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Relationship between some biochemical parameters in healthy and dairy cows with subclinical ketosis

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Abstract

The aim of the presented study is to compare beta-hydroxy butyric acid (BHBA), glucose (GLU) gamma-glutamyl transferase (GGT), phosphorus (P), calcium (Ca), and albumin (Alb) values of healthy animals and animals suffering from subclinical ketosis. The material in this study consisted of 15 healthy cows and 15 cows with subclinical ketosis on a commercial dairy farm located in Bursa (Turkey). Significant differences ($p<0.001$) were observed in BHBA, GLU, and Ca between the two groups. Results of this study might indicate the importance of calcium metabolism in the pathogenesis of the disease.

Keywords

dairy cows, beta-hydroxy butyric acid, calcium, subclinical ketosis

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Introduction

The most important period in the lives of dairy cows is the transition period covering the prepartum period and the succeeding three weeks after parturition. In this period, cows undergo significant hormonal, metabolic, and nutritional processes and prepare for the lactation that follows the parturition. The most important problems during this period are clinical and subclinical ketosis, fatty liver (hepatic lipidosis), displaced abomasum, retentio secundarium, metritis, and mastitis (1, 4).

Although the clinical ketosis, which is commonly observed in Turkey, can be diagnosed, the subclinical ketosis is often overlooked (5). This leads to serious economic losses in the dairy cattle industry as it predisposes for infertility, secondary metabolic and infectious diseases as well as reduced milk yield in the cows with high efficiency, in particular.

This study was aimed at looking for the differences in BHBA, GLU, GGT, P, Ca, and Alb between cows with subclinical ketosis and healthy cows.

Material and Methods

The study was conducted with 30 dairy cows aged between 3-6 years, among whom 15 experienced subclinical ketosis, and 15 were healthy on a farm located in Bursa (Turkey). Housing, feeding, and management conditions were same for all cows. All cows in the study were sampled during the period of 14-20 days following parturition. The clinical examinations (body temperature, pulse rate and respiration, palpation of the lymph nodes, tracheal palpation, lung auscultation and percussion) of all animals in the study were extensively performed. Fifteen cows without ketonuria and with blood BHBA levels between 1 and 1.4 mmol/l were enrolled in Subclinical Ketosis Group (SKG). Another 15 cows without ketonuria and with blood BHBA levels lower than 1 mmol/l were assigned to Control Group (CG). Animals selected for the study were clinically examined for postpartum diseases such as mastitis, metritis, displaced abomasum and laminitis, and those detected to be suffering from any of these diseases were excluded from the study.

Blood samples for glucose analysis were collected before morning feeding. Blood BHBA levels increase after feeding, thus it is suggested to collect blood samples 4-5 h after feeding in order to determine peak concentrations of BHBA (10). Therefore, the blood samples for BHBA, Alb, GGT, Ca, and P were collected 4-5 h after morning feeding between 11.00 and 12.00 a.m., from the coccygeal vein into the plain tubes. After clotting, the samples were centrifuged at 1000 rpm. for 15 min and sera were immediately separated and stored at -20°C until analyses.

Serum glucose, Alb, Ca, P and GGT levels were measured by using an automatic analyzer (Roche Cobas Integra 400; Roche Diagnostics, Indianapolis, IN). Serum BHBA levels were measured according to the manufacturer's instructions by using commercial

ELISA kits (Bovine BHBA ELISA No: CK - E90438, Eastbiopharm, China).

The normal distribution of the serum biochemical values examined in the study was executed by Shapiro-Wilk test. Independent samples t-test was used for normal distribution data, and Mann-Whitney U test was used for non-normal distribution data for statistical evaluation of differences between SKG and CG. Results with normal distribution were given as mean ± SE, while those with no normal distribution appeared as median (minimum-maximum).

Results

The results of the serum biochemical analyzes are shown in Table 1. BHBA levels were higher ($p<0.001$), while serum Ca and GLU levels were lower ($p<0.001$) in SKG compared to CG. On the other hand, Alb, GGT and P levels did not differ between study groups.

Table 1: Serum biochemical values of the animals included in the study *

Parameter	SKG (n=15)	CG (n=15)	p	Reference Ranges (7)
Glucose (mg/ dL)	47.73± 1.71	68.67± 2.38	<0.001	45-75
Albumin (g/ dL)	3.50(2.40-3.80)	3.40(2.60-3.70)	0.389	3.0-3.5
GGT (IU/ L)	25.70(12.90-43.80)	17.60(13.60-26.90)	0.116	6.1-24
Ca (mg/ dL)	9.00± 0.11	9.80± 0.10	<0.001	8.3-12
P (mg/ dL)	6.10± 0.16	6.18± 0.17	0.750	4.5- 6.5
BHBA (mmol/L)	1.10(1.00-1.20)	0.15(0.06-0.46)	<0.001	0-1

*Results with normal distribution were shown as mean ± SE, while those with no normal distribution were shown as median (minimum-maximum); SKG- Subklinical Ketosis group; CG- Control group.

Discussion and conclusions

The adaptation of high milk-yielding cows to negative energy balance is regulated over non-esterified fatty acid (NEFA), ketone bodies and especially glucose (7). In particular, the low glucose level during primary ketosis triggers the mobilization of fat reserves in the body. The blood glucose level in healthy cows is 45-75 mg/dl, whereas this level is 20-40 mg/dl in the cows with ketosis. In the cases with subclinical ketosis, on the other hand, it was reported to be above 40 mg/dl, being at the lowest level in the neural form (7). In the study conducted by Şentürk et al. (5), blood glucose was determined as 44.8 ± 2.3 mg/dl in clinical ketosis animals, as 51.6 ± 2.3 mg/dl in subclinical ketosis animals, and as 68.3 ± 1.8 mg/dl in healthy animals. In this study, the mean glucose value of the SKG was determined as 47.73 ± 1.71 mg/dl and the mean value of the CG as 68.67 ± 2.38 m/dl, which was statistically different ($p<0.001$).

The level of BHBA is more stable in the samples than acetoacetate and acetone (1), hence BHBA is a golden standard in the diagnosis of ketosis (7). Walsh et al. (8) determined the grouping interval as subclinical ketosis with BHBA level 1≤1.4 mmol/L, and Duffield (2) determined this range as 1.2-1.4 mmol/L. In the present study, it was similar to Walsh et al. (8) and the subclinical ketosis with BHBA level is found to be 1≤1.4 mmol/L. A statistically significant difference was found between the two groups for the level of BHBA in the present study ($p<0.001$).

Ketosis and Ca metabolism are directly interrelated. . In general, hypocalcemia may be shaped at varying degrees in the animals with ketosis. In the present study, although the serum Ca levels in the two groups were within the normal limits, there was a statistically significant difference between the SKG and CG ($p<0.001$). Similar to this finding, moderate hypocalcemia was found in subclinical and clinical ketosis cows (2). The present study also showed that Ca levels measured in animals with subclinical ketosis were lower than in the control group.

Yameogo et al. (9) did not find a statistically significant difference between GGT values in clinical and subclinical ketosis animals in a study conducted. Ropstad et al. (3) have reported that severe hepatic lipidosis can occur in chronic cases of ketosis. In the present study, GGT values in animals with subclinical ketosis and control group were within normal limits, and no statistically significant difference was detected. Presumably, this result may indicate that the liver parenchyma was not sufficiently affected in the animals with subclinical ketosis in the study because chronic diffuse hepatic lipidosis did not occur. In the present study, the lack of statistical significance between the values of GGT, Alb and P in the SKG and CG is in line with the results obtained in some previous studies (6).

When the results of the study were evaluated, it was found that BHBA levels were significantly higher in cows with subclinical ketosis than in healthy cows, and that this increase could cause changes in calcium metabolism. Hence, results of this study might indicate the importance of calcium metabolism in the pathogenesis of the disease.

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Odnos između nekih biohemijskih parametara kod zdravih krava i mliječnih krava sa subkliničkom ketozom

Sažetak

Uvod i cilj

Najvažniji period u životu mliječnih krava je tranzicioni period koji obuhvata period prije telenja pa sve do tri sedmice nakon telenja. U ovom periodu krave su izložene značajnim hormonalnim, metaboličkim i nutricionim procesima te pripremi za laktaciju. Najznačajniji problemi tokom ovog perioda su subklinička i klinička ketoza, masna jetra (heaptična lipidoza), dislokacija abomazuma, zaostajanje placente, metritis i mastitis.

Cilj ovog istraživanja je upoređivanje vrijednosti beta hidroksi butrične kiseline (BHBA), glukoze (GLU), gama glutamil trasferaze (GGT), fosfora (P), kalcijuma (Ca) i albumina (Alb) i utvrđivanje njihove povezanosti između zdravih i životinja oboljelih od subkliničke ketoze.

Materijal i metode

Ispitivanje je provedeno na 30 mliječnih krava starosti između 3 i 6 godina na farmi lociranoj u Burzi (Turska). Uslovi smještaja, ishrane i menadžmenta su isti za sve krave. Uzorci od svih životinja uzimani su u peridu od 14 do 20 dana nakon poroda. Klinički pregled (tjelesna temperatura, puls i disanje, palpacija limfnih čvorova, auskultacija pluća i perkusija) svih životinja je detaljno provedena. Petnaest krava bez ketonurije i sa nivoom BHBA u krvi između 1 i 1,4 mmol/l su uvrštene u grupu sa subkliničkom ketozom (SKG). Drugih 15 krava bez ketonurije i sa nivoom BHBA u krvi nižim od 1mmol/l su uvršteni u kontrolnu grupu (CG). Uzorci krvi za analizu glukoze su uzimani prije jutarnjeg hranjenja životinja.

U cilju određivnja maksimalne koncentracije BHBA, uzorci krvi za određivanje BHBA, Alb, GGT, Ca i P su uzimani 4 do 5 sati nakon jutranjeg hranjenja između 11.00 i 12.00 sati, iz kokcigealne vene u obične epruvete.

Nakon zgrušavanja krvi, uzorak je centrifugiran na 1000 rpm, 15 minuta i serum se odmah odvajao i čuvao na -20 °C do analiziranja. Nivo glukoze, Alb, Ca, P i GGT u serumu su mjereni korištenjem automatskog analizatora. Nivo BHBA u serumu je određen korištenjem ELISA testa prema uputstvu proizvođača.Za statističku evaluaciju različitosti između SKG i CG, korišten je T-test za nezavisne uzorke za normalnu distribuciju podataka, a Mann Whitney test je korišten za one podatke koje nije bilo moguće svesti u normalnu distribuciju.

Rezultati i interpretacija

BHBA nivo je veći ($p<0.001$) u SKG u poređenju sa CG. Pored toga nivo serum kalcijuma i glukoze je bio niži ($p<0.001$) u grupi životinja sa subkliničkom ketozom nego u kontrolnoj grupi, dok se nivo albumina i fosfora u krvi nije razlikovao između grupa životinja. Serum GGT je sličan, ali je utvrđeno da je koncentracija GGT veća kod životinja iz grupe sa subkliničkom ketozom nego kod kontrolne grupe iako nema statističke značajnosti između grupa.

Glavni zaključak

Kada se procjene rezultati istraživanja, može se zaključiti da je nađeni nivo BHBA značajno veći kod krava sa subkliničkom ketozom u odnosu na kontrolnu grupu i da ovo povećanje može uzrokovati promjene u metabolizmu kalcija. Rezultati ovog istraživanja ukazuju na značaj metabolizma kalcija u patogenezi subkliničke ketoze.