Clinico-biochemical, oxidative markers and trace elements changes in cows with ketosis

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A B S T R A C T

Ketosis was diagnosed in 10 lactating cows in private farm in Qaluabia government based on the blood level of Non-esterified fatty acid (NEFA) and Beta hydroxy butyric acid (BHBA). Another 10 healthy cows with normal NEFA and BHBA were used as control. Clinically, the affected cows had partial or complete anorexia with reduction of rumen movement. Biochemically, there was a significant (P< 0.05) increase in AST, ALT, creatinine in addition to NEFA and BHBA in ketotic group compared to control. On the other hand, there was significant decrease (P < 0.05) in triacyl glyceride, albumin, copper and zinc. The oxidative stress markers showed significant increase (P <0.05) in MDA concentration and significant decrease in SOD activity. It was concluded that ketosis is related to clinical, biochemical, oxidative stress and reduced serum Cu and Zn levels. It is recommended to add trace elements, specially serum Cu and Zn in the periparturient period.

Keywords: Anti-oxidant, biochemical, copper, ketosis, zinc.

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1. INTRODUCTION

Pregnancy and lactation are physiological statuses considered to modify metabolism in animals and induce stress (Iriadam, 2007).

Ketosis is a major metabolic disease of dairy cows in early lactation, which develops when dairy cows fall into a condition of excessively negative energy balance caused by insufficient dietary intake and generous lactation (Xuet al., 2008). New cows with serum BHBA levels of ≥1800 µmol/L have an expected drain loss of 300 kg for that lactation (Duffield et al., 2009). Subclinical ketosis (serum BHB between 1200 to 1400 micromol/l) in the first or second week after calving is associated with increased risk of displaced abomasum, metritis, clinical ketosis, endometritis, prolonged postpartum an ovulation, increased severity of mastitis, and lower milk production in early lactation. There are several validated and practical tools for cow-side measurement of ketosis (LeBlanc., 2010). The periparturient period is important in terms of its influence on health and subsequent performance of dairy cows, since cows develop serious metabolic and physiological changes during these periods (Tanka et al., 2011).

Ketosis is a common metabolic disorder in high yielding dairy cows. During peak lactation period, high yielding dairy cows enter a stage of negative energy balance due to energy output required for milk production is higher than the energy obtained from the consumed feed. Due to negative energy balance there is decrease in

serum concentration of glucose and insulin that will in-turn leads to mobilization of adipose tissue with which consequently increases serum concentrations of non-esterified fatty acids.

An adequate supply of macro and micronutrients, such as trace element (e.g., Zn, Mn, Cu, Co), is important for ensuring an optimal transition from pregnancy to lactation (Andrieu, 2008). For instance, trace element has critical roles in a variety of physiological process, particularly antioxidant defense, and a deficiency may depress immunity especially in peripartal or transition cows (Spears and Weiss, 2008). it is conceivable that trace element has beneficial effects on oxidative stress and inflammation e.g., can help increase overall health and epithelial tissue integrity while reducing the energy required to maintain these systems and consequently improving available energy concluded by Nayeri et al., (2014).

Bovine ketosis occurs in dairy cows suffer from NEB which results in high mobilization of lipids from body fat reserves, leads to fatty liver infiltration (Djoković et al., 2016). Therefore, the use of hepatic enzyme, could be valuable markers for occurrence of ketosis. This study work aimed to monitor the clinical, biochemical and oxidative markers changes in dairy cows 3-4 weeks postpartum. Further aim was to assess the copper and zinc status during this period.

2. MATERIAL AND METHODS

2.1. Animals and study design:

This study was carried out on 20 Holstein-Friesian dairy cows in a private farm in Qalubia governorate aged between 3–7 years during post parturient period (3rd–4th weeks postpartum). The average daily milk production was 21.51±2.96 kg. According to colorimetric enzymatic method for diagnosis of ketosis which measure the serum BHBA levels, at a cut-off point of serum BHBA ≥1.200 mmol and clinical examination. The cows were classified into 2 groups:

Group 1: included non – ketotic cows (n=10) that were apparently healthy by clinical examination and negative with colorimetric enzymatic method.

Group 2: included ketotic cows (n=10) that were positive with colorimetric enzymatic method. Cows were fed daily on diet consisting of 30 Kg barseem, 10 -15 kg. corn silage, 5 Kg. hay straw and 10–15 kg. concentrates with (17% protein) per animal.

2.2. Sampling:

The blood samples were collected from jugular vein of all cows during postpartum period during the early morning (Kelly,1984). Serum samples were separated, deep frozen and used for biochemical analysis.

2.3. Clinical examination:

Clinical examination of all cows was conducted according to Radostits et al., (2007).

2.4. Biochemical analysis:

The clear non-hemolyzed serum were used for the quantitative determination of glucose, NEFA, BHBA, insulin, cholesterol, TAG, HDL, albumin, globulin, total protein, creatinine, Zn, Cu, AST, ALT, MDA and SOD by using commercial kits.

2.5. Statistical analysis:

All statistical analysis was performed using the Sigma Stat 3.1, statistical software (SPSS Inc., Chicago, IL, USA). The difference between the 2 groups was analyzed by using independent-samples T. Results were presented as means (M) ± standard errors (S.E.) The significance was determined when $P<0.05$.

3. RESULTS

3.1. Clinical findings

Clinical examination revealed partial anorexia (refuse to eat concentrate) was observed in three ketotic cows. Acetone odor was detected in breath in only one clinical cow. Scanty firm feces were observed in two ketotic cows. A reduction in milk yield was observed in all ketotic cows. The body temperature, respiratory rates and pulse rate of ketotic cows were not significantly changed. The ruminal movements showed a significant decrease in ketotic and control cows. The average of daily milk production in healthy cows was 21.51±2.96kg/day, while ketotic cows showed 4.7% reduction in milk production. The ruminal movements of ketotic cows showed a significant decrease (P<0.05) than control. The mucous membrane of ketotic cows was pale compared with rosy red color of control.

3.2. Biochemical changes:

Serum glucose levels showed anon significant ($P \geq 0.05$) decrease in ketotic cows than control. Serum BHBA and NEFA levels revealed a significant ($P<0.05$) increase in ketotic cows than control. the hormonal profile, serum insulin levels showed a significant ($P<0.05$) decreased in ketotic cows than control. The lipid profile, serum TAG levels showed a significant ($P<0.05$) decreased in ketotic cows than control. serum cholesterol levels showed a non-significant increase in ketotic cows than control Serum HDL levels showed a non-significant decrease in ketotic cows than control. Serum TP and globulin levels revealed anon significant decrease in ketotic cows than control. Serum albumin levels showed a significant ($P<0.05$) decrease in ketotic cows than control. Regarding the enzymatic functions, serum AST and ALT activity showed significant ($P<0.05$)) increase in ketotic cows than control. Regarding the kidney functions, serum creatinine levels showed a significant ($P<0.05$) increase in ketotic cows than control. Regarding the serum trace element, zinc and copper showed a significant ($P<0.05$) decrease in ketotic cow than control. Regarding the oxidative stress markers, serum MDA levels showed a significant ($P<0.05$) increase in ketotic cow than control cows, whereas; SOD level showed a significant ($P<0.5$) decrease in ketotic cow than control cows.

4. DISCUSSION

All cases of ketosis were recorded in cows during 3 to 4 weeks post-partum. These results are comparable to previous investigations (Andersson and Emanuelson, 1985 and Oetzel, 2004). This could be due to peak milk production during this period (Holtenius and Holtenius, 1996). The body temperature, respiratory rates and pulse rate of ketotic cows showed no significant change compared with ketotic and control cows. These results are similar to what remarked by Issi *et al*. (2016). Moreover, Asrat *et al*., (2013) noted no significant change in body temperature, respiratory rate and pulse rate in ketotic cows compared with control. On the other hand, the ruminal movements showed a significant decrease in ketotic compared with control cows. The ruminal stasis observed in ketotic cows is similar to that noted by Dar *et al*., (2014). Additionally, Youssef *et al*., (2010), Bali *et al*., (2016) and Ghanem et al., (2016) remarked a significant decrease of ruminal movement in clinical ketotic cows compared with healthy buffaloes. Depressed ruminal motility could be attributed to the excessive generation of ketone bodies. Ketones bodies are reported to affect ruminal motility causing incomplete and depressed ruminal contraction (Lean *et al.*,1992). Inhibition of ruminal stimulants

such as cholecystokinin hormone which stimulates ruminal emptying may explain the presence of ruminal stasis in ketotic buffaloes (Youssef *et al.,* 2010).

Regarding the biochemical analysis of serum glucose levels showed a non-significant decrease in ketotic cows compared with control cows. This result agreed with that recorded by Ghanem et al. (2012). On the contrary, Sun *et al*., (2014) and Li *et al*., (2016). Zhang *et al*., *(*2009) observed that serum glucose levels were lower in ketotic cows than control cows. Decreased blood glucose levels could be attributed to an increased mammary gland activity in lactose synthesis as well as to a decreased hepatocyte activity to synthesize glucose through gluconeogenesis under lipomobilization and lipogenesis in the liver (Djoković *et al.*, 2010 and Djoković *et al.*, 2014). Decreased blood glucose levels could also have attributed to the intake of low energy diet (Bremmer *et al.,* 2000), especially at the early stage of lactation when a high rate of glucose utilization in the mammary gland is required (Nazifi *et al.,* 2008).

Serum NEFA levels showed a significant increase in ketotic cows compared with control cows. This result agreed with Li *et al*., (2016). Moreover, Đoković *et al*., (2012) and Xu *et al*., (2014) observed a significant increase in serum NEFA levels in ketotic cows than control. NEFA concentration reflects the magnitude of fat mobilization from body reserves and reflects the energy and dry matter intake (Adewuyi *et al*., 2005). Blood concentration of NEFA considered as the best indicator of NEB and of the lipomobilization (Civelek *et al*., 2011 and Đjoković *et al*., 2016). The increase of NEFA could be attributed to an increase in lipolysis as a result of stimulation of hormone-sensitive lipase in adipose tissue due to hypoinsulinemia (Lewis *et al*., 2002).

Serum BHBA levels showed significant increase in ketotic cows compared with control cows. This result agreed with that recorded by Zhang et al. 2013; Sun *et al*. 2014;Marutsova *et al*. (2015)and Li *et al*. (2016) in ketotic cows recorded a significant increase in serum BHBA levels in ketotic cows compared with control cows. Dairy cows experience a NEB because the drain of energy for milk production exceeds the energy uptake from the ingested feed stuff; this imbalance leads to mobilization of body fat reserves in the form of fatty acids, this result in an increase in ketone body production in the liver (Zhang *et al*., 2009). Blood BHBA originates from the liver (due to incomplete oxidation of fatty acids) (Oetzel., 2007). Low dry matter intake and increased lactational demand for energy result in propionate deficiency which leads to a lack of oxaloacetate used to convert acetate, butyrate and NEFA to energy in the tricarboxylic acid cycle. As a result, the acetyl-coenzyme A synthesized from acetate, butyrate and NEFA cannot enter into the tricarboxylic acid cycle and combine together to form ketone bodies (acetone, acetoacetate and BHBA) (Goff and Horst.,1997and Kara., 2009). NEFA and BHBA are products of fat catabolism that can supply energy to the body. Their increased levels in blood are symbols of NEB, which predict a great amount of fat mobilization and used as specific indicators for the occurrence of ketosis in cattle (Ospina *et al*., 2010; Asi *et al*., 2011 and González *et al*., 2011).

Serum insulin levels showed a significant decrease in ketotic cows compared with control cows. Decreased serum insulin in ketotic cows is agreeable to that recorded by Sadeghi *et al*. 2011 and Djoković *et al.*

2009 and Xia *et al*. 2010.Moreover, the decreased serum insulin in ketotic cows is agreeable to that recorded by Ahmad *et al*., (2005); and Teli and Ali, (2007) in ketotic buffaloes. Decreased serum insulin levels could be attributed to the decreased ability of β–cells of the endocrine pancreas to synthesize and release insulin (Dokovic *et al.*, 1998 and Djoković *et al*., 2009). Low plasma insulin concentration reduces glucose uptake by non – mammary extrahepatic tissue and makes glucose available for uptake by the mammary gland which is not responsive to insulin (Bauman.,2000). Decreased insulin concentrations also promote the release of NEFA by the adipose tissue through hormone-sensitive lipase (McGuire *et al.,* 1995). Insufficient blood glucose levels induce a decline in plasma insulin, and lipomobilization as NEFA (Block and Sanchez.,2000). Insulin is low in type I diabetes because of a pancreatic defect, but in type II ketosis insulin is low because of chronic hypoglycemia due to a shortage of glucose precursors (Oetzel, 2007).

Serum TG levels revealed anon significant decrease in ketotic cows compared with control group. The non-significant decrease serum TG levels in ketotic cows are agreeable to that recorded by (Djoković *et al*., 2007 and González *et al*., 2011) and Doković *et al*., (2012). TG accumulates in the liver cells of ketotic cows and causes their blood values to decrease (Đoković *et al.*,2005; Djoaković *et al*., 2013 and Đoković *et al*., 2016). Reichel and Sokoi, (1987) and Veenhuizen *et al*., (1991) attributed the decrease in blood TG to the significant increase of FFAs concentrations in the blood that causes an increase of the content of lipids in the hepatocytes (fatty liver). serum cholesterol levels showed a significant increase in ketotic cows compared with control cows. This result is agreeable to that. Anantwar and Singh, (1993) reported that there was an increase in cholesterol levels in ketotic animals. On the contrary Li *et al*., (2016) and Youssef *et al*., (2010) reported decrease cholesterol levels in ketosis could be attributed to mild liver steatosis which causes a decrease in cholesterol formation in the liver (Grummer.,1995). Increased accumulation of triglycerides and cholesterol in hepatocytes in the puerperal ketotic cows probably linked to a depleted liver synthesis of VLDL (Holtenius.,1989)

Serum HDL levels showed a non-significant decreased in ketotic cows compared with control cows. Decreased HDL may be attributed to depressed lipoprotein lipase (LPL) as there is a positive association between LPL and HDL-cholesterol (Cheung *et al.,*2003). Since LPL is insulin dependent, the depression of LPL activity may be due to decrease in blood insulin and insulin resistance which also occur in ketosis *(*Herrera *et al.,* 1990).

Serum TP and globulin levels revealed anon significant decrease in ketotic cows compared with control one. However, albumin levels showed a significant decrease in ketotic cows compared with control cows. The decreased liver synthesis of albumin is induced by the development of fatty liver infiltration (Lubojacka *et al*., 2005).

Serum AST activity showed a significant decrease in ketotic cows compared with control cows. This result is coincided with (Djoković *et al*., 2016) who recorded that AST is a non-specific liver enzyme. However, Li *et al*., (2016) in cows and by Youssef *et al*., (2010) in buffaloes. Sevinc *et al*. (2001), Doković *et al*. (2012), *Xu et al*., (2014) and Shin *et al*., *(*2015) recorded a significant increase in serum AST levels in ketotic cows than healthy cows. Blood activities of AST are correlated

with the degree of fatty infiltration in the liver.

Serum ALT activity showed a significant increase in ketotic cows compared with control cows this result is parallel with that recorded by Li *et al*. (2016) who observed a significant increase of ALT in ketotic cows and in ketotic buffaloes (Youssef et al.,2010 and Bali *et al.*,2016). On the other hand, Stojević *et al*., (2005) considered that the role of ALT in predicting liver damage in ketosis is insignificant.

The significant increase in serum creatinine in ketotic cows was parallel with that recorded by Issi *et al*., (2016) who attributed that to the partial damage of nephrons.

In ketotic cows, serum Zn levels showed a significant decrease than control cows. This result coincided with that recorded by Zhang et al., (2010) who noted that the serum concentrations of Zn were significantly decreased in ketotic cows than control cows. It was observed that, after calving, zinc concentration showed a significant negative correlation with MDA concentration concluded that by Karimi et al., (2015). Therefore, the role of Zn deficiency in ketotic cows could be related to its antioxidant activities. In the antioxidant system, Zn is a component of Cu–Zn SOD. Zinc also induces synthesis of metallothionein, a metal binding protein that may scavenge hydroxide radicals (Spears and Weiss, 2008). In addition, Zinc plays an important role in the synthesis, storage, and secretion of insulin as well as conformational integrity of insulin in the hexameric form which affects the ability of islet cells to produce and secret insulin (Duzguner and Kaya, 2007). Moreover, Zinc deficient animals have reduced serum insulin content. Therefore, insufficient zinc may influence the generation and metabolism of BHBA by reducing the secretion of insulin in dairy cows (Karimi et al., 2015).

Serum Cu levels showed a significant decrease in ketotic cows than control cows. This result coincides with that recorded by Kaya et al., (2016) who noted that Cu level was found significantly lower in ketotic dairy cows compared to healthy dairy cows. On the other hand, there was no significant difference observed for serum Cu concentration between healthy and subclinical ketotic dairy cows (Zhang et al.,2010). The reduction of Cu level in the serum of clinically ketotic cows suggest its role in carbohydrate metabolism (Kozat., 2006). In addition, Cu is involved in the production of antioxidants via its role in the Cu-Zn superoxide dismutase (SOD) and ceruloplasmin (Spears and Weiss, 2008). In dairy cows, low levels of Cu have been observed to be related with low drain quality with expanded physical cell tally, expanded defenselessness to mastitis, diminished sustain admission, loss of multiplication, development sorrow and weakened insusceptible status, diminish fruitfulness, delayed work and fetus removal (Enjalbert et al., 2006).

Regarding the oxidant-antioxidant activity, Serum MDA levels showed a significant increase in ketotic compared with control cows, which is agreeable to that recorded by Li *et al*., (2016) in cows and (Youssef *et al.,* 2010) in ketotic buffaloes. Additionally, Xu *et al.* (2014) recorded a significant increase in serum MDA levels in ketotic cows compared with that of control. A great amount of NEFAs from fat mobilization of dairy cows affected ketosis may produce a great deal of oxygen radical, such as ROS, which can initiate oxidative stress (Schönfeld and Wojtczak, 2008). In humans, increase blood KBs could increase the blood lipid peroxidation which

could be determined by examination of plasma level of MDA (Jain *et al*.,1999). MDA is a degradation product of lipid peroxidation after exposure to ROS and its level in the blood may be considered as an assessing indicator of lipid peroxidation (Turk *et al*., 2008). Additionally, Mudron *et al*., (1999) attributed the increased MDA in the cows with fatty livers to increase in the intensity of hepatic lipoperoxidative processes and a low antioxidative status. Serum SOD levels showed a significant decrease in ketotic cows compared with that of control cows. This result is agreeable to that recorded by Li *et al*., (2016) and that recorded by Al-Qudah, (2011) in pregnancy toxemic ewes. SOD activity of diabetic pregnant women showed significant decrease vs normal pregnant women and non-pregnant group (Djordjevic *et al*., 2004). Decreased SOD levels explained by the severe damage that occurred in the erythrocyte membrane and other cellular structures depending on an inability to fully detoxify oxygen free radicals (Gurdogan *et al*., 2014). However, Xu *et al*., (2014) observed a significantly increased SOD levels in ketotic cows due to enhanced antioxidative ability. Pedernera *et al*., (2010) concluded that oxidants-antioxidants imbalance, an excess of oxidants and/or a depletion of antioxidants, can lead to oxidative stress. Oxidative stress occurs when an imbalance happens between the oxidative system and antioxidative system, which cause cellular damage (Abuelo *et al*., 2013). The oxidative damage could also be a contributing factor for damage to the hepatic cells and release of hepatic enzymes. The reduction in Cu and zn levels could represent contributing factors for reduction of anti-oxidant capapcity since they are members on Cu-zn superoxide dismutase system (Spears and Weiss, 2008).

Finally, Ketosis is an essential metabolic disorder in Holstein- Friesian dairy cows in Egypt which is associated with reduction in milk yield, several biochemical changes and increased oxidative stress markers which reflect the negative impact of ketosis on dairy cows. The disease was also associated with trace element deficiency such as Cu and Zn. Therefore, it is recommended to add trace elements, particularly Cu and Zn during the critical stage of ketosis (3-4 weeks postpartum).

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