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RESEARCH ARTICLE

Changes in blood enzyme activities in ewes with ketosis

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Abstract

Ketosis (pregnancy toxaemia) in dairy ewes is one of the commonest metabolic diseases with substantial health and economic effects for modern sheep husbandry. The investigation was performed on 136 ewes, 106 from the dairy breed Lacaune and 30 – from the meat breed Mouton Charollais. Blood samples were obtained from all animals for determination of β-hydroxybutyrate (BHBA), aspartate aminotransferase (ASAT, U/l), alanine aminotransferase (ALAT, U/l), lactate dehydrogenase (LDH, U/l), alkaline phosphatase (AP, U/l), creatine kinase (CK, U/l) and γ-glutamyltransferase (GGT, U/l). The ewes were divided in three groups depending on their physiological condition, namely: pregnant – from prepartum days 15 to 0; recently lambed – from postpartum days 0 to 15 and lactating – from postpartum days 30 to 45. Target ewes from the three groups were classified as healthy (control, C), affected with subclinical ketosis (SCK) and clinical ketosis (CK). The first group (pregnant; prepartum days 15–0) comprised: control ewes (BHBA < 0.8 mmol/L, n=14); SCK ewes (BHBA from 0.8 to 1.6 mmol/l, n=8) and CK ewes (BHBA >1.6 mmol/l, n=23). The recently lambed group (between postpartum days 0-15) consisted of control group (BHBA < 0.8 mmol/L, n=8); SCK ewes (BHBA 0.8–1.6 mmol/l, n=10); CK ewes (BHBA >1.6 mmol/l, n=12). The lactating ewes between postpartum days 30 and 45 were subdivided into healthy controls (BHBA < 0.8 mmol/L, n=8); SCK (BHBA 0.8–1.6 mmol/l, n=11) and CK (BHBA >1.6 mmol/l, n=12). Mouton Charollais ewes were identically categorised.

Blood BHBA concentrations were increased in Lacaune sheep from the first, second and third groups affected by SCK vs control levels, as well as in animals with CK from all groups vs both controls and SCK. None of Mouton Charollais ewes had blood BHBA levels > 0.8 mmol/L. The activities of ASAT, ALAT, LDH, AP and GGT were increased in Lacaune ewes with subclinical ketosis from the first, second and third groups vs controls. Creatine kinase (CK) activities in all groups of ewes with subclinical and clinical ketosis varied insignificantly close to normal values. In meat-type Mouton Charollais ewes, there were no statistically significant changes in blood ASAT, ALAT, LDH, AP, CK and GGT activities.

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INTRODUCTION

Ketosis (pregnancy toxaemia) in ruminants is a nutritional stress syndrome affecting mainly adult and multiparous animals in good body condition during the last 3 to 6 weeks of gestation (Van Saun, 2000; Schlumbohm and

Harmeyer, 2008). The disease is also known as ovine ketosis, twin-lamb disease, ewe sleepy sickness, lambing sickness or pregnancy paralysis. It could be observed in pregnant cows as a result of negative energy balance (NEB) and twin pregnancies (Gerloff and Herdt, 1984). The economic significance of the disease is determined by reduced milk yields and body weight loss, poor feed conversion, increased culling and mortality rates of offspring and affected animals (Caldeira et al., 2007).

From etiological point of view the disease is associated to impaired metabolism of carbohydrates and fat by the end of pregnancy (Edmondson et al., 2012). The most important cause for the occurrence of this pathology is the negative energy balance due to inadequate feeding, carrying twins or multiples (increased needs of developing fetuses from glucose) or most commonly, to the combined effect of both factors (Van Saun, 2000; Rook, 2000; Schlumbohm and Harmeyer, 2008; González et al., 2011b). As predisposing factors, the number of body weight of fetuses, body condition score of the dam, age, breed, number of lactations, feeding mode, genetic factors, high parasitaemia level, immobilization etc. have been implicated (Rook, 2000; Hefnawy et al., 2011). Another cause outlined by Navarrei and Pugh (2002) is anorexia, occurring as a result of other disease(s) or sudden stress. Ermilio and Smith (2011) reported that sheep and goats pregnant with twins or triplets need from 180 to 240% more energy respectively vs those carrying a single lamb. Nevertheless, Bani Ismail et al. (2008) has observed the condition also in ewes carrying a single but larger fetus, when fed a low-calorie diet. The investigations of Perry et al. (1994) in sheep prior to and after the parturition established that the energy deficiency was higher in early lactation than in late pregnancy. This presumes that similarly to cows, ewes are also susceptible to developing ketosis in early lactation (Baird, 1982).

The data reported in the literature about the prevalence of ketosis among sheep and goats are few. The findings of Van Saun (2000), confirmed also by our studies (Binev et al., 2014), indicate that lactational ketosis is specific only for high-yielding goat breeds and emerges, most frequently in the period before and during peak of lactation. According to the author, in ewes and goats with low milk production, the disease could be seen only sporadically. Schäfer (1991) found out that more than 20% of ewes suffer from subclinical or clinical ketosis during late pregnancy. For a 12-year period (1992-2004) pregnancy toxaemia occurrence in ewes was reported to be between 6.5% and 37% (Al-Mujalli, 2008). With respect to subclinical ketosis, Gupta et al., (2008) established in ewes a prevalence of 14.86% during the pregnancy and 13.51% during the lactation.

It is acknowledged that the use of some blood chemical indices as markers of the physiological, nutritional, metabolic and clinical status is essential for health and welfare management (Găvan et al., 2010; Marutsova et al., 2015). A primary parameter used for detection of pregnancy toxaemia and/or ketosis in ewes and large ruminants is blood BHBA concentration (Kaneko et al., 2008). BHBA is the most stable among the three ketone bodies (Dhanotiya, 2004) and comprises about 85% of the total amount of ketone bodies in sheep with pregnancy toxaemia, the other 15% are distributed between acetone and acetoacetate. Subclinical ketosis in ewes without specific clinical signs of the disease could be detected only via blood BHBA concentrations assay (Duehlmeier et al., 2011).

With respect to diagnosis of SCK in sheep, the following 4 threshold blood BHBA concentrations are mainly discussed in the literature: above 0.5 mmol/l (Feijó et al., 2015), above 0.7 mmol/l (Rook, 2000; Ramin et al., 2007; Moghaddam and Hassanpour, 2008); above 1.0 mmol/l (Smith, 1996) and from 0.8 to 1.6 mmol/l (Ford et al., 1990; Andrews, 1997; Lacetera et al., 2001; Balikci et al., 2009; Anoushepour et al., 2014).

The threshold blood BHBA levels reported in association with clinical ketosis are >1.6 mmol/l (Andrews, 1997; Lacetera et al., 2002); >3.0 mmol/l (Sargison et al., 1994; Kabakci et al., 2003; Balikci et al., 2009) and between 5 and 7 mmol/l (Ford et al., 1990; Henze et al. 1998).

A number of physiological conditions as fasting, parturition and lactation leading to NEB, are related to excessive uncontrolled mobilization of body fat, increased concentrations of circulating ketone bodies (BHBA) and enhanced accumulation of fatty acids in hepatocytes with subsequent morphological and physiological liver alterations (Vazquez-Anton et al., 1994; Djoković et al., 2007). Liver steatosis and hepatocytes' degeneration are accompanied with cell membrane damage and release of cytoplasmic enzymes (AST, GGT and LDH) (Lubojacka et al., 2005). Increased circulating enzyme levels are proportional to the extent of liver fatty infiltration and thus, a useful indicator for evaluation of tissue damage extent (Lubojacka et al., 2005; Djoković et al., 2007). Diagnostics of hepatic lipidosis and susceptibility of cows to ketosis could be done through ultrasonography or biopsy. The analysis of blood biochemical indices is a low-invasive and not expensive method for this purpose (Baird, 1982; Bobe et al., 2004). It is acknowledged that ASAT is not a liver-specific enzyme in ruminants. In cattle, the diagnostic value of the enzyme consists in detection of hepatocytes' injuries, which, together with high blood levels of BHBA and non-esterified fatty acids (NEFA) are associated with hepatic lipidosis (Komatsu et al., 2002; Bobe et al., 2004). Blood BHBA concentrations > 1.2 mmol/l, glucose – 0.7 mmol/l and ASAT >100 U/l are acknowledged as signs for ketosis and hepatic steatosis in cows (Gonzáles et al., 2011a). On the other hand,

serum GGT is believed be a primary marker of hepatobiliary disorders due to cholestasis, which has lately found a wide application for diagnostics of liver diseases (Tennant, 1997).

The purpose of the present study was to evaluate and compare the changes in enzyme activities in dairy and meat type sheep breeds with subclinical and clinical ketosis. The results of the experiments would be useful for diagnosing the disease.

Material and Methods

Animals

The experiments were carried out in a sheep farm in the Republic of Bulgaria in February-March 2014.

Experimental design

A total of 136 ewes (second and third lactation), 106 from the dairy breed Lacaune with 200 l annual lactational yield, average weight 60-80 kg, and 30 from the meat breed Mouton Charollais weighing 70–100 kg were included in the study. All animals were regularly vaccinated and treated against ecto- and endoparasites. They were reared in facilities in compliance with the respective welfare standard for the species. Target sheep were fed rations in concordance with their physiological condition (pregnant, recently lambed and lactating) and norms for roughage and concentrate contents in diets given for each physiological condition. The ewes were divided in three groups depending on their physiological condition, namely: pregnant – from prepartum days 15 to 0; recently lambed – from postpartum days 0 to 15 and lactating – from postpartum days 30 to 45. Blood β-hydroxybutyrate concentrations were assayed in all ewes. In this study, the threshold BHBA values identifying subclinical and clinical ketosis were from 0.8 to 1.6 mmol/l and > 1.6 mmol/l respectively. Lacaune ewes from the three groups were classified as healthy (control, C), affected with subclinical ketosis (SCK) and clinical ketosis (CK).

I. First group (pregnant; prepartum days 15–0) comprised:
1) Control ewes (BHBA < 0.8 mmol/L, n=14);
2) Ewes with subclinical ketosis (BHBA from 0.8 to 1.6 mmol/l, n=8)
3) Ewes with clinical ketosis (BHBA >1.6 mmol/l, n=23).

II. Second group (recently lambed, between postpartum days 0-15) consisted of:
1) Control ewes (BHBA < 0.8 mmol/L, n=8);
2) Ewes with subclinical ketosis (BHBA 0.8–1.6 mmol/l, n=10);
3) Ewes with clinical ketosis (BHBA >1.6 mmol/l, n=12).

III. Third group (lactating ewes between postpartum days 30 and 45):
1) Control ewes (BHBA < 0.8 mmol/L, n=8);
2) Ewes with subclinical ketosis (BHBA 0.8–1.6 mmol/l, n=11)
3) Ewes with clinical ketosis (BHBA >1.6 mmol/l, n=12).

Mouton Charollais ewes were identically categorized.

I. First group (pregnant; prepartum days 15–0):
1) Control ewes (BHBA < 0.8 mmol/L, n=10);

II. Second group (recently lambed, between postpartum days 0-15):
1) Control ewes (BHBA < 0.8 mmol/L, n=10);

III. Third group (lactating ewes between postpartum days 30 and 45):
1) Control ewes (BHBA < 0.8 mmol/L, n=10);

Due to the lack of animals with blood BHBA concentrations > 0.8 mmol/l, SCK and CK groups were not formed.

Blood samples and analyses

Blood samples were collected through puncture of the jugular vein using sterile 21G needles and vacutainers either anticoagulated or with heparin - 5 ml (Biomed, Bulgaria). Samples were obtained in the morning before feeding.

Blood BHBA concentrations were determined in situ using a portable Xpress-I system (Nova Biomedical, UK). Samples for biochemical analysis were transported and stored at 4^0C. Analysis was conducted within 2 hours after sampling. The following indices were determined: Aspartate aminotransferase (ASAT, U/l), Alanine aminotransferase (ALAT, U/l), Lactate dehydrogenase (LDH, U/l), Alkaline phosphatase (AP, U/l), Kreatinkinase (CK, U/l) and γ-glutamyltransferase (GGT, U/l). The biochemical tests were performed using colorimetric method (IFCC 37^0) with test Biolab Diagnostics (France) on an automated biochemical analyser Mindray BS-120 (China).

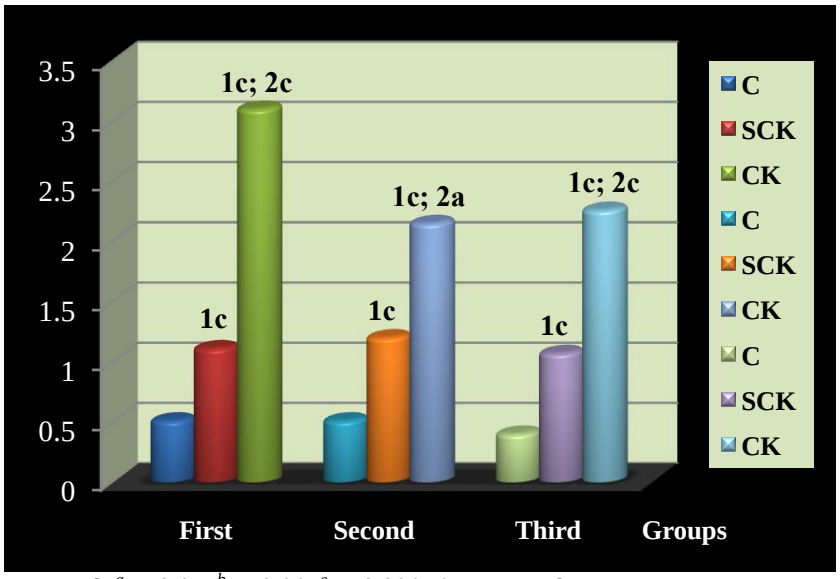
Statistical analysis

Statistical analysis was done with Statistica 6.0 (Windows) software, StatSoft, Inc. (USA, 1993) and ANOVA test. Data are presented as mean (x) ± standard deviation (SD). The level of statistically significance was $p < 0.05$.

Results

The blood BHBA analysis of the three Lacaune sheep groups demonstrated that in control sheep from the first, second and third groups, average BHBA levels were 0.51±0.15 mmol/L; 0.51±0.12 mmol/l and 0.40±0.10 mmol/l respectively.

In ewes from group I (pregnant – prepartum days 15–0) with subclinical ketosis, BHBA in blood was 1.11±0.24 mmol/l – significantly increased vs controls ($p<0.001$). The ewes with subclinical ketosis from group II (recently lambed, postpartum days 0–15) exhibited even higher blood BHBA concentrations – 1.20±0.28 mmol/l vs control (0.51±0.12 mmol/l; $p<0.001$), whereas SCK ewes from group III – 1.07±0.24 mmol/l vs 0.40±0.10 mmol/l in controls ($p<0.001$) (Fig. 1).



Legend: [a]p<0.05; [b]p<0.01; [c]p<0.001; 1-vs control group 1; 2- vs group 2; (C-control group; SCK-with subclinical ketosis; CK-with clinical ketosis)

Fig. 1. Changes in blood β-hydroxybutyrate (BHBA) levels in Lacaune ewes from groups I, II and III with subclinical and clinical ketosis.

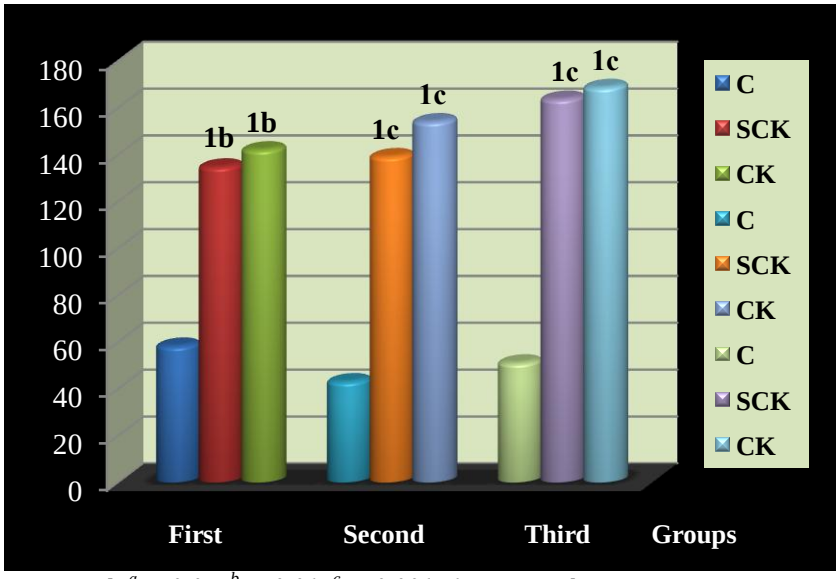
The analysis of changes in this blood parameter in dairy ewes with clinical ketosis from groups I, II and III showed that β-hydroxybutyrate concentrations were statistically significantly higher than respective control and subclinical ketosis groups: 3.10±0.60 mmol/l ($p<0.001$) for group I (pregnant); 2.15±0.63 mmol/l ($p<0.001$ vs control ewes) for group II and 2.26±0.23 mmol/l ($p<0.001$) for group III (Fig. 1).

None of Mouton Charollais ewes had blood BHBA concentrations above 0.8 mmol/l, i.e. none had subclinical or clinical ketosis. In pregnant sheep from the first group, the average BHBA level was 0.44±0.08 mmol/l; in the recently lambed group –0.40±0.17 mmol/l, and in lactating group – 0.18±0.08 mmol/l.

In the three groups of ewes (pregnant, recently lambed and lactating) from the dairy Lacaune breed, the blood activity of aspartate aminotransferase (ASAT) varied close to physiological range and was 58.2±19.4 U/l in pregnant controls. In recently lambed controls, average ASAT activity was 43.0±8.6 U/l, and in lactating controls the average value was comparable (51.0±12.3 U/l, Fig. 2).

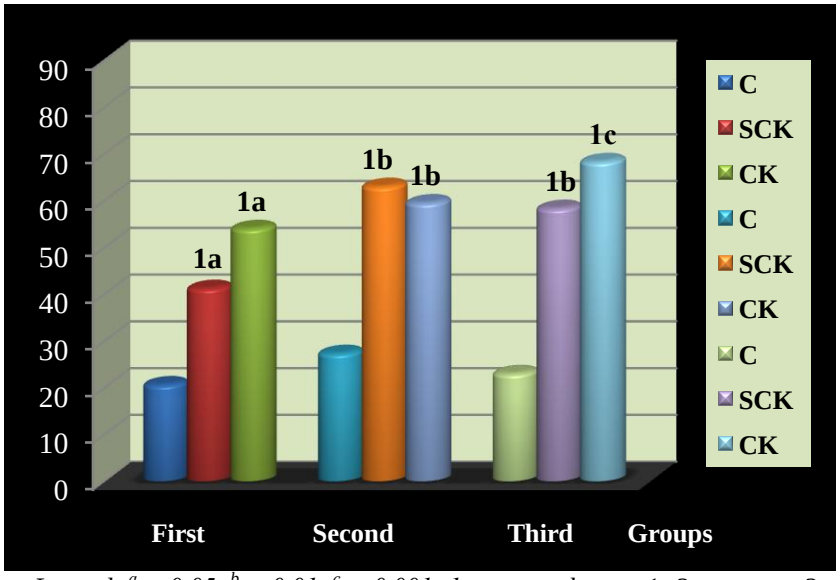
ASAT activity in pregnant dairy ewes (prepartum days 15 to 0) with subclinical ketosis increased statistically significantly vs control values – 134.7±28.4 U/l ($p<0.01$). In SCK sheep from group II (between postpartum days 0 to 15) and group III (postpartum days 30 to 45), blood ASAT level exhibited the same tendency of change with substantial increase vs control values 139.1±25.9 U/l and 163.6±20.9 U/l respectively (Fig. 2).

Lacaune ewes with clinical ketosis from the first (pregnant), second (recently lambed) and third (lactating) groups were considerably higher than respective controls having attained 142.0±20.6 U/I ($p<0.01$) in group I; 154.0±33.5 U/I ($p<0.001$) in group II and 168.6±20.2 U/l ($p<0.001$) in group III (Fig. 2).



Legend: [a]p<0.05; [b]p<0.01; [c]p<0.001; 1-vs control group1; 2- vs group 2; (C-control group; SCK-with subclinical ketosis; CK-with clinical ketosis)

Fig. 2. Changes in blood aspartate aminotransferase (ASAT, U/l), levels in Lacaune ewes from groups I, II and III with subclinical and clinical ketosis.



Legend: [a]p<0.05; [b]p<0.01; [c]p<0.001; 1-vs control group1; 2- vs group 2; (C-control group; SCK-with subclinical ketosis; CK-with clinical ketosis)

Fig. 3. Changes in blood alanine aminotransferase (ALAT, U/l) activities in Lacaune ewes from groups I, II and III with subclinical and clinical ketosis.

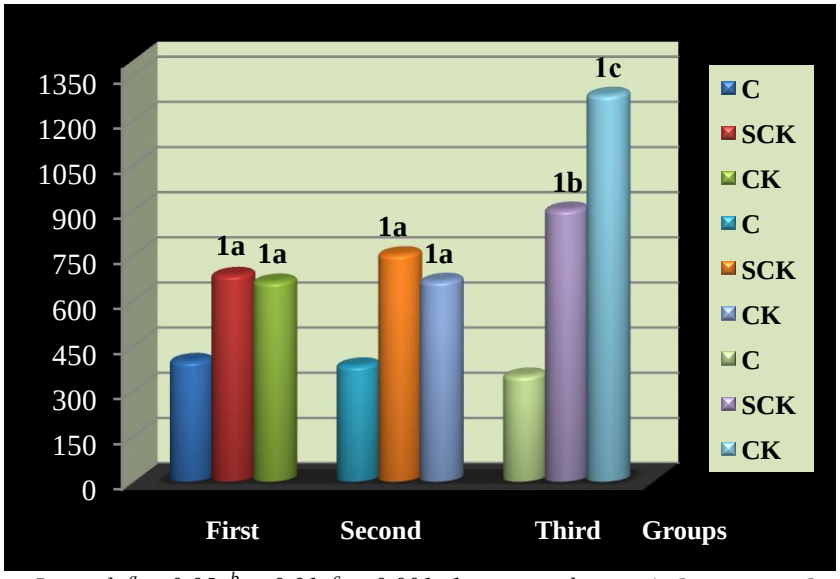
Blood serum analysis for alanine aminotransferase (ALAT) activity in control ewes were close to the reference range and measured 20.6±5.0 U/l (group I), 27.3±6.5 U/l (group II) and 23±4.1 U/l (group III) (Fig. 3).

In pregnant ewes with subclinical ketosis (prepartum day 15 to 0) ALAT activity increased significantly as compared to control determination – 41.2±4.0 U/l ($p<0.05$). In recently lambed (postpartum days

0–15) and lactating (postpartum days 30-45) ewes affected with subclinical ketosis ALAT concentrations in blood exhibited comparable changes attaining 63.0±6.2 U/l ($p<0.01$) in group II and 58.3±2.5 U/l ($p<0.01$) in group III.

ALAT activity in ewes with clinical ketosis from group I (pregnant), II (recently lambed) and III (lactating) was substantially higher than respective controls: 54.0±12.1 U/l ($p<0.05$) for group I; 59.5±8.2 U/l ($p<0.01$) for group II and 68.3±2.5 U/l ($p<0.001$) for group III (Fig. 3).

The analysis of blood in the three groups of ewes (pregnant, recently lambed, lactating) for detection of changes in lactate dehydrogenase (LDH) activities demonstrated levels near the physiological norms as followed: 399.8±56.0 U/l in group I; 385.0±22.6 U/l in group II and 351±26.7 U/l in group III (Fig. 4).



Legend: [a]p<0.05; [b]p<0.01; [c]p<0.001; 1-vs control group1; 2- vs group 2; (C-control group; SCK-with subclinical ketosis; CK-with clinical ketosis)

Fig. 4. Changes in blood lactate dehydrogenase (LDH, U/l) activities in Lacaune ewes from groups I, II and III with subclinical and clinical ketosis.

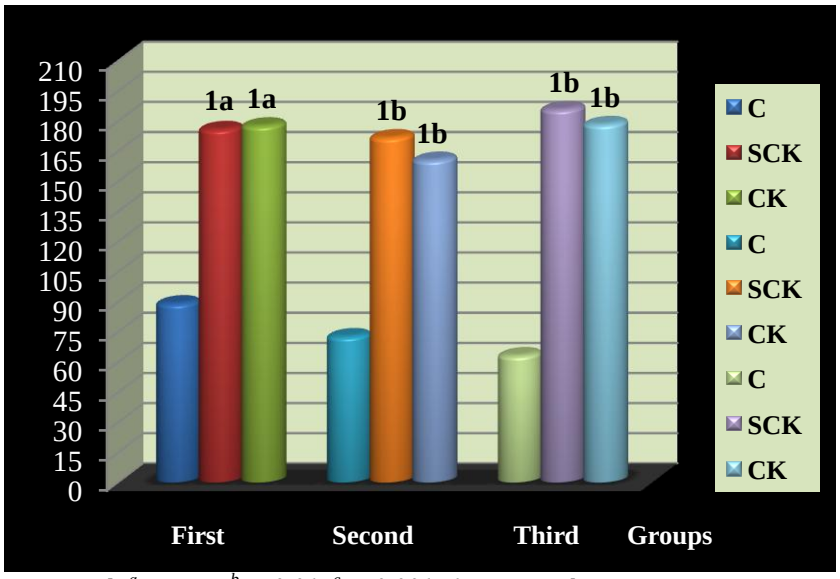
Dairy ewes from group I, (prepartum days 15 to 0), suffering from subclinical ketosis showed significantly higher blood LDH activities than controls 685.8±41.9 U/l ($p<0.05$). SCK sheep from groups II (between postpartum days 0 and 15) and III (between postpartum days 30 and 45) also showed substantially increased blood LDH vs controls: 754.5±125 U/l ($p<0.05$) in recently lambed and 902.6±176 U/l ($p<0.01$) in lactating ewes.

Blood LDH analysis in Lacaune ewes with clinical ketosis also exhibited considerable increase in enzyme activities vs controls - 663.6±122 U/l ($p<0.05$) for the pregnant group; 666.0±130 U/l ($p<0.05$) for the recently lambed group and 1284.3±289 U/l ($p<0.001$) for the lactating group (Fig. 4).

Alkaline phosphatase activity (AP) in blood serum in control ewes from the three groups (pregnant, recently lambed, lactating) corresponded to the norms with average activities of 89.0±16.1 U/l; 72.6±11.8 U/l and 63.1±6.1 U/l in groups I, II and III respectively (Fig. 5).

Pregnant ewes with subclinical ketosis had higher blood AP than controls – 175.8±15.3 U/l ($p<0.05$). A similar tendency was present in recently lambed– 171.7±22.1 U/l ($p<0.01$) and lactating ewes – 185.7±21.5 ($p<0.01$) vs respective controls.

In ewes with clinical ketosis, AP concentrations were very increasing as compared to control groups attaining 177.6±18.5 U/l ($p<0.05$) in the pregnant group, 160.5±16.9 U/l ($p<0.01$) in the second group and 178.3±15.5 U/l ($p<0.01$) in the third, lactating group (Fig. 5).

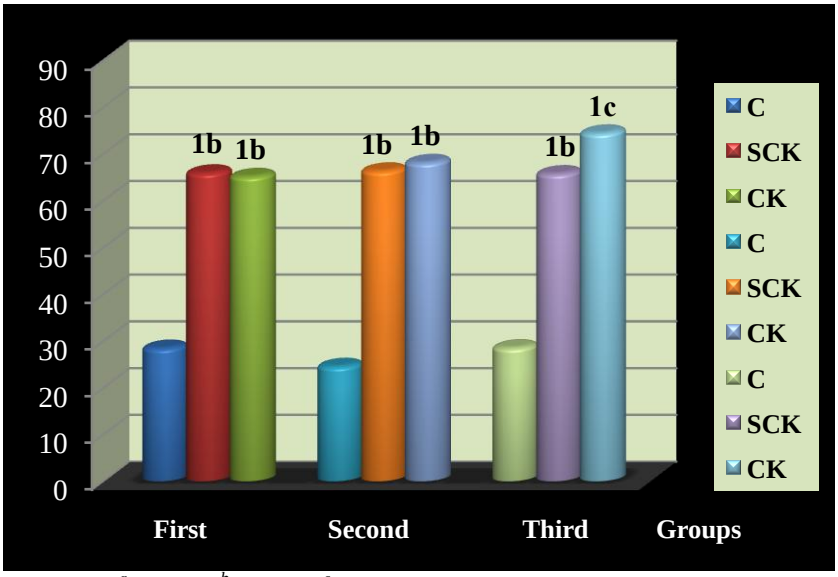


Legend: [a]p<0.05; [b]p<0.01; [c]p<0.001; 1-vs control group1; 2- vs group 2; (C-control group; SCK-with subclinical ketosis; CK-with clinical ketosis)

Fig. 5. Changes in blood alkaline phosphatase (AP, U/l) activities in Lacaune ewes from groups I, II and III with subclinical and clinical ketosis.

The analysis of the activity of γ–glutamyltransferase (GGT) in the three groups of sheep (pregnant, recently lambed, lactating) revealed it was normal in all control Lacaune ewes and averaged 28.4±7.3 U/l in group I; 24.5±7.1 U/l in group II and 28.5±7.0 U/l in group III (Fig. 6).

In ewes with subclinical ketosis, GGT activities increased vs control determinations up to 65.87±9.7 U/l ($p<0.01$) for group I (prepartum days 15 to 0); 66.3±8.0 U/l ($p<0.01$) for group II (postpartum days 0 to 15) and 65.7±9.5 U/l ($p<0.01$) in group III (postpartum days 30–45).



Legend: [a]p<0.05; [b]p<0.01; [c]p<0.001; 1-vs control group1; 2- vs group 2; (C-control group; SCK-with subclinical ketosis; CK-with clinical ketosis)

Fig. 6. Changes in blood γ-glutamyltransferase (GGT, U/l) activities in Lacaune ewes from groups I, II and III with subclinical and clinical ketosis.

In ewes exhibiting clinical signs of ketosis, enzyme GGT activity were similarly higher: 65.12±9.9 U/l ($p<0.01$) in pregnant; 68.16±6.2 U/l ($p<0.01$) in recently lambed and 74.4±7.8 U/l ($p<0.001$) in lactating animals (Fig. 6).

Creatine kinase levels (CK) in all groups of ewes with subclinical and clinical ketosis fluctuated insignificantly close to control values.

The blood activities of ASAT, ALAT, LDH, AP, CK и GGT in ewes from the meat type Mouton Charollais breed in different physiological condition (pregnant, recently lambed and lactating) were not significantly different from ovine reference values and control activities or Lacaune ewes. In pregnant animals (group I) the following enzyme activities were measured: ASAT – 69.3±5.9 U/l; ALAT – 25.0±5.0 U/l; LDH – 336.0±66.9 U/l; CK – 105.6±3.5 U/l; AP – 79.3±13.0 U/l и GGT – 29.6±5.9 U/l.

In recently lambed Mouton Charollais ewes the values of blood enzymes were as followed: ASAT – 57.7±9.5 U/l; ALAT – 17.7±1.5 U/l; LDH – 297.2±74.0 U/l; CK – 113.7±14.4 U/l; AP – 68.2±8.1 U/l и GGT – 21.0±1.4 U/l.

The parameters of liver enzymes in the meat-type ewes from the third (lactating) group averaged 56.0±4.2U/I for ASAT; 19.3±2.5 U/l for ALAT; 397.6±32.0 U/l for LDH; 117.6±22.6 U/l for CK; 72.6±9.2 U/l for AP and 23.0±2.8 U/l for GGT.

Discussion

The pregnancy ketosis in sheep is a metabolic disease, whose origins could be found in the negative energy balance during the last weeks of gestation (González et al., 2011b). Ruminants appear to be well adapted to the deficiency of carbohydrates, being able to synthesize endogenously glucose from non-carbohydrate sources via gluconeogenesis (Kaneko et al., 2008). The susceptibility of ewes to pregnancy toxaemia becomes higher with the growth of the fetus and respectively increased needs from glucose (Kaneko et al., 2008). It is acknowledged that almost 80% of fetal growth occurs during the last 6 weeks of gestation, with 30-40% of maternal glucose being utilized by the fetal–placental unit (Rook, 2000; Kaneko et al., 2008). The reduced blood glucose concentration, respectively insulin secretion entails enhanced mobilization of lipids from the adipose tissue (Moghaddam and Hassanpour, 2008), deposition of triglycerides in the liver parenchyma and stimulation of ketogenesis for maintenance of metabolic homeostasis (Sargison et al., 1994; Firat and Ozpinar, 2002). High blood BHBA concentrations are a compensatory mechanism in response to occurring carbohydrate deficiency and citric acid cycle inhibition (Reece, 2004).

In our experiments, bloods BHBA of 0.8 to 1.6 mmol/l and above 1.6 mmol/l were set as threshold for subclinical and clinical ketosis, respectively. Some researchers accept blood concentrations > 0.7 mmol/l (Rook, 2000; Ramin et al., 2007; Moghaddam and Hassanpour, 2008), others – above 1.0 mmol/l (Smith, 1996) as thresholds for subclinical ketosis and values > 3.0 mmol/l (Sargison et al., 1994; Kabakci et al., 2003; Balikci et al., 2009) from 5 to 7 mmol/l (Ford et al., 1990; Henze et al. 1998) for clinical ketosis in sheep. The values selected by us (of 0.8 to 1.6 mmol/l as indication of subclinical ketosis) are in agreement with data reported by Ford et al., (1990), Andrews (1997), Lacetera et al., (2001), Balikci et al., (2009) and Anoushepour et al. (2014). The chosen threshold for clinical ketosis (>1.6 mmol/l) is comparable to BHBA concentrations outlined by Andrews (1997) and Lacetera et al., (2002).

Our results on the liver enzymes ASAT and ALAT in Lacaune ewes evidenced increased activity in animals with subclinical and clinical ketosis. A positive correlation between blood BHBA and ASAT was found out in affected animals. The high enzyme activity occurred in response of liver parenchyma damage. Usually, short-time feed deficiency of sheep during late pregnancy could provoke a reversible microvesicular degeneration of the liver, sometimes affecting the entire parenchyma. The severe damage of hepatic tissue however correlates with high blood ASAT concentrations in diseased animals (Cal et al., 2009). Our data are comparable to those reported by Kabakci et al., (2003), Balikci et al., (2009), Sargison, (2007) and Yarim and Ciftci, (2008). In their experiments Hefnawy et al., (2011) demonstrated a substantially increased ASAT and ALAT in goats with pregnancy toxaemia at the background of reduced serum total protein, globulins, albumins, cholesterol and total lipids. Albay et al., (2014) also established high ASAT activity in goats with subclinical and clinical ketosis, with considerably higher enzyme activity in ewes with clinical disease. Opposite of our findings, neither Rezapour and Taghinejad Roudabeh (2011) nor Anoushepour et al., (2014) have observed any significant alterations in blood activities of these two liver enzymes.

A high activity of blood LDH was detected in the three groups of ewes with subclinical and clinical ketosis, while CK concentrations were not changed and varied around the reference range values. In goats with spontaneous pregnancy toxaemia, Barakat et al., (2007) found out high blood LDH, CK and ASAT. Peneva and Goranov (1984) established high blood ASAT, ALAT and LDH in ketotic cows, similar to our findings in

pregnant and lactating ewes affected with ketosis. Studies in clinically healthy ewes reported higher postpartum blood CK and LDH (Yokus et al., 2006), whereas Sevinc et al. (1999) provided data about their reduction. Gürgöze et al. (2009) demonstrated higher blood CK during pregnancy as well as increased LDH activity until postpartum day 14, with fluctuations near to the reference values. In the view of Fischbach (2000), the lower intake of protein in many instances results in muscle damage followed by increased activity of muscle enzymes. Therefore, the author affirms that high blood CK activity was a proof for protein deficiency in ewes during late pregnancy, and that increased blood LDH levels reflected the muscle damage during the lactation.

High alkaline phosphatase in all ewes with subclinical and clinical ketosis agrees partly with data reported in the literature. In sheep with spontaneously occurring pregnancy toxaemia, Sargison et al. (1994) ascertained a various extent of AP activity increase. In addition, sheep with high blood levels of this enzyme recovered more slowly as compared to those with lower activities. According to the authors, blood AP and glucose concentrations were useful indices for diagnostics of ovine ketosis. Having studied some liver function parameters in sheep with gestational hyperketonemia, Abd El-Raof and Ghanem (2006) proved substantially higher blood ASAT, ALAT and AP. Cal et al. (2009) did not found any changes in blood AP activity in sheep with ketonemia or any significant correlation between AP levels and the extent of vacuolisation of liver parenchyma; hence, AP could not be used as marker of liver steatosis in ewes with pregnancy toxaemia contrary to what was affirmed by Sargison et al. (1994). Bani Ismail et al., (2008) did not demonstrate altered ASAT and AP activity in goats with subclinical ketosis, which could be due to the subclinical course of disease in pregnant goats or indicate a different metabolism of fat and liver sensitivity among ruminant species. The findings of Peneva and Goranov (1984) evidenced low blood AP in cows with ketosis, correlating negatively with ketonuria. High blood ASAT, AP and GGT were reported by Simonov and Vlizlo (2014) in cows with hyperketonemia. The increased activity of transaminases is a proof that in most cows affected with ketosis, destructive liver changes resulting in increased enzyme release from cell organelles into blood was present.

The statistically significant increase in blood GGT in our studies was in line with the data of Andrews (1997), who affirmed that changed serum activity of ASAT and GGT could indicate liver damage in sheep with pregnancy toxaemia but they did not correlate to the extent of liver damage as evidenced from histological examination. Wierda et al. (1985) believed that serum SDH and GGT activities could be used as prognostic markers of hyperketonemia instead of LDH and AP. In their experiments, Rezapour and Taghinejad Roudabeh (2011) did not report any considerable alterations in GGT activities in sheep experiencing energy deficiency and ketosis.

The lack of changes in blood BHBA and the activities of ASAT, ALAT, LDH, AP, CK and GGT in Mouton Charollais ewes could be attributed to a breed-specific resistance as well as to the lower milk yield.

Conclusion

The economic losses from ketosis might be significant. The mortality rate in affected animals could attain up to 100%, and even if treatment is initiated, the outcome could be fatal due to severe irreversible organ damage. Losses are also incurred by higher death rates in newborn lambs, reduced productivity, poor wool quality and increased labour and medication costs.

A primary and reliable parameter for monitoring negative energy balance and ketosis is blood BHBA concentrations. The analysis of BHBA and other parameters as body condition score evaluation, blood glucose; NEFA should be implemented as important tools for herd health management in high-yielding sheep.

The lack of consistent and sound results suggests that blood enzymatic profile data could be used only as guidelines in ketosis detection. Nevertheless, its diagnostic value for assessment of the extent of morphological and functional damage of the organism remains considerable. The results from the present experiments point at a various extents of liver parenchyma injury, which could entail irreversible damage of hepatocytes if not corrected and together with kidney damage and hyperketonemia, could result in death of affected ewes and their offspring. The comparison of two sheep breeds of a different production type (Lacaune and Mouton Charollais) reared under uniform conditions showed that changed blood BHBA concentrations were established only in Lacaune ewes, whereas in meat-type Mouton Charollais ewes BHBA levels were not altered. With this respect, every result should be interpreted in accordance with individual and breed-specific traits of animals.

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