

# EFFECTS OF SEASON ON METABOLIC PROFILE OF HOLSTEIN FRIESIAN COWS IN POSTPARTUM PERIOD

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<sup>1</sup>Supporting Information

**ABSTRACT:** The aim of the present study was to determine the metabolic profile of Holstein-Friesian cows in the postpartum period, as well as the effect of season on metabolic profile. The postpartum period is essential in the reproductive life of high yielding dairy cows because of its impact on future gravidity. This study included 60 cows up to 15 days after parturition, aged 2-8 years (the largest number of cows was between 3 and 5 years old) with no apparent clinical problems. Cows were sampled in summer season (n=30) and winter season (n=30). Parameters of metabolic profile were determined as follows: glucose, albumin, total protein, cholesterol, bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), urea, calcium, phosphorus and magnesium. Statistical differences were considered significant at the  $p < 0.05$ . Present research showed that all investigated parameters were within a reference range for cattle. Impact of season sampling was determined for glucose, albumin, total protein, cholesterol and phosphorus, while bilirubin, calcium, magnesium, urea as well as activities of ALT, AST and LDH were unaffected by the season of sampling. In conclusion, metabolic status is affected by the season and examination during the postpartum period can provide valuable information of cows' health status, in order to diagnose and moreover prevent postpartum diseases.

**Keywords:** Cows, Climate, Health status, Metabolic profile, Postpartum period.

## INTRODUCTION

Metabolic profile of cows is of a great importance in order to monitor health status, but also to diagnose and prevent metabolic and nutritional diseases in dairy cattle. The concept "metabolic profile" refers to the examination of blood biochemical parameters and is immensely important for the health condition of the herd (Puppel and Kuczyńska, 2016). However, multiple variables should be considered in order to accurately interpret the obtained data, such as physiological state of an animal. The periparturient period, also known as the transition period, lasts from 3 weeks before to 3 weeks after calving (i.e., the pregnant, nonlactating state to the nonpregnant, lactating state) (Erdoğan and Alić, 2020) and is often a disastrous experience for the cow due to metabolic overload (Wang et al., 2014). Dairy cows experience severe metabolic stress after calving, because they cannot meet the enormous energy and protein demands for milk production. The postpartum period is essential in the reproductive life of high yielding dairy cows because of its impact on future gravidity. Immediately after calving a negative energy balance (NEB) occurs; NEB may reduce the conception rate to insemination due to detrimental effect it has on the oocyte that is released after ovulation. Cows under negative energy balance show extended periods of an ovulation. Postpartum anestrus, along with infertility is enhanced by losses of body condition during the early postpartum period associated with negative energy balance (Nigussie, 2018; Mekuriaw, 2023). NEB status can be assessed by changes in blood metabolites. Examination of metabolic status during postpartum period could provide valuable information of cows' health status, in order to diagnose and moreover prevent postpartum diseases.

The aim of the present study was therefore to determine metabolic profile of Holstein-Friesian cows in the postpartum period, as well as the effect of season on metabolic profile.

## MATERIALS AND METHODS

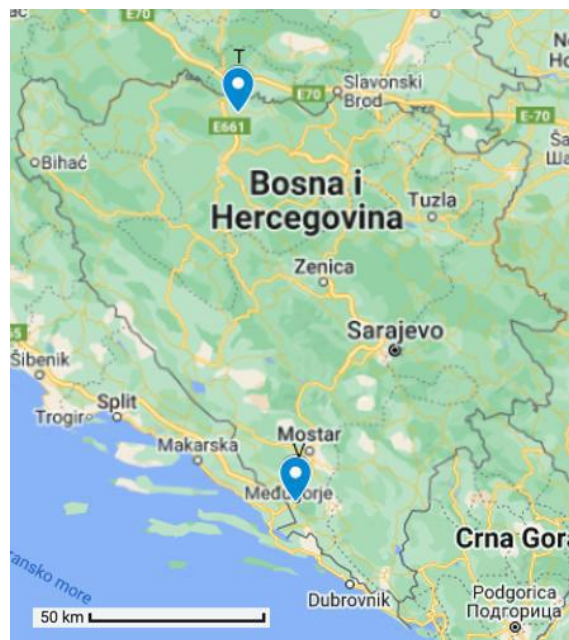
Present study included 60 cows up to 15 days after parturition, aged 2-8 years (the largest number of cows was between 3 and 5 years old) with no apparent clinical problems. The research was conducted in two different geographical areas, specifically in the northern area – farm T and in the southern part of Bosnia and Herzegovina – farm V (Figure 1). Cows were sampled in summer season (June to August; n=30) and winter season (November to February, 2023; n=30). Blood samples were taken in the morning between 09:00 and 11:00 hrs, approximately two hours after feeding, via puncture of a coccygeal vein into two vacutainers. Blood from each animal was taken into two vacutainers. Blood samples for letter assessment of serum parameters was collected using 5-mL vacutainers containing no additives. Blood samples for plasma analyses was collected into 5-mL vacutainers containing sodium heparin. After collection vacutainers were stored

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on ice and transported to the lab, within two hours. Upon arrival, vacutainers with heparin were LC 320, 3000 rpm / 10 min, and plasma was frozen at -20 °C. Blood samples to harvest serum were stored on ice after collection, allowed to clot at 5 °C, and centrifuged at 1,000 × g to harvest serum. Serum was immediately frozen at -20 °C for later analyses.

Parameters of metabolic profile were determined using the “Beckmann DU-64 UV/VIS” spectrophotometer, as follows: glucose, albumin, total protein, cholesterol, bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), urea, calcium, phosphorus and magnesium. Statistical analysis was done using SPSS 10.00 software program. Differences were considered statistically significant at the level  $p < 0.05$ .

**Figure 1.** The image shows two geographical locations in Bosnia and Herzegovina. The northern location is marked with (T) for Topola, while the southern location is marked with (V) for Višići.



## RESULTS AND DISCUSSION

Results are shown in Table 1. In postpartum cows, the metabolic profile is critical for health monitoring, disease diagnosis, treatment, and prognosis. Since glucose is a master regulator of hormones and metabolites that regulate reproductive processes, blood glucose concentrations in postpartum cows are of great significance, especially due to the fact that low blood glucose levels in postpartum dairy cows are linked to infertility. Glucose is a critical nutrient in the postpartum dairy cow since glucose is required for milk synthesis by the mammary gland. A variety of other tissue types, including those involved in reproduction, demand glucose. Moreover, glucose is a valuable parameter for determining dietary sufficiency. Glucose levels of Holstein-Friesian postpartum cows during summer and winter season is given in Table 1. Present study has identified statistically significant higher values of glucose concentrations during summer, compared to winter. The higher glucose levels during summer could be explained by higher supply of forage in summer, compared to the winter. Seasonal effects on blood glucose levels were confirmed in previous studies (Giri et al., 2017; Cerutti et al., 2018). However, obtained values were within the reference range (Table 1).

**Table 1 - Seasonal Variations in Blood Metabolic Parameters of Holstein-Friesian Cows**

Parameter	Summer season	Winter season	Both seasons
Glucose (mmol/L)	3.28±0.08 <sup>a</sup>	3.06±0.06 <sup>b</sup>	3.17±0.05 <sup>ab</sup>
Albumin (g/L)	60.37±1.80 <sup>a</sup>	53.78±1.51 <sup>b</sup>	57.19±1.25 <sup>ab</sup>
Total protein (g/L)	144.65±3.81 <sup>a</sup>	82.32±4.96 <sup>b</sup>	114.03±5.18 <sup>c</sup>
Cholesterol (mmol/L)	2.14±0.17 <sup>a</sup>	3.28±0.21 <sup>b</sup>	2.69±0.15 <sup>c</sup>
Bilirubin (µmol/L)	2.12±0.45	2.74±0.45	2.46 ±0.32
ALT (u/L)	9.15 ±0.55	18.46±1.46	13.73±0.98
AST (u/L)	70.52 ±2.37	60.32±1.54	65.92±1.36
LDH (u/L)	998.01± 8.79	1113.45±20.47	1007.50±11.11
Urea (mmol/L)	5.92±0.13	5.47±0.18	5.81 ±0.35
Calcium (mmol/L)	2.18±0.08	2.01±0.02	2.1±0.04
Phosphorus (mmol/L)	2.29±0.13 <sup>a</sup>	1.73±0.06 <sup>b</sup>	2.04±0.08 <sup>c</sup>
Magnesium (mmol/L)	1.15±0.03	1.04±0.02	1.3±0.03

Means with different superscripts in a row differ significantly ( $p < 0.05$ ). Reference values: Glucose 2.49 – 4.16 mmol/L (Radostits et al., 2000); Albumin 54 – 86 g/L (Forenbacher, 1993); Total protein 73.8 – 106.2 g/L (Olayemi et al., 2001); Cholesterol 1.5 – 6.7 mmol/L (Forenbacher, 1993); Bilirubin 1.2-5.13 µmol/L (Forenbacher, 1993; Radostits et al., 2000; Kaneko, 2008); ALT 4 – 11 U/L (Forenbacher, 1993); 11 – 40 U/L (Radostits et al., 2000; Kaneko, 2008), 9.6 – 35 U/L (Merck Veterinary manual, 2003); AST 35 – 80 U/L (Forenbacher, 1993); LDH 500 – 1500 U/L (Forenbacher, 1993); 692 – 1445 U/L (Radostits et al., 2000; Kaneko, 2008); Urea 1.66 – 6.66 mmol/L (Merck Veterinary manual, 2003); Calcium 2 – 2.8 mmol/L (Merck Veterinary manual, 2003); Phosphorus 1.4 – 2.5 mmol/L (Merck Veterinary manual, 2003); Magnesium 0.7 – 1.2 mmol/L (Merck Veterinary manual, 2003).

Blood proteins are important markers of animal health. Albumin concentration, but also total proteins concentration was found significantly lower during winter season versus summer season (Table 1). Statistically significant higher values obtained in summer season could be explained by the quality of animal nutrition; pasturage used at the examined localities originates from lawns of diverse botanical composition and hence its quality (Hadžimusić and Hrković-Porobija, 2018). Moreover, some types of grasses in the early stages of growth contain large amounts of water and excess protein

and total nitrogen. Higher protein values could also be explained also by possible dehydration of animals. However, albumin concentration changes may indicate deteriorated liver function as a result of inflammatory conditions (Bobbo et al., 2017). Cholesterol concentration determined by present study was found significantly higher during winter season. Similar findings were reported by Dar et al. (2019). Moreover, Yokus and Cakir (2006), observed significantly higher medium cholesterol values ( $4.71 \pm 1.75$  mmol / L) during winter, compared to summer season ( $4.22 \pm 1.16$  mmol / L).

Bilirubin concentration determined by this study was within the reference range. Moreover, this research showed that season has no effect on bilirubin levels. Significant changes in bilirubin concentrations are associated with bile flow disorders (Puppel and Kuczyńska, 2016). The liver enzymes determined by present study (ALT, AST and LDH) were within the normal range and showed no seasonal effect. The impact of pregnancy on the level of AST and ALT activity is quite controversial. Several studies have shown an increase in the activity of liver enzymes during the postpartum period, while some authors on the contrary reported a decrease in activity during the same period (Hadžimusić and Krnić, 2012). Plasma ALT activity is affected by age and muscle activity. Physiological changes of ALT activity are related to pregnancy and the beginning of lactation, when the level of ALT activity is reduced. However, result of our study shown that ALT activity was within the reference range. It is well known that LDH is not an organ-specific enzyme. LDH activity is therefore reported in muscle, heart, kidneys, and liver (Krsmanović et al., 2016). Present research showed LDH activity within reference range, as well no seasonal effect was determined.

Blood urea nitrogen concentration is affected by many factors, such as dietary protein intake and rumen degradability, dietary amino acid composition, liver and kidney function, muscle tissue breakdown (Puppel and Kuczyńska, 2016). Urea concentration determined by study was within the normal range and did not differ significantly during summer and winter season of sampling. In contrast to present study some authors (Giri et al., 2017; Cerutti et al., 2018) reported a seasonal effect on blood urea nitrogen of cattle. However, in contrary with their research, present study included only postpartum cows.

Minerals play a role in almost all living systems, either as structural elements or as regulators of majority of metabolic processes. Calcium is the most abundant mineral in an organism. Since phosphorus and calcium also play a role in bone formation, these two minerals are sometimes considered together. Moreover, hypophosphatemia is often connected with moderate hypocalcaemia (Hadžimusić and Krnić, 2012). In relation to calcium and phosphorus, magnesium metabolism is in many respects specific in. It is well known that magnesium acts as an antagonist to calcium, while its deficiency emphasizes the effect of calcium. Along with hypocalcaemia hypomagnesaemia can cause pasture tetany in cows and goats. Hypocalcaemia may be a major cause for the emergence of hypomagnesaemia. Values of investigated minerals were within the reference intervals for cows, and showed no seasonal effect, except for the phosphorus. While the calcium and magnesium were unaffected by the season, significantly higher concentrations of phosphorus were determined during summer season compared to the winter season. Present result was in agreement with previous studies which reported that phosphorus levels in cows mainly depends on climatic variation and nature of feed (Shrikhande et al., 2008; Kubkomawa et al., 2015; Coates et al., 2019).

## CONCLUSION

In this study, the metabolic profile of postpartum Holstein-Friesian cows was analyzed to understand the seasonal variations in blood glucose, blood proteins, cholesterol, bilirubin, liver enzymes, blood urea nitrogen, and essential minerals. The results highlighted several significant findings that are crucial for optimizing the health and productivity of postpartum dairy cows. Present research showed blood glucose levels higher in the summer compared to winter, although the difference was not statistically significant. Both albumin and total protein concentrations were significantly lower in winter compared to summer. This difference is likely due to the quality of nutrition, as summer pasturage is richer in diverse botanical composition, which enhances protein intake. These findings suggest that improving winter feeding strategies could enhance protein levels and overall cow health. Cholesterol concentrations were significantly higher in winter, aligning with findings from previous studies. Bilirubin and urea levels showed no significant seasonal variation. Liver enzyme activities (ALT, AST, and LDH) also remained within normal ranges and exhibited no significant seasonal effects. Among the minerals studied, only phosphorus showed significant seasonal variation, with higher levels in summer. Calcium and magnesium levels remained stable across seasons.

## DECLARATIONS

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### Authors' contribution

N.Hadžimusić, Dž.Hadžijunuzović-Alagić: Conceptualized and designed the study, conducted the experiments, and drafted the manuscript. Dž. Hadžijunuzović-Alagić., N. Hadžimusić: Analyzed the data, interpreted the results, and revised the manuscript. N. Hadžimusić , Dž. Hadžijunuzović-Alagić: Assisted with sample collection and laboratory analyses, and contributed to the manuscript writing. Dž. Hadžijunuzović-Alagić., N. Hadžimusić: Supervised the project, provided critical feedback, and helped shape the research and manuscript.

### Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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### Ethical regulations

This study did not involve any excessive experimental procedures on animals. The blood sampling conducted was part of the routine health monitoring and systematic veterinary examination of Holstein-Friesian cows. Given that the blood samples were collected as a part of standard veterinary care and did not require any invasive procedures beyond those typically performed during routine check-ups, approval from an ethics committee was not required. All procedures adhered to the established guidelines for animal care and welfare, ensuring minimal stress and discomfort to the animals involved.

### Consent to publish

All authors have reviewed and approved the final manuscript for publication.

### Competing Interests

The authors declare that they have no competing interests.

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