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Research Article

DETECTION OF SUBCLINICAL KETOSIS IN BLOOD OF COWS

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Abstract: The present investigation was carried out on crossbred and Sahiwal cows [Healthy cows (n=146) and Subclinical ketotic (SCK) cows (n=22)] of the institute herd and rural dairy farms around Karnal. The blood samples were tested for presence of ketone bodies during early stage of lactation (5-60 days) postpartum from August 2019 to March 2020. Milk production level (<10kg, 10-20 kg and >20 kg/d) were recorded during the experiment. Blood ketone, plasma glucose and Non Esterified Fatty Acid (NEFA) levels were detected to find out incidence of Subclinical Ketosis. Results showed that the healthy cows had higher (P<0.05) level of glucose and lower BHBA (P<0.01) in comparison to cows suffering from SCK incidence. Milk yield of cows selected from the farm were significantly lower (P<0.01) in comparison to field animals (15.48vs.34.56kg/d). Subclinical ketotic cows of farm produced less milk yield (7.72kg/d) than the healthy cows (P<0.01). BCS of cows and buffaloes of field and farm did not differ between the healthy and SCK animals. Blood glucose level was negatively correlated to NEFA and BHBA level (r=-0.772 and r= -0.578, P<0.01). It can be concluded that the high producer crossbred cows were more vulnerable to SCK due to high milk production during early stage of lactation. Sahiwal cows suffer less from SCK due to medium level of milk production. Factors like high milk production, negative energy balance in transition period makes the animal more susceptible to SCK incidence. Monitoring and screening of animals for the incidence of SCK at monthly intervals should be practiced by the farmers to manage the SCK and to enhance income.

Keywords: *Cows, Glucose, Milk yield, NEFA, Subclinical ketosis*

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Introduction

According to 20th livestock census the total milch cattle in the country is 74.18 million increased by 9.8 % over the previous census. At the onset of lactation, the dairy cow must accommodate a tremendous increase in energy demand by the mammary gland for milk production. Ketosis is one of the most important production disorders occur due to impaired carbohydrate metabolism leads to increased concentrations of betahydroxybutyrate (BHBA), acetoacetate (AcAc) and acetone in blood, milk and urine [1]. Ketosis can either be Clinical or Subclinical ketosis (SCK). Excess of ketone bodies in blood without any clinical signs of ketosis is called as SCK. The circulating ketone body most commonly used to diagnose SCK is blood BHBA. The threshold concentration of BHBA for SCK is 1.2 - 3.0 mmol/L. Cows above this threshold indicate clinical ketosis [2,3]. Ketosis has become a very common metabolic disorder in modern dairy production by causing decrease in milk production and increase in prevalence and duration of fresh cow diseases. Lactating animals have three distinct phase these *viz*., milk production phase, pregnancy phase and transition phase. Though each physiological stage has its own significance, but transition phase is most crucial to determine the enseeing lactation yield and reproductive performance. Transition period is the period from 3 weeks prior to parturition to 3 weeks after parturition [4]. Therefore, this period is especially critical for health and subsequent performance of dairy cows [5-7]. Ketosis can be diagnosed by measuring ketone bodies present in urine, milk, and blood. It is essential to diagnose ketosis in dairy cows, especially during early lactation for prevention and control in advance as well as shut out economic losses. Bovine ketosis is of considerable economic relevance and has been reported to be responsible for downturn in milk production even before two weeks of its clinical symptoms. Serious economic losses have been attributed to the decline of milk production and unattainability of the animals to the peak production potential even after treatment of ketosis [8].

In this study, incidence of subclinical ketosis was determined cows in institute farm and nearby field.

Materials and Methods

Blood samples were collected from the experimental cows in the morning before offering the feed. Blood sample (5 ml) was collected from the jugular vein in disodium EDTA coated vacutainer tubes. To prevent haemolysis the collected blood samples were immediately transported to laboratory and centrifuged at 3000 rpm for 20 minutes to separate out the plasma. Plasma samples were stored at -20ºC in deep freezer till further analysis. The blood BHBA level was estimated by Abbott Blood Glucose and Ketone Monitoring System (Glucometer/Ketometer) using blood β ketone test strips [Fig-1].

Blood samples was collected for blood BHBA level directly from the ear pinna of the experimental animals into the ketone test strip under filed and farm conditions. The applied small amount of blood (1.5 µL) to the end of the ketone test strip and the strip draws the blood into a small sample well. The ketone test strip contains BHBA dehydrogenase, which oxidizes BHBA to AcAc.

This reduces 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 the meter and is directly proportional to the BHBA concentration in the sample. The meter displayed the results as mmol/L after 10 seconds. Plasma Non-esterified fatty acids (NEFA) were estimated using ELISA kits which were procured from the M/S Bioassay technology laboratory. The subclinical ketosis was diagnosed on the basis of plasma beta- hydroxybutyrae (BHBA) and NEFA concentration of >1.2 mmol/L and >0.7 mmol/L, respectively, along with the presence of other signs such as decrease in milk yield and body condition score.

Table-1 *Mean plasma glucose, BHBA, NEFA level and BCS in healthy and subclinical ketotic cows of farm in early lactation*

Parameters	Healthy (n=146)	Subclinical ketosis(n=22)	Overall(n=168)
Glucose(mg/dl)	69.25^{x} ±1.28	42.44^{y} ±0.82	64.68^{x} ±1.64
BHBA (mmol/L)	0.49^{x} ±0.08	1.69^{y}±0.09	0.68^{x} ±0.06
NEFA (mmol/L)	0.19^{x} ±0.05	0.64^{y}±0.01	0.38^{x} ±0.07
BCS (1-5 scale)	2.88^{x} ±0.49	2.66^{y}±0.51	2.69^{x}±0.48

Values with different superscripts x, y in a row differ (P<0.05)

Table-2 *Overall mean milk production of healthy and subclinical ketotic experimental cows during early lactation in farm and field animals*

Parameters	Healthy cows (n=146)		Subclinical ketotic cows (n=22)		Overall (n=168)	
Milk production (Kg)	Farm	Field	Farm	Field	Farm	Field
	15.48^{x}±0.59	34.56^{y}±2.05	7.72^{x} ±0.67	33.66^{y}±2.03	13.76^{x}±0.53	25.46^{y}±0.45
BHBA (mmol/L)	0.36^{x} ±0.27	0.43^{x} ±0.12	1.67^{x} ±0.29	1.29^{y} ±0.21	0.76^{x} ±0.37	0.87^{x} ±0.36
BCS (1-5 scale)	2.99^{x} ±0.52	2.78^{x} ±0.56	2.55^{x} ±0.48	2.77^{x} ±0.37	2.68^{x} ±0.51	2.71^{x} ±0.56

Values with different superscript x, y differ (P<0.05) in a row

Result

The mean values of blood parameters *viz*., plasma glucose, BHBA and NEFA level in healthy and SCK affected cows under the field and farm have been presented in [Table-1&2]. The mean plasma glucose level was significantly higher in healthy farm cows (69.25±1.28mg/dl) in comparison to the subclinically ketosis affected cows (42.44±0.82mg/dl; [Table-1]. However, blood BHBA levels was lower (P<0.05) in healthy (0.49±0.08mmol/L) than in cows suffering from subclinical ketosis (1.69±0.09). Plasma NEFA level was less (P<0.05) in healthy cows in comparison to subclinical ketotic cows (0.19±0.05vs.0.64±0.01mmol/L). The overall mean milk production of healthy and subclinical ketotic cows of field and farm in early lactation revealed that the milk production was significantly lowers (P<0.01) in farm healthy cows in comparison to field cows (15.48vs.34.56kg/day; [Table-2]. The mean blood BHBA level was higher (P<0.01) in field cows in comparison to farm (0.43vs.0.36mmol/L), however BCS of the farm and field cows did not vary [Table-2]. The overall pooled correlation revealed a positive correlation of BHBA with NEFA (r= 0.782; P<0.01) and negative correlation with plasma glucose (r=-0.722; P<0.01). Further plasma NEFA level was negatively correlated to glucose (r= -0.578; P<0.01) [Table-3].

Fig-1 The blood BHBA level was estimated by Abbott Blood Glucose and Ketone Monitoring System (Glucometer/Ketometer) using blood β ketone test strips

Table-3 *Pooled correlation matrix (r) of different parameters of subclinically ketotic cows selected from the farm (n=22, **P≤ 0.01)*

Parameters	BHBA	NEFA	Glucose
BHBA	1		
NEFA	0.782**	1	
Glucose	-0.772**	-0.578**	1

Discussion

In the present study it was found that low producing cows are least susceptible to the subclinical or clinical ketosis in comparison to high producing animals [9-11]. The animals that show the SCK incidence often have blood BHBA level ranging between 1.2 to 2.9 mmol/L [12-14]. The blood BHBA level indicated that SCK affected cows had an average BHBA concentration of 1.69 mmol/L [15] which was similar to values obtained in this study. The lower threshold concentration of BHBA for SCK is 1.2 mmol/L, while other studies have reported lower and upper threshold range between 1.0-1.4 mmol/L and ≥3.0 mmol/L.

A significant decline in the milk yield of healthy vis a vis SCK suffering cows was noticed in present investigation as healthy cows produced more milk in comparison to SCK cows. In earlier studies a decline of 25-60 per cent in milk production have been recorded in bovine clinical ketosis. The possible reason for the decreased milk production could be reduced capacity of the animal to supply the lactogenic precursors to mammary gland than the capacity of gland to produce due to homeorhetic drive for production. Moreover, elevated blood ketones also result in decreased milk production [16]. In ruminants, glucose is synthesized from propionic acid and fulfils the requirement of glucose. The relative demand of glucose during early lactation exceeds the available source leading to negative carbohydrate balance *i.e*., low glucose level, in absence of sufficient energy intake the requirement of glucose increases which causes the mobilization of body fat, accumulation of fat in liver and the rate of ketone body production resulting in ketosis [17].

The higher incidence of SCK observed in farm cows was attributed to more milk yield (P<0.01) in comparison to field cows which were producing medium quantity of milk. Blood glucose level could also be used to assess the SCK incidence along with BHBA as blood glucose level was higher (P<0.01) in healthy cows in comparison to SCK affected cows [18]. The significant negative correlation of milk yield with BHBA level in the study suggest that farmers could assess SCK incidence by monitoring the blood BHBA levels using test stripes which is convenient for use in field conditions [19]. Body condition score is generally measure to assess the energy status of the animal [20,21]. The fact that BCS did not vary in cows and buffaloes of farm and fields, further suggested adequate feeding practices and DMI in these animals. Due to this reason the effect of BCS was not found in this study.

Conclusion

The overall incidence of ketosis amongst early lactating cows was found to be 13.09 per cent, high producer crossbred cows were more vulnerable to SCK due to high milk production during early stage of lactation. Sahiwal cows suffer less from SCK due to medium level of milk production. Factors like high milk production, negative energy balance in transition period makes the animal more susceptible to SCK incidence.

Application of research: The significant finding of experiment suggests that BHBA can be used as standard bimarker to detect SCK and high correlation between Plasma glucose, NEFA and BHBA level determine metabolic imbalance and negative energy balance in transient stage cows. Monitoring and screening of animals for the incidence of SCK at monthly intervals should be practiced by the farmers to manage the SCK and to enhance income generation.

Research Category: Dairy Research

Abbreviations: BHBA: beta- hydroxybutyrae, NEFA: Non-esterified fatty acids

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Study area / Sample Collection: Animal Physiology Division, ICAR-National Dairy Research Institute, Karnal, 132001

Breed name: Cow

Conflict of Interest: None declared

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