



## SHORT COMMUNICATION

### Evaluation of Cobalt, Copper, Manganese, Magnesium and Phosphorus Levels in Cows with Clinical Ketosis

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#### ABSTRACT

The objective of this study was to examine the association between  $\beta$ -hydroxybutyrate (BHBA) and glucose, cobalt (Co), copper (Cu), manganese (Mn), magnesium (Mg), and phosphorus (P) in cows with clinical ketosis and control group. At the beginning; while serum glucose levels, Co, Cu, Mn, Mg and P concentrations in cows with clinical ketosis were lower, BHBA levels were higher than control group. At the 3<sup>rd</sup> day after the treatment, serum Co, Cu, Mn and P concentrations were lower in cows with clinical ketosis than control group. According to these results, mineral level decrease was detected in cows with ketosis. It is thought that there should be more study performed about the contribution of minerals to the treatment of ketosis. This study will provide an insight to the studies will be performed in this respect.

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#### INTRODUCTION

Ketosis is a metabolic disease of highly productive dairy cows occurs in last stage of pregnancy and in first two months of parturition due to disorder of carbohydrate and lipid metabolism (Şahinduran *et al.*, 2010; Mouffok *et al.*, 2013). Serum glucose level decreases with energy deficit (Şahinduran *et al.*, 2010) and to compensate required energy; triglycerides breakdown to fatty acids and glycerine mobilizes from fat reserves (Mouffok *et al.*, 2013).  $\beta$ -hydroxybutyrate (BHBA), acetoacetate and acetone are produced as a result of excessive lipid metabolism which may be measured in blood, urine and milk. In order to diagnose clinical and physiological disorders of ketosis; these ketone bodies are used as indicators (Şahinduran *et al.*, 2010). Ketosis causes increase in circulatory BHBA concentration; decrease in glucose concentration and immunosuppression (Mouffok *et al.*, 2013). Recent reports have focused on the role of minerals in disease resistance in ruminants (Zhang *et al.*, 2011), but little is known about the concentrations of cobalt (Co), copper (Cu), manganese (Mn), magnesium (Mg) and phosphorus (P) in dairy cows with clinical ketosis.

Co is central compound of vitamin B<sub>12</sub> which has essential functions in energy and amino acid metabolism (Kincaid *et al.*, 2003). Serum Cu concentration is closely associated with health of cows (Zhang *et al.*, 2010). Cu is

an essential element and primarily has role in carbohydrate metabolism, physiological processes and various biochemical reactions. Mn is an essential dietary element for ruminants (Kozat, 2006). Ruminants have an effective homeostatic control for Mn levels in blood and tissues. Mn retention by the animal depends on the amount excreted from the bile into the intestines (Herdt *et al.*, 2011). In ketosis, Mg has pathological functions as coenzyme on the metabolism of sugars and ketone bodies and in osteomalasia, Mg has role as a pathological trigger (Reddi, 2014). P deficiency increases risk of subclinical ketosis (Rollin *et al.*, 2009).

This study was performed in order to determine relation between serum BHBA and glucose, Co, Mn, Cu, Mg, P concentrations in clinical ketosis. Recent reviews focused on the role of trace minerals in disease resistance in ruminants and subclinical ketosis (Zhang *et al.*, 2011), but to date, the concentrations of Co, Cu, Mn, Mg and P concentrations in dairy cows with clinical ketosis have not been reported.

#### MATERIALS AND METHODS

In the study, 10 ketotic and 10 healthy cows were used. Cows were examined and subsequent to clinical and laboratory findings (detecting ketone bodies in urine), cows with negative results were determined as control group. Urine samples were taken from animals suspected

with clinical ketosis. Subsequent to urine examination by test strips (test strips-Combur <sup>10</sup>Test<sup>®</sup>M, Roche), animals with positive results were diagnosed as clinical ketosis. Ketotic cows were in the first two months of lactation and their ages were between 4 and 6 years old. 1000 ml of serum dextrose solution 30% intravenously and insulin (0.5 IU/kg/BW) intramuscularly were administered to ketotic cows. In order to evaluate biochemical parameters; blood samples were taken to anticoagulant free tubes from jugular vein of cows before and at the 3<sup>rd</sup> day of treatment. Serum samples were extracted by centrifuging at 3000 rpm (Rotofix 32<sup>®</sup>-Hettich) and biochemical parameters were measured. Moreover, cows were examined for gastro-intestinal parasites and cows did not have any intestinal parasites according to fecal examination.

Serum Co, Cu, Mn, Mg and P concentrations were measured by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) device (Thermo Scientific X2) in Yuzuncu Yil University Science Research and Application Center. Serum glucose and serum D-3-hydroxy butyrate (Randox<sup>®</sup>-UK) levels were measured spectrophotometrically (Photometer 5010<sup>®</sup>-Boehringer Mannheim) according to test kit procedures.

Definitive statistics for characteristics of healthy cows and cows with clinical ketosis were stated as Mean, Standard Deviation, Minimum and Maximum value. Comparison between biochemical parameter groups were performed with Kruskal-Wallis test. In order to determine different groups, Duncan Multiple Range Test was used. Statistical significance level was taken at the level of 5% and SPSS statistics software was used for calculation.

## RESULTS

According to the clinical examination; reduced milk production, loss of appetite, decrease in ruminal contractions and loss of body condition were found. Cows were considered to have clinical ketosis when measured BHBA concentrations were 1.96±0.11 mmol/L. Significantly higher BHBA concentrations (P<0.01) and lower glucose (P<0.01) concentrations were observed (Table 1). Co, Cu, Mn, Mg and P levels were found significantly (P<0.01) lower in ketotic dairy cows compared to healthy dairy cows. In addition, acetone smell was detected on the breath of ketotic cows according to the clinical examination. At the 3<sup>rd</sup> day after treatment, subsequent to clinical examination; acetone smell was not detected. However; serum Co, Cu, Mn and P levels of the cows with clinical ketosis were found lower than the control group (P<0.01).

## DISCUSSION

Minerals, trace elements, and vitamins play important role in prevention of metabolic disorders often seen during this period (Suthar *et al.*, 2013). Serum glucose concentration was lower in clinically ketotic cows than in the healthy ones and there was a negative relationship between negative energy balance and serum BHBA. Serum BHBA concentration in clinical ketosis is 1.76 µmol/L (Şahinduran *et al.*, 2010).

In the current study, serum glucose and BHBA levels in ketotic cows were determined as 28.22±3.56 mg/dL and as 1.96±0.11 mmol/L, respectively. Glucose level was detected lower than healthy cows and BHBA level was detected higher. In the light of these data; the animals were diagnosed as ketosis. In parallel with the decrease in serum glucose level of clinical ketosis group; significant increase in serum BHBA level was determined. Decreased blood glucose concentrations in ketotic cows occurred due to large amount of blood glucose, inadequate glucose uptake and withdrawal by the mammary gland for the synthesis of milk lactose.

**Table 1:** Biochemical parameters in cows with ketosis and healthy cows

Parameters	Control (n=10)	Clinical ketosis (n=10)	
		Before treatment	After treatment (3 <sup>rd</sup> day)
BHBA (mmol/L)	0.20±0.03 <sup>a</sup>	1.96±0.11 <sup>c</sup>	0.27±0.01 <sup>a</sup>
Co (µg/L)	50.48±4.12 <sup>a</sup>	20.65±2.11 <sup>b</sup>	47.98±4.79 <sup>b</sup>
Cu (µg/L)	1232.58±276.43 <sup>a</sup>	535.71±64.21 <sup>b</sup>	873.83±131.65 <sup>b</sup>
Glucose (mg/dl)	67.22±10.56 <sup>a</sup>	28.22±3.56 <sup>b</sup>	56.00±1.14 <sup>a</sup>
Mg (mg/dl)	1.84±0.41 <sup>a</sup>	1.06±0.02 <sup>b</sup>	1.29±0.27 <sup>a</sup>
Mn (µg/L)	53.37±5.38 <sup>a</sup>	35.60±1.72 <sup>b</sup>	47.06±2.60 <sup>b</sup>
P (mg/dl)	6.43±2.85 <sup>a</sup>	4.013±1.30 <sup>b</sup>	5.21±2.85 <sup>c</sup>

Values (Mean±SD) bearing different letters in the same row differ significantly (P<0.01).

Cobalt is an essential element for ruminants in order to synthesis vitamin B<sub>12</sub> which maintains high performance and resistance against diseases in ruminants (Kozat, 2006). In the current study, serum Co level of control group was determined as 50.48±4.12 and 20.65±2.11 µg/L in ketotic cows.

Copper is an essential element and primarily has role in carbohydrate metabolism (Kozat, 2006). In this study; serum Cu levels in ketotic cows were 535.71±64.21 µg/L at the beginning of the treatment and serum Cu levels were 873.83±131.65 µg/L at the 3<sup>rd</sup> day after treatment. Ketosis in dairy cows led to Cu deficiency. Determined Cu levels of ketotic cows in our study are supporting the data determined by the researcher (Zhang *et al.*, 2010).

Mn is an essential element for animals and its deficiency leads to fertility disorders, abortion, joint deformities and abnormalities in cattle (Kozat, 2006). Plasma Mn concentration in cows is 5 to 10 ng/mL in cows (Kincaid *et al.*, 2003).

In this study; serum Mn level in ketotic cows was 35.60±1.72 µg/L before treatment and was 47.06±2.60 µg/L at the 3<sup>rd</sup> day of treatment. Serum Mn levels in ketotic group were found lower than the values for healthy cattle (P<0.01). It can be concluded that according to these data (Kincaid *et al.*, 2003), ketosis in dairy cows led to Mn deficiency.

Mg also plays a role in fat and carbohydrate metabolism, and blood glucose regulation (Zhang *et al.*, 2011). In this study, Mg levels in ketotic cows were found lower than control group (P<0.01).

In cows, the most important reasons of phosphorus deficiency are loss of appetite, displaced abomasum, milk fever and downer cow syndrome associated with the parturition (Kennerman, 2011). In this current study, serum P concentrations were 6.43±2.85 mg/dl in control cows, 4.013±1.30 mg/dl in cows with clinical ketosis

before treatment and  $5.21 \pm 2.85$  mg/dl in cows with clinical ketosis at the 3<sup>rd</sup> day after treatment. The reason of low phosphorus amount in cows with clinical ketosis is excessive phosphorus loss with milk and inadequate phosphorus intake by food in post-parturient period. These data support the researcher's data (Kennerman, 2011).

**Conclusion:** As a result; serum Co, Cu, Mn and P levels of cows with clinical ketosis were found low at the 3<sup>rd</sup> day after the treatment. Despite the treatment, serum glucose and BHBA concentrations were found close to the healthy animals. Measured mineral substance levels were not in the levels of healthy animals. According to these data, mineral level decrease was detected in cows with ketosis. It is thought that there should be more study performed about the contribution of minerals to the treatment of ketosis. This study will provide an insight to the studies will be performed in this respect.

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