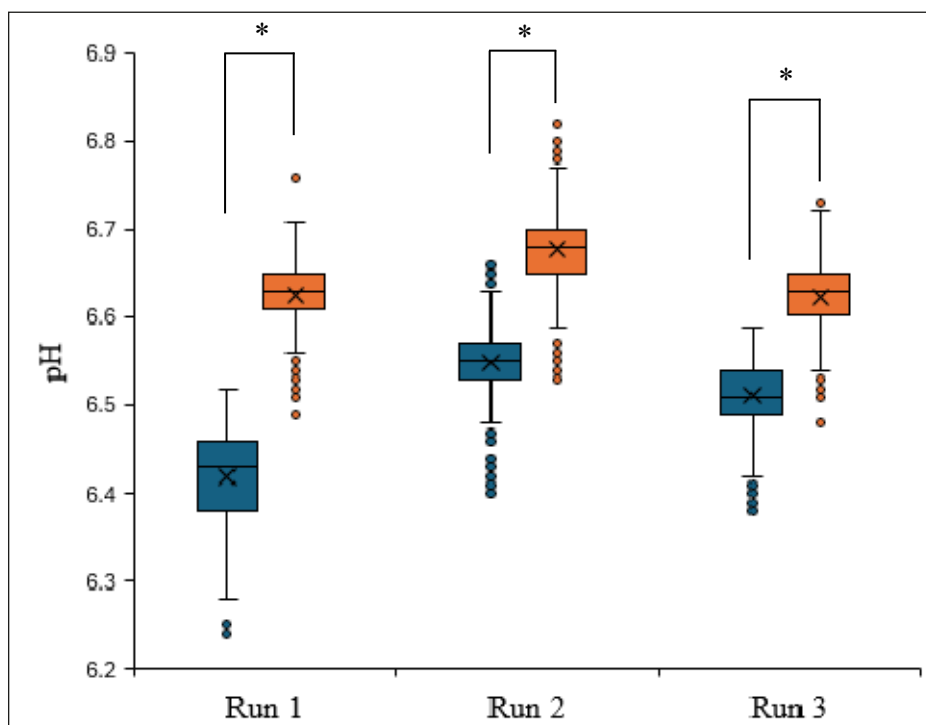


Chart 1: Box plot showing pH values for individual bioreactor runs. The control group is shown in blue, while the tannin-enriched group is shown in orange. Data points of the same colour represent outliers. Whiskers represent the maximum and minimum values excluding outliers. The box boundaries represent the first and third quartiles. The line represents the median, and the cross indicates the mean. * indicates a significant difference ($P < 0.05$) between groups.

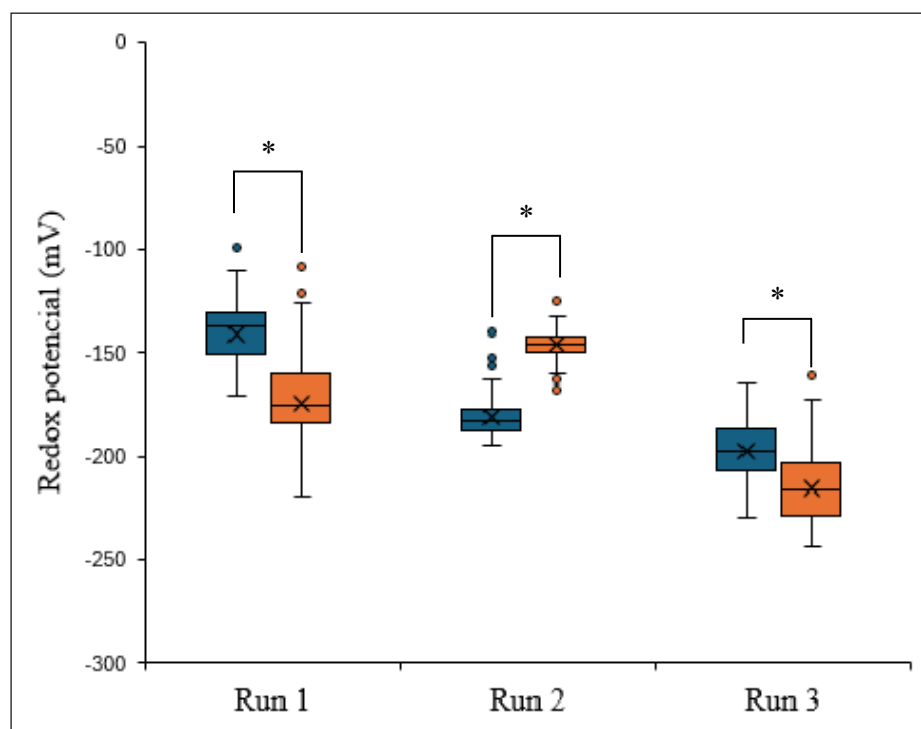


Chart 2: Box plot showing redox potential values (mV) for individual bioreactor runs. The control group is shown in blue, while the tannin-enriched group is shown in orange. Data points of the same colour represent outliers. Whiskers represent the maximum and minimum values excluding outliers. The box boundaries represent the first and third quartiles. The line represents the median, and the cross indicates the mean. * indicates a significant difference ($P < 0.05$) between groups.

The supplementation of tannins significantly influenced several fermentation parameters. The pH of the fermentation medium increased consistently across all experimental runs following tannin addition, although the values remained within the physiological range typical for ruminal conditions. Changes in redox potential were also statistically significant; however, the direction of these changes differed between experimental runs, indicating a possible effect of variations in microbial composition of the inoculum.

During the 1st run the mean pH increased from 6.42 to 6.63 and redox potential decreased from -141 to -174 mV; for the 2nd run from 6.55 to 6.68 for pH and from -197 to -216 mV for redox potential; and for 3rd run pH increased from 6.51 to 6.62 and redox potential decreased from -197 to -216 mV.

A pronounced reduction in dry matter digestibility was observed after tannin supplementation. The digestibility decreased from an average value of 55.2% in the control group to 45.8% in the tannin-enriched group, representing a substantial decline in nutrient availability.

The analysis of VFAs revealed a significant decrease in the concentrations of all major VFAs in response to tannin supplementation. Acetic acid concentration decreased from 982 to 574 µg/mL, propionic acid from 1034 to 558 µg/mL, and butyric acid from 476 to 206 µg/mL. The total concentration of VFAs was similarly reduced, indicating an overall suppression of fermentation activity. Despite these decreases, the ratio of acetate to propionate was not significantly affected.

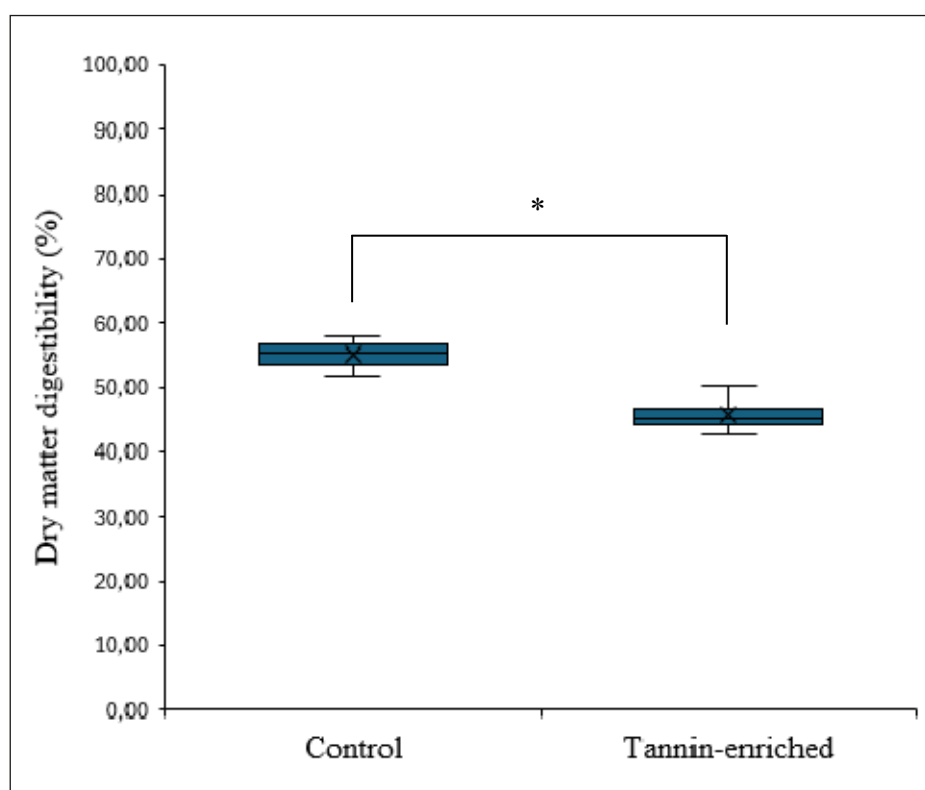


Chart 3: Box plot showing dry matter digestibility (%) for the control and the tannin-enriched group. Whiskers represent maximum and minimum values. The box boundaries represent the first and third quartiles. The line indicates the median, and the cross indicates the mean. * indicates a significant difference ($P < 0.05$) between groups.

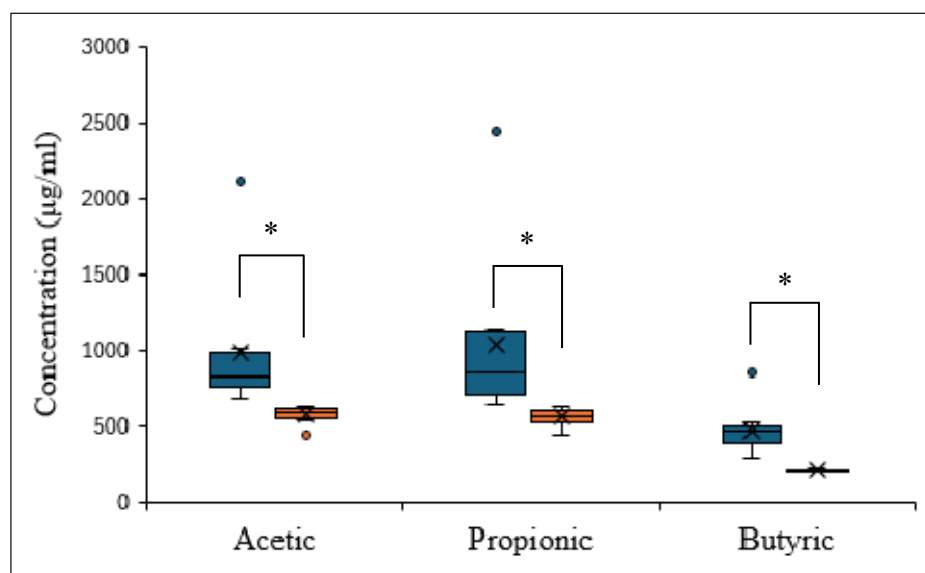


Chart 4: Box plot showing concentrations (µg/mL) of individual VFAs. The control group is shown in blue, while the tannin-enriched group is shown in orange. Points in the same colours represent outliers. Whiskers indicate the maximum and minimum values excluding outliers. The box boundaries represent the first and third quartiles. The line indicates the median. The cross marks the mean. * indicates a significant difference ($P < 0.05$) between groups.

DISCUSSION

During experimentation a significant increase in ruminal pH was observed after the supplementation of tannin into the diet. The elevated pH was most likely a consequence of reduced production of VFAs by the fermentation system. Tannins affect feed digestibility and substrate availability for processes involved in the formation of these metabolites. A reduced concentration or degradation rate of these metabolites in the rumen may therefore influence not only pH but also the ruminal microbiome (Díaz Carrasco et al., 2017). An increase in ruminal pH associated with a higher tannin intake was also observed by (Olafadehan et al., 2016) in goats fed a diet containing 6.8 % tannins in dry matter and by (Díaz Carrasco et al., 2017) using a tannin extract in Holstein cattle.

The effect of tannins on redox potential varied across experimental runs. The simplest explanation for the discrepancy between runs 1 and 3 and run 2 is the use of different inoculum for fermentation initiation in the second run. During preparation of this inoculum, rumen fluid was not mixed with previously digested feed before the experiment, as the rumen fluid had already been supplied pre-filtered. Although the rumen fluid was obtained from the same breed in a different location, this difference may have led to a variation in microbial composition and subsequently influence redox potential during fermentation. These findings slightly differ from those reported by (Dias Batista et al., 2021), who did not observe a significant change in redox potential across different tannin concentrations, however this study measured redox potential only once and thus our significant difference can be caused by superior continuous measurement system. Alongside the present study, this appears to be one of the very few studies investigating the effect of tannins on redox potential.

Dry matter digestibility is primarily influenced by the ability of tannins to bind dietary proteins, thereby protecting them from degradation processes in the rumen. Tannins also reduce cellulolytic and proteolytic activity by affecting the ruminal microbiome, which further contributes to decreased dry matter digestibility (Valcl & Lavrenčič, 2025). The effect of tannins on digestibility is consistent with previous studies. (Valcl & Lavrenčič, 2025) reported decreases in both dry matter and crude protein digestibility depending on tannin type and concentration (5 % and 10 % DM supplementation) in cannulated rams. (Foggi et al., 2022) observed a decrease in organic matter digestibility after feeding cattle with quebracho tannin extract, while (Norris et al., 2020) confirmed a reduction in dry matter digestibility in bulls even at a 3 % tannin supplementation level.

VFAs are a key component of animal metabolism, primarily serving as an energy source (up to 70 % of metabolizable energy) (Bergman, 1990). Their profile is associated with fermentation quality, nutrient partitioning, variation in methane production, and the chemical composition of animal products such as milk (Dijkstra, 1994). A statistically significant decrease in their concentrations indicates a reduction in available energy substrates, which may have a substantial

impact on animal performance *in vivo*. This decrease is again linked to the effects of tannins on digestibility and rumen microbiome. Reduced VFA synthesis may reflect the effect of tannins on feed degradation, as supported by the observed decrease in dry matter digestibility. Our results are not fully consistent with other studies like (Dschaak et al., 2011), who observed a significant effect of tannins *in vivo* only on total VFA concentration and not on individual VFAs or (Norris et al., 2020) who also did not observe significant changes in VFA concentrations *in vivo* following tannin supplementation. These discrepancies may be explained by differences in experimental conditions, including tannin dose and diet composition. The effect of tannins on the acetate-to-propionate ratio was not confirmed in this study, which is consistent with the findings of (Dschaak et al., 2011) and (Norris et al., 2020).

CONCLUSION

The results of this study demonstrate that tannin supplementation at a level of 5% of dry matter significantly affected ruminal fermentation under *in vitro* conditions. The addition of tannins increased pH, altered redox potential, reduced dry matter digestibility, and decreased the production of VFAs. However, the relative proportions of major fermentation products, expressed as the acetate-to-propionate ratio, remained unchanged.

These findings indicate that tannins suppress fermentation primarily through a reduction in microbial degradation of feed substrates. The study contributes to a better understanding of how plant secondary metabolites modulate rumen fermentation and highlights the potential application of tannins in animal nutrition strategies.

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EFFECT OF PARTIAL SORGHUM INCLUSION IN FEED RATION OF DAIRY COWS ON MILK FATTY ACID PROFILE

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ABSTRACT

The aim of this study was to assess the effect of the inclusion of approximately 5 kg of sorghum silage into the feed ration of dairy cows instead of the same amount of legume-grass silages on the fatty acid (FA) profile of milk. The on-farm experiment was conducted on 120 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 based on maize silage and legume-grass silage (Control), while in the second period, legume-grass silage 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 the FA profile, which was determined using gas chromatography equipped with a flame ionization detector. After the sorghum inclusion, the content of palmitic acid (C16) lowered ($P < 0.05$), and the content of pentadecenoic (C15:1), stearic (C18), arachidic (C20) and behenic acid (C22) increased ($P < 0.05$). The diet change also tended to decrease the content of eicosapentaenoic (C20:5n3) and tricosanoic acid (C23) ($0.05 \leq P < 0.1$).

Keywords: alternative feed, eicosapentaenoic acid, palmitic acid, stearic acid

INTRODUCTION

Increasing temperatures and long periods of drought are making it harder for farmers to grow traditional crops for animal feeding. For this reason, alternative crops, that are more drought and heat tolerant compared to maize and grasses, such as sorghum, are beginning to be grown in our conditions. However, it is well known that sorghum contains tannins and cyanogenic glycosides, which are antinutrients (Wang et al. 2024; Suha and Babiker 2015). Furthermore, tannins can impact rumen microbiota, which is responsible for the main lipid metabolism pathways in rumens (biohydrogenation and lipolysis) (Makmur et al. 2022).



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Additionally, around 50 % of fatty acids (FA) in milk originate from the diet of ruminants, therefore the change in the diet can cause major impacts on the resulting fat composition of milk (Mansbridge a Blake 1997). Previous research on sorghum inclusion (Khosravi et al. 2018; Tudisco et al. 2021; Cattani et al. 2017; Yang et al. 2019) are not consistent and report various changes (positive and negative) in the FA profile after sorghum inclusion into the feed ration of dairy cows. The amount of sorghum in our study differs compared to other works, which have a higher sorghum amount (in percentage). For this reason, this work aims to assess how lower sorghum inclusion (around 10% of dry matter) impacts the resulting FA profile of milk.

MATERIAL AND METHODS

Design of experiment, animals and feeding

The design of the experiment is the same as described in Malý et al. (2024) (this being the third experiment). The on-farm experiment was conducted on a group of 120 mid-lactating Czech Fleckvieh cows (Agrospol a.d. Knínice, farm Vanovice), from which 10 representative cows were chosen for a detailed study of milk composition. The trial was divided into two consecutive 3-month periods. Each period consisted of at least a 3-week adaptation period and a 56–60 day collection period in which 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 total mixed ration (TMR) diet was based on maize silage, legume-grass silage and a supplemental mixture (Control), while in the second period, legume-grass silage was partially replaced with sorghum silage (Sorghum). The composition of feed rations is given in Tables 1 and 2. Cows were milked twice daily (at 4 a.m. and 3 p.m.), and milk yield was recorded.

Tab. 36: *Composition of feed ration (in kg/d, on as fed basis)*

Component (kg/d, as fed)	Control	Sorghum
maize silage	20	20.5
legumes-grass silage	10.5	4.5
sorghum silage	-	5.3
sugar beet pulp	8	5.4
molasses	0.6	0.6
supplemental mixture	9.75	10.4
rapeseed meal	1.1	1.25
sum of diets	49.95	47.95

Tab. 37: *Composition of supplemental feeding mixture (%)*

Component	Content (%)
wheat	24.6
barley	10
maize	23
soybean meal	19
rapeseed cake	16
SM Production U ¹	3.7
limestone	1.7
salt	0.5
sodium bicarbonate	1.5

¹SM Production U – vitamin and mineral mixture

Sampling and analysis

The sampling was similar to Malý et al. (2024), as it was the same project, but the third experiment/year. Samples of evening and morning milk were taken from ten cows once per month during the regular milk recording. Samples for FA analysis were frozen (-20 °C) until analysis.

Before FA analysis evening and morning milk was pooled into one sample relative to milk yield. Milk fat was extracted using a modified Bligh and Dyer (1959) extraction procedure. Transesterification of triglycerides in extracted fat to FA methyl esters (FAMES) was done by adding hexane and 0.5M methanolic sodium methoxide solution in a volumetric ratio of 80:3. FAMES were analysed by gas chromatography with flame ionization detection using GC Agilent 8860 (Agilent, United States) under optimised conditions used in Mitchell et al. (2023) (oven program: 60 °C for 4 min to 140 °C @ 2 °C/min and held for 30 min to 160 °C @ 1 °C/min and held for 60 minutes to 190 °C @ 1 °C/min and held for 60 min, detector: FID @ 250 °C) using the ZB-FAME column (Phenomenex, United States) with dimensions 100 m x 0.25 mm x 0.20 µm. The identification of FAs was carried out using the analytical standards (Restek, United States). 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 analysis

Statistical analysis was done using Microsoft Excel (Microsoft). For testing the significance between the groups (Control, Sorghum), the T-test was used.

RESULTS AND DISCUSSION

The results shown here are preliminary, from one sampling term in each experimental group (n = 10).

The resulting FA profile of milk before and after sorghum inclusion is shown in Table 3.

The most abundant saturated fatty acid (SFA) was in both groups palmitic acid (C16), followed by myristic (C14) and stearic (C18) acids, respectively. This is in agreement with Yang et al. (2019) and Cattani et al. (2017). Other authors report slightly higher C14 content compared to C18 in the sorghum group. It is important to note that this is a numerical comparison only without any statistical test. Our results show that after the inclusion of sorghum into the diet of dairy cows, the concentration of palmitic acid has significantly decreased ($P < 0.05$). This is in contrast with Yang et al. (2019), Tudisco et al. (2021), Khosravi et al. (2018) and Cattani et al. (2017), who reported either no change in the content of C16 after the inclusion of sorghum or an increase (Cattani et al. 2017). Another SFA impacted by the inclusion was C18, whose content increased in Sorghum compared to Control ($P < 0.05$). This matches results reported in studies from Yang et al. (2019) and Cattani et al. (2017). Other authors reported no differences in the content of C18 after incorporation of sorghum (Khosravi et al. 2018; Tudisco et al. 2021). Arachidic acid (C20) and behenic acid (C22) were also higher in Sorghum in comparison with Control ($P < 0.05$). Yang et al. (2019) and Khosravi et al. (2018) also reported an increase in C20, but did not observe C22. Other SFA were not affected by the treatment. It should be taken into account that the TMR slightly differs in each work as well as the breed of the cows used compared to our work, so direct comparison with the literature is not possible. Another important factor is the amount of sorghum silage added to the feed ration, which is lower in our work compared to the work of other authors and also varies across studies. Diet and breed have a significant impact on the final FA composition (Hanuš et al. 2018), so this can further explain inconsistencies in scientific literature and also between it and our work.

The major unsaturated fatty acid in both experimental groups in our study is oleic acid (C18:1n9c), followed by linoleic acid (C18:2n6c) and palmitoleic acid (C16:1). This is the same as in work from Yang et al. (2019) and Khosravi et al. (2018). Regarding UFA, the dietary change affected only eicosapentaenoic acid (C20:5n3), whose levels tended to decrease ($0.05 \leq P < 0.1$).

Our results can be considered overall positive. A lower concentration of C16 in the human diet is health beneficial (Hanuš et al. 2018). Hanuš et al. (2018) mentions that C16 increases total blood cholesterol and cardiovascular disease risk. Another important aspect of our study is that incorporating sorghum silage didn't affect most FA, which is very good, so the farmers can include sorghum without it having a negative effect on the FA profile of milk.

Tab. 38: *The content of fatty acids (relative representation) in milk*

	Control ¹		Sorghum ²		P
	mean	SD	mean	SD	
C4	1.13	0.12	1.07	0.11	0.262
C6	1.11	0.10	1.08	0.13	0.554
C8	0.93	0.08	0.95	0.10	0.691
C10	2.90	0.32	3.07	0.39	0.321
C11	0.18	0.11	0.14	0.03	0.285
C12	4.42	0.52	4.53	0.54	0.659
C13	0.26	0.13	0.21	0.04	0.267
C14	13.83	1.06	14.25	0.89	0.349
C14:1	1.38	0.30	1.18	0.33	0.173
C15	2.13	0.85	1.69	0.24	0.149
C15:1	0.23	0.06	0.28	0.04	0.036
C16	35.94	1.52	33.48	2.13	0.008
C16:1	1.97	0.32	1.74	0.39	0.171
C17	0.74	0.16	0.67	0.04	0.180
C17:1	0.31	0.09	0.26	0.05	0.150
C18	7.18	1.37	8.73	1.15	0.014
C18:1n9c	21.16	1.83	22.49	1.87	0.124
C18:2n6t	0.07	0.11	0.03	0.00	0.337
C18:2n6c	2.51	0.32	2.54	0.26	0.795
C18:3n6	0.05	0.00	0.05	0.01	0.495
C18:3n3	0.44	0.05	0.43	0.05	0.783
CLA	0.50	0.09	0.46	0.14	0.495
C20	0.10	0.02	0.13	0.02	0.004
C20:1n9	0.06	0.02	0.05	0.01	0.398
C20:2	0.03	0.00	0.03	0.00	0.490
C20:3n6	0.10	0.02	0.11	0.02	0.331
C21	0.02	0.00	0.02	0.00	0.811
C20:4n6	0.15	0.02	0.15	0.02	0.762
C20:5n3	0.04	0.00	0.04	0.00	0.062
C22	0.03	0.01	0.04	0.00	0.024
C23	0.01	0.01	0.02	0.00	0.058
C24	0.02	0.01	0.02	0.00	0.204
C24:1n9	0.07	0.01	0.06	0.01	0.493

¹ diet based on maize silage and legume-grass silage

² diet based on maize silage and legume-grass silage that was partially replaced by sorghum silage

CONCLUSION

Our results show that sorghum silage can be incorporated into the feed ration of dairy cows without major and negative changes in the fatty acid profile. After the 5 kg inclusion, the content of palmitic acid (C16) decreased ($P < 0.05$), and the content of stearic (C18), arachidic (C20) and behenic acids (C22) increased ($P < 0.05$), with the majority of fatty acids being unaffected by the treatment. These results suggest that sorghum incorporation, especially in regions with higher temperatures and limited rainfall, can be used as a replacement for legumes and grass, without ruining the nutritional value of the fatty acid profile in milk.

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IN OVO FEEDING OF CINNAMON EXTRACT IN BROILER CHICKENS

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ABSTRACT

The study investigated the effect of *in ovo* application of cinnamon extract on the hatchability and performance parameters of broiler chickens. The primary objective was to evaluate the impacts of this early developmental programming on growth performance. Hatching eggs of the Ross 308 were divided into two groups: a control group (without application) and an experimental group supplemented with cinnamon extract. The cinnamon extract, prepared by hydroalcoholic extraction, was injected into the amnion on day 17.5 of incubation as a 0.2 mL solution, delivering a dose of 1.8 mg per egg. The subsequent 35 day feeding trial involved 144 male broiler chickens distributed into the two groups with 6 replicates each (12 birds per replicate). At the end of the fattening period, growth performance parameters (live body weight, carcass yield, and feed conversion ratio), were evaluated. The *in ovo* delivery of cinnamon extract did not significantly affect hatchability ($p > 0.05$) and showed no adverse effects on the growth performance of the broiler chickens ($p > 0.05$). These results demonstrate that the *in ovo* application of cinnamon extract is safe and does not impair growth performance, thereby providing a solid foundation for future research into chicken health status, digestive tract development, and immune system response.

Keywords: cinnamaldehyde; early developmental programming; growth performance; hatchability; phytogenic additives; poultry nutrition



INTRODUCTION

In recent years, the poultry sector has been actively transitioning toward minimizing antibiotic reliance. This pressure comes from rising antibiotic resistance and strict regulations, such as the EU ban on antibiotic growth promoters (Regulation (EC) No 1831/2003). Therefore, researchers are looking for safe, natural alternatives to maintain broiler growth and keep animals healthy. Phytogetic feed additives, such as plant extracts and essential oils, are excellent alternatives. They are popular because they can fight bacteria, reduce oxidative stress, and lower inflammation (Wang et al. 2024).

Using these plant additives with the *in ovo* (IO) method is a promising strategy to improve embryonic development. This method is based on early metabolic programming (Jha et al. 2019). It injects nutrients or bioactive compounds directly into the egg, usually on day 12 or days 17–18 of incubation (DOI). Researchers usually inject the solution into the air cell or the amnion (Das et al. 2021). This intervention stimulates the development of the gut and the immune system before the chicken hatches. As a result, newly hatched chickens have better vitality and a stronger physiological start for future growth (Ebeid et al. 2023).

Based on current knowledge, several phytoiotics show great potential for IO application. These include extracts from black cumin (*Nigella sativa*) (Oke et al. 2021; Oyelola et al. 2023), oregano (*Origanum vulgare*) (Niknafs et al. 2024; Elbaz et al. 2025), thyme (*Thymus vulgaris*), and clove (*Eugenia caryophyllata*) (El-Kholy et al. 2021).

Studies confirm that these extracts bring concrete physiological benefits. Specifically, Oke et al. (2021) observed improvements in both health status and performance parameters of heat-stressed chickens due to the antioxidant effects of black cumin. Similarly, thyme extract administered IO increases body weight (BW) both at hatch and at slaughter age compared to the control group, while improving the feed conversion ratio (FCR) (El-Kholy et al. 2021).

Essential oils (EOs) also fall under this category of promising additives, exhibiting a wide range of biological activities due to their complex mixtures of active compounds. Recently, Khaskheli et al. (2025) found that EOs from ylang-ylang, clary sage, bergamot, lemon myrtle, and black pepper increase the expression of genes related to gut barrier integrity, nutrient transport, and digestive enzyme activity. Meanwhile, EOs from ylang-ylang, turmeric, star anise, clary sage, and black pepper promote the expression of gut immunity markers.

Ceylon cinnamon (*Cinnamomum verum*) is especially interesting due to its strong antioxidant properties. Its main active component, cinnamaldehyde, increases the secretion of digestive enzymes and stabilizes the gut microbiota (Ali et al. 2021). This component works mainly through intestinal TRPA1 receptors. It influences ion channels and secretions, which ultimately improves the morphological development of the intestine (Manneck et al. 2021).

In ovo application is a sensitive intervention into the egg that can alter embryonic homeostasis, so verifying dosage safety for the embryo is a critical first step. Based on the documented antioxidant properties of cinnamon, this study hypothesizes that the IO injection of a hydroalcoholic extract (1.8 mg/egg) will enhance breast meat yield at the end of the 35-day fattening period without compromising other growth variables. Therefore, the primary objective of this study was to evaluate how IO cinnamon extract delivery on 17.5 DOI affects the hatchability of Ross 308 eggs and the subsequent growth performance (live body weight, FCR, and carcass yield) of broiler chickens.

MATERIAL AND METHODS

Extract Preparation

The preparation of the extract was adapted from the method described by Ako-sile et al. (2023). Briefly, 200 g of ground cinnamon (*Cinnamomum verum*) was mixed with 2 L of 36% ethanol. This mixture was macerated for 96 h and then filtered through filter paper. The resulting solution was evaporated in a 55 °C water bath using a rotary vacuum evaporator equipped with a membrane vacuum pump until a constant weight was achieved. After cooling in a refrigerated unit, the pure extract was stored in the dark at 5 °C until IO application.

Hatching Eggs and Incubation

Hatching eggs were obtained from a 34-week-old Ross 308 broiler breeder flock (Czech Republic). Subsequently, the eggs were randomly placed into a BioStream-er 24S setter, where eggshell temperature was monitored. The initial temperature was set at 38.1 °C and gradually decreased to 36.7 °C over the incubation period. At 17.5 DOI, the eggs were candled, and unfertilized eggs or those with dead embryos were discarded. Following the IO treatment, the eggs were transferred to a BioStreamer 8H hatcher, where the temperature was maintained at an average of 36.0 °C until hatching.

In Ovo Treatment

A total of 480 fertile eggs were randomly assigned to two groups: an untreated control group (CG; n = 210) and an experimental group treated with cinnamon extract (EG; n = 270). Based on the findings of Kuka et al. (2023), who reported no differences between uninjected and placebo-injected groups, an untreated control group was selected. A higher number of eggs was intentionally allocated to the EG to ensure a sufficient and representative sample of male chickens for the subsequent growth trial.

The EG was treated via IO injection at 17.5 DOI. A hole was pierced into the shell at the blunt end using a sterile 22G needle. The injection solution was prepared by diluting the stock cinnamon extract with sterile physiological saline (0.9% NaCl) to a final concentration of 9 mg/mL. The application was performed into

the amniotic fluid using a 1-mL syringe equipped with a 23G needle inserted to a depth of approximately 3 cm. Each egg received an injection of 0.2 mL of this solution, delivering a total dose of 1.8 mg of cinnamon extract per egg.

Hatchability and Post-Hatch Management

Hatchability was evaluated for each tray as the percentage of hatched chickens from fertile eggs. After feather sexing, a total of 72 male chickens per group were randomly selected (evenly representing all incubation trays). These birds were distributed into 6 floor pens on wood shaving litter, with 12 chickens per pen. Housing conditions complied with the Ross 308 management guidelines (Aviagen 2025), and water and feed were provided *ad libitum*. The 35-day fattening period included three dietary phases: starter (D1–14), grower (D15–28), and finisher (D29–35). The dietary nutritional composition is presented in Table 1.

Tab. 39: *Nutritional composition of diets (g/kg, on a dry matter basis)*

	Starter	Grower	Finisher
Crude protein	214,44	201,37	175,23
Crude fat	31,94	40,03	43,23
Crude fiber	43,37	34,27	46,80
Crude ash	67,96	56,81	43,28

Growth Performance and Carcass Parameter Evaluation

Cockerels were weighed individually at housing (D1) and weekly until D35 to evaluate BW and body weight gain (BWG) per phase and for the entire trial. Feed intake (FI) was recorded daily per pen to calculate the feed conversion ratio (FCR). Health status was monitored twice daily, and mortality was included in the calculations. At D35, six average birds per group (one per replicate) were weighed and slaughtered to determine carcass weight (excluding neck and feet). Carcass yield was calculated relative to live body weight, and the proportions of skinless breast and thigh muscles were determined based on carcass weight.

Statistical Analysis

Data normality was verified by the Shapiro-Wilk test. Performance (BWG) and feed utilization (FI, FCR) parameters were evaluated via one-way ANOVA with the floor pen as the experimental unit ($n = 6$ per group). For BW, data were analyzed using a linear mixed model (nested ANOVA), with the treatment group as a fixed effect and individual replicates nested within the group as a random effect. This model accounted for the shared pen environment, establishing the replicate as the true statistical unit for individual measurements. Carcass traits were evaluated by one-way ANOVA using one selected bird of average BW from each pen as the statistical unit ($n = 6$ per group). Statistical analyses were performed in Statistica 14. Effects were declared significant at $p < 0.05$, while statistical trends were noted at $p < 0.10$. Results are expressed as means $\pm$ SD (standard deviation).

RESULTS AND DISCUSSION

A crucial indicator of successful embryonic development is the hatchability of fertile eggs, which must not be adversely affected by any IO administration. The results presented in Table 2 demonstrate that IO supplementation with the cinnamon extract solution in the EG did not induce statistically significant changes compared to CG. Although a slightly lower average hatchability was recorded in the EG, this numerical difference was entirely minor from a statistical standpoint, thereby confirming the safety of the applied dose and extract concentration for the developing embryo.

Tab. 40: Hatchability (%) of broiler hatching eggs following in ovo treatment (means $\pm$ SD)

	Group		<i>p</i> -value
	CG (n = 6)	EG (n = 6)	
Hatchability	94.95 $\pm$ 3.65	92.91 $\pm$ 3.46	0.443

No statistically significant differences ($p > 0.05$). Abbreviations: CG = untreated control group; EG = cinnamon extract experimental group (0.2 mL/egg); SD = standard deviation.

Maintaining high hatchability is critical during IO application, as elevated concentrations of EOs can be embryotoxic or restrict oxygen availability by disrupting shell membrane integrity. For instance, EOs from eugenol, clove, tea tree, lemongrass, and thyme are considered unsuitable for IO delivery due to reduced hatchability rates (Khaskheli et al. 2025). Conversely, Bozbay and Göneci (2023) observed no adverse effects on hatchability using a commercial cinnamon extract at doses up to 12 mg/egg, likely due to air cell targeting via a 27G (19 mm) needle. Other cinnamon extract studies (El-Kholy et al. 2021; Akosile et al. 2023) did not report hatchability data, though both also targeted the air cell. In contrast, our study demonstrates that amniotic injection of cinnamon extract at 1.8 mg/egg does not impair the hatching process. This indicates that cinnamaldehyde is well-tolerated at this concentration without inducing fatal metabolic or respiratory disruptions during late incubation.

The development of broiler BW during the 35-day fattening period is presented in Table 3. The data indicate that a single IO application of cinnamon extract had no adverse effects on growth performance. Initial BW at placement (D1) was balanced between groups, and average body weight remained highly consistent throughout the weekly intervals (D7 to D28) without statistically significant deviations. At the end of the trial (D35), the experimental group achieved a numerically higher average weight compared to the control; however, this difference was not statistically significant due to intra-group variability.

In agreement with our findings, Akosile et al. (2023) observed no increase in final BW. In contrast, El-Kholy et al. (2021) reported significantly higher body weight both at hatch and at the end of the fattening period following IO cinnamon extract

Tab. 41: Average body weight (g) of broiler chickens during the 35-day fattening period (means $\pm$ SD)

Age	Group		p-value
	CG (n = 6)	EG (n = 6)	
D1	40.19 $\pm$ 2.71	39.94 $\pm$ 2,87	0.917
D7	160.50 $\pm$ 17.14	160.82 $\pm$ 18.53	0.597
D14	455.19 $\pm$ 56.31	458.55 $\pm$ 51.15	0.582
D21	874.26 $\pm$ 108.15	879.07 $\pm$ 105.87	0.867
D28	1533.27 $\pm$ 158.98	1532.65 $\pm$ 171.15	0.620
D35	2393.75 $\pm$ 254.93	2427.63 $\pm$ 286.84	0.671

No statistically significant differences ($p > 0.05$). Abbreviations: CG = untreated control group; EG = cinnamon ex-tract experimental group (0.2 mL/egg); D = day; SD = standard deviation. Data were analyzed using a linear mixed model based on individual bird weights within each pen ($n = 6$ per group).

application. Enhanced final BW was also recorded by Oke et al. (2021) after black cumin extract administration.

On the other hand, Meijer et al. (2024) failed to confirm any effects of carvacrol, the main component of oregano EO administered into the amnion at 17.5 DOI, on chicken weight at D7, beyond which the fattening of the Ross 308 broilers did not continue. Regarding dietary alternatives, Qaid et al. (2021) described an increase in Ross 308 body weight at D14 and D21 in the case of in-feed supplementation of cinnamon at a dose of 2 g/kg of feed, with the weight of the broilers being comparable to the antibiotic-treated group.

Average FI per chicken across the growth phases and the entire fattening period is presented in Table 4. Statistical analysis confirmed no significant effect of the IO cinnamon extract application on this criterion, with feed consumption remaining balanced between the CG and EG throughout the entire trial.

The balanced feed intake across the groups clearly demonstrates that the pre-natal intervention via IO application of the cinnamon extract did not compromise the post-hatch appetite of the broilers or the mechanisms regulating feed intake.

Tab. 42: Average feed intake (g/bird) of broiler chickens across different growth phases and the entire fattening period (means $\pm$ SD)

Period	Group		p-value
	CG (n = 6)	EG (n = 6)	
D1-14	491.97 $\pm$ 13.49	497.65 $\pm$ 5.62	0.364
D15-28	1586.97 $\pm$ 37.78	1609.14 $\pm$ 50.53	0.409
D29-35	1502.36 $\pm$ 55.07	1496.60 $\pm$ 58.34	0.864
D1-35	3581.29 $\pm$ 79.32	3603.39 $\pm$ 100.79	0.682

No statistically significant differences ($p > 0.05$). Abbreviations: CG = untreated control group; EG = cinnamon ex-tract experimental group (0.2 mL/egg); D = day; SD = standard deviation.;

Average BWG across the growth phases and the entire fattening period are summarized in Table 5. Consistent with the body weight development, no statistically significant differences in weight gains were recorded between the groups. In the final phase and over the entire trial, the EG exhibited a numerically higher average weight gain compared to the CG, though this trend was not statistically significant.

Tab. 43: Average body weight gain (g/bird) of broiler chickens across different growth phases and the entire fattening period (means $\pm$ SD)

Period	Group		p-value
	CG (n = 6)	EG (n = 6)	
D1-14	414.58 $\pm$ 19.36	418.86 $\pm$ 8.40	0.630
D15-28	1078.86 $\pm$ 58.79	1074.84 $\pm$ 37.87	0.891
D29-35	858.79 $\pm$ 84.76	895.58 $\pm$ 44.61	0.369
D1-35	2416.19 $\pm$ 78.65	2453.24 $\pm$ 60.84	0.383

No statistically significant differences ($p > 0.05$). Abbreviations: CG = untreated control group; EG = cinnamon ex-tract experimental group (0.2 mL/egg); D = day; SD = standard deviation.

The results confirm that the single prenatal supplementation with cinnamon extract does not affect the growth intensity of broilers. While continuous dietary administration of cinnamon often leads to a direct stimulation of weight gains through the modulation of the gut environment (Khan et al. 2025), a single IO application at 17.5 DOI primarily demonstrates its biological safety, as it causes neither embryo damage nor subsequent post-hatch growth depression.

The FCR values in relation to dietary treatment are presented in Table 6. No statistically significant differences were detected between the monitored groups during any of the evaluated periods. In the initial phases (D1-28), feed efficiency was identical between both groups, while a numerical decrease in FCR was observed in the EG compared to the CG during the final week (D29-35).

The stable FCR values confirm that prenatal stimulation with cinnamon extract did not impair nutrient utilization for muscle deposition. The slight, non-significant

Tab. 44: Feed conversion ratio of broiler chickens across different growth phases and the entire fattening period (means $\pm$ SD)

Period	Group		p-value
	CG (n = 6)	EG (n = 6)	
D1-14	1.19 $\pm$ 0.08	1.19 $\pm$ 0.02	0.970
D15-28	1.48 $\pm$ 0.11	1.50 $\pm$ 0.08	0.685
D29-35	1.76 $\pm$ 0.16	1.67 $\pm$ 0.06	0.230
D1-35	1.52 $\pm$ 0.03	1.51 $\pm$ 0.04	0.474

No statistically significant differences ($p > 0.05$). Abbreviations: CG = untreated control group; EG = cinnamon ex-tract experimental group (0.2 mL/egg); D = day; SD = standard deviation.

FCR improvement in the EG during the final week correlates with the numerically higher weight gains observed in the same period (Table 5). Although FCR was not significantly altered in our study, literature indicates that IO cinnamon extract can positively influence this parameter. For instance, El-Kholy et al. (2021) confirmed enhanced feed efficiency following IO administration of aqueous cinnamon, thyme, or clove extracts. Furthermore, Akosile et al. (2023) reported improved FCR values after applying hydroalcoholic extracts of cinnamon and clove.

The results of the carcass analysis on day 35 are presented in Table 7. Although no parameters reached strict statistical significance, a pronounced statistical trend was recorded for two key indicators. Specifically, a trend toward an increased carcass yield was observed in the EG compared to the CG. A similar tendency toward higher values in the experimental group was noted for the breast muscle proportion. Conversely, the thigh muscle proportion remained balanced between both groups without statistical differences.

Tab. 45: *Carcass yield and proportions of main meat cuts in broiler chickens at day 35 (%; means $\pm$ SD)*

	Group		<i>p</i> -value
	CG (n = 6)	EG (n = 6)	
Carcass yield	68.09 $\pm$ 0.59	69.80 $\pm$ 2.03	0.077
Breast muscle	29.77 $\pm$ 1.30	31.94 $\pm$ 2.15	0.060
Thigh muscle	21.48 $\pm$ 1.36	20.38 $\pm$ 1.56	0.222

No statistically significant differences ($p > 0.05$). Abbreviations: CG = untreated control group; EG = cinnamon ex-tract experimental group (0.2 mL/egg); SD = standard deviation.

The recorded statistical trends suggest that prenatal stimulation with cinnamon extract may exert an anabolic effect on broiler muscle development, persisting until slaughter age. This positive trend in breast muscle proportion and carcass yield is likely associated with cinnamaldehyde, which enhances nutrient utilization, amino acid digestibility, and nitrogen retention, thereby favoring muscle tissue deposition (Chowdhury et al. 2018).

CONCLUSION

This study demonstrates the biological safety of prenatal cinnamon extract supplementation into the amnion of broiler chickens. The absence of significant differences indicates that the tested substance neither inhibits growth nor induces embryonic metabolic stress. From a commercial perspective, the observed positive trends in carcass traits suggest promising potential for this method. A single prenatal intervention indicated a tendency to stimulate breast muscle development, representing the economically most valuable cut, which is presumably mediated by the antioxidant properties of the extract. This occurred without restricting overall carcass growth. Given the verified safety of this protocol, extensive opportunities for follow-up research open up. Future studies should focus on the

histological development of the intestinal mucosa, gastrointestinal enzymatic activity, cellular oxidative status, and post-hatch microbiota modulation to fully elucidate the mechanism of action and optimize this method for commercial poultry production.

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THE POTENTIAL OF HEMP BIOMASS AS A SOURCE OF ANTIMICROBIAL AGENTS AGAINST STAPHYLOCOCCAL AND STREPTOCOCCAL INFECTIONS IN ANIMALS

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ABSTRACT

Bacterial infections caused by staphylococci and streptococci pose a serious burden on animal production. The growing antimicrobial resistance of these pathogens significantly limits treatment options and underscores the need to search for alternative antimicrobial agents. In this context, *Cannabis sativa* L. is one of the most intensively studied plants due to its high content of secondary metabolites, particularly cannabinoids and terpenoids.

The aim of this study was to compare the *in vitro* antimicrobial activity of ethanol extracts from five industrial hemp cultivars (Bialobrzeskie, Felina 32, Futura 75, mixed, and Santhica 27) against strains of *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Streptococcus dysgalactiae*, including both antibiotic-resistant and susceptible forms. Antibacterial activity was determined using the broth microdilution method and expressed as the minimum inhibitory concentration (MIC).



All five tested extracts exhibited inhibitory activity against all tested strains, with MIC values ranging from 128 to 2048 $\mu\text{g/ml}$. The most effective was the extract from the Santhica 27 variety (MIC 128 $\mu\text{g/ml}$ against *Str. agalactiae* and clinical isolates of *S. aureus*), followed by the Bialobrzieskie and mixed varieties. Extracts from the Felina 32 and Futura 75 varieties exhibited only weak activity. The highest total cannabinoid content was determined in the extract from the Bialobrzieskie variety (21,740.94 $\mu\text{g/g}$), and the lowest in the Futura 75 extract (4,713.11 $\mu\text{g/g}$). Higher antibacterial efficacy correlated with higher cannabinoid content, and the observed effect likely results from the synergistic action of the entire complex of active compounds.

The Santhica 27, Bialobrzieskie, and mixed varieties appear to be the most promising for further research. Follow-up *in vivo* experiments are necessary to verify their therapeutic potential.

Keywords: antibacterial; bacteria; cannabinoids; cannabis; pathogen;

INTRODUCTION

Bacterial infections pose a serious burden on livestock production. According to estimates by the World Organisation for Animal Health, approximately 20% of livestock production is lost annually due to disease, resulting in financial losses estimated at \$358.4 billion per year (Health for Animals 2023). Among the most significant causative agents of bacterial diseases in livestock are *Staphylococcus aureus* and streptococcal species, particularly *Streptococcus agalactiae* and *Streptococcus dysgalactiae*, which are the main causes of mastitis, the most common and economically significant disease of the mammary gland in dairy cows, with estimated costs of approximately \$ 147 per cow per year (Mesquita et al. 2019; Cheng & Han 2020).

The situation is further complicated by the growing antimicrobial resistance (AMR) of these pathogens. In *S. aureus* isolated from pigs, 100 % resistance to tetracycline, 88 % to penicillin, and 64% to clindamycin were reported, with methicillin resistance (MRSA) posing a serious problem in both veterinary and human medicine (Rao et al. 2022). For streptococcal species, increasing resistance to clindamycin, erythromycin, and tetracycline has been documented (Guy et al. 2023). The excessive use of antibiotics in animal production creates reservoirs of resistance and contributes to the spread of AMR encoding genes into the environment, which has global implications for public health (Kirchhelle, 2018; Ahmed et al. 2024).

In response to this situation, interest in natural alternatives to traditional antibiotics is growing. *Cannabis sativa* L. is one of the most intensively studied plants in this context. Industrial hemp varieties produce a broad spectrum of biologically active secondary metabolites, including cannabinoids, terpenoids, flavonoids, and other compounds with proven antimicrobial activity (Paulová et al.

2025a; Sionov & Steinberg 2022). Cannabinoids exhibit activity primarily against Gram-positive bacteria, but also against their biofilms (Appendino et al. 2008; Blaskovich et al. 2021). Importantly, industrial varieties containing $\leq 0.2\%$ THC are permitted for use in animal feed within the EU (European Food Safety Authority (EFSA) 2011), which is a prerequisite for their use in subsequent *in vivo* studies.

However, research to date has focused primarily on the activity of isolated cannabinoids or extracts from medicinal cannabis strains with high cannabinoid content. To date, there has been a lack of comparative studies on the antibacterial potential of ethanol extracts from individual industrial hemp varieties against pathogens relevant to bovine mastitis. The aim of this study was therefore to compare the *in vitro* antibacterial activity of ethanol extracts from five industrial hemp varieties (Bialobrzесьkie, Felina 32, Futura 75, mixed, and Santhica 27) against strains of *S. aureus*, *Str. agalactiae*, and *Str. dysgalactiae*, including antibiotic resistant and susceptible clinical and standard isolates.

MATERIAL AND METHODS

Plant materials

Four varieties of industrial hemp biomass (Bialobrzесьkie, Felina 32, Futura 75, and Santhica 27) obtained from the Czech Hemp Institute (Chraštice, CZ) were tested. The Bialobrzесьkie, Santhica 27, and Felina 32 varieties were harvested in October 2021, while the Felina 32 variety was harvested again in September 2022. In addition, a mixed variant was tested, prepared by combining the Bialobrzесьkie, Santhica 27, and Felina 32.

Preparation of hemp biomass extracts

The dried samples were homogenized and ground into a powder using a grinding bowl, and 0.3 g of each sample was added to 10 mL of 96% ethanol (Penta, Prague, CZ). The resulting mixture was vigorously shaken for 60 minutes on a shaker (IKA KS 130 Basic, Staufen, DE) (800 rpm). The mixture was then filtered under low pressure. The residue from the filter was transferred to a flask, and 10 mL of ethanol was added. The same extraction procedure was repeated twice. After three extractions, the total volume of 30 mL was collected in a single 50-mL plastic tube (VWR, Stříbrná Skalice, CZ).

Quantification of cannabinoid content in extracts

For analysis, 0.5 mL of the ethanol extract was filtered through a nylon filter (0.22 μm ; Labicom, Olomouc, CZ) and diluted 100-fold. After transferring the sample to an HPLC vial, 20 μL was injected into the chromatographic system. Chromatographic separation of 14 cannabinoids (CBDVA – cannabidivarinic acid, CBDV – cannabidivarin, CBDA – cannabidiolic acid, CBGA – cannabigerolic acid, CBG – cannabigerol, CBD – cannabidiol, THCV – tetrahydrocannabidivarin, THCVA – tetrahydrocannabidivarinic acid, CBN – cannabinol, Δ^9 -THC – Δ^9 -tetrahydrocannabinol,

$\Delta 8$ -THC – $\Delta 8$ -tetrahydrocannabinol, CBC – cannabichromene, THCA – tetrahydrocannabinolic acid, CBCA – cannabichromenic acid) was performed according to Czauderna et al. (2024) using a NexLeaf guard column (5 × 4.6 mm), a Shim-Pak column (Shimadzu, 2.2 μ m, 75 × 3 mm), and two Phenomenex C18 columns (Synergi 2.5 μ m, Hydro RP, 100 Å, 100 × 3 mm) on a high-performance liquid chromatography system (Shimadzu, Kyoto, JP) equipped with an SPD-M20A detector. The column temperature was maintained at 40 °C. All samples were analyzed using a linear gradient of 99.9% HPLC-grade acetonitrile (Chem-Lab NV, Zedelgem, BE) with 0.1% formic acid (Lachner, Neratovice, CZ) (v/v; solvent B) and 99.8% HPLC-grade purity methanol (Chem-Lab NV, Zedelgem, BE) (v/v; solvent C). The maximum system pressure was 24, MPA.

Bacterial strains and growth medium

Four strains of *S. aureus* and two strains of the genus *Streptococcus* were used in this study. The standard *S. aureus* strains ATCC 29213 and ATCC 43300 were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). Clinical isolates SA1 and SA3 were provided by Motol University Hospital (Prague, CZ) and identified by MALDI-TOF-MS according to Rondevaldová et al. (2018). *Str. agalactiae* strain DSM 2134 and *Str. dysgalactiae* strain DSM 20662 were obtained from the German Resource Centre for Biological Material (DSM, Braunschweig, GE). Cation-modified Mueller-Hinton broth (Oxoid, Basingstoke, UK) was used for the cultivation of *S. aureus* strains; streptococcal strains were cultured in tryptone soy broth (Oxoid, Basingstoke, UK). All culture media were adjusted to pH 7.6 using Trizma base (Sigma-Aldrich, St. Louis, MO, USA). The tested strains included the resistant reference strain *S. aureus* ATCC 43300 (MRSA – methicillin-resistant *S. aureus*, resistant to methicillin and oxacillin) and the susceptible control strain *S. aureus* ATCC 29213 (MSSA – methicillin-susceptible *S. aureus*).

Broth microdilution method

The antibacterial potential of the extracts was determined using the broth microdilution method in 96-well microtiter plates according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2018), with minor modifications based on Cos et al. (2006) for a more effective evaluation of the antimicrobial potential of natural substances. Serial dilutions of industrial hemp extracts in the range of 16–2048 μ g/mL were prepared in the appropriate culture medium using a manual multichannel pipette. Plates were inoculated with a bacterial suspension at a final concentration of 5×10^5 CFU/mL, adjusted using a McFarland densitometer, and incubated at 37 °C for 24 h under aerobic conditions. The growth of *S. aureus* strains was assessed at 405 nm using an Infinite 200 PRO microplate reader (Tecan, Männedorf, CH). The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the extract that inhibited bacterial growth by $\geq 80\%$ compared to the control without the test substance. An uninoculated well with broth served as the sterile control, and an inoculated well without extract

served as the negative (growth) control. Ethanol used as a solvent showed no inhibitory effect against any of the tested strains. The susceptibility of *S. aureus*, *Str. agalactiae*, and *Str. dysgalactiae* strains to antibiotic controls (oxacillin, penicillin) was verified. All samples were tested in three independent experiments, each performed in triplicate, and the results are expressed as modal MIC values.

Data analysis

Microsoft Excel was used to analyse the data obtained from the spectrophotometer. The calculations were performed in Excel, and the resulting MIC values are presented as the median or mode.

RESULTS AND DISCUSSION

Results

Antimicrobial potential of hemp extract

The results of *in vitro* inhibitory effects of hemp extracts on the tested bacterial strains of *S. aureus*, *Str. agalactiae*, and *Str. dysgalactiae*, are summarized in Table 1.

The lowest MIC value (128 µg/mL) was recorded for the Santhica 27 cultivar against *Str. agalactiae* and both clinical isolates of *S. aureus* (SA1 and SA3). The same inhibitory activity against *Str. agalactiae* was also achieved by extracts of the Bialobrzeskie cultivar and the mixed cultivar. Against standard *S. aureus* strains (ATCC 29213 and ATCC 43300), the Santhica 27 cultivar exhibited a slightly higher MIC (256 µg/mL), followed by the Bialobrzeskie cultivars (256 and 512 µg/mL) and the mixed cultivar (512 µg/mL). An MIC value of 256 µg/mL was similarly observed for the Bialobrzeskie cultivar and the mixed extract against clinical isolates SA1 and SA3, as well as for the Felina 32 and Futura 75 cultivars (MIC 256 and 512 µg/mL) against *Str. agalactiae* and clinical isolates of *S. aureus*. The Felina 32

Tab. 46: Antimicrobial potential of hemp extracts on gram positive tested pathogens (Paulová et al. 2025b)

Bacterium	Strain	Hemp extracts/MIC (µg/ml)				
		Bialobrzesikie	Felina 32	Futura 75	Mixed	Santhica 27
S. aureus	ATCC 29213	512	1024	1024	512	256
	ATCC 43300	256	1024	1024	512	256
	SA1	256	512	512	256	128
	SA3	256	512	512	256	128
S. agalactiae	DSM 2134	128	256	256	128	128
S. dysgalactiae	DSM 20662	1024	2048	2048	1024	1024

Abbreviations: *S. aureus* – *Staphylococcus aureus*; ATCC – American Type Culture Collection; SA1, SA3 – clinical isolates *Staphylococcus aureus*; *S. agalactiae* – *Streptococcus agalactiae*; *S. dysgalactiae* – *Streptococcus dysgalactiae*; DSM – German Resource Centre for Biological Material; MIC – minimum inhibitory concentration (expressed as an average of three independent experiments performed in triplicate)

and Futura 75 cultivars exhibited only weak inhibitory activity against standard *S. aureus* strains (MIC 1024 µg/mL). The most resistant strain tested was *Str. dysgalactiae*, for which all five cultivars showed uniformly weak inhibitory activity with an MIC of 2048 µg/mL. Slight differences compared to previously published data can be attributed to different methodological approaches and differences in the bacterial strains used.

The content of monitored cannabinoids in hemp extracts

Differences in cannabinoid content were observed among the five extracts tested. The total content of the analyzed cannabinoids is shown in Figure 1, and the content of individual cannabinoids is listed in Table 2.

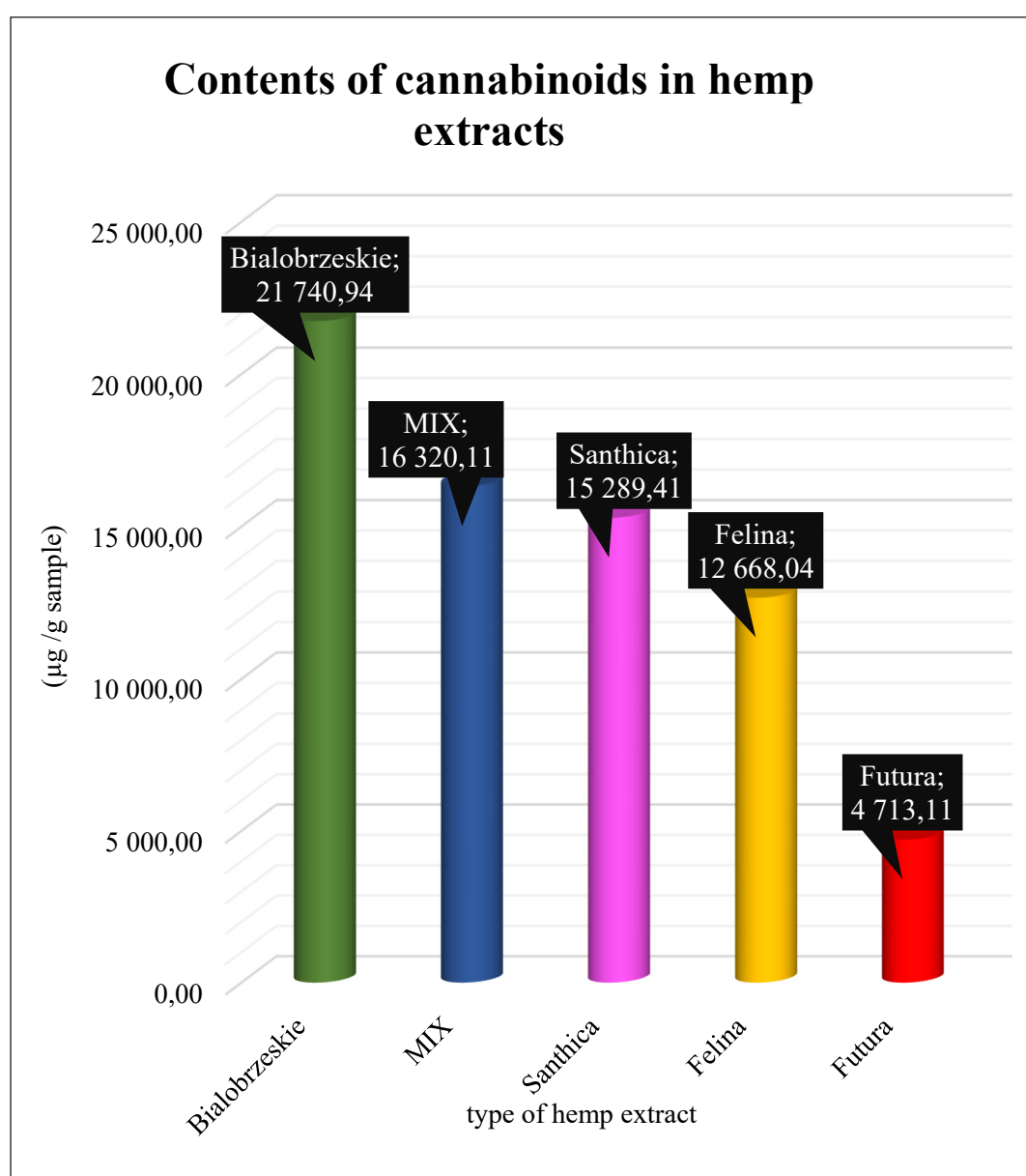


Fig.1: Contents of cannabinoids in hemp extracts (Paulová et al. 2025b)

Tab. 47: Contents of cannabinoids in hemp extracts (Paulová et al. 2025b)

Observed cannabinoids	Contents of cannabinoids in hemp extracts (µg/g vzorku)				
	Bialobrzeskie	Felina 32	Futura 75	Mixed	Santhica 27
CBDVA	360,58	144,94	63,82	258,58	ND
CBDV	ND	8,65	14,9	ND	ND
CBDA	15 208,79	7 404,60	2 776,69	10 206,44	2 650,84
CBGA	471,21	2 727,43	213,81	2 215,25	10 450,78
CBG	89,78	269,63	46 373	232,44	1163,19
CBD	2 839,14	521,72	1257,34	1590,11	370,69
THCV	ND	ND	ND	ND	ND
THCVA	ND	ND	ND	ND	ND
CBN	ND	ND	15,6	ND	ND
Δ9-THC	513,27	ND	40,61	261,37	59,36
Δ8-THC	ND	ND	ND	ND	ND
CBC	ND	301,71	105,28	ND	ND
THCA	1 350,45	314,58	53,32	746,26	194,74
CBCA	907,73	974,79	154,62	809,65	399,81
Total value	21 740,94	12 668,04	4713,11	16 320,11	15 289,41

Abbreviations: CBDVA – cannabidivarinic acid, CBDV – cannabidivarin, CBDA – cannabidiolic acid, CBGA – cannabigerolic acid, CBG – cannabigerol, CBD – cannabidiol, THCV – tetrahydrocannabidivarin, THCVA – tetrahydrocannabidivarinic acid, CBN – cannabinol, Δ9-THC – Δ9-tetrahydrocannabinol, Δ8-THC – Δ8-tetrahydrocannabinol, CBC – cannabichromene, THCA – tetrahydrocannabinolic, CBCA – cannabichromenic acid, ND – not detected.

The most abundant cannabinoid in all analyzed extracts was CBDA, followed by CBD, THCA, and CBCA. In contrast, THCV, THCVA, and Δ⁸-THC were not detected in any of the extracts. CBDV, CBN, and CBC were recorded only in low concentrations. The highest total cannabinoid content was determined in the extract of the Bialobrzeskie cultivar (21,740.94 µg/g), followed by the mixed extract (16,320.11 µg/g) and the extract of the Santhica 27 cultivar (15,289.41 µg/g). A lower cannabinoid content was recorded in the extract of the Felina 32 cultivar (12,668.04 µg/g), and the lowest value was found in the Futura 75 cultivar (4,713.11 µg/g).

Discussion

All tested ethanol extracts from hemp demonstrated some degree of *in vitro* antimicrobial activity against all tested bacterial strains, including both antibiotic-resistant and susceptible strains. However, their efficacy varied significantly—MIC values ranged from 128 to 1024 µg/ml. Although these values are higher than those reported in the literature for individual cannabinoids, our results are partially consistent with recent studies demonstrating the inhibitory effects of crude cannabis extracts on *Staphylococcus* strains, with MICs ranging from 8 to 8,552 µg/ml (Ali et al. 2012; Kaur et al. 2015; Skala et al. 2022; Giselle et al. 2023). Lower MIC values (8 µg/ml) for the ethanol extract against *S. aureus* (ATCC 29213) were

published by Skala et al. (2022). However, these results are difficult to directly compare with our study, as the total cannabinoid content in the extracts by Skala et al. (2022) was substantially higher (245,940 and 329,100 µg/g) than in the extracts from our study, with the contents of individual cannabinoids—particularly Δ9-THC, CBC, and CBG—being up to an order of magnitude higher in their extracts. These differences may explain the lower MIC values, as the antibacterial activity of these cannabinoids is well documented (Sionov & Steinberg 2022). Furthermore, Skala et al. (2022) worked with a medicinal cannabis strain with a relatively high cannabinoid content, whereas our study utilized industrial cannabis varieties with a THC content $\leq 0.2\%$ (EFSA 2011), which are permitted for use in livestock and whose application is therefore relevant for potential subsequent *in vivo* experiments. Giselle et al. (2023) also report values comparable to those in our study for *S. aureus* (ATCC 29213): MIC of the inflorescence extract 115.25 µg/ml and MIC of the root extract 8552.38 µg/ml. For streptococci, MIC values for individual cannabinoids have been determined for various species like *Streptococcus mutans* (MIC 2.5 µg/ml), *Streptococcus sanguis* (MIC 1 µg/ml), *Streptococcus pyogenes* (2–5 µg/ml), and *Streptococcus pneumoniae* (1–2 µg/ml) (van Klinger-en & ten Ham 1976; Aqawi et al. 2021; Blaskovich et al. 2021). The MIC values from our study are higher, which can be attributed to the complex composition of whole extracts, in which individual components may interact with one another or exhibit different mechanisms of action. For comparison, Skala et al. (2022) report an MIC of 128 µg/ml for the ethanol extract against *Str. pyogenes*, which corresponds to the values from our study.

The cannabinoid content in *Cannabis sativa* is influenced by a number of biotic and abiotic factors, such as nutrient availability, growth stage, storage conditions, light intensity, and temperature (Glivar et al. 2020). In our study, differences in cannabinoid content were observed among the five extracts tested, which likely influenced their antimicrobial activity. Higher antimicrobial activity was observed in extracts with higher cannabinoid content (Bialobrzeskie, mixed, and Santhica 27 varieties), while lower activity was recorded in extracts with lower content (Felina 32 and Futura 75 varieties). These findings suggest that cannabinoid content could be one of the key factors influencing the antibacterial efficacy of cannabis extracts. However, it is important to emphasize that the observed activity cannot be attributed solely to the cannabinoids themselves, but rather to the synergistic action of the entire complex of substances contained in cannabis (Giselle et al. 2023).

CONCLUSION

The present study evaluated the *in vitro* antimicrobial activity of ethanol extracts from five industrial hemp varieties (Felina 32, Futura 75, mixed, Santhica 27, and Bialobrzeskie) against strains of *S. aureus*, *Str. agalactiae* and *Str. dysgalactiae*, including both antibiotic-resistant and susceptible forms. All five tested ethanol

extracts exhibited some degree of inhibitory activity against the tested bacterial strains. The extract from the Santhica 27 variety proved to be the most effective, followed by the mixed and Bialobrzeskie varieties. Extracts from the Felina 32 and Futura 75 varieties exhibited only weak antibacterial activity. The results obtained demonstrate that the composition of hemp extracts varies greatly by variety, which is directly reflected in the differing degrees of growth inhibition of individual bacterial species. This variability underscores the need for further research focused on the relationship between the chemical composition of the extracts and their antibacterial effect. In the context of increasing antimicrobial resistance of pathogens and the restriction of preventive antibiotic use in animal production, hemp represents a promising source of natural antimicrobial substances. The Santhica 27, Bialobrzeskie, and mixed varieties appear to be the most promising candidates for further research. However, to verify the therapeutic potential of cannabis extracts in the treatment of staphylococcal and streptococcal infections in livestock, it will be necessary to conduct follow-up in vivo experiments.

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THE EFFECT OF DIETS SUPPLEMENTED WITH PHYTOADDITIVES ON SELECTED GROWTH PERFORMANCE TRAITS IN FATTENED RABBITS

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ABSTRACT

This study assessed the effect of complete diets supplemented with selected phytogetic additives on chosen growth performance traits in fattened rabbits. A total of forty-eight 42-day old weaned HYLA rabbits were randomly assigned to four dietary groups. housed in loose group pens. The feeding trial lasted until 97 days of age. Four complete diets without coccidiostats were formulated: a control diet (C) without additives, and three experimental diets supplemented with 2.0% *Artemisia abrotanum* (E1), a combination of 1.5% *Arthrospira platensis* and 0.5% Sencha green tea (E2), and a combination of 1.5% *Chlorella vulgaris* and 0.5% Matcha green tea (E3). Live weights and feed intake were recorded throughout the experimental period, thereafter feed conversion ratio (FCR) and average daily gain (ADG) were calculated. No statistically significant differences in the monitored growth performance traits were found. The results indicate that the inclusion of *Artemisia abrotanum*, microalgae, and green tea combinations at the tested levels does not negatively affect growth performance, feed palatability, or feed efficiency in growing rabbits. These findings can be used for further updating and optimizing of rabbit diets enriched with bioactive compounds with regard to metabolism and health evaluation of the rabbits.

Keywords: *Oryctolagus cuniculus*; *Artemisia abrotanum*; microalgae; green tea; feed conversion ratio

INTRODUCTION

In recent years, rabbit production has experienced a notable transformation. Beyond traditional meat production, rabbits are increasingly kept as companion animals, making breeders focus more on long-term health and longevity of their animals. Nutrition plays a key role in maintaining overall vitality, supporting



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a healthy gut microbiome, and preventing gastrointestinal disorders. As a result, functional feed additives are becoming popular because they effectively support general health and gut function (El-Sabrout et al., 2023; Plachá et al., 2022). Phytogetic feed additives, such as plant extracts, microalgae, and herbal plants, provide a wide range of bioactive substances (polyphenols, essential oils, and polysaccharides) with reported antimicrobial, antioxidant, and immunomodulatory properties (Dalle Zotte et al., 2016; El-Sabrout et al., 2023; Plachá et al., 2022). Among plant additives, *Artemisia* species contain essential oils and flavonoids capable of enhancing nutrient digestion, improving gut health, and preventing coccidial infections (Popović et al., 2017). Microalgae such as *Arthrospira platensis* and *Chlorella vulgaris* are rich sources of high-quality protein, essential fatty acids, and immunomodulatory pigments that enhance gut immunity and support growth performance (Abdelnour et al., 2019; El Basuini et al., 2023; Saleh et al., 2026). Furthermore, green teas (*Camellia sinensis*), particularly Sencha and Matcha, are rich sources of catechins (e.g., epigallocatechin gallate), which act as strong antioxidants and support healthy gut bacteria (Mohamed et al., 2025). Recent research demonstrates that combinations of Spirulina and Matcha tea can effectively protect against hepatic coccidiosis, support growth performance, and restore digestive integrity in rabbits (Saleh et al., 2026). Although these natural additives mainly aim to promote long-term health and longevity, key growth traits, such as feed intake and feed conversion ratio (FCR), remain essential indicators of feed palatability, nutrient absorption, and overall gut function. Therefore, the aim of this study was to evaluate the effects of diets supplemented with chosen phytogetic additives of principal growth performance traits in fattened young broiler rabbits.

MATERIAL AND METHODS

A total of 48 young, weaned HYLA rabbits aged 42 days were selected for the feeding trial. The experiment commenced immediately after weaning and lasted until the rabbits reached 97 days of age. The rabbits were housed in the experimental facility of the Department of Animal Breeding, Animal Nutrition and Biochemistry, Faculty of Veterinary Hygiene and Ecology, University of Veterinary Sciences Brno. At 55 days of age, all rabbits were vaccinated against rabbit haemorrhagic disease and myxomatosis (BioRabbit RHDV 1,2, Bioveta, Czech Republic; MXT, Dyntec, Czech Republic). The rabbits were housed loose in groups of six per pen, resulting in a total of 12 rabbits per monitored dietary group. Both sexes were represented in all experimental groups. To provide environmental enrichment, individual pens were equipped with tunnels and mirrors. Wood shavings were used as bedding, and plastic rabbit litter boxes were placed within each pen. Ambient temperature and relative humidity were recorded continuously throughout the feeding trial. Throughout the experiment, the overall health status of all rabbits was checked daily. The rabbits were weighed at regular, predetermined weekly intervals to monitor growth rates. Feed intake was recorded too with aim to calculate ADG and FCR.

For the purpose of the experimental trial, four original formulations of complete diets were designed and manufactured by Biokron, s. r. o. (Blučina, Czech Republic). The feed mixtures were formulated without coccidiostats. The rabbits were randomly assigned to four dietary groups: **control group (C)**: basal complete compound feed without added phytoadditives; **experimental group 1 (E1)**: basal complete compound feed supplemented with 2.0% dried Southernwood herb (*Artemisia abrotanum*); **experimental group 2 (E2)**: basal complete compound feed supplemented with 1.5% spirulina algae (*Arthrospira platensis*) and 0.5% Sencha green tea (*Camellia sinensis*); **experimental group 3 (E3)**: basal complete compound feed supplemented with 1.5% chlorella algae (*Chlorella vulgaris*) and 0.5% Matcha green tea (*Camellia sinensis*).

Given the normal distribution of the data, a two-way analysis of variance (ANOVA) was subsequently applied to assess the effects of sex, experimental diet type, and their mutual interaction on rabbit growth.

RESULTS AND DISCUSSION

The effects of complete diets supplemented with selected phytogetic additives (*Artemisia abrotanum*, *Arthrospira platensis*, *Chlorella vulgaris*, and *Camellia sinensis*) on growth performance, weekly body weights, daily feed intake, and feed conversion ratio (FCR) of fattened rabbits are summarized in Table 1 and Table 2. Overall, no significant differences ($P > 0.05$) were observed among the dietary groups or between sexes for any of the evaluated growth parameters, and no diet-sex interactions were recorded.

Throughout the 55-day trial period (D42–D97), initial (960.17–975.58 g) and final live weights (2721.6–2786.3 g) showed no statistically significant differences among experimental groups. ADG followed common patterns ($P > 0.05$), with the highest rates recorded during D63–D69 at 44.25–48.83 g/d. These results confirm that supplementation with 2.0% *Artemisia abrotanum* (E1), 1.5% *Arthrospira platensis* + 0.5% Sencha tea (E2), or 1.5% *Chlorella vulgaris* + 0.5% Matcha tea (E3) does not impair growth. These findings are consistent with previous studies evaluating similar natural feed additives in rabbit nutrition. Specifically, Liu et al. (2019) and Ding et al. (2019) found that dietary inclusion of *Artemisia* spp. supported rabbit intestinal health without compromising LW, or ADG. Similarly, Yin et al. (2024) reported that supplementing post-weaning rabbit diets with green tea polyphenols showed no adverse effects on growth performance parameters. Similarly in dwarf rabbit breed, receiving *Artemisia vulgaris* enriched diets did not affected growth dynamics within young kits raising (Kudělková et al., 2022).

Total FCR (D42–D97) remained uniform across experimental groups (4.35–4.50 g/g), displaying typical age-dependent increases toward the end of the fattening period (Table 2). Although the diets were formulated for pet rabbits - characterized by high fiber and lower protein content compared to commercial fattening diets - the rabbits maintained consistent growth and optimal feed

efficiency. Furthermore, plant secondary metabolites (such as essential oils, tannins, and polyphenols in *Artemisia* and green teas) can occasionally reduce feed intake due to bitterness or altered palatability. However, daily feed intake and FCR in this study were unaffected by any additive combination, confirming that inclusion rates up to 2.0 % preserved feed palatability. This is consistent with findings by Yin et al. (2024), who reported that green tea polyphenols do not impair palatability or feed efficiency in growing rabbits. Similarly, incorporating low levels of microalgae maintained feed intake and digestibility without disrupting overall feed conversion (Abu Hafsa et al., 2021). Overall health status remained excellent, with exceptionally low mortality (only one rabbit died in E2; 2.08 %). This is consistent with findings by Liu et al. (2019), who similarly reported that dietary supplementation with *Artemisia* species in post-weaning rabbits significantly reduced the incidence of diarrhea and strengthened gut barrier integrity without requiring synthetic coccidiostats. The bioactive compounds in *Artemisia*, green tea, and microalgae protect the digestive system by maintaining a healthy gut microbiome (Liu et al., 2019; Gadzama et al., 2025).

Tab. 48: Live weight and average daily gain of fattened rabbits depending on dietary group.

Traits	Units	Diet (x ± SEM)				P
		C	E1	E2	E3	
LW D42	g	960,2 ± 24,41	968,4 ± 16,80	975,6 ± 17,45	970,4 ± 20,31	n.s.
LW D49	g	1103,8 ± 26,82	1124,3 ± 30,39	1114,8 ± 22,43	1110,6 ± 25,19	n.s.
ADG D43-D49	g/d	20,5 ± 1,10	22,3 ± 2,41	19,9 ± 1,36	20,0 ± 2,15	n.s.
LW D55	g	1271,4 ± 30,39	1279,5 ± 38,06	1292,7 ± 27,02	1277,7 ± 25,73	n.s.
ADG D50-D55	g/d	24,0 ± 1,13	22,2 ± 1,55	25,4 ± 1,42	23,9 ± 1,16	n.s.
LW D62	g	1488,8 ± 41,52	1475,8 ± 44,18	1507,7 ± 31,67	1509,4 ± 27,83	n.s.
ADG D56-D62	g/d	31,1 ± 2,13	28,0 ± 1,87	30,7 ± 1,43	33,1 ± 1,21	n.s.
LW D69	g	1798,6 ± 52,98	1787,2 ± 56,64	1828,9 ± 34,51	1851,3 ± 28,58	n.s.
ADG D63-D69	g/d	44,2 ± 2,38	44,5 ± 2,08	45,9 ± 1,47	48,8 ± 1,56	n.s.
LW D76	g	2005,7 ± 63,00	1975,7 ± 67,10	2017,8 ± 42,82	2054,9 ± 37,98	n.s.
ADG D70-D76	g/d	29,6 ± 2,23	26,9 ± 2,01	27,0 ± 2,74	29,1 ± 2,46	n.s.
LW D83	g	2244,9 ± 70,38	2234,1 ± 75,97	2274,0 ± 39,07	2327,8 ± 37,05	n.s.
ADG D77-D83	g/d	34,2 ± 2,04	36,9 ± 1,87	32,8 ± 1,99	39,0 ± 1,92	n.s.
LW D90	g	2497,7 ± 79,06	2483,6 ± 78,58	2577,5 ± 54,93	2562,8 ± 39,71	n.s.
ADG D84-D90	g/d	36,1 ± 2,85	35,6 ± 0,84	43,4 ± 3,69	33,6 ± 1,56	n.s.
LW D97	g	2747,8 ± 85,27	2721,6 ± 89,50	2784,9 ± 72,20	2786,3 ± 49,87	n.s.
ADG D91-D97	g/d	35,7 ± 1,54	34,0 ± 1,95	29,6 ± 2,84	31,9 ± 2,92	n.s.

C, control group; E1, experimental group fed basic diet with 2 % supplementation of *Artemisia abrotanum*; E2, experimental group fed basic diet with 1.5% supplementation of *Spirulina platensis* and 0.5 % Sencha green tea; E3, experimental group fed basic diet with 1.5 % supplementation of *Chlorella vulgaris* and 0.5 % Matcha tea; LW, live weight; ADG, average daily gain; D, day; FCR, feed conversion ratio; n.s., not significant

Tab. 49: Feed conversion ratio (FCR) across different periods and mortality rate of rabbits according to dietary groups.

FCR (g/g)	Diet			
	C	E1	E2	E3
D43-D49	3,51	3,20	3,58	3,56
D50-D55	3.20	3.46	2.97	3.07
D56-D62	3.44	3.81	3.48	3.13
D63-D69	3.27	3.43	3.21	3.16
D70-D76	5.07	5.62	5.64	5.29
D77-D83	4,99	4,77	5,52	4,81
D84-D90	5.19	5.44	4.36	5.79
D91-D97	5.65	5.85	6.70	6.45
D42-D97	4.35	4.50	4.38	4.41
Mortality (indiv.)	0	0	1	0

C, control group; E1, experimental group fed basic diet with 2 % supplementation of *Artemisia abrotanum*; E2, experimental group fed basic diet with 1.5 % supplementation of *Spirulina platensis* and 0.5 % *Sencha green tea*; E3, experimental group fed basic diet with 1.5 % supplementation of *Chlorella vulgaris* and 0.5 % *Matcha tea*; FCR, feed conversion ratio.

CONCLUSION

The dietary inclusion of selected herbs and microalgae in coccidiostat-free diets did not alter growth performance or feed efficiency in young growing rabbits. Consequently, these functional natural ingredients can be safely incorporated into rabbit diets as health-supportive alternatives without compromising growth or feed utilization. Further studies are needed with aim to deepen knowledges and understand mechanisms of action of these bioactive compounds in rabbit diets.

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INFLUENCE OF ORGANIC AND INORGANIC SELENIUM FORMS ON CARCASS TRAITS AND BIOCHEMICAL PARAMETERS IN QUAILS

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ABSTRACT

The aim of the study was to evaluate the effects of different forms of selenium, including selenium nanoparticles, administered as dietary supplements in feed mixtures, on carcass characteristics and the sensory properties of quail meat. A total of 200 Japanese quails were included in the experiment and randomly allocated into four experimental groups (n = 50 per group). The results demonstrated that selenium supplementation, particularly in organic and inorganic forms, exerted beneficial effects on meat quality, especially with regard to oxidative stability, fat content, and protein content. Moreover, the observed effects appeared to be dependent on the specific form of selenium administered, suggesting distinct physiological and metabolic responses associated with different selenium sources.

Keywords: dietary supplementation; oxidative stability; carcass traits; lipid metabolism

INTRODUCTION

Over the past decade, nanotechnology has attracted considerable attention in the fields of diagnostics, medicine, and nutrition. Recently, there has been a growing interest in the potential application of nanomaterials in poultry nutrition. The primary use of nanotechnology in this area involves nano-sized mineral elements, which may reduce the antagonistic interactions commonly observed with conventional inorganic minerals in the gastrointestinal tract and enhance their bioavailability (Gopi et al., 2017). As a result, lower dietary inclusion levels may achieve comparable or even superior biological effects.



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Furthermore, improved bioavailability and utilization of trace elements can positively influence metabolic processes and reduce mineral excretion, thereby minimizing the environmental impact associated with poultry production (Surai et al., 2017). The application of nanoparticles in poultry diets has also been associated with a reduction in pathogenic bacterial populations while promoting the growth of beneficial probiotic microorganisms, which may subsequently improve growth performance and overall health status (Mahmoud, 2012).

Among the various nanomaterials, selenium nanoparticles (SeNPs) have received particular attention because of their unique physicochemical properties, high biological activity, and lower toxicity compared with conventional selenium sources. Previous studies have demonstrated that SeNPs may enhance feed efficiency, growth performance, reproductive functions, and immune responses in poultry and other animal species (Marković et al., 2018). In addition, selenium plays a crucial role in antioxidant defense mechanisms as an essential component of several selenoproteins, including glutathione peroxidase, thereby contributing to the protection of tissues against oxidative damage. Consequently, selenium nanoparticles represent a promising alternative selenium source in poultry nutrition, with potential benefits for animal health, productive performance, and meat quality.

MATERIALS AND METHODS

Experimental Animals and Housing Conditions

A total of 200 Japanese quails (*Coturnix japonica*) were included in the present study. The birds were reared under identical environmental and management conditions in cages measuring 150 × 150 × 200 cm, with 50 quails housed in each cage. Throughout the experimental period, all birds had unrestricted access to drinking water and were fed a standard commercial diet *ad libitum*.

At the beginning of the experiment, the birds were randomly assigned to four experimental groups (n = 50 per group), with two replicates established within each group. The control group (C) received a basal diet without selenium supplementation. Experimental group EXG1 received the basal diet supplemented with selenium nanoparticles (SeNPs). Experimental group EXG2 was supplemented with sodium selenite as an inorganic selenium source, whereas experimental group EXG3 received selenium-enriched yeast as an organic selenium source.

All birds were maintained under identical husbandry conditions throughout the experiment in order to minimize the influence of environmental factors on the evaluated parameters.

Experimental Procedure and Carcass Evaluation

The experimental period lasted 50 days. At the end of the experiment, the birds were slaughtered by severing the jugular vein, and blood samples were collected for subsequent laboratory analyses.

After slaughter, carcasses were processed by removing the head, feathers, internal organs, and distal portions of the limbs. Carcass weight was determined immediately after processing. Carcass yield was calculated as the ratio between live body weight before slaughter and carcass weight and was expressed as a percentage.

Chemical Analysis of Meat

Samples of the breast muscle (*Musculus pectoralis major*) were collected for the determination of the basic chemical composition of the meat. The analyses included the determination of crude protein, fat, moisture, and ash content according to the methodology described by (Marcinčák et al., 2023).

Sensory Evaluation

Sensory properties of the meat were evaluated by a panel of trained assessors. The assessed attributes included appearance, aroma, tenderness, juiciness, and overall acceptability. Samples were prepared under identical conditions prior to evaluation.

Statistical Analysis

The obtained data were statistically evaluated using one-way analysis of variance (ANOVA). Differences among experimental groups were assessed using Tukey's post hoc test. The results were expressed as mean values $\pm$ standard deviation (SD), and differences were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Table 1 presents the values of live body weight, carcass weight, and breast muscle weight on day 50 of the fattening period. On this day, no significant differences were observed in live body weight, carcass weight, or breast muscle weight between the experimental groups and the control group. Furthermore, lower carcass weight values were recorded in the Na_2SeO_3 group, in which quails were supplemented with inorganic selenium, compared with the control group.

Similar findings were reported by (Liu et al., 2015), who did not observe significant improvements in growth performance in chickens supplemented with either organic or inorganic forms of selenium. According to the study by (Reda et al., 2024), dietary supplementation with nano-selenium had no significant effect on carcass characteristics. Likewise, (Sevim et al., 2022) found that dietary nano-selenium supplementation did not influence carcass yield in broiler chickens. (Bakhshalinejad et al., 2019) also reported that dietary nano-selenium supplementation at levels of 0.1 and 0.3 mg/kg had no significant effect on carcass traits in broiler chickens.

Tab. 50: *Effect of selenium supplementation on live body weight and carcass parameters of quails on day 50.*

	Live weight (LW)		Carcass weight (CW)		Carcass yield	Pectoral muscle weight		
	g				% LW	g		%CW
Group	x	sd	x	sd	x	xs	sd	x
Control	289.66 ^a	23.70	185.6 ^a	13.46	64.09	60.33	7.91	32.49
SeNP	278.0 ^{a,b}	21.18	180.0 ^{a,b}	16.64	64.74	58.16	10.19	32.31
Na ₂ SeO ₃	261.82 ^b	12.38	168.0 ^b	9.97	64.16	55.5	3.88	33.03
Yeast Se	279.66 ^{a,b}	17.89	178.0 ^{a,b}	14.81	63.64	59.83	6.79	33.61

Note: SeNP – quails supplemented with selenium nanoparticles; Na₂SeO₃ – quails supplemented with sodium selenite; Se yeast – quails supplemented with selenium-enriched yeast; different superscript letters within a column indicate statistically significant differences at $P < 0.05$.

Table 2 summarizes the results of selected meat quality parameters in quails on day 50 of the fattening period. The moisture content increased ($P < 0.05$) in the experimental group supplemented with inorganic selenium (Na₂SeO₃) compared with all other groups.

The fat content was lower ($P < 0.05$) in all experimental groups compared with the control group, with the most pronounced reduction observed in the group supplemented with the organic form of selenium. A slight decrease in protein content ($P < 0.05$) was recorded in the SeNP group as well as in the group supplemented with the organic form of selenium (Se yeast) compared with the control group and the inorganic selenium group (Na₂SeO₃).

The pH value was lower in the SeNP group and in the group supplemented with the organic form of selenium (Se yeast) compared with the control group and the inorganic selenium group. The MDA concentration was lower ($P < 0.05$) in the experimental groups supplemented with organic selenium and inorganic selenium compared with the control group.

Similar findings were reported by (Reda et al., 2024), who observed a significant reduction in MDA values ($P = 0.0009$) following dietary supplementation with biological nano-selenium in quails compared with the control group. Dietary supplementation with selenium nanoparticles also improved the plasma lipid profile in quails.

(Mohapatra et al., 2014) reported that the inclusion of nano-selenium in the diets of laying hens significantly reduced fat levels. Likewise, (Elsaid, 2015) observed a significant decrease in blood triglyceride concentrations in birds treated with nano-selenium. (Oppenheimer et al., 1991) suggested that the beneficial effects of selenium on the lipid profile may be attributed to enhanced lipolysis induced by selenium intake. Furthermore, selenium supplementation increased the levels of 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂, resulting in reduced concentrations of sterol regulatory element-binding protein-2 and consequently decreased cholesterol synthesis.

Tab. 51: Comparison of selected meat quality parameters in quails on day 50 of the fattening period.

Parameter	SeNP		Na ₂ SeO ₃		Yeast Se		Control	
	x	Sd	x	sd	x	sd	x	sd
Water	69.36 ^b	0.28	70.47 ^a	0.31	69.81 ^b	0.29	69.70 ^b	0.51
Fat	2.52	0.18	2.62	0.13	2.47 ^b	0.18	2.77 ^a	0.08
Ash	1.03 ^a	0.01	1.07 ^b	0.07	1.05	0.02	0.94	0.03
Protein	24.18 ^b	0.40	25.15 ^a	0.80	24.45 ^b	0.40	25.24 ^a	0.44
Ph	6.56 ^b	0.02	6.93 ^a	0.06	6.40 ^b	0.05	6.86 ^a	0.05
MDA	1.05 ^a	0.13	0.691 ^c	0.09	0.829 ^b	0.12	1.01 ^a	0.17

Note: SeNP – quails supplemented with selenium nanoparticles; Na₂SeO₃ – quails supplemented with sodium selenite; Se yeast – quails supplemented with selenium-enriched yeast. Different superscript letters within a column indicate statistically significant differences at $P < 0.05$.

CONCLUSION

In conclusion, no significant differences were observed between the experimental groups and the control group in the evaluated growth and carcass parameters on day 50 of the fattening period, although the group supplemented with inorganic selenium (Na₂SeO₃) exhibited slightly lower carcass weight values. These findings indicate that selenium nanoparticle supplementation did not significantly affect the growth performance of quails during the experimental period. However, further studies are needed to elucidate the potential long-term effects of this selenium source on the health status and productive performance of poultry.

Dietary supplementation with different forms of selenium influenced selected meat quality parameters during the experimental period. On day 50, the group supplemented with organic selenium (selenium-enriched yeast) exhibited lower fat content, reduced pH values, and decreased MDA concentrations, whereas higher moisture content was observed in comparison with the other experimental groups.

Overall, the results suggest that selenium supplementation, particularly in organic and inorganic forms, exerts beneficial effects on quail meat quality, especially with respect to oxidative stability and fat and protein content. The observed effects appear to depend on the chemical form of selenium administered, indicating distinct physiological responses associated with different selenium sources.

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EFFECT OF REPLACING RAPESEED MEAL WITH CALCIUM NITRATE ON RUMEN FERMENTATION AND RUMEN MICROBIOME IN DAIRY COWS

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ABSTRACT

Cattle farming is a major agricultural source of methane emissions originating from rumen fermentation. Methane has a high global warming potential, and its production represents an energy loss for ruminants. Nitrate supplementation is a promising mitigation strategy, as nitrates act as alternative hydrogen acceptors in the rumen, reducing methanogenesis and producing ammonia that can serve as a source of non-protein nitrogen. This study aimed to assess the effect of replacing rapeseed meal with calcium nitrate on rumen fermentation and the composition of the rumen microbiome in dairy cows under in vivo conditions. We hypothesized that calcium nitrate would alter the ratio of volatile fatty acids in rumen fluid, increase the concentration of ammoniacal nitrogen in rumen fluid, and influence the structure of the rumen microbial community. Eight Holstein dairy cows were fed two diets in a crossover design for two 21-day periods (14 days of adaptation and 7 days of sampling). The treatments were: 1) silage-based basal diet + rapeseed meal (40 g/kg TMR) (CONTROL) and 2) silage-based basal diet + 100 g nitrate per cow/d (Bolifor CNF 1%) (NITRATE). Nitrate supplementation did not affect dry matter intake ($P = 0.521$) or any fermentation parameters: VFA ($P = 0.342$); pH ($P = 0.421$); $\text{NH}_3\text{-N}$ ($P = 0.761$). Nitrate supplementation affected alpha diversity in the Chao1 richness index ($P = 0.0164$), and the number of ASVs ($P = 0.0153$); other indices (Shannon and Simpson diversity indices and Pielou evenness index) were not statistically significant. At the phylum level, Firmicutes ($P = 0.844$) and Bacteroidota ($P = 0.945$) were dominant with no significant changes



in their relative abundances. No significant differences were observed in the relative abundance of any major bacterial families, including Methanobacteriaceae (Control 6.2 %, Nitrate 3.9 %, $P = 0.039$). In conclusion, calcium nitrate supplementation affected bacterial diversity and the Methanobacteriaceae family.

Keywords: nitrate; methane; emissions; volatile fatty acids; ruminant nutrition

INTRODUCTION

In recent years, the approach to reducing emissions from ruminants has shifted from a narrow focus on enteric methane production to a broader perspective on optimizing the entire feed ration. Modern strategies aim not only to reduce greenhouse gas emissions but also to increase nutrient utilization efficiency and improve animal performance. Methane (CH_4) production during enteric fermentation in dairy cows represents a significant energy loss for the animal, as approximately 6% of the gross energy in the diet is lost in the form of methane; at the same time, it is a major source of greenhouse gas emissions (Ellis et al., 2008). Reducing methane production is therefore considered an effective way to not only lower greenhouse gas emissions from the dairy industry but also to increase the energy efficiency of dairy cows (Johnson & Johnson, 1995). The goal is to balance environmental sustainability with the economic efficiency of livestock production, while maintaining or improving production parameters such as feed intake, nutrient digestibility, and milk or meat production.

Enteric methane originates primarily from the microbial fermentation of feed in the rumen. Here, plant polysaccharides are primarily broken down to form volatile fatty acids, which represent the main source of energy for ruminants. At the same time, however, hydrogen is produced and utilized by methanogens, with methane formed as a byproduct of fermentation (Mizrahi et al., 2021). Various factors influence its production, but adjusting the feed ration composition using feed additives appears to be the most effective strategy for reducing methane emissions (Hristov et al., 2013).

One promising option is the use of nitrates, which serve as an alternative electron acceptor in the rumen. When nitrates are reduced, competition arises with methanogenic archaea for available hydrogen, leading to a redirection of hydrogen from methanogenesis to nitrate reduction. The final product of this reduction is ammonia, which microorganisms can use to synthesize microbial biomass. Their significance, therefore, also lies in partially replacing the nitrogen component of the feed ration (Latham et al., 2016).

The addition of nitrate to the diet may affect not only methane production but also rumen fermentation, the profile of volatile fatty acids, nitrogen metabolism, and the composition of the rumen microbiome (Olijhoek et al., 2016). In particular, the impact on the microbial community remains poorly understood, and changes in its composition can significantly affect not only the intensity of methanogenesis

but also nutrient utilization efficiency and the stability of the rumen environment (Veneman et al., 2015).

This study examined three hypotheses. It was hypothesized that calcium nitrate would alter the ratio of volatile fatty acids in rumen fluid, increasing the proportion of propionic acid at the expense of acetic acid. Furthermore, it was hypothesized that calcium nitrate would increase the concentration of ammoniacal nitrogen in rumen fluid. Finally, it was expected that calcium nitrate would influence the rumen microbial community structure, specifically by suppressing methanogenic archaea and promoting the growth of bacteria involved in nitrate reduction. This study aimed to describe the effects of replacing rapeseed meal with calcium nitrate on rumen fermentation and rumen microbiome composition in dairy cows.

MATERIAL AND METHODS

The animals were treated in accordance with applicable European and Czech legislation (Directive 2010/63/EU on the protection of animals used for scientific purposes, European Union 2010). All procedures involving animals were approved by the Ethics Committee of the Institute of Animal Science (Prague, Czech Republic). The cows were housed in the experimental barn of the Institute of Animal Science (Netluky, Prague, Czech Republic).

Animals, experimental design, and treatment

Eight Holstein dairy cows were included in the experiment (mean $\pm$ standard deviation: 567 $\pm$ 70 kg body weight, parity 3 $\pm$ 1). The experiment was designed as a crossover trial with two experimental periods, each lasting 21 days. Each experimental period included a 14-day adaptation phase to the diet, followed by 7 days of data and sample collection. The cows were sorted by parity and weight, then randomly assigned to one of two controlled-airflow chambers, each designed for 4 animals (4 open stalls simulating standard housing in a loose-housing system on dairy farms). The chambers were then randomly assigned to one of two dietary treatments.

The cows in the control group were fed ad libitum a total mixed ration (TMR) three times a day (at 5:00 a.m., 11:00 a.m., and 5:00 p. m.). The control total mixed ration contained alfalfa silage (490 g/kg), corn silage (230 g/kg), straw (160 g/kg), wheat (80 g/kg), and rapeseed meal (40 g/kg). The experimental group (nitrate) was fed the same diet, with rapeseed meal replaced by calcium nitrate, and the amount of wheat increased. The experimental diet contained alfalfa silage (490 g/kg), corn silage (230 g/kg), straw (160 g/kg), wheat (110 g/kg), and the commercial product Bolifor CNF 1% containing calcium nitrate. Both diets had the same protein and energy content. In the experimental diet, the dairy cows consumed approximately 100 g of nitrates per head per day. The chemical composition of the substrate (DM basis) was as follows: organic matter (OM) 915 g/kg, crude

protein (CP) 130 g/kg, crude fat 19 g/kg, neutral detergent fiber (NDF) 501 g/kg, and acid detergent fiber (ADF) 326 g/kg.

Sample collection

Feed intake was determined in the respiratory chambers as the difference between the amount of feed offered and the amount of feed remaining over the last 7 days of each experimental period. Samples of the experimental diets and their residues were collected daily and stored at -20 °C. These samples were subsequently thawed and combined into composite samples for each chamber within the individual experimental periods. Samples of the individual components of the basal diet were collected once during the last 7 days of each experimental period. All samples were dried at 60 °C for 48 hours (forced-air oven), then ground to a particle size passing through a 1-mm mesh sieve (Retsch GmbH, Haan, Germany) and analyzed for dry matter (DM), ash, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), starch, and gross energy (GE) according to the methodology described previously (Joch et al., 2022).

Rumen fluid samples (250 ml) were collected from all cows on the 18th day of each experimental period, 3 hours after the morning feeding, using an oral rumen probe. The tip of the oral rumen probe, fitted with a strainer, was inserted to a depth of approximately 180 cm to reach the central part of the rumen, and the sample was collected using a vacuum pump into an Erlenmeyer flask (500 ml). To minimize contamination with saliva, the first 200 ml of rumen fluid was discarded. The samples were placed on ice immediately after collection and transported to the laboratory. The samples were stored at -80 °C for subsequent microbiome analysis. In the laboratory, the pH of the rumen fluid was determined using a portable pH meter (WTW) with a WTW Sentix 41 electrode (WTW, Weilheim, Germany).

To determine volatile fatty acids (VFA) and ammonia nitrogen (NH₃-N), 0.5 ml of rumen fluid was acidified by adding 10 µl of 9 M H₂SO₄. The concentrations of volatile fatty acids (VFA) were determined by gas chromatography by comparison with a calibration mixture of standards (Supelco Volatile Fatty Acid Standard Mix; Merck, Missouri, USA). The analysis was performed on a gas chromatograph (GC-82F, Labio, Czech Republic) equipped with a flame ionization detector (FID) and a Stabilwax capillary column (15 m × 0.53 mm ID × 0.5 µm; Restek, USA) with hydrogen as the carrier gas (Joch et al., 2022). The concentration of NH₃-N was determined using the phenol-hypochlorite reaction (Weatherburn, 1967), with absorbance measured at 625 nm (Libra S22, Biochrom, Cambridge, UK).

DNA extraction, polymerase chain reaction (PCR), and analysis of amplicon sequence variants (ASVs)

DNA extraction was performed using a modified method involving mechanical cell disruption (bead-beating) and column purification. DNA was isolated from 200 µl of a homogenized rumen fluid sample (0.5 ml). The quantity and quality of the DNA were verified using a spectrophotometer (Tecan Group, Männedorf,

Switzerland). The isolated DNA was subsequently adjusted to a uniform molecular concentration (5 ng/μl) and stored at -20 °C until polymerase chain reaction (PCR) was performed.

The V4 region of 16S rRNA was used for amplification using primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The number of PCR cycles was the same for all samples and was kept as low as possible to prevent the formation of chimeric sequences. The success of PCR amplification was verified by gel electrophoresis using a 1% agarose gel stained with SYBR™ Green I Nucleic Acid Gel Stain (Thermo Fisher, Waltham, MA, USA) and subsequently documented using the Gel Doc XR+ System (BioRad, Hercules, CA, USA). All samples were amplified in triplicate. The PCR products were sequenced on the MiniSeq platform (Illumina, San Diego, CA, USA). Subsequent analysis of the resulting amplicons was performed using the DADA2 pipeline and the SILVA database (v128). The obtained data were normalized to the sample with the lowest sequencing depth (30,000 sequences/sample).

Visualization, assessment, and statistical analysis of microbial communities

The relative abundance of bacteria was visualized at various taxonomic levels (taxa with a relative abundance > 0.1% of the total number of sequences) in Python using the “plotly” package. Differences in relative abundance were determined using the Wilcoxon signed-rank test. Differences with $p < 0,05$ were considered statistically significant. Alpha diversity indices (Chao1, Pielou, Shannon, and Simpson) were calculated based on a table of standardized (rarefied) amplicon sequence variants (ASVs) and subsequently analyzed using a paired t-test. Box plots illustrating differences in relative abundance at the family level were created in R software (version 4.3.2) using the “ggplot2”, “ggpubr”, and “ggsignif” packages.

Statistical analysis of fermentation parameters was performed using the PROC MIXED procedure in SAS, with the cow considered as the experimental unit, according to the following model:

$$Y_{(ijkl)} = \mu + T_i + P_j + W(C)_{kl} + e_{(ijkl)}$$

where Y represents the dependent variable, μ the grand mean, T_i the treatment effect, P_j the period effect, $W(C)_{kl}$ is the within-pen cow effect, and $e_{(ijkl)}$ is the residual error. The within-pen cow effect was considered random, while the other effects were fixed. Differences were considered statistically significant at the $P < 0,05$ level.

RESULTS AND DISCUSSION

Fermentation parameters

No statistically significant difference was found between the group of dairy cows fed the control diet (Control) and the group of dairy cows fed the nitrate-supplemented diet (Nitrate) for any of the monitored parameters ($P > 0.05$). Cows fed the nitrate diet consumed 0.3 kg more dry matter per day, but this difference was not statistically significant. The effect of nitrates on dry matter intake and fermentation parameters is shown in Table 1. Nitrate supplementation resulted in an approximately 12% reduction in methane production; however, these data are not presented in this study because methane production measurements were not among the main objectives. The obtained values should be considered indicative only, as methane was measured across the entire respiratory chamber, and the number of replicates was insufficient for a robust statistical analysis.

Tab. 52: Feed intake and rumen fermentation parameters in dairy cows fed a control diet (Control) and a diet containing nitrates (Nitrate)

	Parameters	Control	Nitrate	SEM	P-value
	DMI (kg/day)	17.7	18.0	0.33	0.521
Rumen	VFA (mmol/l)	113	104	4.5	0.342
	Acetic acid	60.8	61.3	0.98	0.769
	Propionic acid	21.8	20.1	1.31	0.510
	Butyric acid	17.0	18.3	0.40	0.135
	Lactic acid	0.50	0.34	0.059	0.165
	pH	6.67	6.74	0.041	0.421
	NH ₃ -N (mg/100 ml)	14.03	14.54	1.365	0.761

SEM – standard error of the mean; DMI – dry matter intake; VFA – volatile fatty acids; NH₃-N – ammonia nitrogen; $P < 0.05$

The administration of calcium nitrate did not alter the ratio of volatile fatty acids (VFA) in rumen fluid, nor did it increase the proportion of propionic acid at the expense of acetic acid, which refutes the first hypothesis. Although there was a numerical decrease in VFA concentration, it was not statistically significant. This result is consistent with the study by Villar et al. (2020), which also failed to demonstrate an effect of nitrates on VFA concentration. On the other hand, Sharifi et al. (2022) report a decrease in VFA concentration with nitrate supplementation, which may be due to reduced dry matter intake at higher dietary nitrate doses (Newbold et al., 2014). However, a decrease in VFA concentration was also observed under in vitro conditions, where the substrate concentration is constant; therefore, the decrease could not be attributed to lower dry matter intake (Guo et al., 2009). One possible explanation is the inhibitory effect of nitrite, an intermediate product of nitrate reduction, on rumen microorganisms, which may reduce fermentation activity and TMK production.

Dry matter intake (DMI) was also not statistically significantly affected by nitrate supplementation, which is consistent with Olijhoek et al. (2016), who found that nitrates do not appear to have a consistent effect on dry matter intake but may increase the animals' susceptibility to a potential decrease in dry matter intake. These differences may be related to feeding strategies and the amount of nitrate in the diet, with a decrease in dry matter intake observed up to 2.4 % dry matter (Newbold et al., 2014). According to Meller et al. (2019), dry matter intake is negatively affected by reduced diet palatability and the metabolic consequences of higher nitrate levels.

The molar proportion of the individual VFA was not affected by nitrate supplementation. The percentage of propionic acid remained unchanged, even though its absolute concentration decreased numerically. In contrast, the proportions of acetic and butyric acids were numerically higher; however, these differences were not statistically significant. Thus, the anticipated shift in the fermentation profile toward an increase in the proportion of propionic acid at the expense of acetic acid was not confirmed. These results are also supported by the studies by Ntokome et al. (2026) and Olijhoek et al. (2016), which also failed to demonstrate a significant effect of nitrates on the VFA profile. However, the meta-analysis by Abdelbagi et al. (2023) describes changes in the VFA profile following nitrate supplementation, specifically an increase in the proportion of acetic acid accompanied by a decrease in the proportion of propionic acid. This discrepancy in results may be related to the fact that during their reduction, nitrates compete for hydrogen not only with methanogenesis but also with propionogenesis, thereby reducing their availability for propionic acid production (Van Zijderveld et al., 2010).

The pH of the rumen fluid did not differ statistically significantly between the groups, given the constant concentration and profile of VFA (6.67 vs. 6.74). Fluctuations in rumen fluid pH reflect changes in the relative contributions of hydrogen flux and individual volatile fatty acids (Latham et al., 2016). Similar results are reported in studies by Van Zijderveld et al. (2011) and Olijhoek et al. (2016), which recorded only a slight numerical increase in pH. Latham et al. (2016) further report that the form of the nitrate salt influences the rates of nitrate reduction and nitrite accumulation, as well as the efficiency of methane reduction, with sodium and potassium salts being more soluble than calcium nitrates. The addition of calcium nitrate may therefore lead to a more uniform reduction process and fewer fluctuations in fermentation activity.

The concentration of ammoniacal nitrogen ($\text{NH}_3\text{-N}$) was not affected by calcium nitrate supplementation, which is inconsistent with the second hypothesis of this study, which predicted an increase in $\text{NH}_3\text{-N}$. This prediction is based on the fact that bacteria in the rumen convert nitrates to nitrites and then to ammonia. At the same time, they serve as an alternative electron acceptor, thereby reducing methane production (Datta et al., 2017). Olijhoek et al. (2016) observed an increase in $\text{NH}_3\text{-N}$ concentration upon isonitrogenous replacement of urea with nitrates, which they attributed to incomplete reduction of microbial protein synthesis by

rumen microorganisms. The study by Wang et al. (2018), however, describes a 33.1% reduction in $\text{NH}_3\text{-N}$ concentration during a similar replacement of the nitrogen source with nitrates. Such an increase in ammonia utilization can redirect more nitrogen, electrons, and energy toward microbial protein synthesis at the expense of methane emissions (Wang et al., 2018). In this study, there was no increase in the concentration of ammoniacal nitrogen in rumen fluid when rapeseed meal was isonitrogenously replaced with nitrate. Nitrates can thus serve as a source of non-protein nitrogen, a finding confirmed by Rebelo et al. (2019), who replaced nitrogen from protein feeds with nitrates and found that isonitrogenous replacement of soybean meal with nitrates did not increase ammonia concentration or adversely affect rumen fermentation. The rumen microbiota in this study can effectively utilize nitrogen derived from nitrates. However, the relatively low nitrate content in the diet (approximately 0.6% of dry matter) may also contribute to this. The measured $\text{NH}_3\text{-N}$ values (approximately 14 mg/100 ml) fall within the optimal range for microbial protein synthesis (approximately 5–20 mg/100 ml). Within this range, sufficient nitrogen is available to microorganisms without excessive accumulation. In conclusion, the availability of ammonia and fermentable energy is particularly crucial for microbial protein synthesis, whereas the nitrogen source itself is of secondary importance (Rebelo et al., 2019). At an isonitrogenous feed ration, the addition of nitrates in the diet represents more of an opportunity to utilize an alternative form of nitrogen than a process leading to a change in the concentration of ammoniacal nitrogen.

Rumen microbiome

Nitrate supplementation affected the alpha diversity of rumen fluid as measured by the Chao1 richness index and the number of ASVs ($P < 0.05$). For the other indices examined—the Shannon and Simpson diversity indices and the Pielou evenness index—no statistically significant difference was found between the groups ($P > 0.05$). The effect of nitrates on alpha diversity is shown in Table 2.

The effect of nitrates on the rumen microbiome of dairy cows is shown in Table 3 and Figure 1. At the phylum level (Figure 1A), the rumen microbiome was not statistically significantly affected by nitrate supplementation. In rumen fluid samples (both Control and Nitrate groups), Firmicutes and Bacteroidota were dominant, while Euryarchaeota, Spirochaetota, and Proteobacteria were present

Tab. 53: *Effect of Nitrates on Alpha Diversity – Diversity Indices, Species Richness*

Index	Control	Nitrate	SEM	P-value
Shannon diversity index	8.71	8.90	0.063	0.0791
Simpson diversity index	0.994	0.0791	0.0004	0.1834
Chao1 richness index	999.4	1102.0	31.69	0.0164*
Pielou evenness index	0.877	0.883	0.0054	0.4700
ASV	986.0	1088.3	30.60	0.0153*

SEM – standard error of the mean; ASV – amplicon sequence variants

in lower relative abundances. A trend toward a decrease in the relative abundance of the Euryarchaeota phylum was observed in the group of dairy cows fed a diet containing nitrate ($P = 0.078$). At the family level, a statistically significant difference was observed only for the family Methanobacteriaceae, whose relative abundance was lower in the nitrate group (control 6.2% vs. nitrate 3.9%, $P = 0.039$).

Tab. 54: *Relative abundance of strains and associated families in the rumen fluid of dairy cows fed the control diet (Control) and the nitrate-containing diet (Nitrate) ($P > 0.05$)*

Phylum	Family	Control	Nitrate	P-value
Firmicutes		57.2	61.7	0.844
	Lachnospiraceae	18.2	18.7	0.844
	Christensenellaceae	9.6	11.1	0.148
	Ruminococcaceae	5.8	8.0	0.195
	Anaerovoracaceae	5.6	4.3	0.460
	Oscillospiraceae	4.8	5.7	0.109
	Hungateiclostridiaceae	2.0	2.1	0.641
	Erysipelotrichaceae	1.9	2.2	0.742
	Eubacterium coprostanoligenes group	1.4	1.5	0.313
Bacteroidota		34.4	31.9	0.945
	Prevotellaceae	24.0	22.3	0.844
	Rikenellaceae	6.8	6.5	0.641
	Bacteroidales RF16 group	1.4	1.2	0.945
Euryarchaeota		5.7	3.72	0.078
	Methanobacteriaceae	6.2	3.9	0.039*
Spirochaetota		1.3	1.6	0.547
	Spirochaetaceae	1.4	1.6	0.742
Proteobacteria		1.3	1.1	0.641
Unclassified	F082	2.2	2.2	0.547

The third hypothesis posited that nitrate supplementation would alter the structure of the rumen microbiome, specifically decreasing the proportion of methanogenic archaea and increasing the abundance of bacteria involved in nitrate reduction.

Alpha diversity of the microbial community was not significantly affected by either the Simpson diversity index or the Pielou evenness index, suggesting no change in the dominance of individual bacterial taxa or in the evenness of their representation. A similar result is reported by Granja-Salcedo et al. (2019), who observed no changes in any of the alpha diversity indices; however, this is inconsistent with the results of this study, which showed changes in the indices of bacterial community species richness—specifically the Chao1 index and the number

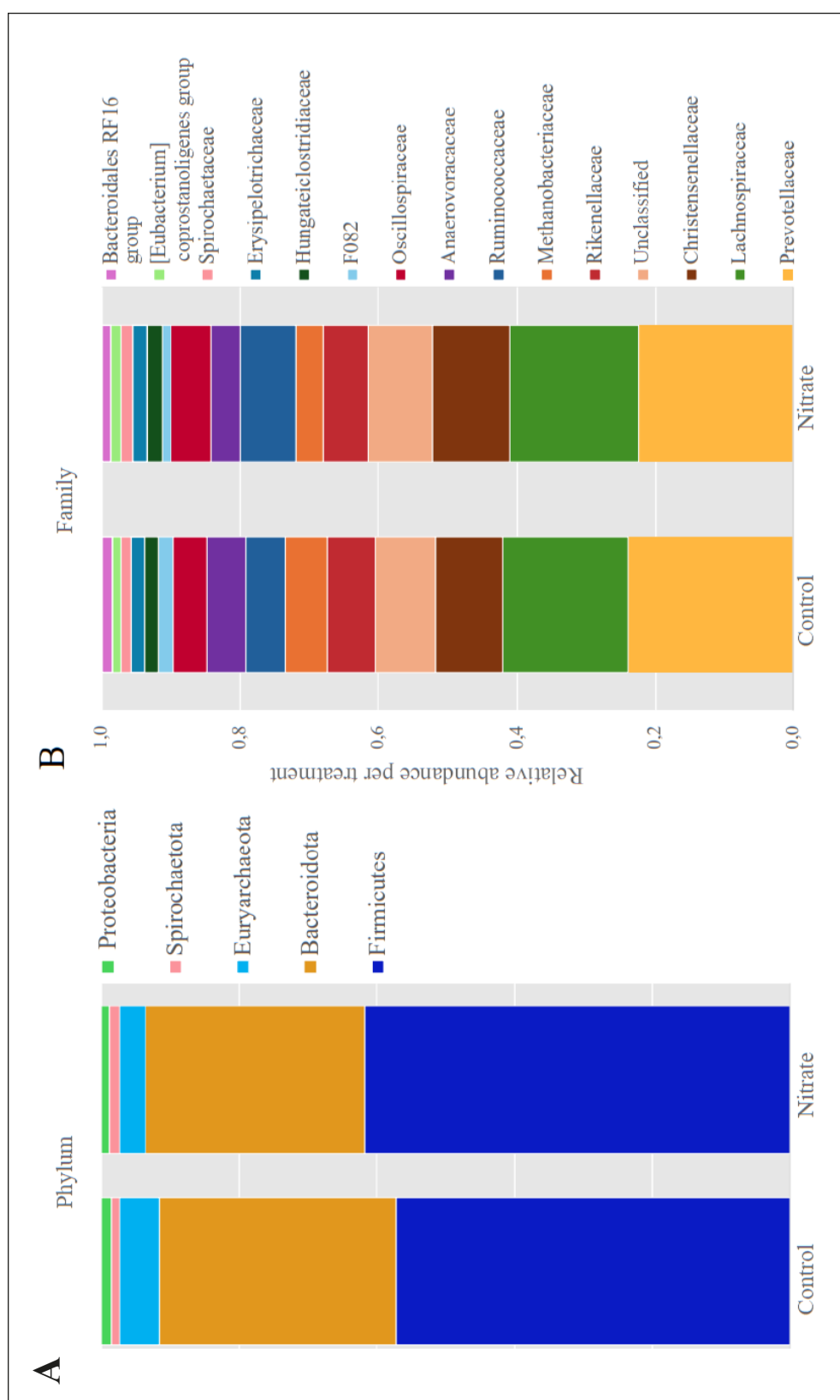


Fig. 1: The effect of nitrate supplementation on the relative abundance of bacteria at the Phylum (A) and Family (B) levels in the rumen fluid of dairy cows. The experimental groups included a control diet (Control; n = 8) and a diet supplemented with nitrate (Nitrate; n = 8)

of ASV (amplicon sequence variants)—as well as a tendency toward an increase in the Shannon index. Methodological differences can explain these differences, as the ASV-based approach enables high-resolution detection of microbial sequences at the single-nucleotide level, whereas OTU (Operational Taxonomic Unit)-based analyses cluster sequences using a predetermined identity threshold (Chiarello et al., 2022). Changes in the Chao1 index and the number of ASV in this study may thus reflect an increase in microbial community species richness, implying a broadening of the spectrum of microbial taxa while maintaining the dominance of major microbial populations.

According to Belanche et al. (2026), such an increase in rumen microbial complexity can lead to positive effects, particularly better adaptation to changes in diet composition. Promoting greater rumen microbial diversity is thus desirable for preventing digestive disorders (Belanche et al., 2026).

The composition of bacterial strains in rumen fluid reflects the dynamics of the rumen microbiome, with Firmicutes and Bacteroidota strains often the most abundant across various conditions (Mao et al., 2015), consistent with this study's results. At a lower taxonomic level, it was further found that within the Firmicutes phylum, the families Lachnospiraceae, Christensenellaceae, and Ruminococcaceae were primarily represented. Within the Bacteroidota phylum, the Prevotellaceae family was dominant.

Among the less abundant phyla, Euryarchaeota, Spirochaetota, and Proteobacteria were particularly well represented, which is generally consistent with the literature (Mao et al., 2015). Granja-Salcedo et al. (2019), however, demonstrated a statistically significant increase in the Proteobacteria phylum following nitrate supplementation. In contrast, this study observed only a slight numerical decrease, without statistical significance. Sharifi et al. (2022) report that an increase in the abundance of the Proteobacteria phylum is associated with reduced methane emissions due to competition for hydrogen (Zhao et al., 2015). However, the differing results also suggest that the response of the Proteobacteria phylum to nitrate supplementation may not be uniform. The slight numerical decrease, without statistical significance, observed in this study may also be explained by the taxonomic composition of the microbiome at the family level, as families typically classified within the phylum Proteobacteria and associated with nitrate reduction (e.g., Succinivibrionaceae or Desulfovibrionaceae) were not detected. This result may also be related to the presence of unclassified taxa (F082), which may include nitrate-reducing bacteria. Zhao et al. (2015) also note that nitrate-reducing bacteria have been reported across various bacterial families, including Selenomonadaceae, Veillonellaceae, Helicobacteraceae, Campylobacteraceae, and Pasteurellaceae, and are thus not confined to a single taxon. Furthermore, these bacteria are present in the rumen in low numbers and at lower taxonomic levels (Zhao et al., 2015; Granja-Salcedo et al., 2019).

A statistically significant decrease was observed in one of the most abundant methanogen families in the rumen, the Methanobacteriaceae, consistent with

findings reported by Granja-Salcedo et al. (2019). In contrast, Zhao et al. (2018) report that the addition of nitrates did not significantly affect the dominant genus *Methanobrevibacter*. However, the abundance of the least prevalent genera, *Methanosphaera* and *Methanimicrococcus*, was significantly affected. Patra & Yu (2013) report that significant changes in methanogen composition may not accompany a reduction in methane production.

Zhao et al. (2015) and Latham et al. (2016) attribute the decline in the *Methanobacteriaceae* family primarily to competition for hydrogen, whereby nitrate is reduced to ammonia—a thermodynamically more favorable reaction—thereby limiting the availability of hydrogen for hydrogenotrophic methanogens. This explanation is also supported by Van Zijderveld et al. (2010), who additionally suggest a possible role for nitrite, a toxic intermediate product of nitrate reduction. However, a direct inhibitory effect on methanogens has not been fully confirmed (Yang et al., 2016). Given the absence of significant changes at the genus and family levels in this study, we can conclude that there was likely no significant accumulation of nitrite, as its accumulation is associated with the inhibition not only of methanogenic archaea but also of other microbial groups (Yang et al., 2016).

In conclusion, the hypothesis regarding a change in the structure of the rumen microbiome was only partially confirmed. A decrease in methanogenic archaea was demonstrated, while an increase in bacteria involved in nitrate reduction was not confirmed.

CONCLUSION

Our study demonstrated that calcium nitrate did not alter the ratio of volatile fatty acids in rumen fluid; thus, the first hypothesis regarding a change in the volatile fatty acid profile was not confirmed, as neither the total concentration nor the relative ratios were statistically significantly affected by nitrate. The second hypothesis regarding an increase in the concentration of ammoniacal nitrogen in rumen fluid was also not confirmed. Given the stable concentration of ammoniacal nitrogen, it can be assumed that the supply of nitrogen to the rumen microbiome via calcium nitrate was sufficient. The third hypothesis regarding the structure of the microbial community in the rumen was only partially confirmed, as suppression of methanogenic archaea was observed. However, there was no change in the overall structure of the microbial community, nor was it possible to confirm or refute the growth of bacteria involved in nitrate reduction.

The observed effect of calcium nitrate on rumen fermentation parameters and the microbiome may represent a potentially effective mitigation strategy for ruminants. Replacing part of the nitrogen in the diet with nitrates offers a solution without a significant economic burden for producers and could yield economic benefits.

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NUTRITIONAL STRATEGIES TO IMPROVE EGG QUALITY: THE ROLE OF HEMP AND FLAX SEED SUPPLEMENTATION IN LAYING HENS

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ABSTRACT

This study evaluated the effects of dietary supplementation with hemp seed and flax seed on laying hen performance, egg quality traits, yolk nutritional composition, and oxidative stability. A total of 240 Lohmann Brown laying hens were assigned to 4 dietary treatments in a 2 × 2 factorial experiment with two levels of non-psychoactive hemp seed (Futura variety; 0 and 40 g/kg) and two levels of non-extruded flax seed (Floral variety; 0 and 40 g/kg). The experiment lasted 12 weeks. The inclusion of flax seed significantly increased both hen-day egg production ($p < 0.001$) and egg mass ($p < 0.001$), while the addition of hemp seed resulted in a higher total egg mass ($p < 0.001$). Laying hens fed the flax seed-enriched diet exhibited a lower feed conversion ratio. The combined hemp seed and flax seed supplementation significantly increased egg weight ($p = 0.002$) and shell percentage ($p = 0.036$). The inclusion of hemp seed significantly elevated yolk γ -tocopherol concentrations ($p < 0.001$) and reduced total cholesterol ($p = 0.042$) and malondialdehyde levels in fresh yolks ($p = 0.003$), indicating improved oxidative stability. Both seed supplements and their combination increased the content of n -6 ($p = 0.022$) and n -3 ($p < 0.001$) polyunsaturated fatty acids. In particular, the levels of α -linolenic ($p < 0.001$) and eicosapentaenoic ($p < 0.001$) acids were elevated. These changes resulted in a more favorable n -6/ n -3 ratio, which was significantly affected by both hemp seed and flax seed supplementation ($p < 0.001$). In addition, all dietary treatments significantly affected the nutritional lipid quality indices, namely the index of atherogenicity and the index of thrombogenicity ($p < 0.001$).



These findings highlight the potential of combined hemp seed and flax seed supplementation as a novel nutritional strategy for producing eggs enriched with health-promoting bioactive compounds and improved nutritional quality.

Keywords: oilseeds; tocopherols; *n*-3 polyunsaturated fatty acids; nutritional indices; oxidative stability

INTRODUCTION

Eggs are an affordable and highly nutritious food, providing an excellent source of high-quality proteins and essential fats (Réhault-Godbert et al. 2019). They are also widely recognized as a valuable source of both fat-soluble and water-soluble vitamins (Adeoye et al. 2023). Owing to their rich nutritional profile, eggs are considered an ideal medium for delivering additional health-promoting bioactive compounds, the concentrations of which can be significantly influenced by the diet of laying hens. The recognized health benefits of *n*-3 polyunsaturated fatty acids (PUFAs), particularly α -linolenic acid (ALA, C18:3, *n*-3) and its long-chain derivatives eicosapentaenoic acid (EPA, C20:5, *n*-3) and docosahexaenoic acid (DHA, C22:6, *n*-3), have stimulated considerable research into increasing the *n*-3 PUFA content of eggs through dietary supplementation with *n*-3 PUFA-rich feed ingredients (Farinon et al. 2020; Usturoi et al. 2025). Seeds and their oils are among the most important dietary sources of *n*-3 PUFAs (Jing et al. 2017), including hemp seed (HS) and flax seed (FS). These oilseeds are natural sources of numerous health-promoting bioactive compounds and may exert distinct effects on laying hen performance as well as on egg quality and yolk nutritional composition. According to Bekhit et al. (2018) and Farinon et al. (2020), HS and FS contain lipids with unique and well-balanced fatty acid (FA) profiles, characterized by favorable *n*-6/*n*-3 PUFA ratios of approximately 3.5:1 and 0.3:1, respectively. Furthermore, the *n*-6/*n*-3 PUFA ratio is considered a critical indicator of dietary fat quality and is recommended to be below 4 (Simopoulos 2008). FS represents a particularly rich source of ALA (Shim et al. 2015). In addition, HS contains considerable amounts of tocopherols (Farinon et al. 2020), which are commonly used in laying hen nutrition to improve the oxidative stability of *n*-3 PUFA-enriched eggs (Ren et al. 2013). Therefore, the present study aimed to evaluate the effects of dietary supplementation with HS from the non-psychoactive variety Futura, non-extruded FS from the variety Floral, and their combination on laying hen performance, egg quality traits, yolk nutritional composition, and oxidative stability.

MATERIAL AND METHODS

Experimental Design and Dietary Treatments

The experiment was performed with a total of 240 Lohmann Brown laying hens aged 50 weeks. The laying hens were housed in three-tier enriched cages within the same air-conditioned facility and randomly allocated to 4 dietary treatments, with 6 replicate cages and 10 laying hens per cage. The 2×2 factorial experiment included two levels of non-psychoactive HS (Futura variety; 0 and 40 g/kg) and two levels of non-extruded FS (Floral variety; 0 and 40 g/kg) in the diet. The first (I) control diet consisted of basal wheat–maize–soybean meal mixed feed without HS and FS. The second (II) and third (III) experimental groups of laying hens were fed diets supplemented with 40 g/kg FS and 40 g/kg HS, respectively. The laying hens from the fourth (IV) experimental group received a diet containing a combination of the above seeds, specifically 40 g/kg HS and 40 g/kg FS. Apart from the respective HS and FS supplementation, the experimental diets for laying hens were based on wheat, maize, and soybean meal as the main energy and protein sources. The diets also contained soybean oil, mineral supplements, synthetic amino acids, and a vitamin-mineral premix. Each enriched cage had an area of 7560 cm². The stocking density, cage equipment (i.e., feeders, drinkers, nests, and perches), lighting, ventilation, and vaccination complied with the requirements of the European Council Directive 1999/74/EC. The technological and microclimatic housing conditions complied with the standard procedure for the Lohmann Brown hybrid, with an average temperature of 20–22 °C and humidity between 50 and 60 %. The light cycle was 14 hours of light and 10 hours of darkness, with a light intensity of approximately 10 lx at the middle tier of the three-tier enriched cage system. Feed and fresh water complying with hygiene standards were supplied *ad libitum*. The experiment lasted for 12 weeks, including a two-week preparation period. The health status of the laying hens and the number of eggs laid were monitored daily. The hen-day egg production (%) and feed conversion ratio (FCR; kg/kg) were calculated weekly for each cage and group. Egg weights (g) were recorded three times per week for all eggs in each replicate. The eggs used for chemical analyses of the yolk were collected at the end of the experiment. Three eggs from each replicate were homogenized to form one sample ($n = 6$).

Physical Analysis of Eggs

For the analysis of physical parameters, eggs from a whole day of production were collected from laying hens at 61 weeks of age. A total of 219 eggs were analyzed. The values were averaged per cage ($n = 6$). The color of the yolk was determined using a DSM yolk color fan (DSM Nutritional Products, Basel, Switzerland) with a scale ranging from 1 to 15. The percentage of shell was determined on the basis of the individual weight of each egg (g) and the corresponding weight of its shell (g). All eggs and their shells were weighed using a digital scale (KERN & SOHN GmbH, Balingen, Germany) with an accuracy of 0.1 g.

Chemical Analyses of Egg Yolks

The concentrations of α -tocopherol, γ -tocopherol, and β -carotene (mg/kg dry matter; DM) in the lyophilized egg yolks ($n = 6$) were determined using a Shimadzu high-performance liquid chromatography system (VP series; Shimadzu, Kyoto, Japan) equipped with a diode array detector (HPLC-DAD), according to the European Standards EN 12822-1 and EN 12823-2. The FA profiles (mg/100 g fresh weight) of the egg yolks ($n = 6$) were determined following chloroform-methanol extraction of total lipids according to Folch et al. (1957). The FAs were converted to methyl esters (FAMES) by alkaline trans-methylation. The resulting FAMES were analyzed by gas chromatography using an HP 6890 gas chromatograph (Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with a flame ionization detector (GC-FID). The cholesterol concentration (g/kg egg yolk) in the egg yolks ($n = 6$) was determined following lipid saponification and extraction of the unsaponifiable fraction with diethyl ether according to ISO 3596:2011. The cholesterol was derivatized to silyl derivatives using trimethylchlorosilane (TMCS) and hexamethyldisilazane (HMDS) silylation reagents (Sigma-Aldrich, Prague, Czech Republic) and subsequently analyzed by GC-FID (Agilent Technologies, Inc., Santa Clara, CA, USA). Lipid peroxidation in the egg yolks ($n = 6$) from fresh eggs and eggs stored for 28 days at 18 °C and 50–55% relative humidity on paper trays was determined using a modified method based on Czauderna et al. (2011). The oxidative stability of the egg yolk lipids in both fresh and stored eggs was expressed as malondialdehyde (MDA) concentration (mg/kg fresh egg yolk), as determined by HPLC-DAD analysis (VP series; Shimadzu, Kyoto, Japan).

Calculations of Nutritional Indices

On the basis of the FA profiles determined, nutritional indices (i.e., index of atherogenicity – IA and index of thrombogenicity – IT) related to the nutritional value of egg yolk lipids were calculated with the formulas provided below.

The formula for calculating the index of atherogenicity (IA) was as follows:

$$IA = [C12:0 + (4 \times C14:0) + C16:0] / \Sigma \text{UFAs}$$

where lauric acid (C12:0), myristic acid (C14:0), and palmitic acid (C16:0) represent the main classes of saturated fatty acids (SFAs), and Σ UFAs denotes the sum of unsaturated fatty acids (UFAs), including monounsaturated fatty acids (MUFAs) and PUFAs.

The formula for calculating the index of thrombogenicity (IT) was as follows:

$$IT = (C14:0 + C16:0 + C18:0) / (0.5 \times \Sigma \text{MUFAs}) + (0.5 \times \Sigma n-6 \text{ PUFAs}) \\ + (3 \times \Sigma n-3 \text{ PUFAs}) + (n-3 \text{ PUFAs} / n-6 \text{ PUFAs})$$

where myristic acid (C14:0), palmitic acid (C16:0), and stearic acid (C18:0) represent the main classes of SFAs; Σ MUFAs represents the sum of MUFAs; and Σ $n-6$ PUFAs and Σ $n-3$ PUFAs denote the sums of $n-6$ PUFAs and $n-3$ PUFAs, respectively.

Statistical Analysis

The experimental data obtained were analyzed with a two-way analysis of variance (ANOVA) with the general linear model (GLM) procedure in the Statistical Analysis System (SAS 2013). The main effects were the level of HS in the diet, the level of FS in the diet, and the interaction effect between these factors (i.e., HS*FS). Differences between the groups were tested with Duncan's multiple range test. The experimental unit was a cage ($n = 6$). The results in Table 1 are presented as the means (a, b,c, d), the standard error of the mean (SEM), and the probability (p) values. All differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The effects of dietary HS and FS supplementation on laying hen performance, egg quality traits, yolk nutritional composition, and oxidative stability are summarized in Table 1.

Performance Characteristics of Laying Hens

The inclusion of FS in the experimental diets (II and IV) of laying hens significantly increased both hen-day egg production ($p < 0.001$) and egg mass ($p < 0.001$). In contrast, HS supplementation had no significant effect on overall hen-day egg production. Similarly, Neijat et al. (2014) reported no significant differences in the overall performance of laying hens when graded levels of either HS (up to 300 g/kg) or HS oil (up to 90 g/kg) were included in the diet compared with laying hens fed the control diet. However, in the present study, the addition of HS to the diets (III and IV) resulted in a higher total egg mass ($p < 0.001$). In addition, laying hens fed the FS-enriched diet exhibited a lower FCR (II; 2.09 kg/kg) compared with those fed the combination of HS and FS (IV; 2.14 kg/kg) or the control diet (I; 2.17 kg/kg). FS is rich in lipids and represents a valuable source of n -3 PUFAs, particularly ALA (Shim et al. 2015), thereby increasing the energy density of the feed. This may reduce feed intake while maintaining production performance, consequently improving FCR. However, published findings regarding the effects of FS supplementation on the performance characteristics of laying hens remain inconsistent. Such inconsistency has also been reported by Imran et al. (2015).

Physical Egg Quality Characteristics

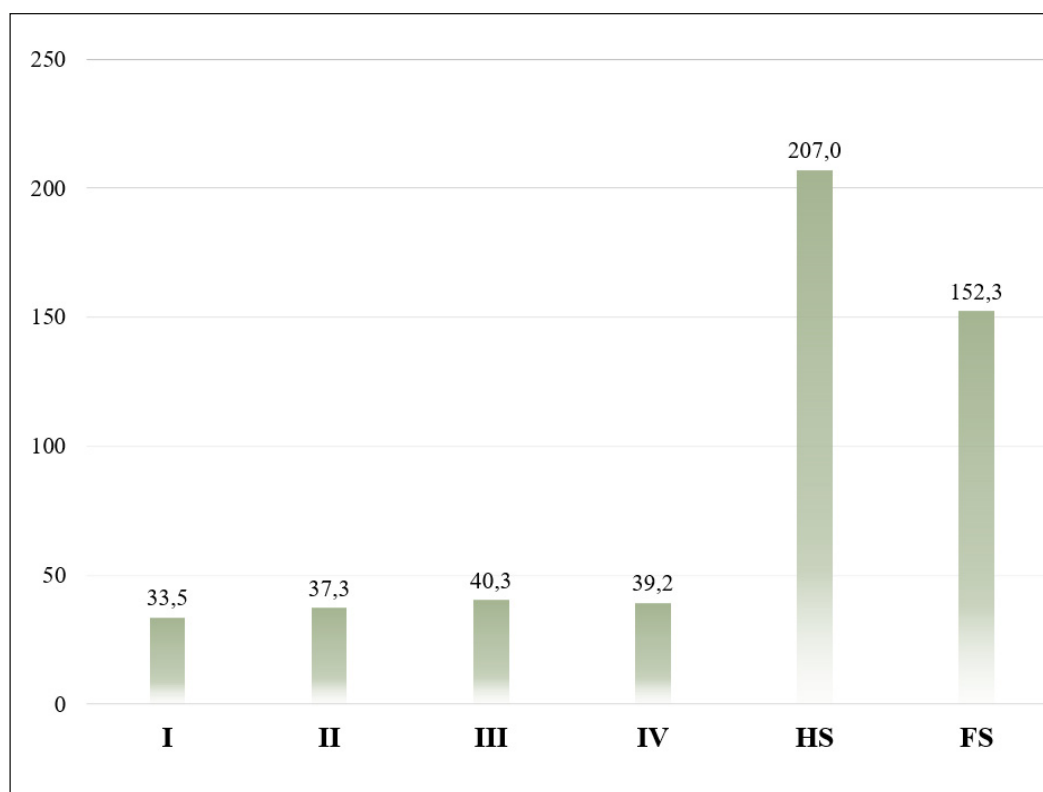
The mixed feed containing both HS and FS (IV) significantly increased egg weight (HS*FS; $p = 0.002$) by almost 2% compared with the control group (I). The highest egg weight was observed in laying hens fed the diet with HS supplementation alone (III; 62.4 g), followed by the combination of HS and FS (IV; 62.3 g) and FS supplementation alone (II; 61.7 g), while the lowest value was recorded in the control group (I; 60.5 g). Similarly, previous studies have reported a positive effect of HS supplementation on egg mass or egg weight (Halle and Schöne 2013; Skřivan et al. 2019). Neither HS nor FS supplementation significantly affected yolk color. A significant HS*FS interaction ($p = 0.014$) was observed. Numerically,

slightly higher yolk color values were found in all seed-supplemented groups (II, III, and IV) compared with the control group (I). The yolk color intensity is primarily determined by the presence of carotenoids, which represent one of the most widespread groups of natural lipophilic pigments with a wide range of bioactive and health-promoting properties (Szmek et al. 2026). Because laying hens are incapable of synthesizing these pigments *in vivo*, they must be entirely supplied through the diet. There are various natural dietary sources of carotenoids. For example, Halle and Schöne (2013) reported that FS meal increased yolk color more markedly than rapeseed meal or HS meal did. Furthermore, in the present study, the combined HS and FS supplementation in the diet (IV) resulted in a significantly higher shell percentage (HS*FS; $p = 0.036$) than that in group III, which received HS alone.

Tocopherol and β -carotene Concentrations in Egg Yolks

While egg yolks are a valuable source of α -tocopherol, the content of γ -tocopherol is negligible. In the present study, the inclusion of HS in the experimental diets (III and IV) of laying hens resulted in elevated levels of γ -tocopherol in egg yolks ($p < 0.001$), with the highest value (62.1 mg/kg DM) observed in group IV, which received the combined HS and FS supplementation. The addition of HS alone in the experimental diet (III) resulted in a γ -tocopherol content of 61.5 mg/kg DM in egg yolks. In contrast, Skřivan et al. (2019) reported that a considerably higher dietary inclusion level of HS (90 g/kg) resulted in a lower γ -tocopherol content in egg yolks (43.3 mg/kg DM). According to Wang et al. (2010), the main factors influencing yolk tocopherol content include nutrition, age, and laying hen genotype. Although rapeseed oil was used in the study by Skřivan et al. (2019), soybean oil served as the primary dietary fat source in our experimental diets. Although soybean oil naturally contains γ -tocopherol and therefore contributed to its overall concentration in the feed, our results suggest that the majority of this compound originated directly from the supplemented HS. According to our HPLC-DAD analysis (Graph 1), HS contained a 1.4-fold higher concentration of γ -tocopherol than FS did (207.0 mg/kg fresh weight vs. 152.3 mg/kg fresh weight, respectively). According to Kriese et al. (2004), γ -tocopherol is the predominant tocopherol isomer in HS. Furthermore, Skřivan et al. (2019) observed that increasing the HS concentration in the diets of laying hens led to elevated concentrations of both α -tocopherol and γ -tocopherol in egg yolks. In the present study, no significant effects of dietary treatments were observed on α -tocopherol concentration. Tocopherols are well known for their strong antioxidant properties, which may contribute to increasing the antioxidant potential and oxidative stability of eggs. Among all tocopherol isomers, α -tocopherol exhibits the highest antioxidant activity and represents the most bioavailable form (Es-Sai et al. 2025) with vitamin E activity in both animals and humans. Unlike other tocopherol isomers, γ -tocopherol effectively neutralizes reactive oxygen species (ROS) and reactive nitrogen species (RNS), thereby providing robust cellular protection against oxidative

damage and lipid peroxidation (Es-Sai et al. 2025). In addition to its strong antioxidant properties, particularly in lipophilic matrices (Porto et al. 2014), γ -tocopherol is also characterized by anti-inflammatory activity through the modulation of pathways involving cyclooxygenase-2 (COX-2) and tumor necrosis factor- α (TNF- α), thereby reducing chronic inflammation and associated health risks (Es-Sai et al. 2025). Finally, a significant HS*FS interaction ($p = 0.029$) was detected for the β -carotene content of egg yolks. The control group (I) showed the highest β -carotene level (0.387 mg/kg DM), whereas the lowest value (0.312 mg/kg DM) was recorded in group III, which received HS alone. The elevated γ -tocopherol concentration observed in egg yolks from laying hens fed the HS-enriched diets (III and IV) may be associated with the antioxidant properties of carotenoids, which can protect this lipophilic compound against oxidative degradation in the upper gastrointestinal tract (Mortensen et al. 2001). This may explain the lowest β -carotene concentration recorded in group III, suggesting that a greater proportion of this carotenoid was utilized for antioxidant protection.



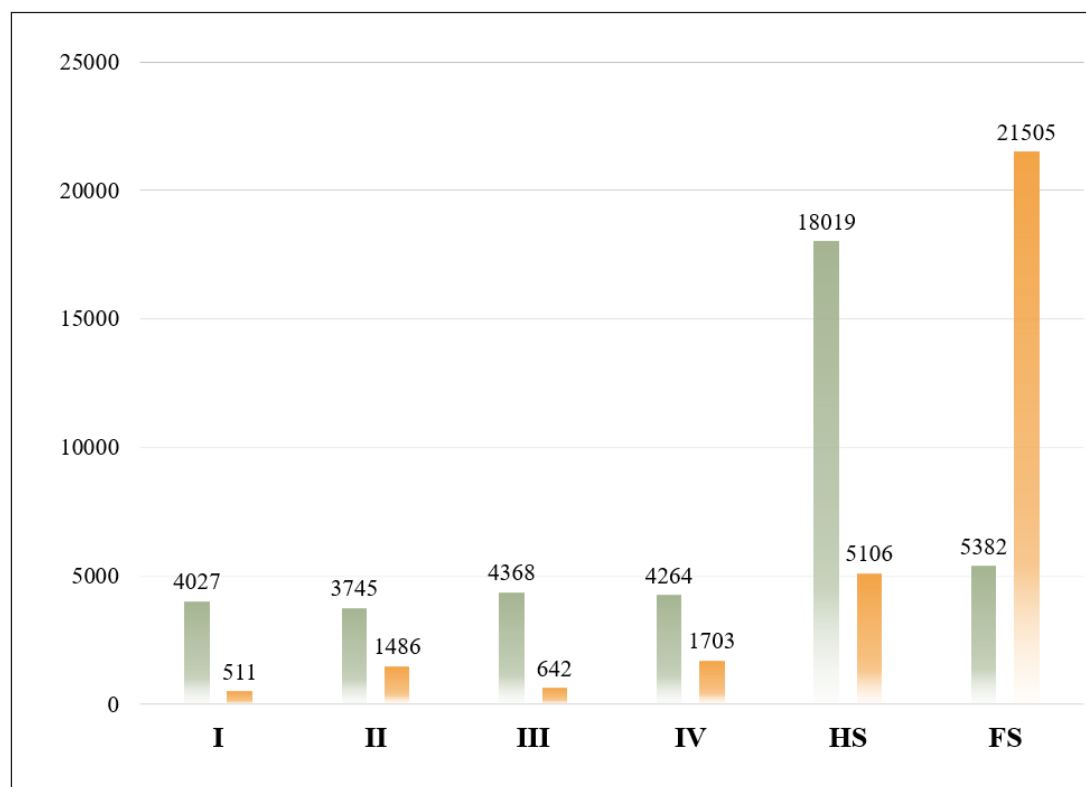
Graph 1: Concentrations of γ -tocopherol (mg/kg fresh weight) in the experimental diets (I, II, III, and IV), HS, and FS

Fatty Acid Composition and Cholesterol Concentrations in Egg Yolks

From a nutritional perspective, pronounced beneficial changes were observed in the lipid profile of egg yolks. Both the individual seed supplements (HS and FS) and, in particular, their combination (HS*FS) increased the content of n -6 ($p = 0.022$) and n -3 ($p < 0.001$) PUFAs. The highest total PUFA content in group IV, receiving combined HS and FS supplementation, was mainly driven by a marked

increase in ALA, with the highest level observed in group IV (866 mg/100 g), reflecting the strong *n*-3 PUFA enrichment effect of FS ($p < 0.001$). According to our GC-FID analysis (Graph 2), FS contained a 4.2-fold higher concentration of *n*-3 PUFAs than HS did (21505 mg/100 g fresh weight vs. 5106 mg/100 g fresh weight, respectively). ALA, the principal *n*-3 PUFA, is an essential nutrient for both birds and mammals, as it cannot be synthesized *de novo* and must be supplied through the diet. Through desaturation and elongation pathways, ALA serves as a precursor for long-chain FAs such as EPA and DHA, which are important for multiple physiological processes and overall health in both animals and humans (Jing et al. 2017; Farinon et al. 2020). The enrichment of eggs with *n*-3 PUFAs through dietary FS and HS supplementation has been widely documented in poultry. FS (Cherian and Quezada 2016) and HS (Rbah et al. 2025) have both been shown to increase ALA deposition in egg yolks. Moreover, Omri et al. (2019) demonstrated that dietary inclusion of FS at 45 g/kg altered yolk FA composition and increased EPA and DHA levels. In agreement with these findings, the present experiment showed that combined HS and FS supplementation in the diet (IV) of laying hens markedly enhanced the deposition of *n*-3 PUFAs in egg yolks, with ALA, EPA, and DHA levels being 2.2-, 1.8-, and 1.6-fold higher than in the control group (I), respectively. The conversion of ALA to EPA and DHA in both animals and humans depends on a balanced *n*-6/*n*-3 PUFA ratio; otherwise, metabolism is shifted toward arachidonic acid (AA, C20:4, *n*-6) synthesis from linolenic acid (LA, C18:2, *n*-6). In general, lipid mediators derived from *n*-3 PUFAs tend to be less pro-inflammatory and more pro-resolving, whereas those originating from *n*-6 PUFAs are typically more pro-inflammatory (de Batlle et al. 2012). Therefore, beyond absolute PUFA levels, the *n*-6/*n*-3 PUFA ratio is a key nutritional indicator associated with the prevention of chronic degenerative diseases (Simopoulos 2008). In the present study, the *n*-6/*n*-3 PUFA ratio in egg yolks decreased from 8.31 (I) to 5.44 (IV). This result was significantly affected by both HS ($p < 0.001$) and FS ($p < 0.001$) supplementation. In previous studies, similar reductions have been reported for both HS- and FS-based dietary interventions. For example, Taaifi et al. (2023) reported a decrease in the *n*-6/*n*-3 PUFA ratio from 8.19 in the control group to 4.89 in laying hens fed 300 g/kg HS by day 84. Consistently, numerous studies have shown that FS and HS supplementation improve yolk lipid profiles and enhance *n*-3 PUFA enrichment in line with current nutritional recommendations.

All dietary treatments also affected nutritional lipid quality indices, namely the IA and IT ($p < 0.001$). These indices are widely used indicators for evaluating the nutritional quality of lipids, including egg yolk lipids, in relation to human health. The IA reflects the atherogenic potential of FAs, indicating their capacity to promote the formation of atherosclerotic plaques in arteries (Ulbricht and Southgate 1991). In contrast, the IT characterizes the thrombogenic potential of FAs, reflecting the tendency to promote clot formation in blood vessels and the balance between pro-thrombogenic FAs (mainly SFAs) and anti-thrombogenic FAs (mainly MUFAs and PUFAs; Ulbricht and Southgate 1991). Consumption of foods



Graph 2: Concentrations of *n*-6 and *n*-3 PUFAs (mg/100 g fresh weight) in the experimental diets (I, II, III, and IV), HS, and FS

with lower IA and IT values has been associated with reduced total and low-density lipoprotein (LDL) cholesterol concentrations in human blood plasma, thereby contributing to improved cardiovascular health (Chen and Liu 2020). Although the IA and IT values in all experimental groups remained below the recommended thresholds (0.5 and 1.0, respectively), their further reduction ($p < 0.001$) in the FS-supplemented groups (II and IV) indicates improved nutritional quality of egg yolk lipids. The lowest IA (0.321) and IT (0.632) values observed in group IV may suggest a synergistic effect of combined HS and FS supplementation (HS*FS; $p < 0.001$). In agreement with our findings, Omri et al. (2019) reported that FS-supplemented diets significantly reduced the IT value. Similarly, Mierliță (2019) observed that dietary inclusion of HS and HS meal decreased the IT value in egg yolks, although no significant effect on IA was detected.

Finally, HS supplementation significantly reduced the total cholesterol content in egg yolks ($p = 0.042$). This finding is consistent with the study by Skřivan et al. (2019), who reported a reduction in egg yolk cholesterol from 12.2 g/kg in the control group to 10.6 g/kg in laying hens fed a diet containing 90 g/kg HS. According to Rbah et al. (2025), the hypocholesterolemic effect of HS is likely associated with several components of its chemical composition, particularly phytosterols (e.g., β -sitosterol), proteins, and insoluble fiber.

Oxidative Stability of Egg Yolks

Lipid peroxidation, a process compromising the nutritional and sensory quality of eggs, was evaluated by determining MDA concentrations in egg yolks. MDA, one of the major oxidation products of PUFAs, is widely recognized as a reliable marker of lipid peroxidation in food products. In fresh eggs, HS supplementation significantly reduced MDA levels in egg yolks ($p = 0.003$), indicating improved oxidative stability of yolk lipids. A similar effect was reported by Mierliță (2019), who observed the lowest MDA concentrations in egg yolks from laying hens fed HS (0.22 mg/kg in fresh eggs and 0.35 mg/kg in eggs after 30 days of storage). According to the author, the unexpectedly low degree of lipid peroxidation despite the elevated n -3 PUFA content may be associated with the relatively high concentration of tocopherols, particularly α -tocopherol, in egg yolks. A similar explanation may also apply to our findings, although HS supplementation in the present study specifically resulted in a marked increase in γ -tocopherol concentration in egg yolks. This tocopherol isomer, the predominant form of vitamin E in HS (Kriese et al. 2004), is considered a highly potent antioxidant in lipophilic matrices (Porto et al. 2014). It may effectively protect n -3 PUFAs against peroxidation, thereby contributing to enhanced oxidative stability of the produced eggs (Es-Sai et al. 2025).

Tab. 55: Effects of dietary HS and FS supplementation on laying hen performance, egg quality traits, yolk nutritional composition, and oxidative stability (n = 6)

Group (diet)	I	II	III	IV	SEM	Probability		
Hemp seed (%)	0	0	4	4		HS	FS	HS*FS
Flax seed (%)	0	4	0	4				
Hen-day egg production (%)	94.3	95.7	94.0	96.2	0.24	NS	<0.001	NS
Feed conversion ratio (kg/kg)	2.17 ^a	2.09 ^c	2.12 ^{bc}	2.14 ^{ab}	0.008	NS	NS	<0.001
Egg mass (g/hen/day)	57.1	59.1	58.7	59.9	0.18	<0.001	<0.001	NS
Egg weight (g)	60.5 ^c	61.7 ^b	62.4 ^a	62.3 ^{ab}	0.12	<0.001	0.017	0.002
DSM yolk color fan	13.5 ^b	13.7 ^a	13.6 ^a	13.6 ^{ab}	0.02	NS	NS	0.014
Shell percentage (%)	10.2 ^{ab}	10.2 ^{ab}	10.1 ^b	10.3 ^a	0.03	NS	NS	0.036
α-tocopherol (mg/kg DM)	181	171	183	184	2.7	NS	NS	NS
γ-tocopherol (mg/kg DM)	54.0	49.0	61.5	62.1	1.45	<0.001	NS	NS
β-carotene (mg/kg DM)	0.387 ^a	0.332 ^{ab}	0.312 ^b	0.348 ^{ab}	0.0107	NS	NS	0.029
LA (mg/100 g)	5901 ^c	6435 ^b	6420 ^b	7313 ^a	97.8	<0.001	<0.001	0.023
ALA (mg/100 g)	394 ^d	635 ^b	463 ^c	866 ^a	33.2	<0.001	<0.001	<0.001
EPA (mg/100 g)	6.09 ^c	7.45 ^b	7.61 ^b	11.24 ^a	0.360	<0.001	<0.001	<0.001
DHA (mg/100 g)	330	403	399	516	13.1	<0.001	<0.001	NS
n-6 PUFAs (mg/100 g)	6495 ^c	7004 ^b	7082 ^b	7991 ^a	104.4	<0.001	<0.001	0.022
n-3 PUFAs (mg/100 g)	782 ^d	1114 ^b	943 ^c	1472 ^a	46.8	<0.001	<0.001	<0.001
n-6/n-3 PUFA ratio	8.31	6.29	7.51	5.44	0.201	<0.001	<0.001	NS
Index of atherogenicity	0.362 ^a	0.350 ^b	0.362 ^a	0.321 ^c	0.0033	<0.001	<0.001	<0.001
Index of thrombogenicity	0.792 ^a	0.712 ^b	0.778 ^a	0.632 ^c	0.0117	<0.001	<0.001	<0.001
Cholesterol (g/kg)	12.0	11.2	11.1	11.0	0.15	0.042	NS	NS
MDA – fresh eggs (mg/kg)	0.564	0.512	0.429	0.403	0.0508	0.003	NS	NS
MDA – stored eggs (mg/kg) ¹	0.603	0.530	0.509	0.528	0.0237	NS	NS	NS

SEM = standard error of the mean; HS = hemp seed; FS = flax seed; HS*FS = interaction between hemp seed and flax seed; NS = not significant; ^{a,b,c,d} in the same row indicate significant differences; DM = dry matter; LA = linoleic acid (C18:2, n-6); ALA = α-linolenic acid (C18:3, n-3); EPA = eicosapentaenoic acid (C20:5, n-3); DHA = docosahexaenoic acid (C22:6, n-3); PUFAs = polyunsaturated fatty acids; MDA = malondialdehyde; ¹ indicates the oxidative stability of egg yolks from eggs stored for 28 days.

CONCLUSION

In conclusion, the present study demonstrated that dietary supplementation with HS and FS significantly enhanced the nutritional profile and oxidative stability of eggs. While FS primarily served as a rich source of *n*-3 PUFAs, particularly ALA, and improved laying hen performance, HS supplementation effectively elevated γ -tocopherol concentrations in egg yolks, reduced yolk cholesterol content, and decreased lipid peroxidation. Importantly, the combined supplementation with HS and FS exerted a synergistic effect, characterized by a higher shell percentage and increased deposition of *n*-3 PUFAs, especially ALA and EPA, in egg yolks, resulting in the most favorable *n*-6/*n*-3 PUFA ratio and improved nutritional lipid quality indices. These findings highlight the potential of combined HS and FS supplementation as a novel nutritional strategy for producing eggs enriched with health-promoting bioactive compounds and improved lipid quality, thereby supporting the development of value-added poultry products.

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BLOOD ANTIOXIDANT STATUS IN COCCIDIOSIS-INFECTED BROILER CHICKENS FED ALLIUM SPP.-BASED SUPPLEMENTS

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ABSTRACT

This study aimed to evaluate the dietary effect of dried onion (*Allium cepa*) and Garlicon, a product rich in propylpropanthiosulfinate (PTS) and propylpropanthiosulfonate (PTSO), on antioxidant indicators in the blood plasma of broiler chickens challenged to *Eimeria* spp. infection. Chickens were divided into four dietary groups with specific treatment (CP, control positive group receiving a control diet with synthetic coccidiostat; CN, control negative group receiving a control diet without supplements; ON1, experimental group receiving a diet supplemented with 7,5 g/kg onion meal; ON2, experimental group receiving a diet supplemented with 30 mg/kg Garlicon). At 21 and 42 days of age, no statistically significant differences were found in catalase and glutathion-S-transferase activity, or malondialdehyde level, indicating no significant effect on lipid peroxidation in blood plasma. A significant increase in glutathione peroxidase (GPx) activity was observed at 21 days of age in the ON1 group (1329.97 ± 62.217 nmol NADPH/min/ml; $P < 0.01$), and in ON2 group (1225.12 ± 96.029 nmol NADPH/min/ml; $P < 0.05$) compared with the CP group (915.42 ± 86.754 nmol NADPH/min/ml). At 42 days, GPx activity remained significantly higher in the ON1 group (1485.07 ± 78.595 nmol NADPH/min/ml; $P < 0.05$) while a highly significant increase was also detected in the CN group (1681.29 ± 58.741 nmol NADPH/min/ml; $P < 0.01$) compared with the CP group (1120.80 ± 102.672 nmol NADPH/min/ml). The results suggest that supplementation with dried onion meal, and Garlicon did not reduce lipid peroxidation in plasma, but it positively affected antioxidant enzyme activity, especially GPx. Further studies dealing the effect of *Allium* spp.-based phytogetic additives on chickens' health and metabolism are needed to evaluate with regards to growth rate dynamics.

Keywords: antioxidants; onion; broilers; glutathione peroxidase



INTRODUCTION

Oxidative stress affects the overall health of chickens in modern production systems. It arises from an imbalance between the formation of reactive oxygen species and the antioxidant defense mechanisms of the organism. In broiler chickens, it can lead to negative effects on growth, health status, immunity, and overall performance. Impaired antioxidant protection further increases their susceptibility to infections (Oke et al., 2024). A current trend in this area of research is the effort to identify suitable alternatives to synthetic additives that support improved performance and health status of broiler chickens, in order to reduce environmental impact caused by residues and to provide potential benefits for consumer health (Adeyemi et al., 2021; Šimek et al., 2026; Zapletal et al., 2026). In this regard, plants of the genus *Allium* are promising, as they are an important source of organosulfur compounds with proven antioxidant, antimicrobial, and immunomodulatory effects, and they are also a source of polyphenols, especially the flavonoid quercetin, a strong antioxidant that is present at higher levels in onion (*Allium cepa*) (Marefati et al., 2021; Cascajosa-Lira et al., 2024; Aditya et al., 2017). Phytochemical compounds derived from these plant species, particularly propyl thiosulfinates (PTS) and propyl thiosulfonates (PTSO), derivatives of propiin, also appear to be highly effective. These compounds are stable, which makes them suitable for use in feed, and they have been shown to possess marked antimicrobial activity (Aguinaga-Casañas et al., 2022; Cabello-Gómez et al., 2022; Cascajosa-Lira et al., 2024). The aim of this study was to compare the effects of dried onion, and Garlicon product, which contains high levels of PTS and PTSO, and to demonstrate their impact on reducing lipid peroxidation and on modulating the activity of antioxidant enzymes in blood plasma.

MATERIAL AND METHODS

The feeding trial (a total of 192 ROSS 308 hybrid chickens) was performed in the approved experimental facility of Department of Animal Breeding, Animal Nutrition and Biochemistry, Faculty of Veterinary Hygiene and Ecology, University of Veterinary Sciences Brno, Czech Republic. Male chicks were divided into four dietary groups (CP, control positive group receiving a control diet with synthetic coccidiostat; CN, control negative group receiving a control diet without synthetic coccidiostat; ON1, experimental group receiving a diet supplemented with 7,5 g/kg onion meal; ON2, experimental group receiving a diet supplemented with 30 mg/kg Garlicon) were used in this study. Garlicon product was provided by DOMCA SAU (Granada, Spain). A two-phase feeding programme was used, with grower and finisher diets provided across all four dietary treatments. With respect to other health evaluation, all chickens were challenged to *Eimeria* spp. Chickens were housed in floor litter pens according to management handbook for ROSS 308 (Aviagen 2025).

At each sampling time (21 and 42 days of age), 32 male broiler chickens ($n = 8$ per dietary group) were randomly chosen for blood sampling. After recording the live weight of the birds, blood samples were obtained via puncture of the *v. basilica* using an aspiration syringe with sodium heparin as an anticoagulant. Each blood sample was centrifuged at 3000 rpm for 10 min at 20 °C to obtain plasma. Then samples were stored at -80 °C until further biochemical and oxidative stress analyses.

Plasma total protein (TP) was measured using an automated analyser (Indiko, ThermoFisher Scientific, USA) with commercially available kit (DiaSys Diagnostic Systems GmbH, Germany). The plasma sample was used to determine catalase (CAT), glutathione peroxidase (GPx), and glutathione S-transferase (GST) activities. MDA, GST, and GPx were measured using a Varioskan Flash multimode reader (Thermo Fisher Scientific, Brno), while CAT activity was measured using a GENESYS 150 UV-visible spectrophotometer. The parameters were measured according to the methodology previously described in the study by Blahová et al. (2013).

Statistic analysis was performed using STATISTICA CZ version 10. After Shapiro-Willk W test, and K-S & Lilliefors test, one-way ANOVA was used. When ANOVA showed significant effect, Tukey *post-hoc* test was used. Significance was considered at $P < 0.05$ (*), and $P < 0.01$ (**).

RESULTS AND DISCUSSION

Tab. 56: Selected antioxidative blood plasma indicators in ROSS chickens with regard to dietary treatment.

Indicators	Dietary groups				P
	CP	CN	ON1	ON2	
	x SEM	x SEM	x SEM	x SEM	
Age 21 days					
LW (g)	701.00 ± 19.168	726.50 ± 28.359	705.00 ± 11.220	733.38 ± 32.734	n.s.
TP (g/L)	20.14 ± 0.680	21.80 ± 1.079	23.54 ± 0.780	23.03 ± 2.661	n.s.
CAT (μmol/min/mL)	15.73 ± 0.764	15.16 ± 2.351	12.32 ± 0.785	13.69 ± 2.012	n.s.
MDA (μmol/L)	0.35 ± 0.046	0.33 ± 0.031	0.37 ± 0.020	0.33 ± 0.052	n.s.
GST (μmol/min/L)	23.34 ± 1.867	24.55 ± 1.604	29.04 ± 1.248	23.38 ± 2.221	n.s.
GPx (nmol NADPH/min/mL)	915.42 ^{Aa} ± 86.754	1122.76 ± 40.905	1329.97 ^B ± 62.217	1225.12 ^b ± 96.029	**
Age 42 days					
LW (g)	2454.75 ^a ± 47.298	2743.25 ^b ± 94.166	2476.00 ^a ± 23.337	2579.75 ± 39.964	*
TP (g/L)	25.21 ± 4.345	27.22 ± 1.863	25.60 ± 2.797	25.77 ± 3.687	n.s.
CAT (μmol/min/mL)	78.94 ± 2.263	79.56 ± 2.251	87.54 ± 2.356	85.75 ± 3.128	n.s.
MDA (μmol/L)	0.26 ± 0.014	0.24 ± 0.034	0.26 ± 0.031	0.24 ± 0.021	n.s.
GST (μmol/min/L)	30.90 ± 2.490	32.89 ± 1.048	29.40 ± 2.046	34.66 ± 1.740	n.s.
GPx (nmol NADPH/min/mL)	1120.80 ^{Aa} ± 102.672	1681.29 ^B ± 58.741	1485.07 ^b ± 78.595	1336.10 ± 107.325	**

CP, control positive; CN, control negative; ON1, experimental group fed onion meal supplementation; ON2, experimental group fed Garlicon supplementation; LW, live weight; TP, total protein; CAT, catalase; MDA, malondialdehyde; GST, glutathion-S-transferase; GPx, glutathion peroxidase; ns, not significant ($P > 0.05$); *, means within row differs significantly at level $P < 0.05$; **, means within row differs significantly at level $P < 0.01$.

As shown in Table 1, no statistically significant effects were observed at either 21 or 42 days of age on CAT and GST activity or on MDA levels as an indicator of ongoing lipid peroxidation. Total plasma protein was also not significantly affected, and the values remained within the physiological range for broiler chickens reported by Zálešáková et al. (2025). Besides that, in 21-day-old chickens, the chosen dietary phytogenic additives did not significantly affect body weight ($P > 0.05$). A statistically highly significant increase in GPx activity, an important antioxidant enzyme, was found in group ON1 as compared to CP group (+414.55 nmol NADPH/min/ml, $P < 0.01$), and compared to ON2, (+309.70 NADPH/min/ml, $P < 0.05$). In 42-day-old chickens, we also found a highly significant increase in GPx activity

in group CN with comparison to CP (+560.49 nmol NADPH/min/ml, $P < 0.01$). Concerning the experimental groups, chickens receiving a diet supplemented with onion meal at rate of 7.5 g/kg of diet (group ON1) reached significantly higher GPx activity as compared to control positive group (+364.27 nmol NADPH/min/ml, $P < 0.05$). The highest final LW was found in chickens of CN groups as compared to CP and ON1 (+288.50 g and +267.25 g, resp., $P < 0.05$) groups. In neither sampling period, similarly to the study by Vossen et al. (2011), were we able to demonstrate the expected effect of the selected phytogetic additives on reducing lipid peroxidation; instead, only the effect of dried onion and Garlicon on the antioxidant enzyme GPx was observed. Our findings are consistent with study of Adeyemi et al. (2021), who reported increased antioxidant enzyme activity in blood plasma and tissues in broiler ROSS 308 chickens supplemented with onion skin waste. The antioxidant effect of PTSO in chickens has not been sufficiently studied, although its antimicrobial effect has been demonstrated to a greater extent (Peinado et al., 2012; Aguinaga-Casañas et al., 2022; Cabello-Gómez et al., 2022) but our findings demonstrate its effect on increasing antioxidant enzyme activity, especially in younger 21-day old chickens. Aditya et al. (2017), in a study in which they supplemented broiler chickens with onion extract, showed that dietary supplementation affected lipid peroxidation in breast muscle but they did not assess antioxidant indicators in blood plasma. The effect of feed supplementation with onion and garlic, as principal representatives of the genus *Allium*, on antioxidant enzymes was also demonstrated by Sallam et al. (2024), who added onion and garlic juice to the drinking water of broiler chickens; in their study, a statistically significant increase in CAT, GST, and superoxide dismutase was observed in homogenized liver tissue, where the activity of these enzymes is higher than in blood plasma.

CONCLUSION

Supplementation of broiler chicken feed mixtures with dried onion meal, and Garlicon did not reduce lipid peroxidation in blood plasma of chickens challenged with *Eimeria* spp. The main finding of the study was GPx increase in with the strongest effect observed for dried onion. Further studies dealing the effect of *Allium* spp.-based phytogetic additives on chickens' health and metabolism are needed to evaluate with regards to growth rate.

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BLACK SOLDIER FLY FAT AS AN ALTERNATIVE LIPID SOURCE IN ANIMAL NUTRITION: COMPARATIVE FATTY ACID PROFILING AGAINST CONVENTIONAL FEED OILS AND FATS

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ABSTRACT

Black soldier fly (*Hermetia illucens*) fat has recently gained attention as a potential alternative lipid source in animal nutrition because of its distinctive fatty acid profile and sustainable production potential. The present study compared the fatty acid composition and selected lipid quality indices of black soldier fly (BSF) fat with representative conventional feed and edible lipid sources, including soybean oil, rapeseed oil, coconut oil, palm oil, chicken fat, pig fat/lard, and marine and freshwater fish-derived lipids. Four BSF fat samples were analysed using gas chromatography with flame ionisation detection (GC-FID), while comparative literature-derived datasets were harmonised for calculation of the atherogenicity index (AI), thrombogenicity index (TI), and hypocholesterolemic/hypercholesterolemic ratio (h/H). BSF fat was characterised by elevated proportions of saturated fatty acids, particularly lauric acid (C12:0), while still retaining moderate levels of monounsaturated and polyunsaturated fatty acids. Among the evaluated lipid sources, BSF fat showed the greatest similarity to coconut oil with respect to its medium-chain saturated fatty acid profile; however, its AI and TI values remained lower because of the higher proportion of unsaturated fatty acids. In contrast, soybean and rapeseed oils exhibited the most favourable lipid quality indices, whereas marine and freshwater fish-derived lipids were distinguished by higher polyunsaturated fatty acid content and lower n-6/n-3 ratios. Overall, the obtained results indicate that BSF fat occupies a unique compositional position among the evaluated lipid sources. Although its fatty acid profile is most similar to coconut oil because of the high proportion of saturated fatty acids, its lipid quality indices suggest an intermediate nutritional position between highly saturated tropical oils and more unsaturated lipid sources. These findings further support the growing interest in BSF-derived lipids as nutritionally relevant alternative lipid sources.



Keywords: *Hermetia illucens*; insect-derived lipids; fatty acid profiling; lipid quality indices; alternative feed fats; animal nutrition; gas chromatography

INTRODUCTION

Black soldier fly (*Hermetia illucens*) larvae have attracted increasing attention as a sustainable nutrient source for livestock production systems due to their efficient biomass conversion, ability to utilise diverse organic substrates, and favourable protein and lipid composition (Lu et al., 2022). In recent years, interest in black soldier fly-derived lipids has expanded considerably, particularly in relation to their possible use as alternative fat sources in animal feeding (Nekrasov et al., 2022; Quigley et al., 2025). Unlike many conventional vegetable oils, BSF fat is typically rich in medium-chain saturated fatty acids, especially lauric acid (C12:0), which is considered one of the characteristic features of insect lipid fraction (Suryati et al., 2023; Srisuksai et al., 2024). Owing to this distinct fatty acid profile, BSF lipids are increasingly being evaluated not only as energy sources, but also as nutritionally and functionally relevant feed ingredients with potential effects on gut health, lipid metabolism, and feed utilisation efficiency (Kim et al., 2020; Khan et al., 2024).

The nutritional and functional properties of dietary fats are strongly influenced by fatty acid composition, particularly by the relative proportions of saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA). Differences in fatty acid distribution can substantially affect oxidative stability, physicochemical behaviour, digestibility, and overall nutritional evaluation of lipids used in feed and food systems (Maheshwari et al., 2025). In black soldier fly-derived lipids, fatty acid composition may additionally vary according to substrate composition, larval developmental stage, environmental conditions, and processing methodology, resulting in considerable heterogeneity among BSF lipid products (Ijdema et al., 2025; Srisuksai et al., 2024). Accurate compositional characterisation therefore represents an important prerequisite for evaluating the practical suitability of BSF-derived fats in animal nutrition. Conventional fat sources used in feed formulations differ markedly in both fatty acid distribution and functional characteristics. Vegetable oils such as soybean and rapeseed oil generally contain higher proportions of unsaturated fatty acids, whereas tropical oils including coconut and palm oil are characterised by substantially greater levels of saturated fatty acids (Kostik et al., 2013; Sabahannur and Alimuddin, 2022). Animal-derived fats and fish lipids further expand this compositional diversity because of differences in oleic acid content, long-chain polyunsaturated fatty acids, and n-6/n-3 fatty acid balance (Özogul et al., 2007; Zhang et al., 2023). Chicken fat, lard, and fish-derived lipids are also commonly used in feed and food systems and represent useful comparative reference materials when evaluating alternative fat sources (Marikkar et al., 2021; Dongmo et al., 2024). Taken together, these lipid

categories provide a broad compositional framework for assessing the relative positioning of BSF-derived fat among conventionally used dietary lipids.

In recent years, several lipid quality indices, including the atherogenicity index (AI), thrombogenicity index (TI), and hypocholesterolemic/hypercholesterolemic ratio (h/H), have increasingly been applied as complementary tools for comparative evaluation of fatty acid quality in edible oils, animal fats, fish lipids, and insect-derived lipid materials (Ulbricht and Southgate, 1991; Santos-Silva et al., 2002; Chen et al., 2020). These indices provide additional insight into the balance between saturated and unsaturated fatty acids and allow broader compositional interpretation beyond individual fatty acid proportions alone. Comparative application of such indices has become increasingly common in studies focused on both conventional and emerging lipid sources (Dongmo et al., 2024; Domiszewski et al., 2026).

Despite growing interest in black soldier fly larvae oils and lipid fractions, their compositional positioning relative to conventional feed and edible lipids remains insufficiently characterised, particularly when evaluated using harmonised fatty acid datasets and derived lipid quality indices. Comparative studies integrating plant oils, animal fats, fish-derived lipids, and other insect lipid fractions within a unified analytical framework are still relatively limited. Therefore, the aim of the present study was to characterise the fatty acid composition and selected lipid quality indices of black soldier fly fat and compare them with representative conventional lipid sources used in feed and food systems.

MATERIALS AND METHODS

BLACK SOLDIER FLY FAT SAMPLES

Four black soldier fly (*Hermetia illucens*) fat samples were included in the study. Three samples originated from a commercially obtained black soldier fly fat product that exhibited visible phase separation into two distinct layers. These fractions were analysed separately and designated as BSF_A and BSF_B, while an additional mixed sample (BSF_C) was prepared by combining both fractions. A fourth independent sample (BSF_D) originated from the same supplier but represented a separate batch obtained independently. Each BSF sample was analysed independently, and fatty acid composition was expressed as percentage of total fatty acid methyl esters (%FAME).

EXTRACTION OF FAT AND PREPARATION OF FATTY ACID METHYL-ESTERS

Black soldier fly lipid samples were processed according to modified STN ISO 5509 and STN ISO 5508 procedures for determination of fatty acid methyl esters (FAME) with minor laboratory-specific modifications related to sample preparation and transmethylation conditions. Lipids were obtained by Soxhlet extraction using petroleum ether as a non-polar extraction solvent following sample homogenisation and preparation according to the laboratory protocol for lipid analysis.

Approximately 200 mg of extracted lipid was dissolved in n-hexane and subjected to alkaline transmethylation using methanolic potassium hydroxide solution. The reaction mixture was intensively mixed and briefly incubated at 60 °C to facilitate cleavage of acylglycerols and formation of fatty acid methyl esters. Subsequently, hydrochloric acid solution was added to promote phase separation. After equilibration, the upper hexane layer containing FAME was collected, filtered through anhydrous sodium sulfate, diluted with n-hexane, and used for gas chromatographic analysis.

GAS CHROMATOGRAPHIC ANALYSIS

Fatty acid methyl esters (FAME) were analysed using gas chromatography with flame ionisation detection (GC-FID) on an Agilent 6890 GC system (Agilent Technologies, USA) equipped with a DB-23 capillary column (60 m × 250 µm × 0.15 µm; Agilent Technologies, USA). Helium was used as the carrier gas under a head pressure of approximately 239 kPa corresponding to a flow rate of 2.225 mL/min. The injector temperature was set to 250 °C and the detector temperature to 280 °C. Samples were injected in split mode (1:10) with an injection volume of 1 µL. Separation of FAME was achieved using the following temperature program: initial temperature 50 °C for 1 min, followed by heating at 25 °C/min to 175 °C and subsequently at 2 °C/min to 230 °C.

The flame ionisation detector was operated using hydrogen (35 mL/min), air (350 mL/min), and nitrogen as make-up gas (30 mL/min). Identification of fatty acids was performed by comparison of retention times with a commercial 37-component FAME standard mixture (Supelco, USA) containing C4–C24 fatty acid methyl esters. Fatty acid composition data for all BSF samples were expressed as percentage of total fatty acid methyl esters (%FAME).

LITERATURE-BASED LIPID DATASETS

Comparative lipid datasets representing conventionally used feed and edible fats were constructed from published studies reporting fatty acid methyl ester (FAME) composition determined by gas chromatography with flame ionisation detection (GC-FID). Only studies providing sufficiently detailed fatty acid composition data suitable for calculation of lipid quality indices were included in the comparative analysis.

Plant-derived lipid datasets included soybean oil, rapeseed oil, coconut oil, and palm oil. Soybean and rapeseed oil datasets were derived from the studies of Kostik et al. (2013) and Toishimanov et al. (2024), whereas coconut and palm oil datasets were obtained from Sabahannur and Alimuddin (2022).

Additional comparative datasets representing animal-derived fat sources included chicken fat, pig fat/lard, and fish-derived lipids. Chicken fat composition data were derived from Marikkar et al. (2021). Pig fat/lard datasets were constructed using fatty acid composition data reported by Zhang et al. (2023), where values were calculated as the mean across all adipose tissue depots reported in

the study in order to minimise depot-specific selection bias and better represent generalised pig fat composition. Fish-derived lipid datasets were constructed using the fatty acid composition data reported by Özogul et al. (2007). Marine and freshwater fish species were evaluated separately, generating two distinct comparative datasets representing mean fatty acid composition of marine fish-derived lipids (FISH_A) and freshwater fish-derived lipids (FISH_B), respectively.

Explicitly reported zero values were retained as analytical zeros, whereas fatty acids described as not detected (N/D) were treated as absent for lipid quality index calculations. Only datasets reporting fatty acid composition as relative percentages of total fatty acids (%FAME) were included in order to ensure methodological comparability among lipid sources. Fatty acids required for calculation of lipid quality indices were harmonised across all datasets prior to comparative evaluation. The construction strategy and origin of all comparative lipid datasets are summarised in Table 1.

Tab. 57: *Construction of comparative lipid datasets used for fatty acid composition and lipid quality index evaluation*

Dataset group	Origin/source	Construction strategy
BSF fat samples (BSF_A-D)	Experimental samples	Individually analysed black soldier fly lipid samples
Soybean oil (SOY_A-B)	Kostik et al. (2013); Toishimanov et al. (2024)	Literature-derived fatty acid profiles
Rapeseed oil (RAPESEED_A-B)	Kostik et al. (2013); Toishimanov et al. (2024)	Literature-derived fatty acid profiles
Coconut oil (COCONUT_A)	Sabahannur and Alimuddin (2022)	Literature-derived fatty acid profile
Palm oil (PALM_A)	Sabahannur and Alimuddin (2022)	Literature-derived fatty acid profile
Chicken fat (CHICK_A)	Marikkar et al. (2021)	Literature-derived chicken fat profile
Pig fat/lard (LARD_A)	Zhang et al. (2023)	Mean fatty acid profile calculated across six adipose tissue depots
Marine fish-derived lipids (FISH_A)	Özogul et al. (2007)	Mean fatty acid profile calculated across marine fish species
Freshwater fish-derived lipids (FISH_B)	Özogul et al. (2007)	Mean fatty acid profile calculated across freshwater fish species

Fatty acids not reported in individual studies weren't included unless biologically justified. The atherogenicity index (AI) and thrombogenicity index (TI) were calculated according to Ulbricht and Southgate (1991), whereas the hypocholesterolemic/hypercholesterolemic ratio (h/H) was calculated according to Santos-Silva et al. (2002).

$$AI = \frac{C12:0 + (4 \times C14:0) + C16:0}{\sum MUFA + \sum n-6 + \sum n-3}$$

$$TI = \frac{C14:0 + C16:0 + C18:0}{0.5(\sum MUFA) + 0.5(\sum n-6) + 3(\sum n-3) + \left(\frac{\sum n-3}{\sum n-6} \right)}$$

$$h/H = \frac{C18:1n9 + C18:2n6 + C20:4n6 + C18:3n3 + C20:5n3 + C22:5n3 + C22:6n3}{C14:0 + C16:0}$$

STATISTICAL ANALYSIS

Statistical processing of the data was performed using Microsoft Excel (Microsoft Corp., Redmond, WA, USA). The study was designed as a comparative evaluation of fatty acid composition and derived lipid quality indices across experimentally analysed BSF fat samples and literature-derived lipid datasets. Descriptive statistics, including mean values and standard deviations (SD), were calculated for fatty acid groups (SFA, MUFA, PUFA), n-6/n-3 ratio, and derived lipid quality indices (AI, TI, and h/H). For BSF fat, descriptive statistics were calculated from four independently analysed samples (BSF_A–D). For literature-derived lipid categories, statistics were calculated from the datasets constructed as described in Section 2.4.

Because the evaluated lipid categories originated from different biological materials and independent literature sources, no inferential statistical analyses (e.g., analysis of variance or significance testing) were performed. The results are therefore presented as descriptive comparative summaries intended to characterise compositional similarities and differences among the evaluated lipid sources rather than to establish statistically significant differences between categories. Categories represented by a single dataset are reported with SD = 0.00, indicating the absence of within-category variation rather than analytical replication.

RESULTS

FATTY ACID COMPOSITION OF BSF FAT AND SELECTED LIPID SOURCES

The fatty acid composition and derived lipid quality indices of black soldier fly (BSF) fat and comparative lipid sources are summarised in Table 2, whereas the distribution of saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA) is illustrated in Figure 1. Overall, the evaluated lipids exhibited distinct compositional patterns associated with their relative proportions of saturated and unsaturated fatty acids.

Tab. 58: Summary lipid composition and lipid quality indices of BSF fat and comparative lipid sources

Lipid source	SFA (%)	MUFA (%)	PUFA (%)	n-6/n-3	AI	TI	h/H
BSF fat	68.88 ± 1.91	16.68 ± 3.25	13.22 ± 1.36	8.42 ± 0.74	3.09 ± 0.35	1.57 ± 0.04	0.42 ± 0.04
Coconut oil^a	87.00 ± 1.41	6.50 ± 0.71	2.00 ± 0.00	12.00 ± 0.00	14.56 ± 0.80	6.71 ± 0.06	0.12 ± 0.01
Palm oil^a	49.00 ± 1.41	39.50 ± 0.71	10.50 ± 0.71	30.00 ± 2.83	0.94 ± 0.05	1.96 ± 0.11	1.14 ± 0.07
Rapeseed oil^b	9.50 ± 2.12	55.00 ± 7.07	30.50 ± 0.71	2.31 ± 0.28	0.06 ± 0.01	0.14 ± 0.04	15.75 ± 3.18
Soybean oil^b	14.50 ± 1.41	25.50 ± 2.12	59.75 ± 1.06	7.52 ± 0.46	0.14 ± 0.01	0.24 ± 0.02	8.17 ± 0.80
Chicken fat^c	32.49 ± 0.00	49.26 ± 0.00	18.26 ± 0.00	18.26 ± 0.00	0.49 ± 0.00	0.88 ± 0.00	2.34 ± 0.00
Pig fat/lard^d	47.51 ± 0.00	42.80 ± 0.00	9.14 ± 0.00	25.33 ± 0.00	0.63 ± 0.00	1.36 ± 0.00	1.71 ± 0.00
Marine fish-derived lipids^e	31.42 ± 4.83	28.65 ± 3.71	39.11 ± 5.04	0.34 ± 0.05	0.54 ± 0.08	0.22 ± 0.04	3.94 ± 0.61
Freshwater fish-derived lipids^e	28.97 ± 3.92	36.44 ± 4.12	33.82 ± 4.57	1.21 ± 0.17	0.42 ± 0.05	0.31 ± 0.05	4.76 ± 0.58

^a Sabahannur and Alimuddin (2022);

^b Dataset constructed from fatty acid profiles reported by Kostik et al. (2013) and Toishimanov et al. (2024);

^c Marikkar et al. (2021);

^d Zhang et al. (2023);

^e Özogul et al. (2007).

Values are presented as descriptive mean ± standard deviation. For BSF fat, statistics were calculated from four independently analysed samples (BSF_A–D). Literature-derived lipid categories were represented either by individual literature profiles or by aggregated profiles constructed from multiple biological observations reported in the original studies (e.g., adipose tissue depots or fish species), as described in Section 2.4. The presented values represent descriptive comparative summaries and should not be interpreted as inferential statistical comparisons among lipid sources.

BSF fat was characterised by elevated SFA content ($68.88 \pm 1.91\%$), primarily due to the high proportion of lauric acid (C12:0), accompanied by moderate MUFA and relatively low PUFA levels. Among the evaluated lipids, coconut oil exhibited the highest SFA content ($87.00 \pm 1.41\%$) and showed the closest compositional similarity to BSF fat with respect to medium-chain saturated fatty acids. In contrast, soybean and rapeseed oils displayed markedly lower SFA proportions and substantially higher PUFA and MUFA contents, respectively (Figure 1).

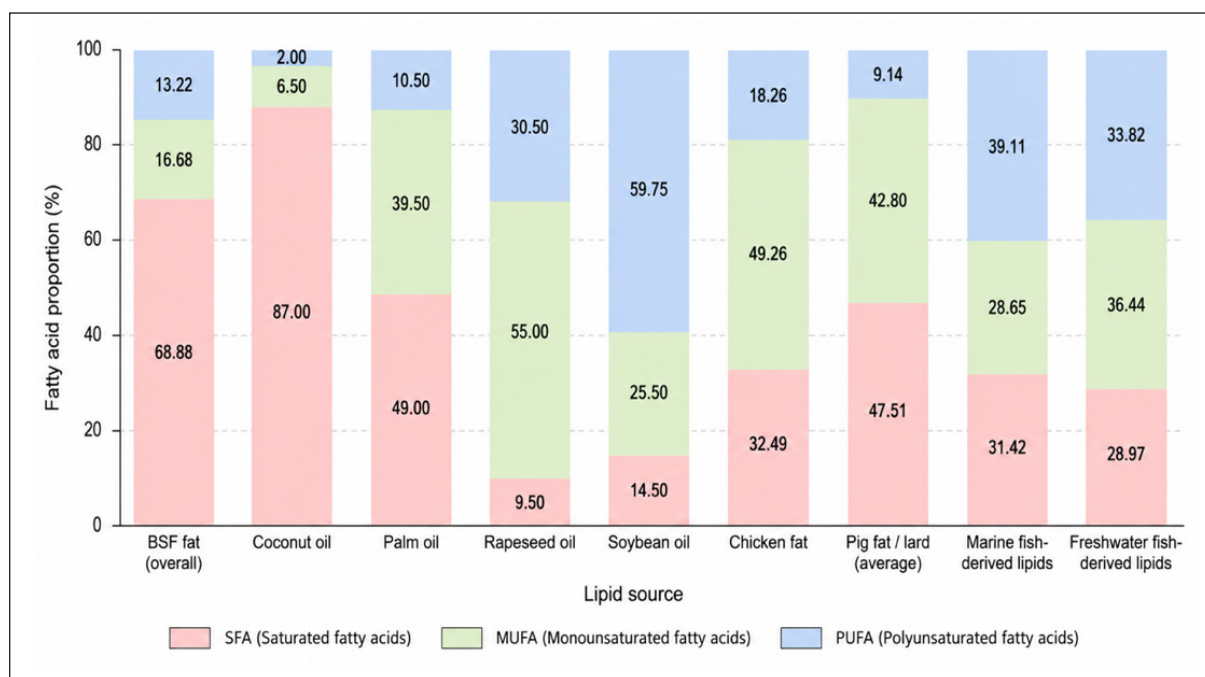


Fig. 1: Distribution of SFA, MUFA, and PUFA across evaluated lipid sources. (% of total FAME)

Chicken fat and pig fat/lard exhibited intermediate compositional characteristics between highly saturated lipids and unsaturated plant oils. Chicken fat was characterised by elevated MUFA content, whereas pig fat/lard contained higher SFA proportions accompanied by moderate MUFA levels. Marine and freshwater fish lipid fractions displayed comparatively higher PUFA content and lower n-6/n-3 ratios than the remaining lipids, reflecting the contribution of long-chain polyunsaturated fatty acids typical of fish-derived oils. Lauric acid (C12:0) predominated in BSF fat and coconut oil, whereas oleic acid (C18:1) represented the dominant fatty acid in rapeseed oil, chicken fat, and pig fat/lard. Soybean oil and fish lipid fractions were characterised by higher proportions of polyunsaturated fatty acids, particularly linoleic acid (C18:2 n-6) and long-chain n-3 fatty acids.

LIPID QUALITY INDICES

Calculated lipid quality indices reflected the marked compositional differences among the evaluated lipid sources (Table 2). Coconut oil exhibited the highest AI and TI values together with the lowest h/H ratio, corresponding to its predominance of saturated fatty acids, particularly medium-chain saturated fatty acids. In

contrast, soybean and rapeseed oil displayed the most favourable index values, characterised by low AI and TI values and elevated h/H ratios associated with their higher proportions of unsaturated fatty acids.

BSF fat exhibited intermediate lipid quality index values positioned between highly saturated tropical oils and more unsaturated plant-derived oils. Although BSF fat was characterised by elevated saturated fatty acid content, its AI and TI values remained substantially lower than those observed for coconut oil, reflecting the contribution of monounsaturated and polyunsaturated fatty acids within the BSF lipid fraction. Chicken fat, pig fat/lard, and fish lipid fractions likewise occupied intermediate positions, with marine and freshwater fish-derived lipids exhibiting comparatively lower TI values and reduced n-6/n-3 ratios associated with their higher long-chain polyunsaturated fatty acid content.

DISCUSSION

The evaluated BSF fat samples showed a fatty acid profile dominated by saturated fatty acids, particularly lauric acid (C12:0), which is commonly regarded as one of the characteristic fatty acids of black soldier fly-derived lipids (Lu et al., 2022; Suryati et al., 2023). Similar findings were reported by Srisuksai et al. (2024), who described BSF oil as a lauric acid-rich lipid source with certain physicochemical similarities to tropical oils. In the present study, BSF fat was compositionally closer to coconut oil than to soybean or rapeseed oil with respect to overall saturation profile and medium-chain fatty acid content. At the same time, however, BSF samples contained noticeably higher MUFA and PUFA proportions than coconut oil, which resulted in substantially lower AI and TI values despite the elevated saturated fatty acid fraction. This suggests that BSF lipids should not be viewed simply as another tropical saturated fat source, but rather as a separate lipid category occupying an intermediate position between highly saturated oils and more unsaturated vegetable oils.

The broader comparative analysis further demonstrated how diverse the fatty acid distribution of commonly used feed and edible fats can be. Rapeseed oil and chicken fat contained predominantly oleic acid and generally exhibited a more MUFA-rich profile than the remaining lipids, whereas soybean oil and fish lipid fractions were characterised by markedly higher PUFA levels. Pig fat/lard showed a more balanced distribution of SFA and MUFA, corresponding well with fatty acid patterns previously reported for porcine adipose tissue lipids (Zhang et al., 2023). Marine and freshwater fish-derived lipids differed from the remaining evaluated fats mainly because of their elevated long-chain polyunsaturated fatty acid content and substantially lower n-6/n-3 ratios, which is consistent with previously described fish lipid profiles (Özogul et al., 2007). Together, these observations illustrate that BSF fat occupies a distinct compositional position among conventional lipid sources rather than closely matching any single plant or animal-derived fat.

Some variability among BSF samples was observed even though all analysed materials originated from the same commercial supplier. In particular, the BSF_D sample contained relatively higher lauric acid and PUFA proportions together with lower MUFA levels compared with the remaining BSF samples. Similar differences in BSF lipid composition have previously been linked to substrate composition, larval developmental stage, environmental conditions, and extraction or processing methodology (Lu et al., 2022; Ijdema et al., 2025; Srisuksai et al., 2024). The observed variation therefore likely reflects the considerable metabolic flexibility of black soldier fly larvae and further supports the idea that substrate manipulation can significantly influence the final lipid profile of BSF-derived products. From a practical perspective, such variability also highlights the importance of compositional standardisation when BSF fat is considered for larger-scale feed applications.

The calculated lipid quality indices reflected the pronounced differences in saturation profile among the evaluated lipid sources. As expected, rapeseed and soybean oil displayed the lowest AI and TI values together with the highest h/H ratios because of their elevated unsaturated fatty acid content, while coconut oil exhibited the least favourable index values due to its predominance of saturated fatty acids (Dongmo et al., 2024). Similar relationships between fatty acid saturation patterns and derived lipid quality indices have been described previously for edible oils, fish lipids, and insect-derived fats (Maheshwari et al., 2025; Domiszewski et al., 2026). BSF fat consistently occupied an intermediate position between highly saturated coconut oil and more unsaturated plant-derived oils. Chicken fat, pig fat/lard, and fish-derived lipids likewise represented intermediate compositional categories, although fish-derived lipids differed because of their substantially lower n-6/n-3 ratios and higher proportions of long-chain omega-3 fatty acids. Nevertheless, AI, TI, and h/H values should be interpreted cautiously, since these indices primarily serve as comparative compositional tools rather than direct predictors of physiological or health-related effects in animals (Chen et al., 2020).

Interest in BSF larvae oil has recently expanded beyond compositional evaluation alone, particularly in relation to their practical use as alternative dietary fat sources in animal nutrition. Schiavone et al. (2017) and Kim et al. (2020) reported that partial or complete replacement of soybean oil with BSF larvae oil in broiler diets did not negatively affect growth performance or feed efficiency, whereas Khan et al. (2024) described acceptable serum biochemical and gut health responses following dietary inclusion of BSF-derived ingredients. Similar findings were reported by Somparn et al. (2024), who evaluated replacement of rice bran oil with BSF larvae oil in broiler diets. In young ruminant nutrition, Quigley et al. (2025) further suggested that BSF oil may represent a suitable alternative to conventional milk replacer oils such as palm or coconut oil. Although the present study was limited to compositional characterisation and literature-based comparison of lipids, the obtained results support the growing view that BSF-derived

lipids may represent a nutritionally relevant alternative fat source worthy of further investigation under practical feeding conditions.

Several limitations should nevertheless be considered when interpreting the present findings. The comparative datasets combined experimentally analysed BSF samples with literature-derived fatty acid profiles originating from studies that differed in biological material, analytical conditions, and sample origin. Although only datasets reporting fatty acid composition as relative percentages of total fatty acids (%FAME) determined by GC-FID were included in order to improve methodological comparability, some degree of inter-study variability remains unavoidable. In addition, the calculated AI, TI, and h/H indices should primarily be regarded as comparative compositional indicators rather than direct indicators of animal health or nutritional efficacy. Future studies should therefore combine compositional lipid characterisation with controlled *in vivo* experiments evaluating digestibility, metabolic responses, animal performance, and product quality following dietary inclusion of BSF or other insect-derived lipids.

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GRASS STRAW AS FEED

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ABSTRACT

Grass straw is produced as a byproduct when growing grasses for seed. Its yields vary and, depending on the grass species, the intensity of inputs into the stand, and weather conditions, range from 2.0 to 12.1 t·ha⁻¹. Grass straw has a wide range of uses, including as a source of thermal energy, a raw material for compost production, a substrate for growing edible mushrooms, shredded and spread over the field, and, finally, as an animal feed. When straw is utilized appropriately, it increases the revenue and profitability of grass seed growers. In terms of nutrient composition, it is a higher-quality feed than cereal straw. Compared to meadow hay, it has a higher fiber content (usually more than 650 g·kg⁻¹ NDF) and a lower crude protein content (26–92 g·kg⁻¹ DM).

Keywords: mineral contents, feed value, perennial ryegrass, italian ryegrass, dry mater content

INTRODUCTION

The growing emphasis on sustainable agriculture and the efficient use of byproducts from crop production is leading to increased interest in alternative roughage feeds for livestock. One such feed is grass straw, which is produced as a byproduct of grass seed production. Although a significant amount of grass straw is produced annually in areas with intensive seed production, its potential in livestock nutrition is not fully utilized (Bohnert et al., 2012).

In terms of nutritional value, grass straw represents a middle ground between high-quality meadow hay and cereal straw. Hay harvested at the optimal stage of growth contains a higher concentration of crude protein (CP), a lower proportion of lignified fiber, and a higher content of metabolisable energy, which is reflected in higher digestibility. In contrast, cereal straw is characterized by a low CP content, a high proportion of lignin, and therefore exhibits lower feed value (Van Soest, 1994). Grass straw typically contains more crude protein and has higher



digestibility than wheat or barley straw; however, its nutritional value is generally lower than that of high-quality hay (Bohnert et al., 2012).

The chemical composition of grass straw is significantly influenced by the grass species, variety, growth stage, seed harvest date, and conditions during crop maturation. Differences between individual species can be substantial, particularly in the content of crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and lignin, which decisively influence nutrient utilization by ruminants and horses (Hejduk, Macháč, 2019; Moore, Jung, 2001). For this reason, grass straw cannot be considered a homogeneous feed, and its nutritional value should be assessed individually based on species and production conditions, considering the proportion of sterile tillers and leaves.

When appropriately supplemented with energy, CP, and mineral nutrients, grass straw can be an economically attractive component of ruminant diets, particularly for beef cattle, dry cows, or sheep with lower nutrient concentration requirements. Compared to cereal straw, it is generally more readily accepted by animals and provides a higher content of digestible nutrients. On the other hand, attention must be paid to the possible presence of endophytic alkaloids in certain grass species, particularly tall fescue and perennial ryegrass, which can negatively affect the health and productivity of animals (Cheeke, 1995; Bohnert et al., 2012).

The aim of this study is to assess the production potential and chemical composition of grass straw from selected grass species grown for seed and to evaluate its potential use in ruminant nutrition. The results are discussed in relation to the quality of hay and cereal straw, which are commonly used roughage feeds.

MATERIAL AND METHODS

This study evaluates the available knowledge on grass straw production and its quality characteristics in relation to its use in livestock feed. There is relatively little published information on this topic, and most of it comes from regions where grass seed production has long been concentrated.

This study primarily draws on the results of the authors' previously unpublished experiments conducted in the Czech Republic. The greatest attention was devoted to Italian and perennial ryegrass, which are among the most important grass species cultivated for seed production not only in Europe but in all temperate regions of the world. Experimental data were collected at the Forage Research Station of Mendel University in Valtín (49.52° N, 15.97° E) and at the Research Station of the Oseva PRO Grass Seed Company in Zubří (49.47° N, 18.07° E) (Hejduk, Macháč, 2019; unpublished data). The experiments were conducted on 10 m² plots, which were harvested using a small plot combine harvester. Additional results were obtained from commercial stands, where samples were collected from a 1 m² area in three replicates.

Grass straw samples were collected immediately after harvest to determine dry matter (DM) content and dry matter yield. For laboratory analysis, the dried

samples were ground and homogenized in a laboratory grinder equipped with a 1.0-mm sieve. Three samples were analysed from each combination of species, location, and year. Chemical analyses included the determination of CP, NDF, and ADF content. Ash content was determined by ignition at 550 °C for six hours. NDF and ADF values were determined using the ANKOM method (2017a, 2017 b). Total nitrogen content was determined by the Kjeldahl method according to AOAC (1984), and crude protein content was subsequently calculated by multiplying the nitrogen content by a factor of 6.25.

RESULTS AND DISCUSSION

The results of the chemical composition of grass straw from individual species are presented in Table 1. Significant differences were found among the evaluated species in crude protein, neutral detergent fibre, acid detergent fibre, and ash content, confirming that the nutritional value of grass straw is significantly influenced by botanical species.

Crude protein content ranged from 26 g·kg⁻¹ DM in red fescue (*Festuca rubra*) and meadow fescue (*Festuca pratensis*) to 92 g·kg⁻¹ DM in Kentucky bluegrass (*Poa pratensis*). A relatively high crude protein content was also found in timothy grass (*Phleum pratense*); 81 g·kg⁻¹ DM, while both ryegrass species reached intermediate values (64–74 g·kg⁻¹ DM). These results are consistent with the findings of Youngberg and Yough (1977), who reported considerable variability in CP content among individual grass species grown for seed. Compared to high-quality hay, which typically contains 120–180 g CP·kg⁻¹ DM (Van Soest, 1994), the crude protein concentration in grass straw was significantly lower. On the other hand, for most of the evaluated species, it was higher than the values typically reported for cereal straw, which range approximately between 20 and 45 g CP·kg⁻¹ DM. These results confirm that grass straw can represent a nutritionally more valuable alternative to cereal straw, especially for species with higher CP content.

The NDF content ranged from 629 to 762 g·kg⁻¹ DM. The lowest concentration was found in meadow foxtail, while red fescue had the highest NDF content. Higher NDF concentrations are generally associated with lower feed intake in ruminants, as they reduce the rate of rumen passage (Mertens, 1997). The values observed for ryegrasses (656–680 g·kg⁻¹ DM) correspond to the data published by Bohnert et al. (2012) for grass straw produced in Oregon. Although the NDF values are higher than those of high-quality hay, they generally remain lower than those of most cereal straws, where the NDF content often exceeds 750 g·kg⁻¹ DM (Van Soest, 1994).

A similar trend was also observed for ADF, with concentrations ranging from 410 g·kg⁻¹ DM in meadow grass to 523 g·kg⁻¹ DM in red fescue. Higher ADF content is associated with a higher proportion of cellulose and lignin, which leads to reduced digestibility of organic matter (Jung, Allen, 1995). The lowest ADF values were found in meadow grass and meadow fescue, suggesting their higher

potential digestibility compared to fescues. In contrast, red fescue exhibited the highest NDF and ADF contents, indicating a high degree of lignification of the tissues following seed harvest and lower nutrient availability.

Tab. 59: *Nutritional composition of grass species (g·kg⁻¹ DM) (trial Czech Republic)*

Species	Crude protein	NDF	ADF	Ash
Timothy	81	629	412	71
Peren ryegrass	64	656	442	68
Italian ryegrass	74	680	442	68
Red Fescue	26	762	523	41
Meadow Fescue	26	671	452	37
Kentucky Bluegrass	92	672	410	60

Ash content ranged from 37 to 71 g·kg⁻¹ DM. The highest concentration was found in timothy, while the lowest content was recorded in meadow fescue. Differences in mineral content may be due not only to the genetic characteristics of individual species but also to variations in the intensity of nutrient uptake from the soil, fertilization levels, rainfall prior to straw harvest, and contamination of the harvested material with soil particles during harvest (Moore, Jung, 2001).

An overall assessment of the chemical composition suggests that there are significant differences in the potential feed value of grass straw among the species studied. From the perspective of ruminant nutrition, based on the above analyses, meadow fescue and ryegrass appear to be the most promising, as they combine a higher crude protein content with a lower ADF concentration. Both species can therefore be considered a more suitable alternative to cereal straw, particularly as a structural component of feed rations. In contrast, red fescue exhibited the lowest CP content and, at the same time, the highest concentration of structural fiber, which limits its usefulness in ruminant nutrition.

Tab. 60: *Grass straw yields by species (trials 2016–2017, Hejduk, Vlk, 2026)*

Species	Range of yield (t·ha ⁻¹)
Timothy	8.1 – 12.1
Perennial Ryegrass	3.3 – 11.4
Italian Ryegrass	3.3 – 11.3
Red Fescue	2.0 – 7.8
Meadow Fescue	5.3 – 9.1
Kentucky Bluegrass	6.9 – 7.8

The results of this study are consistent with the findings of Bohnert et al. (2012) and Niemeläinen et al. (2001), who reported that, in terms of nutritional value, grass straw represents a middle level between high-quality hay and cereal straw. Although it does not reach the quality of a meadow hay, in most cases it provides a higher concentration of crude protein (CP) and better digestibility than cereal straw, making it an interesting byproduct suitable for use in rations for ruminants with lower nutrient concentration requirements.

CONCLUSION

The results of this study confirmed that the chemical composition of grass straw varies significantly among different grass species, which significantly affects its potential use in ruminant nutrition. The highest crude protein (CP) content was found in Kentucky bluegrass and meadow fescue, while the lowest values were observed in red fescue. At the same time, significant differences were found among species in NDF and ADF content, which are key indicators of digestibility.

Compared to high-quality hay, grass straw exhibited a lower concentration of crude protein and a higher content of structural carbohydrates, which is a result of the physiological aging of the plants. Nevertheless, most of the evaluated species were found to have a higher nutritional value than commonly used cereal straw, primarily due to their higher CP content. Grass straw thus represents a promising alternative roughage, particularly for categories of ruminants with lower nutrient concentration requirements, such as beef cattle, dry cows, or sheep.

Greater use of grass straw in livestock nutrition can contribute to increased sustainability and efficiency in agricultural production, while making better use of byproducts from seed production. Supplementing the feed base during dry years when forage is scarce can be particularly important.

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COURSE OF OOCYST SHEDDING IN FAECES OF BROILERS EXPERIMENTALLY CHALLENGED WITH EIMERIA IN RELATION TO DIETARY ADMINISTRATION WITH WORMWOOD

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ABSTRACT

The aim of this work was to assess the effect of feed supplementation with *Artemisia absinthium* (wormwood) meal on the course of *Eimeria* spp. oocyst shedding in broilers following their targeted infection using area under the curve (AUC) analysis. Broilers in the control (C) group were fed basal diets without coccidiostat or wormwood herb. Broilers of the CC group were fed starter and grower basal diets supplemented with coccidiostat. Broilers in the P3 and P6 groups were fed diets containing 3 and 6% of meal from whole aerial parts of wormwood in feed, respectively. Collective faeces per pen base were gathered on days 0, 6, 13, 20 and 27 post infection to obtain the final number of oocysts per one gram of faeces (OPG). Then, the AUC analysis of OPG values was carried out. Dietary administration of the coccidiostat robenidine resulted in lower AUC values ($P < 0.05$) during the main phase of infection and confirmed its high anticoccidial efficacy. Dietary supplementation with wormwood meal did not lead to effective suppression of oocyst shedding ($P > 0.05$). However, a lower total AUC value was observed in the P6 group compared to the P3 group ($P > 0.05$); it suggests a dose-dependent effect of the tested plant-based additive on the intrinsic dynamics of the *Eimeria* infection.

Keywords: meat-type chicken; feed; *Artemisia absinthium*; oocyst; coccidiosis; area under the curve



INTRODUCTION

Traditional methods for coccidiosis control in poultry include anticoccidial chemicals, attenuated vaccines (particularly in the EU), and, alternatively, certain phyto-genic herbal supplements. Anticoccidial chemicals, coccidiostats, coccidioides and ionophores - which have been in use for decades – can be still considered the conventional method for coccidiosis control in poultry farming. Although these chemicals are an effective and economically acceptable solution, the risk of developing resistance and current consumer demands for residue-free products have recently led to the search for and development of alternative strategies for coccidiosis control in animal production (Chapman et al., 2010; Beski 2023).

Using effective phyto-genic products as an alternative to anticoccidial agents can mitigate the risks posed by certain chemical drugs. According to Srinivasu et al. (2020), certain herbs contain bioactive phytochemicals that, due to their natural immunostimulatory effects, are effective both in controlling coccidiosis in poultry and against certain other poultry diseases. The genus *Artemisia* is known for its wide spectrum of pharmacological effects, including mainly antioxidant, anti-inflammatory, immune-boosting and antiparasitic effects. The herb *Artemisia absinthium* L., known as wormwood (WM), is used primarily for its stomachic, cholagogue, antiseptic, antispasmodic, carminative, febrifuge and anthelmintic effects. Because of its rich content of interesting bioactive phytochemicals, this herb could be also a potential supplement in animal feed (Amin et al. 2022; Zapletal et al. 2024).

Despite previous studies demonstrating a number of positive effects of *Artemisia* herbs given in feed to chickens (Abdullah and Al-Barwary 2020; Wang et al. 2024), specific knowledge regarding the effect of WM as a feed additive on the shedding of *Eimeria* oocysts following coccidiosis infection remains still very limited. Therefore, the aim of this work was to assess the effect of feed supplementation with WM meal on the course of *Eimeria* spp. oocyst shedding in broilers following their targeted infection using area under the curve (AUC) analysis.

MATERIAL AND METHODS

Trial was performed on 208 one-day-old male Ross 308 chicks, which were randomly assigned to four dietary treatments, each with four replicate pens and thirteen birds per pen in the accredited stable of the Department of Animal Breeding, Animal Nutrition and Biochemistry, University of Veterinary Sciences (UVS) Brno. All broilers were housed under the same controlled housing conditions that were adjusted according to the Ross 308 guidelines. Feed and water were supplied *ad libitum* for broilers. The trial lasted for 42 days. During the trial period, starter diets were fed between days 1–14, grower diets between days 15–28, and finisher diets between days 29–42. The experiment was approved by the Animal Welfare Committee of UVS Brno.

The broilers in the control (C) group were fed basal diets without coccidiostat or WM herb. The broilers of the CC group were fed starter and grower basal diets supplemented with coccidiostat robenidine (33 mg/kg of feed); the finisher diet for the CC group didn't contain coccidiostat. The broilers in the P3 and P6 groups were fed diets containing 3 and 6% of meal from whole aerial parts of WM in feed, respectively. The P3 and P6 diets didn't contain any commercial coccidiostat. For all dietary groups, diets were designed as isoenergetic and isonitrogenous (Table 1).

Tab. 61: *Analysed nutrients composition (%) of respective diets*

Nutrient (%)	Dietary groups			
	C	CC	P3	P6
Starter diet (day 1 to 14)				
Crude protein	20.5	20.6	20.6	20.4
Crude fibre	3.6	3.5	3.7	4.0
Crude fat	3.1	3.1	3.5	3.3
Crude starch	35.6	35.4	34.5	34.1
Ash	5.9	5.9	5.9	5.8
Calcium	0.80	0.81	0.89	0.92
Inorganic phosphorus	0.59	0.57	0.56	0.55
Metabolizable energy (MJ/kg)	12.8	12.8	12.9	12.7
Grower diet (day 15 to 28)				
Crude protein	19.0	19.0	19.1	19.1
Crude fibre	3.3	3.3	3.2	3.2
Crude fat	8.0	8.0	8.0	8.1
Crude starch	35.2	35.2	35.5	35.1
Ash	5.4	5.5	5.4	5.3
Calcium	0.87	0.89	0.83	0.91
Inorganic phosphorus	0.48	0.49	0.48	0.47
Metabolizable energy (MJ/kg)	14.0	13.9	14.0	14.0
Finisher diet (day 29 to 42)				
Crude protein	18.8	18.7	18.7	18.9
Crude fibre	4.1	4.1	3.7	4.1
Crude fat	6.3	6.3	6.4	6.2
Crude starch	35.5	35.4	34.7	33.9
Ash	5.2	5.2	5.1	5.0
Calcium	0.78	0.76	0.70	0.71
Inorganic phosphorus	0.45	0.46	0.48	0.49
Metabolizable energy (MJ/kg)	13.5	13.5	13.5	13.4

At the age of 16 days, broilers in all dietary groups were infested by tenfold overdosing of live vaccine LIVACOX T (Biopharm Co., Prague, Czech Republic) to induce a mild coccidiosis infection according to procedure earlier used by Yu et al. (2021). Inoculum dose of 1 ml was orally gavaged into the crop to each broiler. Inoculum dose contained 15 000 live oocysts of *E. acervulina*, *E. tenella* and *E. maxima*. Collective faeces per pen base were gathered on days 0, 6, 13, 20 and 27 post infection (PI). The freshest faeces were collected from the pen to contain at least 15 g of faeces without bedding. The McMaster technique was further applied. An aliquot of the faecal sample was transferred in to a McMaster chamber and all the *Eimeria* oocysts were counted manually in both parts of the chamber. The sum was multiplied by a dilution factor (50) to obtain the final number of oocysts per one gram of faeces (OPG). Then, area under the curve (AUC) analysis of OPG values was performed using the trapezoidal rule (Bailer 1988).

The AUC was calculated according to the following equation:

$$\text{AUC} = \sum (y_i + y_{i+1})/2 \times (t_{i+1} - t_i)$$

where: y_i = OPG value at time point t_i ; y_{i+1} = OPG value at the subsequent time point; t_{i+1} = time interval between measurements.

Segmented AUC analysis was additionally performed for individual PI intervals (0–6, 6–13, 13–20, and 20–27 days) to evaluate temporal differences in parasite excretion dynamics among dietary groups.

The OPG values used for the current statistical re-analysis were derived from data previously published by Zapletal et al. (2025) in an IF-ranked peer-reviewed journal. Normality analysis of data distribution for AUC values was performed by a Shapiro-Wilk test; normality was not found. The non-parametric Kruskal-Wallis ANOVA was used to find differences in AUC values among dietary groups within individual PI intervals. Then, pairwise comparisons were performed using Mann-Whitney U tests without multiplicity correction due to the exploratory nature of the study and limited sample size. The significance was considered at the $P < 0.05$ level. All statistical procedures were performed by the STATISTICA CZ, vs. 10 software.

RESULTS AND DISCUSSION

The AUC represents the cumulative oocyst excretion over time and integrates both the magnitude and duration of parasite shedding during the experimental period (Velkers et al., 2012). A segmented AUC analysis of OPG levels revealed differences among the experimental groups during the early and middle phases of infection (Table 2). The results show that a significant effect ($P < 0.05$) of the broiler diet treatment on AUC values was observed from the time of infection (PI day 0) through day 20 PI; as expected, the significantly lowest AUC value (0) was observed in the CC group, which was treated with the coccidiostat robenidine,

throughout all three evaluated periods. Compared to the CC group, a significantly higher AUC value was found during the PI 0–6 day period in the P3 and P6 groups, during the PI 6–13 day period in the C, P3, and P6 groups, and during the PI 13–20 day period in the P3 group. The highest level of total oocyst shedding occurred in all infected groups during the period from PI day 6 to 20, which generally corresponds to the main phase of replication and shedding of *Eimeria* spp. In terms of total AUC, the P3 group exhibited the highest cumulative intensity of oocyst excretion, followed by the P6 and C groups.

The results show that dietary supplementation with WM meal did not lead to effective suppression of oocyst shedding; however, a lower total AUC value was observed in the P6 group compared to the P3 group ($P > 0.05$). These results suggest a dose-dependent effect of the tested plant-based additive on the intrinsic dynamics of the infection.

Published studies focusing on the use of *Artemisia* spp. in experimental and field coccidiosis in broilers have repeatedly reported a reduction in the intensity of oocyst shedding, although the effect is generally less pronounced than that of standard anticoccidial chemicals. Studies using parasitological models of *Eimeria* spp. infection found a reduction in OPG, improvement in intestinal lesions, and partial control of the infection following the administration of *Artemisia annua* or *Artemisia absinthium*. However, under the experimental conditions used, complete inhibition of oocyst shedding was not achieved compared to commercial coccidiostats (Almeida et al. 2012; Almeida et al. 2014; Coroian et al. 2022; Hezil et al. 2024), which is consistent with the results of this work. The mere inclusion of dried WM meal in this work was not associated with the expected reduction in the number of excreted oocysts.

Tab. 62: Dietary effect on oocysts shedding in chicken faeces determined by the area under the curve (AUC) values found in respective periods

PI period (days)	Dietary groups				P-value
	C	CC	P3	P6	
0–6	450 ^{ab}	0 ^b	1 275 ^a	1 313 ^a	0.013
6–13	18 681 ^a	0 ^b	47 119 ^a	29 619 ^a	0.017
13–20	23 931 ^{ab}	481 ^b	53 769 ^a	33 600 ^{ab}	0.024
20–27	6 388	3 413	8 838	6 169	0.423

PI: post infection; ^{a,b} Means within a row with different superscripts letters differ at $P < 0.05$.

The specific representation of the biologically active compounds contained in WM can be influenced by a wide range of factors, including, for example, the geographical origin of the herb, soil type, and altitude of cultivation, as well as harvest time and the duration and method of storage (Amin et al. 2022). In this context, it can be stated that the potential use of essential oil derived from WM, ideally with verified levels of β -thujone, 1,8-cineole, and p-cymene, could serve as a more reliable tool for controlling coccidiosis in poultry farming.

Segmented AUC analysis has proven to be a useful tool for evaluating the time-dependent dynamics of infection, as it integrates both the intensity and duration of oocyst shedding. Compared to the evaluation of individual time points, it allows for a more comprehensive assessment of the overall infectious load during the experiment. Although relatively high variability in OPG values was observed among individual pens (replicates), the AUC analysis confirmed a significant anticoccidial effect of the commercial coccidiostat and simultaneously suggested a partial modulatory effect of a higher dose of WM meal on the course of infection in broilers infested with *Eimeria* spp.

CONCLUSION

The results of this work showed that segmented AUC analysis represents a potential tool for the comprehensive evaluation of oocyst excretion dynamics in broilers experimentally infected with *Eimeria* spp., as it takes into account both the intensity and duration of oocyst shedding. Administration of the coccidiostat robenidine resulted in significantly lower AUC values during the main phase of infection and confirmed its high anticoccidial efficacy. In contrast, dietary supplementation with WM meal did not result in a significant reduction in oocyst shedding compared to the infected control group, although a trend toward decreased total cumulative shedding was observed at the higher dose (6%) compared to the lower dose (3%). The results suggest that the efficacy of wormwood may be influenced by the content of biologically active compounds and the form of the plant material used. Further research should therefore focus on standardized plant extracts or essential oils with a defined composition of active ingredients and on verifying their potential in controlling coccidiosis in poultry.

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ABSTRACTS

EFFECTS OF CORN DISTILLERS DRIED GRAINS AND CORN GLUTEN IN THE DIET OF DAIRY COWS ON RUMEN FERMENTATION AND METABOLISM

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ABSTRACT

The production of bioethanol from crops has led to the large-scale output of nutritionally valuable bioproducts. This study compared the effects of incorporating wet distillers' grains with solubles (WDGS) versus enriched corn gluten (CG) into the diet of Holstein dairy cows on rumen fermentation and blood metabolic profiles. In a 16-week trial on a commercial herd (300 cows), 20 cows were selected into balanced groups (10 per group) using analogical pairs based on lactation number, days in milk, and prior milk yield. One group received 4 kg of WDGS per cow daily within a total mixed ration (TMR), while the second received 4 kg of enriched CG. Blood was sampled from the *v. coccygea* at weeks 0, 7, and 16; rumen fluid was collected orally at weeks 7 and 16.

Experimental diets showed no negative impact on systemic metabolism. At week 16, the only significant blood parameter difference was a higher triacylglycerol concentration in the CG group. All metabolic values remained within physiological reference ranges. In the rumen fluid, a significant difference was detected at week 16 only in the molar proportion of acetic acid, which was higher in the CG group, indicating better fiber digestion and lower dietary acidogenicity. At the trial's conclusion, average rumen pH was low in both groups (CG 5.73 ± 0.30 vs. WDGS 5.56 ± 0.15), indicating subclinical rumen acidosis (SARA). This difference relates to dietary composition, as the WDGS ration contained higher starch (25.1 % vs. 23.1 % DM). In conclusion, both WDGS and CG are highly suitable protein sources for dairy cows. However, WDGS poses a higher risk of acidogenicity, which must be managed during TMR formulation.

Keywords: WDGS; corn gluten; dairy cattle; rumen fermentation; subclinical rumen acidosis (SARA)

FOUNDINGS

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THE EFFECT OF A MICROBIAL INOCULANT ON THE FERMENTATION PROCESS OF MAIZE SILAGE PRODUCED USING NARROW-ROW TECHNOLOGY

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ABSTRACT

Maize biomass grown using narrow-row technology was ensiled with a microbial inoculant containing *L. plantarum*, *Ped. parvulus*, and *Lcb. rhamnosus*. The microbial inoculant was applied to improve the fermentation process and reduce nutrient and dry matter losses during ensiling. Maize grown under narrow-row technology was ensiled in microtubes either without treatment (control) or with the application of the microbial inoculant.

After five months of ensiling, the silages were subjected to laboratory analyses. The following parameters were determined: dry matter, ash, crude protein, crude fibre, acid detergent fibre (ADF), neutral detergent fiber determined with α -amylase (aNDF), pH, water extract acidity (WEA), volatile fatty acids, and lactic acid.

Silage treated with the microbial inoculant had significantly ($P < 0.05$) higher contents of dry matter (360.1 g/kg vs. 350.0 g/kg in the control), crude fibre (220.8 g/kg vs. 205.5 g/kg), ADF (272.4 g/kg vs. 247.98 g/kg), and aNDF (450.83 g/kg vs. 416.36 g/kg). No differences were observed in silage pH (3.87 vs. 3.83 in the control). Water extract acidity values were within the optimal range for maize silage. However, WEA was significantly higher ($P < 0.05$) in silage treated with the microbial inoculant (1,780.9 mg KOH per 100 g of silage) than in the control silage (1,383.9 mg KOH per 100 g of silage).

In contrast, the proportions of lactic and acetic acids showed the opposite trend. The control silages had a higher proportion of lactic acid (79.7%) and a lower proportion of acetic acid (16.33%) than the silages treated with the microbial inoculant (63.86% lactic acid and 33.73% acetic acid). Sensory evaluation showed that all silages exhibited the characteristics of a high-quality fermentation process.

Keywords: microbial inoculant; fermentation quality; maize silage; narrow-row spacing; nutrient preservation

FOUNDINGS

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ALTPROT RESEARCH PROJECT OVERVIEW

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ABSTRACT

The main goal of the ALTPROT project (***Evaluation of the environmental impacts of feeding alternative protein sources in monogastric farm animals***) is to contribute to the reduction of the environmental impacts of animal production by assessing the usability of protein sources - white lupine, extracted meal from sunflower and rapeseed and insect protein (larvae of the black soldier fly) in the nutrition of broiler poultry and pigs, which could serve as an alternative to soy extracted scrap. The monitored outputs will include nutritional, zootechnical, health parameters, but especially zoohygienic parameters, which will represent the content of ammonia and methane in the stable air. All the mentioned sources have their advantages and disadvantages. Therefore, one of the goals of the project is to find a suitable combination of these resources so that their use in replacing soy is full-value.

Within the project, feeding trials were conducted with broiler chickens and pigs to evaluate the potential of selected alternative protein feedstuffs as partial or complete replacements for soybean meal. Individual protein sources as well as their combinations were assessed with respect to production performance, animal health, nutritional value of the diets, and their effects on selected zoohygienic parameters of the housing environment.

The study evaluated diets containing insect protein, white lupin, and oilseed meals. The results were used to develop functional samples and will serve as a basis for future applied outputs, including utility models and certified methodologies. Overall, the project integrates nutritional, production, and environmental assessments of alternative protein sources, contributing to the development of more sustainable feeding systems for monogastric livestock.

Keywords: local protein sources; performance parameters; poultry; pig; nutrition; greenhouse gases; mitigation; global changes; food

FOUNDINGS

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COMPOSITIONAL ANALYSIS OF NON-SALEABLE MILK USED FOR CALF FEEDING

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ABSTRACT

Non-saleable milk is commonly used for calf nutrition. A 2024 survey across 67 dairy herds revealed its administration to heifer calves on 34.3 % of farms and to bull calves on 68.7 %. This study analyzed the nutritional, mineral, vitamin, and microbiological composition of non-saleable milk used in commercial dairy herds.

Representative milk samples (n=36) were collected from 13 dairy farms utilizing non-saleable milk. Analytical parameters encompassed basic macronutrients, somatic cell count (SCC), macro-minerals (Ca, Mg, Na, K), trace elements (Zn, Cu, Fe, Mn, Se), vitamins (A, beta-carotene, E), total plate count (TPC), and antimicrobial inhibitory substances.

Non-saleable milk exhibited high variability. Total solids ranged from 8.9 % to 18.8 % (mean: 13.1 %), driven by fluctuations in fat (0.6–9.5 %; mean: 4.08 %). Lower variability was observed for crude protein (2.3–4.4 %; mean: 3.66 %) and lactose (3.78–4.94 %; mean: 4.61 %). Marked disparities occurred in SCC (56,000–6,509,000 cells/mL; mean: 1,724,000 cells/mL) and TPC (5,000–599,000 CFU/mL; mean: 212,800 CFU/mL), indicating frequent use of mastitic milk and substandard hygiene. Antimicrobial residues were detected in 47.2 % of samples. Comparing 8 L of non-saleable milk against 1 kg of standard milk replacer revealed severe micronutrient deficiencies: iron met only 4.7 % of the standard, zinc 61.2 %, copper 21.8 %, manganese 0.8 %, and vitamin E 4.4 %. Conversely, vitamin A achieved 100 % coverage and selenium reached 93.0 %.

Non-saleable milk used for calf feeding is characterized by macronutrient instability—particularly fat—and severe deficiencies in essential micronutrients (Fe, Zn, Cu, Mn, vitamin E). These results are used to develop a specialized vitamin-mineral supplement for calves that is soluble in milk. Furthermore, high microbial contamination and antimicrobial residues pose substantial biosecurity risks. These findings emphasize the necessity of pasteurization or acidification of non-saleable milk to safeguard calf health.

Keywords: non-saleable milk, calf nutrition, micronutrient deficiency, microbial contamination, antibiotic residues

FOUNDINGS

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INFLUENCE OF DIET ON THE NUTRITIONAL COMPOSITION OF MEALWORM LARVAE

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ABSTRACT

The primary objective of this research was to evaluate the influence of varying substrate compositions on the growth performance and larval dry matter (DM) composition of mealworm (*Tenebrio molitor*) larvae. The study sought to verify how specific deviations from the theoretical nutrient optimum – established in literature as approximately 6–10 % lipids, 20–23 % proteins, and 65–72 % carbohydrates (Kröncke and Benning, 2022; Morales-Ramos et al., 2020) – manifest in both morphological development and the final chemical profile of the larval biomass.

The experiment utilized five-week-old *Tenebrio molitor* larvae obtained from food and feed grade edible insect producer from the Czech Republic. The trial was conducted over an eight-week feeding period in a standardized rearing environment. The larvae were allocated into five experimental groups to test a range of nutritional experimental formulations:

- **OTR:** Wheat bran (control).
- **KOM:** Commercial insect feed (control).
- **EX 1/LC:** Experimental low-carbohydrate/high-protein formulation (32 % crude protein).
- **VL:** Oat flakes.
- **EX 2/HC:** Experimental high-carbohydrate/low-protein formulation (9.5 % crude protein, 85 % nitrogen-free extract).

The results demonstrate a significant impact of dietary composition on the nutritional quality and growth performance of the insect biomass. High crude protein (CP) content in the diet showed a strong positive association ($r = 0.89$) with the resulting larval protein levels. The EX 1/LC group, receiving the 32 % CP in diet, achieved the peak crude protein content (61.63 ± 0.45 %) in its dry matter.

In terms of morphological traits and growth performance (individual weight, length, and width, respectively), the OTR (0.12 ± 0.02 g; 23.23 ± 1.73 mm; 2.74 ± 0.22 mm), KOM (0.11 ± 0.03 g; 23.29 ± 1.58 mm; 2.71 ± 0.25 mm), and EX 1/LC (0.11 ± 0.03 g; 23.06 ± 2.38 mm; 2.65 ± 0.29 mm) groups achieved the most favorable results. Conversely, the VL group consistently exhibited the second lowest values across all measured parameters. The poorest performance was recorded in the EX 2/HC group;

larvae fed this substrate showed significantly lower values in all growth metrics (0.05 ± 0.04 g; 17.37 ± 2.84 mm; 1.75 ± 0.36 mm) compared to the other groups, indicating that extreme deviations toward high carbohydrate concentrations at the expense of protein are detrimental to larval development.

This study confirms that the macronutrient profile of the rearing substrate significantly dictates both the physical growth and the final larval DM composition of mealworm. The findings highlight the inherent larval plasticity, which allows for nutritional “loading” based on the protein-to-carbohydrate ratio of the feed. From a practical and industrial perspective, utilizing protein-rich diets is essential for optimizing yield and protein content. Furthermore, while larvae can survive on diverse substrates, diets characterized by extreme protein deficiencies, such as the EX 2/HC formulation, are commercially non-viable due to severely stunted growth. These results provide a roadmap for the food and feed industry to optimize production efficiency through targeted substrate formulation.

Keywords: *Tenebrio molitor*, larvae, nutrition, growth performance, nutrient composition, crude protein

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