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Lecithin cholesterol acyltransferase (LCAT) activity as a predictor for ketosis and parturient haemoglobinuria in Egyptian water buffaloes

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ABSTRACT

Lecithin cholesterol acyltransferase (LCAT) activity was measured in 48 Egyptian water buffaloes four weeks pre-parturient. The activity was significantly low in 37 buffaloes (77.1%). Four weeks post-partum, clinical examination revealed that 23 buffaloes had the clinical signs of ketosis (K) while 14 had the clinical signs of parturient-haemoglobinuria (PHU). Serum samples were collected from 5 buffaloes of each group (K and PHU) besides 5 clinically healthy buffaloes with normal LCAT (control). Glucose level was significantly reduced in K and PHU groups while the phosphorous (P) level was significantly reduced in PHU group compared to control. There were significant reductions in the total cholesterol, free cholesterol, triglycerides, total protein and albumin in K and PHU groups; whereas, significant increases in AST, GGT, non-esterified fatty acids (NEFA) and beta-hydroxybutyric acid (BHBA) in K and PHU groups were detected. Therefore, LCAT could be a predictor for metabolic disorders in Egyptian water buffaloes.

1. Introduction

Lecithin cholesterol acyltransferase (LCAT) is a serum enzyme that catalyses esterification of free cholesterol to produce cholesteryl ester (CE). Cholesterol for this reaction comes from peripheral tissues and the donor of the acyl group is lecithin from the high density lipoprotein (HDL) particles. LCAT is thus necessary for reverse cholesterol transport from peripheral tissues. The produced CE is transferred to low-density lipoprotein (LDL), and LDL CE is finally taken up by the liver (Brown et al., 1981). LCAT is synthesized in the liver and therefore, its synthesis and/or excretion is impaired in hepatocellular diseases as indicated by decreased activity of LCAT (Tahara et al., 1993).

It has been demonstrated that LCAT activity is reduced in fatty liver in cows (Nakagawa-Ueta and Hatoh, 2000; Uchida et al., 1995) and also in ketosis and left displacement of the abomasum (Nakagawa and Katoh, 1998). The serum CE concentration is similarly decreased in these diseased cows, suggesting that the decrease in LCAT activity is involved in the development of periparturient metabolic disorders. The decrease in LCAT was documented 20 to 11 days before parturition in ketotic cows and was suggested to be a useful marker for detection of cows susceptible to fatty liver-related periparturient diseases in cattle (Nakagawa and Katoh, 1998).

Parturient haemoglobinuria (PHU) is a metabolic disease affecting ruminant in the periparturient period (Chugh et al., 1996). The disease has economic importance in buffalo rearing-countries in general and in India, Pakistan and Egypt in particular (Pirzada and Hussain, 1998). PHU is mainly caused by inadequate dietary phosphorus (Stockdale et al., 2005) and usually results in haemolysis and haemoglobinuria (Ogawa et al., 1987) as a sequence of decreased red cell glycolysis and resultant ATP synthesis. Subnormal concentrations of ATP would predispose red cells to altered structure and function, a loss of normal deformability, and an increase in fragility and haemolysis with resultant haemoglobinuria (Wang et al., 1985).

Although LCAT has been extensively used as a predictor for metabolic diseases in dairy cows (Uchida et al., 1995; Nakagawa-Ueta et al., 1997; Nakagawa and Katoh, 1998; Akamatsu et al., 2007), limited information is available on its importance as a predictor for metabolic disorders in buffaloes as general and in Egyptian water buffaloes in particular. Therefore, the objective of this study was to determine the relationship between LCAT level and the occurrence of metabolic diseases in Egyptian water buffaloes, specially the ketosis and the metabolic PHU. Further objective was to evaluate other related biochemical changes in the periparturient period in the Egyptian water buffaloes.

2. Materials and methods

2.1. Buffaloes

This study was carried out on a total number of 48 Egyptian water buffaloes, aging from 4 to 7 years old with average body

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weigh of 650 kg from a private farm in Dakahlyia governorate, Egypt. Buffaloes were fed on seasonal green fodders, mainly Berseem (*Trifolium alexandrinum*) and concentrate mixture consisted of 29.0% cotton seed cake, 30.0% yellow corn, 29.0% wheat, 6.0% rice bran, 3.0% molasses, 2.0% limestone and 1.0% common salt. In addition, wheat straw was supplied *ad libitum*.

2.2. Clinical examination

All buffaloes were clinically examined every day until 4 weeks after parturition, according to Radostits et al. (2007).

2.3. Collection of serum samples

Four weeks before the expected date of parturition and four weeks after parturition, blood samples were collected from the jugular vein into plain tubes (without anticoagulant). The samples were allowed to clot at room temperature; serum was separated by centrifugation and stored at −20 °C until analyzed (Poso et al., 2000).

LCAT was determined on serum obtained before parturition. LCAT, aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), glucose, total protein, albumin, total cholesterol (TC), free cholesterol (FC), BHBA, NEFA, triglyceride, serum phosphorus and serum calcium were determined on serum obtained after parturition.

2.4. Biochemical analysis

LCAT activity was determined by the use of commercial kit (Dai-Ichi Pure chemicals, Tokyo, Japan) according to the method of Uchida et al. (1995).

Spectrophotometric assays was conducted for colorimetric determination of AST (Reitman and Frankel, 1957), GGT (Yang et al., 1998), glucose (Lott, 1975), total protein and albumin (Dumas et al., 1971), TC, FC, BHBA, NEFA, and triglyceride (Nakagawa and Katoh, 1998), serum phosphorus (Morinal and Prox, 1973) and serum calcium (Glinder and King, 1972). All steps were performed using a selective chemistry analyzer (Abbott Alcyon 3001, USA). The cholesteryl ester (CE) was calculated by subtracting the FC concentration from that of TC as previously described (Nakagawa and Katoh, 1998). The concentration of globulin was calculated by subtracting serum albumin from total protein.

2.5. Detection of ketonuria

The presence of ketone bodies in the urine was detected by commercial kits (Fujisawa pharmaceutical Co., Osaka, Japan) as previously described (Oikawa et al., 1997).

2.6. Study design

According to the combination of LCAT concentration four weeks pre-partum, and clinical findings observed during the first four week post-partum, three groups were built up: group I included five buffaloes selected randomly from buffaloes with low LCAT and red urine (haematuria or haemoglobinuria), group II included five buffaloes selected randomly from buffaloes with low LCAT with ketonuria and emaciation, and group III included five apparently healthy buffaloes selected randomly from buffaloes with normal LCAT and used as control.

2.7. Statistical analysis

Data were expressed as means ± standard error (M ± SE). The comparison between the three groups was conducted by using one-way analysis of variance (ANOVA) by Sigma Stat 3.2 software (SPSS Inc. Chicago, Illinois). Holm-Sidak test was used for determination of the significance between groups. The means were considered significantly different when $P < 0.05$.

3. Results

3.1. LCAT activity

Of the 48 buffaloes screened, the level of LCAT was reduced in 37 (77.1%) as shown in Table 1. The other 11 buffaloes (22.9%) had normal LACT.

Table 1
The activity of LCAT (U) in 48 apparently healthy Egyptian water buffaloes four weeks before parturition.

Apparently healthy buffaloes ($n = 11$)		Buffaloes developed ketosis ($n = 23$)		Buffaloes developed haemoglobinuria ($n = 14$)	
Animal no.	LCAT	Animal no.	LCAT	Animal no.	LCAT
1	920	1	515	1	663
2	909	2	611	2	674
3	908	3	622	3	662
4	968	4	633	4	665
5	949	5	711	5	811
6	901	6	821	6	721
7	960	7	662	7	513
8	988	8	590	8	555
9	967	9	585	9	598
10	995	10	660	10	590
11	998	11	627	11	612
		12	638	12	721
		13	670	13	723
		14	811	14	622
		15	701		
		16	655		
		17	580		
		18	590		
		19	590		
		20	663		
		21	663		
		22	655		
		23	654		
	Mean ± SE 951.18 ± 10.96		Mean ± SE 648.13 ± 14.32		Mean ± SE 651.31 ± 22.44

3.2. Clinical findings

Twenty-three buffaloes (47.9%) exhibited the clinical signs of ketosis, 14 buffaloes (29.1%) showed the clinical signs of PHU whereas 11 buffaloes (22.9%) did not show any clinical findings. Buffaloes with PHU demonstrated signs of haemolytic anaemia including pale mucosas, rapid respiration, haemoglobinuria (Fig. 1a), haematuria with blood clots in the urine (Fig. 1b), frequent urination with dullness and depression (Fig. 1c). On the other hand, buffaloes with ketosis had reduced appetite, emaciation (Fig. 1d) with acetone odor on breath. The presence of ketone bodies in the urine was confirmed by commercial kits.

3.3. Biochemical findings

The serum P level was significantly reduced in buffaloes with PHU (group 1) compared to control (group 3) ($P < 0.001$) but not significantly affected in ketotic buffaloes (group 2). The Ca level was not significantly affected in PHU and ketotic buffaloes compared to control. Blood glucose level was significantly reduced in ketotic and hypophosphatemic buffaloes compared to control ($P < 0.001$). However, glucose level was significantly lower in ketotic buffaloes compared to PHU buffaloes. The total cholesterol, the CE and FC were significantly reduced in ketotic buffaloes compared to control. On the other hand, only the total cholesterol and FC

Fig. 1. Clinical findings of Egyptian water buffaloes suffering from PHU and ketosis. (a) A buffalo with haemoglobinuria, (b) a buffalo with haematuria and blood clot in urine, (c) a hypophosphataemic buffalo with dullness and depression, (d) a buffalo with ketosis showing severe emaciation.

Table 2
Mean and standard error of enzymes, minerals and lipoprotein concentrations in control, ketotic and PHU Egyptian buffaloes measured four weeks post-parturient.

Groups	Control ($n = 5$)	Ketosis ($n = 5$)	PHU ($n = 5$)	P-value
Parameters				
LCAT (U)	$962.2^{a} \pm 16.605$	$521^{b} \pm 14.327$	$624.143^{b} \pm 20.796$	<0.001
AST (U/L)	$70.6^{a} \pm 4.164$	$140^{b} \pm 11.293$	$96.6^{c} \pm 3.784$	<0.001
GGT (U/L)	$7.21^{a} \pm 1.235$	$14.92^{b} \pm 1.486$	$12.49^{c} \pm 1.128$	0.004
Phosphorus (mg/dl)	$5.56^{a} \pm 0.44$	$6.22^{a} \pm 0.421$	$2.5^{b} \pm 0.167$	<0.001
Calcium (mg/dl)	$8.34^{a} \pm 0.136$	$8.66^{a} \pm 0.15$	$8.28^{a} \pm 0.22$	0.285
Glucose (mm(mmol/L))	$3.07^{a} \pm 0.242$	$1.66^{b} \pm 0.125$	$2.2^{c} \pm 0.118$	<0.001
TC (mg/dl)	$53.2^{a} \pm 4.363$	$23.80^{b} \pm 2.311$	$39.80^{c} \pm 1.463$	<0.001
CE (mg/dl)	$25.42^{a} \pm 4.817$	$9.84^{b} \pm 2.614$	$25.84^{a} \pm 1.640$	0.007
FC (mg/dl)	$27.78^{a} \pm 0.901$	$13.96^{b} \pm 0.69$	$13.96^{b} \pm 0.690$	<0.001
TG (mg/dl)	$30.44^{a} \pm 0.653$	$19.82^{b} \pm 0.554$	$24.42^{c} \pm 1.530$	<0.001
NEFA (mg/dl)	$365.6^{a} \pm 13.633$	$634^{b} \pm 20.902$	$494.00^{c} \pm 26.588$	<0.001
BHBA (mmol/L)	$1.0600^{a} \pm 0.0521$	$1.98^{b} \pm 0.0260$	$1.60^{c} \pm 0.176$	<0.001
Total protein	$6.22^{a} \pm 0.183$	$4.42^{b} \pm 0.248$	$4.80^{b} \pm 0.095$	<0.001
Albumin	$2.66^{a} \pm 0.147$	$1.6^{b} \pm 0.0894$	$1.86^{b} \pm 0.051$	<0.001
Globulin	$3.56^{a} \pm 0.136$	$2.82^{a} \pm 0.325$	$2.94^{a} \pm 0.108$	0.064

Means with different superscript letters of the same row denotes significant difference ($P < 0.05$).

were significantly reduced in PHU buffaloes compared to control. The level of NEFA and BHBA were significantly increased in ketotic and HP buffaloes compared to control ($P < 0.001$). However, the NEFA level was significantly higher in ketotic buffaloes compared to PHU buffaloes. The level of TG, total protein and albumin were significantly reduced in ketotic and PHU buffaloes compared to control. The levels of AST and GGT were significantly elevated in ketotic and PHU buffaloes. However, the AST and GGT levels were higher in ketotic than PHU buffaloes. All biochemical parameters are given in Table 2.

4. Discussion

Health and performance management systems for dairy animals focus on the early identification and subsequent prevention of production diseases (Ingvartsen et al., 2003) by either treating the affected animals or by improving the herd diet (Enjalbert et al., 2001). Buffaloes are one of the main sources for milk and meat production in Egypt. Therefore, it was crucial to investigate the early indicators for production-related diseases to help control these diseases before complication. Our result demonstrated that LCAT was significantly lower in 77% of the buffaloes examined. Since LCAT is responsible for esterification of cholesterol to cholesteryl esters (Nakagawa and Katoh, 1998), its reduction suggests that Egyptian water buffaloes have great susceptibility for metabolic abnormalities in the peripartum period, particularly in lipid metabolism. It is well-known that there is a dramatic requirements during late gestation and early lactation that make the dairy animal highly susceptible to the metabolic diseases e.g. ketosis and hepatic lipidosis (Osborne, 2003).

The clinical signs of ketosis and PHU were observed 4 weeks after parturition in buffaloes with low LCAT activity. The signs appeared in ketotic buffaloes were inappetance, emaciation and acetone odor that were consistent with those recorded in Indian buffaloes (Sharma and Rakesh, 2001; Teli and Ali, 2007). These signs were also similar to those observed in cattle (Akamatsu et al., 2007). Ketotic buffaloes represented 47.9% of all examined buffaloes, suggesting a high incidence of ketosis in Egyptian buffaloes. This could be attributed to the bad nutritional system, specially during the periparturient period.

The red urine (haemoglobinuria), the pale mucous membrane, the straining and the dullness and depression were consistent to signs PHU in Iranian river buffaloes (Dalir-Naghadeh et al., 2006), Indian buffaloes (Digraskar et al., 1991) and Pakistani buffaloes (Akhtar et al., 2008). The exact etiology for the haemolytic anaemia observed in PHU buffaloes is not established, although the reduction in P level was incriminated in many other studies (Chugh et al., 1998; Pirzada and Hussain, 1998; Dalir-Naghadeh et al., 2006). It has been demonstrated that phosphorus deficiency leads to impaired or insufficient antioxidant potential of the red blood cells in buffaloes and consequently increasing the oxidant injury and promoting haemolysis (Chugh et al., 1998). Moreover, it is hypothesized that hypophosphataemia results in decreased red blood cell glycolysis and ATP synthesis. Subnormal concentration of ATP predisposes red blood cells to altered structure and function, a loss of normal deformability, and an increase in fragility and haemolysis, with resultant haemoglobinaemia and haemoglobinuria (Akhtar et al., 2007).

The presence of AST in many organs of animals makes serum level a good marker of soft tissue damage but preclude its use as an organ-specific enzyme (Kramer, 1989). However, determining AST activities in dairy cows is most often connected with fatty liver syndrome (Cebra et al., 1997). Moreover, Steen et al. (1997) found that AST activity was greater in cows with ketosis (115 U/L) and hepatic lipidosis (252 U/L) than in healthy cows (70 U/L). The infiltration of hepatic cells with fat increases cell membrane permeability with subsequent release of AST enzyme that serves as a good tool for metabolic liver diseases (Karasai and Schefar, 1984). Therefore, the increased level of AST in ketotic buffaloes in our study could be attributed to the fatty liver changes associated with the negative energy balance occurring in the peripartum period. The activity of GGT activity was also significantly increased in ketotic buffaloes compared to control. Serum concentrations of GGT increases in liver and bile duct malfunctions (Steen et al., 1997) and liver is the main source of serum GGT (Kaneko, 1989). Therefore, the significant increase of GGT in ketotic buffaloes support the occurrence of the liver damage that may be induced by hepatic lipidosis.

Biochemical analysis demonstrated that buffaloes with ketosis had lowered blood glucose level, total cholesterol, CE, FC, triglycerides, total protein and albumin. These changes were consistent to those observed before in buffaloes (Sharma and Rakesh, 2001) and cows (Akamatsu et al., 2007; Yameogo et al., 2008). On the other hand, there were significant elevations in the levels of NEFA and BHBA, which was similar to those observed by Nazifi et al. (2008). The reduction in glucose level may occur in response to energy restriction in the diet (Bremmer et al., 2000) specially at the early stage of lactation when high rate of glucose utilization in the mammary gland is required (Nazifi et al., 2008). In response to low glucose level, fat mobilization is initiated to support the negative energy balance (Dann et al., 2005; Padilla et al., 2005) leading to elevation of NEFA and BHBA, which are importance source of energy when carbohydrate levels are reduced (Duffield, 2000). Many theories have been proposed to explain the elevation of ketone bodies in blood of ketotic animals. Central to these theories is the concept that acetyl-CoA production from fatty acids exceeds its removal by the citric acid cycle or lipogenesis, and hence acetyl-CoA tends to accumulate and contributes to enhanced ketone body synthesis (Kronfeld, 1961; Wieland et al., 1964). The elevation of FC is an indicator for the low activity of LCAT, which is responsible for esterification of FC to produce CE. Since the LCAT is synthesized in the liver (Jonas, 1998), the altered hepatocyte function (due to fatty changes occurring in ketosis) could presumably lead to accumulation of FC in the blood. However, in our study, the FC was unexpectedly reduced by 50% in both diseases (ketosis and PHU). The mechanism by which the FC is reduced in ketosis and PHU, instead of the hepatic dysfunction is unclear. Possible explanation is that the FC concentrations are not only regulated by hepatic tissue but also by several other mechanisms, for example, uptake by HDL from extrahepatic tissues or by the mammary gland in lactating animals (Miller et al., 1991; Nakagawa and Katoh, 1998). This uptake could contribute to the reduction of FC observed in cows affected with ketosis and PHU.

On the other hand, the decrease in serum triglycerides in ketotic buffaloes has been reported in liver injuries and fatty liver syndrome conditions (Reichel and Sokoi, 1987), which is due to low capacity of liver lipoprotein synthesis (Grummer, 1995). Therefore, the low triglycerides and high AST and GGT in our study support the occurrence of hepatic damage in Egyptian water buffaloes affected with ketosis. Since albumin is indicative of the liver's synthetic function (West, 1990), the reduction in total protein and albumin in our study is an indicator for hepatic injury. Moreover, hypoalbuminaemia is a common terminal feature of chronic liver disease, occurring when the functional hepatic mass has been reduced to 20% or less (Dunn, 1992).

The significant reduction of LCAT in PHU buffaloes compared to control healthy buffaloes could be attributed to haemolysis and liver injury. In severe haemolysis, as that occurs in PH, hypoxia develops which reduces the functions of liver (Stogdale, 1981) and therefore decreasing its capacity for synthesis of LCAT (Nakagawa and Katoh, 1998).

The reduction of P level (hypophosphataemia) in buffaloes with haemoglobunirua was consistent with the finding of Akhtar et al. (2007) and Kaya et al. (2008). This result supports the hypothesis that P deficiency plays a major role in the pathogenesis of this disease (Pirzada and Hussain, 1998). It has been reported that P deficiency is associated with decreased erythrocytic glucose-6-phosphate dehydrogenase (G6PD) activity in haemoglobinuric buffaloes that may be partially responsible for a decrease in reduced glutathione, thereby causing oxidative stress to erythrocytes, which results in haemolytic syndrome (Singari et al., 1991).

The glucose level in PHU buffaloes was significantly reduced and the BHBA was significantly increased compared to control. These results indicated that buffaloes with PHU had sub-clinical ketosis without obvious clinical signs. According to Duffield (2000), sub-clinical ketosis is defined as elevated concentrations of circulating ketone bodies in the absence of clinical signs of ketosis. It is clear from biochemical analysis that the changes in lipoproteins were milder (although significant from control) in PHU buffaloes than in buffaloes with clinical ketosis. The total cholesterol was significantly reduced in PHU buffaloes compared to ketotic and control group. This finding disagrees with that of Akhtar et al. (2008) which demonstrated no significant changes in cholesterol in buffaloes with PHU. This could be explained by the concurrent occurrence of hypoglycemia (ketosis) and PHU in our study.

The blood calcium level was not significantly changed in ketosis and PHU compared to control. This result was in line with that of Akhtar et al. (2007) and Khan and Akhtar (2007). It is well-known that Berseem as a green fodder is a rich source of calcium. It was concluded by Khan and Akhtar (2007) that PHU is strongly associated with Berseem feeding in winter season, probably because Berseem has high calcium to phosphorus ratio (>2:1) (Macwilliams et al., 1982). It was also reported that feeding Berseem represents a main risk factor for PHU in buffaloes, specially when no other sources of P are supplemented in the ration (Nagpal et al., 1968).

In conclusion, our results suggested that LCAT could be used as a predictor for pending metabolic disorders in Egyptian water buffaloes in the transition (periparturient) period. Knowing buffaloes susceptible to metabolic disorders may be helpful in formulation of suitable feeding and management systems to avoid the economic consequences of such diseases. Whether or not the changes in LCAT are attributed to the PHU alone or to the concurrent subclinical ketosis requires further investigations. However, to the best of our knowledge, this is the first report to suggest a relationship between LCAT and metabolic disorders in Egyptian water buffaloes.

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