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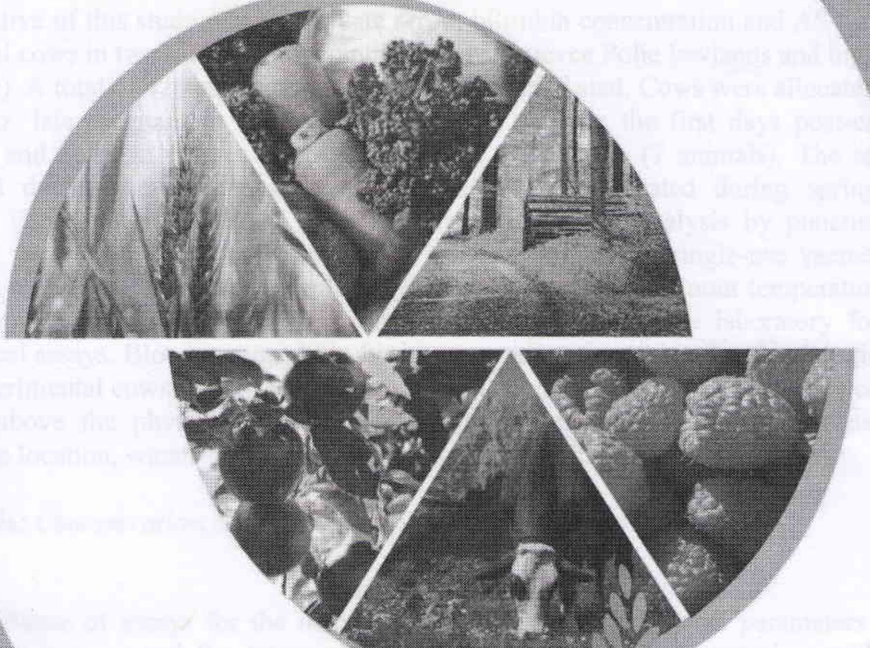

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TOTAL BILIRUBIN CONCENTRATION AND AST ACTIVITY IN SERUM OF SIMMENTAL COWS IN DIFFERENT GEOGRAPHIC REGIONS OF BOSNIA AND HERZEGOVINA

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Abstract

The objective of this study was to evaluate serum bilirubin concentration and AST activity in Simmental cows in two different geographic regions (Lijevce Polje lowlands and high altitude Nevesinje). A total of 42 healthy cows, 21 per farm, were tested. Cows were allocated to three groups viz. late pregnant or dry cows (7 animals), cows in the first days post-calving (7 animals), and cows in the second to third month of lactation (7 animals). The study was conducted during the autumn-winter period (2008) and repeated during spring (2009) involving the same cows. Blood samples were collected for analysis by puncture of the coccygeal vein (vena coccigea) using sterile needles into sterile single-use vacuette blood collection tubes without an anticoagulant. After 24 hour storage at room temperature, serum was separated, deep-frozen, and transported within 48 days to the laboratory for further biochemical assays. Blood (serum) bilirubin concentration was within the physiological range in all experimental cows in both locations. The results related to AST activity showed a slight increase above the physiological limit in all groups of cows, with late pregnant cows (Nevesinje location, winter period) exhibiting significantly higher values.

Key words: *Concentration, Serum, Geographic area, Bilirubin, AST*

Introduction

The importance of assays for the determination of serum biochemical parameters in high-yielding dairy cows and the interpretation of their levels as the supporting evidence for identifying some diseases have been explained in detail by many authors who have addressed this issue for many years (Brugere-Picoux and Brugere, 1987; Lotthammer, 1991; Klinkon et al., 2007; Radojicic et al., 2001; Radojicic et al., 2007; Radojicic, 2008; Samanc et al., 2004). Some researchers have focused on analyzing hepatographic data such as glucose, urea, albumin and bilirubin levels and AST activity, which can serve as indicators of both nutritional and metabolic equilibrium and the functional status of the liver (Kasagic, 2005; Radojicic et al., 2007).

The determination of AST activity as an important indicator of the hepatic status in cows showed stable physiological values of AST activity in the blood serum of older cows after peroral administration of propylene glycol via feed (as an energy precursor, but also as a hepatoprotective) seven days prepartum and seven days postpartum (Kasagic, 2005).

Table 1. Physiological values of bilirubin and AST in the blood of high-yielding dairy cows (according to different authors)

Author	Bilirubin (mmol/l)	AST(U/l)
Kaneko (1989)	2.0-8.0	Up to 60
Blood (1994)	0.1-9.0	80
Lotthammer (1991)	1.0-5.7	80
Radojicic et al. (2007)	0.1-8.0	80

Material and Methods

The research was conducted on two dairy farms. Twenty-one animals aged 3-5 years were selected from each farm and allocated to three groups: late pregnant or dry cows, cows in the first days post-calving and lactating cows, each consisting of 7 animals. The experiment was conducted during the autumn-winter period of 2008 and repeated on the same animals in spring 2009 when many of them belonged to different categories of dairy cattle. The Livac Agricultural Cooperative operating the dairy farm covers about 2.5 ha of land. The cooperative runs its own production of breeding young stock. The high-altitude Nevesinje farm estate employs a closed housing system.

Blood samples were collected for analysis by puncture of the coccygeal vein (vena coccigea) using sterile needles into sterile single-use vacuette blood collection tubes without an anticoagulant. After 24 hour storage at room temperature, serum was separated, deep-frozen and transported within 48 hours to the laboratory for further biochemical assays.

Serum analyses were performed at the accredited Laboratory of Hematology and Biochemistry, Hexalab Institute, Belgrade, Serbia, using a modern spectrophotometer and commonly used standardized reagents, with values calibrated for veterinary use.

The data obtained were statistically analyzed using a factorial analysis of variance and LSD test. Comparisons were made across production statuses and locations. The statistical analysis of the results was performed using the Prisma Pad 4.00 statistics package and MS Excel.

Results and Discussion

Data showing mean values and the factorial analysis of variance are presented in the tables below.

Table 2. Serum parameters across locations and production statuses – comparison I

Location	Production status	$\overline{X}$ for Bilirubin	$\overline{X}$ for AST
Lijevce	Late pregnant	3.04	74.00
	Lactation II/III	1.50	97.58
Nevesinje	Late pregnant	2.50	112.57
	Lactation II/III	1.01	69.31

Parameter	F_{exp}			$LSD_{0,05}$
	Location	Production status	Interaction (Location × Production Status)	Interaction (Location × Production Status)

Bilirubin	1.66	14.35**	0.01	
AST	0.22	0.82	9.43	31.76

*Source: Authors' original data

A statistically highly significant difference was observed in blood bilirubin concentration depending on the production status regardless of location.
The interaction shows a highly significant difference in blood AST concentration in late pregnant cows and cows in the second/third month of lactation in the two locations.

Table 3. Serum parameters across locations and production statuses – comparison II

Location	Production status	$\overline{X}$ for Bilirubin	$\overline{X}$ for AST
Lijevce	Late pregnant	5.56	94.57
	Lactation II/III	1.41	96.14
Nevesinje	Late pregnant	4.26	90.57
	Lactation II/III	1.36	72.86

Parameter	F_{exp}		
	Location	Production status	Interaction (Location × Production Status)
Bilirubin	0.75	20.12**	0.63
AST	5.09**	1.78	2.54

*Source: Authors' original data

A statistically highly significant difference was observed in blood bilirubin concentration depending on the production status regardless of location.
The difference in blood AST concentration was statistically highly significant depending on the location, regardless of the production status.

Table 4. Serum parameters across locations and production statuses – comparison III

Location	Production status	$\overline{X}$ for Bilirubin	$\overline{X}$ for AST
Lijevce	Late pregnant	2.56	72.14
	Lactation II/III	1.24	94.86
Nevesinje	Late pregnant	2.84	77.86
	Lactation II/III	1.42	80.49

Parameter	F_{exp}		
	Location	Production status	Interaction (Location × Production Status)
Bilirubin	1.02	35.98**	0.06
AST	0.37	3.21	2.01

*Source: Authors' original data

Blood bilirubin concentration showed a highly significant difference depending on the production status regardless of location.

Bilirubin; the main coloring matter of the bile, is a breakdown product of hemoglobin in the liver, more precisely the hepatocytes. Total bilirubin includes direct and indirect bilirubin i.e. conjugated and unconjugated bilirubin. Both forms of bilirubin can be separately measured in the serum, but total bilirubin is used for the analysis of the functional ability of the liver. Elevated levels of serum bilirubin imply three aspects of hepatocyte decay, the most important and common being the so-called hepatocellular icterus (icterus hepatocelulare). The production of bilirubin itself comes from the catabolism of hemoglobin or microsomatic cytochromes. Bilirubin is produced in the reticuloendothelial system, spleen, Kupffer cells and bone marrow. There are two forms: conjugated (direct) and unconjugated (indirect) bilirubin. Unconjugated bilirubin is lipid soluble, toxic and bound to plasma albumin. Conjugated bilirubin is insoluble and excreted from the body by the kidneys i.e. in the urine. The liver can process bilirubin concentrations that are manifold higher than the physiological concentration without expending conjugation mechanisms, and only extremely high blood bilirubin concentrations lead to elevated levels of unconjugated bilirubin in the blood. However, in hepatic or hepatocellular jaundice, the damaged hepatocytes are unable to process all the produced bilirubin, and even the processed bilirubin flows back into the bloodstream, leading to elevations in both conjugated and unconjugated bilirubin in the blood. In obstructive jaundice, for instance, the amount of conjugated bilirubin in the blood is directly dependent on the degree of obstruction of the bile ducts.

Bilirubin levels should be assessed as soon after blood sampling as possible. Serum must not be exposed to sunlight. It is not affected by the anticoagulant used.

Samanc et al. (1993) found that serum bilirubin level in older cows was 14.3 µmol/l on average. However, in first-calvers, the value was lower i.e. 6.59 µmol/l on average in early puerperium, and significantly higher than in the last stage of pregnancy, when it was 4.85 µmol/l (Samanc et al., 1993). Djokovic (1989) proved that there is a correlation between fatty liver and elevated blood bilirubin level in ketotic cows compared to healthy cows (x=6,79:5,80 µmol/l). Radojicic et al. (2001) reported an average bilirubin value of more than 8 µmol/l in ketotic cows, which is in agreement with the values reported by Kaneko (1989) and Blood (1994) which can be as high as 9 µmol/l for the upper physiological limit.

Aspartat aminotransferase or AST is not the key enzyme which can on its own serve as an indicator of hepatocyte status or as the so-called biomarker of liver status, but it plays an important role when correlated with increased bilirubin and urea levels and decreased albumin concentration (Brugere-Picoux and Brugere, 1987; Radojicic, 2008). Changes in its amount and activity occur in cases of myopathies, damage and injury to the skeletal striated muscle, heart and liver. Also, these values are increased in hepatopathies, but are compared along with other parameters. For example, in hepatitis, AST values are increased, whereas a more moderate increase is observed in chronic liver cirrhosis. In organophosphate poisoning, toxic liver injury results in the release of high transaminase levels i.e. the increase in serum transaminase activity. Although quite disparate AST values have been reported, the increase of over 80 U/L in cattle and up to 120 U/L in sheep is deemed physiological in a number of studies (Kaneko, 1989; Blood, 1994; Lotthammer, 1991; Brugere-Picoux and Brugere, 1987; Radojicic et al., 2007; Radojicic, 2008).

Conclusions

Aspartat aminotransferase or AST is not the key enzyme which can on its own serve as an indicator of hepatocyte status or as the so-called biomarker of liver status, but its activity when correlated with bilirubin, urea and albumin plays an important role in evaluating the functional activity of the liver (Brugere-Picoux and Brugere, 1987; Radojicic, 2008). Changes in its amount and activity occur in cases of myopathies, damage and injury to the skeletal striated muscle, heart and liver. Also, these values are increased in hepatopathies, but are compared along with other parameters. Although quite disparate AST values have been reported, the increase of over 80 U/L in cattle and up to 120 U/L in sheep is deemed physiological in a number of studies (Kaneko, 1989; Blood, 1994; Lotthammer, 1991; Brugere-Picoux and Brugere, 1987; Radojicic et al., 2007; Radojicic, 2008). In this study, there was a slight increase in AST activity above the physiological limit in all groups of cows, with late pregnant cows (Nevesinje location, winter period) exhibiting significantly higher values i.e. x=112.57.

In many studies, total bilirubin level in the blood serum is considered the most important indicator of liver status and, even, the key biomarker. Also, many authors have studied the importance of changes in the peripartum period i.e. the correlation between bilirubin level and the degree of liver cell damage (Lothammer, 1991; Samanc et al., 1993; Djokovic, 1989; Kovacevic, 2000; Radojicic et al., 2001). These results are supported by the present findings, as the presence of bilirubinemia in this study was within the physiological range in all experimental cows in both locations.

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