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PREVALENCE OF SUB-CLINCIAL KETOSIS AND ITS ASSOCIATED COW LEVEL RISK FACTORS IN LACTATING DAIRY CROSS-BRED COWS IN BANGLADESH

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ABSTRACT

Background: Ketosis is one of the most economically important metabolic diseases commonly found in high milk producing dairy cows worldwide.

Objectives: The main objectives of this study were: (a) to evaluate the relationship between the urinary ketone bodies Rothera's test and the serum glucose level to determine the prevalence of sub-clinical ketosis (SCK) and (b) to explore the cow-level risk factors associated with SCK in cross-bred dairy cows.

Materials and Methods: Two types of cross-bred, Holstein Friesian × Local (HF × L) and Shahiwal × Local (SH × L) of a total of 68 lactating cows of three dairy herds were randomly selected to study the SCK from January to May 2012. Serum glucose level (SGL) was estimated by using Reflotron® Glucose test strip (Roche) and urinary ketone bodies (UKB) by Rothera's nitroprusside test.

Results: An overall 25% prevalence of SCK was detected by Rothera's test of which comparatively higher percentage of SCK was recorded in HF × L (25.9%) than SH × L (21.43%) cross-bred lactating cows. The overall SGL of Rothera's test positive cases varied from 30 to 39 mg / dl with an average of 34.24 ± 2.44 which was significantly ($p < 0.001$) lower than the Rothera's test negative cows (40 to 49 and 46.27 ± 3.77 mg / dl). The SCK detected on Rothera's test was compared with SGL which was 100% sensitivity at ≤ 39 mg / dl which could be considered as a 'cutoff point' of SGL for SCK. Results of the Rothera's test were highly correlated with the concentrations of SGL. The significantly ($p < 0.01$) highest prevalence of SCK was recorded in the fourth parity (66.7%) and fourth week of lactation (46.7%) in comparison to their respective parameters.

Conclusions

The present findings reveal that the prevalence of SCK in lactating dairy cows in Bangladesh is very high and remain undetected and unattended. To prevent the SCK, feeding a good feed stuff and implementing good management at the transition period with regular testing for detecting SCK to reduce the consequences and economic losses caused by SCK in dairy industry in Bangladesh.

Keywords: Serum glucose level, Rothera's test, Subclinical ketosis, Cross-bred dairy cows, Cow-level risk factors

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INTRODUCTION

Ketosis (clinical and subclinical) is a common metabolic disease in fresh dairy cows in modern intensive dairy production when animals are in negative energy balance (NEB) and associated with reduced milk yield, decreased milk protein, reduced reproductive capacity, increased risk of displaced abomasum (DA) and early culling.[1,2] SCK is defined as an excess level of circulating ketone bodies (acetone, acetoacetate and β-hydroxybutyrate (β-HB) in the absence of the clinical signs of ketosis.[3] Macrae [4] reported 2 to 3% prevalence of clinical ketosis (CK) and 30% SCK in first 50 days of lactation in UK, however, it remains under-diagnosed in most herds and caused a significant effect on cow health and production, especially less milk, more disease and poorer fertility, that costing the average herd £257 per cow. The occurrence of SCK in early lactation is usually associated with increased risk of DA, metritis, clinical ketosis, endometritis, prolonged postpartum anovulation, increased severity of mastitis and lower milk production in early lactation.[5] It is a multifactorial disorder of energy metabolism, leads to hypoglycemia and hyperketonemia. Detection of SCK by estimating blood glucose level and detection of ketone bodies in blood, urine and milk has been shown to be a relatively simple and reliable procedure.[6-8] It is imperative to diagnose ketosis in dairy cows at early lactation for treatment in advance and prevention of further losses. There is a need for the use of specific reasons for setting cut-points or threshold values of blood glucose level for SCK in dairy cows. However, there seems to be no published report on the prevalence of SCK in dairy cows from Bangladesh. This paper describes the prevalence of SCK and its cow-level risk factors by comparative evaluation of the serum glucose level and Rothera's test for urinary ketone bodies in cross-bred lactating dairy herds in Bangladesh.

MATERIALS AND METHODS

A cross-sectional study was carried out on randomly selected 68 cross-bred (54 Holstein Friesian (HF) × Local (L) and 14 Shahiwal (SH) × Local (L) lactating cows of Bangladesh Agricultural University Dairy Farm (BAUDF), Mymensingh (n = 24); Idris Dairy Farm, Sherpur (n = 21) and Community Dairy Herd adjacent villages of BAU Campus, Mymensingh (n = 23) during the period from January to May 2012. Random selection was carried out for uniform collection of sample irrespective to herd size and management practices. These randomly selected lactating cows were aged between 3.5 to 14 years, at different lactation stages, parity and level of milk production. Only cows free of clinical ketosis (loss of appetite especially to grain, rumen inactivity, smell of ketone on breath etc.) were selected for this study.

A structured questionnaire was used to collect animal and farm level data on age, breed, parity, previous milk production record, present milk production, lactation stage (week), owner's complain, number of lactating cows in the herd, grazing, milking system and disease history in the selected farms. Data were collected by interviewing the farm owners and in some cases abstracting the farm records.

Examination of urinary ketone bodies by Rothera's test

Urine samples were collected in clean dry glass wide-mouth bottle in the morning and were subjected to Rothera's test for the detection of ketone bodies as described.[9-11] Briefly, 3.0 ml of urine was taken in a clean dry test tube and 1.0 ml of Rothera's reagent (ammonium nitrate 30 g, sodium nitroprusside 2 g and distilled water 80 ml) was added to the test tube, which was then mixed by agitation, holding tube vertically, and then 1.0 ml of concentrate ammonia water was added by the side of the test tube to form a layer above the mixture. It was then allowed to stand for 15 minutes and then the presence of acetoacetic acid or of larger concentrations of acetone is indicated by the development of a permanganate like color (purple color) at the line of contact indicated positive reaction (Photo 1 & 2). The test was read on the basis of color development graded as: No color change: -ve, Faintly purple color: ± ve, Slightly purple color: 1+ve, Purple color: 2+ve, Deep purple color: 3+ve and Very deep purple color: 4+ve with increasing ketone bodices concentrations in the urine as described.[12-14]

Photo 1. Rothera's test of urinary ketone bodies in lactating cow showing a deep purple color 3+ (left tube) and very deep purple color (right tube) at the line of contact between urine and reagent.

Statistical analysis

Data recorded were entered in Microsoft Excel 2010 and transferred to R 2.14.2 (The R Foundation for Statistical Computing, Vienna, Austria). Descriptive statistics were obtained using Data Mining package of the software R 2.14.2. A bi-variable logistic regression was done to find out the significant differences in the prevalence of bovine SCK in terms of breed, parity, lactation stage, milk yield and blood glucose level of dairy cows.

RESULTS

The prevalence of SCK was studied in 68 cross-bred (HF × L = 54 and SH × L = 14) dairy cows during the nine weeks of early lactation period by detecting the urinary ketone bodies by using Rothera's test and serum glucose level. The overall prevalence of bovine SCK was 25% of which 25.9% in HF × L cross-bred and 21.43% in SH × L cross-bred cows (Table 1).

The milk production (liter / day) and serum glucose (mg / dl) levels in both the cross-bred (HF × L and SH × L) UKB positive lactating cows were lower in comparison to UKB negative cows (Table 2). The overall average milk yield of cross-bred lactating cows between UKB positive (6.74 ± 1.11 liter / day) and negative (6.40 ± 1.36 liter / day) groups did not differ significantly, however, the overall average serum glucose level of UKB positive cows (34.24 ± 2.44 mg / dl) was found significantly ($p< 0.01$) lower in comparison to UKB negative cows (46.27 ± 3.77 mg/dl) cows (Table 2).

Table 1. Breed-wise prevalence of sub-clinical ketosis detected by using urinary ketone bodies in cross-breed lactating dairy cows

SN	Cross-bred	No. of cow tested	Lactation age (wk)	UKB testing results No. - ve (%)	No. + ve (%)
01	HF × L	54	01-09 4.09± 2.35	14 (25.9)	40 (74.1)
02	SH × L	14	01-09 4.43 ± 2.28	03 (21.43)	11 (78.57)
	Total	68	01-09 4.16 ± 2.32	17 (25.0)	51 (75.0)

HF × L = Holstein Frisian × Local SH × L = Shahiwal × Local UKB = Urinary ketone bodies

Cow-level risk factors associated with SCK in dairy cows

The results of cow-level risk factors associated with the prevalence of SCK in dairy cows were analyzed by using bi-variable logistic regression are presented in Table 3. The prevalence of SCK was found significantly higher ($p < 0.001$) in fourth parity in comparison to other investigated parity in dairy cows (Table 3). Significantly ($p < 0.01$) higher prevalence of SCK was recorded at 2^{nd}, 3^{rd} and 4^{th} weeks of lactation age in comparison to 1^{st} week of lactation week, whereas no occurrence of SCK between 5^{th} to 9^{th} lactation weeks (Table 3).The higher prevalence of SCK was also associated with higher milk yield at > 7 to 10 liter / day (31.6%) in comparison to lower > 5 to 7 liter / day (26.5%) and 4 to 5 liter (13.33%) milk production (Table 3). The serum glucose (mg / dl) level in dairy cows with the prevalence of SCK (94.4%) was significantly ($p < 0.01$) lower in comparison to non-occurrence of SCK with higher serum glucose levels (Table 3).

DISCUSSION

High reproductive performance in dairy cows is an essential requirement to ensure maximum milk production and satisfactory economic return. In this context, the artificial insemination (AI) was introduced in erstwhile East Pakistan (now Bangladesh) in 1958 with establishment of five district AI centers (DAIC) and subsequently 23 DAIC with 1072 sub-centers in mid-1970 and currently operating all over the country.[15,16] Although AI services were introduced in the

Table 2. Relationship between milk yield and blood glucose level with urinary ketone bodies (UKB) in cross-bred dairy lactating cows

SN	Breed	No. of cows		UKB Positive (n = 17)		UKB Negative (n = 51)	
		tested	+ ve	Milk yield (liter / day)	Serum glucose (mg / dl)	Milk yield (liter/ day)	Serum glucose (mg / dl)
①	HF × L	54	14	06-08 7.04 ± 0.77	30-37 33.86 ± 2.32	04-10 6.63 ± 1.28	40-59 46.48 ± 4.09
②	SH × L	14	03	04-07 5.33 ± 1.53	34-39 36.00 ± 2.65	04-08 5.57 ± 1.37	41-49 45.55 ± 2.25
	Total	68	17	04-08 6.74 ± 1.11	30-39 34.24* ± 2.44	04-10 6.40 ± 1.36	40-59 46.27 ± 3.77

HF × L = Holstein Frisian × Local SH × L = Shahiwal × Local *Significantly ($p < 0.01$) lower

country in 1958, but its impact is yet to be visible and only approximately 10% of total cattle population are cross-bred in Bangladesh.[15,17] Holstein crossbred (Holstein Friesian x Local) and Shahiwal cross-bred (Shahiwal x Local) are the two major crossbred dairy cattle in Bangladesh.[18] However, the cattle cross-breeding program in Bangladesh has focused mainly on biological rather than economic evaluation. Moreover, nutritional status of dairy cows at transition period is not evaluated in relation to metabolic diseases in Bangladesh and inadequate and imbalance levels of nutrition during this period may be associated to metabolic disorders, especially milk fever and ketosis. Ketosis in dairy cows occurs due to a period of negative energy balance (NEB) that occurs almost universally at the early lactation. As lactation continues, dry matter intake increases at a slower rate than milk production and body stores (fat & protein) used to support the milk production.[19,20] Several studies have since described the detrimental effects of ketosis on milk production.[2,21-23]

The SCK is widely prevalent and it may remain undiagnosed in lactating dairy cows and yet have effects on productivity with parallel those elicited by clinical ketosis, and the cost of SCK per cow is estimated to be widely varied as US $ 78 -289 [1,4,24,25] and UK £ 257.[4] Therefore, monitoring of SCK at an early stage of lactation can help to minimize the economic losses on a dairy herd.

Ketone is found when there is an excessive fat metabolism which occurs in various situations: (a) Impaired ability to metabolize carbohydrate, (b) Inadequate carbohydrate intake, (c) Excessive carbohydrate loss and (d) Increased metabolic demand. There are two major changes occur in the blood of ketosis (clinical & SCK) affected cows: (a) Drop in blood glucose level (initiation factors) and (b) Increased in ketone bodies level. The 'cutoff point' of blood glucose level for SCK is considered as ≤ 40 mg / dl. Measurement of blood glucose level can be a major tool to diagnose SCK in bovine.[26] However, the diagnosis of ketosis in dairy

Table 3. Cow-level risk factors associated with the prevalence of bovine subclinical ketosis analyzed by using bi-variable logistic regression

SN	Variables	No. of cows		Prevalence (%)	Z-value	p-value
		Tested	+ ve to UKB			
①	Breed					
	SH × L	14	03	21.43	Reference	-
	HF × L	54	14	25.93	0.346	0.729
②	Parity					
	1	17	02	11.76	Reference	-
	2	24	04	16.67	0.436	0.663
	3	13	04	30.77	1.250	0.211
	4**	09	06	66.67	2.622	0.008
	5	03	01	33.33	0.919	0.358
	6	02	00	00.00	-0.009	0.993
③	Lactation stage (weeks)					
	1	08	01	12.50	Reference	-
	2	10	04	40.00	1.23	0.21
	3	12	05	41.67	1.32	0.19
	4	15	07	46.67	1.53	0.13
	5	05	00	00.00	-0.004	0.99
	6	04	00	00.00	-0.003	0.99
	7	06	00	00.00	-0.004	0.99
	8	04	00	00.00	-0.003	0.99
	9	04	00	00.00	-0.003	0.99
④	Milk yield (liter/day)					
	04-05	15	02	13.33	Reference	-
	>05-07	34	09	26.47	0.996	0.319
	>07-10	19	06	31.58	1.213	0.225
⑤	Serum glucose (mg / dl)					
	> 45 – 59	31	00	00.00	Reference	-
	> 40 – 45	19	00	00.00	-0.006	0.995
	≤ 40***	18	17	94.44	4.256	0.001

**Significant at ($p < 0.01$) ***Significant at ($p < 0.001$)

cows depends on the determination of increased concentrations of ketone bodies (acetone, acetoacetate and β-HB in the blood, urine and milk.[6] The blood β-HB test is considered 'gold standard' test for SCK because this method has stability.[20,24] However, it is not always practical to collect blood samples for determination of β-HB concentration. Moreover, this method

requires special equipment such as spectrophotometer and the procedure is time consuming.[27] In addition, some cows have high β-HB without showing any clinical signs, even with careful observation of attitude and appetite.[28] Alternatively cow-side tests such as Rothera's test (Ketostix urine strip test) have been applied to detect ketosis in dairy cows.[6,7] These tests are more practical for use by farmers and are reliable to detect ketosis in dairy cows.

Nitroprusside test is at least 10 times more sensitive to detect acetoacetate than to acetone and give no reaction at all with β-HB. So, the Rothera's test is used for ketosis detection or measure acetoacetate only, so presence of all ketosis may not be detected.

This study on lactating cross-bred dairy cows succeeded for the first time in Bangladesh to record the prevalence 25% SCK at early lactation by using urinary ketone bodies Rothera's test and serum glucose level. An overall 25% prevalence of SCK in dairy cows recorded in Bangladesh support the 8.9 to 34% prevalence of SCK elsewhere.[29-35] The Canadian and US studies indicate around 30% of cows affected with SCK,[2,5,25] whereas a review of reports on 528 herds in 10 European countries found 21.8% prevalence of SCK with a range from 11.2 to 36.6%.[36] Published research reports show an overall ≥ 10% prevalence of SCK in dairy herds is usually considered as the alarm level for herd-based ketosis testing.[28] Intensive NEB due to high milk production is one of the most important factors that affect the occurrence of SCK.[24] However, the comparatively lower prevalence rate of SCK in dairy cows in Bangladesh in comparison to exotic dairy breeds elsewhere might probably be due to genetically cross-bred cows with comparatively lower milk yield.

Cow-level risk factors on the prevalence of ketosis

The most important cow-level risk factors that may influence the occurrence of SCK include breed, parity and body condition of cows. Many cow-level factors include things about the cow that cannot be changed, such as breed[36,37] and parity.[25,31,38] Though these factors are interesting to examine, they have little bearing on ketosis management.

The SCK was recorded from 1st to 5th parity with a significantly highest prevalence at 4th parity in dairy lactating cross-bred cows. Previous studies have found that the risk of ketosis increases with increasing parity to peak prevalence during the 2nd to 6th lactation.[25,40,41] Most of the authors have reported a higher prevalence of SCK with increasing lactation number.[31,34,36] Whatever the exact reason for the difference, it shows the importance of herd in SCK risk.

The prevalence of SCK was recorded from 1st to 4th week with peak prevalence at 4th week of lactation age in cross-bred dairy cows. The peak prevalence of SCK has been reported between 2nd to 4th weeks of lactation age.[30,31,36,42] The peak prevalence of SCK at the 4th week of post-calving recorded in this study which is supported by earlier reports.[3,43] However, the peak prevalence of SCK in 2nd week and 1st week of lactation have also been reported.[6,31] The SCK is commonly reported for the first two months of lactation since this is the primary risk period.[19] However, the differences in peak occurrence for SCK may reflect differences in etiology, with the occurrence SCK at early lactation reflecting sub-optimal dry cow management, and expression of fatty liver, while later occurrences may indicate deficiencies in lactating cow management.[44]

CONCLUSIONS

The SCK is a widely prevalent metabolic disorder in dairy lactating high milk yielding cows worldwide associated with effect on milk production and possible consequences of several diseases. This is the first report of the prevalence of SCK in lactating dairy cross-bred cows in Bangladesh. It demonstrates that parity, lactation age and milk yield are important risk factors determining prevalence of SCK in dairy herds. Therefore, early diagnosis and proper treatment of affected cows is required to restore the milk yield and stop conversion of SCK to clinical ketosis and other associated disorders. The Rothera's test of urinary ketone bodies and blood glucose levels which could be used as diagnostic methods under local condition and this would lead to early treatment and prevention of SCK at farm levels.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

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