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**Nagesh Guru**

PG Scholar, Department of  
Veterinary Medicine, Veterinary  
College, KVAFSU, Bidar,  
Karnataka, India

**Sandeep Halmandge**

Professor and Head, Department  
of Veterinary Medicine,  
Veterinary College, KVAFSU,  
Gadag, Karnataka, India

**Ravindra BG**

Professor and Head, Department  
of Veterinary Clinical Complex,  
Veterinary College, KVAFSU,  
Shivamogga, Karnataka, India

**Vinay Tikare P**

Associate Professor and Head,  
Department of Veterinary  
Pharmacology and Toxicology,  
Veterinary College, KVAFSU,  
Gadag, Karnataka, India

**Mallinath KC**

Assistant Professor, Department  
of Veterinary Microbiology,  
Veterinary College, KVAFSU,  
Bidar, Karnataka, India

**Vivek Kasaraliker R**

Retired Professor and Head,  
Department of Veterinary  
Medicine, Veterinary College,  
KVAFSU, Bidar, Karnataka,  
India

**Corresponding Author:**

**Nagesh Guru**

PG Scholar, Department of  
Veterinary Medicine, Veterinary  
College, KVAFSU, Bidar,  
Karnataka, India

## Prevalence and biochemical alterations of subclinical ketosis in lactating buffaloes in Bidar

**Nagesh Guru, Sandeep Halmandge, Ravindra BG, Vinay Tikare P,  
Mallinath KC and Vivek Kasaraliker R**

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### Abstract

Ketosis is an important production disease and continues to cause significant economic losses to the farmers. Subclinical ketosis (SCK) causes greater losses than clinical ketosis because it occurs more frequently and often cannot be detected by farmers. In the present study a total of 108 early lactating buffaloes maintained in various organized and unorganized dairy farms in and around Bidar were screened to know the prevalence of subclinical ketosis. The findings of the present study revealed the overall prevalence of Subclinical ketosis in lactating buffaloes based on Precision xtra® blood BHBA meter was 14.81 per cent. Highest prevalence of SCK was recorded in third parity and least in first parity based on blood BHBA test. The biochemical study indicated statistically significant decrease in serum glucose, albumin, calcium and serum phosphorus, further statistically significant increase in AST in Subclinical ketosis affected buffaloes.

**Keywords:** Subclinical ketosis, Buffaloes, Prevalence, Biochemical

### Introduction

Indian buffaloes are important source of milk supply today and yield nearly three times as much milk as cows (Yadav *et al.*, 2017) <sup>[1]</sup>. Among various production diseases, ketosis is an important production disease and continues to cause significant economic losses to the farmers. Subclinical ketosis (SCK) causes greater losses than clinical ketosis because it occurs more frequently and often cannot be detected by farmers (Brunner *et al.*, 2019) <sup>[2]</sup>. As the course of the diseases is often subclinical and commonly goes unnoticed by the farmers, early detection is very important to mitigate further losses (Enjalbert *et al.*, 2001) <sup>[3]</sup>. Diagnosis of individual cases of subclinical ketosis at an earlier point in the course of the disease would permit earlier treatment and help to extenuate further losses. Due to the absence of signs or symptoms for subclinical ketosis, blood, urine and milk tests are used as diagnostic tools to detect ketone bodies concentration in circulation. The volatile nature of acetone and relatively unstable nature acetoacetate make them unreliable as a cow side test. Beta hydroxy butyric acid (BHBA) on the other hand is more stable and is detectable in urine, milk and blood.

### Material and methods

A total of 108 early lactating buffaloes maintained in various organized and unorganized dairy farms in and around Bidar were screened for subclinical ketosis. To diagnose the subclinical ketosis in buffaloes, pen side diagnostic tests was employed *viz.*, enzyme based electrochemical test for blood BHBA using Precision Xtra® blood glucose and ketone monitoring system, the drop of blood was placed on the BHBA strip and results were recorded. The blood samples were collected from six subclinical ketosis affected buffaloes and separated serum for estimation of serum Glucose, AST, Total protein, Albumin, Calcium and Phosphorus by using Semi-automated clinical chemistry analyzer. A total of six apparently healthy early lactating buffaloes which were negative for subclinical ketosis were kept as healthy control group.

## Results and discussion

In the present investigation, a total of 108 apparently healthy buffaloes suspected of subclinical ketosis were screened using a handheld ketone meter (Precision Xtra®), employing a cut-off point of  $\geq 1.0$  mmol/L of  $\beta$ -hydroxybutyrate (BHBA) (Whitaker *et al.*, 1983) [4]. Out of these, 16 buffaloes were found positive, recording an overall prevalence of 14.81 per cent. Similar prevalence rates were also reported by Katta (2003) [5], Padmaja (2009) [6], and Youssef *et al.* (2010) [7]. However, Surender (2018) [8] reported comparatively lower prevalence of subclinical ketosis in recently calved buffaloes. Earlier reports have also documented varying rates of prevalence of subclinical ketosis in buffaloes in India (Gupta, 2012 [9]; Krishna *et al.*, 2014 [10]). Regional and country-wise differences in animal husbandry practices play an important role in ketosis prevalence. Differences in study design and methodology may also account for variation in prevalence estimates. Such wide variation in prevalence might be attributed to agro-climatic conditions, climatic changes, breed susceptibility, stage of lactation, production potential, feeding habits, and managerial practices followed by owners.

In the present investigation, the highest prevalence of subclinical ketosis in buffaloes was recorded in the third parity, followed by fourth parity, second parity, and fifth and above parity, with the lowest prevalence observed in first parity animals. These results were in agreement with McArt *et al.* (2012) [11], who reported that the prevalence of subclinical ketosis was highest during the third parity, followed by the first parity. Similarly, Krishna *et al.* (2014) [10] recorded the highest prevalence of SCK during the fourth lactation, followed by third and second lactations, with the lowest prevalence during the first lactation. Kumar *et al.* (2015) [12] reported that most cases of ketosis occurred during the third lactation in animals aged 3–9 years. Vanholder *et al.* (2015) [13] reported that the risk of developing subclinical ketosis was higher in second parity and older cows compared with heifers. The higher prevalence of SCK in the third parity might be due to the fact that animals at this stage attain their genetically determined peak productivity and are unable to withstand the metabolic shift arising from increased nutritional demands, making them more susceptible to production diseases (Thirunavukkarasu *et al.*, 2010) [14].

The mean BHBA concentration (mmol/L) in the SCK-affected group was significantly higher compared to the healthy control group. The findings of the present study are in accordance with observations reported by McArt *et al.* (2012) [11], Ghanem *et al.* (2016) [15], and Surender (2018) [8]. BHBA is regarded as the gold standard for diagnosing subclinical ketosis in cows and buffaloes during lactation, as it is more stable than acetoacetate and acetone. BHBA is the predominant circulating ketone body and remains relatively stable in whole blood, plasma, and serum (Dohoo and Martin, 1984) [16].

Serum glucose level is considered one of the key indicators of energy status in ruminants. In the present study, blood glucose concentration was significantly lower in subclinical ketosis-affected buffaloes compared to the healthy control group. These results correlate well with the findings of Youssef *et al.* (2010) [7], Padmaja and Rao (2013) [17], and Surender (2018) [8] in subclinical ketotic buffaloes, as well as Hagawane *et al.* (2009) [18] in early lactation buffaloes, and Youssef *et al.* (2010) [7] and Bali *et al.* (2016) [19] in clinically ketotic buffaloes. The reduced blood glucose concentration during early lactation is attributed to the large withdrawal of glucose by the mammary gland for lactose synthesis (Schultz, 1968)

[20]. Hypoglycaemia may also occur due to intake of low-energy diets, particularly during early lactation when glucose demand by the mammary gland is high (Nazifi *et al.*, 2008) [21]. Glucose serves as the principal substrate for lactose synthesis, and approximately 60–80 per cent of circulating glucose is utilized by the mammary gland for milk production (Annison and Linzell, 1963) [22]. Consequently, hypoglycaemia observed in early lactating buffaloes in the present study can be attributed to increased glucose utilization for lactose synthesis.

Aspartate transaminase (AST) activity showed a significant increase in subclinical ketosis-affected buffaloes compared with healthy controls. These findings were in agreement with Youssef *et al.* (2010) [7], Padmaja and Rao (2013) [17], and Bali *et al.* (2016) [19]. However, Surender (2018) [8] recorded a non-significant increase in AST levels in subclinical ketotic buffaloes compared with healthy animals. Furthermore, Ghanem *et al.* (2016) [15] observed a significantly higher AST activity in clinically ketotic cows compared to SCK and healthy cows. Aspartate transaminase is a non-specific liver enzyme, and its increased activity in dairy animals is often associated with fatty liver syndrome (Djokovic *et al.*, 2016) [23]. Serum AST activity correlates with the degree of hepatic fatty infiltration (Dokovic *et al.*, 2012) [24]. During early lactation, cows experiencing severe negative energy balance (NEB) exhibit reduced hepatic capacity to export triglycerides (TAG) as very-low-density lipoproteins (VLDL), leading to hepatic accumulation of TAG and subsequent fatty liver development (Rukkwamsuk *et al.*, 1999) [25], ketosis (Herd, 2000) [26], and increased hepatocellular membrane permeability with subsequent release of AST (Karasai and Schefar, 1984) [27]. Therefore, elevated AST levels observed in subclinical ketotic buffaloes may be attributed to negative energy balance.

The present study revealed no significant difference in total protein levels between healthy control and SCK-affected groups. This finding corroborates reports by Youssef *et al.* (2010) [7], Hussein *et al.* (2015) [28], and Surender (2018) [8]. However, Padmaja and Rao (2013) [17] and Singh (2015) [29] reported significantly lower total plasma protein levels in SCK-affected buffaloes. Similarly, Singh *et al.* (2017) [30] and Paramesh *et al.* (2020) [31] reported a significant decrease in serum total protein levels in subclinical ketotic cows. In contrast, Hagawane *et al.* (2009) [18] reported slightly elevated total protein levels in early lactation buffaloes. Total protein concentration provides information regarding liver and kidney function as well as nutritional status (Stojevic *et al.*, 2005) [32]. The absence of significant differences in total protein levels between groups in the present study indicates no derangement in serum total protein concentration in subclinical ketosis.

In the present study, a significant decrease in albumin levels was observed in the SCK-affected group compared with healthy controls. Similar findings were reported by Padmaja and Rao (2013) [17] and Singh (2015) [29]. These results were also in agreement with Reddy (2015) [33], who reported significantly lower albumin concentrations in SCK-affected dairy cows. However, Youssef *et al.* (2010) [7] and Hussein *et al.* (2015) [28] reported non-significant differences in albumin levels in subclinical ketotic buffaloes, while Akgul *et al.* (2017) [34] and Singh *et al.* (2017) [30] reported no significant difference in albumin levels in subclinical ketotic cows.

The decreased albumin level in SCK-affected buffaloes could be attributed to hepatic insufficiency. Albumin is an indicator of liver synthetic function (West, 1990) [35], and a decreased

concentration of albumin reflects hepatic insufficiency (Whitaker, 2000) [36]. The decreased liver synthesis of albumin may be induced by the development of fatty infiltration of the liver (Lubojacka *et al.*, 2005) [37]. The serum calcium level was found to be low in the affected group, indicating hypocalcaemia. These findings were in accordance with those reported by Hagawane *et al.* (2009) [18], Padmaja and Rao (2013) [17], and Singh (2015) [29] in subclinical ketotic buffaloes, and Bali *et al.* (2016) [19] in clinically ketotic buffaloes. However, Youssef *et al.* (2010) [7] found non-significant changes in calcium levels in post-parturient ketotic and subclinical ketotic buffaloes. This could be attributed to increased loss of base in urine to compensate for ketosis-induced acidosis. The lower level of serum calcium in subclinical ketosis can also be attributed to a high concentration of  $\beta$ -hydroxybutyrate (BHBA), which impairs the absorption and utilization of calcium in dairy cows during the early lactation period (Zhang *et al.*, 2009) [38]. Consequently, the decrease in calcium levels could be a result of impaired absorption of food metabolites from the gastrointestinal tract, excessive losses through urine and

colostrum (especially during excessive milking), and insufficient mobilization from the skeleton (Hagawane *et al.*, 2009) [18].

In the present study, a significantly low level of phosphorus was recorded in the affected group. This finding is in accordance with Youssef *et al.* (2010) [7] and Hagawane *et al.* (2009) [18], who reported significantly low phosphorus levels in subclinical ketotic buffaloes. Moreover, Bali *et al.* (2016) [19] reported significantly low phosphorus levels in clinically ketotic buffaloes, and Ghanem *et al.* (2016) [15] demonstrated a significant decrease in serum phosphorus levels in ketotic cows.

The decrease in serum phosphorus level observed in the present study might be due to the large amount of inorganic phosphorus drained from blood for milk production, hyperparathyroidism induced by low serum calcium levels, and increased urinary losses (Valk *et al.*, 2002) [39]. The lower level of serum phosphorus in ketosis may also be attributed to compensation for ketosis-induced acidosis, which triggers increased excretion of phosphorus via urine (Fatur, 1994) [40].

**Table 1:** Prevalence of subclinical ketosis in lactating buffaloes

Diagnostic test	No. of buffaloes screened	No. of buffaloes found positive	Prevalence (%)
Blood BHBA test using hand held ketone meter (Precision Xtra®)	108	16	14.81

**Table 2:** Parity wise prevalence of subclinical ketosis in lactating buffaloes

Sl. no.	Parity	No. of buffaloes screened	No. of buffaloes found positive	Prevalence (%)
1	First	29	2	6.90
2	Second	15	2	13.33
3	Third	19	6	31.58
4	Fourth	32	5	15.62
5	Fifth and above	13	1	7.69
	Overall	108	16	14.81

**Table 3:** Mean  $\pm$  SE biochemical parameters of healthy and SCK affected lactating buffaloes

Sl. no.	Parameter	Healthy control (N=6)	SCK affected buffaloes (N=6)
1	BHBA (mmol/L)	0.45 $\pm$ 0.08 <sup>a</sup>	1.22 $\pm$ 0.14 <sup>b</sup>
2	Glucose (mg/dL)	51.37 $\pm$ 0.93 <sup>b</sup>	35.18 $\pm$ 3.40 <sup>a</sup>
3	AST (U/L)	103.60 $\pm$ 5.74 <sup>a</sup>	148.80 $\pm$ 12.10 <sup>b</sup>
4	Total protein (g/dL)	6.67 $\pm$ 0.21 <sup>a</sup>	6.58 $\pm$ 0.12 <sup>a</sup>
5	Albumin (g/dL)	2.93 $\pm$ 0.08 <sup>b</sup>	2.23 $\pm$ 0.23 <sup>a</sup>
6	Calcium (mg/dL)	10.76 $\pm$ 0.28 <sup>b</sup>	7.93 $\pm$ 0.27 <sup>a</sup>
7	Phosphorus (mg/dL)	6.07 $\pm$ 0.15 <sup>b</sup>	4.76 $\pm$ 0.30 <sup>a</sup>

Means bearing different superscripts within a row differ significantly ( $p \leq 0.05$ ).

## Conclusion

Hand held ketone meter Precision Xtra® was most specific cow side diagnostic test to detect subclinical ketosis in lactating buffaloes. Overall prevalence of Subclinical ketosis in lactating buffaloes was 14.81 per cent. Results of biochemical study indicated statistically significant decrease in serum glucose, albumin, serum calcium and serum phosphorus, further statistically significant increase in AST in Subclinical ketosis affected buffaloes.

## Conflict of Interest

Not available

## Financial Support

Not available

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