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ENZYME ACTIVITIES IN COW PLASMA DEPEND ON GEOGRAPHICAL LOCATION AND SEASON

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Abstract

This research is significant because it investigates how geographic location and seasonal variations affect enzyme activities in dairy cows, which are crucial indicators of liver health and metabolic status. Understanding these variations is vital for accurately interpreting metabolic profile tests (MPTs) used to monitor cow health, diagnose diseases, and optimize herd management practices. By highlighting the influence of environmental and physiological factors on enzyme levels, this study can help improve dairy farming practices, enhance animal welfare, and ultimately contribute to better productivity and health outcomes in dairy herds. This study aimed to investigate the enzymatic activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP) in Holstein-Friesian cows, with a focus on variations due to geographical location and seasonal changes.

The study involved 480 Holstein-Friesian cows aged between 2 and 9 years, with most being between 3 and 5 years old. The research was conducted in two distinct geographical areas in Bosnia and Herzegovina: the northern region (Farm T) and the southern region (Farm V). Blood samples were collected via puncture of the coccygeal vein during two periods: summer (June-August) and winter (December-February). Samples were stored in 5 mL vacuum blood containers and transported in portable refrigerators at 4 ^{0}C to the Veterinary Faculty in Sarajevo. The blood samples were centrifuged (LC 320, 3000 rpm/10 min) to separate the plasma. Enzyme activities were determined using a spectrophotometer (Beckmann DU-64 UV/VIS). Commercial kits from "Human" (Max-Planck-Ring 21, D-65205 Wiesbaden, Germany) were used to measure the enzyme activities in the plasma. Data analysis was performed using SPSS 10.00 software. Mean values of the examined parameters between different animal groups, based on the sampling season, were compared using the t-test. Differences were considered statistically significant at a $P < 0.05$ significance level.

Significant differences were observed between locations and seasons. Elevated enzyme levels were particularly noted during the winter, suggesting heightened metabolic demands and potential liver stress. The results underscore the impact of environmental factors, such as climate and altitude, on metabolic processes and liver function in dairy cows.

These findings highlight the importance of considering geographical and seasonal factors in the management of dairy herds to optimize health and productivity. Further research is recommended to explore the underlying mechanisms driving these enzymatic variations, which could inform more targeted interventions in dairy farming practices.

***Key words**: Enzyme Activities, Dairy Cows, Geographic Location, Seasonal Variations.*

1. Introduction

The metabolic profile test (MPT) has been utilized for many years on high-production dairy farms as an effective method for monitoring health and preventing diseases in dairy herds (Madreseh-Ghahfarokhi and Dehghani-Samani [16]). Proper interpretation of MPT results requires knowledge of pathological deviations in specific indicators and physiologically induced variations. Studies indicate a connection between the quality and composition of dairy cow feed and the concentration of blood metabolites (Urbutis *et al.*, [25]). Climatic conditions, such as temperature variations, can also affect changes in certain MPT indicators (Mbuthia *et al.*, [18]; Giannone *et al.*, [6]). The physiological state of the animal, particularly the reproductive cycle of cows, significantly impacts biochemical indicators. The metabolism of high-yield dairy cows is often burdened due to their genetic predisposition for high milk production and reproductive demands. Increased energy needs during pregnancy and lactation are often not matched by adequate energy intake, leading to dysfunction in certain organs, primarily the liver.

High activity levels of certain enzymes are observed in the liver, especially in cases of suspected acute or chronic liver disease. Increased AST activity in dairy cows is mainly associated with fatty liver syndrome (Bombik *et al.*, [2]), reduced appetite, and ketosis during early lactation (Delić *et al.*, [3]). AST and LDH are frequently used to diagnose liver diseases in cows (Klein *et al.*, [12]). Some reports suggest that increased AST and LDH activity is a sensitive marker of liver damage, even if the damage is subclinical (Klein *et al.*, [12]). Besides toxic or metabolic liver damage, increased AST and LDH activity also occurs during infections and inflammatory processes. Changes in AST levels are observed in cows during lactation. Unlike AST, ALT activity in horses, pigs, and cattle is not significantly higher during liver damage, even in cases of liver necrosis (Satué *et al.*, [24]). Some authors suggest that serum ALT is a good indicator of hepatocellular necrosis in dogs and cats but is less significant in horses, cattle, pigs, sheep, and goats (Abu Damir *et al.*, [1]). Age and muscle activity influence ALT activity in blood plasma (Khairita *et al.*, [10]). Physiological variations in ALT activity are observed during pregnancy and early lactation when ALT activity levels are reduced. ALP mainly originates from the intestinal mucosa, kidneys, bones, and liver (Lowe *et al.*, [15]). Although ALP activity is present in many cells and tissues, the highest activity is found in liver, bone, kidney, intestinal mucosa, and placenta cells (Makris *et al.*, [17]). Most ALP is located in the cell membrane of hepatocytes (Makris *et al.*, [17]). Physiological increases in this enzyme's activity occur during the animal's growth period (Sammad *et al.*, [23]). Increased alkaline phosphatase activity occurs due to cholestasis. This study aims to determine the activity of these enzymes concerning geographic localization, as well as the season of sampling and feeding regime.

This study aimed to investigate the enzymatic activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP) in Holstein-Friesian cows, with a focus on variations due to geographical location and seasonal changes.

2. Materials and Methods

The study involved 480 Holstein-Friesian cows aged between 2 and 9 years, with the majority being between 3 and 5 years of age. The research was conducted in two distinct geographical areas: the northern region on Farm T (45°01'43.4"N 17°18'19.0"E) (n = 240), and the southern part of Bosnia and Herzegovina on Farm V (43°03'52.7"N 17°42'38.2"E) (n = 240). Sampling took place during two periods: the summer (June - August) (n = 240), and the winter (December-February) (n = 240).

The animals in the northern region were kept on a modern farm, which is a key factor in production and animal welfare. Maintaining a dynamic balance between biotic and abiotic factors is crucial for the health and productivity of animals (Vučemilo *et al.*, [26]). On Farm T, Radio Frequency ID (RFID) technology was used, where each animal had a chip to monitor its activity, recording data related to reproduction, lactation, and medical history. Animals on Farm V were not kept under the same modern conditions as Farm T but were managed under similar zootechnical conditions. The feeding systems on both farms did not differ significantly and followed these protocols:

- Winter Feeding: High-yielding cows received 30 kg of silage, 15 kg of haylage, and 10 kg of a concentrate mixture containing 18 - 20% protein. Dry cows were fed 20 kg of silage, 10 kg of haylage, and 3 - 4 kg of concentrate with 18 - 20% protein. Postpartum cows were given ad libitum silage, 2 kg of concentrate mixture, 3 kg of hay, and 0.05 kg of soybean meal. All categories received 0.05 kg of livestock salt and 0.05 kg of livestock chalk.
- Summer Feeding: High-yielding cows were given 20 kg of corn silage, 15 kg of haylage, 2.5 kg of hay, 6 kg of concentrate mixture, and 6 kg of brewers' grains. The diet included 0.05 kg of livestock salt, 0.05 kg of livestock chalk, and 0.15 kg of mineral-vitamin supplements. Dry cows were fed 25 kg of corn silage, 1 kg of soybean meal, 2 kg of bran, 2 kg of concentrate mixture, 0.05 kg of livestock salt, and 0.05 kg of livestock chalk. Postpartum cows received ad libitum silage, 2 kg of concentrate mixture, 3 kg of hay, and 0.05 kg of soybean meal, livestock salt, and livestock chalk.

Blood samples were collected via puncture of the coccygeal vein and stored in 5 mL vacuum blood containers. The samples were transported in portable refrigerators at 4 ^{0}C to the Veterinary Faculty in Sarajevo. The blood was immediately centrifuged (LC 320, 3000 rpm/10 min) to separate the plasma.

Enzyme activities were determined using a spectrophotometer (Beckmann DU-64 UV/VIS) with commercial kits from "Human" (Max-Planck-Ring 21, D-65205 Wiesbaden, Germany).

Data analysis was performed using SPSS 10.00 software. Mean values of the examined parameters between different animal groups, based on the sampling season, were compared using the t-test. Differences were considered statistically significant at a $P < 0.05$ significance level.

3. Results and Discussion

According to the literature, the level of alanine aminotransferase (ALT) activity ranges from 4 to 11 U/L (Kim *et al.*, [11]) 11 to 40 U/L (Radostits *et al.*, [20]; Kaneko [9]), and 9.6 to 35 U/L (Merck Veterinary Manual, [19]). Krnić and colleagues [13], determined the ALT activity in lactating cows, with a mean value of 23.50 ± 1.42 U/L. The mean value for cows 5 - 10 days prepartum was 15.00 ± 1.40 U/L, and the mean value for cows 1 - 5 days postpartum was 27.00 ± 7.22 U/L. The authors concluded that these values fall within the reference range. The mean value determined in our study was 13.43 ± 0.58 U/L during the summer period at location T. Significantly higher values were recorded at the same location during the winter period, with a mean value of 24.31 ± 0.70 U/L (Figure 1).

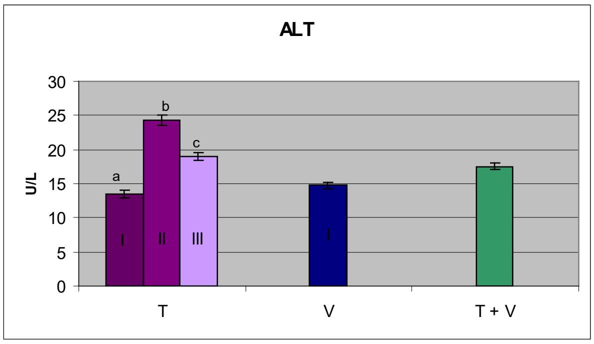

Figure 1. Level of ALT activity (U/L) in the blood plasma of cows by location and season. All values represent the mean ± standard deviation. The labels T and V refer to the locations: T = northern part of Bosnia and Herzegovina, V = southern part of Bosnia and Herzegovina. The labels I, II, and III represent different seasons, where I = summer period, II = winter period, and III = I + II. a, b = values within the same location that have different letters are statistically significant ($p < 0.05$). * = statistically significant difference ($p < 0.05$) between the same sampling periods of different locations

This statistical significance can be explained by the high variability of liver enzymes, which may be due to their instability in blood plasma (Reynolds *et al.*, [21]). Elevated levels of this enzyme occur in hepatocellular disorders, viral infections, and muscle injuries. Variations in ALT levels in animals can also indicate an intensification of metabolic processes and an adverse impact of negative energy balance.

At location V, during the only summer sampling, the mean ALT value was 14.73 ± 0.51 U/L, which did not significantly differ from the same sampling period at the other location (Figure 1).

The AST activity values for cows provided by the authors are 35 - 80 U/L (Forenbacher [5]), and 78 - 132 U/L (Radostits *et al.*, [20]; Kaneko [9]). The range determined by our research based on the summer period at location T was 21.6 - 125.44 U/L, with a slightly narrower range in the winter period at the same location, 27.0 - 102.64 U/L. The analysis of the total sample from location T during both seasons (summer + winter) resulted in a range of 23.06 - 115.18 U/L. According to the research by Đoković *et al.*, [4], the mean activity of this enzyme in healthy cows was 92.80 ± 29.5 U/L. The investigated AST activity at location V ranged from 20.66 to 97.02 U/L. A statistically significant difference ($p < 0.05$) was observed at location T between the summer and winter periods (Figure 2).

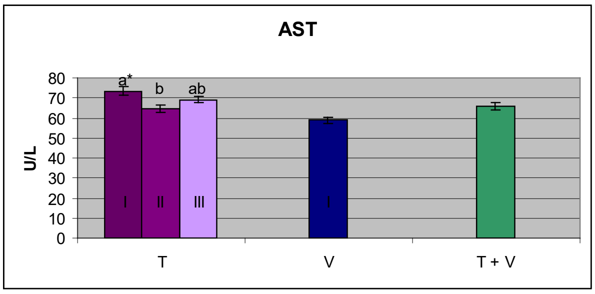

Figure 2. AST activity (U/L) in the blood plasma of cows by location and season. All values represent the mean ± standard deviation. The labels T and V refer to the locations: T = northern part of Bosnia and Herzegovina, V = southern part of Bosnia and Herzegovina. The labels I, II, and III represent different seasons, where I = summer period, II = winter period, and III = I + II. a, b = values within the same location that have different letters are statistically significant ($p < 0.05$). * = statistically significant difference ($p < 0.05$) between the same sampling periods of different locations

As can be seen in Figure 2, a statistically significant difference was also recorded between the summer sampling periods at different locations. This difference could be due to variations in the chemical composition of the soil, and thus the nutritional value of the feed in different areas. The variability of feed composition is linked to climate, type of feed, soil type, and weather conditions, which are also defined by different altitudes (Godde *et al.*, [7]). Yokus and Cakir [27], did not observe statistically significant differences in AST levels in cattle based on season. The statistical significance we recorded in this study can be explained by the high variability of enzymes, resulting in their instability in blood plasma (Reynolds *et al.*, [21]). Similar to ALT, AST in animals can indicate an intensification of metabolic processes and an adverse impact of negative energy balance.

The mean LDH activity values determined by our research were slightly higher than the quite variable literature values, with reference ranges of 500 - 1500 U/L (Forenbacher [5]), 692 - 1445 U/L (Radostits *et al.*, [20]; Kaneko [9]), and 309 - 938 U/L (Merck Veterinary Manual [19]). Our research determined the intervals as follows: at location T during the summer period, 614.56 - 1383.04 U/L with a mean value of 998.80 ± 17.91, which was significantly lower compared to the winter sampling period (1237.21 ± 42.35) at the same location, with a range of 317.13 - 2157.29 U/L (Figure 3).

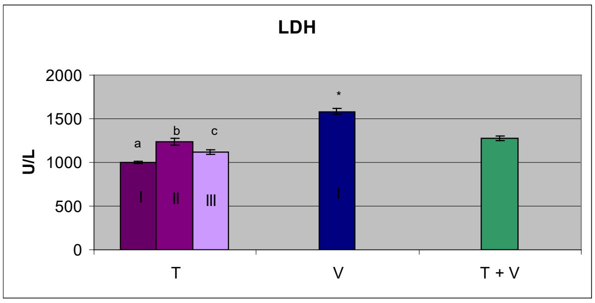

Figure 3. LDH activity (U/L) in the blood plasma of cows by location and season. All values represent the mean ± standard deviation. The labels T and V refer to the locations: T = northern part of Bosnia and Herzegovina, V = southern part of Bosnia and Herzegovina. The labels I, II, and III represent different seasons, where I = summer period, II = winter period, and III = I + II. a, b = values within the same location that have different letters are statistically significant ($p < 0.05$). * = statistically significant difference ($p < 0.05$) between the same sampling periods of different locations

Determining this enzyme's activity at location V was only conducted in the summer period, and the values were significantly higher compared to the same period at location T, ranging from 946.17 to 2222.77 U/L (Figure 3). The study by Hodžić *et al.*, [8], also showed high mean values of this enzyme in clinically healthy cows. Sako *et al.*, [22], recorded slightly higher LDH values in cows, ranging from 1230 to 2074 U/L. Variations in LDH activity are often due to different physiological statuses of cows (Yokus and Cakir [27]). Increased activity of this enzyme occurs in infectious, inflammatory, toxic, or metabolic liver damage. In chronic liver diseases or slow-progressing liver diseases, the activity of this enzyme can drop below the lower reference value if a small number of hepatocytes are damaged, resulting in a significantly reduced hepatocellular mass (Lechtenberg and Nagaraja [14]). LDH activity is elevated in hepatic lipidosis, venous congestion, and diseases causing distension of the forestomachs and abomasum.

Available literature data for alkaline phosphatase activity levels vary significantly. Forenbacher [5], reports a range of 20 - 70 U/L, Radostits *et al.*, [20], report 0.0 - 500 U/L, Merck Veterinary Manual [19], reports 18 - 153 U/L, and Kaneko [9], reports 0.0 - 488 U/L. The study by Yokus and Cakir [27], showed different mean ALP values depending on the physiological status of the animal: lactating cows had a mean ALP value of 199.57 ± 13.66 U/L, early lactating cows 291.64 ± 71.56 U/L, mid-pregnancy cows 402.21 ± 76.12 U/L, and highly pregnant cows had the highest mean value of 522.43 ± 22.17 U/L. The overall mean ALP value determined by these authors was 353.96 ± 170.63 U/L. The alkaline phosphatase activity values in our study were complete for location T, while they were determined only during the summer period at location V (Figure 4).

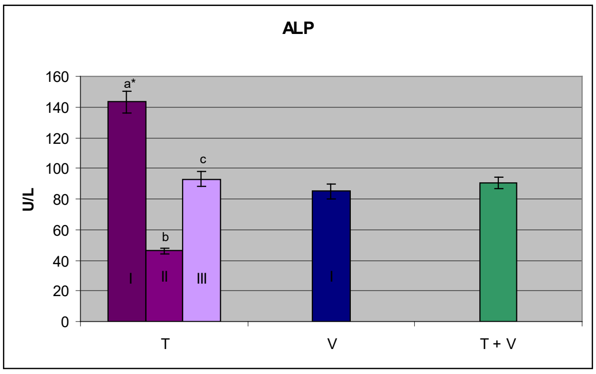

Figure 4. ALP activity (U/L) in the blood plasma of cows by location and season. All values represent the mean ± standard deviation. The labels T and V refer to the locations: T = northern part of Bosnia and Herzegovina, V = southern part of Bosnia and Herzegovina. The labels I, II, and III represent different seasons, where I = summer period, II = winter period, and III = I + II. a, b = values within the same location that have different letters are statistically significant ($p < 0.05$). * = statistically significant difference ($p < 0.05$) between the same sampling periods of different locations

The widest value range, based on our research, was determined at location T during the summer sampling period, and it was 0.0 - 296.13 U/L. The narrowest range, based on our research, was also from location T during the winter period, and it was 0.0 - 92.32 U/L. The alkaline phosphatase activity determined in our research showed significant differences between different sampling periods. The ALP values for cows during the summer period at location T were significantly higher compared to the winter sampling period (Figure 4). Yokus and Cakir [27], found that ALP activity varies depending on the physiological state, as well as seasonal variations. Statistically significant higher ALP values were also determined between the same sampling periods at different locations. This difference is likely due to differences in the chemical composition of the feed in different areas. The variability of feed composition is related to climate, type of feed, soil type, and weather conditions, which are also defined by different altitudes.

Significantly higher alkaline phosphatase values during the summer period at location T may also be due to increased animal movement, as animals were taken to pasture in the farm area. Increased animal movement during the summer period results in a faster metabolism, and therefore higher ALP levels.

Reduced ALP activity in ruminants may be associated with zinc deficiency (Lowe *et al.*, [15]). Many drugs stimulate ALP synthesis in the liver. However, due to insufficient knowledge of the factors influencing ALP synthesis, it is challenging to determine the actual reason for the increase in this enzyme's value, i.e., whether its activity is increased primarily due to increased synthesis or secondarily as a liver response to drug-induced cytokines or due to drug-induced liver damage (Kaneko [9]).

The study provides comprehensive insights into the enzymatic activities of ALT, AST, LDH, and ALP in Holstein-Friesian cows across different locations and seasons, highlighting the influence of environmental and physiological factors on these biochemical parameters:

- The ALT activity levels observed in our study ranged from 4 to 40 U/L, aligning with the literature values. Notably, the mean ALT values were significantly higher during the winter period at location T, likely due to the high variability and instability of liver enzymes in blood plasma. These elevated levels can indicate hepatocellular disorders, viral infections, and muscle injuries, reflecting an intensification of metabolic processes and a negative energy balance.
- AST activity values varied significantly between seasons and locations. Our findings, which ranged from 20.66 to 125.44 U/L, showed significant differences between summer and winter periods at location T and between different locations during the summer. These variations could be attributed to differences in soil chemical composition and feed nutritional value, influenced by climate, soil type, and altitude.
- The LDH activity levels in our study were higher than some literature values, with significant seasonal variations at location T. The winter period exhibited higher mean LDH values, potentially due to the physiological status of the cows and increased metabolic demands during this season. Elevated LDH levels are associated with infectious, inflammatory, toxic, or metabolic liver damage.
- ALP activity values varied widely, with significantly higher values during the summer period at location T compared to the winter period. These differences may be due to increased animal movement during summer, leading to higher metabolic rates. Variations in ALP activity can also be linked to physiological states, zinc deficiency, and the influence of drugs on liver enzyme synthesis.

4. Conclusions

- Overall, the results underscore the importance of considering environmental and physiological factors when interpreting enzymatic activity levels in dairy cows. These findings can inform better management practices, ensuring optimal animal health and productivity.
- Future research should further explore the underlying mechanisms driving these variations to develop targeted interventions for improving dairy cow health and performance.
- The investigated enzymes are significant indicators of various diseases. Increased enzyme activity in the blood is usually a sign of cell and tissue damage, as well as intensified metabolic processes. However, the sampling season, animal age, and energy status are also factors influencing the values of ALT and AST enzymes [28].

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