CLINICO-BIOCHEMICAL STUDIES OF KETOSIS IN BUFFALO (*BUBALUS BUBALIS*)

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ABSTRACT

The study was conducted on 18 clinical cases of ketosis presented in the Teaching Veterinary Clinical Complex, Faculty of Veterinary Science and Animal Husbandry (F.V.Sc & A.H), R.S. Pura and areas around R.S. Pura Tehsil. Clinical and haemato-biochemical parameters were studied in the affected animals. A minor decrease in mean body temperature with an increase in severity of ketosis was noted. The severe cases had diminished rate of ruminal motility and prominent clinical signs were sudden and unexpected drop in milk production, depraved appetite, wasting and depression. Biochemical parameters viz. plasma glucose, total plasma protein, LDL and HDL-cholesterol, calcium and magnesium decreased significantly.

Keywords: ketosis, buffalo, biochemical parameters

INTRODUCTION

Ketosis is a common metabolic disorder frequently observed in dairy cows during the early lactation period characterized by increased levels of ketone bodies in the blood, urine, and milk. In buffalo, ketosis remains one of the major diseases that decrease the productivity (Ghanem and El-deeb, 2010). Ketosis can be clinical or subclinical depending on the subjectivity of the clinical signs. It is generally accepted that clinical ketosis occurs in ruminants when they are subjected to demands on their resources of glucose and glycogen that cannot be met by their digestive and metabolic activity. Clinical ketosis has visible clinical symptoms and typically occurs within the first six to eight weeks post-calving, resulting in anorexia, licking and blindness, hard dry feces, rapid loss of condition, and decreased milk production (Youssef *et al.,* 2010). In addition, the milk fat yield of ketotic cows is increased due to the availability of beta-Hydroxybutyric acid (BHBA) and fatty acids. Clinical ketosis is easy to diagnose by its clinical

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symptoms. The present study was conducted to study the clinico-biochemical parameters in buffaloes with clinical ketosis.

MATERIALS AND METHODS

The study was undertaken for 8 months duration. Buffaloes with history of anorexia, hard dry feces, rapid loss of condition, and decreased milk production drop in milk production presented at Teaching Veterinary Clinical Complex of the college, constituted the cases for this study. A total of 18 cases (Murrah, Jaffarabadi and few Non-descript) were found to be suffering from ketosis diagnosed by history, clinical signs and by urine and milk nitroprusside tests viz. Modified Rothra's, Ross Modification of Rothra's test and Multidiagnostic strip reaction (supplied by Siemens, India). Three buffaloes were used as control. Blood samples from the buffaloes were collected from jugular vein. Plasma was separated by centrifugation at 3000 rpm for 15 minutes and was stored at -20^{o}C. About 2 ml of blood was collected for glucose estimation. Blood glucose, total serum protein, albumin, Alanine transaminase(ALT), Aspartate Aminotransferase (AST), Blood urea nitrogen, Createnine, HDL- Cholesterol, LDL-Cholesterol, calcium, phosphorus, and magnesium were estimated using UV-Spectophometer by employing standard kits. The statistical analysis was done as per the method described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The present study revealed varying degrees of frequency of occurrence of symptoms in ketosis with sudden decline in milk yield being present in 100 per cent of the cases (Table 1). This was followed by selective feeding (79.14%), wasting (49.91%), depression (36.67%), complete anorexia (29.37%), acetone smelling breath (19.35%), dry mucus coated feces (12.90%) and signs of central nervous system involvement (6%) of the cases of bubaline ketosis. The pattern of signs observed in this study was similar to clinical profile described by other workers (Roy and Ghorui, 2000; Radostits *et al.,* 2007).

The present study elucidated that ketosis was predominantly accompanied by a drop of 3.44±0.1 litres milk/animal/day estimating 34.92 on per cent basis. Decline of 25-60 percent in milk production in bovine clinical ketosis has also been placed on record by, Swain and Tripathy (1987) and Mir and Malik (2003). The possible reason for the decreased milk production could be reduced capacity of the animal to supply the lactogenic precursors to mammary gland than the capacity of the gland to produce due to homeorhetic drive for production (Lean *et al.,* 1992). Moreover, elevated blood ketones also result in decreased milk production (Andersson and Lundstrom, 1985). In the present study depressed ruminal motility was recorded in ketotic buffaloes and this could be attributed to excessive generation of ketone bodies, as ketones bodies are reported to effect ruminal motility causing incomplete and depressed ruminal contraction (Lean *et al*., 1991). In severe cases mean rumen motility was as low as 1.77±0.11 against 2.17±0.19 per two minutes in mild cases.

In the present study a significant decrease ($P<0.05$) in Glucose (36.64 mg/dl), Total protein (5.52 g/dl), Albumin(2.03 g/dl) and A:G ratio (0.57) was observed when compared with healthy control (Table 2). The decrease of glucose level may occur in response to intake of low energy diet specially at

the early stage of lactation when high rate of glucose utilization in the mammary gland is required (Nazifi *et al.,* 2008). Hypocalcemia can exert an additional depressive effect on endogenous glucose production, hence, aggravating hypoglycemia (Schlumbohm and Harmeyer, 2003). Decrease in plasma glucose level in ketosis has also been by Youssef *et al,* (2010) in lactating buffaloes. 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. In the energy deficient ketotic animals labile pool of body protein also serves as an important source for energy synthesis of milk lactose and milk protein (Radostits *et al.,* 2007). This protein catabolism for an increased rate of gluconeogenesis may be the reason for a reduction in total plasma protein levels. Similar results have also been recorded by Youssef *et al.,* (2010).

Buffaloes suffering from ketosis showed a non significant increase in blood urinary nitrogen (22.27mg/dl) level and createnine (2.18 mg/dl) (Table 2). The high levels of blood urea results from either increased breakdown of tissue or dietary protein or impaired excretion. Significantly higher average values of AST (144.81 U/L) and ALT (144.8 U/L) were observed in ketotic buffaloes when compared to healthy control (Table 2). Although AST is non-specific liver enzyme estimation of its activity in dairy cows is most often associated with fatty liver syndrome (Cebra *et al.,* 1997). AST has been found to increase significantly in ketotic cows compared with healthy ones (Youssef *et al.,* 2010). 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). Consequently, in the present study, the elevated serum AST in ketotic buffalo compared with control ones could be due to negative energy balance. Similarly, ALT has been found to increase in liver and bile duct malfunctions (Steen *et al.,* 1997). Consequently, in the present study, the high AST and ALT support the occurrence of hepatic damage in ketotic buffalo.

Significantly lower values of Cholesterol were observed in buffaloes 84.9 mg/dl, when compared with the control group (Table 2). The low cholesterol observed could be attributed to mild liver asteatosis which cause reduction in cholesterol formation in the liver (Grummer, 1995). However, Anantwar and Singh (1993) reported that there was an increase of cholesterol levels in ketotic animals. The decrease of serum cholesterol in ketotic buffaloes is similar to clinical conditions caused by liver injuries and fatty liver syndrome in cattle (Marcos *et al.,* 1990). HDL and LDL-cholesterol level (64.90 mg/dl and 30.42 mg/dl, respectively) showed a significant decrease in ketotic buffalo in comparison to control groups (Table 2). These results coincide with those of Turk *et al.* (2008) and Youssef *et al.* (2010). These results may be attributed to moderate liver steatosis, which causes reduction in cholesterol level. In contrast to present study, Youssef *et al.* (2010) observed there was non-significant decrease in LDL-cholesterol levels in ketotic buffalo in comparison to the normal ones.

Significantly lower levels of plasma calcium, phosphorus and magnesium were observed in ketotic buffaloes 8.14 mg/dl, 4.77 mg/dl and 1.90 mg/dl respectively when compared with the control group (Table 2). The decrease of phosphorus and magnesium level coincided with the findings obtained by Ziogas *et al.* (2007) and Youssef *et al.* (2010). Insufficient phosphorus supply in the diet, prolonged anorexia, and increased urinary phosphorus excretion due to hyperparathyroidism could explain presence of hypophosphatemia in this

Table 1. Important clinical signs observed in clinically ketotic buffaloes (n=18).

S. NO.	Clinical Signs	Percent animals
1.	Nervous signs	6
2.	Constipation	12.90
3.	Acetone smell in breath	19.35
4.	Complete anorexia	29.37
5.	Depression	36.67
6.	Wasting/ woody appearance	49.91
7.	Selective feeding (partial anorexia)	79.14
8.	Sudden drop in milk yield	100

Table 2. Plasma biochemical and mineral values in ketotic buffaloes.

Parameters	Control	Ketotic animals
Glucose (mg/dl)	58.4±2.86	36.64±1.19*
T Protein (g/dl)	7.44±0.26	5.52±0.13*
Albumin (g/dl)	3.13±0.15	2.03±0.05*
A:G ratio	0.71±0.01	0.57±0.007*
ALT (IU/L)	31±3.1	43.90±3.22*
AST (IU/L)	118.2±4.07	144.8±2.19*
Cholesterol (mg/dl)	179.11±7.18	84.9 ±3.16*
LDL-Cholesterol (mg/dl)	48.4±4.60	30.42±1.05*
HDL-Cholesterol (mg/dl)	86.5±2.97	64.90±1.62*
Creatinine (mg/dl)	1.71±0.05	2.18±0.05
BUN (mg/dl)	18.3±1.24	22.27±1.10
Ca (mg/dl)	10.8±0.41	8.14±0.09*
P (mg/dl)	5.80±0.19	4.77±0.07
Mg (mg/dl)	2.20±0.05	1.90±0.03*

Means marked with asterisk (*) differ significantly ($p<0.05$) from the control group value in a column.

condition. However, the decrease of magnesium level could be attributed to ketonuria which results in decrease tubular resorption or to the need of magnesium that regulate metabolism for milk secretion. In reference to the fall in calcium levels it is suggested that there might be increased loss of base in urine to compensate ketosis induced acidosis. Besides, Cote *et al.* (1969) indicated that reduced feed intake may also lead to secondary hypocalcemia.

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