

COMPARATIVE EVALUATION OF KETONE BODIES IN BLOOD AND URINE FOR THE DETECTION OF SUBCLINICAL KETOSIS IN POSTPARTUM BUFFALOES

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ABSTRACT: Buffaloes are one of the major contributors of milk production in Pakistan. Like other dairy animals, it also faces a variety of metabolic disorders right after parturition. Among these problems ketosis is a major one in high producing buffaloes when energy demands (e.g. high milk production) exceed energy intake and result in a negative energy balance. When large amount of body fat is utilized as an energy source to support production, fat is sometimes mobilized faster than the liver can properly metabolize it. If this situation occurs, ketone production exceeds ketone utilization by the animal, and ketosis results. At early stage Sub-clinical ketosis occurs which if not detected and controlled proceeds to clinical ketosis that has economic impact in terms of both production and reproduction losses. Early detection of sub-clinical ketosis is of vital importance to minimize the subsequent losses associated with this condition. Many studies have been conducted in past to diagnose sub-clinical ketosis in cattle, but a very little data is available for buffaloes. This study was aimed to compare digital ketometer and conventional urine strips method to detect Ketone bodies in blood and urine samples respectively for the early diagnosis of subclinical ketosis in buffaloes. For this purpose, blood (n=100) and urine (n=100) samples were collected from buffaloes at post-partum period, from three different herds and were processed for the presence of ketone bodies. The data obtained from both cow-side detection assays was analyzed statistically. The results of this research study laid the foundation of early detection of sub-clinical ketosis in buffaloes that was made possible using the cow-side Abott Optium Neo Ketometer.

Key Words: Buffaloes, Ketosis, BHBA, Blood, Urine.

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INTRODUCTION

Pakistan is an agricultural country having a large livestock population that is playing a pivotal role in the agricultural sector which contributes 58.3% of the agricultural value (GDP). Livestock contribute significant in economic growth of the country by production meat, milk, eggs, manure, fiber, hides and horns. According to the Economic Survey of Pakistan 2019-20, over 8 million families in the village areas are involved with the livestock business for driving most of their income. From the last few decades, livestock has contributed a major role to value addition in agriculture. A total value addition of livestock has increased from Rs1430 billion (2018-19) to Rs1466 billion (2019-20) representing an annual growth rate of 2.5%.

Total milk produced by livestock in the country is estimated over 61.69 million tons. Out of this, 49.7 million tones are estimated to be available for humans use. Livestock in Pakistan busy to produce 77.6 million hides for the leather industry and contributes to 5% of total exports of the country. Most of the production processes needs technology to meet standards of production whereas, the production processes in Pakistan depend on traditional methods and livestock in the

country faces several production and health related challenges. Milk and meat are the two main products of livestock which can boost the economic growth of any country. In Pakistan public organizations, and from the last few decades private companies are also financing in this sector to develop the appropriate marketing. However, Pakistan is planning to expand its market by increasing trade and economic activities with neighboring countries in exporting Halal meat and its products.

Because of increasing demands for milk and milk products in areas of high population pressure, modern techniques for increasing milk production per animal are employed. Nutrition requirements of animals are increased due to increased milk production. If the nutrition requirements of animals are not met, situation of negative energy balance may arise. Under such scenario, there will be higher concentration of non-esterified fatty acids (NEFA) in the serum, in order to provide energy source (Allen and Piantoni, 2013). However, when the number of triglycerides reaching the liver exceed the capacity of oxidation, partial oxidation of NEFA occurs, resulting in increased production of ketone bodies, mainly β -Hydroxybutyric acid (BHBA). Elevated concentration of ketone bodies is indicative for ketosis.

This metabolic problem can lead to huge economic losses to the dairy industry, in the form of decrease in milk as well as reproductive efficiency (Walsh *et al.*, 2007). Therefore, early detection and treatment of ketosis is very important to maintain the health and production of the animals.

Buffalos (*Bubalus bubalis*) are major dairy animal of Pakistan and due to their significance in agriculture and milk production these are called “Black Gold”. Buffalo breeds of Pakistan are well known throughout the world and are reared for triple purpose (milk, meat and draught). About 28.4 million buffaloes are present throughout the country, which are contributing approximately 22 million tons of milk per annum (Anonymous, 2006). Nili-Ravi buffalo is the high milk producing breed with 1800-2500 liters production per lactation (Hasnain and Usmani, 2006). Buffaloe milk is enriched by high butter fat (6.7 %) and total solid (16.6%) nutrients preferred by customers (Fischer, 1975). Buffalo milk is quite suitable for making value added milk products i.e., soft cheese, condensed milk products such as khoa and rabdi (local words), fermented milk products like ice cream and kulfi, milk powder and various sweets. Pakistan has big share among buffalo’s meat producers in the world followed by India, China, Thailand and Vietnam. Buffaloes share over 55% in meat production of the country. Buffalo meat is lean (40% less cholesterol in comparison to bovine meat) with low saturated fat than beef. (Lemcke, 1997).

Buffalo is one of the major contributors of milk production in Pakistan. However, like other dairy animals, it also faces a several production and health related challenges, amongst these metabolic disorders after parturition are very common. High milk producer buffaloes at time of peak production period if fed low energy ration may experience negative energy balance which may results mobilization of body fat reservoirs to meet the energy demand which may lead to the development of metabolic disorder, such as ketosis (Herdt, 2000).

Appearance of high level of non-esterified fatty acids (NEFA) in the blood/serum to support energy requirement may results in higher production of ketones mostly mainly BHBA (β -Hydroxybutyric acid) if the quantity of triglycerides in the liver exceeds the oxidation capacity. This may result in partial oxidation of non-esterified fatty acids causing elevated concentration of ketones in the serum (ketosis).

Ketosis is a metabolic disorder that occurs in cattle when energy demands (e.g. high milk production) exceed energy intake and result in a negative energy balance. Ketotic cattle often have low blood glucose (blood sugar) concentrations. When large amounts of body fat are utilized as an energy source to support production, fat is sometimes mobilized faster than the liver can properly metabolize it. If this situation occurs,

ketone production exceeds ketone utilization by the cow, and ketosis results.

This condition develops more likely in late pregnancy when appetite of the animal is at its lowest level and the energy demands of the growing calf are at its peak. In the first few weeks of lactation there is a gap between energy intake and output, because the cattle is not able to eat enough to match the energy lost in the milk, so negative energy balance occurs. Ketotic cattle usually shows following symptoms:

- Reduced milk yield
- Decreased appetite
- Weight loss
- Fever
- Acetone smell in breath or milk
- Dull skin coat
- In some cases nervous signs are shown including incoordination, salivation, licking, chewing and aggression etc.

Sub-clinical ketosis is an important metabolic condition, having economic impact in terms of both production and reproduction losses. It is due to an abnormal ketone concentration in blood circulation without clinical signs (Andersson, 1988). Early detection of sub-clinical ketosis is of vital importance to minimize the subsequent losses associated with this condition. Many studies have been conducted in past to diagnose sub-clinical ketosis in cattle, but very little data is available for buffaloes. Ketosis is a common metabolic disease in high-lactating buffaloes which occurs in the first 60 days post-partum and prevalent from 6.9 up to 34% however peak incidences are observed within first 14 days [Voyvoda and Erdogan, 2010; Dohoo and Martin, 1984; Duffield, 2006; 8]. Sub clinical ketosis causes longer calving intervals and impaired milk production which resulted in huge economic losses in the dairy industry [Dohoo and Martin, 1984; Fass, 2010; Gustafsson and Emanuelson, 1996; Geishauser *et al.*, 2001; Duffield, 2003]. Moreover, buffaloes with subclinical ketosis may develop higher risks cystic ovaries development, displaced abomasum and clinical ketosis (Dohoo and Martin, 1984; Duffield 1984; Fass, 2010; Gustafsson and Emanuelson, 1996).

Ketosis has been reported to cause huge economic losses to dairy industry in terms of declining the productive and reproductive efficacy of farm animals (Geishauser *et al.*, 1998; Oetzel, 2004; Walsh *et al.*, 2007; LeBlanc, 2006; Allen and Piantoni, 2013). Therefore, it is dire need to detect and treat the ketosis to maintain the health, reproductive efficiency and production of the farm animals. For this, early diagnosis of subclinical ketosis would allow early treatment hence may help to mitigate further losses (Enjalbert *et al.*, 2003; Geishauser *et al.*, 2001). Higher levels of ketones (acetoacetate, acetone and β -hydroxybutyrate) in urine, blood and milk without any clinical signs is called

subclinical ketosis (Voyvoda and Erdogan, 2010; Andersson, 1984; Geishauser *et al.*, 2000). Measuring β -hydroxybutyrate concentration of serum is helpful for evaluating herd health and feeding management (Iwersen *et al.*, 2009).

Varying cow side tests are existed for monitoring of ketosis in dairy herd. Yet, the quantitative determination of β -hydroxybutyrate depends on special laboratory equipment and needs blood sampling, centrifugation, separation plasma or serum samples, freezing of samples (serum, plasma) and transportation of frozen samples for lab analysis (Iwersen *et al.*, 2009). Thus, these methods are laborious and costly. Therefore, numerous rapid/immediate and cost-effective tests have been tested. However, there are certain limitations in their use; therefore, the current study aims to compare efficacy of digital ketometer and conventional urine strips method to detect Ketone bodies in blood and urine samples respectively.

MATERIALS AND METHODS

Study was conducted at three buffalo farms as follows:

- 1- Livestock Production and Research Institute (LPRI) Bahadur-Nagar, Okara
- 2- Buffalo Research Institution (BRI), Pattoki, Punjab.
- 3- Mian Manzoor Buffalo Dairy Farm, Faisalabad, Punjab.

High milk producing buffaloes (with milk production 12 kg/day or above), during their peak production postpartum period (i.e., 1st week to 2 months after parturition) were included from each dairy farm. The health parameters under examination were including animal's age, parity, stage of lactation, feed and management practices and milk production. The clinical parameters including rectal temperature, pulse and respiration rates, ruminal motility, etc. were also recorded at the time of blood sampling. For this a total of 100 samples were collected, from buffaloes having history of recent parturition with good milk yield.

Two types of samples were collected from each buffalo i.e.

- 1- Blood was collected from jugular vein under strict antiseptic measures.
- 2- Urine sample was collected in clean labeled container from freshly voided urine or through stimulating micturition.

Blood samples were tested immediately. Urine samples were shifted to clean sterile test tubes for later detection of ketone bodies. Evaluation of ketone bodies was conducted in blood and urine.

RESULTS

Correlation of ketosis with reduction in milk production: The reduction in milk production of the four animal groups (as shown in Table 4.1) was compared with the average ketone body levels in their blood.

Group 4 showed the most decreased milk production at 5-6 kg along with the highest blood ketone levels (1.44 mmol/L). The correlation between blood ketone levels and reduction in milk production was higher in group 4 as compared with group 1 ($p < 0.05$). Correlation in Group 1 was higher than Group 2, whose correlation was higher than Group 3 ($p < 0.05$). But the correlation between blood ketone levels and reduction in milk production in Group 3 was not significant when compared to Group 4 ($p > 0.05$).

Correlation of ketosis with decrease in feed intake: The reduction in feed intake of the three animal groups (as shown in Table 4.2) was compared with the average ketone body levels in their blood.

In Group 1 with no change in feed intake, the blood ketone levels were lowest among other groups (0.46 mmol/ml). The co-relation between reduced feed intake and increased blood ketone levels was significant in all groups when compared with other groups. Group 1 more than Group 2, Group 2 more than Group 3 and Group 3 was more than Group 1 ($p < 0.05$).

Correlation of ketosis with blood glucose level: The blood glucose level of the four animal groups was compared with the average ketone body levels in their blood. Group 4 had the highest blood glucose levels (51-60 mg/dl) but group 1 has the highest blood ketone levels (1.48 mmol/L). The correlation between blood glucose level and increased blood ketone levels was not significant in Group 1 when compared with Group 2 ($p > 0.05$). The correlation in Group 2 was more than Group 3, Group 3 was more than Group 4 and Group 4 was more than Group 1 ($p < 0.05$).

Correlation of ketosis with post-calving period: The post-calving period of the three animal groups was compared with the average ketone body levels in their blood. Group 2 had the lowest blood ketone levels among other groups (0.50 mmol/ml). The co-relation between post-calving period and increased blood ketone levels in Group 2 when compared with Group 3 was not significant ($p > 0.05$). The co-relation between post-calving period and increased blood ketone levels was significant in Group 1 when compared with Group 2 and Group 3 was more than Group 1 ($p < 0.05$).

Correlation of ketosis with body condition score: The body condition score (BCS) of the three animal groups was compared with the average ketone body levels in their blood. Group 3 had the highest BCS and the

blood ketone levels among other groups (3.75 and 1.19 mmol/ml respectively). The co-relation between body condition score and increased blood ketone levels in Group 1 was significant when compared with Group 2 ($p>0.05$). The co-relation between body condition score and increased blood ketone levels was also significant in Group 2 when compared with Group 3 and Group 3 when compared with Group 1 ($p<0.05$).

Correlation of ketosis with urine ketone levels: The Urine Ketone Levels of the three animal groups measured

by Urine Strips were compared with the average ketone body levels in their blood. Group 3 had the highest Urine and the blood ketone levels among other groups at 1.1-1.5 and 1.30 mmol/L respectively. The co-relation between urine ketone levels and increased blood ketone levels in Group 1 was significant when compared with Group 2 ($p>0.05$). The co-relation between body condition score and increased blood ketone levels was also significant in Group 2 when compared with Group 3 and Group 3 when compared with Group 1 ($p<0.05$).

Table 1: Correlation of ketosis with reduction in milk production.

Groups	N	Reduction in milk production (kg)	Average ketone level in blood (mmol/ml)	SD	Comparison	P value
1	30	0	0.5	0.06	G1 vs G2	0.029
2	25	1-2	0.7	0.07	G2 vs G3	0.004
3	25	3-4	1.12	0.07	G3 vs G4	0.089
4	20	5-6	1.44	0.08	G4 vs G1	< 0.001

Table 2: Correlation of ketosis with decrease in feed intake.

Groups	N	Decrease in feed intake (kg)	Average ketone level in blood (mmol/L)	SD	Comparison	P value
1	35	No decrease	0.46	0.03	G1 vs G2	< 0.001
2	40	1-4	0.86	0.09	G2 vs G3	0.004
3	25	5-8	1.1	0.10	G3 vs G1	< 0.001

Table 3: Correlation of ketosis with blood glucose level.

Groups	N	Blood glucose level (mg/dl)	Average ketone level in blood (mmol/L)	SD	Comparison	P value
1	20	21-30	1.48	0.12	G1 vs G2	0.078
2	25	31-40	1.24	0.09	G2 vs G3	0.003
3	30	41-50	0.96	0.05	G3 vs G4	0.002
4	25	51-60	0.52	0.03	G4 vs G1	< 0.001

Table 4: Correlation of ketosis with post-calving weeks.

Groups	N	Post-calving Period (weeks)	Average ketone level in blood (mmol/L)	SD	Comparison	P value
1	35	1-2	1.07	0.12	G1 vs G2	< 0.001
2	35	3-4	0.5	0.07	G2 vs G3	0.219
3	30	5-6	1.19	0.17	G3 vs G1	< 0.001

Table 4: Correlation of ketosis with the body condition score.

Groups	N	BCS	Average ketone level in blood (mmol/L)	SD	Comparison	P value
1	35	3	0.54	0.03	G1 vs G2	< 0.001
2	30	3.5	0.97	0.09	G2 vs G3	0.003
3	35	3.75	1.19	0.16	G3 vs G1	< 0.001

Table 5: Correlation of ketosis with urine ketone levels.

Groups	N	Urine ketone level	Average ketone level in blood (mmol/L)	SD	Comparison	P value
1	35	< 0.5	0.45	0.05	G1 vs G2	< 0.001
2	35	0.5-1	0.80	0.08	G2 vs G3	< 0.001
3	30	1.1-1.5	1.30	0.11	G3 vs G1	< 0.001

DISCUSSION

Ketosis, which is the abnormal high levels of ketone bodies in body tissues and fluids, may present as a primary disease or in association with other pathological conditions in dairy animals (Patel and Patel., 2017). In high-yielding buffaloes, negative-energy balance occurs due to increased demand of maintaining blood glucose levels which leads to hypoglycemia and ketonemia. This has devastating effects on animals like nervous disorders and also effects the farm economics due to decreased milk production and failure of animals to return to their normal potential (Teli *et al.*, 2007; Thirunavukkarasu *et al.*, 2010). Clinical ketosis is most common manifestation which usually occurs 6-8 weeks after parturition. It is easier to diagnose due to visible signs and symptoms like rapid loss in body condition, decreased milk production, acetone-like smell from breath and nervous disorders. Ketosis also results in hematological changes like neutropenia and increased cholesterol levels. (Youssef *et al.*, 2010). Subclinical ketosis, however, is difficult to diagnose due to lack of obvious signs and symptoms. But the economic losses are considerable in both forms (Purohit *et al.*, 2013).

The most common diagnostic test used to diagnose clinical and sub-clinical ketosis in cattle and buffaloes is the Rothera's test performed on milk or urine. This test is based on strips composed of sodium nitroprusside (Geishauser *et al.*, 1998). However, this method was found effective only against the acetoacetate and acetone. The most stable ketone body in blood is β -hydroxybutyric acid (BHBA) and thus detection of BHBA in blood is considered as the gold standard for detection of ketosis in dairy cows and buffaloes (Oetzel and Garrett, 2007). Commercially available strips for detection of BHBA in urine or milk have good specificity but are not that sensitive resulting in false positive results (Krogh *et al.*, 2011). Irrespective of the ketone body being detected, measuring them in blood is the only way sub-clinical ketosis can be detected. In recent years, hand-held digital ketometers which were predominantly used for humans have been introduced in dairy industry to detect the level of BHBA in blood (Fiorentin *et al.*, 2017). This study was designed to compare the results of commercial urine strips and hand-held digital ketometer for the detection of clinical and sub-clinical ketosis in three Buffalo farms of Punjab, Pakistan. It was also

objectified to provide a cut-off value obtained by digital ketometer to differentiate between clinical and sub-clinical ketosis.

Reduced milk production is often associated with clinical and sub-clinical ketosis. During early phases of lactation, increased concentration of BHBA in the serum of dairy animal alters the metabolism, thus directly effecting the milk production in the udder (Duffield *et al.*, 2009; Chapinal *et al.*, 2012). High milk producing animals are more prone to developing ketosis due to increased physiological demand and transitional changes (Sordillo *et al.*, 2009). This correlation between reduced milk production and ketosis was also exploited in the present study. The animals were divided in four groups based on reduction in milk production. The correlation with ketosis was compared among four groups. The correlation was significant in most groups which coincided with similar studies indicating milk losses as high as 73-89 kg in 305d lactation which were directly relation to sub-clinical ketosis (Ospina *et al.*, 2010). Only Group 3's reduced production was not significantly correlated with ketosis when compared with Group 4. Although the decreased in milk production and blood ketone levels are significantly higher. This indicates that there are other causes than ketosis like abomasum displacement, increased parity and post-partum problems which cause reduced milk production in dairy cattle and buffaloes (Raboisson *et al.*, 2014).

Decreased feed intake is a common management problem occurring in dairy herds. It directly effects the production performance of animals and causes metabolic disorders like ketosis. This is due to the decrease in feed nutrients putting pressure on the body thus giving rise to various ketone bodies originating from adipose tissues (Kupczyński *et al.*, 2020). In the present study, animals were divided in three groups based on the amount of decreased feed intake in kilograms. Group 1 with no decrease, Group 2 with 1-4 kg decrease and Group 3 with 5-8 kg decrease in normal feed intake. The blood ketone levels of all the groups increased with decrease in feed intake. All the groups were compared with each other and the correlation between blood ketone levels and decreased feed intake was significant in all. These results were in line with previous study which indicated the highest feed intake decrease (up to 7 kg) during the first occurrence of ketosis (Bareille *et al.*, 2003). Another study monitored metabolic and signaling gene networks in liver of dairy cows and showed that feed restrictions of

up to 50% can increase the levels of non-esterified Fatty Acids and BHBA in blood, thus enhancing the correlation between decreased feed intake and ketosis (Loor *et al.*, 2007).

The blood glucose levels are high in post-partum dairy animals as this transition state is linked to insulin resistance because the placenta is less insulin sensitive and this mechanism helps the fetus to acquire maximum glucose (Hammon *et al.*, 2009). This results in increased blood glucose levels in post-partum cows and buffaloes altering their metabolic state, thus giving rise to possible metabolic disorders like peri-parturient hemoglobinuria and ketosis (Weber *et al.*, 2013). In this study, animals were divided into four groups based on the increasing concentration of blood glucose levels in mg/dl. All the groups were compared to one another and Group 1 had a non-significant ketosis correlation with blood glucose levels when compared with Group 2. A study also showed similar results in which low levels of cortisol were found instead of glucose in dairy cattle suffering from ketosis (Forslund *et al.*, 2010). In other comparisons, the correlation was significant between increasing blood glucose levels and decreasing ketone levels. These results were comparable to another study which demonstrated that blood glucose levels decrease when the animals are infused with BHBA primarily due to the inhibition of gluconeogenesis (Zarrin *et al.*, 2017).

Post-calving period is marked by substantial changes in the metabolic system of dairy animals to support the increasing demand of milk production. This makes it inevitable for the animals to go in a negative energy balance. This puts stress on the animal and when coupled with excessive decrease in dry matter intake, animal experiences a non-adaptive negative energy balance and both clinical and sub-clinical ketosis (Kaufman *et al.*, 2016; Abuajamieh *et al.*, 2016). In the current study, animals were divided into three groups based on weeks post-calving. The highest levels of blood ketone levels were found in Group 3 (5-6 week post-calving) which may be due to the fact that animals attain peak production during this time and is continuously in negative energy balance (Wathes *et al.* 2007). Group 2 (3-4 weeks post-calving) had the lowest blood ketone levels and when compared with group 3, the correlation with post-calving period was non-significant. Although the classic type of ketosis is usually seen during this period (Holtenius *et al.*, 1996), it could be possible that samples were taken before the ketone bodies reached to their peak. In other comparisons, the correlation between increasing post-calving period and level of ketone bodies was significant, coinciding with the findings of previous studies (Berge *et al.*, 2014).

Body condition score (BCS) is an important parameter for assessing the nutritional management of dairy animals. It is highly variable and changes continuously throughout the lactation and reproductive

cycles of cattle and buffaloes. During the negative energy balance, the adipose tissues and muscle mass is depleted to cope with the increasing demand of energy, thus decreasing the body condition score (Schulz *et al.*, 2014). In this study, animals were placed in three groups on basis of their body condition score measured on a 5-point scale. Average ketone levels in blood were directly proportional to the increase in BCS of the three groups. These results were comparable to another study which reported an increased incidence of ketosis in dairy animals of BCS more than 3.5. This was due to the fact that they were more prone into going to the negative energy balance because of their high production (Gillund *et al.*, 2001). The results were also compared between groups and all three comparisons significantly correlated increased body condition score to the development of ketosis. This implies that high body condition score renders the animals more prone to the development of post-partum disorders due to more room of production and increased negative energy balance (Koeck *et al.*, 2014).

Finally, the efficacy of measuring ketone bodies with urine strips and digital ketometer was assessed in all the animals. Urine strips usually only measure the acetoacetate in the urine and not the BHBA which are the actual indicator of ketosis (Federici *et al.*, 2006). The baseline value for the cattle has been set at 1.2-1.4 mmol/L (Oetzel *et al.*, 2004) but the baseline value for buffalo has not been established to our knowledge yet. The animals were divided into three groups based on their urine ketone levels. There was a direct correlation between urine ketone bodies and blood ketone bodies among all the groups. This is comparable to another study which found that the urine ketone levels and blood ketone levels were directly proportional to each other (Faruk *et al.*, 2020). The comparisons between the three groups were also made and the correlation between the three groups was significant with respect to urine ketone levels and blood ketone levels.

Level of ketone bodies was standardized in buffaloes having different level of Parity, Milk production, Age, Body condition score, post calving days (Shehroz khan, unpublished data). Highest level of ketone was recorded in highly producing animals (0.83 ± 0.07) whereas lowest at highest parity (0.50 ± 0.10). Level of ketone bodies ranges from 0.50-0.83 in buffaloes.

Buffaloes with mild reduction in milk production, slightly decreased feed intake and a minor decrease in blood glucose level having overall normal health parameters were considered at subclinical stage of ketosis. Buffaloes with slight decrease in feed intake up to 4 kg (group 2, table 4.2) and 5-8 kg decrease in feed intake (group 3, table 4.2) were having average blood ketone values 0.86 mmol/L and 1.1 mmol/L respectively. Buffaloes with mild decrease in milk production up to 4

kg (group 2 and 3, table 4.1) were having highest level of average blood ketone value 1.12 mmol/L. Buffaloes with mild decrease in blood glucose level 41-50 mg/dL (normal 51-60) were having average ketone value 0.96 mmol/L. So groups of buffaloes sub clinically effected with ketosis have range of ketone bodies from 0.86 mmol/L to 1.12 mmol/L.

Conclusion: Digital Ketometer is useful for rapid and early detection of clinical as well as subclinical ketosis in buffaloes. Early detection of subclinical ketosis is helpful in preventing economical losses due to ketosis.

Digital Ketometer has advantage over conventional urine strips as

- 1) It gives digital value of ketone bodies while conventional urine strips only shows color change.
- 2) It takes less time (only 10 seconds) to analyze blood for ketone bodies.
- 3) It detects BHBA that is most stable ketone body in blood.

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