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Original article

CHANGES IN BLOOD ENZYME ACTIVITIES AND SOME LIVER PARAMETERS IN GOATS WITH SUBCLINICAL KETOSIS

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Summary

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The aim of the present study was to establish the changes in blood enzyme activities and some liver parameters in goats from the Saanen breed in different physiological conditions with subclinical ketosis (SCK). A total of 113 dairy goats with yearly milk yield of 680 L, in their 2nd to 3rd lactation were included in the study. The goats were divided in three groups: pregnant; recently kidded and lactating. Blood samples were obtained from all animals for determination of β-hydroxybutyrate (BHBA), glucose (mmol/L), 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), γ-glutamyltransferase (GGT, U/L), total protein (TP, g/L), albumin (ALB, g/L) and total bilirubin (TB, μmol/L). The animals were classified as healthy (control) and with SCK according to their blood BHBA levels. Blood BHBA concentrations indicative for clinical ketosis (BHBA <1.6 mmol/L) were not established in goats from the three groups. Blood biochemical analysis of activities of ASAT, ALAT, LDH, AP, CK and GGT in goats from the three groups with SCK demonstrated a various extent of statistically significant hyperenzymaemia vs control groups. The concentrations of total bilirubin were statistically significantly elevated vs healthy groups, while blood glucose, total protein and albumin values decreased, in result of the impaired liver function in goats with SCK.

Key words: β-hydroxybutyrate, biochemical parameters, dairy goats, enzymes, ketosis

INTRODUCTION

Ketosis in goats is a nutritional stress syndrome affecting dairy breeds (Saanen, Bulgarian White Dairy goat, Toggenburg etc.). The disease affects more often adult over-conditioned goats or those with a poor body condition status (although the condition can occur even in ideally conditioned goats on an adequate ration) and carrying more than one foetus. Ketosis commonly occurs in does during the late stage of pregnancy or early lactation (Schlumbohm & Harmeyer, 2008; Abba *et al.,* 2015). The main economic losses in ketotic goats and sheep result from the production losses, treatment costs, death of affected animals etc. (Caldeira *et al.,* 2007).

The primary predisposing cause of ketosis in small ruminants is inadequate nutrition during late gestation, as a result of insufficient energy density of the ration and decreased rumen volume as a result of foetal growth. Mobilisation of fat reserves is increased pre-partum as a way to assure adequate energy for the increased demands of the developing foetuses and forthcoming lactation. In a period of negative energy balance (NEB), increased mobilisation may complicate the liver function and result in hepatic lipidosis (González *et al.,* 2011). Under these conditions, the body of the animals is depleted of carbohydrates that are used to produce glucose. Glucose is essential for proper functioning of many tissues and organs (brain, muscles, etc.) but it is largely used by the foetuses. The growing foetuses continuously remove large quantities of glucose and amino acids for their growth and energy requirements. Each foetus requires 30–40 g of glucose per day in late gestation. That is why pregnancy toxaemia most often affects ewes/does pregnant with twins or triplets. It has been found that sheep and goats pregnant with twins or triplets require 180 to 240% more energy compared to pregnant with a single foetus (Ermilio & Smith, 2011). The cause of the disease is also anorexia resulting from animal obesity, other illness or sudden stress in the transition period (Navarrei & Pugh, 2002).

Ketosis as a metabolic disorder is characterised by reduced gluconeogenesis activity in the liver, development of hepatic lipidosis, acetone-smelling breath, ketonuria, ketolactia and changes in the some biochemical indicators of the blood – ketonaemia, hypoglycaemia etc. (Schlumbohm & Harmeyer, 2008; Djoković *et al.,* 2013).

Preparing the metabolic profile of the animals in the herd by determining biochemical parameters in the blood is essential for early diagnosis and prevention of metabolic diseases in ruminants, in particular ketosis (pregnancy toxaemia). A part of that test is determining the quantities of BHBA, glucose, total protein and albumin, total bilirubin and enzyme activities in the serum (ASAT, ALAT, LDH, AP, CK and GGT) (Kirovski *et al.,* 2008).

The levels of blood BHBA are used for evaluation of the degree of NEB and lipid mobilisation in dairy goats, ewes and cows (Sordillo & Raphael, 2013). With respect to diagnosis of SCK in goats, the following 2 threshold blood BHBA concentrations are mainly discussed in the literature: >0.7 mmol/L (Ramin *et al.,* 2007) and above 0.8 mmol/L (Bani Ismail *et al.,* 2008; González *et al.,* 2011). The threshold blood BHBA levels reported in association with clinical ketosis (CK) are >1.6 mmol/L (Ramin *et al.,* 2007; Bani Ismail *et al.,* 2008; Albay *et al.,* 2014). Deviations in BHBA values in goats suggest the presence of inadequate energy supply and occurrence of postpartum metabolic disorders (Koyuncu & Altınçekiç, 2012).

Determination of blood glucose levels, total protein, albumin, bilirubin and enzymes is important for assessing the functional status of the liver in ruminants. The amount of glucose precursors in ruminants varies depending on the stages of lactation, food intake, fat mobilisation and energy balance. Hypoglycaemia and hypoproteinaemia occur in dairy goats, sheep and cows with ketosis as a result of fatty dystrophy in the liver and kidneys (Bertoni & Trevisi, 2013; Djoković *et al.,* 2013). The high activities of AST, ALT, GGT, GDH, AP and CK in the blood of dairy animals are also indicative of liver damage (fatty liver syndrome), acute and

chronic diseases of liver and hepatobiliary system diseases connected with cholestasis, as well as low appetite and muscle damage (Gürgöze *et al.,* 2009; Vasava *et al.,* 2016).

The aim of this study was to investigate dairy goats in different physiological conditions (pregnant: from pre-partum days 15 to 0, recently kidded: from postpartum days 0 to 15 and lactating: from postpartum days 30 to 45) with diagnosed SCK to detect changes in blood enzyme activities (ASAT, ALAT, LDH, AP, CK and GGT) and some liver parameters (glucose, total protein, albumin and total bilirubin).

MATERIALS AND METHODS

A total of 113 goats (2^{nd} and 3^{rd} lactation) from the dairy Saanen breed with 680 l annual lactational yield and average weight 50-60 kg were included in the study. All goats were regularly vaccinated and treated against ecto- and endoparasites. They were reared in facilities in compliance with the respective welfare standard for the species. Goats were fed rations in concordance with their physiological condition (pregnant, recently kidded and lactating).

The target goats were divided in groups depending on their physiological condition, namely:·I group – pregnant (from pre-partum days 15 to 0);·II group – recently kidded (from postpartum days 0 to 15);·III group – lactating (from postpartum days 30 to 45).

Blood chemical test to determine the amount of BHBA was performed in all goats, on the basis of the results they were classified as healthy (control, BHBA <0.8 mmol/L) and affected with SCK (BHBA from 0.8 to 1.6 mmol/L). The BHBA study data was reflected in our previous article (Marutsova & Binev, 2017). The number of animals in each group was as followed: I group (n=27, pregnant), of which 17 healthy (control) and 10 goats (37%) with SCK; II group (n=28, recently kidded), of which 16 healthy controls and 12 (43%) with SCK; III group (n=58, lactating), of which 30 healthy and 28 (48.3%) with SCK.

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

Blood BHBA and glucose concentrations were determined *in situ* using a portable Xpress-I system (Nova Biomedical, UK). Samples for biochemical analysis were transported and stored at 4 ^{o}C. Analysis was conducted within 2 hours after sampling. The following parameters were assayed: 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), γ-glutamyltransferase (GGT, U/L), total protein (TP, g/L), albumin (ALB, g/L) and total bilirubin (TB, μmol/L). The biochemical tests were performed using colorimetric method (IFCC 37^{0}) with Biolab Diagnostics (France) kits on an automated biochemical analyser Mindray BS-120 (China) and Integra 400 plus Roche (F. Hoffmann – La Roche Ltd., Switzerland).

Statistical analysis

Statistical analysis was done with ANOVA test (Statistica 6.0, StatSoft, Inc. USA, 1993). Results were presented as mean ± standard deviation (SD). The level of statistically significance was $P<0.05$.

RESULTS

The blood glucose levels in control goats were within the respective physiological ranges: 3.30±0.83 mmol/L for group I; 3.02±0.61 mmol/L for group II and 3.07±0.78 mmol/L for group III (Fig. 1). In goats from groups with SCK, blood glucose values were significantly lower than controls: 2.1±0.14 mmol/L ($p<0.01$) for group I (pregnant); 1.90±0.56 mmol/L ($p<0.001$) for group II (recently kidded) and 1.83±0.67 mmol/L ($P<0.001$) for group III (lactating) (Fig. 1).

The blood total bilirubin in goats from control groups (pregnant, recently kidded and lactating) were within the reference interval: 6.53±0.3 μmol/L for group I; 6.18±0.1 μmol/L for group II and 6.35±0.5 μmol/L for group III (Fig. 2). Blood chemistry analysis in goats with SCK showed statistically significant

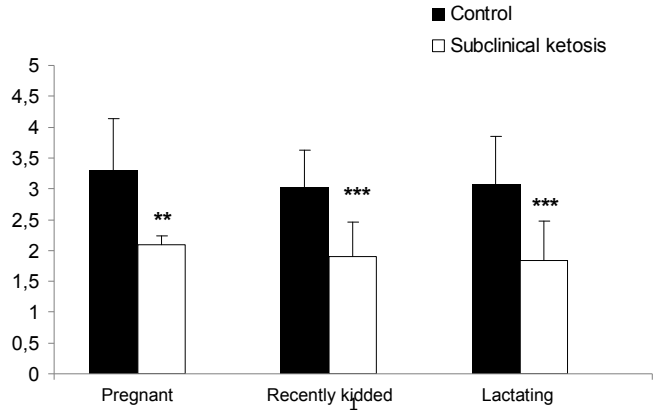

Fig. 1. Changes in blood glucose levels in healthy control goats (17 pregnant; 16 recently kidded and 30 lactating) and goats with subclinical ketosis (10 pregnant; 12 recently kidded and 28 lactating); **P<0.01; ***P<0.001 vs controls.

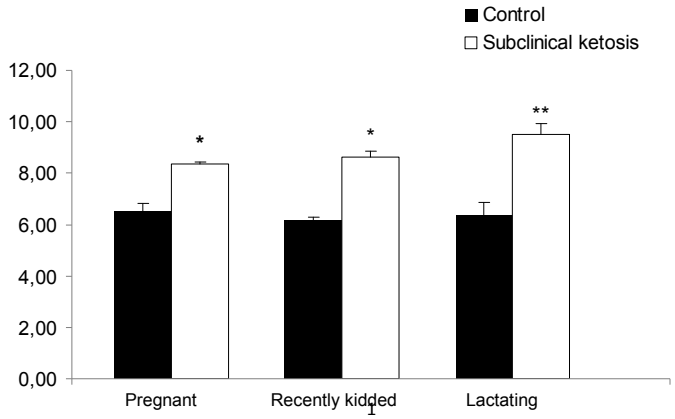

Fig. 2. Changes in blood total bilirubin levels in healthy control goats (17 pregnant; 16 recently kidded and 30 lactating) and goats with subclinical ketosis (10 pregnant; 12 recently kidded and 28 lactating); *P<0.05; **P<0.01 vs controls.

bilirubinaemia: 8.34±0.1 μmol/L ($p<0.05$) for group I; 8.64±0.2 μmol/L ($p<0.05$) for group II and 9.52±0.4 μmol/L ($P<0.01$) for group III (Fig. 2).

The analysis of blood in the three control groups for detection of changes in total protein and albumin quantities demonstrated levels close to the reference ranges (Table 1). Blood total protein and albumin in goats from group I (pregnant) with SCK were statistically insignificantly decreased vs control levels, while in goats from groups II (recently kidded) and III (lactating), they decreased significantly ($P<0.05$) (Table 1).

The analysis of blood enzyme constellation (ASAT, ALAT, LDH, AP, CK and GGT) in the three control groups of Saanen goats with different physiological state showed values close to reference ones (Table 1). The analysis of enzyme activities in goats with SCK from groups I (pregnant – from pre-partum days 15 to 0), II (recently kidded – from postpartum days 0 to 15) and III (lactating – from postpartum days 30 to 45) demonstrated various extent of statistically significantly differences vs control groups (Table 1).

DISCUSSION

Pregnancy and lactation are the two periods influencing biochemical blood indicators reflecting the metabolic needs of animals (Albay *et al.* 2014). In order to predict such disorders and eventual subclinical diseases it is necessary to determine ranges of biochemical parameters in a dairy herd, by creating metabolic profiles. The basic and the most important blood parameter used to diagnose and determine the type of ketosis (subclinical and clinical) in ruminants is blood BHBA concentration (Lacetera *et al.,* 2002).

Our data show that goats were suffering only from SCK throughout the gestation, kidding and lactation. The goats from the three groups (pregnant, recently kidded and lactating) did not suffer from CK as their blood BHBA levels were <1.6 mmol/L. Our results showed levels <1.2 mmol/L which correspond with the studies of Bani Ismail *et al.* (2008); González *et al.* (2011) and Albay *et al.* (2014). High BHBA levels in blood are a compensatory mechanism in response to occurring carbohydrate deficiency (especially oxalacetate deficiency) and Krebs cycle inhibition (Ingvartsen, 2006). The increased BHBA concentration in blood reveals incomplete oxidation of non-esterified fatty acids (NEFA) in the tricarboxylic acid cycle at the time of NEB (Doepel *et al.,* 2002). BHBA increased during pregnancy, after parturition and during lactation (30–45 days), following the increase of NEFA; this suggests that NEFA provides the substrate for BHBA synthesis (Marutsova & Binev, 2017).

Affected goats in this study exhibited hypoglycaemia. Glucose metabolism in ruminants depends on the degree of uptake from food, as well as the available glucose precursors and gluconeogenesis. In adequately fed ruminants, the principal glucose precursors in the liver are propionate and amino acids (Sordillo & Raphael, 2013). During starvation, liver or skeletal muscles glycogen directly and indirectly maintains glucose levels in the body (Kuhla *et al.,* 2011). Blood glucose concentrations in goats with SCK decresed statistically significantly as a result of depletion of glycogen from the liver. It was confirmed by other authors (Rani *et al.,* 2015). In contrast, Lima *et al.* (2012) and Souto *et al.* (2013) reported hyperglycaemia in later stages of pregnancy toxaemia when the foetuses were dead.

Table 1. Changes in blood total protein, albumin, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (AP), γ-glutamyltransferase (GGT), lactate dehydrogenase (LDH) and creatine kinase (CK) levels in goats from groups I, II and III with SCK (mean ± SD)

	Group I (pregnant; 15–0 days pre-partum)		Group II (recently kidded; 0–15 days postpartum)		Group III (lactating; 30–45 days postpartum)	
	Control (n=17)	Subclinical ketosis (n=10)	Control (n=16)	Subclinical ketosis (n=12)	Control (n=30)	Subclinical ketosis (n=28)
Total protein (g/L)	72.5±3.2	67.2±2.1	72.3±4.9	55.8±3.4*	74.0±3.0	58.3±4.1*
Albumin (g/L)	37.3±1.4	30.9±2.1	36.8±1.7	22.2±2.4*	37.3±2.0	24.7±3.5*
ASAT (U/L)	74.5±5.6	176.0±4.6*	66.8±2.6	165.3±5.4*	83.9 ±6.1	153.6±7.2*
ALAT (U/L)	20.0±1.3	46.6±2.6*	20.5±1.2	59.5±3.4**	20.2±3.3	68.7±2.5**
AP (U/L)	68.2±9.5	272.1±12.3**	75.8±5.1	325.6±7.2**	84.3±10.8	936.9±36.5***
GGT (U/L)	23.6±1.0	47.9±1.1*	32.8±1.3	52.2±1.4*	27.5±1.8	66.6±2.3*
LDH (U/L)	152.4±8.4	744.1±14.2***	102.3±8.4	724.6±10.8***	121.8±11.7	704.1±12.9***
CK (U/L)	96.0±8.3	258.3±6.4**	95.5±5.3	198.6±6.6*	92.2±4.9	171.6±8.6*

*P<0.05; **P<0.01; ***P<0.001 vs control goats.

The changes in the development of SCK in the goats of the three groups (pregnant, recently kidded and lactating) showed that the liver function was disturbed, as could be seen from the statistically significantly increased ($P<0.05$) blood total bilirubin. Established bilirubinaemia would be due to the fact that the dystrophic changes in the liver mechanically obstruct bilirubin secretion in bile. As a result of the destruction of hepatocytes the permeability of bile and blood capillaries is also impaired, which facilitates the passage of bilirubin into the bloodstream. Similar observations were reported by other authors (Djoković *et al.,* 2013) as well.

Impaired function of the liver was evidenced by decreased serum levels of total protein and albumin (hypoproteinaemia, $P<0.05$) in goats with SCK. Values of total protein may be influenced to a large extent by the condition of the liver, as the plasma proteins are synthesised there. Albumin is the major protein in blood, where it makes up about 60% of the total protein. The main functions of albumin include maintenance of colloid-osmotic pressure, binding and transport of physiologically important substances, including lipids, amino acids, metal and calcium ions, and others. The amount of albumin may decrease, when conditions interfere with its production by the liver, increase protein breakdown or increase protein loss via the kidneys (in result of the dystrophic changes in them). The decrease in total protein in the early lactation can also be explained by redirecting albumin and globulins from the blood to the udder. The hypoproteinaemia in dairy goats with hyperketonaemia is due to the dystrophic processes and fatty infiltration of the liver and kidneys (Hefnawy *et al.,* 2011; Abba *et al.,* 2015).

Blood enzymatic assays performed in dairy goats with SCK in different physiological states demonstrated highly statistically significantly increased levels in comparison to control groups.

Our results on the liver enzymes ASAT and ALAT in goats evidenced increased activity in animals with subclinical ketosis. The high enzyme activity occurred in response of liver parenchyma damage. Usually, short-time feed deficiency of goats during late pregnancy could provoke a reversible microvesicular degeneration of the liver, sometimes affecting the entire parenchyma. The severe damage of hepatic tissue correlates with high blood ASAT concentrations in diseased animals (Vasava *et al.,* 2016). In their experiments Hefnawy *et al.* (2011) and Albay *et al.,* (2014) demonstrated a substantially increased ASAT and ALAT in goats with ketosis. Our data are comparable to those reported by Hefnawy *et al.* (2011), Albay *et al.* (2014) and Rani *et al.* (2015). Opposite to our findings, Gupta *et al.* (2008) reported decreased values of ASAT and ALAT in small ruminants with ketosis.

A high activity of blood LDH was detected in the three groups of goats with SCK. The high enzyme activity could be associated to cell damage of some parenchymal organs (liver, kidneys and hearth). It points at muscle damage during the lactation period. In goats with spontaneous pregnancy toxaemia, Barakat *et al.* (2007) and Abba *et al.* (2015) found out high blood LDH, ASAT and CK. The activity of blood creatine kinase was also increased in our study in goats with SCK. 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 (CK and LDH).

Unlike literature data related to changes in AP and GGT in sheep with ketosis, those concerning goats are very scarce. Our experiments proved increased values of AP in the three groups of animals (pregnant, recently kidded and lactating). Simonov & Vlizlo (2015) reported an increased release of AP in the circulation from the epithelium of the bile ducts in severe liver damage associated with cholestasis. In sheep with gestational hyperketonaemia, Sargison *et al.* (1994) and Abd El-Raof & Ghanem (2006) ascertained substantially higher blood ASAT, ALAT and AP activity. Cal *et al.* (2009) did not found any changes in blood AP activity in sheep with ketonemia. Bani Ismail *et al.* (2008) did not demonstrate altered ASAT and AP activity in goats with SCK, 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.

High GGT level in blood is a diagnostic marker of hepatobiliary system disease and is used in diagnosing liver disease (Djoković *et al.,* 2013). In the present study, blood GGT was significantly elevated. Increased activity of GGT and ASAT is used as an indicator of liver damage in ketotic goats, being proportional to the extent of histological changes.

In conclusion, blood biochemical analysis of activities of ASAT, ALAT, LDH, AP, CK and GGT in goats with SCK demonstrated statistically significant elevations. Goats with hyperketonaemia also exhibited bilirubinaemia, hypoglycaemia and hypoproteinaemia. The results from the present study point at various extents of liver parenchymal injury, which could entail irreversible damage of hepatocytes if not corrected and together with kidney damage and hyperketonaemia, could result in death of affected goats. The analysis of BHBA and other parameters as blood glucose, total bilirubin, total protein, albumin and enzymes should be implemented as important tools for herd health management and can be used for the diagnosis of subclinical ketosis in high-yielding goats.

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