



E-ISSN: 2320-7078

P-ISSN: 2349-6800

JEZS 2018; 6(4): 1146-1150

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Received: 14-05-2018

Accepted: 16-06-2018

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Clinico-biochemical alternation in bovine ketosis

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Abstract

The study was aimed to study clinico-biochemical profile of bovine ketosis in Kashmir valley. During the study, 309 milk-cows of mixed population were screened for suspected ketosis out of which 28 were positive for clinical ketosis and 81 for subclinical ketosis and 08 animals were kept as healthy control. The body temperature, respiration rate and heart rate in affected animal was within the normal range, however rumen motility was decreased. The most consistent clinical signs were sudden drop in milk yield (100.00%) and selective feeding (75.00%). Wasting (35.71%), complete anorexia (25.00%), acetone smell in breath/milk (17.85%), depression (39.28%), constipation (14.28%), dry and scant faeces (21.42%), disinclination to move (3.57%) and nervous signs (3.57%) were observed in less than 50% of cases. The multi-diagnostic strip reaction (Siemens) in ketotic cows showed (+++) in eight cases, (++) in four cases and (+) in nine cases. Whereas urobilinogen, blood, specific gravity, bilirubin, nitrites and glucose were normal in all cases of ketosis in cattle, although presence of leucocytes (7/28), proteinuria (6/28) and lowered urine pH were seen in four cases. There was a significant decrease in blood glucose level in clinical ketosis compared to control group. The plasma NEFA and BHBA levels were significantly increased in both clinical and subclinical ketosis.

Keywords: BHBA, bovine ketosis, Kashmir valley, ketone bodies and NEFA

1. Introduction

Ketosis is a major metabolic disorder caused by period of negative energy balance (NEB) that occurs almost universally at the beginning of lactation [1]. High demands for energy, fat, and protein to support lactation are coupled with a decrease in dry matter intake around the time of calving [2]. As lactation continues, dry matter intake increases at a slower rate than milk production and body stores are used to support milk production [1]. Breakdown of body stores, especially fats to support lactation occurs in many species, but when excessive catabolism is present, pathologic levels of blood ketone bodies can accumulate. Build-up of ketone bodies in the blood can cause inappetance and further exacerbate the problem. Ketosis is characterized by relatively high concentration of ketone bodies (hyperketonemia) (acetoacetate, beta-hydroxy butyrate and acetone) with a concurrent decrease in blood glucose (hypoglycaemia) level, hypoinsulinemia, decreased blood thyroxine, and hepatic glucagon and increased hepatic triglycerides and increased non-esterified fatty acids (NEFA) in plasma [3-6]. Decrease of dry matter intake around parturition, increase demands for glucose and insufficient propionate production during the early postpartum period could explain the occurrence of ketosis at postpartum period [7]. Primary ketosis diagnosed based on the ketotic clinical signs viz. gradual loss of appetite, mucous coated faeces, sudden reduced milk yield and body weight, sweetish smell in breath, urine and milk, sub normal body temperature and decreased ruminal contraction examined the presence of ketone bodies in the urine of the animals by using Rothera's test and strip test [8].

High incidence of clinical and subclinical ketosis causes economic loss to the dairy farmers due to loss of milk production as well as sharp drop in the SNF content of milk and failure of affected animals to return to normal production after recovery [9]. Financial losses are from decreased milk, decreased body weight, cost of treatment, disposal of cows that have recurring cases and possibly death. If treatment, prevention and other costs could be determined, an annual loss approaching \$150 million for US dairymen is probable [10]. Losses caused by undiagnosed subclinical ketosis exceed losses caused by clinical ketosis [11]. Keeping in view all this, present study was aimed to determine alterations in biochemical parameters of bovine ketosis in Kashmir valley.

2. Materials and Methods

The study was conducted on recently calved crossbred cows at Veterinary Clinical Service Complex, FVSc and AH, Shuhama; District Budgam and Mountain Livestock Research Institute, Mansbal during the study period. During the study, 309 milk-cows of mixed population were screened for suspected ketosis. After obtaining complete history of milk yield, stage of lactation, age, the analysis of urine and milk samples of 309-test-cows was done for the presence of ketone bodies and positive cases were categorized as primary ketosis cases. Complete clinical examination of the suspected animals was made which included rectal temperature, pulse rate, respiration rate, colour of mucous membrane and ruminal movements. Cowside urine and milk nitroprusside tests (Rothra's test) and Multidiagnostic strip reaction (Siemens) were employed for diagnosis of ketosis. Briefly, 10 ml of urine and 10 ml of milk was collected from all the recently calved cattle (309) and were subjected to Rothra's test for the confirmation of the ketosis. Cases testing positive for Rothra's test were classified as ketotic animals and were included in the study. If clinical signs were evident the cases were categorized as clinical ketotic animals (28 animals) and if the clinical signs were not so evident the animals were categorized as subclinical ketotic (81 animals). Eight healthy animals were used as control for comparison of clinic-biochemical alternation in ketosis

Four ml of blood was collected from clinical cases of ketosis from jugular vein using sterile syringes. Two ml of this was transferred to heparinized vacutainer for the estimation of NEFA and BHBA and two ml was transferred in vacutainer for glucose estimation by commercially available kits. The blood samples were centrifuged immediately at 3000 rpm for 30 minutes to separate out the plasma.

2.1 Statistical analysis

The statistical analysis of the data was done to compare the efficacy of drugs within and between the groups. The data were subjected to one way ANNOVA and simple "t" test by using SPSS software. The results were evaluated at 1% and 5% level of significance.

3. Results

The body temperature ($101.86 \pm 0.11^\circ\text{F}$), respiration rate ($20.28 \pm 0.71/\text{minute}$) and heart rate (65.50 ± 1.04) in all the clinical cases was within the normal range. Rumen motility was slightly reduced ($2.32 \pm 0.17/2$ minute), being 1 in eight cases, 2 in three cases and 3 in 17 cases. All the cases of ketosis were meticulously observed for clinical signs. The most common clinical signs observed included sudden drop in milk yield, followed by partial anorexia and selective feeding. The ketotic cows first refused to eat concentrates and then had reduced appetite for green and dry fodder. The other clinical symptoms were rapid deterioration of body condition, muscle wasting, complete anorexia, acetone smell in breath and milk, depression, nervous signs, and disinclination to move and eat, faeces were dry, firm, scanty and mucus coated in some animals. All these signs were not observed in all the clinical cases but varied as shown in Table 1. The most consistent clinical signs were sudden drop in milk yield (100.00%) and selective feeding (75.00%). Wasting (35.71%), complete anorexia (25.00%), acetone smell in breath/milk (17.85%), depression (39.28%), constipation (14.28%), dry and scant faeces (21.42%), disinclination to move (3.57%) and nervous signs (3.57%) were observed in less than 50% of cases.

The clinical diagnosis was confirmed by demonstration of ketone bodies in urine, milk and blood. All the clinical ketotic cases (28) were screened by on spot urine -milk nitroprusside tests and multi-diagnostic ketostix. Urine and milk samples were clear and had no color change or turbidity. However, characteristic colour reactions were noticed when they were subjected to modified Rothra's test. The multi-diagnostic strip reaction (Siemens) in ketotic cows showed (+++) in eight cases, (++) in four cases and (+) in nine cases. Whereas urobilinogen, blood, specific gravity, bilirubin, nitrites and glucose were normal in all cases of ketosis in cattle, although presence of leucocytes (7/28), proteinuria (6/28) and lowered urine pH were seen in four cases (table 2)

There was a significant decrease in blood glucose level in clinical ketosis (36.68 ± 1.65 mg/dl) when compared to control group (45.83 ± 3.2 mg/dl). However blood glucose levels in subclinical ketosis (40.62 ± 0.98) were only marginally decreased. The blood glucose level in clinical ketosis was significantly lower than subclinical ketosis (Table 3).

The plasma NEFA level was significantly increased in both clinical (0.902 ± 0.011 mmol/L) and subclinical ketosis (0.89 ± 0.007 mmol/L) when compared to control group (0.538 ± 0.038 mmol/L). Plasma NEFA levels in clinical and subclinical ketosis were at par with each other (Table 3).

The plasma BHBA level was significantly increased in clinical (1.27 ± 0.033 mmol/L) and subclinical ketosis (0.939 ± 0.007 mmol/L) as compared to control group (0.755 ± 0.021 mmol/L). There was also a significant difference in BHBA levels between clinical and subclinical ketosis (Table 3).

4. Discussion

The body temperature, respiration rate and pulse rate in all the ketotic animals were 101.86 ± 0.11 , 65.50 ± 1.04 and 20.28 ± 0.71 respectively which are normal for the species. Urine and milk was clear and gave positive reaction to the Rothra's test. Similar observation has been reported in ketotic animal by [5, 12, 13]. Our findings are also in agreement with, [14] who reported normal temperature, pulse and respiratory rate in clinical cases of ketosis.

Ruminal movements were marginally decreased in amplitude and number, but were still in normal range (2.32 ± 0.17). The decrease in amplitude of rumen movements could be attributed to the effect of excessive ketone bodies which have inhibitory effect on rumen wall. [15] Reported that excessive generation of ketone bodies have inhibitory effects on the ruminal wall, as it remains partly empty causing incomplete and depressed ruminal contraction. Decreased ruminal movements in number and amplitude in bovines have also been placed on record by [2, 5].

Ketosis is most commonly seen as gradual loss of appetite and decrease in milk production over several days. As feed intake is decreased, weight is lost rapidly, because of protein and fat mobilization and thus milk production drops [14]. The main clinical signs observed in the present study were sudden drop in milk yield, partial anorexia/selective feeding, depression and wasting and were consistently repeated in more than 50 per cent of the cases studied. As diverse clinical symptoms are observed in Ketosis but varying degrees of frequency of occurrence of these symptoms were observed in present study. Amongst all the clinical symptoms, sudden drop in milk yield ranked on top being repeated in 100 per cent of the cases, followed by selective feeding (partial anorexia) (75%), depression (39.28%), wasting (35.71%), complete anorexia

(25%), dry and scant feaces (21.42%), acetone smell in breath and milk (17.85%), constipation (14.28%) and nervous signs and disinclination to move (3.57%) each. The pattern of signs observed in this study was similar to clinical profile described by other workers [16-18, 15, 19, 9].

These clinical findings may be attributed to feeding practices, individual characteristics, general environment, hyperketonemia and consequently the decline in feed intake which results in rapid mobilization of adipose tissue and protein storage, which provide gluconeogenic amino acids to support hepatic glucose production [20, 21]. In Ketosis, the capacity of the animal to supply the lactogenic precursors to mammary gland is reduced than the requirements of the gland to produce due to homeorhetic drive for production [22]. Moreover, elevated blood ketones has been reported to result in decreased milk production [23]. The present study elucidated that ketosis was predominantly accompanied with a drop of 5.19 ± 0.19 litres milk/animal/day amounting to 50.81 on per cent basis. Decline of 25-60 per cent in milk production in bovine clinical ketosis has also been placed on record by [24, 25, 12].

Rothera's test in the present study showed the development of slightly purple, moderate purple, dark purple and black purple colour with increasing ketone bodies and was graded as single to + to +++++. These finding corroborate with the earlier reports [26, 14]. According to [5] the clinical ketosis showed a pink to maroon color on Rother's test which was a strong reaction. The commercially available strip test results showed various colors ranging from buff-pink, moderate pink, pink and maroon color corresponding to quantities 1-5, 5-10, 10-50 and above 50 mg/dl of acetoacetic acid present in the urine respectively. Similar finding were reported by [14].

In the cases of subclinical ketosis, no characteristic clinical signs were observed. However the blood biochemical profile indicated hypoglycemia, increased NEFA and BHBA levels. [27] Defined subclinical ketosis as elevated concentration of circulating ketone bodies in the absence of clinical signs [21].

In the present study hypoglycemia was the consistent and predominant biochemical aberration. The mean plasma glucose level in the clinical and subclinical ketosis was 34.75 ± 1.39 and 40.62±0.98 mg/dl respectively which was lower when compared to healthy control group (45.83±3.2 mg/dl). The results of the present study are in accordance with a number of similar studies [28, 29, 30] where the authors also reported a decrease in blood glucose in cows with ketosis. The hypoglycemia in cows with ketosis can be ascribed to the decreased glucogenesis [31]. Lowered plasma glucose concentration might be due to large amount of blood glucose withdrawal by the mammary gland for the synthesis of milk lactose [30]. [32, 33] also suggested that negative energy balance and stress condition immediately after parturition might also be the cause of reduced blood glucose level. Glucose is the principal substrate of lactose and 60-80 per cent of blood

glucose is utilized by the mammary glands for milk production [34]. A heavily milking animal may lose over 1,000 g of lactose per day in its milk [35] and the relative demand of glucose during early lactation exceeds the available source leading to negative carbohydrate balance [36]. It has been recorded that the decrease in glucose output by the liver could lower blood glucose concentration and decreased insulin secretion, which in turn leads to increased lipid mobilization from adipose tissue and increased rate of hepatic fatty acid uptake [3], leading to subsequent increase in NEFA and BHBA levels. Hypocalcemia can also exert an additional depressive effect on endogenous glucose production, hence, aggravating hypoglycaemia [37]. Severity of the clinical syndrome in ketosis is proportional to the degree of hypoglycemia which is the principal metabolic disturbance in ketosis [5].

In response to decreased glucose levels, fat mobilization is initiated to support the negative energy balance [7, 38] leading to elevation of NEFA and ketone bodies which are important source of energy when carbohydrate levels are reduced.

Blood BHBA is considered as gold standard test for diagnosis of ketosis as BHBA is being more stable ketone body than acetone or acetoacetate. The increase in BHBA and NEFA in ketotic animals could be ascribed to increased fat mobilization from the adipose tissue during the early lactation period to support the negative energy balance, when blood glucose levels are low due to the initiation of milk production, resulting in increased production of acetylo-CA which results in increased production of ketone bodies [39]. Our finding is in agreement with [21].

5. Conclusion

This can be concluded that sudden drop in milk yield and selective feeding are most consistent sign in bovine ketosis. Rother's test and multi-diagnostic strip reaction can be used for screening of animals for ketosis. Hypoglycaemia, hyperketonemia along with increase NEFA are consistent biochemical changes observed in animals with ketosis.

Table 1: Important clinical signs observed in clinically ketotic cows (n=28)

S. No.	Clinical signs	No. of animals	Per cent animals
1	Disinclination to movement	1	3.57
2	Dry and scanty feaces	6	21.42
3	Nervous signs	1	3.57
4	Constipation	4	14.28
5	Complete anorexia	7	25.00
6	Acetone smell in breath/milk	5	17.85
7	Depression	11	39.28
8	Wasting/woody appearance	10	35.71
9	Selective feeding (partial anorexia)	21	75.00
10	Sudden drop in milk yield	28	100.00

Table 2: Assessment of urine using multi-diagnostic strip reaction in ketotic cows

Parameter	Uro-bilinogen	Bilirubin	Ketone	Blood	Protein	Nitrate	Leucocytes	Glucose	Specific gravity	pH
Reaction	3.4 (25/28) 17 (3)	- (28/28)	+++ (8/28) ++ (4/28) + (9/28) - (7/28)	- (28/28)	+++ (4/28) + (2/28) - (20/28)	+(6/28) - (22/28)	++ (3/28) ++ (4/28) + (2/28) - (16/28)	- (28/28)	1.02± 0.002	7.88±0.073

Table 3: Effect of ketosis on Biochemical profile of cows

Parameter	Control (n=08)	Subclinical ketosis (n=81)	Clinical ketosis (n=28)
Glucose (mg/dl)	45.83±3.200 ^a	40.62±0.986 ^a	34.75±1.39 ^b
NEFA (mmol/L)	0.538±0.038 ^a	0.890±0.007 ^b	0.902±0.011 ^b
BHBA (mmol/L)	0.755±0.021 ^a	0.928±0.005 ^b	1.276±0.033 ^c

The values bearing different superscript (small letters) in a row differs significantly ($p \leq 0.05$)

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