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SUBCLINICAL AND CLINICAL KETOSIS IN SHEEP – RELATIONSHIPS BETWEEN BODY CONDITION SCORES AND BLOOD β-HYDROXYBUTYRATE AND NON-ESTERIFIED FATTY ACIDS CONCENTRATIONS

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ABSTRACT

The study included a total of 136 sheep from two breeds (Lacaune and Mouton Charollais) to establish changes in the values of body condition score (BCS), β-hydroxybutyrate (BHBA) and non-esterified fatty acids (NEFA) in their blood and their relationship in the development of subclinical (SCK) and clinical (CK) ketosis. The ewes were divided in three groups (pregnant, recently lambed and lactating). Blood concentrations of BHBA and NEFA were assayed in all ewes. The body condition was scored. Blood BHBA concentrations were increased in Lacaune sheep from the three 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 concentrations >0.8 mmol/l. The evaluation of BCS of ewes with SCK and CK showed a trend of decrease compared to the control groups. The quantities of NEFA in sheep of the three groups with SCK were statistically significantly elevated vs control groups, while in sheep with CK - decreased, vs both controls and SCK. In meat-type Mouton Charollais ewes, values of NEFA ranged around the reference.

Key words: *Ketosis, BCS, BHBA, NEFA, ewes*

INTRODUCTION

Ketosis is among the most significant health problems in high-producing dairy livestock operations (cows, sheep and goats) at both national and global scale. Acetonaemia (pregnancy toxaemia) in sheep is a nutritional stress syndrome affecting dairy breeds (Lacaune, Assaf, Awassi, Stara Zagora dairy sheep breed, Blackhead Pleven etc.). Adult, multiparous animals in good body condition are affected during the last 3 to 6 weeks of gestation (Van Saun, 2000; Schlumbohm and Harmeyer, 2008), as well as during early and late lactation (Ferris et al., 1969; Van Saun, 2000; Marutsova, 2016). Contrary to the considerable body of evidence on SCK and CK in dairy cattle, data about this disease is sheep comprise mainly pregnancy toxaemia and at a lesser extent, postpartum lactational ketosis. Some researchers observed pregnancy toxaemia rates in sheep between 6.5% and 37% (Al-Mujalli, 2008), while others (Gupta et al., 2008) reported a prevalence of SCK of 14.86% during the pregnancy and 13.51% during lactation in ewes. The main economic losses in ketotic sheep result from the death of affected animals, respectively their progeny, medication costs, production losses and triggering of secondary diseases.

Blood BHBA concentrations reflect the magnitude of negative energy balance (NEB) and lipid mobilisation in dairy animals, hence there are diagnostic markers for SCK and CK (Panousis et al., 2012; Sordillo and Raphael, 2013). Threshold blood BHBA in sheep with SCK is rather variable – from 0.5 mmol/l to 1.6 mmol/l (Andrews, 1997; Balikci et al., 2009; Anoushepour et al., 2014; Feijó et al., 2015), while in sheep with CK – from 1.6 mmol/l to 7 mmol/l (Andrews, 1997; Lacetera et al., 2002; Balikci et al., 2009). Some authors (Panousis et al., 2012) reported higher BHBA levels in sheep with pregnancy toxaemia as compared to lactating sheep.

Deviations in BCS and BHBA values in small ruminants are indicative for NEB and for emergence of metabolic disturbances after the parturition (Andrews, 1997; Koyuncu and Altınçekiç, 2012). Body condition is scored using a 5-point scale: from 1.0 to 5.0 (Jefferies, 1961; Russel, 1984; Thompson and Meyer, 1994). Some researchers (Fthenakis et al., 2012; Karagiannisa et al., 2014) outlined 3.0 – 4.0 as the optimum BCS of sheep during the reproduction period; values of 2.5 – 4.0 during early and mid-gestation. At the time of lambing, optimum BCS should be 3.0–3.5 in ewes carrying singletons and 3.5–4.0 in those carrying twins, while BCS at weaning of lambs – 2.0 or higher.

Higher blood NEFA concentrations are reported in fasting sheep, sheep with twin vs those with singleton pregnancy (Schlumbohm and Harmeyer, 2008). In dairy sheep with ketosis, blood BHBA < 1.0 mmol/l, high serum NEFA and low blood glucose levels are reported (Moallem et al., 2015). Unlike these data, others (Ferris et al., 1969) did not establish any significant changes in blood glucose in sheep with pregnancy toxaemia.

The reported contradictory data about body condition scores in pregnant and lactating sheep with SCK and CK and its relationship with blood BHBA and NEFA values were the incentive for these experiments.

MATERIALS AND METHODS

Animals. The studies carried out in two sheep farms of the southern part of Bulgaria. 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. All ewes were regularly vaccinated and treated against ecto- and endoparasites. They were reared in facilities in compliance with the respective welfare standard for the species. Sheep of both breeds were fed rations in concordance with their physiological condition (pregnant, recently lambed and lactating).

Experimental design. The ewes were divided in three groups depending on their physiological condition, namely: pregnant (from pre-partum days 15 to 0); recently lambed (from postpartum days 0 to 15) and lactating (from postpartum days 30 to 45). Sheep of the three groups, respectively, of the two breeds we performed a chemical blood test to determine the level of BHBA as a result of which they were classifies as healthy (control, BHBA < 0.8 mmol/l), affected with SCK (BHBA from 0.8 to 1.6 mmol/l) and CK (BHBA > 1.6 mmol/l).

Group I from the dairy breed Lacaune included 45 animals – 14 healthy (control), 8 (18%) with SCK and 23 (51%) with CK. The second group (n=30) comprised 8 healthy controls, 10 (33%) with SCK and 12 (40%) with CK. The third group consisted of 31 sheep: 8 healthy, 11 (35.5%) with SCK and 12 (38.7%) with CK.

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

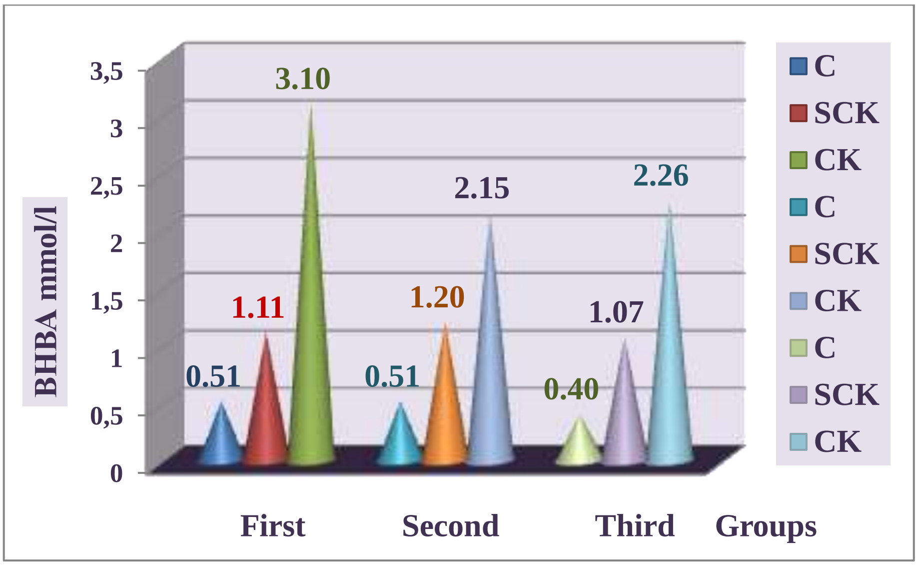
Blood samples and analyses. Blood samples for determination of BHBA and NEFA were obtained in the morning, before feeding, through puncture of the jugular vein using sterile 21G needles and vacutainers (with heparin and without anticoagulant, 5 ml, Biomed, Bulgaria). Blood BHBA concentrations were determined in situ using a portable Xpress-I system (Nova Biomedical, UK). The values of NEFA in the blood serum determination using NEFA ELISA Kit (Changhay Crystal Day Biotech Co., LTD., China) and ELISA Reader Sunrise (Tecan, Switzerland).

Body condition scoring (BCS). The scoring of body condition of Lacaune sheep was done using a five-point scale (1.0 – 5.0) at 0.5-point intervals between 2.0 and 4.0 (1, 2, 2.5, 3, 3.5, 4 and 5) (18, 19). Sheep were examined by palpation of the loin region after the last rib. Sheep from the meat breed Mouton Charollais were not scored as they did not exhibit blood BHBA > 0.8 mmol/l, i.e. no SCK and CK were present.

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

RESULTS

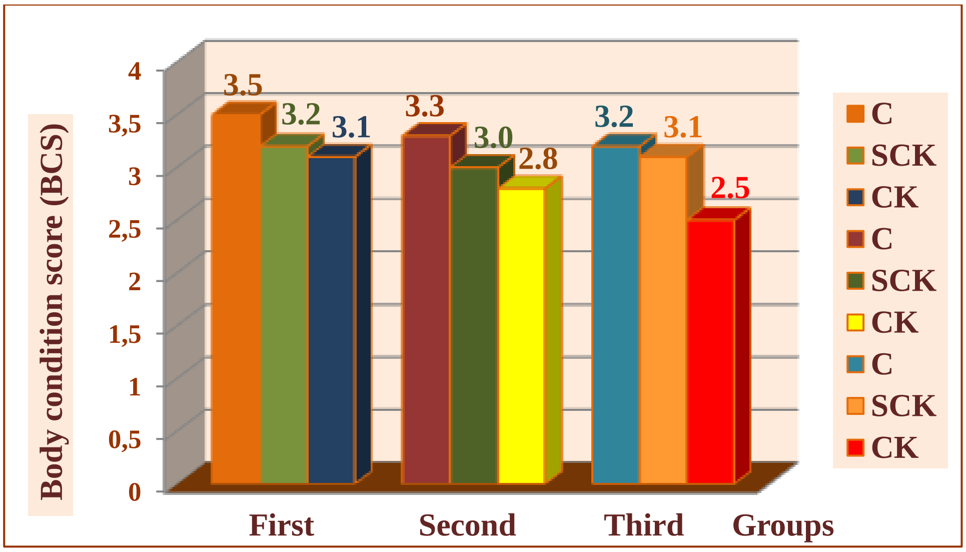
Blood BHBA analysis in the three control groups of Lacaune sheep were within the reference range. Sheep from the three groups with SCK had statistically significantly higher BHBA concentrations than control groups (Fig.1; $p < 0.001$). Sheep from the three groups (pregnant, recently lambed and lactating) with CK had BHBA levels in blood substantially higher than both controls and SCK (Fig.1; $p < 0.001$). 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 the first group, 0.40 ± 0.17 mmol/l in the second group and 0.18 ± 0.08 mmol/l in the third group).



(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.

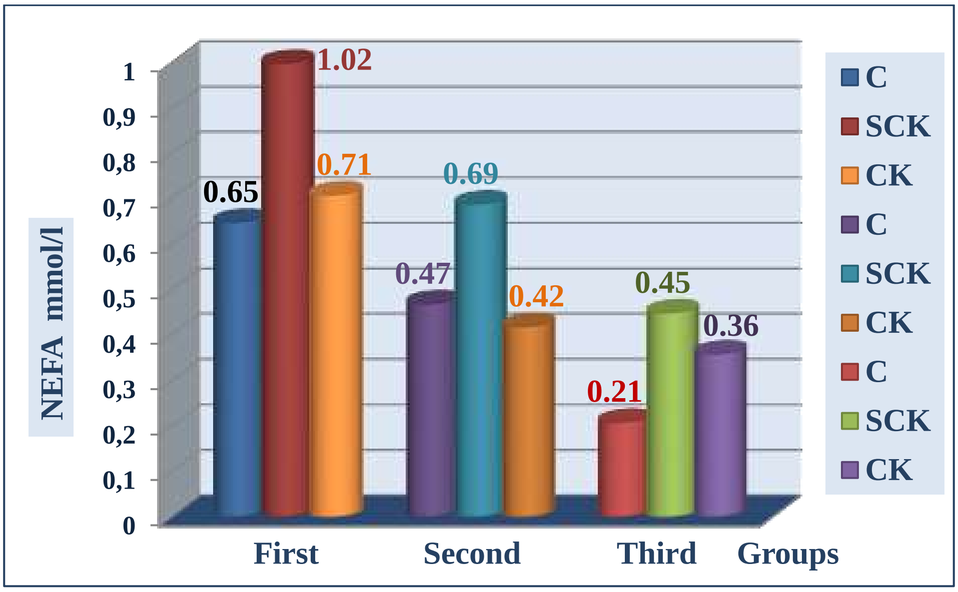
Body condition scores of Lacaune sheep from the three groups are presented on Figure 2. According to analyses, BCS of control sheep were within the reference ranges. The BCS of sheep from the three groups with subclinical ketosis showed a tendency towards insignificant decrease vs control groups. The BCS of sheep with clinical ketosis was further lower compared to the sheep with subclinical ketosis (Fig. 2).



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

Figure 2. Evaluation of body condition scores of Lacaune ewes from first, second and third group with subclinical and clinical ketosis.

Blood serum NEFA concentrations of the three control groups ranged within the reference interval (Fig. 3). In the first, second and third groups with SCK, blood NEFA values were statistically significantly higher than controls (Fig. 3; $p < 0.05$). All sheep with clinical ketosis exhibited lower blood NEFA concentrations than those with subclinical ketosis (Fig. 3; $p < 0.05$). Blood chemistry analyses in Mouton Charollais sheep did not show any substantial deviations in blood NEFA values compared to the reference values. In this breed, blood NEFA was 0.32 ± 0.01 mmol/l in the first group; 0.28 ± 0.02 mmol/l in the second group and 0.45 ± 0.03 mmol/l in the third group.



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

Figure 3. Changes in blood non-esterified fatty acids (NEFA) levels in Lacaune ewes from first, second and third group with subclinical and clinical ketosis.

DISCUSSION

High blood BHBA concentrations in animals with CK and CK are a mechanism for compensation of occurring carbohydrate deficiency and the inhibition of the citric acid cycle (Ingvartsen, 2006). In cases of excessive mobilization of fats accompanied by formation of large amounts of acetyl CoA, fatty acids are not completely metabolized via the citric acid cycle and as a result, acetyl CoA is converted to acetoacetate which is either reduced to BHBA by BHBA-dehydrogenase or is spontaneously decarboxylated to acetone (Roche et al., 2013; Allen and Piantoni, 2013). Non-esterified fatty acids provide the substrate for BHBA synthesis in line with our studies. The increased BHBA concentration indicates incomplete oxidation of NEFA in the citric acid cycle at the time of NEB (Doepel et al., 2002). The rate of ketone bodies formation is proportional to the extent of lipolysis and oxidation of fatty acids (Roche et al., 2013).

Our results demonstrated that sheep from the dairy Lacaune breed were affected by SCK and CK during the pregnancy, at parturition and during the lactation when blood BHBA concentrations were around 1.10 – 1.20 mmol/l in SCK and 2.15 – 3.10 mmol/l in CK. Mouton Charollais sheep did not suffer from both forms of ketosis as their blood BHBA levels were not over 0.8 mmol/l. These data are comparable to those of Andrews (1997); Lacetera et al. (2002); Balikci et al. (2009) and Anoushepour et al. (2014). Blood BHBA < 0.8 mmol/l in sheep are not only threshold values for SCK, but could be accepted as indicating a good transition from pregnancy to lactation, a belief supported by other authors as well (Allen and Piantoni, 2013). The deficiency of energy in sheep was greater during early lactation than in late pregnancy. This presumes that during the early lactation, sheep are prone to ketosis (Perry et al., 1994). The deviations from optimum BCS result in development of metabolic diseases and failure to attain the maximum lactational milk yields (Roche et al., 2015). The lower BCS of Lacaune sheep with SCK and CK correlated negatively to higher blood BHBA. This relationship resulted from the additional suppression of the appetite from higher blood ketone bodies concentrations. The weight loss during the early postpartum period influenced highly the risk for development of ketosis, reduction of milk yield, impaired reproduction performance and early embryonic death. These assumptions are in line with the experiments of López-Gatius et al. (2002). Both our results and those of other researchers (Roche et al., 2015) allowed assuming that obesity of dairy animals at drying-off and during the dry period has an important role in the occurrence of clinical ketosis. The established changes in blood serum NEFA as marker of systemic NEB were not unidirectional – in dairy ewes with SCK from the different groups (pregnant, recently lambed, lactating) NEFA levels were increased vs controls attaining up to 1.02 mmol/l in ewes. These results support the thesis that the lipolysis, assisted by insulin resistance in the period of early lactation, occurred at a higher rate so the net quantity of NEFA was substantially higher that the amount that could be converted in the liver (Doepel et al., 2002; Allen and Piantoni, 2013; Roche et al., 2013). The extent of lipid mobilization and the decreased appetite determine whether the levels of ketone bodies in dairy animals would be normal, or they would develop SCK and/or CK (Allen and Piantoni, 2013) pre-partum (Joshi et al., 2006) and postpartum (Roche et al., 2013). In the different groups of ewes with CK, NEFA levels decreased and attained 0.36 mmol/l. Fat is accumulated in the liver without maximum stimulation of gluconeogenesis. Fatty acids that are not completely oxidized are either converted into ketone bodies or are reesterified to triglycerides, resulting in fatty liver due to the low capacity of the ruminant liver to synthesize VLDL for transport of triglycerides (Holtenius and Holtenius, 1996). These statements are also supported by our research.

CONCLUSION

The performed field studies with ewes from the daily and met-type breed allowed concluding that the main blood biochemical parameter of blood, indicating the form of ovine ketosis, was the β-hydroxybutyric acid (BHBA). The sheep from the daily Lacaune breed suffered from SCK and CK throughout the gestation, lambing and lactation, while those from the Mouton Charollais breed were not affected (BHBA < 0.8 mmol/l). Non-esterified fatty acids in the blood changed inconsistently – the levels were increased in ewes with SCK whereas reduced in those affected with CK. Lower BCS of sheep with SCK and CK correlated negatively to higher blood BHBA concentrations, therefore BCS is suggested as an important tool in the management of feeding programmes for dairy sheep herds.

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