

## **Blood Biochemical Parameters and Enzyme Activity in Beef Cattle**

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**Abstract:** The objective of this study was to evaluate characteristic blood biochemical parameters and enzyme activity in two groups of beef cattle: cattle aged 6 months (n=11) and cattle aged 9 months (n=11). No statistical difference ( $p>0.05$ ) in blood glucose levels was observed between the two study groups, suggesting preserved liver function. Blood triglyceride and total cholesterol levels were within the physiological range in both groups of cattle and were not statistically different ( $p>0.05$ ), clearly indicating preserved lipid mechanism. There was no statistically significant difference ( $p>0.05$ ) in the blood levels of total proteins, albumin and urea between the two groups, with total protein level being, however, slightly below the physiological range, likely suggesting the slightly decreased synthetic capacity of hepatocytes in both study groups. Blood bilirubin levels were not statistically different ( $p>0.05$ ). They were found to be somewhat above the physiological level in cattle aged 9 months, indicating the slightly decreased excretory capacity of the liver. Blood AST and GGT activities were not statistically different ( $p>0.05$ ) between the test groups of cattle and they were within the physiological range, suggesting preserved hepatocellular function. In contrast to the above activities, blood LDH activities were statistically significantly higher ( $p<0.01$ ) in the older cattle than in the young cattle, primarily indicating an increased degree of skeletal muscle degeneration. The results of this study suggest that the evaluated

biochemical parameters and activities of enzymes involved in energy metabolism in the blood may be used as good indicators of the metabolic status of beef cattle.

**Key words:** beef cattle, proteins, lipids, bilirubin, enzymes.

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## Introduction

During intensive fattening, cattle are pushed to their physiological limits. This results in a substantial load on an animal organism, specifically the digestive organs, liver and the reproductive organs and, consequently, in abrupt changes in energy metabolism that are likely to induce severe uncontrolled disorders of organic matter metabolism (Šamanc 2009).

When high levels of compound feed are fed, beef cattle commonly develop ruminal acidosis (increased lactate levels), negatively affecting the maintenance of an acid-base equilibrium in the organism and the morphology and function of parenchymatous organs, particularly the liver, and the striated muscle (Mori *et al.* 2007, Šamanc 2009).

The increased metabolic activity of the digestive organs and the liver and mild acidosis associated with intensive fattening of beef cattle cause slight damage to the hepatic parenchyma by disturbing its physiological and morphological integrity as induced by diets predominantly composed of feed concentrate (Lupi *et al.* 2005, Mori 2007, Šamanc 2009).

During the increased metabolic load on the organism associated with intensive fattening, the values of blood parameters are within the physiological range. The increased metabolic load on the liver of beef cattle can sometimes lead to fat accumulation in the liver and disturb the morphological and functional integrity of hepatocytes, resulting in decreased blood levels of some liver-synthesized indicators of liver function (glucose, total proteins, albumin, globulin, cholesterol, triglycerides, urea). Moreover, the excretory function of hepatocytes is reduced and, accordingly, the levels of certain metabolic products in the blood (bilirubin, ammonia, bile acids) are likely to increase (Herdt *et al.* 1983, Philips *et al.* 1987, Holtenius 1989, Grummer 1995, Reynolds *et al.* 2003, Sevinc *et al.* 2003, Lubojacka *et al.* 2005, Lupi *et al.* 2005, Liker *et al.* 2006, Mori 2007, Boonprong *et al.* 2007).

Severe damage of parenchymatous organs and muscles involve cell membrane damage accompanied by the release of cytoplasmic enzymes specific to energy metabolism (AST, GGT, LDH), the activity thereof in the blood being elevated (Tietz 1987, Jovanović *et al.* 1993, Pechova 1997, Lubojacka *et al.* 2005, Liker *et al.* 2006, Boonprong *et al.* 2007). Mori *et al.* (2007) report that the elevated activity of AST, GGT and LDH in the blood of fattening cattle is the result of the acidosis-induced liver cell damage. Tietz 1987 note that increased LDH levels primarily indicate the degree of striated muscle and myocardial damage.

Given the increased metabolic loads on the digestive organs and liver in fattening beef cattle, the objective of this study was to determine blood parameters i.e. indicators of the functional status of the liver, being the following: glucose, triglycerides, total cholesterol, total proteins, albumin, urea, bilirubin, as well as the blood enzyme activity (AST, GGT, LDH) in beef cattle.

### Material and methods

**Test animals.** Beef cattle (n=22) were randomly selected from the Simmental herd (Farm, Trbušani, Čačak) for analysis. The animals were allocated to one of the following two groups: Group A, comprising beef cattle aged 6 months (194d±16d) (n=11), and Group B, made up of cattle aged 9 months (281d±18d) (n=11). The body weight of the test cattle was about 250 kg and 350 kg (groups A and B, respectively). Studies were conducted in mid-September, during the same season. The test animals were kept in a closed free-stall barn. The diet and the housing facilities used were adapted to research purposes. Ration composition was suited to the energy requirements of beef cattle depending on the fattening stage. Group A cattle were fed daily rations containing 3.3 kg concentrate and 8 kg maize silage. The older cattle (Group B) were offered 4.5 kg concentrate and 13 kg maize silage. Concentrate feed formulation is given in Table 1.

Tab. 1. Formulation of concentrate feed fed to beef cattle.

Maize	76.5%
Wheat, barley	10%
Soybean meal	5%
Sunflower meal	5%
Dicalcium phosphate	1%
Premix	2.5%

**Blood sampling.** Blood samples were taken from all tested cows by jugular vene-puncture between 10:00 a.m. and noon, four to six hours after feeding. Approximately 20 ml of blood was collected into each of two test tubes per puncture. After spontaneous coagulation at room temperature, blood serum was separated by centrifugation at 3000 rpm. Serum samples were transported to the biochemical laboratory where they were stored until analysis.

**Biochemical analysis of blood sera.** The levels of glucose (Cat. No. 11803), triglycerides (Cat. No. 11828) and urea (Cat. No. 11536) were assessed by an enzymatic spectrophotometric assay. Spectrophotometry was used to evaluate the blood levels of total cholesterol (Cat. No. 11805), total proteins (Ct. No. 11500), albumin (Cat. No. 11547) and bilirubin (Cat. No. 11515) and the activities of liver-specific enzymes: AST-aspartat-aminotransferase (Cat. No. 11830), GGT-gamma-glutamyltransferase (Cat. No. 11584), and LDH-lacto-dehydrogenase (Cat. No. 11552). All biochemical blood parameters were assayed using a Cobas Mira device at the Medicus Biochemical Laboratory in Kraljevo.

**Statistical analysis of data.** The obtained data were subjected to statistical analysis by ANOVA-procedure. An analysis of variance and an LSD test were used to evaluate the probability of the statistical significance of differences between mean values of blood parameters from the two groups of cattle tested. The probability levels used were  $p < 0.05$  and  $p < 0.01$  (Microsoft STATISTICA ver.5.0 Stat. Soft. Inc. 1995).

## Results

Table 2 shows results on the blood levels of glucose, triglycerides, total cholesterol, total proteins, albumin, urea, and bilirubin, and blood AST, GGT and LDH activities in beef cattle.

Tab. 2. Blood levels of glucose, triglycerides, total cholesterol, total proteins, albumin, urea and bilirubin and blood AST, GGT and LDH activities in beef cattle aged 6 months (Group A) and in those 9 months of age (Group B) and the statistical significance between the mean values obtained.

Group	A	B	P<0.05	P<0.01
n	15	15		
Glucose (mmol/l)	3.21 ± 0.35	3.29 ± 0.24		
Triglycerides (mmol/l)	0.15 ± 0.05	0.14 ± 0.05		
Total cholesterol (mmol/l)	1.77 ± 0.36	1.85 ± 0.31		
Total proteins (g/l)	56.83 ± 4.78	54.95 ± 5.54		
Albumin (g/l)	31.86 ± 3.57	29.93 ± 2.03		
Urea (mmol/l)	5.33 ± 1.25	5.06 ± 1.20		
Bilirubin (µmol/l)	5.14 ± 1.86	6.54 ± 2.36		
AST (IJ/l)	73.34 ± 9.52	70.33 ± 21.51		
GGT (IJ/l)	13.06 ± 2.34	14.18 ± 3.21		
LDH (IJ/l)	1974 ± 397	3039 ± 604		A:B

Table 2 shows that there were no statistically significant differences in blood glucose levels between the two groups of cattle ( $p>0.05$ ). Likewise, differences between the groups regarding the blood values of total lipids, total cholesterol, total proteins and albumin were not statistically significant ( $p>0.05$ ). The blood levels of bilirubin and urea, the end products of metabolism, were within the physiological range and showed no statistically significant differences ( $p>0.05$ ). Blood activities of the liver enzymes AST and GGT were within the physiological range in both groups of fattening cattle, but no statistically significant differences ( $p>0.05$ ) were observed. The estimated values of LDH were significantly higher ( $p<0.01$ ) in beef cattle aged 9 months than in those 6 months of age.

## Discussion

Glucose is among major blood parameters defining the energy metabolism in cattle. The measured blood glucose levels in all groups of cattle were within the physiological range (2.2-4.0 mmol; Šamanc 2009). The cattle showed no statistically significant differences in blood glucose values. The above results are in agreement with literature data (Philips *et al.* 1987, Grummer 1995, Lupi *et al.* 2005, Liker *et al.* 2006, Mori 2007, Boonprong *et al.* 2007) and suggest the preserved gluconeogenic capacity of the liver in the test animals.

Lipid metabolism parameters include blood triglyceride and total cholesterol levels. Somewhat lower blood triglyceride levels were measured in cattle aged 9 months, whereas the total cholesterol values were slightly higher but statistically insignificant ( $p>0.05$ ) as compared to those in the other group of cattle. The results

indicated that the blood levels of triglycerides and total cholesterol (triglycerides 0.1-0.3mmol/l; total cholesterol 1.5-5 mmol/l, Šamanc , 2009) were within the physiological values in both groups of cattle and suggested the preserved liver function within fat metabolism. The data are in agreement with the results obtained by other authors (Pechova *et al.* 1997, Sevinc *et al.* 2003, Lupi *et al.* 2005, Liker *et al.* 2006, Mori 2007, Boonprong *et al.* 2007).

Nitrogen metabolism parameters involve determination of the blood levels of liver-synthesized total proteins, albumin and urea, the values thereof declining in cases of hepatopathy (Jovanović *et al.* 1993, Lubojacka *et al.* 2005). Albumin is an indicator of the synthetic capacity of the liver. The decline in blood albumin levels to values as low as 20% is induced by chronic liver diseases in cattle (Sevinc *et al.* 2003). The values of total proteins were slightly below the physiological limit, whereas those of albumin and urea were within the physiological range (total proteins 60-80 g/l; albumin 30-40 g/l; urea 1.66-6.66 mmol/l, Šamanc, 2009) in all groups of cattle. There were no statistically significant ( $p>0.05$ ) differences in the above blood parameters between the two groups of cattle tested. The values of total protein below the physiological limit in both groups seemed to suggest a somewhat reduced synthetic capacity of the hepatocytes in the test animals. Similar findings were reported by other authors (Philips *et al.* 1987, Grummer 1995, Pechova *et al.* 1997, Lubojacka *et al.* 2005, Lupi *et al.* 2005, Liker *et al.* 2006, Mori 2007, Boonprong *et al.* 2007).

Blood bilirubin value is a highly sensitive indicator of the functional capacity of liver cells. Reynolds *et al.* (2003) reported a positive correlation between blood bilirubin values and liver fat content. The blood bilirubin values determined in the present study were within the physiological range (0.85- 5.60  $\mu\text{mol/l}$ ; Šamanc 2009) in the group of beef cattle aged 6 months. The values above the physiological limit were found in cattle aged 9 months, but no statistically significant ( $p>0.05$ ) differences were observed between the two groups of cattle, suggesting the impaired excretory capacity of the liver cells in the older cattle. These data conform to those reported in literature (Vasilev 1979, Jovanović *et al.* 1993, Liker *et al.* 2006). Rosenberger (1979) infers that blood bilirubin levels of up to 8.55  $\mu\text{mol/l}$  are indicative of a liver function disturbance or a hemolytic process and that those above 8.55  $\mu\text{mol/l}$  always suggest liver pathology.

The activities of blood enzymes related to energy metabolism are important blood parameters used in estimating the degree of cell damage in parenchymatous organs. The activities of the blood enzymes AST, GGT and LDH were evaluated in this study. AST is found within the cytoplasm and mitochondria of different tissues and organs and its highest activity occurs in the heart muscle, skeletal muscle and the liver. The AST activity is the most sensitive indicator of hepatic dystrophy in cattle (Pechova *et al.* 1997, Lubojacka *et al.* 2005, Liker *et al.* 2006, Boonprong *et al.* 2007). Kupczynski *et al.* (2002) reported increased blood AST activities in dairy cows and beef cattle during intensive fattening i.e. during high metabolic load, resulting in pathological changes, reproduction disorders, parenchymatous organ damage and energy metabolism disorders. The blood AST activities in this study were within the physiological range (45-110 IU/l, Šamanc 2009) and showed no statistically significant differences ( $p>0.05$ ) in the test cattle, suggesting the preserved morphological and functional integrity of the hepatocytes.

The blood GGT activity in both groups of cattle was within the physiological range of 10-20 IU/l (Šamanc 2009), with no statistically significant differences ( $p>0.05$ ) observed, suggesting that the activity of the blood enzyme remained unchanged in both groups of cattle. GGT is a membrane-bound enzyme located in microsomes. It is found primarily in the liver, kidney and the small intestines. The increase in the activity of this enzyme is associated with the damage in the cellular structure of hepatocytes (Jovanović *et al.* 1993, Kupczynski *et al.* 2002, Lubojacka *et al.* 2005, Liker *et al.* 2006, Boonprong *et al.* 2007).

LDH is not an organ-specific enzyme. High levels of LDH are present in the muscle, heart, kidney and the liver. LDH is released into the bloodstream in acute cell damage of these organs (Šamanc 2009). In the present study, the activity of LDH was statistically significantly higher ( $p<0.01$ ) in cattle aged 9 months than in the younger group of beef cattle. Moreover, it was substantially higher than the physiological value (500-1500 IU/l; Šamanc 2009), likely indicating cell membrane damage in myofibrils and/or hepatocytes and release of the enzyme into the bloodstream. Tietz (1987) reported an association between elevated levels of LDH and the degree of striated muscle and myocardial damage.

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### Conclusion

No statistical differences ( $p>0.05$ ) in blood glucose levels were observed between the two groups of cattle tested, suggesting preserved liver function.

Blood triglyceride and total cholesterol levels in both groups of cattle were within the physiological range and were not statistically different ( $p>0.05$ ), clearly indicating the preserved lipid mechanism in cattle.

There was no statistically significant difference ( $p>0.05$ ) in the blood levels of total proteins, albumin and urea between the groups tested, with the total protein level being slightly below the physiological range, likely suggesting the slightly decreased synthetic capacity of hepatocytes in both study groups.

Blood bilirubin levels were not statistically different ( $p>0.05$ ). They were found to be somewhat above the physiological level in the older group of cattle (aged 9 months), indicating the slightly decreased excretory capacity of the liver in this group of cattle.

Blood AST and GGT activities did not show statistically significant differences between the study groups and were within the physiological range, suggesting preserved hepatocellular function. In contrast, blood LDH activities were statistically significantly higher ( $p>0.01$ ) in the older cattle than in the young cattle, primarily suggesting an increased degree of striated muscle damage.

The obtained results suggest that the evaluated blood organic parameters and activities of enzymes involved in energy metabolism may be used as good indicators of the metabolic status of beef cattle.

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## **VREDNOSTI BIOHEMIJSKIH PARAMETARA I ENZIMSKA AKTIVNOST U KRVI KOD JUNADI U TOVU**

-originalni naučni rad -

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### **Rezime**

Cilj rada je bio da se odrede odgovarajući biohemijski parametri, kao i enzimatska aktivnost u krvi kod grupe junadi u tovu starosti 6 meseca (n=11) i grupe junadi u tovu starosti 9 meseci (n=11). Koncentracije glukoze u krvi nisu se statistički razlikovale ( $p>0.05$ ) između ispitivanih grupa junadi, što ukazuje na očuvanu funkciju jetre. Vrednosti triglicerida i ukupnog holesterola u krvi kod obe grupe junadi su bile u fiziološkim granicama, bez statističko značajne razlike ( $p>0.05$ ), što jasno ukazuje na očuvan metabolizam masti kod ispitivanih grupa junadi. Vrednosti ukupnih proteina, albumina i ureje u krvi, nisu se međusobno statistički značajno razlikovale ( $p>0.05$ ) između ispitivanih grupa junadi, ali su vrednosti ukupnih proteina bile nešto niže od fizioloških vrednosti, što može ukazati na neznatno smanjenu sintetsku sposobnost ćelija jetre kod obe grupe junadi. Koncentracije bilirubina u krvi kod ispitivanih junadi nisu se međusobno statistički razlikovale ( $p>0.05$ ), vrednosti bilirubina u krvi kod grupe junadi (9 meseci) bile su nešto veće od fizioloških vrednosti, što može ukazati na nešto smanjenu ekskrecionu sposobnost jetre. Aktivnosti AST,GGT u krvi se nisu međusobno statistički razlikovale ( $p>0.05$ ), između ispitivanih grupa junadi i bile su u fiziološkim granicama, što ukazuje na očuvanu funkciju hepatocita, dok su aktivnosti LDH u krvi bile statistički visoko značajno veće ( $p<0.01$ ) kod starije grupe junadi u odnosu na drugu grupu, što može prevashodno ukazati na povećan stepen oštećenja skeletne musculature. Na osnovu rezultata ispitivanja može se zaključiti da ispitivani biohemijski parametri krvi i aktivnosti za energetske metabolizam specifičnih enzima u krvi mogu biti dobri indikatori stanja metabolizma kod junadi u tovu.