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Effects of *Stevia rebaudiana B.* and *Hippophae rhamnoides* on dairy cow metabolism and milk quality

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ABSTRACT

The present study aimed to evaluate the effects of the phytogenic feed additives *Stevia rebaudiana* Bertoni and *Hippophae rhamnoides* L. on the serum biochemical profile and physicochemical characteristics of milk in dairy cows. Thirty clinically healthy Black-and-white dairy cows were assigned to three experimental groups (n = 10) using the analog-group method: a control group (C), a *Stevia rebaudiana* group (SR) receiving 100 g/day of dried stevia leaves, and a *Hippophae rhamnoides* group (HR) receiving 100 g/day of dried sea buckthorn leaves. No formal randomization procedure was applied. The experimental period lasted 30 days. Serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides were determined on Days 0 and 30. Milk was analyzed on Day 30. Supplementation had no statistically significant effect on milk fat, protein, lactose, total solids, solids-not-fat, density, pH, titratable acidity, ash content, or freezing point ($p > 0.05$). No significant changes were observed in serum AST activity, urea concentration, or glucose levels. Although ALT activity increased significantly in both supplemented groups, the recorded values remained within physiological reference limits. Both supplements significantly reduced total cholesterol, LDL cholesterol, and triglyceride concentrations while increasing HDL cholesterol levels ($p < 0.05$-0.01). Under the conditions of this 30-day study, the supplements were not associated with adverse changes in the measured biochemical variables or physicochemical characteristics of milk and were associated with a more favorable serum lipid profile.

Keywords: *Stevia rebaudiana; Hippophae rhamnoides;* phytogenic additives; dairy cows; serum biochemistry.

INTRODUCTION

The production of high-quality milk while maintaining optimal animal health and reducing the use of antimicrobial agents has become one of the major priorities of modern dairy farming. The intensification of dairy production, together with the increasing demands for high milk yield, imposes substantial metabolic stress on dairy cows, particularly during early and mid-lactation. As a consequence, increased generation of reactive oxygen species (ROS), disturbances in lipid and energy metabolism, alterations in hepatic function, and impairment of the animals' natural defense mechanisms may occur. These physiological changes can adversely affect animal health, productive performance, and milk quality (Sordillo & Aitken, 2009). At the same time, the growing prevalence of antimicrobial resistance (AMR) has emerged as one of the greatest challenges facing public health, veterinary medicine, and food production. The One Health concept recognizes the close interconnection between human, animal, and environmental health and emphasizes the need to reduce antimicrobial use through effective preventive strategies. In line with this approach, the European Union Veterinary Medicinal Products Regulation (Regulation (EU) 2019/6) promotes the development and application of innovative natural alternatives aimed at improving animal health while decreasing reliance on antimicrobial agents (European Parliament & Council, 2019). Within this framework, phytogenic feed additives have attracted increasing attention as safe and sustainable nutritional strategies for supporting animal health and productive performance. Phytogenic additives comprise plant materials, extracts, or their bioactive constituents, which are rich in polyphenols, flavonoids, terpenes, organic acids, essential oils, and other secondary metabolites with well-documented biological activities. Numerous experimental and review studies have demonstrated that these compounds enhance antioxidant capacity, modulate immune responses, support liver function, improve lipid metabolism, and positively influence ruminal fermentation. Furthermore, phytogenic feed additives have been reported to improve nutrient utilization efficiency, metabolic performance, and certain indicators of milk production, highlighting their potential as valuable tools for sustainable ruminant nutrition (Windisch et al., 2008; Hashemi & Davoodi, 2011; Kholif & Olafadehan, 2021; Nastoh et al., 2024). Among the phytogenic plants with considerable potential for application in dairy cattle nutrition, *Stevia rebaudiana* has received particular attention. In addition to its widespread use as a natural sweetener, *S. rebaudiana* represents a rich source of steviol glycosides, phenolic acids, flavonoids, vitamins, and other antioxidant compounds. These bioactive constituents have been shown to possess antioxidant, anti-inflammatory, hypolipidemic, hepatoprotective, and immunomodulatory properties by reducing lipid peroxidation, enhancing cellular antioxidant defense systems, and supporting normal hepatic function (Chatsudthipong & Muanprasat, 2009; Lemus-Mondaca et al., 2012; Gupta et al., 2013; Nenova et al., 2025). In livestock species, dietary supplementation with stevia-derived products has been associated with improved antioxidant status, a more favorable lipid profile, and enhanced metabolic adaptation to physiological stress (Pirgozliev et al., 2021; Pirgozliev et al., 2022; Xiong et al., 2022; Nenova & Enchev, 2022). Another promising phytogenic source is *Hippophae rhamnoides*, which is characterized by an exceptionally rich phytochemical composition. Its leaves and berries contain substantial amounts of vitamin C, tocopherols, carotenoids, polyphenols, flavonoids, phytosterols, and essential fatty acids. Owing to this complex composition, *Hippophae rhamnoides* exhibits pronounced antioxidant, anti-inflammatory, immunomodulatory, and hepatoprotective properties. Experimental studies have demonstrated that dietary supplementation with *Hippophae rhamnoides* in farm animals improves antioxidant status, stabilizes lipid metabolism, enhances resistance to physiological stress, and may positively influence productive performance (Beveridge et al., 1999; Suryakumar & Gupta, 2011).

Despite these promising findings, published in vivo studies evaluating the effects of *Stevia rebaudiana* and *Hippophae rhamnoides* in dairy cows remain limited. Consequently, their influence on serum biochemical parameters and the physicochemical characteristics of milk has not yet been comprehensively established. Additional controlled studies under commercial production conditions are therefore required to evaluate the potential of these phytogenic plants as functional feed additives in sustainable dairy production systems.

The aim of the present study was to evaluate the effects of dietary supplementation with *Stevia rebaudiana* and *Hippophae rhamnoides* on the serum biochemical profile and physicochemical characteristics of milk in Black-and-white dairy cows.

MATERIALS AND METHODS

Experimental Animals and Housing Conditions

The experiment was conducted at the experimental dairy farm of the Agricultural Institute–Shumen, Northeastern Bulgaria (43.28° N, 26.93° E). Thirty clinically healthy Black-and-white dairy cows, comparable in lactation stage, age, body weight, and milk yield, were enrolled in the study. All animals were housed under identical management and environmental conditions in a free-stall barn with unrestricted access to fresh drinking water (*ad libitum*). Cows were milked twice daily according to the routine management practices of the farm. Throughout the experimental period, all animals received the same basal diet consisting of 20 kg of corn silage, 2 kg of alfalfa hay, 2 kg of wheat straw, and 5 kg of concentrate per cow per day. The concentrate mixture consisted of ground corn (40%), barley (33%), wheat bran (15.9%), corn meal (9%), limestone (1.1%), salt (0.5%), and a vitamin–mineral premix (0.5%). The ration supplied 13.47 milk feed units (MFU) and 1,228 g of crude protein per cow per day.

Experimental Design

The cows were assigned to three experimental groups (n = 10 per group) using the analog-group method. Animals were matched as closely as possible according to age, lactation stage, body weight, and milk production and were then allocated to the control, *Stevia rebaudiana*, or *Hippophae rhamnoides* group to obtain comparable baseline groups. No formal randomization procedure was applied.

- **Control group (C):** cows received only the basal diet.
- **Stevia group (SR):** cows received the basal diet supplemented with 100 g/day of dried and ground *Stevia rebaudiana* B. leaves.
- **Hippophae rhamnoides group (HR):** cows received the basal diet supplemented with 100 g/day of dried and ground *Hippophae rhamnoides* leaves.

The experimental period lasted 30 consecutive days.

Plant Material

The dried leaves of *Stevia rebaudiana* Bertoni and *Hippophae rhamnoides* L. were prepared using a standardized processing protocol. Plant material was harvested at the stage of maximum physiological maturity, naturally dried under controlled environmental conditions, and subsequently milled to obtain a homogeneous particle size ranging from 0.25 to 3.0 mm. The residual moisture content was maintained below 10%, ensuring the stability of bioactive compounds throughout the experimental period. The selection of both plant species was based on their high concentrations of phenolic compounds, flavonoids, carotenoids, and other antioxidant constituents that have been reported to exert beneficial effects on the metabolic and physiological status of farm animals.

Blood Sampling and Biochemical Analysis

Blood samples were collected from the jugular vein on Day 0 (before dietary supplementation) and Day 30 (end of the experimental period). Samples were collected into vacuum tubes without anticoagulant, centrifuged at 3,000 rpm for 10 min to obtain serum, and stored at -20°C until analysis. Serum biochemical analyses were performed using an EXICO C200 automated biochemical analyzer (EXICO Medical Systems, Sweden) with commercially available diagnostic reagent kits, according to the analyzer and reagent manufacturers' instructions. The following parameters were determined: alanine aminotransferase (ALT), aspartate aminotransferase (AST), glucose, urea, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides.

Milk Sampling and Analysis

Individual milk samples were collected on Day 30 during the morning milking. After thorough homogenization of the milk obtained from each cow, a 100-mL sample was collected, transported at 4°C, and analyzed within 24 h. Milk fat, protein, lactose, total solids, solids-not-fat (SNF), density, pH, titratable acidity, ash content, and freezing point were determined using a calibrated MilkoScan infrared milk analyzer (FOSS, Hillerød, Denmark), according to the manufacturer's operating and calibration procedures. Ash content and freezing point were recorded from the analyzer outputs generated using its validated calibration equations.

Statistical Analysis

Statistical analyses were performed using STATISTICA version 13.0 (TIBCO Software Inc., Palo Alto, CA, USA). Data are presented as mean ± standard deviation (SD). The normality of data distribution was assessed using the Shapiro-Wilk test.

For serum biochemical variables measured on Days 0 and 30, the effects of treatment (Control, *Stevia rebaudiana*, and *Hippophae rhamnoides*), time, and their interaction (treatment × time) were evaluated using a linear mixed-effects model (LMM), with animal identity included as a random effect to account for within-animal correlation. When significant fixed effects were detected, pairwise comparisons were performed using Tukey-adjusted post hoc tests. Because milk was sampled only once, on Day 30, milk variables were analyzed by one-way analysis of variance (ANOVA), with treatment as the fixed factor, followed by Tukey's post hoc test. Statistical significance was accepted at $p < 0.05$.

RESULTS

The effects of dietary supplementation with *Stevia rebaudiana* and *Hippophae rhamnoides* on the serum biochemical profile and the Day-30 physicochemical characteristics of milk in Black-and-white dairy cows were evaluated after a 30-day feeding period. Serum biochemical responses are presented in Tables 1 and 2, whereas the Day-30 milk characteristics are summarized in Table 3.

Table 1 presents liver enzyme activities (ALT and AST) and indicators of energy and protein metabolism (glucose and urea) on Days 0 and 30. Table 2 summarizes the serum lipid profile on Days 0 and 30. Table 3 presents the physicochemical characteristics of milk measured on Day 30.

Serum variables were evaluated using a linear mixed-effects model with treatment, time, and treatment × time interaction as fixed effects and animal as a random effect. Day-30 milk variables were evaluated by one-way ANOVA followed by Tukey's post hoc test.

DISCUSSION

The present study demonstrated that 30-day dietary supplementation with *Stevia rebaudiana* Bertoni and *Hippophae rhamnoides* L. was not associated with adverse changes in the measured biochemical variables or the physicochemical characteristics of milk evaluated on Day 30. Significant treatment × time interactions were observed for ALT and the serum lipid profile, whereas no significant effects were detected for AST, glucose, or urea.

The maintenance of stable serum urea concentrations throughout the experimental period further suggests that dietary supplementation did not interfere with nitrogen metabolism or protein utilization. Serum urea is widely used as an indicator of the dietary protein-to-energy balance and ruminal nitrogen utilization efficiency, and values within the normal physiological range indicate adequate nutritional status and efficient microbial protein synthesis (National Academies of Sciences, Engineering, and Medicine [NASEM], 2021). Likewise, the slight increase in serum glucose observed in the supplemented groups was not statistically significant, indicating that the phytogenic additives did not markedly influence carbohydrate metabolism under the conditions of the present study. Similar observations have been reported for phytogenic feed additives rich in polyphenols and flavonoids, which improve metabolic efficiency and antioxidant status without causing clinically relevant alterations in routine serum biochemical variables (Windisch et al., 2008; Steiner, 2009).

The most pronounced metabolic response to dietary supplementation was observed in the serum lipid profile. Both *Stevia rebaudiana* and *Hippophae rhamnoides* significantly reduced total cholesterol, LDL cholesterol and triglyceride concentrations while increasing HDL cholesterol. According to the linear mixed-effects analysis, significant treatment × time interactions were detected for all evaluated lipid variables, indicating that these changes were attributable to the dietary treatments rather than to temporal variation alone. The improvement in lipid metabolism occurred without concomitant disturbances in liver enzymes or protein metabolism, suggesting that the investigated phytogenic additives exerted targeted metabolic effects while maintaining overall physiological homeostasis.

The hypolipidemic effects observed in the present study are consistent with the known biological activities of stevia and sea buckthorn. Both plants are rich sources of polyphenols, flavonoids and other bioactive compounds that regulate lipid metabolism through multiple mechanisms, including suppression of hepatic cholesterol synthesis, stimulation of fatty acid β-oxidation, enhancement of reverse cholesterol transport and reduction of oxidative stress (Chatsudthipong & Muanprasat, 2009; Ramos-Tovar & Muriel, 2017; Ramos-Tovar et al., 2019; Suryakumar & Gupta, 2011). Experimental studies have likewise demonstrated that supplementation with stevia-derived products improves antioxidant capacity while reducing serum cholesterol and triglyceride concentrations in livestock species (Xiong et al., 2022; Nenova et al., 2025). Similarly, the lipid-modulating properties of *Hippophae rhamnoides* have been attributed to its high content of flavonoids, carotenoids, tocopherols, phytosterols and unsaturated fatty acids, which collectively contribute to improved lipid metabolism and protection against oxidative damage (Wang et al., 2022).

Taken together, these findings indicate that both phytogenic feed additives improved lipid metabolism without adverse changes in the measured indicators of liver function or general metabolic status during the 30-day study. These responses support further investigation of plantderived bioactive compounds as nutritional strategies for supporting metabolic health in dairy cattle. Dietary supplementation with *Stevia rebaudiana* and *Hippophae rhamnoides* did not significantly affect the physicochemical characteristics of milk measured on Day 30. Milk fat, protein, lactose, total solids, solids-not-fat, density, pH, titratable acidity, ash content, and freezing point remained within the normal physiological ranges for clinically healthy dairy cows.

The absence of significant changes in milk composition is consistent with previous reports demonstrating that phytogenic feed additives primarily influence metabolic efficiency, antioxidant status and animal health rather than the basic chemical composition of milk (Steiner, 2009; Windisch et al., 2008). Similarly, Paglia et al. (2021) reported that supplementation with a phytogenic blend containing cardol, cardanol and ricinoleic acid did not significantly alter the principal physicochemical characteristics of milk. More recently, Kalaitsidis et al. (2025) observed that dietary supplementation with *Stevia rebaudiana* improved oxidative stability and modified the milk fatty acid profile without affecting milk fat, protein or lactose concentrations. These observations suggest that the biological activity of stevia is expressed mainly through modulation of metabolic and antioxidant pathways rather than changes in the gross composition of milk.

Published information regarding the use of *Hippophae rhamnoides* in dairy cattle remains limited. Nevertheless, the plant is recognized as an exceptionally rich source of flavonoids, carotenoids, tocopherols and other phenolic compounds with well-documented antioxidant and hypolipidemic properties (Suryakumar & Gupta, 2011; Wang et al., 2022). The present findings indicate that supplementation with sea buckthorn maintained normal milk composition while contributing to an improved serum lipid profile, suggesting that its primary effects are associated with metabolic regulation rather than direct modification of milk constituents.

The improvement in lipid metabolism observed in the present study, together with the absence of adverse changes in the measured liver-related variables and unchanged Day-30 milk quality, supports further evaluation of both plant species as functional phytogenic feed additives for dairy cattle. Longer studies with larger animal populations and additional health and performance endpoints are required before broader conclusions regarding their practical use can be drawn.

Several limitations of the present study should be acknowledged. The feeding trial was limited to 30 days and involved a relatively small number of animals, which may have reduced the ability to detect subtle physiological responses. Furthermore, only routine biochemical indicators and physicochemical characteristics of milk were evaluated. Future studies should include longer experimental periods, larger animal populations and additional biomarkers related to oxidative status, inflammatory response, rumen fermentation and milk fatty acid composition in order to further elucidate the mechanisms underlying the biological effects of *Stevia rebaudiana* and *Hippophae rhamnoides*.

Overall, dietary supplementation with *Stevia rebaudiana* and *Hippophae rhamnoides* for 30 days was associated with an improved serum lipid profile and was not associated with adverse changes in the measured liver-related, protein metabolism, or Day-30 milk physicochemical variables. These findings are limited to the variables and duration evaluated and warrant confirmation in longer and larger controlled studies.

CONCLUSIONS

The present study demonstrated that 30-day dietary supplementation with *Stevia rebaudiana* Bertoni and *Hippophae rhamnoides* L. was safe and well tolerated in Black-and-white dairy cows. Supplementation did not adversely affect liver function, protein metabolism or the physicochemical characteristics of milk, and all evaluated variables remained within their physiological reference ranges. According to the linear mixed-effects analysis, significant treatment × time interactions were observed for the serum lipid profile, whereas no significant effects were detected for AST, glucose or urea.

The most important finding was the improvement in the serum lipid profile, characterized by reduced concentrations of total cholesterol, LDL cholesterol, and triglycerides, together with increased HDL cholesterol levels. These changes occurred without adverse alterations in the other measured biochemical variables or Day-30 milk characteristics.

The present findings expand the limited knowledge regarding the use of *Stevia rebaudiana* and *Hippophae rhamnoides* in dairy cattle nutrition. Nevertheless, the conclusions are restricted to the measured variables and the 30-day experimental period. Studies involving larger animal populations, longer supplementation periods, different stages of lactation, and additional indicators of oxidative status, inflammation, animal performance, and milk composition are required to confirm these observations.

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CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Radena Nenova: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing; **Stanimir Enchev:** Supervision, Methodology, Validation, Resources, Writing – review & editing; All authors have read and approved the final version of the manuscript.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ETHICS AND PERMIT APPROVALS

All procedures involving animals were conducted in accordance with the applicable Bulgarian and European Union legislation on animal welfare and with the institutional animal-care guidelines of the Agricultural Institute - Shumen. The trial was conducted under standard farm management conditions. Jugular blood samples were collected by a qualified veterinarian using routine aseptic venipuncture and appropriate restraint, and milk samples were obtained during routine morning milking.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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AI USE STATEMENT

None.

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Table 1. Effects of dietary supplementation with *Stevia rebaudiana* Bertoni and *Hippophae rhamnoides* L. on liver enzymes and indicators of energy and protein metabolism in Black-and-white dairy cows.

Variable	Group	Day 0 (Mean ± SD)	Day 30 (Mean ± SD)	Treatment (p)	Time (p)	Treatment × Time (p)
ALT (U/L)	Control	29.0 ± 1.30^{a}	29.5 ± 1.35^{a}	n.s.	n.s.	<0.001
	SR	28.5 ± 1.20^{a}	39.6 ± 1.55^{b}			
	HR	28.8 ± 1.22^{a}	40.2 ± 1.58^{b}			
AST (U/L)	Control	133.0 ± 10.5	132.5 ± 10.2	n.s.	n.s.	n.s.
	SR	132.0 ± 10.5	128.5 ± 9.8			
	HR	132.8 ± 10.6	128.8 ± 9.6			
Glucose (mmol/L)	Control	3.30 ± 0.30	3.32 ± 0.29	n.s.	n.s.	n.s.
	SR	3.30 ± 0.30	3.45 ± 0.28			
	HR	3.29 ± 0.30	3.48 ± 0.28			
Urea (mmol/L)	Control	3.05 ± 0.30	3.10 ± 0.31	n.s.	n.s.	n.s.
	SR	3.10 ± 0.32	3.05 ± 0.30			
	HR	3.08 ± 0.30	3.02 ± 0.28			

Values are presented as mean ± SD (n = 10 cows per group). Statistical analysis was performed using a linear mixed-effects model with treatment, time and treatment × time interaction as fixed effects and animal as a random effect. a,b Values within a variable not sharing the same superscript differ significantly ($p < 0.05$). SR = *Stevia rebaudiana*; HR = *Hippophae rhamnoides*; n.s. = not significant.

Table 2. Effects of dietary supplementation with *Stevia rebaudiana* Bertoni and *Hippophae rhamnoides* L. on the serum lipid profile of Black-and-white dairy cows.

Variable	Group	Day 0 (Mean ± SD)	Day 30 (Mean ± SD)	Treatment (p)	Time (p)	Treatment × Time (p)
Total cholesterol (mmol/L)	Control	2.20 ± 0.19^{b}	2.18 ± 0.18^{b}	n.s.	n.s.	<0.001
	SR	2.10 ± 0.18^{b}	1.80 ± 0.16^{a}			
	HR	2.11 ± 0.18^{b}	1.82 ± 0.16^{a}			
Triglycerides (mmol/L)	Control	0.24 ± 0.03^{b}	0.25 ± 0.03^{b}	n.s.	n.s.	<0.001
	SR	0.25 ± 0.03^{b}	0.20 ± 0.02^{a}			
	HR	0.24 ± 0.03^{b}	0.19 ± 0.02^{a}			
HDL cholesterol (mmol/L)	Control	1.38 ± 0.12^{a}	1.37 ± 0.11^{a}	n.s.	n.s.	<0.001
	SR	1.37 ± 0.11^{a}	1.52 ± 0.12^{b}			
	HR	1.36 ± 0.11^{a}	1.54 ± 0.12^{b}			
LDL cholesterol (mmol/L)	Control	0.66 ± 0.07^{b}	0.65 ± 0.07^{b}	n.s.	n.s.	<0.001
	SR	0.64 ± 0.07^{b}	0.46 ± 0.05^{a}			
	HR	0.65 ± 0.07^{b}	0.45 ± 0.05^{a}			

Values are presented as mean ± SD (n = 10 cows per group). Statistical analysis was performed using a linear mixed-effects model with treatment, time and treatment × time interaction as fixed effects and animal as a random effect. a,b Values within a variable not sharing the same superscript differ significantly ($p < 0.05$). SR = *Stevia rebaudiana*; HR = *Hippophae rhamnoides*; n.s. = not significant.

Table 3. Physicochemical characteristics of milk after 30-day dietary supplementation with *Stevia rebaudiana* Bertoni and *Hippophae rhamnoides* L. in Black-and-white dairy cows.

Parameter	Control (n = 10)	SR (n = 10)	HR (n = 10)	*p-value*
Milk fat (%)	3.74 ± 0.23	3.78 ± 0.21	3.81 ± 0.20	0.74
Protein (%)	3.28 ± 0.11	3.31 ± 0.10	3.33 ± 0.12	0.69
Lactose (%)	4.71 ± 0.08	4.73 ± 0.07	4.74 ± 0.08	0.61
Total solids (%)	12.43 ± 0.32	12.49 ± 0.30	12.55 ± 0.29	0.72
Solids-not-fat (%)	8.69 ± 0.12	8.71 ± 0.11	8.73 ± 0.10	0.76
Density (g/cm^3)	1.0302 ± 0.0007	1.0304 ± 0.0006	1.0305 ± 0.0007	0.64
pH	6.67 ± 0.04	6.68 ± 0.03	6.68 ± 0.04	0.82
Titratable acidity (°T)	16.6 ± 0.6	16.5 ± 0.5	16.4 ± 0.5	0.57
Ash (%)	0.72 ± 0.02	0.72 ± 0.02	0.73 ± 0.02	0.81
Freezing point (°C)	−0.536 ± 0.005	−0.537 ± 0.004	−0.538 ± 0.005	0.70

Values are presented as mean ± SD (n = 10 cows per group) measured on Day 30. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. No statistically significant differences were observed among groups ($p > 0.05$). SR = *Stevia rebaudiana* group; HR = *Hippophae rhamnoides* group.