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Evaluation of Some Haematological and Biochemical Parameters Before and After Treatment in Cows with Ketosis and Comparison of Different Treatment Methods

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Abstract: In the present study, therapeutic effectiveness of dexamethasone, prednisolone and insulin were compared in ketotic dairy cows. Furthermore, the importance of some haematological and biochemical parameters in the diagnosis of ketosis was also evaluated. For this purpose, 21 holstein dairy cows with ketosis and 7 clinically healthy cows (control) were used. All the animals with ketosis divided into 3 groups equally. Each animal in first group received 750 mL of 30% serum dextrose Intravenouse (IV) and a dose of 4 mg/100 kg dexamethasone (IM) for 2 consecutive days. While, each animal in second group was injected with 750 mL of 30% serum dextrose Intravenouse (IV) plus a dose of 0.2 mg kg^{-1} prednisolone Intramuscular (IM) daily for 2 days. Furthermore, 750 mL of 30% dextrose Intravenouse (IV) and 200 IU insulin were administered to the each animal in third group daily for 2 days and twice 48 h intervals, respectively. All the animals in Control Group were not received any treatment during the study. Urine samples and blood samples were collected from all the animals before and after treatment and then used to determine some haematological and biochemical parameters. It was concluded that ketosis causes significant alterations in some haematological and biochemical parameters in dairy cows. These parameters can be used for early diagnosis of ketosis in dairy cattle, which may help to reduce the economical losses of dairy industry. Furthermore, the application of dextrose plus insulin was found to be more effective on the treatment of ketotic cows than other treatment methods applied. Therefore, the simultaneous use of dextrose and insulin can be recommended for the treatment of cows with ketosis.

Key words: Cow, ketosis, haematological and biochemical values, treatment, evaluation

INTRODUCTION

Ketosis is a common metabolic disease in high producing dairy cows. It is caused by a negative energy balance and typically occurs within 2 months after calving (Grummer, 1993; Radostits *et al*., 2000). It is well-known that high amounts of nutrition are needed for the rapidly growing fetus and mammary tissue during late gestation and early lactation period. Due to decreased feed intake, cows mobilize body reserves to meet nutrient requirements for milk synthesis during early lactation. Thereby, the ketones, acetacetate, acetone and β-Hydroxybutiric Acid (BHBA), produced as a result of excessive fat metabolism. These ketone bodies can all be measured in milk, urine and blood and are useful as indicators of physiological imbalance and clinical ketosis (Radostits *et al*., 2000; Enjalbert *et al*., 2001).

Studies indicated that ketosis is accompany with a number of metabolic changes including hyperketonemia, hypoglycaemia, hypoinsulinemia, decreased blood thyroxine and hepatic glucagons, increased hepatic triglycerides and increased Nonesterfied Fatty Acids (NEFA) in plasma (Grummer, 1993; Moore, 1997; Radostits *et al*., 2000; Hayirli, 2006). Moreover, increases in blood activities of aspartate Aminotransferase (AST), alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP) due to impaired liver function have been reported in ketotic cows (Grummer, 1993; Dann *et al*., 2005). It has also been stated that alterations in blood Phosphor (P), potassium (K) and Calcium (Ca) occur in cows with ketosis (Radostits *et al*., 2000).

It is well-known that ketosis can affect milk production (Rajala-Schultz *et al*., 1999; Fleischer *et al*., 2001), reproduction (Andersson *et al*., 1991) and be associated with an increased frequency of left displaced abomasum (Geishauser *et al*., 1997) or decreased nonspecific immunity (Sartorelli *et al*., 2000). Therefore, the disease causes considerable amount of economical

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losses to the dairy industry because of impaired milk production, decreased productive efficiency, increased involuntary culling and increased treatment cost. Because of the economic consequences, the detection of cows with ketosis is important in order to treat individual cows or to improve the diet (Enjalbert *et al*., 2001). It is known that ketosis is a disease caused by the disturbance of carbohydrate and lipid metabolism; thus, it has been treated with various sugar preparations because of its complex etiology. For this purpose insulin and corticosteroids alone or in combination with glucose have been used to eliminate negative energy balance in cows with ketosis (Shpigel *et al*., 1996; Hayirli *et al*., 2002; Hayirli, 2006).

The objective of this study was to evaluate the therapeutic effect of dexamethasone, prednisolone and insulin in ketotic dairy cows. Furthermore, diagnostic importance of some biochemical and haematological parameters were also investigated before and after treatment of cows with ketosis.

MATERIALS AND METHODS

Animals: Twenty-eight Holstein dairy cattle were used in this study. Twenty-one of these animals were brought by their owners to the Veterinary Medical Teaching Hospital, with suspected ketosis. These cows were in early lactation (2-37 days after parturition). The remaining 7 clinically healthy cattle were obtained from a Dairy Farm. Milk yield was approximately 8,000 kg/year/cow and was similar in healthy and diseased cows. Routine clinical examination was carried out and body temperature, heart and respiratory rate were recorded for each animal before and after treatment. Urine samples were collected from each animal and then Rothera test and spin react-10 test (test strips) were applied to diagnose ketosis. Clinical ketosis (diagnosed via positive results of the Rothera test in the urine, high concentration of β-hydroxybutyrate in the serum and clinical signs of disease, such as decrease of milk yield or loss of appetite) was diagnosed in 21 dairy cows that were allocated in to 3 groups equally for treatment by use of 3 procedures. Seven healthy dairy cattle were also used as control group.

Each animal in first group received 750 mL of 30% serum dextrose Intravenous (IV) and a dose of 4 mg/100 kgbw dexamethasone Intramuscularly (IM) for 2 consecutive days. Whereas each animal in second group was injected with 750 mL of 30% serum dextrose IV plus a dose of 0.2 mg kg^{-1} prednisolone IM daily for 2 days. Furthermore, 750 mL of 30% dextrose (IV, for 2 days) and 200 IU insulin were administered to the each animal in third group daily for 2 days and twice 48 h intervals, respectively. All the animals in control group were not received any treatment during the study.

Haematology: Peripheral blood samples were collected in the morning (before feeding) before and after the treatments in dipotassium Ethylenediamine Tetra Acetic acid (EDTA)-coated evacuated tubes and used to establish total White Blood Cell (WBCs) and total Red Blood Cell (RBCs) electronically (MS9 blood cell counter, Melet Schloesing Laboratories, France).

Clinical biochemistry: Blood samples were collected before and after the treatments in tubes without anticoagulant and used to separate serum samples. These sera were then used to establish the concentrations of Ca, P, glucose and the serum activity of ALP, AST, ALT and Gammaglutamyl Transferase (GGT) using an auto analyser (VET TEST 8008, IDEXX Laboratories, inc Westbrook ME 04092 USA).

Serum concentrations of vitamin A and Vitamin E were determined by the methods of Suzuki and Katoh (1990), using a spectrophotometer (Shimadzu UV-1601, Japon). Blood concentration of D-3-Hydroxybutyrate level (BHBA) was determined using a commercially available test kit (Randox Laboratories LTD. United Kingdom) spectrophotometrically according to the manufacturer instructions. Furthermore, thyroxin (T4) concentration was determined with an Abbott Axsym System (USA).

Statistical analysis: All the values were expressed as the mean and the standard deviations of the mean (mean±SD). The paired t-test was used to analyze the significance of the differences of the deviations from pre-treatment values within each group. Student's t-test was then used to compare the significances of the differences of each parameter between the groups.

RESULTS AND DISCUSSION

Clinical findings: Decrease in appetite and milk yield, weight loss, depression and firm faeces were the common clinical findings observed in cows with ketosis. There were no significant differences in body temperature, heart and respiratory rate between values obtained before and after treatments.

Haematological findings: There were no significant differences in the number of RBCs and WBCs between ketotic animals and control animals (Table 1).

Table 1: Hematological and biochemical parameters of dairy cows with ketosis and healthy cows

Parameters	Ketotic cows (n = 21)	Healthy cows (n = 7)
BHBA (mmol L^{-1})	1.76±0.6*	0.88±0.04
T4 (nmol L^{-1})	18.69±2.30*	46.12±4.44
Cortizol (nmol L^{-1})	7.71±1.27*	15.48±1.33
Glucose (mmol L^{-1})	1.24±0.39*	2.82±0.60
Ca (mmol L^{-1})	1.79±0.41*	2.93±0.16
P (mmol L^{-1})	0.79±0.28*	1.95±0.31
AST (U L^{-1})	166.00±18.24*	94.85±10.63
ALT (U L^{-1})	68.66±11.71*	29.57±6.32
ALP (U L^{-1})	465.57±193.26*	122.71±59.89
GGT (U L^{-1})	55.95±25.08*	22.75±31.05
Vitamin A (μg dL^{-1})	73.69±29.48	84.33±14.04
Vitamin E (μg dL^{-1})	555.42±16.28	641.42±82.75
WBC ($\times 10^3$ mL^{-1})	12.81±4.09	9.77±3.18
RBC ($\times 10^6$ mL^{-1})	6.76±0.97	6.66±0.88

*: Significant differences in values between cows with ketosis and healthy cows (Control Group) is indicated by asterisk ($p<0.05$)

Clinical chemistry: There were significant increases in the blood concentration of BHBA ($p<0.001$) and in the serum activity of AST ($p<0.001$), ALT ($p<0.001$), GGT ($p<0.05$) and ALP ($p<0.001$) in ketotic cows, compared to the control cows. There was no significant difference in the concentrations of vitamin A and vitamin E in cows with ketosis compared to those of Control Group. The concentrations of thyroxine ($p<0.001$), cortisol ($p<0.001$), glucose ($p<0.001$), P ($p<0.001$) and Ca ($p<0.001$) were significantly lower in ketotic cows than in the control group (Table 1).

In dexamethasone group, there were significant differences in the concentrations of glucose ($p<0.001$), Ca ($p<0.01$), P ($p<0.01$), BHBA ($p<0.01$) and in the serum activity of AST ($p<0.01$), GGT ($p<0.01$) and in the number of WBCs ($p<0.05$) between the values obtained before and after the treatment. There were no significant changes between the remaining parameters obtained before and after the treatment (Table 2). In prednisolone group, significant changes were obtained in the concentrations of glucose ($p<0.05$) and in the serum activity of ALT ($p<0.05$) compared with the values obtained before and after the treatment. There were no significant changes between the remaining parameters obtained before and after treatment (Table 2). In insulin group, the concentrations of cortisol ($p<0.05$), glucose ($p<0.001$), Ca ($p<0.001$), P ($p<0.01$), BHBA ($p<0.001$) and the activity of AST ($p<0.001$), ALT ($p<0.001$), ALP ($p<0.001$) and GGT ($p<0.001$) were significantly different compared with the values obtained before treatment and those of values obtained after the treatment. There were no significant changes in the concentrations of T4, vitamin A and vitamin E and in the number of WBCs and RBCs between the values obtained before and after the treatment (Table 2).

The comparison of the values between the groups suggested that normalization of abnormal parameters were found to be more pronounced in insulin treated cows compared with other treatment groups. The normalization of parameters in dexamethasone group was closed to those of insulin group. However the most of these parameters were not significantly changed, except glucose, BHBA and ALT, in prednisolone group (Table 2). All the animals in insulin treated group completely recovered at the end of the study. However, an animal in dexamethasone group and three cattle in prednisolone group needed to treat for further one more day according to the clinical improvements and normalization of biochemical abnormalities. Result of the present study, therefore, indicated that the application of dextrose plus insulin was more effective on the recovery of ketotic cows than other treatment methods applied.

The ketotic syndrome is related to high energy requirement for milk production, which often can not be met in high producing cows by the normal intake of energy during late gestation and early lactation (Radostits *et al*., 2000). It may develop over several days or even weeks in cows with negative energy balance. It is possible to detect the disease in its early stage before the cow develops strong clinical symptoms and thereby limit the farmer's economic losses and cow's malaise. Ketosis in cows is accompany with several metabolic abnormalities, which can all be measured and used as indicators of physiological imbalance and clinical ketosis (Grummer, 1993; Enjalbert *et al*., 2001). Determination of these parameters may also be use to manage and choice the necessary treatment of cows with ketosis. Therefore, in the present study, diagnostic values of some biochemical and haematological parameters were investigated. Furthermore, therapeutic effectiveness of dextrose plus dexamethasone or dextrose plus prednisolone administration were compared with those of dextrose plus insulin in ketotic dairy cows.

In the present study, significant increases in the blood concentration of BHBA and in the serum activity of AST, ALT, GGT and ALP were obtained in ketotic cows, whereas the concentrations of thyroxine, cortisol, glucose, P and Ca were significantly lower in these animals. There were high positive correlations between cortisol and glucose, AST and GGT, GGT and BHBA values, while high negative correlations between cortisol and BHBA and glucose and BHBA values were obtained. Moreover, there were no significant changes in the concentrations of vitamin A and vitamin E and in the haematological values between ketotic and healthy cows.

Beta-hydroxybutyric acid concentrations of blood that are <1000 μmol L^{-1} have been considered to be normal and greater values than this are accepted as either

Table 2: Hematological and biochemical parameters of cows in Control Group and in dexamethasone, prednisolone and Insulin Group

	Dexamethasone group (n = 7)		Prednisolone group (n = 7)		Insulin group (n = 7)		Control group (n = 7)	
Parameters	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
BHBA (mmol L^{-1})	1.42±0.25*	0.99±0.18	1.80±0.63	1.16±0.56^{e}	2.08±0.71*	0.99±0.14^{f}	0.88±0.04	0.87±0.05
T4 (nmol L^{-1})	17.34±1.92	17.92±2.08^{c}	18.55±2.93	19.32±2.81^{e}	18.1±2.02	18.81±2.06^{f}	46.12±4.44	45.04±5.79
Cortizol (nmol L^{-1})	8.05±1.18	9.13±0.87^{c}	7.34±1.26	8.48±1.43^{e}	9.42±1.06*	7.91±1.45^{f}	15.48±1.33	15.45±1.35
Glucose(mmol L^{-1})	1.47±0.25*	2.19±0.40^{bc}	1.26±0.40*	1.78±0.38^{de}	0.98±0.4*	3.00±0.38	2.82±0.6	2.79±0.53
Ca (mmol L^{-1})	2.02±0.11*	2.18±0.12^{bc}	1.71±0.56	2.17±0.31^{de}	1.64±0.37*	2.83±0.17	2.93±0.16	2.92±016
P (mmol L^{-1})	0.59±0.23*	1.29±0.44^{c}	0.98±0.09	1.12±0.2^{de}	0.79±0.34*	1.78±0.68	1.95±0.31	1.94±0.30
AST (U L^{-1})	167.28±3.58*	126±21.32^{c}	168.71±27.1	150.71±27.14^{e}	162±12.70*	130±3.65^{f}	94.85±10.63	93.57±11.02
ALT (U L^{-1})	71.14±10.36	59.71±11.65^{bc}	64.71±15.07*	47.85±10.68^{bde}	70.14±9.75*	37.57±4.85^{f}	29.57±6.32	29.28±4.07
ALP (U L^{-1})	309.71±98.59	295.42±193.39^{c}	501.57±181.31	467.85±61.55^{be}	585.42±74.66*	461.57±32.62^{f}	122.71±59.89	118.22±54.73
GGT (U L^{-1})	49±6.70*	33.28±9.53^{bc}	64.85±39.07	35.85±29.26^{b}	54±19.28*	17.57±1.61^{f}	22.75±31.05	10.64±2.43
Vit. A (μg dL^{-1})	74.68±36.12	86.26±47.07	68.35±17.63	70.61±16.37^{a}	78.03±35.01	93.23±40.31	84.33±14.04	84.15±13.97
Vit. E (μg dL^{-1})	0.49±0.13	0.49±0.14	0.56±0.15	0.61±0.16^{a}	0.60±0.19	0.61±0.13	0.64±0.08	0.60±0.08
WBC ($\times 10^{3}$ mL^{-1})	11.72±3.63*	20.26±9.14^{c}	12.47±3.71	16.43±9.65^{a}	14.23±5.00	12.94±3.96	9.77±3.18	9.70±3.15
RBC ($\times 10^{6}$ mL^{-1})	6.86±0.92	6.60±0.35	6.83±1.41	6.57±1.37^{a}	6.59±0.52	6.47±0.53	6.66±0.88	6.72±0.82

*: Significance of the deviations between pre-treatment and post-treatment values within each group is indicated by asterisk ($p<0.05$). $^{a-d}$: Significant differences in post-treatment values between the treatment groups are indicated by superscript letters as followed ($p<0.05$); ^{a}: Dexamethasone-Prednisolone Group, ^{b}: Dexamethasone-Insulin Group, ^{c}: Dexamethasone-Control Group, ^{d}: Prednisolone-Insulin Group, ^{e}: Prednisolone-Control Group, ^{f}: Insulin-Control Group

subclinical or clinical ketosis (Geishauser *et al*., 1998). In the present study, BHBA concentrations of ketotic cows were >1000 μmol L^{-1}, indicating the presence of clinical ketosis in these animals. It has been reported that decrease in glucose out put by the liver could lower blood glucose concentrations and decrease in insulin secretion, which, in turn, could lead to increased lipid mobilization from adipose tissue and increase rate of hepatic fatty acid uptake and ketogenesis (Grummer, 1993). In the present study, a significant reduction in thyroxine level was detected in ketotic cows, as reported elsewhere in cattle with ketosis (Kaneko, 1997a; Rijnberk and Mol, 1997; Radostits *et al*., 2000). However, in contrast to the finding, cortisol concentration has been reported to be normal in cows with ketosis (Breves *et al*., 1980). Thyroxin has an important role on the carbohydrate metabolism due to increase glucose turn over and absorption (Kaneko, 1997a). Furthermore, glucocorticoids, like cortisol, supply glucose to the organism by the transformation of proteins (Rijnberk and Mol, 1997). Thus, reduction in the concentrations of thyroxin and cortisol obtained in the present study may impair glucose metabolism and associate with hypoglycemia obtained in the present study.

In this study, high ALP, ALT, ALP and GGT activities detected and also reported in ketotic cows by others (Grummer, 1993; Dann *et al*., 2005). Increase in the activities of liver enzymes may have been associated with cholestasis and thus, with the disruption of normal hepatobiliary circulation. In this study, hypocalcaemia was detected in the cows with ketosis. Therefore, the high ALP activity may also have been osseous in origin, accompanying an increased mobilization of Ca from the body stores in response to hypocalcaemia (Kramer and Hoffmann, 1997). The increased AST activity detected in the present study may reflect liver and/or muscle disorders in the ketotic cows. Aspartate aminotransferase activity may also be high in cattle with hepatic lipidosis, passive venous congestion and diseases that cause distension of the fore stomachs and abomasums (Moore, 1997; Kramer and Haffmann, 1997). Thus, its high activity here may also have been related to the presence of hepatic lipidosis, as reported elsewhere in ketotic cows (Grummer, 1993). In the present study therefore, increased ALP, ALT and GGT and AST activity in ketotic cows may indicate impaired hepatic function and/or hepatobiliary circulation.

Reduction in the concentrations of Ca and P obtained in the present study can be due to decreased Ca uptake because of any illness such as ketosis which may affect the appetite and decrease its absorption (Moore, 1997). Additionally, Ca level can also be reduced due to increased loss of base in the urine to compensate for the acidosis reported in cows with ketosis (Radostits *et al*., 2000).

Following treatment with insulin, the concentrations of cortisol, glucose, Ca and P were significantly increased, in contrast to the presence of significant reductions in the activity of AST, ALT, ALP, GGT and concentrations of BHBA. The normalization of parameters in dexamethasone group was closed to those of insulin group. However, most of these parameters were not significantly changed, except glucose, BHBA and ALT, in prednisolone group. The comparison of the values between the groups suggested that normalization of abnormal parameters were found to be more marked in insulin treated cows compared with other treatment groups. All the animals in insulin treated group completely recovered at the end of the

study. However, an animal in dexamethasone treated group and three cattle in prednisolone group needed to treatment for further one more day. Clinical improvements, such as the recovery of appetite, normalization of blood glucose, Ca, P and cortisol concentrations and the activities of AST, ALT and GGT and disappearance of BHBA in blood were remarkable in cows with ketosis that were administered dextrose plus insulin. It is well-known that insulin promotes uptake of glucose by cells of peripheral tissues and accelerates carbohydrate metabolism. Insulin also increases the activities of hepatic enzyme and carbohydrate utilization in liver (Kaneko, 1997b). Studies indicated that administration of glucose plus insulin in ketotic cows resulted with a marked increase in blood concentrations of glucose and insulin, whereas ketone bodies, fatty acids and glucagon concentrations decreased significantly compared with those of cows receiving glucose alone. It was also reported that low doses of insulin aid utilization of parenteral glucose and inhibit lipolysis without significantly inhibiting gluconeogenesis (Hayirli *et al*., 2002; Hayirli, 2006). Therefore, in the present study, fat deposition and ketone body formation were likely reduced in the dextrose plus insulin group, due to these effects of insulin on carbohydrate and lipid metabolisms.

CONCLUSION

Significant changes were obtained in some haematological and biochemical parameters in cows with ketosis. These parameters can be used for early diagnosis and management of treatment in individual cows with ketosis, which may help to reduce farmer's economical losses caused by the disease. The comparison of the values between the groups suggested that normalization of abnormal parameters were found to be more pronounced in insulin treated cows compared with other treatment groups. It was concluded that the simultaneous use of dextrose and insulin can be recommended for the treatment of cows with ketosis.

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