

EFFECTS OF DIETARY SUPPLEMENTATION WITH *NIGELLA SATIVA* L. ON PRODUCTIVITY AND HEALTH IN DAIRY COWS

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Original scientific paper

Abstract: The aim of this study was to evaluate the effects of *Nigella sativa* seeds supplementation on the productive performances and health status of lactating dairy cows. The study was conducted at the Experimental Farm of the Research and Development Institute for Bovine Balotesti, Romania, between November and December 2023, on a number of 40 Romanian Black and Spotted dairy cows divided into two groups: the experimental group E (n=20 heads) and the control group C (n=20 heads). The experimental group received 20 g of *Nigella sativa* seeds/head/day for 28 consecutive days. Milk and blood samples were collected at day 0 and day 28. Statistical comparisons were performed using Student's t-test. Results revealed statistical differences for lymphocytes (P=0.0479), glucose (P=0.0473), uric acid (P=0.0222), and amylase (P=0.0032) in the experimental group (E) after the 28-days trial. *Nigella sativa* seeds, due to their bioactive and antioxidant constituents, demonstrated promising potential as a natural dietary additive for enhancing dairy cattle physiological stability.

Key words: *Nigella sativa*, dairy cows, productive performances, blood profile.

Introduction

Globally, there is growing interest in using medicinal plants as feed additives to enhance ruminant productivity and health. *Nigella sativa* L., commonly known as black cumin, is an annual herb of the *Ranunculaceae* family native to Southwest Asia, the Mediterranean basin, and parts of Africa (Akram Khan and Afzal, 2016; Hannan et al., 2021). Its seeds have been traditionally used for both medicinal purposes and as a nutritional supplement in human and veterinary diets. *Nigella sativa* seeds contain approximately 26.7 % protein, 28.5 % fat, 24.9 % carbohydrates, 8.4 % crude fibre, and 4.8 % ash, along with appreciable levels of copper, phosphorus, zinc, iron, and provitamin A (Ahmad et al., 2013; Srinivasan,

2018). The biological activities of black cumin are largely attributed to its major bioactive compounds (Alberts et al., 2024): thymoquinone (antioxidant, anti-inflammatory, and anticancer properties), thymohydroquinone (a powerful acetylcholinesterase inhibitor), and thymol (virucidal effect). Due to its high nutritional value and content of polyunsaturated fatty acids (omega-6 and omega-3), phytochemicals, flavonoids, and saponins (Abbas et al., 2024), *Nigella sativa* can function as an antioxidant by inhibiting non-enzymatic lipid peroxidation, thus enhancing immunity and animal productivity (El-Naggar et al., 2018). These antioxidant properties are considered to be linked to a dimer of thymoquinone, respectively, nigellone (Abd El-Hafeez et al., 2014). Several studies have highlighted the positive effects of *Nigella sativa* supplementation in ruminant diets, including improved nutrient digestibility and milk production (Matthäus and Özcan, 2011; Obeidat, 2020; Shahbazi et al., 2022). Moreover, *in vitro* data from Medjekal et al. (2017) also suggest that *Nigella sativa* exhibits anti-methanogenic properties, with methane emissions reduced by 20%. Beyond its nutritive value, black cumin exhibits broad-spectrum antimicrobial, antifungal, antiviral, and antiparasitic activities (Forouzanfar et al., 2014), with efficacy against both Gram-negative and Gram-positive pathogens (Ishtiaq et al., 2013; Pandey et al., 2025), and can favourably modulate haematological and biochemical parameters in small ruminants (Zanouny et al., 2013).

This trial aims to evaluate the effects of *Nigella sativa* seeds supplementation on the productive performance and health status of lactating dairy cows, in order to enhance milk production efficiency and overall animal health.

Materials and Methods

The study was conducted at the Experimental Farm of the Research and Development Institute for Bovine Balotesti (44°36'46"N 26°4'43"E), Romania, between November-December 2023, following the practices and standards approved by Romanian Law no. 43/2014 and the Council Directive 2010/63/EU regarding the protection and handling of animals used for scientific purposes. A total of 40 multiparous Romanian Black and Spotted dairy cows were divided into two equal groups: the experimental group E (n=20 heads) and the control group C (n=20 heads). The experimental group received 20 g *Nigella sativa* seeds/head/day for 28 consecutive days. The *Nigella sativa* seeds were purchased from certified suppliers. No adaptation period was implemented before the start of the experiment. All animals were transitioned directly to the experimental diet on Day 1 of the study, ensuring uniform exposure to the dietary treatment from the outset. The cows that were included in the study were balanced for age, live weight, and milk yield (E group: 3.5 years old, 500-600 live weight, with an average of 21.96 kg of milk/head/day; C group: 4 years old, 500-600 live weight, with an average of

20.85 kg of milk/head/day). The cows were housed under tied stanchion barn conditions. All cows were fed twice daily, the diet/head/day consisting of 5 kg alfalfa hay, 30 kg corn silage, and 5 kg concentrates, salt, and water *ad libitum*. The chemical composition (dry matter, organic matter, crude protein, crude fat, crude fiber, nitrogen-free extract, and crude ash) of the feeds used in the diet of the dairy cows (alfalfa hay, corn silage, and concentrate) was determined using the classical WEENDE system of feed analysis. The chemical composition of black cumin (*Nigella sativa*) seeds was obtained from published literature sources (Ahmad et al., 2013; Srinivasan, 2018). The energy and protein values of the feeds and black cumin seeds were calculated based on the determined chemical composition and regression equations established, which are widely applied in Romanian nutritional research for ruminants (Stoica and Stoica, 2001). The dietary formulation was based on the nutritional requirements established by the National Research Council (2001), ensuring compliance with the recommended standards for lactating dairy cows based on their milk production and body weight. The nutritional value of the feeds and the composition of the diets used in the study are presented in Tables 1 and 2.

Table 1. The nutritional value of the feeds used in the study (g/kg forage)

| Nutritive crude value | DM, kg | MNU | DIPN, g | DIPE, g | Ca, g | P, g |
|------------------------------------|--------|------|---------|---------|-------|------|
| Alfalfa hay | 0.87 | 0.64 | 93 | 75 | 10 | 1.9 |
| Corn silage | 0.26 | 0.21 | 13 | 17 | 1.2 | 0.5 |
| Concentrates | 0.90 | 0.98 | 123 | 85 | 9.7 | 6.5 |
| <i>Nigella sativa</i> seeds | 0.94 | 0.96 | 95 | 74 | 10 | 5 |

DM=dry matter; MNU=milk nutrition units; DIPN=digestible intestine protein allowed by the nitrogen content of the fodder; DIPE=digestible intestine protein allowed by the energy content of the fodder; Ca=calcium; P=phosphorus.

Table 2. The diet used in dairy cows feed during the study

| Feed | Kg | 15-18 | 13-15 | 1200-1350 | 1200-1350 | 90 | 54-60 |
|--------------------------|--------------|--------------|--------------|----------------|-----------------|--------------|-------------|
| | | DM, kg | MNU | DIPN, g | DIPE, g | Ca, g | P, g |
| Alfalfa hay | 5 | 4.35 | 3.2 | 465 | 375 | 50 | 9.5 |
| Corn silage | 30 | 7.8 | 6.3 | 390 | 510 | 36 | 15 |
| Basic ratio input | 35.00 | 12.15 | 9.5 | 855 | 885 | 86 | 24.5 |
| Concentrates | 5.0 | 4.5 | 4.9 | 615 | 425 | 48.5 | 32.5 |
| Total (C) | 40.00 | 16.65 | 14.4 | 1.470 | 1.310 | 134.5 | 57 |
| <i>Nigella sativa</i> | 0.02 | 0.01 | 0.01 | 1.9 | 1.48 | 0.20 | 0.10 |
| Total (E + C) | 40.02 | 16.66 | 14.41 | 1.471,9 | 1.311,48 | 134.7 | 57.1 |

C=diet supplemented with 0 g *Nigella sativa* seeds/had/day; E=diet supplemented with 20 g *Nigella sativa* seeds yeast/had/day; DM=dry matter; MNU=milk nutrition units; DIPN=digestible intestine protein allowed by the nitrogen content of the fodder; DIPE=digestible intestine protein allowed by the energy content of the fodder; Ca=calcium; P=phosphorus.

Cows were milked twice daily (5:00 AM and 5:00 PM), milk samples (30 ml) were collected during morning and evening milking, before feeding, and stored at 4°C until analysis. Milk quality assessments, including fat (%), protein (%), and lactose (%), were performed using the Ekomilk 120 ultrasonic analyzer. Milk and blood samples were collected at the start of the experiment (day 0) and the end of the experiment (day 28). Blood samples were collected from the mammary vein in vacutainer tubes with K₃EDTA for hemoleukogram (2 ml/tube, and chilled to +4 °C), and sterile vacutainer tubes (9 ml/tube, centrifuged at 6000 rpm, 8 minutes) for biochemical examination, at 2–4 hours post-morning feeding. For cortisol analysis, blood samples were clotted at room temperature for 30–60 minutes, then centrifuged at 3000 rpm (~1500 × g) for 15 minutes at 20–22 °C. Red blood cell (10⁶/μl), hemoglobin (g/dl), hematocrit (%), platelets (10³/μl), white blood cell (10³/μl), and leukocyte differential count: lymphocytes (%), monocytes (%), total neutrophils (%), were performed using an automated analyser Abacus Junior Vet 5. Glucose (mg/dl), fructosamine (μmol/l), total proteins (g/dl), blood urea nitrogen (mg/dl), creatinine (mg/dl), uric acid (mg/dl), alanine aminotransferase (IU/L), gama-glutamyl transferase (IU/L), alkaline phosphatase (IU/L), and amylase (IU/L), were determined using the automated biochemical analyzer Spotchem EZ SP-4430. For serum cortisol analysis (ng/ml), ELISA kits (Cortisol ELISA Kit, DiaMetra) were used. Statistical analyses were carried out using Minitab Statistical Software (version 17). The data were expressed as mean ± standard error of the mean (SEM) and coefficient of variation (CV, %). To test the influence of *Nigella sativa* seeds on the productive performances and blood parameters studied, the Student t-test was used.

The statistical model was:

$$Y_{ijk} = \mu + T_i + e_{ijk}$$

where:

Y_{ijk} = dependent variables

μ = overall mean

T_i = fixed effect of treatment i

e_{ijk} = residual experimental errors

Differences were considered statistically significant at $P \leq 0.05$, and trends $P \leq 0.1$

Results and Discussion

The animals included in the study achieved an average daily intake ranging between 16.65–16.66 kg DM/head/day, 14.40–14.41 MNU, 1.470–1.471.9 g DIPN, 1.310–1.311.48 g DIPE, 134.5–134.7 g Ca, and 57–57.1 g P, values that fall within standard nutritional requirements relative to body weight and milk yield. The results regarding the effects of *Nigella sativa* seeds on milk yield and milk composition are presented in Table 3. Milk yield was not affected by the addition of *Nigella sativa* seeds (E: 20.32 ± 0.86 kg/head/day vs. C: 19.03 ± 1.14 kg/head/day, $P=0.1907$) over the 28-day trial ($P>0.05$). For fat percentage, the experimental group ($3.92 \pm 0.34\%$) and the control group ($3.65 \pm 0.16\%$) differed slightly, with a nonsignificant difference ($P=0.2810$). Milk protein levels were also similar at the start of the trial ($4.09 \pm 0.07\%$ vs. $3.95 \pm 0.11\%$) and remained comparable after 28 days ($3.97 \pm 0.14\%$ vs. $3.88 \pm 0.14\%$), with no significant changes ($P=0.3333$). Lactose percentage changed slightly at the end of the period ($4.66 \pm 0.05\%$ in the experimental group compared to $4.51 \pm 0.11\%$ in the control group), without statistical significance ($P=0.1606$). Contrary to our findings, several studies that investigated the use of *Nigella sativa* seeds in the diets of ruminants (sheep, goats, and water-buffalo) have reported beneficial effects on the efficiency of feed utilization as a result of improved nutrient digestibility coefficients, as well as an improvement in milk production (Randa, 2007; Sanad, 2010; Zounouny et al., 2012; Odhaib et al., 2018). Moreover, Mohamed et al. (2003) demonstrated that dietary supplementation with 100 mg *Nigella sativa* seeds/kg of body weight increased the milk fat content in goats and sheep. In a study conducted by Safaa (2005), the use of 5 g *Nigella sativa* seeds/head/day led to a significant increase in milk fat concentration and milk protein concentration in lactating ewes. Likewise, Sanad (2010) found that using 50 mg or 100 mg *Nigella sativa* seeds/kg of body weight significantly increased the fat and protein concentrations in milk from lactating water buffalo. Furthermore, after longer intervals of administering 80 g *Nigella sativa* seed powder /head/day, Goswami et al. (2018) reported an increase in milk production in dairy cows. In contrast, El-Saadany et al. (2008) reported that the percentage of fat and the percentage of protein in milk did not increase significantly when the diet of lactating goats was supplemented with *Nigella sativa* seeds. We therefore assume that the shorter administration interval in our pilot study may have limited the full expression of *Nigella sativa*'s physiological effects in the experimental group. In this study, *Nigella sativa* seeds were administered for 28 days at a dosage of 20 g seeds/head/day. While this dosage aligns with values reported in the literature, the relatively brief duration may have constrained the manifestation of its potential benefits. Previous studies have typically employed longer supplementation periods, ranging from 30 to 90 days, depending on species and experimental design. In this context, although the dosage used in our study

falls within the recommended range (20–50 g/day), the shorter administration period may have been a limiting factor in capturing the full spectrum of *Nigella sativa*'s beneficial effects on performance and health indicators in dairy cows.

Table 3. Effects of *Nigella sativa* seeds on milk yield and milk composition in dairy cows

| Parameters | E group | | C group | | P |
|--------------------------------|------------|-------|------------|-------|----------|
| | X±SEM | CV | X±SEM | CV | |
| Milk yield, kg/head/day | | | | | |
| Start (0 day) | 21.96±1.08 | 21.98 | 20.85±0.99 | 21.38 | P=0.2141 |
| Final (28 days) | 20.32±0.85 | 18.73 | 19.03±1.14 | 26.82 | P=0.1907 |
| Fat, % | | | | | |
| Start (0 day) | 4.64±0.14 | 13.84 | 4.66±0.16 | 15.78 | P=0.4537 |
| Final (28 days) | 3.92±0.34 | 38.75 | 3.65±0.16 | 20.57 | P=0.2810 |
| Protein, % | | | | | |
| Start (0 day) | 4.09±0.07 | 8.13 | 3.95±0.11 | 12.77 | P=0.1513 |
| Final (28 days) | 3.97±0.14 | 16.15 | 3.88±0.14 | 16.25 | P=0.3333 |
| Lactose, % | | | | | |
| Start (0 day) | 4.62±0.04 | 4.63 | 4.67±0.03 | 3.54 | P=0.1991 |
| Final (28 days) | 4.66±0.05 | 5.72 | 4.51±0.11 | 11.48 | P=0.1606 |

E=experimental group; C=control group.

The haematological parameters (red blood cells, hematocrit, white blood cells, monocytes, and neutrophils) were not influenced ($P>0.05$) by the addition of *Nigella sativa* seeds in the diet of the dairy cows (Table 4). Although the lymphocyte percentage in the experimental group remained stable at 54.19% throughout the study, the statistically significant difference ($P = 0.0479$) compared to the control group (59.26%) suggests that *Nigella sativa* seeds supplementation may have contributed to maintaining lower lymphocyte levels. However, since no intra-group change was observed, this effect may reflect pre-existing differences rather than a direct immunomodulatory impact. For the platelets, the experimental group had a significantly lower number of platelets (E: $255.65 \pm 11.16 \times 10^3/\mu\text{l}$) compared with the control group (C: $309.10 \pm 17.5 \times 10^3/\mu\text{l}$), with a significant P-value of 0.0090. After 28 days, although both groups showed lower counts compared to the start, the difference was statistically nonsignificant (E: $241.65 \pm 13.9 \times 10^3/\mu\text{l}$ vs. C: $277.95 \pm 18.9 \times 10^3/\mu\text{l}$, $P=0.0952$). The early significant reduction in platelet count in the experimental group (E) could indicate an anti-inflammatory or immunomodulatory response, which may equilibrate with time. This finding is in line with previous studies that have reported beneficial changes in immune parameters following *Nigella sativa* supplementation in ruminants (Sadarman et al., 2021). For instance, Habeeb and El Tarabany (2012) have shown that supplementing diets with different levels of *Nigella sativa* seeds significantly increased the concentration of hemoglobin and leukocytes in lactating goats.

Table 4. Effects of *Nigella sativa* seeds on hematological parameters in lactating dairy cows

| Parameters | E group | | C group | | P |
|---|-------------|-------|-------------|-------|-----------------|
| | X±SEM | CV | X±SEM | CV | |
| Red blood cells, 10⁶/μl | | | | | |
| Start (0 day) | 6.69±0.11 | 7.85 | 6.76±0.14 | 9.72 | P=0.3590 |
| Final (28 days) | 6.74±0.99 | 6.59 | 6.56±0.13 | 9.36 | P=0.1570 |
| Hemoglobin, g/dl | | | | | |
| Start (0 day) | 10.16±0.15 | 6.67 | 9.76±0.22 | 19.16 | P=0.0616 |
| Final (28 days) | 10.02±0.14 | 6.33 | 9.73±0.18 | 8.36 | P=0.1567 |
| Hematocrit, % | | | | | |
| Start (0 day) | 28.39±0.41 | 6.56 | 27.83±0.59 | 9.48 | P=0.1795 |
| Final (28 days) | 28.22±0.34 | 5.51 | 27.33±0.59 | 9.68 | P=0.1215 |
| Platelets, 10³/μl | | | | | |
| Start (0 day) | 255.65±11.6 | 20.24 | 309.10±17.5 | 25.37 | P=0.0090 |
| Final (28 days) | 241.65±13.9 | 25.66 | 277.95±18.9 | 30.42 | P=0.0952 |
| Withe blood cells, 10³/μl | | | | | |
| Start (0 day) | 9.09±0.62 | 30.75 | 9.45±0.50 | 24.05 | P=0.3245 |
| Final (28 days) | 9.30±0.42 | 20.81 | 9.13±0.39 | 19.38 | P=0.3912 |
| Lymphocytes, % | | | | | |
| Start (0 day) | 54.60±1.98 | 15.20 | 58.21±2.36 | 19.30 | P=0.1633 |
| Final (28 days) | 54.19±2.52 | 20.81 | 59.26±1.93 | 14.55 | P=0.0479 |
| Monocytes, % | | | | | |
| Start (0 day) | 7.20±0.89 | 55.57 | 8.48±0.61 | 32.60 | P=0.1141 |
| Final (28 days) | 5.71±0.89 | 70.05 | 5.12±0.94 | 82.82 | P=0.6761 |
| Neutrophils, % | | | | | |
| Start (0 day) | 34.5±1.81 | 23.42 | 36.91±2.23 | 27.00 | P=0.2339 |
| Final (28 days) | 36.87±2.10 | 23.64 | 39.67±1.64 | 19.90 | P=0.1299 |

E=experimental group; C=control group.

The descriptive statistic for serum biochemical indicators is presented in Table 5. The glucose levels were similar between the two groups (E: 54.00±0.93 mg/dl vs. 55.2±0.72 mg/dl; P=0.1569) at day 0. After 28 days, a significant increase in glucose levels was found in the experimental group (56.25±1.59 mg/dL) compared with the control group (51.35±2.03 mg/dl; P=0.0473). This increase may point toward improved energy metabolism or altered insulin sensitivity associated with the supplementation. The fructosamine levels, which reflect average blood glucose concentration over the preceding 2-3 weeks (Shohat et al., 2021), did not differ significantly at either time point. Similarly, total protein levels remained stable between the groups, suggesting no adverse effects on protein metabolism. Blood urea nitrogen and creatinine are valuable markers for protein metabolism and renal function, respectively (Avila et al., 2025). In our study, the obtained values did not show significant differences (P>0.05) at the end of the trial. This could indicate that kidney function was maintained despite the dietary changes. For the uric acid, there was a significant difference at 28 days for the

experimental group showing lower uric acid levels (E: 0.82 ± 0.03 mg/dl vs. C: 0.92 ± 0.02 mg/dl; $P=0.0222$).

Table 5. Effects of *Nigella sativa* seeds on biochemical parameters in lactating dairy cows

| Parameters | E group | | C group | | P |
|--|-------------------|--------|------------------|-------|-----------------|
| | X \pm SEM | CV | X \pm SEM | CV | |
| Glucose, mg/dl | | | | | |
| Start (0 day) | 54.00 \pm 0.93 | 7.74 | 55.2 \pm 0.72 | 5.84 | P=0.1569 |
| Final (28 days) | 56.25 \pm 1.59 | 13.88 | 51.35 \pm 2.03 | 16.17 | P=0.0473 |
| Fructosamine, μmol/l | | | | | |
| Start (0 day) | 210.6 \pm 2.06 | 4.37 | 208.5 \pm 4.49 | 9.63 | P=0.3366 |
| Final (28 days) | 201.75 \pm 1.89 | 4.19 | 201.6 \pm 2.60 | 5.76 | P=0.4823 |
| Total-Protein, g/dl | | | | | |
| Start (0 day) | 7.34 \pm 0.08 | 4.89 | 7.53 \pm 0.12 | 7.16 | P=0.1355 |
| Final (28 days) | 7.13 \pm 0.07 | 4.39 | 7.11 \pm 0.09 | 6.00 | P=0.4008 |
| Urea Nitrogen, mg/dl | | | | | |
| Start (0 day) | 6.65 \pm 0.35 | 24.04 | 6.35 \pm 0.36 | 25.69 | P=0.2581 |
| Final (28 days) | 9.6 \pm 0.46 | 21.48 | 9.7 \pm 0.61 | 28.20 | P=0.4526 |
| Creatinine, mg/dl | | | | | |
| Start (0 day) | 0.93 \pm 0.02 | 10.57 | 0.90 \pm 0.02 | 14.55 | P=0.2301 |
| Final (28 days) | 1.06 \pm 0.02 | 9.87 | 1.05 \pm 0.02 | 9.52 | P=0.3705 |
| Uric acid, mg/dl | | | | | |
| Start (0 day) | 0.76 \pm 0.01 | 7.68 | 0.77 \pm 0.02 | 11.75 | P=0.3469 |
| Final (28 days) | 0.82 \pm 0.03 | 17.49 | 0.92 \pm 0.02 | 13.48 | P=0.0222 |
| ALT, IU/L | | | | | |
| Start (0 day) | 64.5 \pm 2.56 | 17.76 | 69.15 \pm 3.42 | 22.12 | P=0.1452 |
| Final (28 days) | 71.2 \pm 2.23 | 14.00 | 58.5 \pm 1.44 | 11.02 | P=0.0004 |
| GGT, IU/L | | | | | |
| Start (0 day) | 17.95 \pm 1.30 | 32.46 | 16.00 \pm 1.17 | 32.57 | P=0.1305 |
| Final (28 days) | 24.65 \pm 2.42 | 43.86 | 18.85 \pm 2.66 | 63.22 | P=0.0302 |
| ALP, IU/L | | | | | |
| Start (0 day) | 56.8 \pm 3.78 | 29.80 | 54.35 \pm 3.79 | 31.18 | P=0.2550 |
| Final (28 days) | 56.7 \pm 4.14 | 32.68 | 49.85 \pm 3.00 | 26.92 | P=0.0747 |
| Amylase, IU/L | | | | | |
| Start (0 day) | 326.20 \pm 15 | 20.57 | 353.3 \pm 15.3 | 19.40 | P=0.1134 |
| Final (28 days) | 312.55 \pm 12.1 | 17.24 | 261.4 \pm 11.5 | 19.67 | P=0.0032 |
| Cortisol, ng/ml | | | | | |
| Start (0 day) | 27.07 \pm 8.58 | 141.74 | 29.71 \pm 6.46 | 97.22 | P=0.3650 |
| Final (28 days) | 15.97 \pm 6.32 | 176.90 | 23.54 \pm 6.48 | 123.2 | P=0.1998 |

E=experimental group; C=control group; ALT=alanine aminotransferase; GGT=gama-glutamyl transferase; ALP=alkaline phosphatase.

Lower uric acid levels could be associated with changes in purine metabolism or an enhanced antioxidant status (Zhang et al., 2022). ALT and ALP are liver enzymes that serve as markers used in the determination of liver function (Ojediran et al., 2024). In our study, at the end of the trial, the experimental group showed

significantly higher ALT levels (E: 71.2 ± 2.23 IU/L vs. C: 58.5 ± 1.44 IU/L; $P=0.0004$). Similarly, GGT levels increased significantly in the experimental group at 28 days (24.65 ± 2.42 IU/L vs. 18.85 ± 2.66 IU/L; $P=0.0302$), while changes in ALP were not statistically significant. While elevated liver enzymes could be a sign of hepatic stress, within the physiological context, the increases in ALT and GGT might be part of an enhanced metabolic process attributed to the bioactive compounds in black cumin. Amylase levels significantly dropped in the experimental group at 28 days (312.55 ± 12.1 IU/L vs. 261.4 ± 11.5 IU/L; $P=0.0032$). This may indicate an effect on pancreatic enzyme activity or improved carbohydrate digestion efficiency. Cortisol levels, which are a stress indicator (Grelet et al., 2022), showed a reduction in the experimental group compared to the control group (E: 15.97 ± 6.32 ng/ml vs. C: 23.54 ± 6.48 ng/ml) without statistical significance ($P=0.1998$) at 28 days. This suggests that there might be a trend toward reduced stress levels under supplementation. In a trial conducted by Forslund et al. (2010), the average cortisol values were between 10 nmol/l and 30 nmol/l (3.62 ng/ml–10.87 ng/ml) in healthy dairy cows. Similarly, Nedic et al. (2017) reported serum cortisol values between 4 ng/ml and 26 ng/ml in Holstein dairy cows kept under intensive systems, limits being in accordance with the ones registered by us.

Conclusions

The dietary inclusion of *Nigella sativa* seeds in the lactating dairy cows' diet did not significantly affect milk yield or milk composition over the 28-day trial. Hematological assessments revealed that most blood parameters (red blood cells, hemoglobin, leukocytes, neutrophils, and monocytes) were not influenced by the inclusion of *Nigella sativa* seeds. However, a significant reduction in the lymphocyte percentage was observed in the E group, suggesting a potential immunomodulatory effect. Biochemical analyses showed significant differences in several metabolic markers, including increased glucose levels, liver enzyme activities (ALT and GGT), reduced amylase activity, and lower uric acid concentrations following supplementation. These changes indicate that *Nigella sativa* may influence energy metabolism, liver function, and enzymatic activity in a manner that could be reflective of adaptive metabolic responses to its bioactive components. Overall, *Nigella sativa* can be used as a dietary supplement in dairy cow nutrition without negatively impacting milk production or health. Future research should aim to determine the optimal dosage and evaluate the long-term effects of *Nigella sativa* supplementation in dairy cattle, in order to fully realize its potential as a functional feed additive for improving health, productivity, and metabolic resilience in modern dairy production systems.

Efekti dodavanja u obrok crnog kumina (*Nigella sativa* L.) na produktivnost i zdravlje mlečnih krava

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Rezime

Cilj ove studije bio je da se proceni efekat dodavanja u obrok semena crnog kumina (*Nigella sativa*) na produktivne performanse i zdravstveno stanje mlečnih krava u laktaciji. Studija je sprovedena na eksperimentalnoj farmi Instituta za istraživanje i razvoj goveda Balotești, Rumunija, između novembra i decembra 2023. godine, na 40 rumunskih crno-šarenih mlečnih krava podeljenih u dve grupe: eksperimentalna grupa E (n=20 grla) i kontrolna grupa C (n=20 grla). Eksperimentalna grupa je primala 20 g semena crnog kima/grlo/dan tokom 28 uzastopnih dana. Uzorci mleka i krvi su prikupljeni 0. i 28. dana. Statistička poređenja su izvršena korišćenjem Studentovog t-testa. Rezultati su pokazali statističke razlike za limfocite (P=0,0479), glukozu (P=0,0473), mokraćnu kiselinu (P=0,0222) i amilazu (P=0,0032) u eksperimentalnoj grupi (E) nakon 28-dnevnog ispitivanja. Seme crnog kumina (*Nigella sativa*), zbog svojih bioaktivnih i antioksidativnih sastojaka, pokazalo je obećavajući potencijal kao prirodni dodatak ishrani za poboljšanje fiziološke stabilnosti mlečnih krava.

Ključne reči: *Nigella sativa*, mlečne krave, produktivne performanse, krvni profil.

Acknowledgement

The results of this research were financed by the Research and Development Institute for Bovine Balotesti, project no. 5309/2023.

Conflict of interest

The authors declare that they have no conflict of interest.

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Received 8 July 2025; accepted for publication 20 September 2025