

Effect of chromium propionate supplementation on lactation performance and blood parameters of Simmental cows in mid-lactation under heat stress

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ABSTRACT

Nutritional management of high-producing dairy cows under heat stress conditions presents a significant challenge due to the increasing issue of global warming. The study aimed to explore the effects of Cr supplementation in the form of chromium propionate (CrP) on feed intake, lactation performance, and selected biochemical serum parameters in mid-lactation Simmental dairy cows under heat stress conditions. The experiment was conducted during July and August and lasted for 8 wk. The average temperature-humidity index (THI) was 76.45 ± 2.80 during the experimental period, indicating that the cows were under heat stress. The added CrP provided $0.34 \text{ mg Cr kg}^{-1} \text{ DM}$ of used ration. The supplementation of diet with CrP increased DM intake (4.11%, $P < 0.01$) and the yield of milk (4.48%, $P < 0.01$), 4% fat-corrected milk (4.08%, $P < 0.01$), milk fat (4.46%, $P < 0.01$), protein (4.21%, $P < 0.01$), lactose (3.79%, $P < 0.01$), and total solids (3.51%, $P < 0.01$). Additionally, CrP supplementation increased blood glucose concentrations (8.39%, $P < 0.01$), while reducing blood urea N (BUN) levels (14.85%, $P < 0.05$) and beta-hydroxybutyrate (BHB) levels (17.14%, $P < 0.05$). The use of CrP as additional dietary source of Cr significantly improved productive performances of dairy cows under heat stress conditions. It was indicated that supplemented Cr could have positive effect on glucose and N metabolism, as well as on energy metabolism of lactating cows at moderately high THI values.

Key words: Cattle, chromium propionate, lactation performance, temperature-humidity index.

INTRODUCTION

Climate change represents one of the main threats to the survival of various species, ecosystems, and the sustainability of agricultural production worldwide, especially in tropical and temperate countries (Abbas et al., 2020).

The dairy industry is faced with the heat stress as a significant challenge as dairy cows are susceptible to the negative impacts of heat (Shan et al., 2020). The temperature-humidity index (THI) is the most common indicator of the degree of heat stress in dairy cows (Wang et al., 2023). Researchers typically use $\text{THI} \geq 72$ as the threshold at which cows begin to suffer from heat stress (Becker and Stone, 2020). Heat stress negatively affects the appetite center in the hypothalamus, thereby directly reducing DM intake (DMI), leading to a negative energy balance (Collier et al., 2017) and consequently impairing the health, production, and reproduction of the animals (Sammad et al., 2020). The impact of elevated temperatures on blood glucose levels shows a significant reduction in blood glucose levels in dairy cows (Baumgard and Rhoads, 2013), along with a simultaneous increase in beta-hydroxybutyrate (Collier et al., 2017). In addition to physical cooling methods, Sun et al. (2019) suggest nutritional strategies to minimize the negative impacts of heat stress.

Chromium (Cr) is an essential trace element and it is most stable in the trivalent state (Cr³⁺), which is the active component in glucose tolerance factor (GTF) (Wo et al., 2023). The dominant physiological role of Cr is to enhance the action of insulin (Al-Saiady et al., 2004). Chromium increases glucose uptake into cells, thereby improving the binding of insulin to extracellular receptors, which causes the mobilization of the insulin-dependent glucose transporter type 4 (GLUT4) (Nishimura et al., 2021). The need for Cr increases under physiological stress (Keshri et al., 2019). Since the feed ingredients that are commonly available to dairy cows have low concentrations of Cr (Malik et al., 2023), the addition of Cr supplements to the feed during the hot summer months could improve metabolic status and performances of lactating cows (Shan et al., 2020).

Inorganic Cr has a low absorption rate in animals and is rarely used for research on the function of Cr in the metabolism of dairy cows. In contrast, organic forms of Cr, such as Cr yeast or chromium propionate (CrP), have been developed as dietary supplements with the aim of increasing Cr absorption and milk yield in dairy cows (Spears et al., 2012). NASEM (2021) recommends that CrP should not be added in amounts greater than 0.5 mg Cr kg⁻¹ DM. Previous studies, though limited, have shown that different forms of Cr can have a positive impact on feed intake, milk yield, and blood biochemical parameters in cows under heat stress (Shan et al., 2020; Wo et al., 2023; Wang et al., 2023).

We believed that an appropriate dose of CrP could positively affect lactation performance by increasing feed intake and reducing the harmful effects of heat stress. Therefore, the aim of this study was to evaluate the effect of increased CrP intake on lactation performance, DMI, and certain blood biochemical parameters in Simmental dairy cows in mid-lactation under heat stress.

MATERIALS AND METHODS

This study was conducted at "DMN" dairy farm near Požarevac, 100 km southeast of Belgrade, Serbia (44°33'13" N, 21°17'05" E). All procedures on animals were carried out in accordance with the Animal Welfare Act (2009; 41/2009). The experiment was approved by the Ethics Committee for Animal Experimentation of the Faculty of Agriculture, University of Belgrade, No. 323-07-05873/2022-05.

Animals, experimental design and diets

The experiment was designed as one factorial arrangement with two treatments. Eighteen primiparous Simmental cows and 52 multiparous Simmental cows were divided equally into two physically separated groups (n = 35 each) based on days in lactation, previous milk yield and parity (averaging 115 d in milking, milk yield of 29.1 kg d⁻¹, and parity of 2.75): A control group without CrP supplementation (CON) and a group with CrP supplementation (CrP). Each group consisted of nine primiparous cows and 26 multiparous cows. All cows were housed in free-stalls in a mechanically ventilated barn and had free access to fresh water and total mixed ration (TMR). The cows were offered a TMR two times a day, at 07:00 and 19:00 h for *ad libitum* intake. The pre-trial period lasted 1 wk during which all cows consumed the same TMR, without CrP supplementation. The experiment was conducted during July and August and lasted for 8 wk. During experimental period, for CrP group, 198 g ground corn and 0.34 mg Cr kg⁻¹ DM (2 g KemTRACE 0.4% Cr, Kemin Industries, Des Moines, Iowa, USA) were premixed and added to the TMR, while CON group received 200 g ground corn added to the TMR. Feeds offered and orts were measured and recorded daily to calculate feed intake. Samples of TMRs and orts were collected three times a week during the experimental period, for DM determination, and then grouped by period, frozen (-20 °C) and stored for further laboratory analyses.

Analytical procedure

The TMRs and orts samples were analyzed in the Laboratory for the Animal Nutrition at the Faculty of Agriculture, University in Belgrade. The samples were dried at 55 °C in a forced-air oven for 48 h, and were ground to pass a 1 mm screen on a small-sample mill (Kinematica PX-MFC 90D, Maltes, Switzerland). The samples of TMRs and orts were analyzed according to the AOAC official methods (AOAC, 2002). The DM content was determined by drying at 105 °C for 16 h (method 967.03). Ash was determined by combustion the sample at 540-600 °C for 2 h (method 942.05). The crude protein (CP) content was determined by the Kjeldahl method using K₂SO₄/Cu catalyst-Kjeltabs S 3.5, on the Kjeltac Auto 1030 Analyzer-Tecator System (method 2001.11; FOSS, Hillerød, Denmark). Ether extract (EE) content was measured using diethyl-ether in the Soxhlet apparatus

(method 920.39). The crude fiber (CF) was determined according to the Henneberg and Stohmann method (method 978.10). The content of neutral detergent fiber (NDF) was determined according to the Method 2002.04 using heat-stable α -amylase (A3306; Sigma-Aldrich, St. Louis, Missouri, USA) without using sodium sulfite and without correcting ash content. The acid detergent fiber (ADF) was determined without correcting ash content (method 973.18). Calcium, P and Cr levels were determined by inductively coupled plasma mass spectrometry (ICP-MS) using Thermo iCAP Q (Thermo Scientific, Bremen, Germany) with previous digestion of samples using START D microwave digestion system (Milestone S.r.l., Sorisole, Italy) in accordance with study of 10.1088/1755-1315/333/1/012050. Nitrogen-free extract (NFE) and net energy value for milk production (NE_L) were calculated using the CVB Feed Table (CVB, 2021). The rations were formulated using the CVB model: CVB Table Booklet Feeding of Ruminants 2008 (CVB, 2008), as well as the CVB Feed Table (CVB, 2021). Table 1 shows the components of the total mixed ration used for feeding the cows in the experimental and control groups.

Table 1. Ingredients and chemical composition of the basal diet. ¹Encapsulated N source (INBERG, Belgrade, Serbia). ²Premix provided Fe 1102 mg kg⁻¹; Cu 404 mg kg⁻¹; Mn 2154 mg kg⁻¹; Zn 2848 mg kg⁻¹; I 37 mg kg⁻¹; Se 11 mg kg⁻¹; Co 15 mg kg⁻¹; vitamin A 551 100 IU kg⁻¹; vitamin D3 110 220 IU kg⁻¹; vitamin E 1445 mg kg⁻¹; biotin 7.5 mg kg⁻¹; niacin 726 mg kg⁻¹; antioxidant 3340 mg kg⁻¹. ³NFE: N-free extract = 1000 - (moisture + Ash + CP + EE + CF). ⁴ NE_L : Net energy value for milk production calculated according to CVB (2021).

Item	Content (%DM)
Ingredients	
Alfalfa hay	11.16
Alfalfa haylage	7.59
Rye haylage	7.21
Corn silage	21.07
Wheat straw	6.49
Ground corn	21.61
Ground wheat	7.04
Soybean meal	8.30
Rapeseed meal	1.14
Molasses	3.16
Glycerol	1.69
IncapsPro ¹	0.34
NaHCO ₃	0.63
MgO	0.21
CaCO ₃	1.14
Ca(H ₂ PO ₄) ₂	0.34
NaCl	0.46
Premix ²	0.42
Chemical composition (\pm SD)	
Dry matter	51.46 (1.12)
Crude protein (CP)	14.66 (0.36)
Crude fiber (CF)	16.22 (0.57)
Acid detergent fiber (ADF)	19.22 (0.71)
Neutral detergent fiber (NDF)	28.63 (1.13)
Ether extract (EE)	2.62 (0.19)
NFE ³	9.05
Ash	8.91 (0.19)
Ca	0.95 (0.08)
P	0.39 (0.09)
Cr, mg kg ⁻¹	0.17 (0.06)
NE_L ⁴ , MJ kg ⁻¹	6.27

Ambient temperature and relative humidity

The ambient temperature (T) and relative humidity (RH) were recorded three times per day at 06:00, 14:00 and 22:00 h using Data logger Testo 174H (Testo Ltd., Hampshire, UK) that was positioned in the center of the barn, 1.5 m above the ground. The THI was calculated based on the equation according to Wo et al. (2023): $THI = (1.8 \times T + 32) - (0.55 - 0.0055 \times RH) \times (T - 26.8)$, where T is the ambient temperature (°C) and RH is the relative humidity (%).

Collection and analysis of milk and blood samples

The cows were milked two times daily (07:00 and 19:00 h) and their individual milk yields were recorded during each milking using milking control (DemaTron 70, GEA Farm Technologies, Düsseldorf, Germany). Milk samples were collected three times during experimental period, in third, fifth and eighth week from two consecutive milkings in plastic sterile bottles volume 48 mL. The milk samples were transported in an icebox to the laboratory at 4 °C, 30 min after sampling. The milk composition (fat, protein, lactose and total solids) was determined by infrared analysis (LactoScope Filter C4+, Delta Instruments, Drachten, The Netherlands). The produced kilograms of fat, protein, lactose, and total solids were calculated based on the percentages obtained from the milk composition analysis and multiplied by the volume produced per animal (Calvache et al., 2024).

Blood samples from 15 cows each group were taken from the *venae coccigea* and were transferred into vacutainer tubes containing a clot activator (10.0 mL, BD Vacutainer, Plymouth, Devon, UK) for biochemical analyses. The blood samples were taken approximately 1 h before feeding in the morning, and the samples were transported to a laboratory within 60 min in an icebox. Blood samples were allowed to clot spontaneously. After clotting, samples were centrifuged at 1500x g for 10 min to separate the serum, were decanted into graduated polypropylene tubes (1.5 mL, Eppendorf AG, Hamburg, Germany), and analyzed.

Analyses of biochemical indicators included the determination of glucose (mmol L^{-1}), total protein (g L^{-1}), albumin (g L^{-1}), Ca (mmol L^{-1}), P (mmol L^{-1}), blood urea N (BUN; mmol L^{-1}), total bilirubin ($\mu\text{mol L}^{-1}$), triacylglycerols (TAG; mmol L^{-1}), beta-hydroxybutyrate (BHB; mmol L^{-1}), aspartate aminotransferase (AST; U L^{-1}) and gamma-glutamyltransferase ($\gamma\text{-GT}$; U L^{-1}). Biochemical indicators were analyzed using the methods/kits: Glucose (glucose dehydrogenase, GDH-NAD method), total protein (biuret reaction); albumin (bromocresol green method), Ca (arsenazo colorimetric method), P (colorimetric method), BUN (urease/glutamate dehydrogenase method); total bilirubin (diazotized sulfanilic acid method), TAG (glycerol phosphate oxidase/peroxidase), BHB (enzymatic method), AST and $\gamma\text{-GT}$ (International Federation of Clinical Chemistry and Laboratory Medicine [IFCC] method). Analyses were performed using a biochemical analyzer (BioSystem A15, BioSystems SA, Barcelona, Spain).

Statistical analysis

The Student's t-test using the JASP v.0.15 (JASP Team, 2021) was conducted to assess the effects of CrP supplementation on DMI, milk production characteristics and blood indicators of dairy cows. The Shapiro-Wilk's test used to test the assumption of the normal distribution of analyzed data. Overall differences between treatment means were considered to be significant at $P < 0.05$ and trend at $P < 0.1$. The parameters of descriptive statistics were also determined.

RESULTS

The daily environment data are shown in Figure 1A. The mean THI was used to construct a temperature and humidity index curve (Figure 1B).

As illustrated in Figure 1, the THI remained above 72 (76.45 ± 2.80) throughout the entire 8 wk experimental period, suggesting that the cows were under heat stress.

As shown in Table 2, DMI was higher (4.11%, $P < 0.01$) using supplementary CrP in the ration. The feed efficiency ratio (as appears in Table 2) for milk production was not affected by added CrP.

Supplementation of CrP to the diet of dairy cows under heat stress ($P < 0.01$) increased milk yield as well as 4% FCM yield (Table 2). Milk yield and 4% FCM yield were higher by 4.48% and 4.08%, respectively. There was nonsignificant effect on the chemical composition of milk, but the yield of milk fat, protein, lactose, and total solids were also higher ($P < 0.01$) for cows fed ration with supplementary Cr. This increase was 4.46%, 4.21%, 3.79%, and 3.51% for milk fat, protein, lactose, and total solids, respectively.

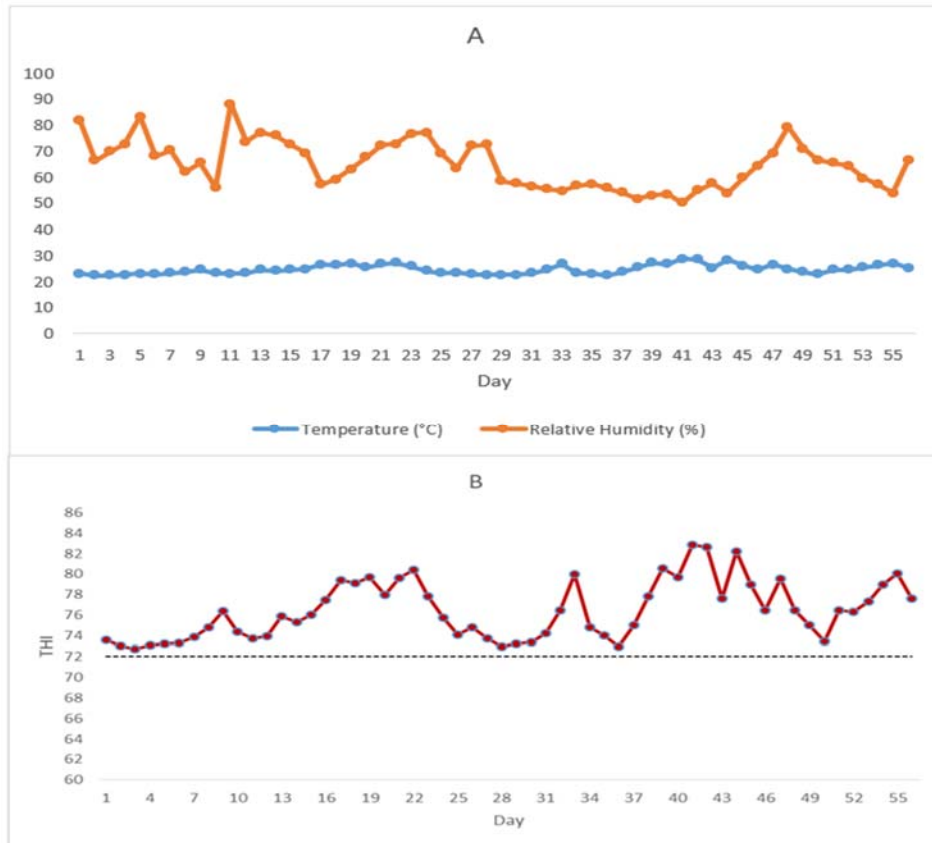


Figure 1. Environmental data and temperature and humidity index (THI) during the study period: Daily mean of temperature and relative humidity in the barn (A); daily mean THI during the experimental period (B).

Table 2. Effect of chromium propionate (CrP) supplementation on DM intake (DMI), milk yield, chemical composition of milk and feed efficiency. CON: Control group without chromium propionate supplementation (n = 35); CrP: chromium propionate group receive additional CrP (8 mg Cr cow⁻¹ d⁻¹) (n = 35); FCM: Fat corrected milk. Feed efficiency: 4% FCM/DMI.

Item	Treatment		P-value
	CON	CrP	
DMI, kg d ⁻¹	21.66 ± 0.45	22.55 ± 0.84	<0.01
Actual milk yield, kg d ⁻¹	27.91 ± 2.96	29.16 ± 3.38	<0.01
4% FCM yield, kg d ⁻¹	28.17 ± 0.93	29.32 ± 0.70	<0.01
Milk fat			
%	4.03 ± 0.49	4.05 ± 0.43	0.78
kg d ⁻¹	1.12 ± 0.04	1.17 ± 0.03	<0.01
Milk protein			
%	3.40 ± 0.31	3.43 ± 0.31	0.51
kg d ⁻¹	0.95 ± 0.03	0.99 ± 0.02	<0.01
Lactose			
%	4.71 ± 0.13	4.72 ± 0.15	0.81
kg d ⁻¹	1.32 ± 0.04	1.37 ± 0.03	<0.01
Total solids			
%	13.24 ± 1.06	13.28 ± 0.85	0.74
kg d ⁻¹	3.70 ± 0.12	3.83 ± 0.09	<0.01
Feed efficiency	1.29 ± 0.05	1.28 ± 0.07	0.76

Table 3 illustrates concentrations of analyzed blood biochemical parameters in both groups. Supplementation with CrP increased the serum glucose concentrations by 8.39% ($P < 0.01$), while decreased the BUN by 14.85% ($P = 0.02$) and BHB 17.14% ($P = 0.04$). Other biochemical parameters in the blood serum did not show the differences between the CON and CrP groups.

The majority of the monitored biochemical blood parameters were within physiological ranges. However, the values of albumin and gamma-glutamyl transferase (GGT) were above the reference values, which are 30-40 g L⁻¹ for albumin and 4.9-25.7 U L⁻¹ for GGT.

Table 3. Effect of chromium propionate (CrP) supplementation on blood serum parameters in heat-stressed dairy cows. CON: Control group without CrP supplementation (n = 35); CrP: chromium propionate group receive additional CrP (8 mg Cr cow⁻¹ d⁻¹) (n = 35); BUN: blood urea N; TAG: triglyceride; BHB: beta-hydroxybutyrate; AST: aspartate aminotransferase; γ -GT: gamma-glutamyltransferase.

Item	Treatment		P-value
	CON	CrP	
Glucose, mmol L ⁻¹	2.98 ± 0.29	3.23 ± 0.28	<0.01
Total protein, g L ⁻¹	74.75 ± 5.09	74.05 ± 4.75	0.62
Albumin, g L ⁻¹	42.21 ± 4.85	42.93 ± 4.16	0.46
Ca, mmol L ⁻¹	2.30 ± 0.18	2.32 ± 0.15	0.57
P, mmol L ⁻¹	1.98 ± 0.30	1.90 ± 0.26	0.18
BUN, mmol L ⁻¹	6.06 ± 0.98	5.16 ± 0.98	0.02
Total bilirubin, μ mol L ⁻¹	2.46 ± 0.85	2.58 ± 0.76	0.50
TAG, mmol L ⁻¹	0.17 ± 0.06	0.17 ± 0.05	0.94
BHB, mmol L ⁻¹	0.70 ± 0.33	0.58 ± 0.18	0.04
AST, U L ⁻¹	83.06 ± 13.38	78.76 ± 13.13	0.13
γ -GT, U L ⁻¹	33.68 ± 10.87	30.64 ± 8.87	0.13

DISCUSSION

Many studies typically use a THI = 72 as an indicator to assess heat stress in dairy cows (Shan et al., 2020; Wo et al., 2023), while some others report THI = 68 as the threshold at which the first signs of heat stress occur (Collier et al., 2017). In this study, the average daily THI values were above 72 throughout the entire experimental period, indicating that the cows were under heat stress.

One of the main indicators of heat stress in dairy cows is a decrease in DM intake (DMI) (Bin-Jumah et al., 2020). Some earlier research show that supplementation with various amounts of Cr increases DMI in lactating cows (Mirzaei et al., 2011; Vargas-Rodriguez et al., 2014). In the study with Holstein cows 143 d in milking of Wang et al. (2023), it is noted that DMI increased quadratically with increasing dietary content of CrP. Our study results are consistent with Al-Saiady et al. (2004), where a group of cows consuming Cr yeast (CY) had a 9% higher DMI compared to the control group. In contrast, Yasui et al. (2014) in trial with Holstein cows showed that CrP at 8 mg d⁻¹ cow⁻¹ had no effect on DMI during the prepartum period, early lactation and mid-lactation (Garcia et al., 2017). These studies indicate that the effects of Cr on DMI are mostly observed under heat stress conditions. Cows under heat stress may increase glucose metabolism and Cr requirements, leading to Cr deficiency (Bin-Jumah et al., 2020). One reason for increased DMI in this study could be explained by the need to compensate for Cr deficiency.

Some earlier studies on the effects of Cr supplementation on the productive performance of Holstein dairy cows have reported inconsistent results. Wang et al. (2023) reported that CrP supplementation at levels of 4 and 8 mg d⁻¹ head⁻¹ increases DMI, which contributes to higher milk yield, but increasing the dose to 12 mg d⁻¹ head⁻¹ leads to a decrease in DMI and milk yield. Shan et al. (2020) state that 10 mg d⁻¹ head⁻¹ Cr is the threshold for Cr supplementation. The higher DMI obtained in our research agrees with earlier findings. The effects of

the inorganic form Cr on milk yield are present with supplementation at levels of 20 mg d⁻¹ head⁻¹ (Zade et al., 2014), which can be explained by its lower absorption (Spears et al., 2017). Under heat stress conditions, the main effect of higher DMI of Simmental cows on ration with added Cr is determined significant increase in milk yield. The positive impact of Cr supplementation on blood glucose levels was also found. Baumgard and Rhoads (2013) reported that the reduction in milk production in cows under heat stress can be attributed to lower lactose synthesis, which is closely related to lower glucose concentration under heat stress (Stewart et al., 2022). This can be explained by the fact that blood glucose is the main precursor of lactose synthesis, and lactose concentration determines osmotic pressure and milk yield (Baumgard and Rhoads, 2013). Wo et al. (2023) report that the increased milk yield caused by Cr yeast supplementation may be due to enhanced N utilization, as a lower concentration of urea N was observed in the blood serum of cows received additional Cr. The lower BUN in conducted research is probably a result of improved utilization of consumed N from Cr supplemented diet. However, there are also studies in which Cr had no effect on milk yield (Yasui et al., 2014; Vargas-Rodriguez et al., 2014; Leiva et al., 2017).

The chemical composition of milk in the conducted trial did not differ between the CON and CrP groups, but the yields of fat, protein, lactose, and total solids were significantly higher in the CrP group. These results are consistent with the study by Wu et al. (2021) where the concentrations of individual components did not differ, and the yields increased linearly with higher levels of Cr methionine. Additionally, Soltan (2010) reported that Cr supplementation did not affect the chemical composition of milk in Holstein cows under heat stress. However, Wo et al. (2023) noted higher percentages of lactose and protein in cows in mid-lactation fed Cr yeast under heat stress conditions.

Dairy cows experiencing heat stress have a reduced ability to mobilize lipids compared to moderate temperatures, and rely more on glucose for energy (Slimen et al., 2016). Trivalent Cr, an active component of the glucose tolerance factor (GTF) (Nishimura et al., 2021), plays an important role in maintaining appropriate blood glucose concentrations (Zhang et al., 2014). Wo et al. (2023) reported that serum glucose in the Cr yeast group was 11% higher, which is similar to the results from our study. On the other hand, there are many studies that have not found effects of Cr supplementation on this parameter under heat stress conditions (Soltan, 2010; Shan et al., 2020; Wang et al., 2023), as well as under normal conditions (Yasui et al., 2014).

Blood urea N as an indicator can reflect the effects of ration on NH₃-N production and utilization in the rumen and N metabolism in the liver (Yari et al., 2010). In this study, a decrease in BUN concentration was observed in the blood serum of cows fed with CrP. This could be due to increased microbial activity and greater incorporation of NH₃-N into microbial protein synthesis in the rumen (Wang et al., 2023). Wo et al. (2023) recorded an 18% lower BUN value in cows fed ration with Cr yeast. On the other hand, Mousavi et al. (2019) found no differences in BUN concentration in calves under heat stress fed diet with Cr-methionine. These conflicting results may be a consequence of differences in physiological stage, type of stress, as well as the form or duration of Cr supplementation.

In this trial, it was observed that Cr supplementation reduced BHB concentration during an 8 wk trial period in mid-lactation Simmental cows. The decrease in BHB levels with Cr supplementation in lactation cows may be associated with increased gluconeogenesis (Yang et al., 1996). However, Yasui et al. (2014) in their study did not find differences in BHB concentration that could be attributed to Cr supplementation. Under heat stress conditions, there is no data on the effect of Cr on BHB concentration. This could be one of the potential parameters for further scientific research.

Liver enzymes, AST and GGT, were determined to assess the liver condition of the sampled cows. Elevated serum GGT levels reflect liver damage in dairy cattle, although the significant effect of parity on this parameter could be a result of greater productive stress in multiparous cows compared to primiparous cows (Cozzi et al., 2011).

CONCLUSIONS

This study demonstrates that supplementation of chromium propionate (CrP) that provides 0.34 mg Cr kg⁻¹ DM increases milk yield by enhancing DM intake. Although, no changes were observed in the milk composition, there is an observed increase in milk fat, protein, lactose, and total solids production. Additionally, under heat stress conditions, CrP plays a crucial role in regulating blood glucose levels, as well as reducing urea and beta-

hydroxybutyrate. Based on the results of this study, the use of CrP during summer months improves productive performances and metabolic parameters of mid-lactation Simmental cows.

Author contribution

Conceptualization: B. Stojković, B. Stojanović. Methodology: B. Stojković, B. Stojanović, V.D. Formal analysis: B. Stojković, S.S., A. Ivetić. Investigation: B. Stojković. Resources: B. Stojković, V.D., A. Ivetić. Data curation: B. Stojković, A. Ignjatović, I.G. Writing-original draft: B. Stojković. Writing-review & editing: B. Stojković. Supervision: B. Stojković, B. Stojanović. Project administration: B. Stojković, B. Stojanović. Funding acquisition: B. Stojković, B. Stojanović. All co-authors reviewed the final version and approved the manuscript before submission.

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