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Evaluation of Efficacy of Propylene Glycol in the Treatment of Subclinical Ketosis and its effect on Plasma Concentration of Various Metabolic Parameters

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ABSTRACT

Sub clinical ketosis is the excessive production of ketone bodies in blood leading to reduced reproductive performances and decreased milk yield without showing any clinical sign of ketosis. Since a lot of metabolic parameters are affected during the subclinical ketosis, which are having their roles in normal functioning of the body. Therefore the study was conducted to check the efficacy of Propylene glycol (PG) which was given @ 200 ml per day orally for 5 days in the treatment of subclinical ketosis and to study its effect on various metabolic parameters. A significant decrease was noted in the mean plasma Beta Hydroxyl Butyric Acid (BHBA) and Non Esterified Fatty Acid (NEFA) values, along with a significant increase in the mean plasma glucose, calcium and total plasma proteins levels after treatment. The mean plasma inorganic phosphorus levels were within the normal range. A non significant decrease was observed in the mean plasma copper and zinc levels, where as a increase was noted in iron levels from the late pregnancy upto the early lactation period. However, a increase was noted in the mean values of copper and zinc after treatment with PG. Marked improvement was recorded in the oxidative stress parameters after feeding of PG It was concluded from the present study that Propylene glycol (PG) was given @ 200 ml per day orally for 5 days results in decrease in plasma NEFA and BHBA levels, indicating its effectiveness for treatment of sub clinical ketosis.

Keywords: Subclinical ketosis, BHBA, NEFA, propylene glycol, oxidative stress

Subclinical ketosis (SCK) is defined as the excess of circulating ketone bodies in blood without the presence of clinical signs of ketosis. The SCK is more important than clinical ketosis, because clinical signs are not evident to recognize the disease and the animals continue to produce at a reduced rate resulting in significant economic losses. This condition of SCK mainly occurs due to the incomplete oxidation occurring in the liver during the early lactation period producing acetyl- CoA, which acts as a main precursor for the production of ketone bodies (acetone, acetate and Betahydroxy butyrate) resulting in development of subclinical ketosis (Grummer, 1993). Sub clinical ketosis has been found to be responsible for number of reproductive problems, decreased milk yield, displaced abomasum, fatty liver, mastitis, metritis and clinical ketosis (Ospina *et al.,* 2010). The mechanism by which propylene glycol prevents ketosis is by increasing the oxidation of acetyl-CoA via the Tricarboxylic acid (TCA) cycle, as Acetyl- CoA is the main precursor responsible for producing ketone bodies, and by increasing the production of glucose via gluconeogenesis which stimulates insulin secretion from the pancreas, thereby reducing the fatty acids mobilization from the adipose tissues and hence, substrate for hepatic ketogenesis (Brockman and Laarverd, 1986).

Copper, iron and zinc are essential elements, having a

pivotal role in the growth, reproduction and production of the animals (Ranjan *et al.,* 2006), as Cu is involved in the production of antioxidants via its role in the Cu-Zn superoxide dismutase (SOD) and ceruloplasmin (Spears and Weiss, 2008). In dairy cows, low levels of copper, iron and zinc has been found to be associated with low milk quality with increased somatic cell count, increased susceptibility to mastitis, reduced feed intake, loss of reproduction, growth depression and impaired immune status, decrease fertility, prolonged labour and abortion (Enjalbert *et al*., 2006)

As a lot of work has been done to study the effects of minerals on dairy cows reproduction, growth and production status, but very limited work has been done to study the effect of propylene glycol in the treatment of subclinical ketosis and its effect on various haematological, biochemical and its effects on various minerals.

Therefore the study was planned to study the effect of propylene glycol on the various haematological, biochemical and various other minerals, along with its efficacy in the treatment of sub clinical ketosis.

MATERIALS AND METHODS

The study was conducted in a well organized dairy farm in six crossbred dairy cows in their early lactation period suffering from subclinical ketosis (Blood BHBA > 1.2 mmol/L). Propylene glycol (PG) @ 200ml/day orally was given to these cows for 5 days along with the concentrate feed and fed separately from the forages. Blood samples were collected from these cows in four stages i.e. (i) Far off dry period (FOD)- >10 days following dry off and not < 30 days prior to calving, (ii) close up dry period (CUD)- Between 3 and 21 days prior to calving, (iii) Fresh - 3 to 30 days in milk and (iv) one month after the feeding of propylene glycol to evaluate the effect of PG on various haematological indices viz. haemoglobin (Hb), Packed cell volume (PCV), total erythrocyte count (TEC) and Total leucocyte count (TLC); biochemical constituents (total plasma protiens (TPP), albumin, plasma urea nitrogen (PUN), creatinine, glucose, Beta- hydroxy butyric acid (BHBA) and Non esterified fatty acid (NEFA) and plasma minerals concentrations viz, calcium (Ca), magnesium (Mg), plasma inorganic phosphorus (Pi), sodium (Na), potassium (K), copper (Cu), Iron (Fe) and Zinc (Zn).

Body condition scoring (BCS)

Body condition score assessment was done as per the methods of Ferguson *et al.* (1994).

Haematological analysis

For the haematological pupose, blood was collected in plastic vials containing disodium EDTA as anticoagulant and various parameters(Hb, PCV, TEC and TLC) were estimated on Seimens Advia 2120 Hematology Analyzer, USA.

Biochemical parameters analysis

Various biochemical parameters (TPP, albumin, PUN, creatinine and glucose) were accessed on semi automatic Biochemistry analyzer (DT analyzer) using kits provided by Ortho Clinical Diagnostics, UK. Both plasma BHBA and NEFA levels were estimated in the ELISA plates with the help of kits provided by Diasys Diagnostics systems, Germany.

Plasma minerals analysis

Copper, iron and zinc

Two ml of each of the plasma sample was digested with 10 ml of double distilled nitric acid over a hot plate and heated below 80ºC till digestion, followed by one cycle of hydrogen peroxide AR (2.0 ml of 30%), until volume reduced to 1-2 ml. The digested samples were diluted with double glass distilled water and the volume of the digestate was made 10 ml, and Cu, Fe and Zn contents were estimated by Atomic Absorption Spectrophotometer (Perkin Elmer Analyst 700, USA).

Calcium, magnesium, sodium and potassium

0.1 ml of plasma samples were mixed with 9.9 ml of 0.1 % of lanthanium chloride and the concentrations of minerals viz. Ca, Mg, Na and K were estimated by Atomic Absorption Spectrophotometer (Perkin Elmer Analyst 700, USA).

Plasma inorganic phosphorus

Plasma Pi was determined by using method given by Tausky and Shorr (1953) and the readings were taken using spectrophotometer (Perkin Elmer Lamda 25 UV/ VIS Spectrometer, USA).

Oxidative stress parameters

Oxidative stress parameters such as Lipid peroxidation (LPO), Superoxide Dismutase (SOD) and Reduced Glutathione (GSH) were analyzed by the methods of Placer *et al.* (1966), Nishikimi *et al.* (1972) and Hafeman *et al.* (1974) respectively.

Statistical analysis

Mean, standard error of mean of various parameters were estimated and test of significance (one way analysis of variance ANOVA), paired t-test for comparing within group were performed using SPSS for Windows (version 16.0; Microsoft).

RESULTS AND DISCUSSION

Haematological parameters

The mean haematological parameters were within the normal physiological ranges, though marked variations were observed during different phases of periparturient period. The mean Hb and PCV values showed a significant ($p<0.05$) decrease from far off dry period (FOD) period upto the fresh period, where as a non significant increase was noticed in the mean TEC and TLC levels from late pregnancy upto early lacatation period (Table 1), whereas post treatment the mean PCV levels showed a significant ($p<0.05$) increase and mean Hb and TEC levels showed a non-significant increase. However no significant difference was noted in mean TLC levels after feeding of propylene glycol (Table 2). This improvement in haematological parameters ((significant increase in PCV, and non significant increase in Hb and TEC) recorded after treatment could be due to the improved metabolic status after the PG feeding and also could be due to increase in dry matter intake as compared to the parturition period. Similar to our studies Sahoo *et al.* (2009) in his studies also reported a significant increase in haematological parameters following treatment in cows with subclinical ketosis.

Table 1: Haematological parameters in cows affected with subclinical ketosis (Mean± SE)

Parameters	FOD	CUD	Fresh
Hb(g/dl)	11.80±0.52^{a}	10.76±0.53^{ab}	9.16±0.38^{c}
PCV (%)	37.16±1.60^{a}	33.85±1.44^{ab}	28±2.11^{c}
TEC(x10^{6}/µl)	5.62±0.23^{a}	6.14±0.23^{a}	5.91±0.35^{a}
TLC(x10^{3}/µl)	9.18±0.40^{a}	9.72±0.45^{a}	12.56±1.76^{a}

Values bearing different superscript (a, b, c) in a row differ significantly ($p<0.05$)

Table 2: Effect of Propylene glycol feeding on haematological parameters (Mean± SE)

Parameter	Pre treatment	Post treatment
Hb(g/dl)	9.16±0.38^{a}	10.68±0.65^{a}
PCV (%)	28±2.11^{a}	35.58±2.60^{b}
TEC(x10^{6}/µl)	5.91±0.35^{a}	5.94±0.21^{a}
TLC(x10^{3}/µl)	12.56±1.76^{a}	9.33±0.38^{a}

Values bearing different superscript (a, b) in a row differ significantly ($p<0.05$)

Biochemical parameters

The mean total plasma proteins and glucose levels were within the normal range, though a significant ($p<0.05$) decrease was noticed from late pregnancy upto early lactation period (Table 3), whereas a significant ($p<0.05$) increase was noted in both the parameters following PG feeding. This decrease in mean plasma glucose and total proteins levels from the late pregnancy upto the early lactation period might be due to low energy diet and also due to reduce dry matter intake during the early lactation period, (Nazifi *et al*., 2008) due to which there is fat mobilization which occurs in response to negative energy balance resulting in increase in mean plasma BHBA and NEFA levels (Padilla *et al.,* 2005).

The mean plasma PUN, BHBA and NEFA values showed significant increase ($p<0.05$) from late pregnancy to early lactation period (Table 3), and a significant decrease ($p<0.05$) in the plasma BHBA and NEFA and a non significant decrease in mean plasma PUN value after PG feeding (Table 4), whereas, no significant difference was noted in the mean plasma albumin and creatinine values from late pregnancy to early lactation period and after

feeding of PG (Table 3 and 4). Similar findings were reported by Doepel (2002) and Singh *et al.* (2014) who has also reported a significant increase in PUN levels in the peri-parturent period in dairy cows. During the periparturient period, when there is increase in the demand of protein and energy, required for foetal growth and colostrums synthesis, then during this period energy is supplemented by the increase in the catabolism of amino acids resulting in the increase in urea production (Bell, 1995) which was the case in our study as well, as there was decrease in the glucose levels during the periparturient period, resulting in significant decrease in total plasma proteins concentration and thus increased PUN levels.

A number of studies (Zhang *et al*., 2010; Ribeiro *et al*., 2013), have also reported results similar to our findings where they classified a cow affected with SCK on the basis of blood BHBA levels above 1.2 mmol/L. The possible reason for this increase in ketone bodies production is due to 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)

Similarly a decrease in plasma BHBA and NEFA levels and a increase in plasma glucose levels after PG feeding is due to the reason that the PG helps in completion of TCA cycle by providing pyruvate and propionate, which are the major end products of PG metabolism, which in turn helps in increasing the supply of glucose via gluconeogenesis and thus reducing the fat mobilization, which in turn help in decreasing the plasma BHBA and NEFA levels (Brockman and Laarveld, 1986) (Table 3 and 4).

Table 3: Plasma biochemical parameters in cows affected with subclinical ketosis (Mean± SE)

Parameters	FOD	CUD	Fresh
TPP (g/dl)	7.38±0.25^{a}	6.96±0.20^{a}	6.33±0.22^{b}
Albumin (g/dl)	2.50±0.13^{a}	2.48±0.12^{a}	2.50±0.18^{a}
PUN (mg/dl)	7.50±0.56^{a}	11.33±0.76^{b}	15.33±1.02^{c}
Creatinine (mg/dl)	1.11±0.09^{a}	1.13±0.07^{a}	1.25±0.15^{a}
Glucose(mg/dl)	71.16±7.64^{a}	58±3.41^{ab}	41.83±1.10^{c}
BHBA (mmol/L)	0.67±0.07^{a}	0.74±0.06^{ab}	1.33±0.02^{c}
NEFA (mmol/L)	0.24±0.04^{a}	0.34±0.02^{ab}	0.60±0.02^{c}

Values bearing different superscript (a, b, c) in a row differ significantly ($p<0.05$)

Table 4: Effect of Propylene glycol feeding on plasma biochemical parameters (Mean± SE)

Parameter	Pre treatment	Post treatment
TPP (g/dl)	6.33±0.22^{a}	7.46±0.33^{b}
Albumin (g/dl)	.50±0.18^{a}	2.60±0.24^{a}
BUN (mg/dl)	15.33±1.02^{a}	14.50±1.70^{a}
Creatinine(mg/dl)	1.25±0.15^{a}	1±0.20^{a}
Glucose(mg/dl)	41.83±1.10^{a}	54±2.03^{b}
BHBA (mmol/L)	1.33±0.02^{a}	0.72±0.06^{b}
NEFA (mmol/L)	.60±0.02^{a}	0.30±0.03^{b}

Values bearing different superscript (a, b) in a row differ significantly ($p<0.05$)

Plasma minerals concentrations

Plasma minerals concentrations were within the normal physiological ranges, with a significant decrease in plasma calcium concentrations, non significant decrease in plasma inorganic phosphorus, along with a non significant increase in plasma magnesium levels from the FOD period upto the fresh period; however post feeding the mean plasma Ca levels showed a significant increase where as the plasma magnesium and phosphorus levels showed a non significant increase (Table 5 and 6). Similar results were reported by Stokes *et al.* (2001) who also reported an increase in the plasma calcium levels after the PG feeding. This decline in plasma calcium levels in the late gestation and in the early lactation period is due to the increased transfer of calcium for the development of foetal skeleton and for the milk synthesis during the early lactation period, which along with decreased dry matter intake is not balanced by increase in rate of absorption from gut or mobilization from bone (Sansom *et al*., 1983).

Similarly a non significant decrease in the mean plasma Pi levels and a non-significant increase in the plasma Mg levels might be due to the decrease in the plasma Ca concentrations, resulting in parathyroid hormone secretion which results in elevated renal threshold for calcium, resulting in similar increase in renal threshold for magnesium and increasing the urinary loss of phosphorus (Thilsing-Hansen *et al*., 2002) (Table 5 and 6).

The mean plasma Na and K levels showed a non significant decrease from the late pregnancy upto the early lactation period, while the mean plasma Na levels further

decreased non significantly, where as the plasma K levels increased non significantly after PG feeding (Table 5 and 6). The possible reason for the decrease in the levels of these electrolytes is their utilization for the foetus growth during late pregnancy and also due to the transfer of these electrolytes in milk during early lactation (Deshpande *et al*., 1998).

The mean plasma Cu, Fe and Zn levels were within the normal physiological ranges; however the plasma Cu and Zn levels decreased non significantly and the mean plasma Fe levels increased non significantly from the late pregnancy upto the early lactation period, where as non significant differences were recorded in Cu, Fe and Zn levels after the PG feeding (Table 5 and 6). This decrease observed in Cu and Zn levels during the periparturient period could be another reason for the decrease in the SOD levels as observed in our another study (Singh *et al.,* 2014), in which a decrease was recorded in the SOD levels from the FOD period upto the fresh period as Cu is involved in the antioxidants production via Cu-Zn SOD (Spears and Weiss, 2008), and also due to the higher Fe levels which causes inhibition of the intestinal Cu absorption (Noaman, 2013). Similar to our results Zhang *et al.* (2010) reported a significant decrease in the plasma Zn levels and no effect on plasma Cu levels in between the healthy and SCK affected cows.

Similarly reduced mean plasma Zn concentration around the peripartum might be due to the fact that during the late term fetuses accumulate Zn at a rate of about 12mg/day (House and Bell, 1993) and also during early lactation Zn was required for the colostrum synthesis (Kincaid and Cronrath, 1992).

Table 5: Plasma minerals concentrations in cows affected with subclinical ketosis (Mean± SE)

Parameters	FOD	CUD	Fresh
Ca (mg/dl)	11.12±0.40^{a}	10.65±0.56^{ab}	8.98±0.59^{c}
Mg (mg/dl)	3.88±0.24^{a}	3.73±0.39^{a}	4.14±0.19^{a}
Pi (mg/dl)	4.21±0.26^{a}	4.32±0.17^{a}	4.20±0.24^{a}
Na (mmol/l)	127.28±6.13^{a}	123.54±4.27^{a}	118.97±7.43^{a}
K (ppm)	5.31±0.52^{a}	5.21±0.17^{a}	4.15±0.44^{a}
Cu (ppm)	0.95±0.11^{a}	0.84±0.12^{a}	0.86±0.07^{a}
Fe (ppm)	1.35±0.34^{a}	2.03±0.69^{a}	2.69±0.88^{a}
Zn (pmm)	0.88±0.20^{a}	0.68±0.13^{a}	0.61±0.07^{a}

Values bearing different superscript (a, b, c) in a row differ significantly ($p<0.05$)

Table 6: Effect of Propylene glycol feeding on plasma minerals concentrations (Mean± SE)

Parameter	Pre treatment	Post treatment
Ca (mg/dl)	8.98±0.59^{a}	11.23±0.40^{b}
Mg (mg/dl)	4.14±0.19^{a}	4.17±0.25^{a}
Pi (mg/dl)	4.20±0.24^{a}	4.91±0.23^{a}
Na (mmol/l)	118.97±7.43^{a}	113.56±4.26^{a}
K (ppm)	4.15±0.44^{a}	4.21±0.08^{a}
Cu (ppm)	0.86±0.07^{a}	0.92±0.07^{a}
Fe (ppm)	2.69±0.88^{a}	1.24±0.58^{a}
Zn (pmm)	0.61±0.07^{a}	0.96±0.24^{a}

Values bearing different superscript (a, b) in a row differ significantly ($p<0.05$)

Oxidative stress parameters

A significant increase was seen in LPO levels and a significant decrease was noted in SOD and GSH levels from FOD to fresh period in cows affected with SCK, whereas after feeding with Propylene glycol non significant decrease in LPO levels, and a significant increase was recorded in SOD and GSH levels. (Table 7 and 8) Similar to our study Karimi *et al.* (2015) reported a increase in LPO levels in post calving period in cows affected with subclinical ketosis, this increase in the MDA levels in cows affected with SCK is due to the increase amount of stress imposed upon these cows due to the effect of subclinical ketosis, as during this period there is increased production of oxidative radicals and as the, antioxidant system of body cannot deal with the increased lipoperoxide production during the first week after calving resulting in increase in oxidative stress in the animal body (Konvicna *et al.,* 2015).

Similarly a decrease was noted in the SOD and GSH levels in SCK cows depicting a decrease in the antioxidant defence in the body during subclinical ketosis. As it is well known, SOD is a Cu/Zn-dependent enzyme and erythrocyte GSH-Px is a Se-dependent enzyme, so a reduction in zinc and copper availability in the early postpartum period of dairy cows might explain the reduction of SOD activity during early lactation period (Michiels *et al.,* 1994).

Table 7: Oxidative Stress parameters in cows affected with subclinical ketosis (Mean± SE)

Parameters	FOD	CUD	Fresh
LPO (n mol/g Hb)	215.31 ± 33.27^{a}	314.44 ± 33.71^{bd}	352.89 ± 31.61^{cd}
SOD (U/mg Hb)	86.37 ± 6.68^{a}	74.78 ± 1.10^{b}	56.86 ± 1.80^{c}
GSH (mM)	2.96 ± 0.22^{a}	2.46 ± 0.14^{b}	1.75 ± 0.09^{c}

Values bearing different superscript (a,b,c,d) in a row differ significantly ($p<0.05$)

Table 8: Effect of Propylene glycol feeding on oxidative stress parameters (Mean± SE)

Parameter	Pre treatment	Post treatment
LPO (n mol/g Hb)	352.89±31.61^{a}	291.20±16.78^{a}
SOD (U/mg Hb)	56.86±1.80^{a}	70.27±1.44^{b}
GSH (mM)	1.75±0.09^{a}	2.24±0.09^{b}

Body condition score (BCS)

The mean BCS showed a significant decrease from the FOD (3.70±0.07) upto the fresh period (2.70±0.10) and post treatment the mean BCS (3.16±0.08) again showed a significant increase from the early lactation period. This decrease in BCS observed in SCK cows could be due to increase mobilisation of body fat reserves after parturition for the energy requirements for maintenance and lactation (Fonesca *et al*., 2004) where as after treatment a marked improvement was noticed in SCK cows in the body condition score.

Milk yield

A significant increase was noticed in the milk yield one month after the treatment (18.83±0.40) with propylene glycol in comparison to pre treatment yield (14.66±0.42). This decrease in milk yield in cows affected with SCK may be due to the higher production of ketone bodies, which alters milk production (Anderson, 1988), where as an increase in milk production due to the PG feeding could be due to the increase glucose availability and reduced production of ketone bodies which helps in increased milk production synthesis (Fonesca *et al.,* 2004).

CONCLUSION

It was observed in the present study that PG was effective in the treatment of SCK and also significant alterations were noticed in various metabolic parameters (haematological, biochemical, minerals and oxidative stress) in cows affected with subclinical ketosis.

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