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Evaluation of renal and hepatic functions in cattle with subclinical and clinical ketosis

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Abstract: The purpose of this study was to research the changes in renal and hepatic functions of high productive dairy cows diagnosed with subclinical and clinical ketosis. The material of the study consisted of 30 dairy cows of high milk production. Diagnosis of ketosis was made according to anamnesis, physical examination, urine ketone bodies, and serum β-hydroxybutyric acid levels. The animals were divided according to their serum β-hydroxybutyric acid levels into control, subclinical, and clinical ketosis groups, each of which included 10 cows. Serum glucose, urea, creatinine, total protein, aspartate amino transaminase (AST), and total bilirubin levels were determined with an automatic biochemistry analyzer using commercial test kits OptiumXceed; a handheld meter was used for the detection of serum β-hydroxybutyric acid levels. The serum β-hydroxybutyric acid levels were 450 ± 0.05, 1310 ± 0.03, and 3920 ± 0.37 mmol/L in the control, subclinical, and clinical ketosis groups respectively, and there were significant differences among the 3 groups ($P < 0.001$). The intergroup significant differences of some biochemical parameters (glucose, total protein, urea, creatinine, AST, and total bilirubin) were determined. New studies must be conducted to determine the glomerulus filtration rate in animals (particularly those with subclinical ketosis) with normal serum creatinine concentrations and suspected kidney disease.

Key words: Ketosis, dairy cow, β-hydroxybutyric acid, biochemical parameters

1. Introduction

Ketosis is a disease leading to economic losses because of its treatment costs and the decrease in milk yield and fertility (1–3). It is a subacute and chronic carbohydrate metabolism disorder caused by a negative energy balance as a result of a failure to meet the growing energy need in the first months of lactation in highly productive dairy cows. It is characterized by the consumption of liver glycogens and other glucose reserves, a decrease in glyconeogenetic activity, fatty degeneration in the liver, hypoglycemia, ketonemia, ketonuria, ketolactia, and an increase in ketone bodies in the respiratory air (1–4).

Ketosis arises primarily from insufficient intake of appropriate feeds or secondarily from the prevention of feed intake due to other diseases, including retentio secundunarium, metritis, mastitis, traumatic reticuloperitonitis, and left displaced abomasum (1–3). It can also be classified as subclinical or clinical according to the existence of clinical symptoms and the levels of ketone bodies in the blood, urine, and milk (3). Its clinical form has digestive and nervous presentations (2).

Gul and Grunder (5) reported increases in bilirubin, aspartate amino transaminase (AST), and serum bile acids due to liver degeneration in dairy cows with ketosis. It was reported by Li et al. (6) that renal functions were slightly disrupted in dairy cows with subclinical ketosis and new studies were needed to explore the extent of this disruption.

The purpose of the present study was to research the changes in renal and hepatic functions of highly productive dairy cows diagnosed with subclinical and clinical ketosis.

2. Materials and methods

The study involved 30 high milk yielding dairy cows aged between 3 and 7 years. The breeds Brown Swiss (5 heads), Holstein (18 heads), and Simmental (4 heads), and their crossbreeds (3 heads) were represented. The diagnosis of ketosis was based on anamnesis, physical examination, the determination of ketone bodies in the urine, and serum β-hydroxybutyric acid. The animals were divided according to their serum β-hydroxybutyric acid levels into control, subclinical, and clinical ketosis groups, each of which included 10 cows. The cows in the control and subclinical ketosis groups were from various facilities in Elazığ's central district, while those in the clinical ketosis group were selected from animals admitted to the clinics

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of Fırat University's Veterinary Faculty, Department of Internal Medicine, for examination, diagnosis, and treatment. For all the groups, we selected animals that were within 2 months postpartum.

Blood samples of the animals were taken from the *V. jugularis* into sterile gel glass tubes for serum biochemical analysis and β-hydroxybutyric acid level measurement. The sera were separated after centrifugation for 10 min at 3000 rpm. Serum samples were kept at –20 °C until analysis.

Serum glucose, urea, creatinine, total protein, AST, and total bilirubin levels were determined with an automatic biochemistry analyzer (Mindray BS-200, China) using commercial test kits OptiumXceed (Abbott Diabetes Care Ltd., USA); a handheld meter was used to determine the serum β-hydroxybutyric acid level.

Urine samples were taken either from animals urinating spontaneously or by urinary catheter (Breslau uterine catheter, Germany) and they were immediately checked with urine test strips (Combur 9 Test, Roche, Switzerland).

The significance of the intergroup difference was calculated through one-way ANOVA with SPSS for Windows 21.0. The data are shown as means ± standard error and $P < 0.05$ was considered significant.

3. Results

General clinical examination findings (body temperature, heart and respiratory frequencies, and rumen movement) of the cattle in the control, subclinical, and clinical ketosis groups are given in Table 1. The serum β-hydroxybutyric acid levels, the arithmetic means, the minimum–maximum values, and the intergroup significant differences in the glucose, total protein, urea, creatinine, AST, and total bilirubin levels are presented in Table 2.

The clinical findings, appetite, and general status of the dairy cows diagnosed with subclinical ketosis were normal. According to their anamnesis, however, there was a low milk yield and postpartum weight loss; moreover, some of the animals had mastitis and metritis and needed several inseminations for successful conception. The anamnesis of the cows with clinical ketosis indicated that they were passive and choosy in eating (especially roughage like straw), and lost too much weight. Physical examinations of the animals revealed normal body temperature and heart and respiratory frequencies, decreased rumen movements, dry stool that was covered with mucus, and respiratory air with acetone odor. In some animals, neural symptoms such as excessive aggression, leaning the head against the trough, teeth gnashing, and empty chewing activity were observed.

Urine pH, density, protein, bilirubin, urobilinogen, ketone, glucose, blood, hemoglobin, and leukocyte were normal in the control and subclinical ketosis groups. The clinical ketosis group had ketonuria (+++) and proteinuria (+, ++, +++).

4. Discussion

If a cow is not able to reach the energy level necessary for milk yield and physiological functioning from the rations after giving birth, a negative energy balance emerges in the first period of lactation. This negativity in the energy balance has an important role in the incidence of metabolism diseases such as ketosis, steatosis, hepatitis, and abomasum displacement (1,7,8).

Metabolism diseases, infertility problems, and decreases in milk yield cause important economic losses in facilities raising dairy cattle. Therefore, it is economically significant to diagnose metabolism diseases earlier, follow

Table 1. Arithmetic means of the general clinical examination findings and significance of intergroup differences and minimum–maximum values of the cattle in the study groups.

Parameters	Control group Mean ± SE	Subclinical ketosis group Mean ± SE	Clinical ketosis group Mean ± SE	Sig	P
Body temperature (°C)	38.42 ± 0.23 (38.0–38.7)	38.44 ± 0.23 (38.0–38.7)	38.50 ± 0.18 (38.2–38.8)	0.705	–
Heart rate (/min)	68.80 ± 3.15 (64–72)	69.200 ± 3.29 (64–72)	69.20 ± 4.63 (64–76)	0.963	–
Respiratory rate (/min)	25.20 ± 1.93 (24–28)	26.80 ± 2.69 (24–32)	24.80 ± 1.68 (24–28)	0.108	–
Rumen movement (/5 min)	9.60 ± 0.51^{a} (9–10)	9.40 ± 0.66^{a} (8–10)	5.00 ± 0.66^{b} (4–6)	0.000	***

–: $P > 0.05$; ***: $P < 0.001$

^{a,b} : the difference between the average values with different letters in the same column is significant ($P < 0.05$).

Table 2. Serum β-hydroxybutyric acid level, arithmetic means of some biochemical values, significance of intergroup differences, and minimum–maximum values of the cattle in the study groups.

Parameters	Control group Mean ± SE	Subclinical ketosis group Mean ± SE	Clinical ketosis group Mean ± SE	Sig	P
β-Hydroxybutyric acid (mmol/L)	450 ± 0.05[a] (200–800)	1310 ± 0.03[b] (1200–1400)	3920 ± 0.37[c] (2600–5400)	0.000	***
Glucose (mg/dL)	53.40 ± 1.62[a] (45.00–62.00)	44.00 ± 1.72[b] (32.00–49.00)	37.00 ± 1.96[c] (27.00–45.00)	0.000	***
Total protein (g/dL)	7.10 ± 0.28[a,b] (6.23–8.10)	7.62 ± 0.21[a] (6.52–8.58)	6.72 ± 0.09[b] (6.08–7.16)	0.021	*
Urea (mg/dL)	13.10 ± 0.69[a] (10–17)	21.30 ± 1.72[b] (13–32)	32.90 ± 3.24[c] (21–48)	0.000	***
Creatinine (mg/dL)	1.12 ± 0.05[a] (0.89–1.45)	1.35 ± 0.04[b] (1.13–1.48)	1.35 ± 0.046[b] (1.24–1.72)	0.005	**
AST (U/L)	66.20 ± 1.54[a] (58–74)	80.70 ± 3.96[b] (63–97)	116.20 ± 5.86[c] (96–147)	0.000	***
Total bilirubin (mg/dL)	0.09 ± 0.016[a] (0.02–0.20)	0.17 ± 0.04[a,b] (0.02–0.35)	0.31 ± 0.07[b] (0.12–0.78)	0.014	*

*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$

[a, b, c]: the difference between the average values with different letters in the same column is significant ($P < 0.05$).

them up, and evaluate the cows' fertility and nutritional status (1,9,10).

The average values of the heart and respiratory frequencies and body temperatures of the animals in the control, subclinical, and clinical ketosis groups were within their physiological limits, which was in accordance with findings reported in the literature (2,4,11–13). We found that the number of rumen movements decreased significantly ($P < 0.001$) in the clinical ketosis group; they were within the physiological limits in the control and subclinical ketosis groups.

Findings such as inappetence, a decrease in milk yield, decreases in the number of rumen movements and in their contraction force, weight loss, depression, acetone smell in the respiratory air, shaped and mucus-covered stool, documented during the examination of animals in the clinical ketosis group, are similar to reports related to the digestive form of clinical ketosis (2–4,11–14). Some neural symptoms like excessive aggression, leaning the head against the trough, teeth gnashing, and empty chewing activities are indicative of the nervous form of ketosis (2–4).

Because ketosis (whose primary metabolic disorders are hyperketonemia and hypoglycemia) does not have pathognomonic symptoms, ketone bodies in the blood, urine, and milk (acetone, acetoacetic acid, β-hydroxybutyric acid, or isopropanol), and blood glucose amounts are used for its diagnosis (1,3). It has been reported in recent years that β-hydroxybutyric acid quantification in serum or plasma is the gold standard test to diagnose and distinguish ketosis (1,3,10,15,16). If the serum β-hydroxybutyric acid level is less than 1000 μmol/L, it is considered normal; if it is between 1000 and 1400 μmol/L, subclinical ketosis is diagnosed; and if it is above 2500 μmol/L, clinical ketosis is diagnosed (2). The β-hydroxybutyric acid levels of the animals included in the study were used to separate the control, subclinical, and clinical ketosis groups. The serum β-hydroxybutyric acid levels were 450 ± 0.05, 1310 ± 0.03, and 3920 ± 0.37 mmol/L for the control, subclinical, and clinical ketosis groups, respectively, and there were significant differences among these 3 groups ($P < 0.001$). The values found in the study and the significant differences among the groups corroborate results reported in other studies (1,3).

The β-hydroxybutyric acid concentration, which is a ketone body, is especially important for the subclinical ketosis determination between days 5 and 50 of lactation after giving birth (7,15,17). That is why samples from all the animals were taken within 2 months postpartum.

The normal glucose level in cattle is 45–75 mg/dL (1,3). Blood glucose levels below the normal value, as found within the first month of lactation, show that the animals

have a negative energy balance and are exposed to the risk of ketosis (8,18,19). In primary ketosis cases, blood glucose concentrations decrease and liver glycogen depletion and fat infiltration into the liver are frequently observed (20). This decrease is somewhat slighter in secondary ketosis cases. In many cases of secondary ketosis it was reported that the glucose concentration was above or within normal limits (21). The glucose levels were normal in the healthy cattle included in the present study; however, those values decreased significantly in the cattle with subclinical and clinical ketosis, and the differences between them were significant ($P < 0.001$).

The normal protein value in cattle is between 5.7 and 8.1 g/dL (1,3). Even though the average total protein values were within normal limits in all the groups in the present study, they were found quite low in the cattle with clinical ketosis ($P < 0.05$). The parameter tended to be lower in cattle with clinical ketosis, and that may be attributed to inappetence and hepatic and renal disorders (3).

Evaluation of the glomerular filtration rate is the most important part of the diagnostic approach in animals with suspected kidney disease because it is directly related to renal tissue (17). Serum creatinine and urea determinations are the classic parameters used in glomerular function evaluation (3,22,23).

Blood serum concentration of urea (3,19,24,25), which emerges as the end product of protein metabolism and is eliminated through the kidneys in healthy cattle, ranges between 12 and 15 mg/dL (18). Creatinine, the best indicator of kidney function among the nitrogenous substances in the blood, endogenously develops as the end product in muscle metabolism and is eliminated only through the kidneys (24). The blood serum creatinine amount is considered normal in cattle at up to 1.5 mg/dL (1,3).

It was reported (19) that diagnosis only through the determination of urea levels is not sufficient when kidney functions are evaluated, and it is more useful to evaluate creatinine levels together with urea levels. According to other reports, however, a significant increase in urea and serum creatinine concentrations occurs when the number of functional nephrons decreases by 75% (22,25). Therefore, creatine clearance and sodium sulfonate half-life tests are suggested to determine the glomerulus filtration rate (19).

Significant ($P < 0.001$) differences were detected in terms of urea concentrations among all 3 study groups, and the average values in cattle with subclinical and clinical ketosis were above the physiological limits. It was reported (25) that serum or plasma urea values could be lower than the normal levels due to insufficient protein in the diet, hunger (inanition), or liver destruction. The urea levels detected in the subclinical and clinical ketosis groups in the present study were higher, possibly due to a decrease in feed intake, which is seen as the main clinical indication in animals with ketosis, liver degeneration, and protein-deficient rations.

Although the average serum creatinine levels in the study groups were below the 1.5 mg/dL physiological upper limit specified for healthy animals, the values in the cows with subclinical and clinical ketosis were significantly ($P < 0.01$) higher than those in the healthy group. Moreover, some individual values in the group diagnosed with clinical ketosis were above 1.5 mg/dL.

Proteins, which are melted in the blood serum under normal conditions, are not exposed to glomerular filtration, but they are eliminated through urine by experiencing glomerular filtration in nephropathy cases (proteinuria, albuminuria) (24). No change was detected during the urine examinations of animals in the control and subclinical ketosis groups; however, nephropathy, which developed in the animals with clinical ketosis, could also be encountered in animals of the subclinical ketosis group when proteinuira detection in the clinical ketosis group animals at different levels (+, ++, +++) was evaluated together with urea and creatinine values.

Because many enzymes are influential in the regulation of vital events, increases and decreases in their activities lead to imbalances in normal functions. Besides enabling the diagnosis of some diseases, blood enzyme levels are also important to reveal disease severity and determine disease prognosis (19). The detection of liver destruction is difficult in cattle due to the nonspecificity of clinical symptoms and the mostly anicteric course of the disease (2).

It is known that a significant increase may occur in serum AST activities, depending on muscle tissue damage (heart, skeletal muscles, and other organs) (26) apart from liver failure (21). Thus, evaluation of the total bilirubin level is recommended together with AST activity for detecting liver function disorders (3,5,14,27,28). Gul and Grunder (27) emphasized in a study including 470 cattle that high AST and total bilirubin levels were sufficient to detect liver damage in cattle.

According to findings reported in other studies (5,29,30), the total bilirubin concentration increases together with serum AST activity in subclinical and clinical ketosis cases. Additionally, the increase in serum AST activity was reported to be higher in clinical ketosis than in subclinical ketosis cases (29). In the present study serum AST activity in the animals of the control study group was within the physiological limits (43–127 U/L) (3); it increased significantly ($P < 0.001$) in the groups with subclinical and clinical ketosis in terms of the average values in all 3 groups. Serum AST values in all groups were similar to those reported in other studies (5,29,30).

Although the average total bilirubin values in the animals of all groups were within the physiological limits (0.01–0.47 mg/dL) (3), the average values in the clinical ketosis group were significantly higher ($P < 0.05$) than those in the control group; some individual values were even above the physiological limits in the group with clinical ketosis. The average values of total bilirubin, which were found in the cows with clinical ketosis, were mostly in agreement with those reported in other studies (1,3,5,16) about cows with azotemia.

The increases in AST activity and total bilirubin levels in animals included in the sick study groups show the existence of liver damage in ketosis patients, in line with findings reported in other studies (3,5,16).

The results of the present study show that β-hydroxybutyric acid and glucose are good indicators for the diagnosis of ketosis in cattle, and the increases in serum AST activity and total bilirubin values of the cattle diagnosed with subclinical and clinical ketosis may indicate the existence of a functional disorder or liver damage. Additionally, the creatinine values within normal limits (high in some animals with creatinine ketosis) were interpreted as an indication that the large majority of nephrons were not damaged; the high urea levels in the animals with subclinical and clinical ketosis indicated kidney destruction. However, because proteinuira and ketonuria can occur without an increase in urea levels, it is thought that even the animals with subclinical ketosis may have slight nephropathy.

In conclusion, new studies must be conducted to determine the glomerulus filtration rate in animals (particularly those with subclinical ketosis) with normal serum creatinine concentrations and suspected kidney disease.

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