Therapeutic Evaluation of Glucogenic Precursors in Sub-Clinical Ketosis in Buffaloes

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Abstract

The study evaluates biochemical changes and treatment aspects in Sub-Clinical Ketotic (SCK) buffaloes within two months of calving. Among 247 screened buffaloes, 25 were found suffering from sub-clinical ketosis. Ten apparently healthy buffaloes (group I) with negative urinary ketone bodies and blood BHBA levels below 1.0 mmol/L served as control group (GI). Twenty buffaloes were positive for sub-clinical ketosis and presence of ketones in urine with blood BHBA levels more than 1.0 mmol/L were divided equally into two treatment groups (group II and group-III). Buffaloes of group II were treated with Calcium propionate for five days and buffered Phosphorus for two days. The mean blood BHBA (mmol/L) before treatment was 1.55±0.35 which significantly decreased to 0.66±0.21 after treatment. There was a significant ($p<0.05$) decrease in mean serum glucose, phosphorus and total cholesterol levels with significant increase ($p<0.05$) in BUN levels. However, these values turned to normal after therapy with a non-significant ($p<0.05$) increase in milk yield by 25.35 percent on seventh day. Urine analysis was negative for ketones. Buffaloes of group III were treated with Propylene glycol @ 200ml oral daily for five days and Inj. Butaphosphan and Cyanacobalamin once daily @ 5ml/100 kg I/m for two days. The mean blood BHBA (mmol/L) before treatment was 1.67 ± 0.44 and significantly decreased to 0.49± 0.18 after treatment. There was a significant ($p<0.01$) decrease in mean serum glucose, calcium and significant ($p<0.10$) decrease in total cholesterol levels as compared to healthy control group. Significant ($p<0.01$) increase in milk yield by 82.61 percent and urine analysis was negative for ketones after treatment. Concisely, the results indicate that combination of Propylene glycol and Inj. Butaphosphan and Cyanacobalamin was found to be highly effective for management of sub-clinical ketosis in buffaloes.

Keywords: Buffalo; butaphosphan; phosphorus; propylene glycol; sub-clinical ketosis

Introduction

Most of the cattle in developing countries like India are fed on low quality crop residues and agriculture by-products, which have got low inherent nutritive value and digestibility. The shortage of feed resources and poor nutritive value is of major concern to low productivity of dairy animals. Production diseases play a major role in high producing milch animals among which Sub clinical ketosis is one of the main conditions causing huge economical loss to the farmer. NEB (Negative energy balance) usually starts three weeks before parturition and remains up to three weeks after parturition *i.e.* transition period (Moreira *et al*., 2015). Mostly, high yielding dairy cattle suffer from state of negative energy balance during transition period. High production performances also cause high nutrition demands, which exceed the animal's metabolic capacities, which usually leads to an aberrant physiological state known as NEB (Irena *et al*., 2015) and can be due to fewer intakes of dry matter and inadequate feed utilization during transition period. NEB is the initial stage of SCK without any loss of milk production and best biomarkers for NEB and SCK are blood BHBA concentration in post partum period and NEFA concentrations in pre partum period. Finding blood BHBA concentration is an easy approach rather than NEFA concentrations.

The maintenance of adequate concentrations of glucose in the blood is critical for the regulation of energy metabolism. Ruminant absorbs very little dietary carbohydrate as hexose sugar, because dietary carbohydrates are fermented in the rumen to short chain fatty acids, principally acetate (70 percent), propionate (20 percent) and butyrate (10 percent). Consequently, glucose needs in ruminants must largely be met by gluconeogenesis. Propionate and amino acids are the major precursors for gluconeogenesis with glycerol and lactate of lesser importance (Lean *et al.,* 1992).

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In the present study propylene glycol and calcium propionate are used as glucogenic precursors along with phosphorus injections in SCK buffaloes to study the impact on certain blood and serum parameters.

Materials and Methods

In present study Ross modified Rothera's test was employed to detect ketone bodies in urine (Kaneko, 1997). Precision Xtra blood glucose and ketone monitoring system (Abbott Laboratories) and precision Xtra blood beta ketone test strips (Abbott Diabetes Care Inc, 1360 South Loop Rd, Alameda, USA) were used to detect blood BHBA. Ketone test strip contains enzyme beta hydroxybutyrate dehydrogenase, which oxidizes BHBA to acetoacetate and this reduces nicotinamide adenine dinucleotide (NAD^+) to NADH and NADH is then reoxidized to NAD^+ by an electron transfer mediator molecule. The electrical current generated by this conversion is measured by meter and is directly proportional to BHBA concentration in sample and results are displayed as mmol/L. Buffaloes with urine samples positive for ketone bodies were selected for the present study and were randomly divided into two groups *viz.* II, III consisting of 10 animals each and two different therapeutic regimens were administered. However, Group I consisted of apparently healthy animals which were between zero to two months of calving and negative for urinary Nutrocal and blood BHBA levels were less than 1.0 mmol/L. Buffaloes belonging to Group II were treated with calcium propionate (Nutrocal[a]) 100gms twice daily for 5 days and Buffered phosphorus inj. (Novizac[b]) (Sodium phosphate 30 mg and Disodium hydrogen phosphate 230 mg) 25 ml i/m daily for two days. Buffaloes belonging to Group III were treated with Propylene glycol (Glucaboost[c]) 200 ml daily orally for 5 days and inj. Butaphosphan and Cyanocobalamin (Synkomet[b]) 5 ml/100 kg b.wt. i/m daily for two days.

Results and Discussion

The comparative means of blood BHBA, serum glucose, calcium, magnesium, phosphorus, AST, total protein, albumin, total cholesterol, BUN and serum creatinine levels of healthy and group II were 0.49±0.19 and 1.55±0.35 mmol/L, 61.7±6.09 and 42.3±4.97 mg/dL, 9.36±0.51 and 9.16±0.46 mg/dL, 2.34±0.15 and 2.23±0.24, 5.21±0.54 and 3.26±0.57, 109±18.06 U/L and 111±10.93, 8.16±0.61 and 8.0±0.31 g/dL, 2.49±0.44 and 2.39±0.15 g/dL, 114.2±11.8 and 96.6±14.86 mg/dL, 13.57±2.97 mg/dL, 1.23±0.15 and 1.36±0.30, respectively. There was significant ($p<0.01$) differences in blood BHBA and serum glucose and phosphorus levels, significant ($p<0.05$) difference in total cholesterol, BUN levels of group II animals compared to healthy before treatment. All buffaloes belonging to group II were treated with Calcium propionate (Nutrocal) 100gms twice daily for 5 days and Buffered phosphorus injection (Novizac) 25ml i/m daily for 2 days. There was a significant ($p<0.01$) improvement in blood BHBA, serum glucose and phosphorus levels. The comparative means of blood BHBA, serum glucose, calcium, magnesium, phosphorus, AST, total protein, albumin, total cholesterol, BUN and serum creatinine of healthy and group II after therapy were 0.49 ± 0.19 and 0.66 ± 0.21 mmol/L, 61.7±6.09 and 60.10±8.91 mg/dL, 9.36±0.51 and 9.65±0.71 mg/dL, 2.34±0.15 and 2.3±0.16 mg/dL, 5.21±0.54 and 4.54±0.55 mg/dL, 109±18.06 and 96.9±11.82U/L, 8.16±0.61 and 8.42±0.36 g/dL, 2.49±0.44 and 2.43±0.15 g/dL, 114.2±11.8 and 104.8±13.72 mg/dL, 13.57±2.97 and 14.7±1.49 gm/dL,1.23±0.15 and 1.36±0.20 mg/dL respectively. There was no significant difference in all the biochemical parameters of group II compared to healthy except for phosphorus levels. Even though the phosphorus levels were increased significantly after therapy, still there was a significant difference to healthy group and urine analysis for ketones was negative in all animals of group after treatment.

The comparative means of blood BHBA, serum glucose, calcium, magnesium, phosphorus, AST, total protein, albumin, total cholesterol, BUN and serum creatinine of healthy and group III before therapy were 0.49±0.19 and 1.74±0.5 mmol/L, 61.7 ±6.09 and 41.5±8.18 mg/dL, 9.36±0.51 and 8.8±0.40 mg/dL, 2.34±0.15 and 2.15±0.42, 5.21±0.54 and 4.41±0.40, 109±18.06 and 116±3.56 U/L, 8.16±0.61 and 7.96±0.61 g/dL, 2.49±0.44 and 3.3±0.89 g/dL, 114.2±11.8 and 103.1±17.4 mg/dL, 13.57±2.97 and 11.01±3.74 mg/dL, 1.23±0.15 and 1.2±0.12 respectively. There was a significant ($p<0.01$) increase in blood BHBA and significant ($p<0.01$) decrease in serum glucose, calcium,

a - Brand of Kemin Industries, Chennai
b - Brand of Intas Animal Health, Ahmedabad
c - Brand of Natural Remedies, Bengaluru

Table 1: Mean blood and serum parameters before treatment

S.no.	Parameters	Group-I (control)	Group-II	Group-III
1	Blood BHBA (mmol/L)	0.49±0.19	0.66±0.21	0.49±0.18
2	Glucose (mg/dL)	61.7±6.09	60.10±8.91	63.1±6.79
3	Calcium (mg/dL)	9.36±0.51	9.65±0.71	9.57±0.36
4	Magnesium (mg/dL)	2.34±0.15	2.3±0.16	2.25±0.23
5	Phosphorus (mg/dL)	5.21±0.54	4.54±0.55•	5.04±0.31
6	AST (U/L)	109±18.06	96.9±11.82	115.9±12.04
7	Total protein (g/dL)	8.16±0.61	8.42±0.36	8.05±0.52
8	Albumin (gm/dL)	2.49±0.44	2.43±0.15	2.92±0.66
9	Total Cholesterol (mg/dL)	114.2±11.8	104±13.72	115.7±13.39
10	BUN (mg/dL)	13.57±2.97	14.7±1.49	11.52±2.85
11	Creatinine	1.23±0.15	1.36±0.20	1.16±0.08

• Significant at p<0.05, •• Significant at p<0.01

Table 2: Mean blood and serum parameters after treatment

S.no.	Parameters	Group-I (control)	Group-II	Group-III
1	Blood BHBA (mmol/L)	0.49±0.19	1.55±0.35••	1.74±0.51••
2	Glucose (mg/dL)	61.7±6.09	42.3±4.97••	41.5±8.18••
3	Calcium (mg/dL)	9.36±0.51	9.16±0.46	8.8±0.40••
4	Magnesium (mg/dL)	2.34±0.15	2.23±0.24	2.15±0.42
5	Phosphorus (mg/dL)	5.21±0.54	3.26±0.57•	4.41±0.40•
6	AST (U/L)	109±18.06	111±10.93	116.3±3.56
7	Total protein (g/dL)	8.16±0.61	8.0±0.31	7.96±0.61
8	Albumin (g/dL)	2.49±0.44	2.39±0.15	3.3±0.89
9	Total Cholesterol (mg/dL)	114.2±11.8	96.6±14.86•	103.1±17.4
10	BUN (mg/dL)	13.57±2.97	16.69±3.21•	11.01±3.74
11	Creatinine (mg/dL)	1.23±0.15	1.36±0.30	1.2±0.12

• Significant at p<0.05

phosphorus and total cholesterol levels of group III animals when compared to healthy group before therapy. All buffaloes belonging to group III were treated with Propylene glycol (Glucaboost[c]) 200ml daily orally for 5 days and inj. Butaphosphan and Cyanacobalamin (Synkomet[b]) 5 ml/100 kg b.wt. i/m daily for two days. There was significant improvement in blood BHBA, serum glucose, calcium, phosphorus and total cholesterol. The comparative means of blood BHBA, serum glucose, calcium, magnesium, phosphorus, AST, total protein, albumin, total cholesterol, BUN and serum creatinine of healthy and group III after therapy were 0.49±0.19 and 0.49±0.18 mmol/L , 61.7±6.09 and 63.10±6.79 mg/dL, 9.36±0.51 and 9.57±0.36 mg/dL, 2.34±0.15 and 2.25±0.23 mg/dL, 5.21±0.54 and 5.04±0.31 mg/dL, 109±18.06 and 115.9±12.04 U/L, 8.16±0.61 and 8.05±0.52 g/dL, 2.49±0.44 and 2.92±0.66 g/dL, 114.2±11.8 and 115.7±13.39 mg/dL, 13.57±2.97 and 11.52 ± 2.85 gm/dL,1.23 ± 0.15 and 1.16 ± 0.08 mg/dL

respectively. There was no significant difference in all the biochemical parameters of group III compared to healthy and urine analysis for ketones was negative in all the animals of the group after treatment.

After therapy serum glucose, total protein levels increased significantly ($p<0.05$), whereas blood BHBA and serum AST decreased significantly ($p<0.05$).These findings are in accordance with Praveena (2011), Padmaja and Rao (2013) and Srinivasulu (2014). Propionic acid will be released from NutroCal[c] during ruminal fermentation, some of it gets absorbed across the rumen wall into blood and transported to the liver where it is converted to glucose via gluconeogenesis (Van Soest *et al.*, 1994) as shown by numerical increase in serum blood glucose levels from feeding Nutrocal[c] during the seven days of treatment. The glucose is used to meet some of the animal's demand to energy by providing oxaloacetate, which is used to convert acetate to energy as adenosine triphosphate (Van Soest, 1994). Animals not receiving the glucogenic precursors (supplemental diet) would have to meet this demand in energy through mobilisation of body fat reserves resulting in increased concentrations of NEFA and ketone bodies in blood, milk, urine (Veenhuizen *et al.,* 1991; Studer *et al.,* 1993; Grummer *et al.,* 1994; Smith *et al.*, 1997). Waterman *et al.* (2002) reported that feeding Nutrocal[a] to grazing beef cows reduced milk fat content. Evidence (Goff *et al.*, 1996) also exists that shows that feeding calcium propionate to periparturient dairy cows lowers NEFA and reduces subclinical ketosis. Daryl *et al.* (2013) suggested the use of propylene glycol drench for 5 days to treat SCK. Urine samples were subjected to Ross modified Rothera's test on days 1, 2, 3, 4, 5 and 7 of all the buffaloes which showed negative for ketone bodies and blood BHBA levels were reached below 1.0 mmol/L on 7th day after therapy. Propylene glycol may be used to diminish negative energy balance after calving and limit risk of ketosis and fatty liver. It may also affect glycogenic effect in a distinctive process. Propylene glycol gets is metabolised in the rumen and produced lactic and propionic acid that transform into glucose through hepatocytes (Cozzi *et al.,* 1996). Propionate plays a main glycogenic effect as a volatile fatty acid in the rumen and can reduce the BHBA and non-esterified fatty acids (NEFA) during the first two days post-partum (Goff *et al.,* 1996). In the same way, propylene glycol administered orally can diminish the concentrations of NEFA and BHBA (Christensen *et al.,* 1997). Gagandeep *et al.* (2017) concluded that when propylene glycol supplemented at 200 ml/day orally for 5 days resulted in decreased BHBA levels, indicating its effectiveness for the treatment of subclinical ketosis. Application of butaphosphan only in combination with cyanocobalamin exhibits the expected positive effects on the metabolism of early lactating cows with subclinical ketosis (Nuber *et al.,* 2016). Catasol, containing butaphosphan and Vitamin B_{12} (at 5ml/100 kg. b.wt. I/M) was very effective in treatment of subclinical ketotic cows which resulted in increase of healthy animals and milk production (Deniz, 2010). High producing dairy animals in early lactation may have a relative/actual deficiency of cyanocobalamin. Cyanocobalamin is the synthetic form of Vitamin B_{12}. Methylmalonyl coenzyme A (CoA) mutase, a mitochondrial enzyme, involved in the conversion of proppionate to succinyl-CoA, which enters in TCA cycle is an important gluconeogenic substrate (Kennedy *et al.,* 1992; Taoka *et al.,* 1994) and is Vitamin B_{12} dependent. An insufficient supply of Vitamin B_{12}, especially in early lactation, could possibly lead to decreased function of Methylmalonyl coenzyme A (CoA) mutase and hinder energy production from propionate, which results in a less active cycle, potentially decreasing gluconeogenesis. A less active TCA cycle would lead to buildup of acetyl-CoA derived from fatty acid beta-oxidation, which would alternatively be used for ketone body synthesis, leading to enhanced ketogenesis in animal. Stress in parturition causes the hormone cortisol to be released into the blood stream, which can impair immune response and increase susceptibility to diseases. Vitamin B_{12} is associated with enhanced liver metabolism and healthy immune response. About half of the propionate that reaches ruminant liver is converted to glucose by a series of enzymatic reactions which require biotin, Vitamin B_{12}, niacin, pantothenic acid and riboflavin. Vitamin B_{12} is essential in metabolism of post-parturient dairy animals. Vitamin B_{12} may be of importance in hepatic protein metabolism (Padmaja, 2013).

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Conclusions

Present study concludes that both treatments the mentioned were effective for treatments SCK. However, combination of Propylene glycol (Glucoboost[c]) 200ml daily orally for five days and Inj. Butaphosphan and Cyanacobalamin (Synkomet[b]) 5 ml/100 kg b.wt intramuscularly daily for two days was found to be more effective in treatment of subclinical ketosis in buffaloes. Propylene glycol was found to be more effective compared to Calcium propionate as glucogenic precursors for treating buffaloes affected with SCK.

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