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Prevalence and Alterations in Metabolic Indices and Cowside Test in Subclinical Ketosis of Prepartum Transition Dairy Cows

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ABSTRACT

Subclinical ketosis is the overproduction of ketone bodies in the blood that causes decreased milk production and reproductive effectiveness without exhibiting any overt clinical signs of ketosis during the transition period of dairy cows. Therefore, the study was conducted to find the alterations of metabolic parameters and the usefulness of cow side tests in the early detection of subclinical ketosis in prepartum itself so that the occurrence of clinical ketosis can be prevented. The prevalence of subclinical ketosis in prepartum was 16.66 percent (36/216) among prepartum dairy cows. Cow side test revealed an increase in BHBA level, a decrease in glucose value, and the presence of ketonuria compared with the respective days of a control group. A significant increase in the concentration of serum metabolites such as non-esterified fatty acid (NEFA) and beta hydroxybutyric acid (BHBA), along with increased body condition score, decreased reticulo-rumen mortality and rumen fill score and reduced rumen protozoa score were recorded. The serum biochemical changes recorded were a decrease in serum glucose, triglyceride, cholesterol direct bilirubin, calcium, and phosphorous and an increase in BUN, Aspartate aminotransferase (AST). An elevation of acute phase protein Haptaglobin was also recorded in subclinical ketosis cows. This levels can be used as risk indicators to predict the occurrence of subclinical ketosis in prepartum transition cows.

HIGHLIGHTS

- Subclinical ketosis is more prevalent in Cross bred HF cows of fifth calving during winter months.
- Alterations in biochemico-metabolic profile documented in prenatal cows with subclinical ketosis.
- Urine Ketometer and BHBA levels in ELISA detection of SCK in cows.

Keywords: Prepartum, Subclinical ketosis, NEFA, BHBA, Cowside, AST

Ketosis is a common serious metabolic disease in the transition period of dairy cows. All the peripartum dairy cows during the transition period have insulin resistance, reduced feed intake, reduced immune function, negative energy balance (NEB), lipolysis, weight loss, and hypocalcemia in early lactation (Sundrum, 2015). Especially, during the late gestation period growing fetus occupies greater space in the abdominal cavity, thereby reducing the rumen space and leading to reduced feed intake, but there is high energy demand for the growing fetus, which results in a negative energy balance (Hummel *et al*., 2021).

To adapt to the NEB, cows mobilize fat from body reserves to provide free fatty acids as energy fuel. A large amount of fatty acids enters the liver hepatocyte

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to disturb hepatic metabolism and produce an excess amount of ketone bodies, thus the occurrence of ketosis (Gathercole *et al*., 2013). It is often divided into clinical and subclinical ketosis based on the presence or absence of clinical manifestations. The excess of circulating ketone bodies in blood without the appearance of clinical ketosis symptoms is known as subclinical ketosis (SCK). Since there are no obvious clinical symptoms to identify the condition and the animals continue to produce at a reduced rate, leading to severe economic losses, the SCK is more crucial than clinical ketosis. The primary cause of SCK is the liver's incomplete oxidation of acetyl-CoA during the early lactation period, which leads to the development of subclinical ketosis and the production of ketone bodies (acetone, acetate, and betahydroxy butyrate) (Cantrell and Mohiuddin, 2020). Subclinical ketosis has been linked to a number of reproductive issues, including mastitis, a displaced abomasum, fatty liver, and reduced milk production.

BHBA is the gold standard test for the diagnosis of subclinical ketosis in cattle as it is more stable in blood than acetone or acetoacetate. Several authors stated that cows with blood BHBA concentrations of 1.2 to 1.4 mmol/L and ≥3 mmol/L were threshold for subclinical and clinical ketosis, respectively.

In addition to the effects on disease events, subclinical ketosis has been found to decrease milk yield in early lactation (Mcart *et al*., 2012) and may adversely affect reproduction. However, subclinical ketosis has no obvious clinical symptoms, is difficult to prevent and diagnose, and is harmful to the health of dairy cows. Therefore, exploring early warning indicators and techniques for dairy cow ketosis is an important measure to prevent huge economic loss to dairy farmers.

Several authors studied and reported subclinical and clinical ketosis only in early lactation of dairy animals. The aim of the present study was to analyze metabolic indicators as a risk factor in the detection of subclinical ketosis during the prepartum period, thereby prevention of postpartum clinical ketosis and reducing economic loss.

MATERIALS AND METHODS

The study was conducted in selected small and large-scale dairy farms at Krishnagiri district, Tamil Nadu. Multiparous pregnant cows during transition period (30 days prior to calving) were selected and subjected to assessing the blood BHBA and glucose level with a portable handheld ketometer and glucometer (Free style Optimum NeoH) from a coccygeal vein. The BHBA value of ≥1.2 - ≤ 1.4 mmol/L was taken as positive for subclinical ketosis. The cows which having BHBA levels below 1.2 mmol/L were taken as a negative control group.

The urine sample was collected from all the transition cows through the free catch method in a sterile vial. The sample was subjected to semi-quantitative estimation of urine glucose and urine ketone levels using the strip method.

All these animals were subjected to clinical examination, which included clinical signs, body condition score (5 point scale method), rumen fill score (1 to 5), rumen protozoa score (1-3). Rumen fluid was collected by puncturing the rumen at the left para lumbar fossa by using 18G needle with a syringe of about 3-5ml. And examined under microscope. According to the presence of concentration of protozoa number at single field, the score was given more than 30 protozoa per low power field graded as +++(3), if the protozoa are 10-30 motile and crowded they are graded as ++ (2), 1-10 protozoa per low power field graded as + (1), Nil (0) protozoa per low power field was graded as (0).

The 10ml of blood samples was collected from the jugular vein from which 2ml in ethylene diamine tetra acetic acid EDTA and 8ml clot activator vacutainer were collected respectively for complete hematological parameters such as (hemoglobin (g/dL), packed cell volume (PCV %), red blood cells (x 10^6/ cmm), white blood cells (/ cmm), platelets count and differential count) and serum biochemical parameters such as (blood urea nitrogen (mg/dL), creatinine (mg/dL), aspartate aminotransferase (IU/L), alanine aminotransferase (IU/L), glucose (mg/dL), total bilirubin, direct bilirubin, alkaline phosphatase (ALP), triglycerides, cholesterol, total protein, albumin, globulin, calcium, phosphorous, magnesium, sodium, potassium and chlorides) by using automated Haematology analyzer (Exigo H400 Vet) and Biochemistry Analyser, (FUJI DRY-CHEM NX600V) respectively.

Serum metabolites Viz Bovine-specific BHBA (MyBIOSource Inc, USA), NEFA (BT LAB Bioassay Technology Laboratory) and acute phase protein Haptoglobin (KRISHGEN Bisystems, GENLISA™

Bovine Haptoglobin) concentration were estimated by using ELISA method, and the analysis was performed according to the manufacturer's instructions.

The data were subjected to statistical analyses with IBM SPSS version 21.0 (IBM Corporation, USA). The data were expressed as mean ± standard error. The basic data, energy metabolism parameters, and liver functions in control and SCK groups were analyzed with independent 't' tests.

RESULTS AND DISCUSSION

Overall prevalence of subclinical ketosis in prepartum transition cows was recorded as 16.66% out of 216 multiparous pregnant cows screened (36/216). On 30th day 52.78% (19 cows), and 15th day of prepartum, 47.22% (17 cows) were recorded. The incidence of SCK was higher in Holstein Friesian crossbred 58.33% (21/36) when compared to Jersey crossbred 41.67% (15/36) cows. According to parity, the incidence was more common in 4th calving 52.78% (19/36) followed by 3rd calving 38.89% (14/36) and 5th calving 8.33% (3/36). Season-wise prevalence SCK was higher in the winter months 47.22% (17/36) followed by 38.89% and 13.89% in the rainy and summer months, respectively.

Reduced feed intake was noticed in 69.44 percent (26/36) of cows with subclinical ketosis. There is a highly significant increase ($p<0.001$) in body condition score (Fig. 2) and a highly significant decrease ($p<0.001$) in reticulo-rumen motility, rumen fill score, and rumen protozoa score were seen on both the days of pre-partum cows when compared to respective healthy control groups (Table 1).

Cowside tests showed a significant increase in blood BHBA (Fig. 1) and urine ketone bodies (Fig. 3) and a decrease in blood glucose of SCK when compared to respective healthy control groups (Table 2). The reason for this increase in ketone body production is due to the increased synthesis of acetyl-CoA, due to the incomplete oxidation of NEFA, which acts as a main precursor for the production of ketone bodies (Adewuyi *et al*., 2005). Increased ketone excretion in urine might be due to hyperketonemia caused by negative energy balance. The kidneys filter ketone bodies from the bloodstream, leading to their excretion in urine. The detection of ketone bodies in urine is commonly performed using test strips or dipsticks, which change color in the presence of ketones, providing a qualitative assessment of the cow's metabolic status (Duffield *et al*., 2009).

Table 1: Clinical parameters of Group I, Group II and Group II pre partum transition dairy cows

Parameters	Group I (Healthy Control) (n=36)	Group II (SCK) (n=19)	Group III (SCK) (n=17)
	Pre Partum -30th day	Pre Partum -30th day	Pre Partum -15th day
Body Condition Score	$3.53^{a} \pm 0.02$	$3.90^{b} \pm 0.03$	$3.89^{b} \pm 0.04$
Reticular Rumen Motility/5min	$6.67^{a} \pm 0.08$	$4.32^{b} \pm 0.22$	$4.65^{b} \pm 0.19$
Rumen Protozoa Score	$3.00^{a} \pm 0.01$	$2.42^{b} \pm 0.12$	$2.12^{b} \pm 0.08$
Rumen Fill Score	$4.00^{a} \pm 0.01$	$2.21^{b} \pm 0.09$	$2.24^{b} \pm 0.11$

Notes: Different alphabets in superscript denotes significance (P value <0.01).

Table 2: Cow side test of Group I, Group II and Group II pre partum transition dairy cows

Parameters	Group I (Healthy Control) (n=36)	Group II (SCK) (n=19)	Group III (SCK) (n=17)
	Pre Partum -30th day	Pre Partum -30th day	Pre Partum -15th day
Blood BHBA (mmol/L)	0.76 ± 0.03	1.30 ± 0.03	1.32 ± 0.02
Blood Glucose (mg/dL)	59.94 ± 0.51	41.21 ± 1.79	38.06 ± 1.41
Urine ketone (mg/dL)	2.09 ± 0.88	13.42 ± 2.51	14.41 ± 1.96

Notes: Different alphabets in superscript denotes significance (P<0.01).

No remarkable changes in hematological values were observed in subclinical ketosis cows, as shown in Table. 3. There was a highly significant ($p<0.01$) difference in biochemical parameters, as shown in Table 4. Significant reduction in blood glucose, triglycerides, cholesterol, total protein, and albumin and increase in AST were suggestive of mild hepatic involvement in SCK.

Fig. 1: 15th day prepartum cow BHBA level 1.2 mmol/L

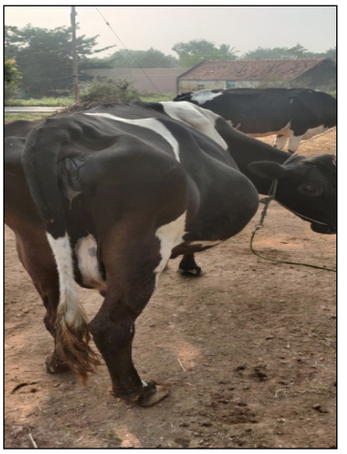

Fig. 2: Body Condition Score (>3.5)

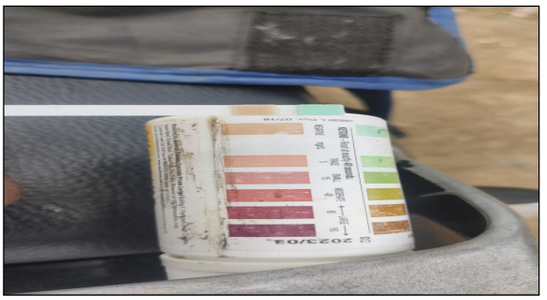

Fig. 3: Urine Ketone test (Keto-Diastix)

The glucose level reduced in late pregnancy might be due to low energy diet and also due to reduced dry matter intake during the transition period (Nazifi *et al*., 2008). Decreased triglycerides in SCK might be due to triglycerides accumulating in the liver cells of ketotic cows and causing their blood value to decrease (Djokovic *et al*., 2016). Decreased serum cholesterol levels could be attributed to mild liver steatosis, which causes a reduction in cholesterol formation in the liver (Grummer, 1995 Li *et al*., 2016). Decrease in calcium and phosphorous in SCK cows could be due to increased loss of base in the urine to compensate for acidosis reported in cows with ketosis, as suggested by (Radostits *et al*., 2007). Further high concentration of BHBA impairs the absorption and utilization of calcium in dairy cows with subclinical ketosis. Low serum calcium levels and increased urinary loss of phosphorous were also reported by stated by Valk *et al*. (2002). Alterations in liver function indices were also reported by Wang *et al.* (2021).

Highly significant increases (p>0.01) in serum metabolites such as NEFA and BHBA and acute phase protein haptoglobin were also recorded in cows with SCK. The NEFA warning value in prepartum was 0.2mmol/L, as stated by Ardavan *et al*. (2011) as cows enter NEB after calving, a switch toward the increasing use of fatty acids exists as an energy source to conserve limited glucose supplies. This is promoted by several endocrine signaling pathways: IGF1 and leptin concentrations fall, insulin signaling is blocked, and growth hormone and catecholamine secretion promote lipolysis. The rate of body tissue mobilization is influenced by energy input (dry matter intake [DMI]), energy stores (BCS), and energy output (fetal growth or milk production) in the critical period around calving Wathes *et al*. (2013).

Elevated levels of BHBA in SCK cows due to the increased concentration of NEFA reflect the magnitude of mobilization of fat from storage and hence indirectly mirror dry matter intake (DMI). The level of BHBA

Table 3: Mean ± S.E value of hematological parameters of Group I, Group II and Group II pre partum transition dairy cows

Parameters	Group I (Healthy Control) (n=36)	Group II (SCK) (n=19)	Group I (Healthy Control) (n=36)	Group III (SCK) (n=17)
	Pre Partum -30th day	Pre Partum -30th day	Pre Partum -15th day	Pre Partum -15th day
Hb (g/dl)	10.59 ± 0.14[a]	10.81 ± 0.12[b]	10.61 ± 0.13	10.86 ± 0.09
PCV(%)	32.83 ± 0.37	32.95 ± 0.27	32.32 ± 0.33	32.00 ± 0.19
RBC x 10^6/µl	6.31 ± 0.06	6.24 ± 0.11	6.29 ± 0.06[a]	5.44 ± 0.07[b]
Platelets Cells/L	329666.33 ± 1117.93	326070.05 ± 4479.99	329030.83 ± 1156.25	327968.71 ± 1823.17
WBC Cells/Cumm	7690.61 ± 210.05	8030.26 ± 60.79	7928.61 ± 76.34	7884.12 ± 101.42
Neutrophils Cells /Cumm	2893.86 ± 82.47	3102.74 ± 69.22	2992.55 ± 45.06	2981.47 ± 50.78
Lymphocytes Cells /Cumm	4308.42 ± 164.63	4351.05 ± 218.08	4433.36 ± 123.34	4272.88 ± 237.87
Eosinophils Cells /Cumm	147.58 ± 8.80	156.47 ± 11.48	149.94 ± 7.68	157.82 ± 12.16
Monocytes Cells /Cumm	123.61 ± 7.64	122.47 ± 9.43	125.69 ± 6.86	120.88 ± 10.12
Basophils Cells /Cumm	97.30 ± 8.63	86.89 ± 8.21	99.36 ± 8.14	97.71 ± 8.70

Notes: Different alphabets in superscript denotes significance (P<0.01).

Table 4: Serum Biochemical parameters of Group I, Group II and Group III pre partum transition dairy cows

Parameters	Group I (Healthy Control) (n=36)	Group II (SCK) (n=19)	Group I (Healthy Control) (n=36)	Group III (SCK) (n=17)
	Pre Partum -30th day	Pre Partum -30th day	Pre Partum -15th day	Pre Partum -15th day
Glucose (mg/dL)	58.29 ± 1.48[a]	39.42 ± 1.06[b]	60.08 ± 1.44[a]	37.06 ± 1.01[b]
BUN (mg/dL)	11.31 ± 0.19[a]	15.83 ± 0.17[b]	12.58 ± 0.26[a]	16.27 ± 0.16[b]
Creatinine (mg/dL)	1.49 ± 0.06	1.47 ± 0.02	1.43 ± 0.05	1.44 ± 0.01
Triglycerides (mg/dL)	23.58 ± 0.75[a]	13.01 ± 0.09[b]	23.00 ± 0.78[a]	13.87 ± 0.12[b]
Cholesterol (mg/dL)	149.05 ± 2.06[a]	84.44 ± 0.79[b]	154.65 ± 3.77a	84.86 ± 0.95[b]
Total bilirubin (mg/dL)	0.39 ± 0.001[a]	0.55 ± 0.01[b]	0.39 ± 0.002a	0.54 ± 0.01[b]
Direct bilirubin (mg/dL)	0.35 ± 0.004	0.32 ± 0.02	0.35 ± 0.005	0.31 ± 0.03
AST (IU/L)	105.07 ± 2.91[a]	112.79 ± 0.55[b]	101.45 ± 2.48[a]	113.73 ± 0.82[b]
ALT (IU/L)	28.04 ± 1.48	28.05 ± 0.38	26.24 ± 1.43	26.82 ± 0.65
ALP (IU/L)	253.04 ± 26.39	314.15 ± 25.63	250.03 ± 26.19	315.41 ± 3.14
Total protein (g/dL)	6.78 ± 0.26	6.49 ± 0.07	6.51 ± 0.03	6.39 ± 0.06
Albumin (g/dL)	3.23 ± 0.02	3.20 ± 0.03	2.93 ± 0.01	2.88 ± 0.02
Globulin (g/dL)	3.26 ± 0.02	3.25 ± 0.09	3.58 ± 0.02	3.51 ± 0.03
Calcium (mg/dL)	11.19 ± 0.14[a]	7.94 ± 0.07[b]	11.01 ± 0.13[a]	8.07 ± 0.02[b]
Phosphorus (mg/dL)	6.52 ± 0.14[a]	5.15 ± 0.02[b]	6.69 ± 0.14[a]	5.19 ± 0.003[b]
Magnesium (mg/dL)	2.33 ± 0.02	2.39 ± 0.02	2.34 ± 0.03	2.32 ± 0.02
Sodium (mmol/L)	134.34 ± 0.96	132.11 ± 1.13	135.94 ± 0.85	135.00 ± 1.19
Potassium (mmol/L)	4.72 ± 0.11	4.62 ± 0.11	4.96 ± 0.11	4.94 ± 0.07
Chloride (mmol/L)	103.47 ± 0.77	102.84 ± 1.13	102.90 ± 0.74	104.06 ± 1.12

Notes: Different alphabets in superscript denotes significance (P<0.01)

Table 5: Serum Metabolites and Acute phase protein parameters of Group I, Group II and Group II pre partum transition dairy cows

Parameters	Group I (Healthy Control) (n=36)	Group II (SCK) (n=19)	Group I (Healthy Control) (n=36)	Group III (SCK) (n=17)
	Pre-Partum -30th day	Pre-Partum -30th day	Pre-Partum -15th day	Pre-Partum -15th day
NEFA (mmol/L)	0.38 ± 0.006^{a}	0.92 ± 0.01^{b}	0.38 ± 0.007^{a}	0.87 ± 0.03^{b}
BHBA (mmol/L)	0.76 ± 0.02^{a}	1.30 ± 0.01^{b}	0.85 ± 0.02^{a}	1.32 ± 0.03^{b}
Haptaglobin (g/L)	0.37 ± 0.005^{a}	0.78 ± 0.01^{b}	0.38 ± 0.006^{a}	0.76 ± 0.03^{b}

Notes: Different alphabets in superscript denotes significance (P<0.01).

reflects the completeness of oxidation of fat in the liver. In the dairy cow, the majority of glucose is produced in the liver via the tricarboxylic acid cycle and originates mainly from propionic acid produced by the rumen microflora. When insufficient glucose precursors are available, there is a lack of substrate to adequately process the mobilized fat resulting in incomplete oxidation of the mobilized body fat and excessive production of ketone bodies (Herdt, 2000). Haptoglobin is an important biomarker that can be used to assess the presence of inflammation and metabolic stress in dairy cows. It is an acute-phase protein produced by the liver in response to inflammation and tissue injury. When cows are in a state of subclinical ketosis, there may be low-grade inflammation and metabolic stress (Bertoni *et al*., 2008).

CONCLUSION

In conclusion, the levels of elevated -the-threshold levels of NEFA, BHBA, and haptoglobin and decrease in glucose, AST, triglycerides, and cholesterol in prenatal dairy cows were indicators for subclinical ketosis. Both ketometer and serum ELISA detection of BHBA were highly useful in the detection of SCK. Early detection of subclinical ketosis in pre-partum may be helpful to reduce the occurrence of clinical ketosis and associated diseases in the transition period, thereby reducing the economic loss to dairy farmers.

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