

CLINICAL AND HEMATOLOGICAL STUDIES IN SHEEP WITH SUBCLINICAL AND CLINICAL KETOSIS

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ABSTRACT

The investigation was performed on 136 ewes, 106 (Lacaune) and 30 (Mouton Charollais). The ewes were divided in three groups: pregnant; recently lambing and lactating. Sheep of the three groups, we performed a chemical blood test to determine the level of β -hydroxybutyrate. Clinical examination was performed on all animals by routine methods of clinical diagnostics. In haematological studies were monitored parameters of red and white blood count.

The investigation of the sheep from the three groups with subclinical ketosis (SCK) showed that clinical parameters varied within the reference ranges. The studied red and white blood cell indices in Lacaune sheep with SCK and clinical ketosis (CK) indicated erythropenia, oligochromemia, reduced haematocrit, leukocytosis and lymphocytosis. The meat breed Mouton Charollais did not exhibit any changes in studied haematological parameters. The sheep from the dairy breed Lacaune were affected with SCK and CK during the pregnancy, parturition and lactation while those from the meat breed Mouton Charollais did not suffer.

Key words: ketosis, β -hydroxybutyrate, clinical and hematological parameters, ewes.

Introduction

In sheep, ketosis is observed during the last 6-4 weeks of pregnancy (pregnancy toxemia) and after lambing (Van Saun, 2000). The condition is caused by the negative energy balance (NEB), occurring from increased demand for glucose of developing fetuses (Van Saun, 2000; Schlumbohm and Harmeyer, 2008). Some predisposing factors are the number of body weight of fetuses, body condition of the dam, number of lactations, age and breed, feeding, stressors etc. (Hefnawy et al., 2011).

Subclinical ketosis (SCK) is a pathological state associated to increased systemic levels of ketone bodies but without the ketosis-specific clinical signs (Duffield et al., 1997). From health and economic point of view, the SCK leads to lower milk yield (McArt et al., 2013), reproductive disorders, prolonged duration and severity of mastitis (Suriyasathaporn et al., 2000) and/or clinical ketosis (LeBlanc et al., 2005).

Clinical ketosis (CK) is manifested with loss of appetite, dehydration, depression, decreased milk secretion. Affected sheep stay away from the herd, exhibit purposeless movements, and often press their heads against the feeder or wall. They show weak chewing muscles, seizures and tremor of the head and neck, opisthotonus, grinding with teeth, amaurosis, ataxia, sternal recumbency and loss of the wool along the entire length of the back, liquid faeces, coma and death (Henze et al., 1998; Van Saun, 2000). Kabakci et al. (2003) and Balikci et al. (2009) established that sheep carrying twins, toxicosis was manifested with neurological signs (convulsions and vision disturbances) as compared to sheep pregnant with a single foetus which did not show such signs. A scent of acetone was perceived in exhaled air in all sheep with pregnancy toxemia, and in some sheep with CK – bradypnea (8 min^{-1}), abdominal breathing, hypothermia (36°C) and tachycardia (120 min^{-1}). Barakat et al. (2007) and Balikci et al. (2009) did not establish changes in body temperature, respiratory and heart rates in sheep with SCK and CK. In previous studies of ours

(Binev et al., 2014; Marutsova et al., 2015) in goats and cows with SCK, significant alterations in their clinical status were not observed, and in cows with CK – gastrointestinal signs.

Hematological and blood biochemical analysis results are inconsistent and not always reliable as markers of metabolic and clinical status disturbances in sheep with CK and SCK. Barakat et al. (2007) and Gupta et al. (2008) found out erythropenia in goats and sheep with ketosis, while Iriadam (2007) did not observe any substantial changes in hemoglobin content in lactating goats. Iriadam (2007) and Abba et al. (2015) reported leukocytosis accompanied with lymphopenia, eosinopenia and monocytopenia in goats with SCK during the first three weeks of the lactation.

The main ketone bodies in blood are BHBA, acetoacetic acid (AcAc) and acetone. A number of researchers (Herdt, 2000; Sordillo and Raphael, 2013) outline BHBA as a parameter for evaluation of NEB and lipolysis extent in dairy animals and a primary numerical parameter of ketosis, as its concentrations define the states of SCK and CK.

Various threshold blood BHBA levels in sheep are commented in the literature. Lacetera et al. (2002); Balikci et al. (2009) and Anoushepour et al. (2014) accept BHBA concentrations up to 0.8 mmol/l as normal; those from 0.8 to 1.6 mmol/l – indicative for SCK and those over 1.6 mmol/l – for CK. In this study, we used the same threshold values.

The aim of the present study was to perform a comparative evaluation of the changes in clinical parameters, hematological indices and blood BHBA in two sheep breeds (one dairy and one meat-type) with SCK and CK.

Materials and methods

Animals. A total of 136 ewes (2nd and 3rd 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.

Experimental design. The sheep from studied sheep farms were divided into three groups according to their physiological condition: group I – pregnant sheep (between pre-partum days 15 and 0); group II – recently lambed (from postpartum days 0 to 15) and group III – lactating (from postpartum days 30 to 45). Blood chemical analysis of BHBA concentrations was performed in all sheep in order to classify them as control (C, BHBA <0.8 mmol/l), affected with SCK (BHBA from 0.8 to 1.6 mmol/l) and CK (BHBA >1.6 mmol/l). Target groups of sheep from both breeds were reared under equal conditions and fed the same ration.

Group I from the dairy breed Lacaune included 45 animals – 14 healthy (control), 8 with SCK and 23 with CK. The second group (n=30) comprised 8 healthy controls, 10 with SCK and 12 with CK. The third group consisted of 31 sheep (8 healthy, 11 with SCK and 12 with CK).

The three groups of the meat breed Mouton Charollais included 10 animals each. All of them did not exhibit blood BHBA concentrations indicative for either SCK or CK, e.g. they were healthy.

Clinical investigation. All sheep were submitted to examination of the rectal body temperature, heart rate, respiratory and rumen contraction rates using routine clinical diagnostic procedures.

Blood samples and analyses. Blood samples were collected through puncture of the jugular vein using sterile 21G needles and vacutainers with K₂EDTA (3 ml, Biomed, Bulgaria). Samples were obtained in the morning before feeding and were stored and transported at 4°C. Analysis was performed within 24 hours after sampling. Blood BHBA concentrations were determined in situ using a portable Xpress-I system (Nova Biomedical, UK). The following indices were determined:

RBC ($10^{12}/l$), HGB (g/l), HCT (%), MCV (fl), MCH (pg), MCHC (g/l), RDW (%), RDW_a (fl), WBC ($10^9/l$), PLT ($10^9/l$) and MPV (fl). The parameters of the Differential Blood Count – LYM ($10^9/l$), MON ($10^9/l$) and GRA ($10^9/l$). Hematological investigations were analyzed on an automated analyser Exigo EOS Vet (Boule Medical AB, Sweden).

Statistical analysis. Statistical analysis was done with Statistica 6.0, StatSoft, Inc. (USA, 1993) and ANOVA test. Results were presented as mean (x) \pm standard deviation (SD). The level of statistical significance was $p < 0.05$.

Results

The results from the clinical investigations in the three groups of Lacaune sheep are shown in Table 1. The studied clinical parameters in control and SCK sheep from group I, II and III were within the reference ranges. The clinical exam of pregnant sheep (group I) with CK revealed reduced appetite, enhanced thirst and depression. In some sheep (21.7%), locomotor disturbances of progressing nature followed by lying down, tremor of the head and neck, and bruxism were present. As the disease progressed, ataxia, sternal recumbency, abortions, coma and death were observed. Sheep from the second and third group affected with CK exhibited polypnea, tachycardia, rumen atony, hind limb locomotor weakness, weakness of chewing muscles, salivation, seizures with clonic head movements, liquid faeces and dehydration with reduced skin elasticity.

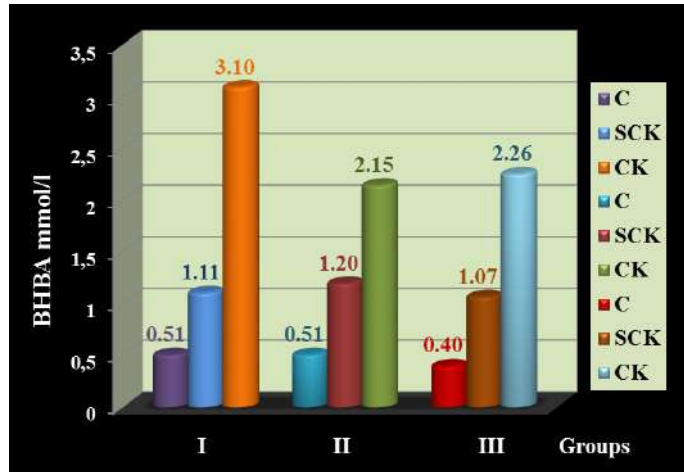
Table 1: Changes in clinical parameters of Lacaune ewes from first, second and third group with subclinical and clinical ketosis (average mean \pm standard deviation).

Parameters Groups	Study period (days)								
	Group 1 (15–0 days prepartum)			Group 2 (0–15 days postpartum)			Group 3 (30–45 days postpartum)		
	C	SCK	CK	C	SCK	CK	C	SCK	CK
Temperature (°C)	39.6 \pm 0.5	38.7 \pm 0.8	38.5 \pm 0.3	39.3 \pm 0.7	38.8 \pm 0.5	38.6 \pm 0.8	39.5 \pm 1.0	39.2 \pm 1.2	39.0 \pm 0.9
Heart rate (min ⁻¹)	76.5 \pm 0.8	78.8 \pm 0.6	81.2 \pm 1.2	74.8 \pm 0.7	76.4 \pm 0.5	79.5 \pm 0.8	75.8 \pm 0.6	77.1 \pm 0.8	79.3 \pm 0.8
Respiratory rate (min ⁻¹)	19.7 \pm 0.7	25.8 \pm 0.6	28.2 \pm 0.9	22.6 \pm 0.5	24.8 \pm 0.7	27.4 \pm 0.5	21.3 \pm 0.5	28.4 \pm 0.6	30.0 \pm 0.4
Rumen contractions (min ⁻⁵)	11.6 \pm 0.6	10.4 \pm 0.3	9.2 \pm 0.2	12.6 \pm 0.7	9.8 \pm 0.5	9.2 \pm 0.4	12.2 \pm 0.6	9.54 \pm 0.3	8.0 \pm 0.5

Legend: ^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$; 1-vs. control groups; C – control group; SCK – with subclinical ketosis; CK – with clinical ketosis

The physical examination of Mouton Charollais sheep from the three groups (pregnant, recently lambled and lactating) did not show any deviations from norms.

Blood BHBA analysis in the three control groups of Lacaune sheep were within the reference range (Fig. 1). Sheep from the first group with SCK had statistically significantly higher BHBA concentrations than controls: 1.11 \pm 0.24 mmol/l ($p < 0.001$); those from group II: 1.20 \pm 0.28 mmol/l, ($p < 0.001$), and from group III – 1.07 \pm 0.24 mmol/l ($p < 0.001$) (Fig. 1). Sheep from groups I, II and III with CK had BHBA levels in blood substantially higher than both controls and SCK – 3.10 \pm 0.60 mmol/l ($p < 0.001$), 2.15 \pm 0.63 mmol/l ($p < 0.001$) and 2.26 \pm 0.23 mmol/l ($p < 0.001$) respectively (Fig. 1).



(C-control group; SCK-with subclinical ketosis; CK-with clinical ketosis)

Figure 1: Changes in blood β -hydroxybutyrate (BHBA) levels in Lacaune ewes from first, second and third group with subclinical and clinical ketosis.

Mouton Charollais sheep did not exhibit blood BHBA higher than 0.8 mmol/l, i.e. no SCK and CK was present (0.44 ± 0.08 mmol/l in group I, 0.40 ± 0.17 mmol/l in group II and 0.18 ± 0.08 mmol/l in group III).

The haematological analysis of red and white blood cell picture in the three control Lacaune groups showed that all parameters were within the respective physiological ranges (Table 2).

In SCK sheep from the first group, erythrocyte counts, hemoglobin and hematocrit decreased insignificantly vs controls. Leukocyte and lymphocyte counts increased considerably and attained $11.13 \pm 2.3 \times 10^9/l$ ($p < 0.05$) and $4.85 \pm 1.0 \times 10^9/l$ ($p < 0.05$). In sheep with SCK from groups II and III, significant changes consisting in erythropenia, oligochromemia, reduced hematocrit, leukocytosis and lymphocytosis were observed. The other red blood parameters were within the reference limits (Table 2). Haematological analysis data in Lacaune sheep with CK from the three groups showed more pronounced changes, even more significant vs controls – erythropenia, oligochromemia, reduced hematocrit, leukocytosis (Table 2).

There were no statistically significant differences than reference values in red and white blood cell parameters in any of studied Mouton Charollais sheep.

Table 2: Changes in the hematological parameters in Lacaune ewes from first, second and third group with subclinical and clinical ketosis (average mean ± standard deviation).

Parameters	Study period (days)											
	Group 1 (15-0 days prepartum)				Group 2 (0-15 days postpartum)				Group 3 (30-45 days postpartum)			
	C	SCK	CK	C	SCK	CK	C	SCK	CK	C	SCK	CK
RBC (x10 ¹² /l)	8.83±2.1	7.88±0.5	6.39±2.3 ^{fb}	11.69±3.4	8.48±3.0 ^{lc}	8.03±0.4 ^c	9.44±3.4	7.73±1.7 ^{fb}	6.96±1.1 ^{lc}			
HGB (g/l)	113.00±8.9	98.66±5.5	87.66±8.9 ^{fb}	149.00±8.7	92.00±8.5 ^{fb}	88.00±4.1 ^c	116.80±5.3	86.16±4.9 ^{fb}	80.00±5.5 ^{fb}			
HCT (%)	30.10±4.3	25.51±1.6	20.50±3.2 ^{fa}	40.66±3.9	30.66±3.0 ^{fa}	23.00±1.4 ^c	31.66±2.1	20.65±3.5 ^{fb}	19.66±1.7 ^{fb}			
MCV (fl)	33.95±2.9	33.70±1.9	35.16±0.4	35.06±1.9	35.36±0.9	34.65±3.1	33.32±2.4	31.80±2.8	33.86±1.5			
MCH (pg)	12.76±0.8	12.56±0.5	12.86±0.1	12.70±0.5	12.88±0.4	12.85±0.07	12.30±0.8	11.98±0.7	12.63±0.2			
MCHC (g/l)	377.0±15.2	373.33±12.2	366.66±3.5	364.5±12.0	363.16±8.6	351.5±3.5	369.8±4.5	372.75±0.9	373.33±10.6			
RDW (%)	21.55±1.4	20.81±0.8	20.03±0.1	20.90±0.2	20.88±0.5	20.65±0.07	21.54±0.9	21.42±1.0	21.90±1.0			
RDWa (fl)	20.03±1.4	19.30±0.8	20.00±0.4	20.40±1.6	20.80±0.7	21.65±0.4	19.46±1.2	19.45±1.4	19.90±0.8			
WBC (x10 ⁹ /l)	8.88±1.1	11.13±2.3 ^{fa}	12.70±3.3 ^{fb}	8.13±1.9	12.50±2.8 ^{fb}	13.30±1.7 ^{lc}	7.46±1.4	10.05±1.6 ^{fb}	13.33±1.3 ^{lc}			
LYM (x10 ⁹ /l)	3.90±0.5	4.85±1.0 ^{fa}	4.63±0.4 ^{fa}	3.80±0.1	5.77±0.9 ^{fb}	6.60±0.2 ^c	4.06±0.8	5.97±0.6 ^{fb}	6.25±0.6 ^{lc}			
MON (x10 ⁹ /l)	0.63±0.1	0.83±0.1	0.86±0.2	0.60±0.1	0.68±0.2	0.55±0.07	0.66±0.1	0.60±0.2	0.90±0.2			
GRA (x10 ⁹ /l)	4.35±0.6	5.45±1.3	6.20±0.6	3.70±1.0	5.07±0.2	5.00±0.2	2.74±0.7	3.41±1.0	4.53±1.3			
PLT (x10 ⁹ /l)	197.30±18.2	173.80±13.3	254.66±26.3	291.30±22.5	394.50±19.2	312.00±28	335.40±28.2	274.00±18.6	489.00±32.8			
MPV (fl)	5.51±0.4	5.53±0.4	5.36±0.4	5.25±0.07	5.05±0.3	5.20±0.1	5.14±0.3	5.20±0.5	5.13±0.1			

Legend: ^ap<0.05; ^bp<0.01; ^cp<0.001; I-vs. control groups; C – control group; SCK – with subclinical ketosis; CK – with clinical ketosis

Discussion

The observed field cases of ketosis in high-yielding dairy sheep conformed the reports of many authors (Van Saun, 2000; Schlumbohm and Harmeyer, 2008), that this condition was provoked by overfeeding with concentrate feed and deficiency of easily digestible carbohydrates in diets especially during the late gestation and early lactation, which are accompanied by substantial changes in the maternal organism.

With regard to its clinical manifestation, ketosis is defined as subclinical and clinical by numerous authors (Herdt, 2000; Gordon et al., 2013); we also agree with their opinion. Dairy sheep breeds (Lacaune) could be affected with SCK and CK during all physiological states (pregnancy, lambing, lactation). The sheep with SCK did not show any deviation in general clinical parameters except for rumen movements, whose strength and frequency decreased at a various extent and were close to the lower limits secondary to alkalization of rumen and duodenal content by increased ammonia and biogenic amines concentrations as reported elsewhere (Kabakci et al., 2003; Balikci et al., 2009). The established changes in the general clinical status in sheep with CK were localized in the alimentary, respiratory and nervous systems. Enhanced heart and respiratory rates in sheep points at functional damage of the cardiovascular and respiratory systems from one part, and could be due to rumen metabolites subsequently to CK on the other. Respiratory and heart failure reflect on the color of visible mucous coats that from pale rose-red become diffusely red and before the lethal outcome, have an icteric tint. The rumen movements in the course of development CK decreased both in strength and frequency compared to control groups. These results are in line with other reports (Kabakci et al., 2003; Balikci et al., 2009).

A main chemical blood parameter used as an early marker for SCK and CK in ruminants is blood BHBA concentration (Lacetera et al., 2002). This was confirmed by our studies as well. We found out that the sheep from the dairy breed Lacaune suffered from SCK and CK during the pregnancy, lambing and lactation when blood BHBA was about 1.10–1.20 mmol/l (in SCK) and 2.15–3.10 mmol/l (in CK). Sheep from the meat breed Mouton Charollais were not affected with both forms of ketosis as indicated by the lack of individual blood BHBA concentrations over 0.8 mmol/l. High BHBA levels in blood are a compensatory mechanism in response to occurring carbohydrate deficiency and Krebs cycle inhibition (Ingvarsen, 2006). In cases of excessive lipolysis accompanied by production of large acetyl CoA amounts, the tricarboxylic acids cycle is not capable to convert entirely fatty acids. Consequently, acetyl CoA is metabolized to acetoacetate, which is reduced to BHBA through BHBA dehydrogenase or is spontaneously decarboxylated to acetone (Roche et al., 2013). The increased BHBA concentration in blood reveals the incomplete oxidation of non-esterified fatty acids (NEFA) in the tricarboxylic acid cycle at the time of NEB (Grummer, 1993; Doepel et al., 2002).

The established erythropenia, resp. oligochromemia and reduced hematocrit in sheep with SCK and CK could be attributed to the body NEB during pregnancy, lambing and intensive lactation. The negative energy balance activates the function of adrenal glands with resulting increase in catecholamines, cortisol and endorphins levels (Antonov, 2000; Găvan et al., 2010). These suggestions are in agreement with data reported in sheep (Gupta et al., 2008), goats (Abba et al., 2015) and cows (Belić et al., 2010). The leukocytosis and lymphocytosis in the three groups of sheep with SCK and CK could be associated to the presence of acute and chronic inflammations (mastitis, endometritis, etc.) during the postpartum period (Găvan et al., 2010). The enhanced lipolysis,

ketogenesis and hypoglycemia could contribute to erythropenia, leukocytosis with lymphocytosis (Belić et al., 2011), or these could be a sequel to stress manifested with increased glucocorticosteroids (cortisol) (Burton et al., 2005; Abba et al., 2015). Our data were therefore comparable to results reported by others (Hoeben et al., 1999).

Conclusion

In conclusion, the values of studied clinical parameters in the three groups of Lacaune sheep with SCK were within the reference ranges, while sheep affected with CK exhibited deviations from the general and specific clinical status. The primary blood biochemical marker of the form of ketosis in sheep is BHBA. Its amount in blood defined pregnant and recently lambing sheep as being affected with either SCK or CK. The sheep from the dairy Lacaune breed suffered from SCK and CK during the pregnancy, lambing and lactation, while those from the meat breed Mouton Charollais were healthy (BHBA <0.8 mmol/l). The studied red blood cell parameters in Lacaune sheep with SCK and CK showed erythropenia, oligochromemia, reduced hematocrit, leukocytosis and lymphocytosis.

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