



Alterations in biochemical parameters and hepatic ultrasonography with reference to oxidant injury in ketotic dairy cows

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ABSTRACT

This study aimed to evaluate the clinical, biochemical and hepatic ultrasonographic changes in ketotic dairy cows. For that purpose, we examined 42 lactating Holstein- Friesian cows with ages from 3- 10 years old during the post parturient period (up to 6 weeks postpartum). The cows were classified into control healthy (C=20), subclinical ketotic cows (SCK=17) and clinical ketotic cows (CK=5). Clinically, anorexia and reduction in milk yield were observed in CK cows. The ruminal movements showed a significant depression ($P<0.05$) in CK than SCK and control. The serum glucose, insulin and cortisol showed a highly significant decrease ($P<0.001$) in CK and SCK than control. The serum NEFA and BHBA showed a highly significant increase ($P<0.001$) in CK and SCK than control. Serum cholesterol and HDL levels showed a significant ($P<0.01$) decrease in SCK and CK cows than control. Serum triglycerides (TG) and very low density lipoprotein (VLDL) were significantly decreased ($P<0.01$) in CK than control. The serum activity of AST, ALT and GGT were significantly increased ($P<0.05$) in CK cows than control. Serum Ca and P levels were significantly decreased ($P<0.05$) in CK cows than SCK and control. Regarding the oxidative stress biomarkers, serum level of malondialdehyde (MDA) showed a highly significant ($P<0.001$) increase in CK cows than SCK and control whereas, serum superoxide dismutase (SOD) level was significantly decreased ($P<0.05$) in CK cows than control. Hepatic ultrasonography of ketotic cows revealed varying degrees of fatty infiltration (focal and diffuse fatty infiltration) appeared as increased hepatic echogenicity with a blurring of hepatic blood vessels. It is concluded that ketosis induced clinical, biochemical and ultrasonographical changes in lactating cows. Oxidant injury could be implicated in the pathogenesis of the disease.

KEYWORDS: Anti-oxidant, biochemical, ketosis, subclinical ketosis, ultrasonography

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1. INTRODUCTION

Ketosis has been recognized as one of the main important metabolic disorders in dairy cows. It is caused by impaired metabolism of carbohydrate and volatile fatty acids that lead to excessive production of ketone bodies: acetoacetic acid, BHBA and their decarboxylation product such as acetone and isopropanol (Radostits et al., 2007). Ketosis can be classified into SCK and CK. CK is characterized by an increase in blood, urine, or milk ketone bodies in conjunction with other visible signs, such as inappetence, obvious rapid weight loss, and dry manure, CK has different clinical forms mainly digestive and nervous forms (Rosenberger and Rinds, 2006). Ketosis in dairy cows is associated with lipolysis due to negative energy balance (NEB) which leads to fatty liver infiltration. When fat infiltrates the liver, hepatocyte degeneration involves cell membrane damage and hepatocyte destruction, and the levels

of enzymes that indicate liver injury are generally increased (Lubojacka et al., 2005 and Đjoković et al., 2016). During ketosis, the large amount of NEFAs emerged from fat mobilization of dairy ketotic cows may induce oxygen radical such as reactive oxygen species (ROS), which can initiate oxidative stress (Schönfeld and Wojtczak, 2008). MDA is one of the final products of polyunsaturated fatty acid peroxidation which is caused by increasing level of ROS and is commonly known as a marker of oxidative damage. Oxidative stress occurs when an imbalance happens between oxidative system and antioxidative system, which can cause cellular damaged (Abuelo et al., 2013). Ultrasonographic examination of hepatic lipidosis in cows revealed an increased coarseness of echoes, increased echogenicity of the liver parenchyma near the abdominal wall, weakening of echo as distance

increases from abdominal wall and poor or no visualization of hepatic vessel (Braun et al., 1996). Because ketosis represents a major economic problem in dairy farms due to the adverse effect on milk production, this study aimed to monitor alterations in the clinical, biochemical and oxidant/antioxidant parameters in ketotic lactating cows. In addition, the hepatic changes were evaluated by ultrasonography.

2. MATERIAL AND METHODS

2.1. Animals and experimental design

This study was carried out on 42 lactating Holstein- Friesian cows of different ages 3- 10 years old during post-parturient period (up to 6 weeks postpartum) with average of daily milk production (28.47 ± 2.96) kg/day which varies from 13 – 50 kg/day, These cows were located in eight dairy farms in four governorates (Qalubia, Dakhalia, Menofia and Ismailia governorates), according to the clinical signs and the colorimetric measurement of the serum BHBA levels (Duffield, 2000) at a cut-off point of serum BHBA ≥ 1.200 mmol /L, these cows were classified into 3 groups. Group 1 included 20 cows (control) that are apparently healthy by clinical examination and negative with colorimetric enzymatic method. Group 2 included 17 cows that are apparently healthy by clinical examination and positive with colorimetric enzymatic method (SCK group). Group 3 included 5 cows that are clinically diseased (CK) and positive with colorimetric enzymatic method.

2.2. Ration.

Cows were fed daily on diet consisting of 25 Kg darawa or 25 Kg barseem, 12 -18 kg. corn silage, 4 Kg. hay straw and 12kg -18kg concentrates with (16% protein) per animal.

2.3. Management System.

All cows were kept in a free-stall barn and were kept under the same environmental condition.

2.4. Samples.

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

2.5. Clinical examination.

Determination of body temperature, pulse, respiratory rates and ruminal movement, as well as examination of mucous membranes was conducted according to Radostits et al. (2007).

2.6. Biochemical analysis.

The clear non-hemolyzed serum were used for the quantitative determination of glucose, NEFA, BHBA, cortisol, insulin, cholesterol, TG, HDL, low Density lipoproteins (LDL), albumin, total protein, urea, creatinine, Ca, P, AST, ALT, GGT, MDA and SOD by using commercial kits.

2.7. Hepatic ultrasonography examination.

The hepatic ultrasonography was performed as previously described (Braun et al., 1996). In brief, the hair was clipped on right side imaged on the right lateral abdomen below the diaphragmatic attachment from the 5th to 12th intercostal space and just caudal to the 13th rib. Gel was applied and B-mode ultrasonography performed using grey-scale equipment (Imago, France) with a 3.5 MHz convex transducer.

2.8. Statistical analysis.

All statistical analysis was performed using the Sigma Stat 3.1, statistical software (SPSS Inc., Chicago, IL, USA). Difference between groups was analyzed by using one way analysis of variance (ANOVA) and post hoc turkey test. Results were presented as means (M) \pm standard errors (S.E.) The significance was determined when $P < 0.05$.

3. RESULTS

3.1. Clinical findings

As shown in Table 1, the complete anorexia was observed in one CK cows. While, partial anorexia (refuse to eat concentrate) was observed in four CK cows. Acetone odor in breath was detected only in one CK cow. Scanty firm feces were observed in three CK cows. A reduction in milk yield was observed in all CK cows. The average of daily milk production in healthy cows was 28.47 ± 2.96 kg/day, while SCK showed 5.6% reduction in milk production and CK cows showed 9.1% reduction in milk production. The body temperature, respiratory and pulse rate of CK did not show significant changes compared to control. The ruminal movements of CK cows showed a significant decrease ($P < 0.05$) than SCK and control. The mucous membrane of CK and SCK cows was pale compared with rosy red color of control (Table 2).

3.2. Biochemical analysis.

As demonstrated in Table 3, the serum glucose levels showed a highly significant ($P < 0.001$) decrease in CK cows and SCK than control, serum glucose levels showed a significant ($P < 0.05$)

decrease in CK cows than SCK. Serum NEFA and BHBA showed a highly significant ($P<0.001$) increase in SCK and CK cows than control, serum NEFA showed a significant ($P<0.05$) increase in CK cows than SCK, serum BHBA showed highly significant ($P<0.001$) increase in CK cows than SCK. Regarding the hormonal profile, serum insulin and cortisol levels showed a highly significant ($P<0.001$) decrease in CK cows and SCK than control, serum insulin levels showed a significant ($P<0.05$) decrease in CK cows than SCK. Regarding the lipid profile, serum TG levels showed a significant ($P<0.05$) decrease in CK cows than SCK and control, serum cholesterol levels showed a significant ($P<0.01$) decrease in SCK and CK cows than control. Serum HDL levels showed a significant ($P<0.01$) decrease in CK and SCK than control. Serum LDL levels showed a significant ($P<0.01$) decrease in SCK than control. Serum VLDL levels showed a significant ($P<0.05$) decrease in CK cows than SCK and control. Regarding the protein profile, serum TP and globulin levels showed a significant ($P<0.05$) decrease in CK cows than SCK. Serum albumin levels showed insignificant change in CK cows

than SCK and control. Regarding the enzymatic functions, serum AST, GGT and ALT activity showed a significant ($P<0.05$) increase in CK cows than control, serum AST showed a significant ($P<0.01$) increase in CK cows than SCK. Regarding the kidney functions, Serum urea levels showed a significant ($P<0.05$) decrease in SCK and CK cows than control. Serum creatinine levels showed insignificant changes in CK cows and SCK than control. Regarding the minerals serum levels, Serum Ca and P levels were significantly decreased ($P<0.05$) in CK than SCK and control. Regarding the oxidative stress biomarkers, serum MDA levels showed a highly significant ($P<0.001$) increase in CK cows than SCK and control. serum SOD levels showed a significant ($P<0.05$) decrease in CK cows than control.

3.3. Hepatic ultrasonographic changes in ketotic cows.

Ultrasonographic examination of liver in ketotic showed varying degrees of fatty infiltration (focal and diffuse fatty infiltration) which result in increased hepatic echogenicity with blurring of hepatic blood vessels (Figure 2)



Figure 1 Hepatic ultrasonograms in control healthy cows showing normal gray echogenicity 1. Abdominal wall 2. Liver parenchyma 3. Portal vein. (Imaged through 11th ICS by convex transducer 3.5 MHz Imago).



Figure 2 Hepatic ultrasonogram in ketotic cows 1. Abdominal wall 2. Fatty infiltration (increased echogenicity, blurring of hepatic blood vessels) (Imaged through 11th ICS by convex transducer 3.5 MHz Imago)

Table 1 Clinical findings in clinical ketotic cows

No. of affected cows	Clinical finding	Percentage
1	Complete anorexia	20%
4	Selective feeding (partial anorexia)	80%
1	Acetone odor in breath	20%
3	Scanty firm feces	60%
5	Reduction in milk yield	100%

Table 2 Clinical parameters in control, SCK and CK cows

Parameter	Control (n=20)	SCK cows (n=17)	CK cows (n=5)
Body temperature (°C)	38.7±0.11 ^a	38.6±0.06 ^a	38.8±.07 ^a
Respiratory rate/minute	18.5±0.36 ^a	17.7±0.35 ^a	18.85±.26 ^a
Pulse rate/minute	57.8±2.1 ^a	58.2±1.89 ^a	60.1±1.6 ^a
Rumen contractions (2minute)	2.5±0.1 ^a	2.47±0.1 ^{ab}	1.6±.24 ^b

Means with different superscripts indicate significant difference at $P < 0.05$

Table 3 Biochemical changes in control, SCK and CK cows

Parameter	Control (n=20)	SCK cows (n=17)	CK cows(n=5)
Glucose (mg/dl)	50.9±2.28 ^a	36.4±1.21 ^b	30.08±1 ^c
NEFA (mmol/l)	.176±.027 ^c	1.05±.05 ^b	1.298±.13 ^a
BHBA (mmol/l)	.501±.02 ^c	1.75±.093 ^b	3.02±.07 ^a
Insulin (uIU/ml)	27.6 ±1.19 ^a	16.52±.91 ^b	11.84±.7 ^c
Cortisol (nmol/l)	29.7±2.4 ^a	16.6± 1.08 ^b	9.9±1.4 ^c
TG(mg/dl)	184.06±10.6 ^a	169.4±6.9 ^a	126.6 ±13.97 ^b
Cholesterol (mg/dl)	170.6 ±8.9 ^a	122.6±6.04 ^b	114.9±7.9 ^b
HDL(mg/dl)	60.4± 3.8 ^a	44.8 ± 2.9 ^b	34.81±2.14 ^b
LDL (mg/dl)	73.9±7.6 ^a	43.93± 5.5 ^b	54.8± 9.06 ^{ab}
VLDL (mg/dl)	36.8± 2.1 ^a	33.8± 1.3 ^a	25.32±2.9 ^b
Total protein (g/dl)	8.6±.83 ^{ab}	9.8±.53 ^a	7.04±.6 ^b
Globulin (g/dl)	6.3±.54 ^{ab}	7.25±.33 ^a	5.12±.41 ^b
Albumin (g/dl)	2.3±.27 ^a	2.58±0.14 ^a	1.92±.23 ^a
AST (U/L)	95.9± 4.2 ^b	87.5±5.6 ^b	126.5±13.6 ^a
GGT (U/L)	13.7±2.5 ^b	21.3±3.08 ^{ab}	34.62±7.8 ^a
ALT(U/L)	13.4±.97 ^b	17.04±.72 ^{ab}	19.8±2.2 ^a
Urea (mg/dl)	96.3±4.5 ^a	81.4±2.7 ^b	74.6±1.9 ^b
Creatinine (mg/dl)	.6±.11 ^a	.75±.15 ^a	.87±.13 ^a
Ca (mg/dl)	8.6±.44 ^a	8.4±.52 ^a	5.07±.37 ^b
P (mg/dl)	5.6± .45 ^a	4.8±.24 ^a	3.46±.27 ^b
MDA (nmol/ml)	2.7±.6 ^b	3.2±.2 ^b	5.9±.3 ^a
SOD(U/ml)	44.8±9.4 ^a	30.8±3.2 ^{ab}	18.4±2.8 ^b

Means with different superscripts indicate significant difference at $P < 0.05$

4. DISCUSSION

Clinical examination showed anorexia (partial and complete anorexia) and scanty firm feces in CK cows. These clinical signs are similar to those previously observed in ketotic cows (Issi et al., 2016) and ketotic buffaloes (Bali et al., 2016). Decreased milk yield in SCK and CK cows is similar to previously recorded in cows (Issi et al., 2016) and in buffaloes (Youssef et al., 2010). Hypoglycemia due to ketosis results in drop in lactose synthesis which leads to reduction in milk production (Lean et al., 1992). Moreover, elevated blood ketones also result in decreased milk production (Andersson and Lundstrom, 1985).

Detection of Acetone odor in breath of ketotic cows is similar to that observed in cattle (Ghanem et al., 2012 and Dar et al., 2014) and in buffaloes (Ghanem and El-Deeb, 2010). Although it is not a constant clinical signs of ketosis as acetone odor in breath was detected only in one CK cow in this study which was comparable to other study (Youssef et al., 2010).

The body temperature, respiratory rates and pulse rate of CK cows showed insignificant changes compared with SCK and control. These results are similar to what remarked by Issi et al. (2016). Moreover, several authors noted a non - significant change in body temperature, respiratory rate and pulse rate in CK cows compared with

control (Asrat et al., 2013 and Dar et al., 2014). The ruminal movements showed a significant decrease in CK cows compared with SCK and control. The ruminal stasis observed in ketotic cows is similar to that previously noted by Dar et al. (2014). Additionally, a significant decrease of ruminal movement in CK buffaloes compared with healthy buffaloes was previously remarked (Bali et al., 2016). Depressed ruminal motility could be attributed to excessive generation of ketone bodies. Ketone bodies are reported to affect ruminal motility causing incomplete and depressed ruminal contraction (Andersson and Lundstrom, 1985).

Regarding the biochemical analysis of serum, serum glucose levels showed a significant decrease in SCK and CK cows compared with control. This result is in agreement with those previously recorded in ketotic cows by Sun et al. (2014). Moreover, Youssef et al. (2010) and Bali et al. (2016) recorded a significant decrease in serum glucose levels in CK buffaloes than healthy buffaloes. Decreased blood glucose levels are attributed to an increased mammary gland activity in lactose synthesis as well as to a reduced hepatocyte activity to synthesize glucose through gluconeogenesis under lipomobilization and lipogenesis in the liver (Đjoković et al., 2014). Decreased blood glucose levels may be attributed to intake of low energy diet (Bremmer et al., 2000), especially at the early stage of lactation when high rate of glucose utilization in the mammary gland is required (Nazifi et al., 2008). Serum NEFA levels showed a significant increase in SCK and CK cows compared with control. This result is in agreement with (Li et al., 2016). 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 (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 a significant increase in SCK and CK cows compared with control. This result is in agreement with those previously recorded in ketotic cows by Li et al. (2016) and in CK and in SCK buffaloes by Youssef et al. (2010). Dairy cows experience a NEB because the drain of energy for milk production exceeds the energy uptake from the ingested feed stuffs. 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 is converted to ketone bodies (acetone, acetoacetate and BHBA) (Kara, 2009). NEFA and BHBA are products of fat catabolism that can supply energy to body. Their increased levels in blood are symbols of NEB, which predict a great amount of fat mobilization (Gonzales et al., 2011).

Serum insulin levels showed a significant decrease in SCK and CK cows compared with control. Decreased serum insulin in SCK is in agreement with those previously recorded in ketotic cows by Sadeghi et al. (2011). Low plasma insulin concentration reduces glucose uptake by non – mammary extra hepatic tissue and makes glucose available for up take 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 mobilization of triacylglycerol deposits as NEFA (Block and Sanchez, 2000). Insulin is low in type I diabetes because of a pancreatic defect, but in type I ketosis insulin is low because of chronic hypoglycemia due to a shortage of glucose precursors (Oetzel, 2007).

Serum cortisol levels showed a significant decrease in SCK and CK cows compared with control. Decreased blood cortisol levels in ketotic cows are similar to those previously observed in ketotic cows (Forsslund et al., 2010). Under low blood cortisol levels ketotic cows undergoing a NEB and increased lipomobilization from body reserves, the ability of liver cells to synthesize glucose from gluconeoplastic precursors is substantially decreased. The ketogenic and lipogenic processes in the liver are intensified, blood levels of NEFA and ketonic bodies increase, and hypoglycemia develops (Đjoković et al., 2013). Lower blood cortisol levels were attributed to dysfunction of the hypothalamus-adenohypophysis-adrenal cortex axis in dairy cows.

Serum TG levels showed a significant decrease in SCK and CK cows compared with control. A significant decreased serum TG levels in ketotic cows is in agreement with to those previously recorded in ketotic cows by Đoković et al. (2012). TG accumulates in the liver cells of ketotic cows

and causes their blood values to decrease (Đjoković et al., 2016). Veenhuizen et al. (1991) attributed the decrease in TG in blood to significant increase of FFA concentrations in the blood causes an increase of the content of lipids in the liver cells (fatty liver). Serum cholesterol levels showed a significant decrease in SCK and CK cows compared with control. This result is in agreement with those previously recorded in ketotic cows by Li et al. (2016). Decreased Serum cholesterol levels could be attributed to mild liver steatosis which cause reduction in cholesterol formation in the liver (Grummer, 1995). Increased accumulation of TG and cholesterol in hepatocytes in the puerperal ketotic cows probably linked to a depleted liver synthesis of VLDL (Moore and Roberts, 1998). Serum HDL levels showed a significant decrease in SCK and CK cows compared with control. This result is in agreement with those previously recorded by Li et al. (2016). Farid et al. (2013) explained the decrease in serum HDL is a result of impaired hepatic secretion of apolipoprotein A, the basic protein for the synthesis of HDL. Decreased serum HDL may also be related to the lower cholesterol levels seen in ketotic cows, as HDL consists of about 60% cholesterol (Rayssiguier et al., 1998). Decreased HDL may be attributed to depressed lipoprotein lipase (LPL) as there is a positive association between LPL and HDL (Cheung et al., 2003). Since LPL is insulin dependent, the depression of LPL activity may be due to hypoinsulinemia and insulin resistance (Herrera et al., 1990). Serum LDL levels showed no significant decrease in CK cows compared with control. SCK showed a significant decrease compared with control. Decreased serum LDL levels in ketotic cows are in agreement with Bali et al. (2016) in buffaloes. Miyamoto et al. (2006) attributed the reduction of serum LDL levels due to fatty liver. Moreover, Farid et al. (2013) explained the decrease in serum LDL as a result of decreased VLDL secretion and decreased conversion to LDL. The decreased LDL levels could be attributed to the increased rate of LDL catabolism (Spady et al., 1985). Serum VLDL levels showed a significant decrease in CK cows compared with SCK and control. Decreased VLDL level in CK cows compared with control is coincided with that previously recorded (Li et al., 2016). Moreover, decreased synthesis and secretion of VLDL in the liver of ketotic cows was observed by Yamamoto et al. (2001). VLDL decreases in cows with fatty liver (Katoh, 2002). Low secretion of VLDL in ruminants indicates the development of ketosis (Kleppe et al., 1998).

Serum TP levels showed a significant decrease in CK cows compared with SCK and control.

Decreased serum TP levels in ketotic cows is in agreement with those previously recorded by Xu et al. (2014) who attributed this reduction to the abnormal status of liver function of ketotic cows. Serum globulin levels showed a significant decrease in CK cows compared with SCK. This result is in agreement with those previously recorded in ketotic cows by Gonzales et al. (2011) who found a negative correlation between serum BHBA and serum globulins. Serum albumin levels showed insignificant decrease in CK cows compared with control. This result is in agreement with those previously recorded in cows by Xu et al. (2014). Decreased liver synthesis of albumin is induced by the development of fatty liver infiltration (Lubojacka et al., 2005).

Regarding the enzymatic activities, the AST activity showed a significant increase in CK cows compared with SCK and control. This result is in agreement with those previously recorded in ketotic cows by Li et al. (2016). Moreover, Youssef et al. (2010) observed a significant increase in serum AST activities in CK buffaloes than SCK and healthy buffaloes. Although AST is non-specific liver enzyme estimation of its activity in dairy cows is most often associated with fatty liver syndrome (Đjoković et al., 2016). Serum activities of AST are correlated with the degree of fatty infiltration in the liver (Đoković et al., 2012). Serum concentration of AST increases due to fat accumulation in the liver which results in high hepatocytes membrane permeability (Karasai and Schefar, 1984). Moreover, Stojević et al. (2005) found that higher concentrations of AST in dairy cattle are associated with fatty liver syndrome, lower dry matter intake and ketosis signs. GGT serum activities showed a significant increase in CK cows compared with control. This result is in agreement with those previously recorded in ketotic cows by Sahinduran et al. (2010) and in ketotic buffaloes (Ghanem and El-Deeb, 2010). Liver is the main source of serum GGT (Kaneko, 1989). Steen et al. (1997) attributed the increase of serum GGT levels to liver and bile duct malfunctions. Fatty liver infiltration and the hepatocyte degeneration involve cell membrane damage and hepatocyte destruction coupled to the release of cytoplasm enzymes (GGT) (Lubojacka et al., 2005). Serum ALT showed a significant increase in CK compared with control. This result is in agreement with that was previously recorded in ketotic cows by Li et al. (2016). The significant increase of serum ALT activities in ketotic cows may indicate impaired hepatic function (hepatic lipidosis and/or disruption hepatobiliary circulation (Sahinduran et al., 2010). On the contrary, Stojević et al. (2005) considered that the

role of ALT in predicting liver damage in ketosis is insignificant.

Regarding the kidney functions, serum urea levels showed a significant decrease in SCK and CK cows compared with control. This result is in agreement with that previously recorded in ketotic cows by Shin *et al.* (2015). Urea is indicators of hepatic functionality and decrease in its concentration may suggest fat infiltration into the liver (Gonzales *et al.*, 2011). On the contrary, Elitok *et al.* (2010) observed that serum urea levels in ketotic cows were significantly higher compared with control and attributed that to possible liver damage. Creatinine serum levels showed no significant change in CK cows than SCK and control. This result is lined with that previously recorded in ketotic buffaloes (Bali *et al.*, 2016). On the other hand, Issi *et al.* (2016) noted a significant increase in creatinine serum level in ketotic dairy cows than control and attributed that to partial damage of nephrons. So, we concluded that ketosis had no effect on the kidney in this study as there was a non-significant increase in serum creatinine in CK cows and SCK compared with control.

Regarding the serum mineral changes, Ca serum levels showed a significant decrease in CK cows compared with SCK and control. This result is coincided with those recorded in ketotic cows (Simonov and Vlizlo, 2015).

Decreased Ca levels can be attributed to increased loss of base in the urine to compensate for the acidosis reported in cows with ketosis (Radostits *et al.*, 2007). Moreover, Walsh *et al.* (2007) attributed hypocalcemia in ketotic cows to disorders of vitamin D metabolism due to damage of organs, involved in its metabolism (liver). Serum P levels showed a significant decrease in CK cows compared with SCK and control, which is in agreement with those previously in buffaloes by Bali *et al.* (2016). Youssef *et al.* (2010) attributed the decrease in P levels to inadequate phosphorus supply in the diet, prolonged anorexia, and increased urinary phosphorus excretion due to hyperparathyroidism.

Regarding the oxidant-antioxidant activity, MDA serum levels showed a significant increase in CK cows compared with SCK and control. The significant increased MDA levels in CK cows compared with control is in agreement with those previously recorded in cows by Li *et al.* (2016). Moreover, Youssef *et al.* (2010) recorded a significant increase in MDA level in CK buffaloes than normal buffaloes. Additionally, Xu *et al.* (2014) recorded a significant increase in serum MDA levels in ketotic cows compared with 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). MDA is a degradation product of lipid peroxidation after exposure to ROS and its level in blood may be considered as an assessing indicator of lipid peroxidation degree (Turk *et al.*, 2008). SOD serum levels showed a significant decrease in CK cows compared with control. This result is in agreement with those previously recorded in ketotic cows by Li *et al.* (2016). Decreased SOD levels explained by the serious damage that occurred in the erythrocyte membrane and other cellular structures depending on inability to fully detoxify oxygen free radicals (Gurdoga *et al.*, 2014). However, Xu *et al.* (2014) observed a significant increased SOD levels in ketotic cows due to enhanced antioxidative ability. Pedernera *et al.* (2010) concluded that imbalance in oxidants-antioxidants imbalance, an excess of oxidants and/or a depletion of antioxidants, can lead to oxidative stress which cause cellular damage. The oxidative damage could also be a contributing factor for damage of hepatic cells and release of hepatic enzymes that were observed in our study.

Ultrasonographic examination of normal liver showed a homogenous gray granular echotexture of parenchyma and clear distinction of vessels. Ultrasonographic examination of liver in ketotic cows revealed hyper- echogenicity (increased hepatic brightness with higher hepatic fat contents). As hepatic fat accumulation increased the liver appears more echogenic and brighter on the ultrasound screen and blurring of hepatic blood vessels (Braun, 2009). With increased liver fat content, beam attenuation and backscattering, fine echogenicity and vascular blurring increased, of which beam attenuation was the most prominent change at higher fat infiltration (Tharwat *et al.*, 2012). High echogenicity of fat is due to its lower acoustic impedance in contrast with that of the normal liver tissue (Braun *et al.*, 1996). Acorda *et al.* (1994) and Braun *et al.* (1996) attributed blurring of hepatic blood vessels to the swollen hepatic tissue that compressing the blood vessels and to the increase in the scattered echoes in the hyperechoic areas of the diseased liver, these echoes projected on the vessels resulting in deteriorating of the contrast between the hepatic parenchyma and hepatic vessels. Therefore, our results suggested the use of hepatic ultrasonography as a potential non-invasive supplementary tool for diagnosis of hepatic fatty infiltration in ketotic cows in conjunction with other biochemical markers.

5. CONCLUSION

Ketosis, either clinical or subclinical, is an important metabolic disorder in Holstein-Friesian dairy cows in Egypt which associated with reduction in milk yield, several biochemical changes and associated with increased oxidative stress markers which reflect the negative impact of ketosis on dairy cows. The hepatic ultrasonography could be used as a potential non-invasive method for diagnosis of fatty liver infiltration in ketotic cows in collaboration with other biochemical parameters

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