Global Veterinaria 9 (1): 01-07, 2012
ISSN 1992-6197

Markers Used for Prediction of Ketosis and Milk Fever in Dairy Buffaloes at Kaliobeia Governorate

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Abstract: The present study was carried out to, determine the activity of plasma lecithin cholesterol acyltransferase which is the enzyme responsible for production of cholesteryl esters. 45 buffaloes on 4, 3 and 2 weeks before parturition indicated significant decrease in 25 animals (55.5%) while it was normal in 20 animals(45.5%) clinical examination of these animals two weeks post parturient revealed that 16 buffaloes had clinical signs of ketosis while 9 animals had clinical signs of milk fever. Serum samples were collected from 5 buffaloes of each ketotic and milk fever groups beside 5 clinically healthy (normal LCAT) as a control group. Results of blood analysis indicated that glucose level was significantly reduced in ketotic animals with increase of keton bodies while calcium and phosphorous levels were reduced in buffaloes affected to milk fever compared with ketotic and control animals, however total protein, albumin, CE, FC and TC were reduced in ketotic and milk fever cases. The level of AST, ALT and NEFA were elevated in ketotic and milk fever compared to control. Therefore LCAT could be a predicator for metabolic disorders in buffaloes. LCAT activity during the prepartuim period is suggested to be a useful marker for detection of animals susceptible to fatty liver-related postpartiurent diseases such as ketosis and milk fever.

Key words: LCAT · Milk Fever · Ketosis · Dairy Buffaloes

INTRODUCTION

High-yielding dairy buffaloes are susceptible to several postpartum diseases, including ketosis, retained placenta, milk fever and downer syndrome these diseases are suggested to originate from fatty liver developed during the non lactating period [1].

Control of metabolic diseases including ketosis and milk fever is crucially important because affected animals even after recovery have high incidences of reproductive and infectious diseases. 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 doner of the acyle 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 which is finally taken up by the liver [2].

LCAT is synthesized in the liver and therefore, its synthesis and/or excretion is impaired in hepatocellular diseases as indicated by decreased activity of LACT [3]. It has been demonstrated that LCAT activity is reduced in fatty liver in cows [4] and also in ketosis and left displacement of the abomasums [5]. The serum concentration is similarly decreased in these diseased cows, suggesting that the decrease in LCAT activity is involved in the development of periparturient metabolic disorders.

It has been reported that the most sever cases of ketosis usually occurred from 10 days to 10 weeks after calving because the stimulus for milk production is at its maximum and the demand of the mammary gland for glucose is often greater than the glucose available in blood and this imbalance increased hepatic ketogenes is [6,7]. 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 [5].

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Milk fever is a metabolic disorder affects buffaloes and causing some economic losses due to decreases milk production, cost of treatment and sometimes death [8].

The occurrence of postpartum diseases, especially milk fever is suggested to originate from fatty liver developed during the non-lactating period, over feeding during the non-lactating period and reduced feed intake and stress near parturition accelerate the release of non-esterified fatty acids (NEFA) from adipose tissues, resulting in an excess uptake of NEFA by liver. The incorporated NEFA are esterified into triglyceride, suppressed liver function due to fatty infiltrations of the liver are thought to be closely associated with the development of postpartum diseases [9]. Over feeding of cows during the dry period for longer than normal and when the ration is rich in protein poor in fiber is believed to be the common cause of fat syndrome [10]. LCAT has been extensively used as a predicator for metabolic diseases in dairy cows [4,5, 11]. Therefore, the objective of this study was to determine the relationship between LCAT level and the occurrence of metabolic diseases, especially ketosis and milk fever. Also, it aimed to evaluate other related biochemical changes.

MATERIALS AND METHODS

Animals: In this study, 45 dairy buffaloes aging from 4-7 years old belonging to a private dairy farm at Kaliobeia governorate were used. The animals fed on barseem, rice straw and concentrate mixture. All animals were clinically examined every day until the 4th weeks after parturition for ketosis, milk fever or any clinical related signs [10].

Samples

Faecal Samples: Were collected for microscopical examination either to prove or to roll out parasitic infestation [12].

Urine Samples: Fresh urine samples were collected from all animals into dry test tubes for examination of ketone bodies (Rothera's tests) [13].

Serum Samples: four blood samples were collected from each animal on the fourth, third and second weeks before the expected date of parturition for determination LCAT activity and the fourth sample was taken two weeks after parturition for biochemical analysis. Blood samples were collected from jugular vein into plan tubes, the samples were left to clot at room temperature; serum was separated by centrifugation and stored at-20°C until analyzed [14].

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*. [15]. Also, colorimetric determination of aseparate amino transferase (AST), alanine amino transferase (ALT) [16] glucose [17], total protein and albumin [18] total cholesterol (TC), free cholesterol (FC) and triglyceride [5], Ketone bodies [19], serum phosphorus [20] and serum calcium [21] were carried out. The cholesteryl ester (CE) was calculated by subtracting the (FC) concentration from that of TC as previously described [5].

Study Design: According to the estimated activity of LCAT during pre-partum and the clinical findings observed during first four weeks post-partum three groups each of 5 buffaloes were built up. Group I was selected randomly from that had low LCAT activity with ketonuria and emaciation, the group II was also randomly selected from buffaloes with low LCAT activity, showing sternal recombancy and tremor in head and neck and group III was randomly selected from healthy buffaloes with normal LCAT activity to be used as control.

Statistical Analysis: Data were expressed as means ± standard error (M±SE). The comparison between the three groups was conducted by one way analysis of variance [22].

RESULTS

Clinical Findings: Daily postpartum examination of animals with low LCAT activity showed that sixteen buffaloes (35.5%) exhibited the clinical signs of ketosis. They had reduced appetite, emaciation with acetone odor on breath. the presence of ketone bodies in the urine Nine animals (20%) showed clinical signs of milk fever whereas they suffered from excitement and tremors of head and neck, others showed sternal recumbency and head turned on flank, subnormal temperature, lateral recumbency and comatosed.

Biochemical Findings: The obtained results for LCAT activity of 45 examined buffaloes were low in 25 animals (55.5%) as shown in table 1. The other 20 animals (54.5%) had normal LCAT levels. Table 2 reveals that the serum calcium and phosphorous levels were significantly ($P \leq 0.05$) decreased in buffaloes with milk fever as compared with that of control and the levels were not affected in the ketotic buffaloes.

Table 1: The activity of LCAT(U) in 45 apparently healthy dairy buffaloes 30,21 and 14 days before parturition

days before parturation	Apparently healthy buffaloes n=20			Buffaloes suffer from ketosis n=16			Buffaloes suffer from milk fever n=9		
	LCAT-30	LCAT-21	LCAT-14	LCAT-30	LCAT-21	LCAT-14	LCAT-30	LCAT-21	LCAT-14
number	20	20	20	16	16	16	9	9	9
Mean	932	925.15	919.65	606	595.38	571.63	618.22	609.56	598
SE	± 4.66	±4.06	±3.67	± 10.9	±8.85	±11.1	±13.63	±13.03	±16.9
range	905-971	890-960	890-952	514-701	515-660	505-630	556-701	582-703	525-702

Table 2: Serum biochemical changes in dairy buffaloes suffered from ketosis and milk fever two weeks post partiurition

Parameters	Apparently healthy N=5	Suffered from ketosis N=5	Suffered from milk fever N=5
LCAT(U)	908.8±3.73^{c}	546.4±7.68^{a}	581±12.37^{b}
AST (U/L)	48.8 ±2.74 ^{a}	88.16±3.19 ^{c}	76.3±1.37 ^{b}
ALT (U/L)	29.04±0.845 ^{a}	50.94±1.39 ^{c}	42.68±1.69 ^{b}
Glucose ((mmol/l)	3.58±0.303 ^{b}	1.93±0.261 ^{a}	3.00±0.049 ^{b}
Calcium (mg/dl)	8.43±0.136 ^{b}	8.40±0.071 ^{b}	3.21±0.087 ^{a}
Phosphorous (mg/dl)	6.84±0.221 ^{b}	6.88±0.235 ^{b}	5.04±0.144 ^{a}
TC (mg/dl)	53.00±0.727 ^{c}	25.30±0.540 ^{a}	30.54±0.379 ^{b}
FC (mg/dl)	27.99±0.376 ^{b}	14.01±0.059 ^{a}	13.72±0.326 ^{a}
CE (mg/dl)	25.00±0.984 ^{c}	11.28±0.522 ^{a}	16.82±0.586 ^{b}
TG (mg/dl)	30.53±0.069 ^{c}	19.87±0.031 ^{a}	24.41±0.27 ^{b}
NEFA (mg/dl)	364.94±1.234 ^{a}	609.20±9.39 ^{c}	489.74±1.54 ^{b}
Ketones (mg/dl)	8.24±0.095 ^{a}	19.52±0.229 ^{b}	8.19±0.087 ^{a}
Albumin (g/dl)	2.77±0.083 ^{c}	1.66±0.051 ^{a}	1.87±0.009 ^{b}
Globulin (g/dl)	3.63±0.055 ^{b}	2.85±0.0159 ^{a}	2.95±0.024 ^{a}
Total protein (g/dl)	6.41±0.088 ^{c}	4.51±0.060 ^{a}	4.82±0.029 ^{b}

Values with different litters within the same row differed significantly at ($P \leq 0.05$).

Blood glucose level was significantly ($P \leq 0.05$) decreased in ketotic and milk fever affected buffaloes. The total cholesterol, CE and FC were significantly ($P \leq 0.05$) decreased in both ketotic and milk fever buffaloes as compared to that of the control group. The level of NEFA and ketones were significantly ($P \leq 0.05$) increased in ketotic buffaloes as compared to milk fever and the control group. The level of TG, total protein and albumin were significantly ($P \leq 0.05$) decreased in ketotic and milk fever affected buffaloes as compared to the control group. The level of AST and ALT were significantly ($P \leq 0.05$) elevated in ketotic and milk fever affected buffaloes.

DISCUSSION

Health and performance management systems for dairy animals focus on the early identification and subsequent prevention of production diseases [23] by either treating the affected animals or by improving the herd diet [24]. Buffaloes are the main sources of 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.

The present study demonstrated that LCAT was significantly low in (55.5% of examined animals) on the 4th week before parturition. These findings were in accordance with those of Mohamed *et al*. [25]. In the same time the reduced activity of this enzyme before day-21 from parturition was reported by Hisami and Norio[26] and Hirohisa *et al.* [7] who reported reduced LCAT enzyme activity 14 day before parturition. Since LACT is responsible for estrification of cholesterol to cholesteryl esters [5], its reduction suggested that Egyptian buffaloes have great susceptibility for metabolic abnormalities in postpartum period particularly in lipid metabolism. It is well known that is a lack of requirements during late gestation and early lactation make the dairy animals susceptible to the metabolic diseases e.g. ketosis and lipidosis [27]. The observed clinical signs of ketosis were seen after parturition in buffaloes with low LCAT activity were inappetance, emaciation, sharp drop of milk production and acetone odor. These signs were similar to those observed by Sharma and Rakesh [28] and Teli and Ali [29]. These clinical findings maybe attributed to feeding practices, individual characteristics, general environmental factors, hyperketonemia and consequently the decline in feed intake which resulted in rapid

mobilization of adipose tissues and protein storage, which provide gluconeogenic amino acids to support hepatic glucose production as reported by Fouda and Gehad [30]. On the other hand, the clinical examination of the other group of diseased buffaloes revealed a variety of clinical signs such excitement, restlessness, tremor of head and neck, sternal recumbency with head turned into the flank region and subnormal temperature (36.5°C), cold extremities, loss of anal reflex with constipation. Also, lateral recumbency and the animal became comatozed in later stage. These signs were similar to those recorded by Yamagishi and Naito [31], Abd El-Raof and Mobarak [32] and Radostitis *et al,* [10] who suggested that susceptibility appear to be related to the extremely high turnover of fluid, salts and soluble organic materials during the early period of lactation. Biochemical analysis revealed that buffaloes with ketosis had lowered blood glucose level, total cholesterol, FC, triglyceride, total protein and albumin (Table 2). These changes were agreed with those reported by Sharma and Rakesh [28], Akamatsu *et al*. [11] and Yameogo *et al*. [33]. On the other hand, there were significant elevation in the levels of NEFA and ketons. This findings were similar to those observed by Nazifi *et al*. [34]. This reduction in glucose level may occur in response to energy restriction in the diet [35] especially at the early stage of lactation whereas high rate of glucose utilization in the mammary gland is required [34]. In response to low glucose level, fat mobilization is initiated to support the negative energy balance [36,37] leading to elevation of NEFA and ketons, which are important source of energy when carbohydrate level are reduced as reported by Duffield [38] who said that sub-clinical ketosis is defined as elevated concentrations of circulating keton bodies in the absence of clinical signs of ketosis. The elevation of FC is an indicator for low activity of LCAT which is responsible for FC esterification to produce CE since LCAT is synthesized in liver [39]. The ketotic buffaloes under study reveled significant reduction in triglycerides which could be attributed to increased lipid uptake by hepatic cells leading to the development of hepatic lipidosis, with concurrent reduction in hepatic out put of triglycerides which consequently reduces the level of circulating triglycerides [40] these results were in agreement with those reported by Smith *et al*. [41] and Ali [42] who proved that during ketosis in dairy cattle, there is usually increased mobilization of non-estrified fatty acids from adipose tissues, which leads to deposition of triacylglycerides within the hepatocyts and consequently reduces the levels of triglycerides [43]. Moreover, Grummer [44] reported low capacity of liver lipoprotein synthesis in ketotic animals.

Regarding the activity of liver enzymes AST and ALT as shown in table 2, there were significant increasein these enzymes in animals with ketosis as compared to healthy one. These results agree with that reported by Nagamania *et al*. [45], Steen *et al*. [46] and Omran *et al*. [47]. The infiltration of hepatic cells with fat increase cell membrane permeability with subsequent release of enzymes that serves as a good tool of metabolic diseases [6,10,48]. Therefore the increased activity of AST in ketotic buffaloes in this study could be attributed to the fatty liver changes associated with the negative energy balance occurring in the peripartum period. The reduction of total protein and albumin reported in the present study is an indicator for hepatic injury. Similar findings were previously reported by West [49] and Radostitis *et al.* [50]. The reduced LCAT activity in milk fever appeared to be due to fatty liver [5,15] and CE concentration which is the product of LACT reaction is also decreased in cows with fatty liver [5]. In addition the patterns of increase in the NEFA concentration and AST activity were similar to those in ketotic animals, however postparturient diseases such as ketosis and milk fever are thought to be derived from fatty liver developed during the non lactating stage [51,52]. The blood calcium level was not significantly changed in ketotic cases as compared to the control group. This result was in line with that of Akhtar *et al.* [53] and Khan and Akhtar [54]. Biochemical analysis of sera from paretic buffaloes showed highly significant decrease of in serum calcium and phosphorus levels. These results were in agreement with those recorded by Abd EL-Raof and Mobark [32], Mullen, [55] and Berger and Gerber [56].

In conclusion, LCAT activity was unaltered during peripartum period at least in healthy animals. A reduction in LCAT activity was observed prior to the occurrence of ketosis and milk fever. LCAT activity during the preipartum period is suggested to be a useful marker for detection of animals susceptible to fatty liver-related post-parturient diseases which may be helpful in formulation of suitable feeding and management systems to avoid the economic consequences of such diseases.

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