



ISSN NO. 2320-5407

Journal Homepage: -www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/2098
DOI URL: <http://dx.doi.org/10.21474/IJAR01/2098>



INTERNATIONAL JOURNAL OF
ADVANCED RESEARCH (IJAR)
ISSN 2320-5407
Journal Homepage: <http://www.journalijar.com>
Journal DOI:10.21474/IJAR01

RESEARCH ARTICLE

BODY CONDITION SCORING AND CHANGES IN SOME BLOOD BIOCHEMICAL PARAMETERS IN COWS WITH SUBCLINICAL AND CLINICAL KETOSIS.

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Manuscript Info

Manuscript History

Received: 24 September 2016
Final Accepted: 26 October 2016
Published: November 2016

Key words: ketosis, BCS, BHBA, NEFA, dairy cows.

Abstract

Studies were conducted in highly productive dairy cows in connection with the establishment of the changes in the values of body condition score (BCS), β -hydroxybutyric acid (BHBA) and non-esterified fatty acids (NEFA) in their blood. For this purpose were used 158 Holstein cows with yearly milk yield of 9000–11000 L in their 1st to 4th lactation. The cows were divided into 3 groups: I group (pregnant cows); II group (recently calved) and III group (lactating cows). Blood concentrations of BHBA were assayed in all cows and on the basis of results, they were classified as healthy (control, C), affected with subclinical ketosis (SCK) and clinical ketosis (CK). The levels of NEFA were determined in the blood of the targeted animals. Evaluation of BCS of the cows was conducted at 5 points system.

It was found that the quantity of BHBA in cows from groups I, II and III with SCK signs was statistically significantly elevated vs control cows and ranged from 1.57 ± 0.55 mmol/l to 1.73 ± 0.61 mmol/l, ($p < 0.001$). There were no BHBA values > 2.6 mmol/l, e.g. CK in cows from the first group. In cows of the groups II and III with CK signs, blood BHBA was statistically significantly increased vs control cows and vs animals with SCK and were 4.27 ± 1.29 mmol/l and 4.75 ± 1.36 mmol/l ($p < 0.001$), respectively.

The evaluation of BCS of cows with SCK signs, showed a trend falsity decrease compared to the control groups and were between 3.0 ± 0.41 - 3.25 ± 0.36 . In cows from groups II and III with CK signs BCS decreased and were 2.75 ± 0.32 for the second group and 2.51 ± 0.31 for the third group ($p < 0.05$).

In cows of the groups I, II and III with SCK quantities of NEFA were from 0.48 ± 0.03 mmol/l to 0.84 ± 0.03 mmol/l, while that of the second and third groups with CK were between 0.35 ± 0.01 mmol/l and 0.68 ± 0.02 mmol/l.

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Introduction:

Ketosis of high-yielding dairy cows is among the most significant health issues in dairy farming both at a national and global scale. The higher the productivity of cows, the greater their susceptibility to metabolic disorders,

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including ketosis and fatty liver dystrophy (Herdt, 2000; Ingvarstsen, 2006; Oltenacu and Broom, 2010; Suthar et al., 2013, Marutsova, 2016).

The body condition of high-yielding cow's changes throughout the lactation, but until 1970, no method for assessment of body energy reserves was available (Stockdale, 2001). Lowman et al. (1973) were the first to develop for body condition scoring system for dairy cows using a scale from 1.0 – 5.0; it was later improved and modified by other researchers (Edmonson et al., 1989; Ferguson et al., 1994). Body condition scoring is based on the visual assessment and palpation of specific areas of the cow's body by the investigator. A number of authors affirm that cows that are obese at the time of calving and during the lactation, were at a higher risk for development of metabolic diseases as ketosis and fatty liver, retained placenta, mastitis, metritis etc. (Gillund et al., 2001; Hoedemaker et al., 2009; Nogalski et al., 2012; Samiei et al., 2015; Roche et al., 2015, Marutsova, 2016).

Gillund et al. (2001); Roche et al. (2009) and Allen and Piantoni (2013) supported the hypothesis that the body condition score (BCS) of cows at calving should be within 3.0 – 3.25 (using a scale from 1.0 to 5.0), while Samarütel et al. (2006); Nogalski and Górak (2008) and Lean et al. (2013) recommend a BCS of 3.5. Cows with BCS values > 3.5 presented 2.5 times higher risk from ketosis. For cows, Ospina et al. (2010) accepted BCS values of 4.0 for pregnant heifers, 3.5 – for pregnant cows and lactating heifers, and 3.25 for dairy cows.

Several research teams (Oetzel, 2007; Voyvoda and Erdogan, 2010; Panousis et al., 2012; McArt et al., 2013) used blood BHBA for evaluation of the degree of negative energy balance (NEB) and lipid mobilization in dairy animals (Sordillo and Raphael, 2013), and outlined it as a primary quantitative parameter of ketosis, using BHBA concentrations to define subclinical and clinical ketosis (SCK and CK).

In cows with SCK, some authors (Whitaker et al., 1999; Wittwer, 2000; Oetzel, 2007; Duffield et al., 2009; Seifi et al., 2011; McArt et al., 2013; Djoković et al., 2013) accepted threshold blood BHBA from 1.0 mmol/l to 1.4 mmol/l, and for cows with CK – over 2.0 mmol/l (Duffield, 2000; Oetzel, 2004; Ospina et al., 2010; González et al., 2011). Pre-partum blood BHBA values >0.5 mmol/l and postpartum BHBA >1.0 mmol/l were believed to indicate enhanced lipid mobilization and appearance of ketosis in cows (Wittwer, 2000).

Palmitic, stearic and the monounsaturated oleic acid are in the group of NEFA in the periparturient period (Roche et al., 2013; Sordillo and Raphael, 2013). According to Whitaker et al. (1999) and Hiss et al. (2009) normal NEFA concentrations are <0.3 mEq/l in pregnant cows, and <1.0 mEq/l at calving. After the third day of lactation, blood BHBA should be <0.7 mEq/l. Nogalski et al. (2012) reported that cows with pre-partum blood BHBA of 0.52 mmol/l and NEFA 0.29 mmol/l, develop ketosis in the postpartum period, demonstrating increased values of BHBA (1.59 mmol/l) and NEFA (1.09 mmol/l).

At the herd level, NEFA values >0.4 mmol/l and BHBA >1.4 mmol/l in more than 10% of tested animals are accepted as reliable biomarkers of NEB and SCK in the herd (Duffield, 2000; Oetzel, 2004). Djoković et al. (2013) found out high BHBA (>1.2 mmol/l) and NEFA (>0.4 mmol/l) concentrations, accompanied with hypoglycemia in cows with subclinical ketosis during early lactation (94.4%). Gillund et al. (2001); Hayirli et al. (2002); Nogalski et al. (2012) and Akbar et al. (2015) established that cows with BCS \geq 4.0 were at higher risk for developing ketosis and had higher blood plasma BHBA and NEFA levels.

The inconsistent literature data about the body condition score of cows and its relationships with blood BHBA and NEFA in high-producing dairy cows with SCK and CK were the incentive of this study.

Material and Methods:

Animals:

Studies were performed in dairy farms in the Republic of Bulgaria. A total of 158 Holstein cows with yearly milk yield of 9000–11000 L, in their 1st to 4th lactation and average body weight 450–550 kg were included in the study. Target cows were fed rations in concordance with their physiological condition (pregnant, recently calved and lactating) and norms for roughage and concentrate contents in diets given for each physiological condition.

Experimental design:

The cows were divided into 3 groups according to their physiological condition: I group – pregnant cows (from day 15 to day 0 pre-calving); II group – recently calved (from day 0 to 15 postpartum) and III group – lactating (from day 30 to 45 postpartum). Blood concentrations of BHBA were assayed in all target animals and on the basis of

results, they were classified as healthy (control, BHBA <1.2 mmol/l), affected with SCK (BHBA from 1.2 to 2.6 mmol/l) and CK (BHBA >2.6 mmol/l).

The first group included 21 pregnant cows (9 healthy; 12 – with SCK). Blood BHBA concentrations indicative for CK were not established in this group. The second group comprised 90 recently calved cows (55 healthy, 27 – with SCK and 8 – with CK). The third group (47 lactating cows) included 24 healthy (control) cows; 15 animals affected with SCK and 8 – with CK.

Blood samples and analyses:

Blood samples for determination of BHBA and NEFA were obtained in the morning, before feeding, through puncture of the coccygeal 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., Bioassay Technology Laboratory, China) and ELISA Reader Sunrise (Tecan, Switzerland).

Body condition scoring (BCS):

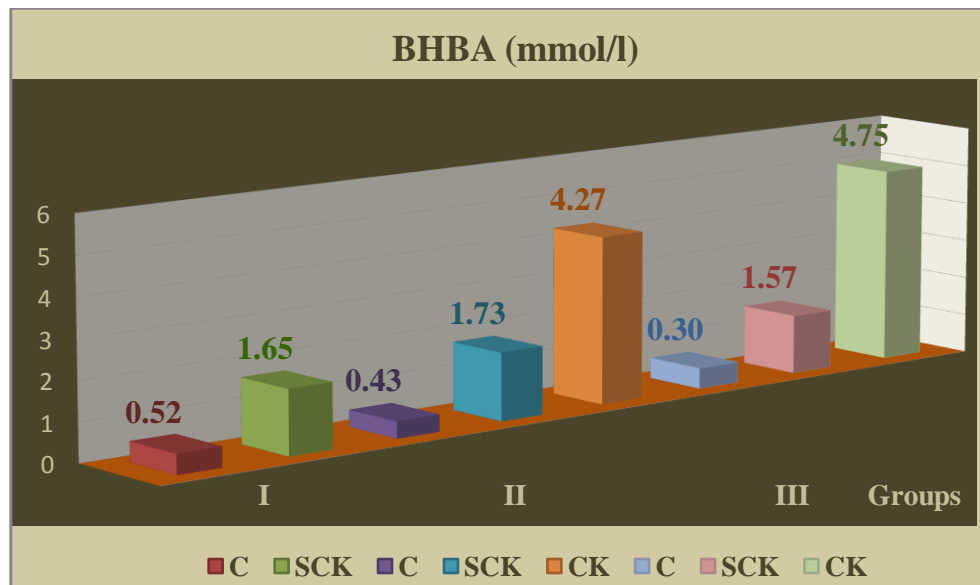
Body condition scores were evaluated using a 5-point scale (1.0-5.0, at intervals of 0.25). The cows were scored visually by two investigators according to the procedure of Edmondson et al. (1989) and Ferguson et al. (1994).

Statistical analysis:

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

Results:

Blood BHBA in control cows were 0.52 ± 0.09 mmol/l for group I (pregnant); 0.43 ± 0.25 mmol/l for group II (recently calved) and 0.30 ± 0.16 mmol/l for group III (lactating) (Fig. 1).



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

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

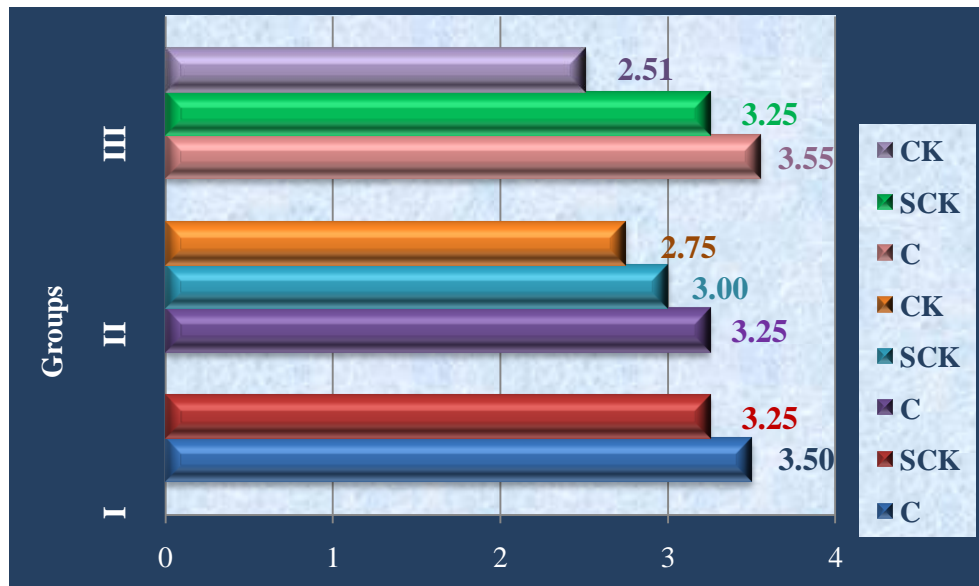
The amount of blood BHBA in cows from group I (pregnant – from pre-partum days 15 to 0) with SCK (1.65 ± 0.63 mmol/l) was statistically significantly increased vs that of the control group (0.52 ± 0.09 mmol/l; $p < 0.001$). In the second group of cows with SCK, blood BHBA concentrations were again considerably higher than controls (1.73 ± 0.61 mmol/l and 0.43 ± 0.25 mmol/l, $p < 0.001$), as well as in group III with SCK – 1.57 ± 0.55 mmol/l as

compared to controls (0.30 ± 0.16 mmol/l, $p < 0.05$) (Fig. 1). The BHBA analysis of cows from group I did not exhibit concentrations higher than 2.6 mmol/l, e.g. clinical ketosis was not present.

The analysis of blood of recently calved and lactating cows with clinical ketosis showed statistically significantly increased BHBA levels 4.27 ± 1.29 mmol/l for group II, $p < 0.001$ and 4.75 ± 1.36 mmol/l for group III, $p < 0.001$) vs control cows (0.43 ± 0.25 mmol/l for group II and 0.30 ± 0.16 mmol/l for group III) and vs SCK cows (Fig. 1).

The body conditions scores of cows from the three groups (pregnant, recently calved and lactating) is illustrated on Fig. 2. BCS of all control groups was within the reference range – 3.50 ± 0.24 for group I; 3.25 ± 0.28 group II and 3.55 ± 0.27 for group III.

The BCS of cows with subclinical ketosis tended to decrease insignificantly vs control groups measuring 3.25 ± 0.36 for group I; 3.0 ± 0.41 for group II and 3.25 ± 0.27 for group III. In recently calved and lactating cows with CK, these values were further lower: 2.75 ± 0.32 and 2.51 ± 0.31 respectively ($p < 0.05$) (Fig. 2).



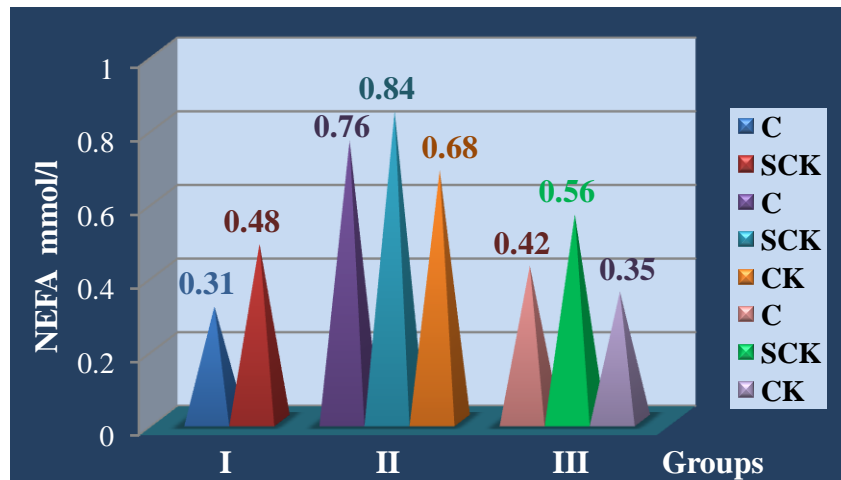
(C – control group; SCK – with subclinical ketosis; CK – with clinical ketosis)

Fig. 2: Evaluation of body condition scores of cows from groups I, II and III with subclinical and clinical ketosis.

Blood serum NEFA in control cows from the three groups were within the reference range – 0.31 ± 0.02 mmol/l for group I; 0.76 ± 0.05 mmol/l for group II and 0.42 ± 0.01 mmol/l for group III (Fig. 3).

In pregnant cows with SCK blood NEFA levels were increased as compared to controls up to 0.48 ± 0.03 mmol/l ($p < 0.05$); in group II average NEFA value was 0.84 ± 0.03 mmol/l whereas in group III – 0.56 ± 0.04 mmol/l ($p < 0.05$) (Fig. 3).

The blood NEFA concentrations in cows with clinical ketosis from groups II and III changed in the same direction showing reduction vs controls: 0.68 ± 0.02 mmol/l ($p < 0.05$) in group II, and 0.35 ± 0.01 mmol/l ($p < 0.05$) in group III (Fig. 3).



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

Fig. 3: Changes in blood non-esterified fatty acids (NEFA) levels in cows from groups I, II and III with subclinical and clinical ketosis.

Discussion:

A primary blood biochemical parameter used as an early marker of ketosis in cows, in blood BHBA (Oetzel, 2007; Kaneko et al., 2008). BHBA concentrations reflect the extent of oxidation of esterified fatty acids in the liver (LeBlanc et al., 2010; Sordillo and Raphael, 2013). The analysis of blood BHBA could help to diagnose subclinical and clinical ketosis in high-yielding dairy animals (Duffield et al., 2009; Seifi et al., 2011; González et al., 2011; McArt et al., 2013; Albay et al., 2014), without specific clinical signs of disease (Duehlmeier et al., 2011). These assumptions are supported by our experiments as well.

The investigations of pregnant cows from group I showed that they were affected by subclinical but not by clinical ketosis, as BHBA levels in their blood did not exceed 2.6 mmol/l. High-yielding cows experience SCK and CK in the postpartum period and during peak lactation (groups II and III), when blood BHBA attained >1.5 mmol/l in subclinical and 4 mmol/l in clinical ketosis. These data are supported by the studies of Seifi et al. (2011); González et al. (2011) and McArt et al. (2013). High blood BHBA are a mechanism for compensation of occurring carbohydrate deficiency and the inhibition of the citric acid cycle (Ingvarsen, 2006). It is acknowledged that oxaloacetic acid and coenzyme A are important products in this cycle. Oxaloacetic acid is a product of carbohydrate degradation in tissues. It is an acceptor that stimulates the aerobic oxidation of acetylated radicals such as activated acetic acid. The end products of this process are CO₂, water and energy. The deficiency of oxaloacetic acid results in accumulation of activated acetic acid in the animal body. The interaction of two molecules of activated acetic acid yields the main ketone body – acetoacetic acid. The latter after dehydrogenation is converted to β-hydroxybutyric acid and after decarboxylation – to acetone. 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 (Herdt, 2000; 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). BHBA is a regulator of lipolysis in the adipose tissue (Van Hove et al., 2003).

The reduced BCS of cows with subclinical and clinical ketosis exhibited negative relationship with the higher blood BHBA concentrations. This is a sequel of the additional suppression of the appetite at the background of high blood ketone bodies levels. Cows losing body weight during the first month of lactation are at a higher risk from development of ketosis, reduced milk yields, dislocated abomasum, impaired reproductive performance and early embryonic death. These postulations are in line with the studies of Gearhart et al. (1990) and López-Gatius et al. (2002). BCS provides information about the actual body reserves which the animal uses to cope with states associated with NEB, stress and disturbed nutrition (Ferguson et al., 1994; Prodanović et al., 2012; Roche et al., 2013). Poor body condition is established at the time of early lactation, when cows mobilize their body fat to satisfy

the energy demands of lactation (Ferguson et al., 1994; Kim and Suh, 2003). The precalving increase in BCS results in increased loss of body weight at the beginning of lactation reduced dry matter intake and decreased milk yields (Lean et al., 2013; Roche et al., 2015).

According to others (Ruegg and Milton, 1995; Pugh, 2002) body weight loss during the lactation and metabolic diseases are not related. Our results, similarly to those of other authors (Markusfeld et al., 1997; Roche et al., 2015) suggest that obesity of dairy animals at drying off and during the dry period play a pivotal role for clinical ketosis, so the BCS is an important tool aiding the management of nutritional regimens of dairy herds.

The established changes in serum NEFA levels as indicator of systemic negative energy balance are not unidirectional – in dairy animals with SCK from the different groups (pregnant, recently calved, lactating) they increased vs controls attaining 0.84 mmol/l, especially in recently calved cows. 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 than the amount that could be converted in the liver (Doepel et al., 2002; Hayirli, 2006; Allen and Piantoni, 2013; Roche et al., 2013). NEFA could be directly used as a source of fuel for tissues, for milk fat synthesis or to be utilized by the liver (Herd, 2000; Sordillo and Raphael, 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 subclinical and/or clinical ketosis (Allen and Piantoni, 2013) pre-partum (Joshi et al., 2006) and postpartum (Gillund et al., 2001; Ospina et al., 2010; Roche et al., 2013). In the different groups of cows with CK, NEFA levels decreased and attained 0.35 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). NEFA are one of markers for the extent of fat mobilization from body depots and are assumed to be a reliable parameter for evaluation of NEB, development of fatty liver infiltration and occurrence of ketosis (Ingvarsen, 2006; Nogalski and Górak, 2008; Huzzey et al., 2011).

Conclusion:

The results from the present studies demonstrated that blood BHBA was a primary parameter serving for discrimination of the forms of ketosis in cows. Its amount in the blood showed that pregnant and recently calved cows were healthy, with SCK and CK. The pregnant cows from group I were affected by SCK, not by clinical ketosis (BHBA <2.6 mmol/l). In the postpartum period and during intensive lactation (groups II and III), cows were affected by both forms of ketosis. The BCS is an important tool aiding the management of nutritional regimens of dairy herds. It provides information about the actual body reserves which the animal uses to cope with states associated with NEB, stress and disturbed nutrition. The changes in blood NEFA concentrations were in opposite directions – they increased in cows with subclinical ketosis while were reduced in cows affected by clinical ketosis.

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