

Balji, Yu., Abakanova, G., Sultanayeva, L., Mayer, Y., Kravchenko, O., Zamaratskaia, G. (2026). Optimization of extruded enriched feed additives production process and their effect on blood parameters of Holstein cows (2026). *Agriculture and Forestry*, 72 (2): 123-135. <https://doi:10.17707/AgricultForest.72.2.09>

DOI: 10.17707/AgricultForest.72.2.09

**Yuriy BALJI<sup>1,2</sup>, Gulim ABAKANOVA<sup>1</sup>, Leila SULTANAYEVA<sup>1,2</sup>,  
Yevgeniy MAYER<sup>1,2</sup>, Oksana KRAVCHENKO<sup>3</sup>, Galia ZAMARATSKAIA<sup>3</sup>**

## **OPTIMIZATION OF EXTRUDED ENRICHED FEED ADDITIVES PRODUCTION PROCESS AND THEIR EFFECT ON BLOOD PARAMETERS OF HOLSTEIN COWS**

### **SUMMARY**

Feeding strategies improve livestock productivity and product quality. This study optimized extruded enriched feed additive (EEFA) production and evaluated short-term blood effects in primiparous Holstein cows. EEFA (corn-based, 1.5% plant extract) process optimization (die 2-8 mm, moisture 5-30 L) identified 8 mm die and 15-17% moisture as optimal (650 kg/h, low losses). NIRS showed reduced moisture (8%) with concentrated protein (10.2%), fat (4%), fiber (3.8%), ash (2.8%). In a 20-day trial, 37 primiparous Holstein cows (60 days post-calving, 450-470 kg BW, 28-29 kg milk/day) received a standard basal ration or basal ration with 3 kg EEFA/cow/day as-fed (2.6-2.7 kg DM, two 1.5-kg portions; convenience allocation without randomization or baseline balance check). No hematological changes occurred. Biochemical effects included higher ALT/cholesterol, lower urea ( $P < 0.05$ ), suggesting metabolic shifts. Further studies should also include animal health monitoring, rumen function assessment, and detailed nutritional evaluation to better understand the mechanisms behind the observed metabolic changes.

**Keywords:** extrusion, probiotics, feed, cows, blood test.

### **INTRODUCTION**

Ensuring a balanced healthy diet is essential for animal health, productivity, and the quality of products (Shahid *et al.*, 2020). High-quality feed improving both the safety and nutritional value of food of animal-derived foods (Prache *et al.*, 2022). Grains from cereals, legumes, and forage crops are rich sources of essential nutrients, but some components (certain fibers, complex

---

<sup>1</sup>Yuriy Balji, Gulim Abakanova, Leila Sultanayeva, Yevgeniy Mayer, Faculty of Veterinary and Animal Husbandry Technology, Kazakh Agrotechnical Research University named after S. Seifullin; LLP «NFT-KATU», Astana, REPUBLIC OF KAZAKHSTAN;

<sup>3</sup>Oksana Kravchenko, Galia Zamaratskaia (corresponding author: [galia.zamaratskaia@slu.se](mailto:galia.zamaratskaia@slu.se)), Department of Molecular Sciences, Swedish University of Agricultural Sciences, Uppsala, SWEDEN;

Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

Received: 01/03/2026

Accepted: 27/04/2026

carbohydrates) are not easily digested, resulting in lower nutrient availability (Humer and Zebeli, 2017).

In ruminants, most dietary starch is fermented in the rumen, producing volatile fatty acids, primary energy source. However, a portion of starch can escape ruminal fermentation and reach the small intestine, where digestion and absorption are limited (Nozière *et al.*, 2010). The efficiency of starch utilization depends on grain type, processing, and diet composition. Therefore, processing technologies that improve nutrient availability and feed digestibility are important in enhancing animal health and production efficiency (Tretola *et al.*, 2025). Extrusion is a modern leading grain processing method (Risvahadi *et al.*, 2023). During extrusion, starch gelatinization and partial depolymerization due to temperature, pressure, and shear forces; improving digestibility, reduced crystallinity, enhancing feed palatability (Huang *et al.*, 2022). Although endogenous enzymes are inactivated, the polysaccharide breakdown occurs through thermo-mechanical cleavage of glycosidic bonds, producing lower-molecular-weight fragments including dextrins (Camire *et al.*, 1990). In addition, extrusion inactivates anti-nutritional factors - trypsin inhibitors and urease (Świątkiewicz *et al.*, 2021), while improving the water absorption index, solubility index, and starch gelatinization (Gandhi *et al.*, 2020). Extrusion influences physical properties of feeds, reducing humidity, bulk density, and angle of repose due to the expansion caused by starch gelatinization (Igorov *et al.*, 2021). Processing parameters, such as temperature, screw speed, and moisture content, create trade-offs between flowability, energy efficiency, and final product quality (McGuire *et al.*, 2022). When properly optimized, extrusion improves protein and starch digestibility, reduces anti-nutritional compounds, and minimizes nutrient losses, while improving the texture, taste, and aroma of the feed, contributing to better feed intake (Giallongo *et al.*, 2015). These mechanisms, promote improved nutrient absorption, productivity, and nitrogen utilization, improving the quality and quantity of products (Claassen *et al.*, 2016). To further enhance the nutritional value, to strengthen immunity, enhance nutrient use, and antibiotic-free animal production natural phytobiotic additives based on plant extracts containing flavonoids, phenolic acids, and essential oils are used (Liu *et al.*, 2025). In this context, this study had two objectives: (1) to optimize the technological conditions of extruded enriched feed additive (EEFA) production, focusing on die diameter, moisture content, and process efficiency; and (2) to evaluate the short-term influence of the optimized EEFA on hematological and biochemical blood parameters of primiparous Holstein cows.

## MATERIAL AND METHODS

**Process optimization.** The optimization of manufacturing processes was carried out at LLP “NFT-KATU» and the effectiveness of EEFA in the diet of primiparous cows during lactation was evaluated at “Agro-firm Rodina” LLP in the Akmola region. EEFA used in the experiment was produced by “NFT-KATU” LLP (BioFeed, 2025) and was composed of a corn extrudate base,

enriched with a plant extract containing ~ 55% fatty acids (stearic, oleic, palmitic), 2.5% organic acids (glycolic, malic, citric), 1.9% carbohydrates (glucose, fructose, ribose), 1% essential oils, 2% phenolic acids, 6.8% flavonoids (pinostrobin, pinocembrine, chrysin), added vitamins (A and C), microelements (iodine, zinc, manganese, copper, cobalt), and amino acids (asparagine, alanine, valine, leucine). The extract was incorporated into the corn extrudate at 1.5% of the final product. Accordingly, the final EEFA contained ~ 0.83% fatty acids, 0.10% flavonoids, 0.04% organic acids, 0.03% phenolic acids, 0.03% carbohydrates, and 0.02% essential oils (as-fed). EEFA was produced using corn as the primary raw material (160 kg per batch). Enrichment was achieved by incorporating a liquid extract before extrusion: 50-350 mL of the phytobiotic additive were homogenized with 5-30 L of water and mixed with the dry corn mass in a horizontal mixer to ensure uniform distribution of the bioactive components. The mixture was extruded using a single-screw laboratory unit equipped with 2-8 mm dies, then dried and granulated to obtain the final feed additive used in the feeding experiment.

Process optimization was performed on a technological line comprising a PD-2000 pneumatic crusher, an EP-350 extruder, a PG-600 granulator, and an SG-800 mixer, with bunkers equipped with discharge screws and auxiliary equipment (Agrotechservice-12, Kazakhstan, 2020). The entire system was interconnected with the electric grid, utilizing a three-phase, 380-volt power supply. Two parameters were systematically varied: die diameter (2, 3, 5, 8 mm) and the amount of water added to the mixture (5-30 L per batch), were chosen because they strongly influence on mass plasticity, extrusion pressure/temperature, equipment load, pellet integrity, and drying requirements. For each combination of die diameter and moisture content, were recorded: throughput ( $\text{kg h}^{-1}$ ), current strength (I, A) for energy consumption assessment, process losses, and pellet quality (Table 1).

**Table 1.** Treatment combinations used for optimization of extruded enriched feed additive production

| Parameter                     | Treatment 1 | Treatment 2 | Treatment 3 | Treatment 4 |
|-------------------------------|-------------|-------------|-------------|-------------|
| Die diameter (mm)             | 2           | 3           | 5           | 8           |
| Water (L)                     | 5-30        | 5-30        | 5-30        | 5-30        |
| Phytobiotic supplement (ml)   | 50-350      | 50-350      | 50-350      | 50-350      |
| Resource-saving eco-food (kg) | 160         | 160         | 160         | 160         |

Optimal conditions were determined as stable operation without critical stops, high throughput, moderate energy consumption (avoiding peaks of 50-

60 A), losses < 5 %, and dense, non-caking pellets with minimal fines. The most efficient operation was achieved at a die diameter of 8 mm and moisture content of 15-17 %, ensuring maximum productivity (up to 650 kg h<sup>-1</sup>) with moderate energy consumption (30-40 A). EEFA batches produced under these optimized conditions were used in the feeding trial. The proximate composition (moisture, crude protein, ether extract, crude fiber, ash, and starch) was analyzed by NIRS (Foss, Denmark, 2016).

**Feeding trial.** In a 20-day trial, 37 primiparous Holstein cows (60 days in milk, 450-470 kg body weight, 28-29 kg milk/day) were allocated to two groups via convenience sampling based on farm routine, productivity, and physiological state to minimize production disruption: no randomization or baseline balancing was performed. The control group (n=20) received the farm's standard basal ration (18.9 kg DM/day; Table 2). The experimental group (n=17) received the same basal ration plus 3 kg/day EEFA (as-fed; two 1.5-kg portions; 2.6-2.7 kg DM), increasing total DM intake to 21-22 kg/day. All cows were housed in free-stall conditions, milked twice daily, fed twice daily, and had ad libitum water access. The sample size was determined by the number of eligible animals available at the commercial farm.

**Table 2.** Ingredient composition and calculated nutrient composition of the basal total mixed ration

| Feed component                | Daily intake (kg)      |                           |
|-------------------------------|------------------------|---------------------------|
| Corn silage (30-35% DM*)      | 21.81                  |                           |
| Wet brewers' grains           | 7.76                   |                           |
| Alfalfa haylage               | 5.00                   |                           |
| Wheat meal                    | 3.47                   |                           |
| Barley haylage                | 3.00                   |                           |
| Corn meal                     | 3.00                   |                           |
| EnergoKAN 3 (BVMK)            | 1.60                   |                           |
| Dried distillers' grains      | 1.20                   |                           |
| Rapeseed meal                 | 0.80                   |                           |
| Sunflower meal                | 0.70                   |                           |
| Alfalfa hay                   | 0.20                   |                           |
| BufferKAN 2%                  | 0.15                   |                           |
| Premix 1%                     | 0.15                   |                           |
| Nutritional value             | Total intake (per day) | Concentration (per kg DM) |
| Dry matter (DM), kg           | 18.9                   |                           |
| Metabolizable energy (ME), MJ | 232.4                  | 12.3                      |
| Crude protein (CP), g         | 3189.9                 | 168.8                     |
| Digestible protein, g         | 1993.9                 | 105.5                     |
| Degradable protein, g         | 2323.8                 | 123.0                     |
| Undegradable protein, g       | 776.7                  | 41.1                      |
| Neutral detergent fiber, g    | 7905.0                 | 418.3                     |
| Crude fiber, g                | 3593.4                 | 190.1                     |

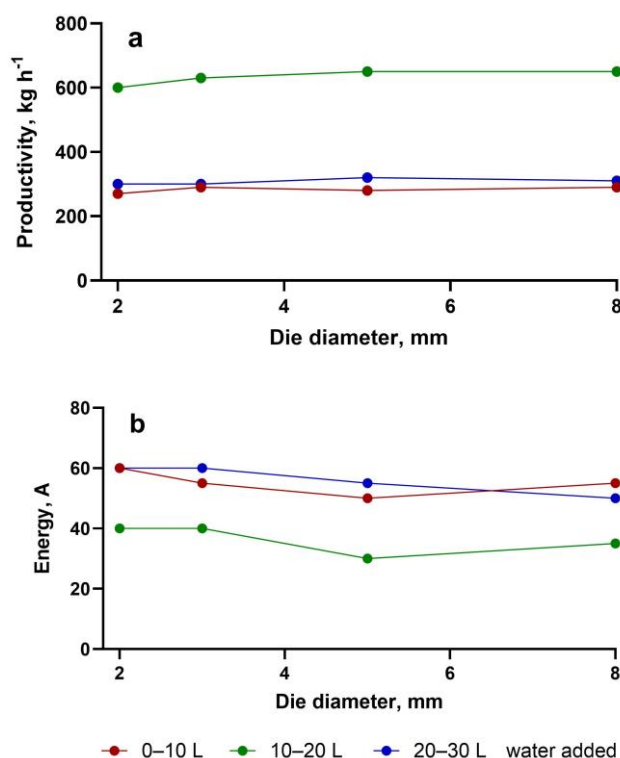
\*Values were based on reference data from Aubakirov and Tlepov (2023)

**Sample collection and biochemical analyses.** Blood samples were collected from all primiparous cows before the start of the experiment (day 0) and after the 20-day feeding period. To standardize physiological conditions, sampling was performed in the morning before feeding and milking (fasted). Blood was drawn from the coccygeal vein using a sterile vacuum system. Blood was collected into K<sub>3</sub>EDTA tubes for hematological analysis and into clot activator tubes for serum biochemical analysis. Blood in serum tubes was allowed to clot at room temperature for 30 min and centrifuged at  $1500 \times g$  for 15 min. Whole blood and serum aliquots were stored at +4 °C and transported refrigerated to the laboratory on the same day and analyzed within 24 h. All analyses were conducted at an accredited testing laboratory (Research Diagnostic Centre "Diagnostic Group" LLP). Biochemical parameters were analyzed using a NeoChem 25 biochemical analyzer (NeoMedica, Belgrade, Serbia). Hematological parameters (WBC, RBC, hemoglobin, platelets) using a Micro CC-20 VET hematological analyzer (DFI, Taiwan).

**Statistical analysis.** The statistical analysis was performed with the Statistical Analysis System (Version 9.4, SAS Institute, Cary, NC, US), using a mixed linear model (PROC MIXED). The model included fixed effects of treatment (with and without dietary supplement), time (at the start and the end of the experiment), and their interaction. A random effect of individual animals was also included in the model. Residual degrees of freedom were calculated using the default method (between-within approach). Model assumptions of normality (Shapiro-Wilk test) and homoscedasticity (residual plots) were verified for all parameters; no transformations were required. The data are presented as Least squares means (LS means) with standard errors (SE) and pairwise comparisons (PDIF). Differences were considered significant at  $P < 0.05$ .

## RESULTS AND DISCUSSION

**Process optimization.** Changing the proportion of EEFA in the formulation did not affect equipment productivity or energy consumption. In contrast, it depended mainly on die diameter and added water (Figure 1). With low water addition (0-10 L), productivity decreased to  $\sim 300 \text{ kg h}^{-1}$ , losses increased to  $\sim 50\%$ . At intermediate moisture (10-20 L), productivity increased markedly (up to about  $650 \text{ kg h}^{-1}$ ) with minimum losses. At high water addition (20-30 L), productivity was slightly higher than at low water addition but clearly lower than at 10-20 L; pellets were wet, prone to clumping, prolonged and complicated drying (Figure 1a). Increasing die diameter increased throughput at all moisture levels, with a difference of approximately  $50 \text{ kg h}^{-1}$  between die sizes within each water range. Both insufficient (0-10 L) and excessive (20-30 L) water addition led to higher energy consumption (50-60 A), whereas intermediate moisture (10-20 L) was associated with lower current draw (30-40 A) across die diameters (Figure 1b). The optimal conditions for EEFA pellet production were an 8 mm die with 15-17% moisture content, providing 600-650  $\text{kg h}^{-1}$  throughput, 30-40 A energy use, and  $\leq 5\%$  losses.

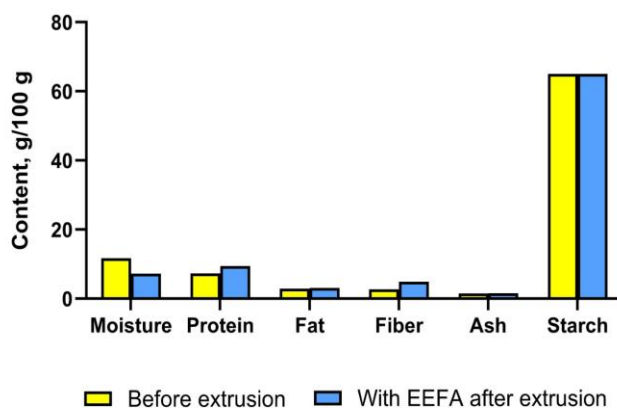


**Figure 1.** a) Effect of die diameter and water addition on (a) productivity (kg h<sup>-1</sup>) and (b) energy consumption (A) during extrusion of enriched feed additive. Lines show water addition levels: 0-10 L, 10-20 L, and 20-30 L per batch

This study evaluated the effects of optimized manufacturing conditions for extruded supplements enriched with phytobiotics on hematological and biochemical blood parameters in dairy cows. Die diameter and the amount of water added, rather than the proportion of EEFA in the formulation, were the main factors that determined equipment performance. Both too little and too much added water reduced process efficiency. Low water levels lowered throughput and increased fines and losses, likely because the mass was poorly plasticized and internal friction increased. High water levels improved performance somewhat but required more drying due to higher pellet moisture (Farha and Hussein, 2023). A similar pattern has been reported in corn extrusion for poultry feed, where increasing water flow rate tended to reduce production rate and energy consumption, while also affecting extrudate physical characteristics (Amornthewaphat *et al.*, 2005). Studies in poultry feed manufacturing have similarly shown that die-hole size, pressure, and temperature are key operating parameters influencing pelleting efficiency and throughput

capacity, confirming the importance of process optimization for stable equipment performance (Amin and Sobhi, 2023).

Composition of non-extruded corn and EEFA. Process optimization and formulation adjustments changed the chemical composition of the EEFA pellets (Figure 2). Moisture content decreased by 38.4%, whereas crude protein, ether extract, crude fiber and ash increased by 28.7%, 6.8%, 84.2% and 3.9%, respectively. The optimized EEFA contained 8.0% moisture, 10.2% crude protein, 4.0% ether extract, 3.8% crude fiber, 2.8% ash, and approximately 77% starch on a dry matter basis. Starch content decreased slightly by 1.6% compared with the pre-extrusion mixture.



**Figure 2.** Chemical composition of non-extruded corn and extruded enriched corn feed additive (EEFA). Bars show values before extrusion and after extrusion for moisture, crude protein, ether extract, crude fiber, ash, and starch

The increases in crude protein, crude fiber, ether extract, and ash should be interpreted primarily because of moisture reduction during extrusion than increases in nutrient mass. Thermo-mechanical processing altered particle size and matrix structure, influencing NIRS spectra and contribute to higher readings, as shown for extruded and ground feeds. NIRS was chosen because it allows rapid, non-destructive analysis and has been validated for feed materials like EEFA (Muramatsu *et al.*, 2015). No wet chemistry verification was performed; however, the NIRS instrument was calibrated against standard feed matrices to ensure reliable compositional estimates. Lower final moisture reduces the risk of microbial spoilage, extends the safe storage period of compound feeds, provided that is not causing excessive fines and dust. In dairy cows, a recent meta-analysis reported that including extruded ingredients in rations improved dry matter and crude protein digestibility and supported higher milk yield (Riswahadi *et al.*, 2023). The modest decrease in starch content together with a high final starch level is consistent with gelatinization and partial depolymerization (shorter-chain carbohydrates and small amounts of maltose), with rapid inactivation of

endogenous amyolytic enzymes, indicating thermomechanical rather than enzymatic transformation (Huang *et al.*, 2022).

### Effects of EEFA on primiparous cow blood biochemistry

The hematological and serum biochemical parameters for animals fed with and without EEFA are presented in Tables 3 and 4. No statistically significant differences were observed in any of the measured hematological parameters (WBC, RBC, HGB, PLT) between treatment groups or over time ( $P>0.05$ ).

**Table 3.** Hematological parameters of primiparous Holstein cows before and after EEFA supplementation (data are presented as LS means and standard error).

| Indicator                           | Norm       | Before the start of the experiment |                               | At the end of the experiment |                              | p-value, treatment | p-value, time | p-value, treatment $\times$ time |
|-------------------------------------|------------|------------------------------------|-------------------------------|------------------------------|------------------------------|--------------------|---------------|----------------------------------|
|                                     |            | Without EEFA                       | With EEFA                     | Without EEFA                 | With EEFA                    |                    |               |                                  |
| WBC, $\times 10^9$ uL <sup>-1</sup> | 5.0-16.0   | 16.2 $\pm$ 5.6                     | 18.3 $\pm$ 4.3                | 16.6 $\pm$ 5.5               | 12.7 $\pm$ 4.4               | 0.869              | 0.626         | 0.566                            |
| RBC, $\times 10^6$ uL <sup>-1</sup> | 5.00-10.10 | 6.4 $\pm$ 1.4                      | 4.6 $\pm$ 1.06                | 4.2 $\pm$ 1.3                | 4.4 $\pm$ 1.06               | 0.617              | 0.299         | 0.375                            |
| HGB, g dL <sup>-1</sup>             | 90-139     | 105.0 <sup>a</sup> $\pm$ 15.2      | 105.2 <sup>a</sup> $\pm$ 11.8 | 74.7 <sup>b</sup> $\pm$ 15.2 | 88.8 <sup>b</sup> $\pm$ 11.7 | 0.662              | 0.084         | 0.560                            |
| PLT, $\times 10^9$ L <sup>-1</sup>  | 120-600    | 286.3 $\pm$ 84.4                   | 305.4 $\pm$ 65.3              | 233.7 $\pm$ 84.3             | 168.2 $\pm$ 65.3             | 0.769              | 0.255         | 0.596                            |

WBC, white blood cell count; RBC, red blood cell count; HGB, hemoglobin; PLT, platelets.

LS means with different superscripts statistically differ at  $p<0.05$

**Table 4.** Blood biochemical parameters in primiparous Holstein cows before and after EEFA supplementation (data are presented as LS means and standard error).

| Indicator                         | Norm       | Before the start of the experiment |                             | At the end of the experiment |                              | p-value, treatment | p-value, time | p-value, treatment $\times$ time |
|-----------------------------------|------------|------------------------------------|-----------------------------|------------------------------|------------------------------|--------------------|---------------|----------------------------------|
|                                   |            | Without EEFA                       | With EEFA                   | Without EEFA                 | With EEFA                    |                    |               |                                  |
| ALT, units L <sup>-1</sup>        | 6.9-35     | 32.8 <sup>a</sup> $\pm$ 2.3        | 10.7 <sup>b</sup> $\pm$ 2.3 | 33.3 <sup>a</sup> $\pm$ 2.3  | 35.0 <sup>a</sup> $\pm$ 2.3  | 0.006              | <0.001        | <0.001                           |
| AST, units L <sup>-1</sup>        | 45-110     | 93.3 $\pm$ 16.2                    | 97.5 $\pm$ 16.2             | 72.3 $\pm$ 16.2              | 109.2 $\pm$ 16.2             | 0.320              | 0.716         | 0.220                            |
| Total protein, g dL <sup>-1</sup> | 6.2-8.2    | 6.7 $\pm$ 0.1                      | 6.7 $\pm$ 0.1               | 6.4 $\pm$ 0.1                | 6.2 $\pm$ 0.1                | 0.776              | 0.002         | 0.496                            |
| Cholesterol, mg dL <sup>-1</sup>  | 64.0-200.0 | 94.1 <sup>a</sup> $\pm$ 8.4        | 133 <sup>b</sup> $\pm$ 8.4  | 122 <sup>b</sup> $\pm$ 8.4   | 139.2 <sup>b</sup> $\pm$ 8.4 | 0.013              | 0.060         | 0.210                            |
| Urea, mmol L <sup>-1</sup>        | 2.8-8.8    | 3.4 <sup>a</sup> $\pm$ 0.5         | 1.7 <sup>b</sup> $\pm$ 0.5  | 6.2 <sup>a</sup> $\pm$ 0.5   | 4.6 <sup>b</sup> $\pm$ 0.5   | 0.013              | 0.005         | 0.895                            |

LS means with different superscripts statistically differ at  $p<0.05$

Among serum biochemical parameters, ALT was significantly affected by EEFA ( $P=0.006$ ) and by time ( $P<0.001$ ). A significant treatment  $\times$  time interaction was also observed ( $P<0.001$ ), with an increase in ALT occurring only in the EEFA group after administration.

Cholesterol concentrations were higher in the EEFA group than in the control group overall ( $P=0.013$ ). Cholesterol increased significantly over time

only in the control group, while no significant change was observed in the EEFA group, and the treatment  $\times$  time interaction was not significant. Urea concentrations were lower in the EEFA group both before and after administration, whereas urea increased in the control group over time. Both treatment ( $P=0.013$ ) and time ( $P=0.005$ ) effects were significant, with no interaction.

No significant differences were observed for AST or total protein, although total protein decreased slightly over time in both groups ( $P=0.002$ ) without a treatment-specific effect.

The inclusion of plant bioactive compounds and extruded forages in the ration may help address nutrient deficiencies by supporting a more balanced diet (Sultanayeva *et al.*, 2023). Changes in biochemical parameters (ALT, urea, and cholesterol) provided further insight into the physiological response to EEFA, likely due to the combined effects of plant-derived compounds. The increase in ALT in the EEFA group remained within normal reference ranges and was not accompanied by changes in AST, suggesting a metabolic rather than pathological cause, possibly related to shifts in liver energy metabolism. Flavonoids and organic acids in EEFA may influence liver enzyme activity through their roles in antioxidant defense, mitochondrial function, and lipid metabolism (Kumprechtová *et al.*, 2022).

Lower urea concentrations in cows receiving EEFA may indicate improved nitrogen utilization, consistent with reports that flavonoids, phenolic acids, and certain fatty acids can reduce ruminal proteolysis, modulate ammonia production, and enhance microbial protein synthesis. This may reflect a greater proportion of nitrogen being incorporated into microbial biomass rather than converted into urea.

Higher cholesterol levels may reflect enhanced lipid absorption or increased hepatic synthesis, potentially related to fatty acids that can influence very low-density lipoprotein (VLDL) production and lipoprotein metabolism. As values remained within normal ranges, this change is unlikely to indicate pathology (Veshkini *et al.*, 2022).

Thus, EEFA may influence hepatic, nitrogen, and lipid metabolism in dairy cows through antioxidant effects, modulation of hepatic enzyme pathways, and changes in microbial fermentation and energy balance. The absence of changes in AST and total protein supports the safety of EEFA at the tested inclusion rate. Further research could improve the efficiency of extruded feed and feed additive production, enhancing both the quality and yield of livestock products. Table 5 summarizes how the effects observed in the present study align with or differ from those reported in previous studies.

### **Study limitations and future research**

The study has several limitations that need to be acknowledged: the duration of the animal trial (20 days) was relatively short for evaluating physiological and metabolic responses in dairy cows; the experiment was constrained by the available farm conditions. Only blood metabolites were measured, but no performance-related data (milk yield/composition, DMI,

BW/BCS, feed efficiency, rumen function, behavior) were collected. Therefore, conclusions regarding the effectiveness of the feed additive must be interpreted with caution. Future research should be based on a longer-term feeding trial with balanced treatment groups, randomization, comprehensive zootechnical data collection, animal health monitoring, rumen function assessment, and detailed nutritional evaluation to better understand the mechanisms behind the observed metabolic changes.

**Table 5.** Comparison of effects between the present study and previous studies

| Parameter     | Effect in the present study                                                        | Reported effect in previous studies                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|---------------|------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cholesterol   | Higher in EEFA group overall; significant increase over time only in control group | <p>Lower in mid-lactating Holstein-tropical crossbred cows fed with 100 g of shade-dried plantain powder, 100 g of shade-dried lemongrass powder, or 50 g each of plantain and lemongrass powder (Rahman <i>et al.</i>, 2024).</p> <p>Lower in heat-stressed early lactating Holstein cows fed 100 g/head/day of the Ovum® herbal mixture supplement (10% nuture betaine and 90% of four herbal plants: flaxseed, fenugreek, curcumin, and peppermint), (Saleh <i>et al.</i>, 2023)</p> <p>Lower in lactating Holstein dairy cows fed with an essential oils blend made of clove, oregano, and juniper essential oils in equal proportions (Al-Suwaiegh <i>et al.</i>, 2020).</p> <p>No effect in transition Holstein cows fed <i>Myrtus communis</i> L. extract (Ozcinar <i>et al.</i>, 2026).</p> |
| Urea          | Lower in EEFA group overall; significant time effect, no interaction               | <p>No effect in mid-lactating Holstein-tropical crossbred cows fed with 100 g of shade-dried plantain powder, 100 g of shade-dried lemongrass powder, or 50 g each of plantain and lemongrass powder (Rahman <i>et al.</i>, 2024).</p> <p>No effect in transition Holstein cows fed <i>Myrtus communis</i> L. extract (Ozcinar <i>et al.</i>, 2026).</p> <p>Lower in mid-to-end lactating Holstein cows fed with a commercial blend of essential oils, primarily menthol (&gt;80%) with smaller amounts of eugenol and anethol (Braun <i>et al.</i>, 2019).</p>                                                                                                                                                                                                                                     |
| Total protein | No effect of EEFA                                                                  | <p>No effect in mid-to-end lactating Holstein cows fed with a commercial blend of essential oils, primarily menthol (&gt;80%) with smaller amounts of eugenol and anethol (Braun <i>et al.</i>, 2018).</p> <p>No effect in transition Holstein cows fed <i>Myrtus communis</i> L. extract (Ozcinar <i>et al.</i>, 2026).</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| ALT           | Higher in EEFA group                                                               | No effect in transition Holstein cows fed <i>Myrtus communis</i> L. extract (Ozcinar <i>et al.</i> , 2026).                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| AST           | No effect of EEFA                                                                  | Lower in transition Holstein cows fed <i>Myrtus communis</i> L. extract (Ozcinar <i>et al.</i> , 2026).                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |

## CONCLUSIONS

EEFA supplementation in this short-term trial was associated with changes in selected blood biochemical indicators in primiparous Holstein cows, whereas hematological parameters were not affected. Because the experiment was brief and did not include performance or rumen measurements, the findings should be interpreted as preliminary and not generalized beyond the conditions studied.

## FUNDING

Financial support for this study was provided by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan under project AP23484620 “Development and implementation phytogenics and phytobiotics for prevention and treatment of animal mastitis with assessment of quality and safety milk”.

## ACKNOWLEDGEMENTS

The authors thank LLP "NFT-KATU" for providing the material and technical base for extruded additive production, "Kazakh Agrotechnical Research University named after S. Seifullin" for supporting the research, and "Agro-firm Rodina" LLP for providing the animals used in this study.

## REFERENCES

- Al-Suwaiegh, S. B., Morshedy, S. A., Mansour, A. T., Ahmed, M. H., Zahran, S. M., Alnemr, T. M., Sallam, S. M. A. (2020). Effect of an Essential Oil Blend on Dairy Cow Performance during Treatment and Post-Treatment Periods. *Sustainability*, 12(21), 9123. <https://doi.org/10.3390/su12219123>
- Amin, S. A. S., Sobhi, N. (2023). Process optimization in poultry feed mill. *Scientific Reports* 13, 9897. doi:10.1038/s41598-023-36072-w
- Amornthewaphat, N., Lerdsuwan, S., Attamangkune, S. (2005). Effect of extrusion of corn and feed form on feed quality and growth performance of poultry in a tropical environment. *Poultry Science* 84, 1640–1647. doi:10.1093/ps/84.10.1640
- Aubakirov, Kh. A., Tlepov, A. A. (2023). Feeding of farm animals. *Almaty: Otan*, 264 p.
- BioFeed (2025). Available at: <https://biofeed.kz/en/product/21> (accessed December 2, 2025).
- Braun, H. S., Schrapers, K. T., Mahlkow-Nerge, K., Stumpff, F., Rosendahl, J. (2019). Dietary supplementation of essential oils in dairy cows: evidence for stimulatory effects on nutrient absorption. *Animal: an international journal of animal bioscience*, 13(3), 518–523. <https://doi.org/10.1017/S1751731118001696>
- Camire, M. E., Camire, A., Krumhar, K. (1990). Chemical and nutritional changes in foods during extrusion. *Critical Reviews in Food Science and Nutrition* 29(1), 35–57. doi:10.1080/10408399009527513
- Claassen, R. M., Christensen, D. A., Mutsvangwa, T. (2016). Effects of extruding wheat dried distillers grains with solubles with peas or canola meal on ruminal fermentation, microbial protein synthesis, nutrient digestion, and milk production in dairy cows. *Journal of Dairy Science* 99, 7143–7158. doi:10.3168/jds.2015-10808
- Farha, A. S., Hussein, A. O. (2023). Effect of die length, moisture content and particle size on some mechanical and physical properties of poultry feed pellets. *Kirkuk University Journal for Agricultural Sciences* 14(3), 201–211. doi:10.58928/ku23.14322
- Gandhi, N., Singh, B., Singh, P., Sharma, S. (2020). Functional, rheological, morphological, and micro-structural properties of extrusion-processed corn and potato starches. *Stärke* 73, 2000140. doi:10.1002/star.202000140
- Giallongo, F., Oh, J., Frederick, T., Isenberg, B., Kniffen, D. M., Fabin, R. A., Hristov, A. N. (2015). Extruded soybean meal increased feed intake and milk production in dairy cows. *Journal of Dairy Science* 98, 6471–6485. doi:10.3168/jds.2015-9786
- Huang, X., Liu, H., Ma, Y., Mai, S., Li, C. (2022). Effects of extrusion on starch molecular degradation, order–disorder structural transition and digestibility – a review. *Foods* 11(16), 2538. doi:10.3390/foods11162538

- Humer, E., Zebeli, Q. (2017). Grains in ruminant feeding and potentials to enhance their nutritive and health value by chemical processing. *Animal Feed Science and Technology* 226, 133–151. doi:10.1016/j.anifeedsci.2017.02.005
- Iegorov, B., Tsiundyk, O., Lapinska, A., Fihurska, L. (2021). Using raw potato peel in the production of extruded feed additive. *Food Science and Technology* 15(2), 85–92. doi:10.15673/fst.v15i2.2028
- Kumprechtová, D., Chabrilat, T., Guillaume, S., Kerros, S., Kadek, R., Indrová, E., Illek, J. (2022). Effect of plant bioactive compounds supplemented in transition dairy cows on the metabolic and inflammatory status. *Molecules* 27(18), 6092. doi:10.3390/molecules27186092
- Liu, L., Wang, P., Liu, S., Yan, M., Zhang, Q., Clark, E., Wang, J. (2026). Meta-analyses of the global impact of non-antibiotic feed additives on livestock performance and health. *Journal of Advanced Research* 76, 1–14. doi:10.1016/j.jare.2025.03.009
- McGuire, C., Siliveru, K., Ambrose, K., Alavi, S. (2022). Food powder flow in extrusion: role of particle size and composition. *Processes* 10(1), 178. doi:10.3390/pr10010178
- Muramatsu, K., Massuquetto, A., Dahlke, F., Maiorka, A. (2015). Factors that affect pellet quality: a review. *Journal of Agricultural Science and Technology* 9(2), 717–722.
- Nozière, P., Ortigues-Marty, I., Loncke, C., Sauvant, D. (2010). Carbohydrate quantitative digestion and absorption in ruminants: from feed starch and fibre to nutrients available for tissues. *Animal* 4(7), 1057–1074. doi:10.1017/S1751731110000844
- Ozcinar, U., Uyarlar, C., Orman, M. E., Çetingül, İ. S., Fatima, S., Bayram, İ. (2026). Effects of Myrtus (*Myrtus communis* L.) Extract Supplementation in the Diet on Metabolic, Immune, and Performance Parameters of Dairy Cows During the Transition Period. *Animals* 16(4), 632. <https://doi.org/10.3390/ani16040632>
- Prache, S., Adamiec, C., Astruc, T., Baéza-Campone, E., Bouillot, P. E., Clinquart, A., Feidt, C., Fourat, E., Gautron, J., Girard, A., Guillier, L., Kesse-Guyot, E., Lebre, B., Lefèvre, F., Le Perchec, S., Martin, B., Mirade, P. S., Pierre, F., Raulet, M., Rémond, D., Sans, P., Souchon, I., Donnars, C., Santé-Lhoutellier, V. (2022). Review: quality of animal-source foods. *Animal* 16(Suppl. 1), 100376. doi:10.1016/j.animal.2021.100376
- Rahman, M. A., Redoy, M. R. A., Shuvo, A. A. S., Chowdhury, R., Hossain, E., Sayem, S. M., Rashid, M. H., & Al-Mamun, M. (2024). Influence of herbal supplementation on nutrient digestibility, blood biomarkers, milk yield, and quality in tropical crossbred cows. *PloS one* 19(11), e0313419. <https://doi.org/10.1371/journal.pone.0313419>
- Risyahadi, S. T., Martin, R. S. H., Qomariyah, N., Suryahadi, S., Sukria, H. A., Jayanegara, A. (2023). Effects of dietary extrusion on rumen fermentation, nutrient digestibility, performance and milk composition of dairy cattle: a meta-analysis. *Animal Bioscience* 36(10), 1546. doi:10.5713/ab.23.0012
- Saleh, A. A., Soliman, M. M., Yousef, M. F., Eweedah, N. M., El-Sawy, H. B., Shukry, M., Wadaan, M. A. M., Kim, I. H., Cho, S., Eltahan, H. M. (2023). Effects of herbal supplements on milk production quality and specific blood parameters in heat-stressed early lactating cows. *Frontiers in veterinary science*, 10, 1180539. <https://doi.org/10.3389/fvets.2023.1180539>

- Shahid, M. S., Raza, T., Wu, Y., Mangi, M. H., Nie, W., Yuan, J. (2020). Comparative effects of flaxseed sources on egg ALA deposition and hepatic gene expression in Hy-Line Brown hens. *Foods* 9(11), 1663. doi:10.3390/foods9111663
- Sultanayeva, L., Karkehabadi, S., Zamaratskaia, G., Balji, Y. (2023). Tannins and flavonoids as feed additives in the diet of ruminants to improve performance and quality of the derived products: a review. *Bulgarian Journal of Agricultural Science* 29(3), 522–530.
- Świątkiewicz, M., Witaszek, K., Sosin, E., Pilarski, K., Szymczyk, B., Durczak, K. (2021). The nutritional value and safety of genetically unmodified soybeans and soybean feed products in the nutrition of farm animals. *Agronomy* 11(6), 1105. doi:10.3390/agronomy11061105
- Tretola, M., Lin, P., Eichinger, J., Manoni, M., Pinotti, L. (2025). Review: nutritional, safety, and environmental aspects of former foodstuff products in ruminant feeding. *Animal* 19(Suppl. 2), 101512. doi:10.1016/j.animal.2025.101512
- Veshkini A, Hammon HM, Vogel L, Viala D, Delosi re M, Tr scher A, D jean S, Cecilian F, Sauerwein H, Bonnet M. (2022). Plasma proteomics reveals crosstalk between lipid metabolism and immunity in dairy cows receiving essential fatty acids and conjugated linoleic acid. *Scientific Reports* 12, 5648. doi:10.1038/s41598-022-09437-w