IJAS Iranian Journal of Applied Animal Science

# Evaluation of Total Antioxidant, Total Calcium, Selenium, Insulin, Free Triiodothyronine and Free Thyroxine Levels in Cows with Ketosis

Research Article

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Received on: 29 Jan 2017
Revised on: 16 Mar 2017
Accepted on: 31 Mar 2017
Online Published on: Sep 2017

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Online version is available on: www.ijas.ir

## ABSTRACT

Ketosis is an important metabolic disease of high milk-producing cows. There are significant changes in many metabolite and hormonal concentrations in metabolic diseases. This study was carried out to assess the concentrations calcium (Ca), selenium (Se), total antioxidant (TAOC), insulin, free triiodothyronine ($fT_3$) and free thyroxine ($fT_4$) in cows with subclinical and clinical ketosis. This study included 20 dairy cows within the first two months of lactation, aged between 4-8 years. Cows with ß-hydroxybutyrate acid (BHBA) concentrations 1.20 mmol/L were considered healthy, whereas 1.20 and 1.50 mmol/L were considered subclinical and 1.60-2.20 mmol/L were classified as clinically ketotic. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), low density lipoprotein (LDH), glucose, Ca, plasma TAOC capacity and BHBA concentrations were performed spectrophotometrically. Serum insulin, free triiodothyronine and free thyroxine concentrations were measured using the chemi-luminescence method. Serum Se concentrations were measured using an Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). In conclusion, significant changes were noted in decreased concentrations of TAOC, Ca, Se, $fT_3$, $fT_4$ and insulin in cows with subclinical and clinical ketosis. The study identified important parameters, changes in the levels of these parameters will be important in determining the treatment and prognosis of the disease. Their use may also help reduce the economic losses suffered by dairy farmers as a result of the disease.

KEY WORDS calcium, dairy cows, insulin, ketosis, selenium, thyroid.

## INTRODUCTION

Ketosis is a metabolic disease of cows with high milk yields, which occurs in the last stage of gestation and in the two months after parturition (Kennerman, 2004). Late gestation and early lactation, more energy is required than is consumed resulting in mobilization of body reserves (Djoković *et al.* 2009; Sahinduran *et al.* 2010). Triglycerides are mobilised from fat reserves and decompose into fat acids and glycerol (Kennerman, 2004). While glycerol participates directly in glucose synthesis, fat acids complexed with serum albumin and are transported to the liver. After the transport of free fat acids to the mitochondria and their decomposition as a result of oxidation, acetyl coenzyme A is formed (Katoh, 2002). Energy deficiency causes inadequate oxaloacetate; as a result, acetyl coenzyme A cannot participate in the tricarboxylic acid (TCA) cycles, which leads to an increased amount of ketone bodies (Kennerman, 2004; Katoh, 2002). Excessive lipid metabolism leads to the production of acetoacetate, acetone and ß-hydroxybutyrate acid (BHBA). Detecting increased levels of these in blood, urine or milk is used to diagnose the disease. At the same time the animal develops hyperketonaemia, she also undergoes hypoglycaemia, low thyroxine and

high non-esterified plasma fat acids are reported. In addition, an increase in the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase activities due to liver failure is reported (Sahinduran *et al.* 2010). Changes in calcium (Ca) concentrations are also reported in cows with ketosis (Sahinduran *et al.* 2010; Saldago Hernández *et al.* 2009). In addition to metabolic changes, hormonal changes (Katoh, 2002) have also been reported in ketosis as well as lower blood insulin concentrations than is found in healthy cows at the first stage of lactation (Djoković *et al.* 2009). Thyroid hormones have a great impact on the basic metabolic rate and are body weight and energy expenditure (Mullur *et al.* 2014). Working in conjunction with growth hormone and insulin, protein synthesis is stimulated and nitrogen excretion is reduced. In this way, growth, metabolism and their production may be affected (Kozat, 2007). Changes in free triiodothyronine ($fT_3$) and free thyroxine ($fT_4$) have been reported in a number of studies (Kennerman, 2004; Saldago Hernández *et al.* 2009; Sahinduran *et al.* 2010). Other research suggests that Se levels should be at a specific for the transformation of serum $fT_4$ to $fT_3$ (Kozat, 2007).

In metabolic diseases, free radical out bursts caused by oxidative stress lead to a decrease in neutralising antioxidant capacity (Sahoo *et al.* 2009). In studies on cows with high triglycerides and non-esterified fat acids, high levels of reactive oxygen metabolites and a low level of total antioxidant capacity have been observed (Katoh, 2002; Haces *et al.* 2008; Wang *et al.* 2010). Free fat acids not only cause oxidative stress but also lead to deterioration in endogenous antioxidant defence by lowering the level of intracellular glutathione peroxidase (GSH-Px) (Sahoo *et al.* 2009). Selenium (Se) is a key component of various selenoproteins, which play a role in the enzymatic functions required for oxidation reduction and thyroid hormone metabolism in order to maintain homeostasis (Stranges *et al.* 2010). Se is present in many selenoproteins. There is a positive relationship between plasma Se concentration and GSH-Px activity (Kozat, 2007; Pavlata *et al.* 2002).

The present study was undertaken to investigate possible associations between levels of serum Ca, Se, insulin, $fT_3$, $fT_4$, TAOC capacity and BHHA concentrations in dairy cows and blood concentrations of glucose and some other metabolites.

## MATERIALS AND METHODS

### Animal material

A total of 86 Simmental cows (4-8 years old) from one commercial dairy herd were included in the study. This research was conducted with a total of 30 dairy cows, including 10 healthy cows, 8 with subclinical and 12 with clinical ketosis. All cows enrolled in study were within the first 2 months after parturition. All cows aged between 4-8 years. The cows that had had subclinical ketosis, with milk yield ranging from 20 to 32.5 kg/day of milk at the time of diagnosis, whereas cows with clinical ketosis were producing from 12 to 22.5 kg/day at diagnosis. This study was carried out during the early spring housing period in April. All animals were routinely treated against endoparasites and no parasite eggs were observed during faecal examination.

Systemic examination of the cows was carried out blood and urine samples were obtained from the animals thought to have the disease. The findings were then examined clinically and systematically. Rothera and spin react 100 tests (test strips–Combur $^{10}$Test®M, Roche, İstanbul, Turkey) were performed. Cows that were positive for the Rothera and spin react 100 tests were taken into study. Animals that were positive according to Rothera and spin react 100 tests, but whose BHHA concentrations were low in laboratory data (plasma D-3-Hydroxy butyrate concentrations) were excluded from the study. These tests yielded positive results and clinical ketosis were defined as plasma BHHA concentrations $\geq$ 1.60 mmol/L. In addition, eight cows with no ketosis symptoms were diagnosed with subclinical ketosis according to evaluation of plasma D-3-Hydroxy butyrate concentrations which are 1.20 mmol/L and serum glucose concentrations which are 35.63 ± 0.73 mg/dL. Threshold values of plasma D-3-Hydroxy butyrate concentrations in subclinical ketosis in this study classified as to on the basis of threshold values to separate healthy cows from cows with SCK are reported by researchers (Voyvoda and Erdogan, 2010; Sakha *et al.* 2007).

Cows with subclinical ketosis were administered 500 mL of 30% serum dextrose solution (Dekstrovet 30%, İ.E. Ulagay Pharmaceutical Trading Co, İstanbul, Turkey), administered intravenously. According to body weight, 0.5 IU/kg insulin (Humulin®M 70/30 100 IU Flakon, Lilly Pharmaceutical Trading Co, İstanbul, Turkey) was administered via intramuscular injection. For cows with clinical ketosis, the amounts were, respectively, 1000 mL of 30% serum dextrose solution, administered intravenously, and 0.5 IU/kg insulin, administered via intramuscular injection for 2 consecutive days.

Blood samples were obtained from all the animals in order to evaluate biochemical parameters. Samples were taken from the jugular vein and placed in anticoagulated and coagulant-free blood tubes. The serum and plasma from the blood samples was extracted after centrifuging at 3000 rpm. Biochemical parameters from the serum and plasma were then measured. Blood samples were taken from all cows before treatment and after treatment (3$^{rd}$ day) for biochemical parameters. Control cows should have been moni-

tored and sampled more frequently to be sure that they did not experience ketosis.

Serum AST, ALT, LDH, glucose concentrations were performed spectrophotometrically (Photometer 5010®Boehringer Mannheim Gmbh, Germany) according to the test kit methods. Plasma TAOC capacity (Rel Assay Diagnostics® Research& Clinical Chemistry, United Kingdom) and plasma D-3-Hydroxy butyrate (Randox Laboratories Ltd., United Kingdom) were also analysed spectrophotometrically (Photometer 5010®Boehringer Mannheim Gmbh, Germany) according to the test kit procedures. Serum insulin, free triiodothyronine and free thyroxine levels in healthy cows and cows with ketosis were examined using the chemi-luminescence method by hormone device (Architect i2000-USA), according to ABBOTT commercial test kit procedures. Similarly, Se concentrations were measured using an Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) device (Thermo Scientific X2, Switzerland).

**Statistical analysis**

Definitive statistics for healthy cows and cows with subclinical and clinical ketosis were stated at mean, standard deviation, minimum and maximum values. A comparison of biochemical parameter groups was performed with the Kruskal-Wallis test. In order to determine different groups, the Duncan Multiple Range Test was used. Spearman correlation multipliers were calculated in order to determine the association between these variables. The level of statistical significance was set at 5% (SPSS, 2011).

## RESULTS AND DISCUSSION

The clinical examination of cows with clinical ketosis revealed a 50-60% decrease in milk yield, loss of appetite, decrease in ruminal movements and weakness. In dairy cows with subclinical ketosis, indigestion symptoms were found less often but milk yield also decreased. When the biochemical parameters of cows with subclinical ketosis were compared with the control group before treatment, AST, ALT and LDH activities and plasma BHBA concentrations were high ($P<0.001$, $P<0.05$, $P<0.001$ and $P<0.001$, respectively). Serum glucose levels, Ca values, $fT_3$, $fT_4$, Se and plasma TAOC amounts were low ($P<0.001$, $P<0.001$, $P<0.01$, $P<0.001$, $P<0.001$ and $P<0.001$, respectively). Insulin values did not change and were closer to those in the control group ($P>0.05$). On the third day after treatment, when the biochemical parameters of the cows with subclinical ketosis were compared with the control group, although Se values were lower than in the control group ($P<0.05$), other parameters were closer to those in the control group.

When the biochemical parameters in cows with clinical ketosis were compared with the control group before treatment, AST, ALT and LDH activities and plasma BHBA concentration were higher ($P<0.001$, $P<0.01$, $P<0.001$ and $P<0.001$, respectively). Serum glucose levels, Ca values, $fT_3$, $fT_4$ and TAOC levels and Se concentrations were lower ($P<0.001$, $P<0.01$, $P<0.001$, $P<0.001$, $P<0.001$ and $P<0.001$, respectively). However, insulin levels did not change. On the third day after treatment, when the biochemical parameters of cows with clinical ketosis were compared with the control group, plasma BHBA concentration had returned to normal levels ($P>0.05$). Although glucose levels had increased, they remained lower than in the control group ($P<0.001$). AST, ALT and LDH activities remained high and these increases were statistically significant ($P<0.01$, $P<0.05$ and $P<0.001$, respectively). Although serum Ca, $fT_3$, $fT_4$ and TAOC levels were higher, these levels were lower than in the control group ($P<0.05$, $P<0.01$, $P<0.001$ and $P<0.001$, respectively). Again, although serum insulin levels in cows with clinical ketosis were closer to those in the control group ($P>0.05$), serum Se concentration levels remained lower ($P<0.001$) (Table 1).

We then analysed the pre-treatment parameters of cows with subclinical ketosis. The results showed a positive correlation between BHBA and LDH concentration ($P<0.05$, $r=0.835$) and a negative correlation between $fT_3$ and LDH ($P<0.01$, $r=-0.919$). A negative correlation was shown between glucose and LDH ($P<0.05$, $r=-0.719$), with a positive correlation between glucose and Se ($P<0.01$, $r=0.839$). The results showed a positive correlation between AST and LDH ($P<0.01$, $r=0.966$) and a negative correlation between AST and selenium ($P<0.05$, $r=-0.796$). There was a negative correlation between LDH, Ca, $fT_3$ and Se ($P<0.01$, $r=-0.954$; $P<0.01$, $r=-0.922$; $P<0.05$, $r=-0.791$). The results showed a positive correlation between Ca, $fT_3$ and Se ($P<0.01$, $r=0.843$; $P<0.05$, $r=0.734$, respectively). Correlation between $fT_3$ and Se was also deemed positive ($P<0.05$, $r=0.780$). Biochemical parameters for cows with subclinical ketosis were also taken on the third day after treatment. Analysis of these parameters showed a negative correlation between serum BHBA and Ca concentration ($P<0.01$, $r=-0.870$). An increase in Ca concentration was shown along with a decrease in BHBA.

When the parameters in cows with clinical ketosis were analysed, a positive correlation was found between serum AST and serum BHB ($P<0.05$, $r=0.675$). A negative correlation was found between serum Se and BHB ($P<0.05$, $r=-0.699$). In addition, the findings showed a negative correlation between serum glucose levels and serum AST levels ($P<0.05$, $r=-0.673$).

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

| Variable | Control (n=10) (Mean±SE) | Subclinical ketosis (n=8) (Mean±SE) | | Clinical ketosis (n=12) (Mean±SE) | |
|---|---|---|---|---|---|
| | | BT | AT (3rd day) | BT | AT (3rd day) |
| BHBA (mmol/L) | 0.22±0.03[a] | 1.25±0.14[b] | 0.25±0.04[a] | 1.76±0.21[b] | 0.28±0.03[a] |
| Glucose (mg/dL) | 69.80±3.55[a] | 35.63±0.73[b] | 68.00±2.65 | 27.75±1.07[b] | 54.92±0.57[b] |
| AST (U/L) | 51.86±1.92[a] | 74.9±0.52[b] | 59.38±0.58[d] | 85.39±1.57[b] | 68.03±0.72[c] |
| ALT (U/L) | 23.43±3.07[a] | 27.91±2.39[d] | 25.34±1.28 | 35.50±4.62[c] | 28.96±3.22[d] |
| LDH (U/L) | 1404±27[a] | 1907±19[b] | 1515±17[d] | 2068±52[b] | 1674±26[b] |
| Calcium (mg/dL) | 9.37±0.06[a] | 7.81±0.10[c] | 9.04±0.11[d] | 6.78±0.13[c] | 8.64±0.09[d] |
| $fT_3$ ( pg/dL) | 2.52±0.04[a] | 2.03±0.01[c] | 2.38±0.01[d] | 1.71±0.04[b] | 2.16±0.02[c] |
| $fT_4$ ( ng/dL) | 0.86±0.03[a] | 0.67±0.10[b] | 0.82±0.03 | 0.69±0.05[b] | 0.70±0.10[b] |
| TAOC (mmol/L) | 4.78±0.07[a] | 3.13±0.04[b] | 4.55±0.10 | 2.59±0.06[b] | 3.53±0.05[b] |
| Insulin (pmol/L) | 3.54±0.21 | 2.95±0.46 | 3.28±0.34 | 2.75±0.26 | 2.93±0.41[a] |
| Se (µg/L) | 69.82±1.17[a] | 54.75±0.59[b] | 63.63±1.03[d] | 43.83±1.34[b] | 57.58±0.50[b] |

BT: before treatment; AT: after treatment; BHBA: ß-hydroxybutyrate acid; AST: aspartate aminotransferase; ALT: alanine aminotransferase; $fT_3$: free triiodothyronine; $fT_4$: free thyroxine and TAOC: total antioxidant.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SE: standard error.

There was a positive correlation between serum glucose levels, $fT_3$ and TAOC levels (P<0.01, r=−0.793; P<0.01, r=−0.807). The findings also showed an increase in fT3 and TAOC levels, in parallel with the increase in serum glucose levels. There was a negative correlation between serum AST activities, $fT_3$ and TAOC levels (P<0.05, r=−0.651; P<0.05, r=−0.697, respectively) and an increase in $fT_3$ and TAOC levels, in parallel with the decrease in serum AST activities. There was a positive correlation between serum Ca values, $fT_3$ and TAOC levels (P<0.01, r=−0.769; P<0.01, r =−0.803, respectively) and an increase in $fT_3$ and TAOC levels, in parallel with the increase in serum Ca values. The findings also showed a positive correlation between serum $fT_3$ and TAOC levels (P<0.01, r=−0.788) and an increase in $fT_3$ levels, in parallel with the increase in TAOC levels. On the third day after treatment**,** the analysis of parameters from cows with clinical ketosis showed a positive correlation between serum BHB and serum AST (P<0.05, r=0.637) and a negative correlation between serum BHB and serum $fT_3$ (P<0.01, r=−0.798). There was a negative correlation between serum AST activities, serum Ca values, fT3 and TAOC levels (P<0.05, r=−0.587; P<0.01, r=−0.782 and P<0.05, r=−0.629, respectively). A decrease in serum AST activities levels was found, in parallel with the increase in serum Ca values, $fT_3$ and TAOC levels. Although a negative correlation was found between ALT and serum Ca values (P<0.01, r=−0.716), a positive correlation was found between $fT_3$ and plasma TAOC values (P<0.05, r=0.701).

Energy deficiency occurs in dairy cows with high milk yields between the second and sixth weeks of lactation as the animals need to maintain tissue functions and because the required energy is more than the amount of energy obtained from feed (Kennerman, 2004; Saldago Hernández *et al.* 2009). To obtain the required energy when a negative energy balance occurs, triglycerides are broken down to fat acids and glycerine by mobilising fat reserves (Kennerman, 2004; Sahoo *et al.* 2009). Hepatic ketogenesis increases as a result of fatty acid oxidation and gluconeogenesis. BHBA levels increase due to inadequate oxidation of fatty acids to the TCA cycles and an increase in ketone bodies (Kennerman, 2004). An abnormal increase in plasma ketone bodies may lead to clinical and subclinical ketosis (Goldhawk *et al.* 2009; Zhang *et al.* 2013). In dairy cows with subclinical ketosis, the required energy amount to produce milk two weeks after calving is approximately 30% higher (Goldhawk *et al.* 2009). It is important to pay attention to the subclinical form of the disease because lack of appetite, body weight loss and a sudden decrease in milk production are significant issues from an economic perspective (Sahoo *et al.* 2009). Tests are performed to diagnosis SCK which are evaluate concentrations of BHBA, acetoacetate, and acetone. But among of them the measurement of BHBA concentration level plays importance role for diagnosis of subclinical ketosis (Zhang *et al.* 2013). In another studies, a diagnosis of subclinical ketosis is reached after measuring plasma glucose, non-esterified fatty acids and the concentration of ketone bodies in blood, milk and urine (Kennerman, 2004; Sahinduran *et al.* 2010; Goldhawk *et al.* 2009; Gartner *et al.* 2009). Findings have shown that serum glucose levels are low and BHBA levels high in cows with subclinical ketosis compared with healthy cows (Zhang *et al.* 2011), while evaluation of glucose concentration is not a very good indicator for the energy status of dairy cows. In another study of cows with subclinical ketosis, serum glucose concentrations in cows with subclinical ketosis were significantly lower (P<0.01) and BHBA concentrations significantly higher (P<0.01) (Zhang *et al.* 2013; Xu and Wang, 2008). In the current study, no distinctive symptoms were found. However, according to the laboratory data, serum glucose levels were measured as 35.63 ± 0.73 mg/dL and BHBA levels as 1.25 ± 0.14 mmol/L. Glu-

cose levels were lower than those in healthy cows, whereas BHBA levels were higher. In the light of these findings, the animals were diagnosed with ketosis. In parallel with the decrease in serum glucose levels in the clinical ketosis group, a significant increase in serum BHBA levels was found. In this study, although the glucose levels in the control group were physiological, glucose levels in cows with subclinical and clinical ketosis were lower than the values reported for the healthy cows. An increase in serum glucose levels in both groups of cows with ketosis was found after the third day of glucose administration. In parallel with this increase, serum BHBA levels had decreased. In cows with both subclinical and clinical ketosis, serum glucose levels were lower and serum BHBA levels were higher before treatment. This occurred by ketogenesis due to energy deficit.

In ketosis, free radical damage to living cells has a significant effect on the antioxidant systems of dairy cows (Spears and Weiss, 2008). At the same time, ketone body metabolism is an important source of free oxygen radicals (Çelik and Karagul, 2005). In studies showing the role of antioxidants on erythrocyte oxidative stress, antioxidants have been used as an additional treatment. In this study, too, oxidative stress was decreased in conjunction with conventional treatment. The erythrocyte lipid peroxide levels of cows in the group that received both conventional and antioxidant treatment were significantly lower (Sahoo *et al.* 2009). In this study, the TAOC levels of both the subclinical and clinical ketosis groups were shown to be lower than those in the control group ($P<0.001$). On the third day after treatment, although TAOC levels of cows with subclinical ketosis were closer to the control group's TAOC levels ($P>0.05$), the TAOC levels of cows with clinical ketosis remained low ($P<0.01$). The lower levels of TAOC in the group with ketosis when compared with the control group supports the data of other researchers (Sahoo *et al.* 2009; Çelik and Karagul, 2005).

In healthy cows, Ca concentrations decrease several days before parturition as Ca is used to synthesise colostrum. Usually, Ca does not reach normal levels until some days after parturition (Arslan and Tufan, 2010). Some studies have reported low insulin concentrations in cows with hypocalcaemia (Forslund *et al.* 2010). Hypocalcaemia occurs in cows with subclinical ketosis and has been reported as being more severe in cows with clinical ketosis (Saldago Hernández *et al.* 2009). Toxaemia occurs due to energy deficit in pregnant sheep; in contrast to increases in BHBA levels, a decrease has been found in their glucose and Ca concentrations (Moghaddam and Hassanpour, 2008). Similar to the serum Ca levels of groups with subclinical and clinical ketosis before treatment were recorded as 7.81 ± 0.10 and 6.78 ± 0.13 mg/dL, respectively. In both groups, serum Ca levels were lower than in the control group ($P<0.01$). A positive correlation was found between the decrease in serum Ca levels and serum glucose levels before the treatment of cows with clinical ketosis ($P<0.05$, $r=0.624$).

In dairy cows, physiological signs can change rapidly. Metabolic profile analysis shows that AST and ALT activities increase at a greater rate at the time of parturition than before parturition (Avcı and Kızıl, 2013). The reason for the increase is thought to result from cellular damage caused by lipid mobilisation related to the energy deficit that occurs close to the time of parturition (Avcı and Kızıl, 2013; Elitok *et al.* 2006). In this study, the increase in serum AST, ALT and LDH levels in cows with subclinical and clinical ketosis before treatment in comparison with the control group was statistically significant ($P<0.01$). This is in line with the increase in serum AST, ALT and LDH activities reported by other researchers (Avcı and Kızıl, 2013; Elitok *et al.* 2006).

Insulin is a peptide hormone secreted from β cells in the Langerhans islet and it has an anabolic effect (Kennerman, 2004). Insulin deficiency leads to acceleration in lipolysis, an increase in ketogenesis and a decrease in the usage of ketone bodies in muscles (Henze *et al.* 1998). The endocrine system, especially the pancreas, may play a role in the development of ketosis in ruminants. The decrease in plasma insulin concentrations triggers lipolysis and increases plasma-volatile fat acid concentration (Brockman, 1979). It has been reported that the serum insulin level of cows with ketosis is lower than the serum insulin level of healthy cows (Djoković *et al.* 2009). In a different study, in order to treat ketosis, the administration of 500 IU insulin as an addition to 30 % dextrose solution resulted in more effective treatment (Saldago Hernández *et al.* 2009). Similar data were obtained in a study performed on sheep with ketosis (Henze *et al.* 1998). In this study, the serum insulin levels of cows with clinical ketosis were lower than in the control group. Insulin data in the study match the data from other studies and support the findings of other researchers (Saldago Hernández *et al.* 2009; Henze *et al.* 1998; Brockman, 1979).

Thyroid hormones play a crucial role in the growth, development and function of most vertebrate tissues such as brain, bone, adipose tissue, skeletal muscles (Cassar-Malek *et al.* 2007), and thermoregulation (Kennerman, 2004). Although circulatory $T_4$ is higher, it is accepted as a prohormon (Kennerman, 2004; Cassar-Malek *et al.* 2007). $T_3$ hormone, which has biological activity in peripheral tissues, is formed at about 80% during transformation of $T_4$ (Brockman, 1979). Circulatory $T_3$ is formed through type 1 deiodinase enzyme by deionisation of $T_4$ (Kennerman, 2004; Haces *et al.* 2008; Cassar-Malek *et al.* 2007). Type 1

deiodinase enzyme is one of the seleno-enzymes (Cassar-Malek *et al.* 2007), which requires the Se element for activation (Haces *et al.* 2008; Cassar-Malek *et al.* 2007; Forrer *et al.* 1998). In lambs with Se deficiencies, serum $T_3$ levels are lower than serum $T_3$ levels in healthy lambs (Haces *et al.* 2008). In oxen with Se deficiency, transformation of $T_4$ to $T_3$ has been reported as corrupted (Rowntree *et al.* 2004). In cases of weight gain in humans, an increase in serum $T_3$ can be seen. This is lowered where weight decreases (Rosenbaum *et al.* 2000). In another study, low circulatory thyroid hormone levels have been reported in cows with energy deficiency (Blum *et al.* 1983). In this study, the serum $T_3$ and $T_4$ levels of cows with subclinical and clinical ketosis were lower than in the control group ($P<0.001$). The transformation level of $T_4$ to $T_3$ before treatment was particularly low in cows with clinical ketosis, where $T_3$ and $T_4$ values were higher than the values before treatment. The $T_3$:$T_4$ ratio increased in cows with clinical ketosis. The reason for the increase in $T_3$:$T_4$ ratio levels results from increased Se serum levels in cows after treatment. Further thorough investigation should be carried out to confirm these results (Kennerman, 2004; Haces *et al.* 2008; Cassar-Malek *et al.* 2007; Contreras *et al.* 2002; Forrer *et al.* 1998; Rowntree *et al.* 2004; Rosenbaum *et al.* 2000; Blum *et al.* 1983).

Several antioxidants and trace minerals play important roles in the immune function. These antioxidants affect the health of pregnant dairy cows in the peripartum period (Spears and Weiss, 2008). Se participates in the GSH-Px enzyme structure, which metabolises the hydrogen peroxide and lipoperoxydes formed during normal cell metabolism. It also protects the cell from the harmful effects of free radicals (Haces *et al.* 2008; Spears and Weiss, 2008). Se is an essential trace element for antioxidant and thyroid hormone processes (Rowntree *et al.* 2004). In order to determine serum Se concentrations in cattle, a number of studies have been carried out (Gerloff, 1992; Rowntree *et al.* 2004). These have found 80-300 µg/L to be normal, 30-70 µg/L to be critical l and 2-25 µg/L to be deficient (Blum *et al.* 1983). Another study suggests that 40 µg/L of serum Se level indicates deficiency, that 40-70 µg/L is critical and that concentrations higher than 70 µg/L are adequate (Gerloff, 1992). In our study, serum Se levels in healthy cows were measured as 69.82 ± 1.17 µg/L. Before treatment, serum Se levels in cows with subclinical ketosis were 54.75 ± 0.59 µg/L. In cows with clinical ketosis, the levels were 43.83 µg/L. On the third day after treatment, serum Se levels in cows with subclinical ketosis were 63.63 ± 1.03 µg/L.

For cows with clinical ketosis, this was 57.58 ± 0.50 µg/L. Serum Se levels in groups with both clinical and subclinical ketosis were lower than the values found in healthy cattle. It can be concluded, therefore, that ketosis leads to Se deficiency in dairy cows, according to these data. In the same time, the Se levels of cows with subclinical and clinical ketosis were lower than in the control group ($P<0.001$).

BHBA levels increased in parallel with the low Se levels of the group with ketosis. A negative correlation was found between Se and BHBA ($P<0.001$ $r=0.919$). There was a positive correlation ($P<0.05$, $r=0.780$) with serum Se concentration in cows with subclinical and clinical ketosis before treatment. Low levels of serum $fT_3$ were found as a result of low Se concentration. This situation suggests an increase in oxidative stress and a decrease in antioxidants in ketosis.

## CONCLUSION

Serum insulin, thyroid hormone and Ca levels in subclinical and clinical ketosis cow were lower than in the control group on the third day after the treatment. The evaluation of insulin, thyroid hormones, Se and Ca parameters in addition to the current routine parameters will help reduce yield losses caused by ketosis. As these parameters are directly associated with yields, regulating these parameters will help prevent losses. Examining Se, insulin, thyroid hormone and Ca levels together with total antioxidant levels will help prevent yield losses and increase the chance of treatment and prognosis. In addition, we think that this study will provide an insight for future research into ketosis.

## ACKNOWLEDGEMENT

This research was carried out in the Department of Internal Medicine at Faculty of Veterinary Medicine at Yuzuncu Yil University. All authors interpreted the data and revised the manuscript and approved the final version. The research was supported by the Yuzuncu Yil University Scientific Research Projects Directorate as 2012-VF-B028 project.

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