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Comparison of physiological parameters in healthy and sub-clinical ketosis affected cows before and after treatment with propylene glycol

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Abstract
The study was undertaken to study the comparison of physiological parameters in Healthy and Sub-clinical Ketosis affected cows before and after treatment with propylene glycol in and around Bengaluru. Recently calved cows (within 0 – 2 months) were subjected to blood BHBA estimation and cases with blood BHBA level between the range of 1400 µmol/L to 2500 µmol/L were selected as positive for subclinical ketosis. Whereas, blood BHBA level less than 1000 µmol/L were selected as negative for subclinical ketosis and these animals were selected as control group.
Animal found positive for subclinical ketosis were treated with Propylene glycol 200 ml per day orally for 5 days. There was no significant difference in the values of temperature, pulse and respiratory rates of subclinical ketosis animals when compared to healthy group before and after treatment and there was a significant decrease in Rumeno-reticular motility of subclinical ketosis cows compared with healthy group before treatment. Following therapy non-significant increase in rumeno-reticular motility was observed. The blood BHBA in subclinical ketosis animals were significantly ($P \leq 0.05$) higher compared to the healthy animals before treatment. After treatment the blood BHBA significantly ($P \leq 0.05$) decreased compared to the values of before treatment. When healthy animals were compared with SCK animals there was non-significant decrease in milk yield per day before treatment and after treatment milk yield was significantly increased which could be due to increase in the blood glucose, reduction in BHBA and also due to the improvement in the overall metabolic status of the animal.

Keywords: Subclinical ketosis, BHBA, propylene glycol

Introduction

Subclinical ketosis may be defined as a preclinical stage of ketosis characterized by an elevated ketone body level without clinical signs such as loss of appetite, hard feces, or dullness. Consequently, it can be confirmed only by qualitative or quantitative analysis of body fluids (Anderson, 1988) [1]. The disease runs sub-clinically; therefore it might be called as silent profit robber on account of its impact on the profitability of dairy farm by milk production loss (around 300kg per lactation), reproduction disturbances (low conception rate, increased artificial insemination), high risk for developing abomasum displacement, metritis, mastitis and clinical ketosis (Deniz, 2011) [3].

More than 90% of subclinical ketosis cases occur in the first and second months after calving. During this period, approximately 40% of all cows are affected by subclinical ketosis at least once, although the prevalence is highest in the first and second weeks after calving (Geishauser *et al.*, 2001) [6]. Subclinical ketosis can be detected by analyzing blood glucose, blood non-esterified fatty acids (NEFA) and ketone bodies in blood, milk and urine (Anderson, 1988) [1]. Hence the present study was undertaken to identify sub clinical ketosis affected cows and to know the effect of subclinical ketosis on physiological parameters in cross bred cows.

Material and Methods

Recently calved cows (0 – 2 months) belonging to the Veterinary College dairy farm Bengaluru, outdoor patients brought for treatment at Veterinary College Hospital, Bengaluru and individual animals shown by owners at their holdings in and around Bengaluru were examined for subclinical ketosis during the year October and November 2019. These cows were subjected to blood BHBA estimation and cases with blood BHBA level between the ranges of 1400 µmol/L to 2500 µmol/L were selected as positive for subclinical ketosis. Cows within two months of calving which were apparently healthy and blood BHBA level less than

1000 µmol/L were selected as negative for subclinical ketosis and these animals were selected as control group.

Blood BHBA: Blood BHBA was estimated by using Freestyle Optium Neo H Blood glucose and ketone monitoring system (Abbott Laboratories, UK) and Freestyle Optium H blood beta ketone test strips (Abbott Laboratories, UK) as described by Schade DS and Eaton RP (1982).

A small drop of blood from ear vein was instilled on a disposable ketone test strip of the meter and after few seconds results were recorded and expressed in mmol/L and convert it to µmol/L by multiplying the results with 10^{-3}.

Detailed clinical examination was carried out for each animal as per the standard methods suggested by Kelly (1984) [8]. The rectal temperature of all the animals was recorded in ^{0}F. The pulse rate was recorded over the middle coccygeal artery and expressed as rate per minute, respiration was counted by observing the nostril movements and heart rate was recorded in beats per minute. The rumeno-reticular motility was recorded by directly placing the fist on left flank and rumen motility counted for three minutes. The milk yield per day of these animals were also recorded.

Animals were divided into 2 groups of 6 animals in each group

Group I or Control group: Recently calved apparently healthy cows, showing blood BHBA level lower than 1000 µmol/L. No treatment was given.

Group II: Animal found positive for subclinical ketosis were treated with Propylene glycol 200 ml per day orally for 5 days.

Results and Discussion

Blood beta hydroxy butyric acid (BHBA): In the present study, the mean ± SE of BHBA on 0th, 3rd, 7th and 14th day were 633.33±55.78µmol/L, 616.67±40.14µmol/L, 683.33±47.73µmol/L and 666.67±49.44µmol/L, respectively. No significant difference was observed between days in Group I.

The mean ± SE of BHBA in Group II on 0th, 3rd, 7th and 14th day were 1683.33±60.09µmol/L, 1083.33±94.58µmol/L, 816.67±47.73µmol/L and 716.67±47.73µmol/L, respectively. There was a significant ($P \leq 0.05$) decrease in BHBA was observed between 0th to 3rd day ,0th to 7th day, 0th to 14th day, 3rd to 7th and 3rd to 14th day. Whereas between 7th to 14th day failed to show any significant difference.

Temperature (ºF): In the present study, the mean ± SE of temperature on 0th, 3rd, 7th and 14th day were 101.15±0.11, 101.45±0.15, 101.37±0.14 and 101.15±0.1921, respectively. There was no significant difference observed between days in Group I.

The mean ± SE of temperature in Group II on 0th, 3rd, 7th and 14th day were 101.12±0.13, 101.20±0.08, 101.15±0.19 and 101.08±0.24, respectively. There was no significant difference observed between days in Group II.

Pulse rate (Per minute): In the present study, the mean ± SE of pulse rate per minute on 0th, 3rd, 7th and 14th day were 58.67±0.84, 59.00±0.86, 59.00±0.45 and 59.67±0.61, respectively. There was no significant difference observed between days in Group I.

The mean ± SE of pulse rate per minute in Group IV on 0th, 3rd, 7th and 14th day were 58.67±0.67, 59.67±1.09, 59.33±0.84 and 59.33±0.67,respectively. There was no significant difference was observed between days in Group II.

Respiration rate (Per minute): In the present study, the mean ± SE of respiration rate per minute on 0th, 3rd, 7th and 14th day were 20.00±0.73, 20.67±0.67, 20.67±0.42 and 20.67±0.67, respectively. There was no significant difference was observed between days in Group I.

The mean ± SE of respiration rate per minute in Group II on 0th, 3rd, 7th and 14th day were 19.33±0.42, 19.00±0.45, 19.33±0.67 and 19.00±0.45, respectively. There was no significant difference was observed between days in Group II.

Rumeo-reticular motility (Per 2 minute): In the present study, the mean ± SE of rumeno-reticular motility per 2minutes on 0th, 3rd, 7th and 14th day were 2.50±0.22, 2.67±0.21, 2.50±0.22 and 2.67±0.21, respectively. There was no significant difference was observed between days in Group I.

The mean ± SE of rumeno-reticular motility per 2minutes in Group II on 0th, 3rd, 7th and 14th day were 1.67±0.21,1.83±0.17, 1.83±0.17 and 2.17±0.17,respectively. There was no significant difference was observed between days in Group II.

Milk yield (Liters per day): In the present study, the mean ± SE of milk yield per day on 0th, 3rd, 7th and 14th day were 14.83±1.23, 14.83±1.19, 14.58±1.01 and 14.83±1.11 respectively. There was no significant difference observed between days in Group I.

The mean ± SE of milk yield per day in Group II on 0th, 3rd, 7th and 14th day were 13.08±0.94, 13.33±0.93, 13.92±0.87 and 14.17±0.81, respectively. There was a significant ($P \leq 0.05$) increase in milk yield was observed between 0th to 7th day, 0th to 14th day, 3rd to 7th day and 3rd to 14th day. Further no significant difference ($P \geq 0.05$) noticed between 0th to 3rd day and 7th to 14th day in Group II.

Table 1: BHBA and physiological parameters in Group I and Group II

Group	Parameters	0th day	3rd day	7th day	14th day
Group – I	BHBA	633.33±55.78^{ax}	616.67±40.14^{ax}	683.33±47.73^{ax}	666.67±49.44^{ax}
Group – II	BHBA	1683.33±60.09^{bx}	1083.33±94.58^{by}	816.67±47.73^{az}	716.67±47.73^{az}
Group – I	Temperature	101.15±0.11^{ax}	101.45±0.15^{ax}	101.37±0.14^{ax}	101.15±0.19^{ax}
Group – II	Temperature	101.12±0.13^{ax}	101.20±0.08^{ax}	101.15±0.19^{ax}	101.08±0.24^{ax}
Group – I	Pulse	58.67±0.84^{ax}	59.00±0.86^{ax}	59.00±0.45^{ax}	59.67±0.61^{ax}
Group – II	Pulse	58.67±0.67^{ax}	59.67±1.09^{ax}	59.33±0.84^{ax}	59.33±0.67^{ax}
Group – I	Respiration rate	20.00±0.73^{ax}	20.67±0.67^{ax}	20.67±0.42^{ax}	20.67±0.67^{ax}
Group – II	Respiration rate	19.33±0.42^{ax}	19.00±0.45^{ax}	19.33±0.67^{ax}	19.00±0.45^{ax}
Group – I	Rumeno-reticlar motility	2.50±0.22^{ax}	2.67±0.21^{ax}	2.50±0.22^{ax}	2.67±0.21^{ax}
Group – II	Rumeno-reticlar motility	1.67±0.21^{bx}	1.83±0.17^{bx}	1.83±0.17^{ax}	2.17±0.17^{ax}

Group – I	Milk yield	14.83±1.23[ax]	14.83±1.19[ax]	14.58±1.01[ax]	14.83±1.11[ax]
Group – II	Milk yield	13.08±0.94[ax]	13.33±0.93[ax]	13.92±0.87[ay]	14.17±0.81[ay]

a, b, c, d Mean values in a row with different superscripts differ significantly ($P \leq 0.05$)
w, x, y, z Mean values in a column with different superscripts differ significantly ($P \leq 0.05$)

Discussion

Blood beta hydroxy butyric acid: There was a significant increase in blood BHBA in animals suffering with SCK animals when compared with healthy animals. Similar findings reported by Rodriguez-Jimenez *et al.* (2018) [11] and (Djokovic *et al.*, 2019) [4]. This could be due a dramatic increase in energy requirements during the late pregnancy and early lactation making dairy cows highly susceptible to negative energy balance. A majority of cows cannot meet their energy requirements for milk production and are forced to mobilize body fat to meet their energy needs.

When large amount 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 when gluconeogenic precursors are limiting. Ketone bodies provide energy to peripheral tissues when carbohydrates are limiting. The circulating ketone bodies are acetoacetate (AcAc), beta hydroxybutyrate (BHB) and acetone (Ac), where acetoacetate (AcAc) is the parent ketone body, which can be reduced to beta-hydroxybutyrate (BHB) in an enzymatic reaction or decarboxylated to acetone (Ac) in a spontaneous non-enzymatic reaction In subclinical ketosis affected cows, BHBA is the predominant circulating ketone body and is relatively stable in whole body, plasma or serum (Dohoo and Martin, 1984) [5].

Physiological Parameters: The physiological parameters such as temperature, pulse rate, respiration rate and rumeno-reticular motility were recorded in each animal.

The mean ± SE values of temperature, Pulse rate and respiration rate in healthy and subclinical ketosis (before and after treatment) in group II were within their physiological limits and no significant difference was noticed. The results of the present study agree with findings of Padmaja and Rao (2013) [10], Sainath (2015) [12], Constable *et al.* (2017) [2] and Mohammed (2019) [9] who also observed that there was no significant difference in these values in animals suffering with subclinical ketosis (before and after treatment) and healthy animals.

When mean ± SE of rumeno-reticular motility of control group was compared with, Group II there was significant decrease in rumeno-reticular motility was noticed which was in accordance with the authors Padmaja and Rao (2013) [10], Sainath (2015) [12], Constable *et al.* (2017) [2] and Mohammed (2019) [9]. This could be due to the fact that there would be decrease in contractile strength of rumen as a result of gradual decrease in serum calcium level (Huber *et al*., 1981) [7] and also there would be excessive generation of ketone bodies that affect ruminal motility causing incomplete and depressed ruminal contraction.

In Group II where SCK affected animals were treated with propylene glycol, there was non-significant increase in the post treatment values when compared to pre-treatment giving an indication that rumeno-reticular motility has no significant role in subclinical ketosis which was also recorded by Surender (2019) [16]. However, Sainath (2015) [12] observed a significant ($P \leq 0.05$) decrease before therapy and a significant ($P \leq 0.05$) increase after therapy. Therefore significant difference in rumino-reticular motility may depend on the severity of subclinical ketosis (Surender, 2019) [16].

Milk yield per day: When healthy animals were compared with SCK animals there was statistically non-significant decrease in milk yield per day. Some of the authors reported significant decrease in milk yield in animals suffering with either clinical or subclinical form of ketosis (Constable *et al.*, 2017) [2]. The decreased milk yield in early lactation might be due to hypoglycemia in subclinical ketosis which caused drop in lactose synthesis and hence reduced milk production (Simensen *et al.*, 1990) [14] and may be due to the higher production of ketone bodies, which alters milk production (Anderson, 1988) [1].

In Group II there was significant increase of milk yield per day from 0th day to 14th day of therapy. This is in accordance with Singh *et al.* (2017) [15]. The increase in milk production due to the propylene glycol feeding will increases glucose availability and reduced production of ketone bodies which helps in increased milk production synthesis.

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