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Detection of Subclinical Ketosis in Urine of Cattle and Buffaloes

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ABSTRACT

Background: Subclinical ketosis (SCK) in dairy cows is a common metabolic disorder and a strong determinant of the health and performance of cows throughout lactation. Considerable amount of milk loss takes place with SCK incidence in dairy animals. Greater benefits are to be gained by monitoring the cows for SCK. The current study was aimed to find out incidence of SCK by urine test strip (Keto-Diastix).

Methods: The present investigation was carried out on crossbred and Sahiwal cows and Murrah buffaloes (n=180) of the institute herd and rural dairy farms (n=97) around Karnal. The urine samples were tested for subclinical ketosis (SCK) 5-60 days postpartum from August 2019 to March 2020. Meteriological data on wet and dry bulb temperature and ambient temperature was collected and THI was calculated. Data of SCK incidence was classified based on stages of early lactation (5-20, 21-40 and 41-60d), parity (I, II, III and above), seasons (hot-humid, autumn, winter and spring) and milk production level (<10kg, 10-20 kg and >20 kg/d). Milk yield and environment variables were recorded during the experiment. Urine ketone and glucose level was detected by keto-Diastix to find out incidence of SCK.

Result: Mean level of ketone bodies in urine was higher (P<0.01) in high yielding cows as compared to the medium yielders; however it did not vary in buffaloes. Ketone bodies in urine were high (P<0.01) in crossbred cows than that of buffaloes and Sahiwal cows. Urine glucose level did not vary among cows and buffaloes. It is concluded that high producing crossbred cows are more vulnerable to SCK between 41-60d of lactation and in III parity and above due to peak milk production. Sahiwal cows and buffaloes suffer less from SCK probably due to medium level of milk production. Factors like high milk yield, higher parity and hot-humid stress makes the animal more vulnerable to SCK. The screening of urine samples of animals at monthly intervals may be practiced by the farmers to monitor the incidence of SCK.

Key words: Cows and buffaloes, Ketone bodies, Parity, Stage of lactation, Season, Milk yield.

INTRODUCTION

Animal husbandry is traditionally an integral part of Indian rural economy to support the livelihood of medium and poor farmers. India ranks first in milk production (>180 million ton) due to large number of dairy livestock's with low procured productivity. In the recent years, the cross-breeding practices are being followed to produce high yielding cows to meet the growing demand of milk and its products. In the climate change scenario and global warming, the importance of indigenous cows is equally recognized vis a vis crossbreds. High producing cows often suffer from enormous losses of body nutrients to support milk production due to inadequate nutrient intake and unfavorable environment. The cost for production diseases is very important as infectious diseases, infertility, lameness ultimately reduce the farmer's profitability (Mulligan and Doherty, 2008). Dairy animals have 3 distinct phases namely milk production, pregnancy and transition phase. Though each physiological stage has its own significance, but transition phase is most crucial to determine the ensuing lactation milk yield and reproductive performance (Sharma *et al*., 2011). Production diseases may be considered 'a man-made problem' due to combined strain of high production and modern intensive dairy husbandry' (Herdt, 2006). Over-conditioned dry cows are more vulnerable for ketosis and fatty liver, which may suppress immunity directly or through an excessive negative energy balance route (Ingvartsen *et al*., 2003).

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Ketosis is one of the metabolic disorders that may lead to less milk yield and income loss to the dairy farmers. Detection of ketosis in a body fluid like blood or urine at subclinical stage is an important management tool to prevent the incidence of ketosis in dairy animals. The present investigation was carried out to know the comparative incidence of SCK in urine samples of crossbred and indigenous cows and the Murrah buffaloes under field and farm conditions.

MATERIAL AND METHODS

The present investigation was carried out at Livestock Research Centre of ICAR-National Dairy Research Institute Karnal and the Dairy Farms located around Karnal District.

A total of 55 crossbred Karan Fries cows, 50 Sahiwal cows and 75 Murrah buffaloes of the institute herd in early stage of lactation (5-60 days) were included for the experiment. In addition to this, 70 cows and 27 buffaloes were selected from the field dairy farms located in Indri, Tikri, Sangoha, Basant Vihar, Chauhan dairy, Nirmal dairy and Mehla dairy farm. The data on milk yield, stage of lactation, parity, season and the disease incidence, like retention of placenta, dystocia, mastitis were also collected for experimental animal. The experimental data was classified based on milk yield: Low (<10kg), Medium (10-20kg) and High (>20kg); stage of lactation: 5-20 d, 21-40d, 41-60d; parity: I, II, III and above; seasons: Hot-humid (August), Autumn (September-November), Winter (December-February) and Spring (March). Urine samples (25ml) were collected in a clean flask for the detection of ketone bodies and glucose level. SCK was detected by a urine test strip (Keto-Diastix). The change in color of the test strip was recorded after immersing in urine sample for 40 seconds (Photo 1). The colour so developed was measured by comparing with standard colour index printed on the bottle for ketone concentration (mg/dL) and glucose concentration (g/dL). The test strip score color for ketone ranged from <5 to ≥80 mg/dL Interpret <5 (Negative), 5 (Trace), 15 (Small), 40 (Moderate), 80 (Large), ≥80 (More than large). The urine glucose concentration (g/dL) ranged from <0.1 to ≥2 g/dL interpret <0.1 (Negative), 0.1 (Trace), 0.25 (+), 0.5 (++), 1 (+++), e"2 (++++) (Table 1). The statistical analysis of the data was carried out by Stat3 software using 3-way analysis of variance (ANOVA). Mean and standard error was calculated and significance was tested at 1 and 5% level. Correlations among the various attributes were determined using Pearson correlation matrix.

RESULTS AND DISCUSSION

The incidence of SCK was maximum (P<0.05) in winter in comparison to hot-humid season in the farm cows (30.23 vs. 26.31%). However, in field cows the incidence of SCK was low (10.71%) than the farm cows in winter season. No incidence of SCK was found in hot-humid season in the cows selected from the field (Fig 1). The incidence was low *viz.,* 10.52 and 8.0% during the autumn season in field and farm cows, respectively. In spring season the SCK was 11.11 % in the farm cows, but no incidence was found in the field cows. The incidence of SCK was undetectable during 5-40 and 41-60 days postpartum in Sahiwal cows, however crossbred farm cows exhibited significantly higher (P<0.05) incidence (20/55) than Sahiwal (2/50). Buffaloes did not

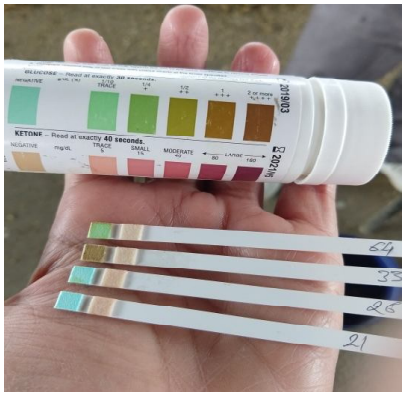

Photo 1: Keto-Diastix strips having two different chemical pads showing change in colour

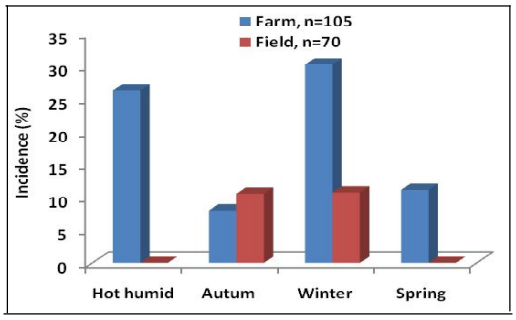

Fig 1: Incidence of subclinical ketosis during different season.

Table 1: Standard colour chart for glucose and ketone concentration in urine.

Colour of strip	Glucose (g/dL)	Interpretation	Colour of strip	Ketone (mg/dL)	Interpretation
	<0.1	Negative		<5	Negative
	0.1	Trace		5	Trace
	0.25	+		15	Small
	0.5	++		40	Moderate
	1	+++		80	Large
	≥2	++++		≥80	More than large

show the incidence of SCK and disorders like retention of placenta, dystocia and mastitis. The overall incidence of SCK in the farm animals in early lactation of 5-20, 21-40, 41-60 days increased steadily and was 6.89, 10.34 and 34.28%, respectively (Table 2). No incidence of SCK was found during 5-20 days of lactation in field animals. Parity-wise incidence of SCK in the farm animal indicated a gradual rise with advancing parity from I to III and above parity. Under field conditions, no incidence of SCK was detected in Ist parity. The incidence of SCK was 2 out of 34 in the I parity farm buffaloes, however no incidence was found in buffaloes under field conditions. Sahiwal cows suffered from SCK in III parity only (2/20) but no incidence was reported in I and II parity cows.

The milk production level did not influenced SCK incidence in Indigenous Sahiwal cows and Murrah buffaloes. The incidence was scanty in Sahiwal (2/29) and buffaloes (2/55) producing milk yield between 10-20 kg/d (Table 3). Contrary to this, incidence was significantly more (P<0.05) in crossbred cows producing milk yield of 10 to 20 kg/d (11/36). Thus higher milk producing cows exhibited maximum SCK incidence in farm animals. In field animals the milk yield of cows and buffaloes ranged between 9.3 to 32.64 kg/d (av.20.97kg/d) and 8.51 to 12.56 kg/d (av.10.53kg/d). Mean urine ketone bodies level was lower (P<0.05) in buffaloes as compared to cows (4.14 mg/dl vs. 7.86 mg/dl ; Fig 2). It was found that high yielding cows producing 28.50 to 32.64 kg milk/d had higher (P<0.01) level of urine ketone bodies (10.95-12.25mg/dl) in comparison to av.4.65 mg/dl level in medium producers (av.11.44 kg/d). Field buffaloes producing milk yield of 8-10kg/d was found to have av.4.14mg/dl urine ketone bodies. Further, mean urine glucose level did not vary between the cows and buffaloes. In the present study, it has been observed that the low producing cows are least susceptible to SCK or clinical ketosis in comparison to high producing animals (Singh, 2002; Chakrabarti., 2006; Samiei *et al*., 2013). Since season has indirect effect on SCK incidence due to management factor and change in quality of feed and fodders, the effect of Hot-humid season was more on SCK incidence in comparison to the rest of the seasons. Temperature with high relative humidity (RH) further exacerbates the stress level (Marai *et al*., 2007). The high THI in this study might have contributed to higher SCK incidence in high producing cows due to impaired immunity, inadequate fodder and increase in physiological reactions indicating stress on animals (Waldron, 2010). THI adversely influence milk yield of cattle and a decrease milk yield by 0.2 kg/unit of THI over 72 (Ravagnolo *et al*.,2000; Thirunavukkarasu *et al*., 2011). The high temperature in summer elevates physiological reactions like respiration rate, pulse rate, rectal temperature and reduce feed intake in cattle (Valente *et al*., 2015; Aleena *et al*., 2018). The decline in feed intake, metabolism, body

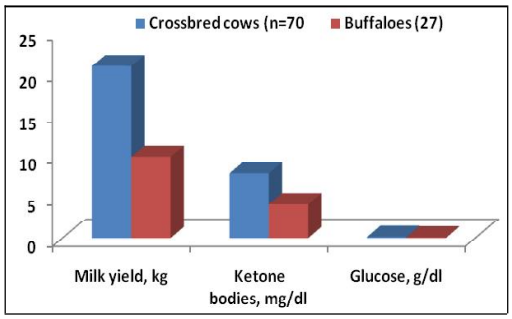

Fig 2: Mean milk yield urine ketone bodies and glucose level in cows and buffloes.

Table 2: Incidence of SCK (%) during different stages of lactation and parity in field and farm animals.

Stage of lactation (days)	Subclinical ketotic animals					Incidence (%)	
	Farm			Field			
	Crossbred cows (n=55)	Indigenous cows (n=50)	Buffaloes (n=75)	Cows (n=70)	Buffaloes (n=27)	Farm (n=180)	Field (n=97)
5-20	6 (27)	0 (21)	0 (39)	0 (13)	0 (9)	6.89 (87)	0.0 (22)
21-40	6 (16)	0 (17)	0 (25)	2 (37)	0 (9)	10.34 (58)	4.34 (46)
41-60	8 (n=12)	2 (n=12)	2 (n=11)	3 (n=20)	0 (n=9)	34.28 (n=35)	10.34 (n=29)
Parity I	1 (n=15)	0 (n=13)	0 (n=15)	0 (n=14)	0 (n=8)	2.32 (n=43)	0.00 (n=22)
II	6 (n=19)	0 (n=17)	0 (n=26)	2 (n=36)	0 (n=10)	12.90 (n=62)	4.34 (n=46)
III and above	8 (n=21)	2 (n=20)	2 (n=34)	3 (n=20)	0 (n=9)	16.00 (n=75)	10.34 (n=29)

Table 3: Effect of milk production on the incidence of subclinical ketosis (%) in cows and buffalsoes.

Level of milk production (Kg)	Farm			Field		% incidence
	Crossbred cows (n=55)	Sahiwal cows (n=50)	Buffaloes (n=75)	Crossbred cows (n=70)	Buffaloes (n=27)	Total animals (n=277)
<10	3 (n=7)	0 (n=21)	0 (n=20)	0 (n=12)	0 (n=6)	3 (4.54%) (n=66)
10-20	11 (n=36)	2(n=29)	2 (n=55)	0 (n=18)	2 (n=21)	17 (10.69%) (n=159)
>20	4 (n=12)	0 (n=0)	0 (n=0)	5 (n=40)	0 (n=0)	9 (17.30%) (n=52)

weight and milk yields is an physiological adjustment to alleviate the heat imbalance and the drop in the lactation yield is proportional to the length of the heat stress (Habeeb *et.al*., 2018a). Further, the availability of feed quality and energy become one of the rate limiting factors for the milk secretion in summer season (Liinamo *et al*., 2012; Manzanilla-Pech *et al*., 2014). Therefore high yielding cows suffer more from ketosis than the low yielders (Chakrabarti, 2006). Since milk yield gradually increases with the advancement of parity, the high parity (III) animal had more SCK incidence than the animals of I and II parity which resulted in a significant decline in milk yield (15.48 vs 7.72Kg/d) in healthy vis a vis SCK cows in this study. Thus a decline of 25-60 percent in milk production in bovine clinical ketosis may occur in cows (Kumar *et al*., 2015). The inadequate energy intake in summer attenuate requirement of glucose which causes mobilization of body reserves, fatty liver and increase in rate of ketone body production resulting in ketosis (Benedixen *et al*., 1987). The higher incidence of SCK during 41- 60d of early lactation was due to the peak yield and the associated negative energy balance which is most often attained during these days of lactation (Mir and Malik., 2002). This corroborated the higher incidence of SCK in farm cows producing higher milk yield >20kg/day in comparison to medium milk yield <10kg/day. Due to this reason factors like stage of lactation, (41- 60d), number of parity (III and above) and hot- humid (HH) season influenced the SCK incidence in this study. The higher incidence of SCK in HH season was due to high THI>80 thereby suggesting adoption of appropriate measures like adequate shelter and nutrient availability and the timely detection of SCK incidence by dairy farmers. The buffaloes and Sahiwal cows were least affected by above factors due to their thermal tolerance adoptability and medium milk production level (Behera *et al*., 2020). Further buffaloes and cows can withstand rigors of high temperatures if they are provided with adequate shelter, *ad lib* green fodder and water availability (Ludri and Singh, 1987; Yadav *et al*., 2016).

CONCLUSION

The higher incidence of SCK in crossbred cows was mainly due to their vulnerability to high temperature which is reflected by higher urine ketone bodies and lower glucose levels. The Factors like season, parity and the level of milk production renders them more susceptible to SCK incidence. The animals kept under farm conditions are more vulnerable to SCK due to higher milk yield during 41-60d of lactation whereas Sahiwal cows and buffaloes were least susceptible to ketosis.

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