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PREVALENCE AND RISK FACTORS OF SUBCLINICAL MILK FEVER AND KETOSIS IN LACTATING CROSS-BRED DAIRY COWS WITH THEIR THERAPEUTIC MANAGEMENT IN BANGLADESH

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ABSTRACT

Background: Bovine Milk fever (MF/hypocalcaemia) and ketosis (CK/hypoglycemia and hyperketonemia) both in clinical and sub-clinical forms are the most important metabolic diseases caused by metabolic disorders of calcium and carbohydrate respectively that affect mainly high milk yielding dairy cows worldwide. Sub-clinical form may be more costly due to comparatively high prevalence and consequence of high risk of decreased productive and reproductive performances with increased reproductive and other disorders.

Objectives: The objectives of this study were to determine the prevalence of sub-clinical hypocalcaemia (SCHC) and sub-clinical ketosis (SCK) and to investigate important potential risk factors for SCHC and SCK with their therapeutic management in lactating cross-bred dairy cows.

Materials and Methods: A cross sectional study was conducted on 220 dairy crossbred (HF × L = 190, SH × L = 20 and JS × L = 10) cows maintained in nine dairy farms and one smallholder farm during the period from July to November 2016. The parity (1 to 8), lactation stages (1 to 13 weeks), body condition score (BCS), breed (3 crossbreds), age (3.5 to 14 years) and milk yield (liter/day) were evaluated as possible risk factors. The serum calcium, inorganic phosphorus, magnesium and glucose concentrations of the 220 dairy cows were determined by using imported commercial kits. Dairy cows with serum calcium concentrations ≤ 8.0 mg/dl and serum glucose ≤ 44.0 mg / dl with positive ketone tests but not showing any clinical signs were considered SCHC and SCK respectively.

Results: The overall prevalence of SCHC was 30.0%, of which 32.11% were recorded in HF × L, 15.0% in SH × L and 20.0% in JS × L cross-bred cows. The overall prevalence of SCK was 25.0%, of which 27.37% in HF × L, 10.0% in SH × L and 10.0% in JS × L cross-bred cows. The SCHC was recorded 10 times greater than MF and SCK 6 times greater than CK in Bangladesh. The hypocalcemia and hypophosphatemia with hypermagnesemia status were recorded in SCHC affected lactating cows which were more significantly ($p < 0.05$) higher (46.67%) at 4th parity and lower (16.67%) at 1st parity. The significantly ($p < 0.01$) higher prevalence of SCK was recorded at the 4th (53.33%) in comparison to other parity especially lowest at 1st (2.78%) and 2nd (4.0%) parity. The significantly ($p < 0.1$) highest prevalence of SCHC and SCK were recorded at high milk yield during the 1st (94.44%; 77.78%) and 2nd (66.67%; 56.67%) weeks of lactation period than the higher lactation stages respectively. The effects of BCS on the milk yield and the prevalence of SCHC and SCK are presented and discussed. Encouraging results with increased blood calcium and glucose levels were obtained on the therapeutic response of SCHC with oral calcium and SCK with oral propylene glycol.

Conclusions: The SCHC and SCK have detrimental effects on cow health, productivity and reproduction and also predisposes to other diseases and disorders. The efficient balanced ration, periodic screening blood, milk and urine for determination of concerned biochemical constituents and ketone bodies considering risk factors could help to early detection of SCHC and SCK to limit their effects in dairy cattle. The high prevalence of SCHC and SCK recorded in this study should be viewed as a potential health risk to the transition cows that requires further research.

Keywords: Sub-clinical milk fever, Sub-clinical ketosis, crossbred cows, prevalence, risk factors, calcium, magnesium, phosphorus, glucose, ketone bodies, therapeutic management, Bangladesh

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INTRODUCTION

Bangladesh is a tropical country with a total of 24.086 million cattle population which includes 80 to 90% indigenous and 10 to 15% cross-bred cattle,[1-3] of which 3.53 million lactating and 2.61 million dry cows.[4] In the last two decades, a heavy demand of milk due to high rate of urbanization of people in Bangladesh have initiated to establish a large number of mini dairy farms with mainly high yielding crossbred cows throughout the country. Currently, there are approximately 58,590 DLS registered mini dairy farms in Bangladesh.[5] Most of these private dairy farms in Bangladesh are small in size with 73% contain less than 11 cows and 17% has 11 to 20 cows. Of these dairy farms, 65% are reared by stall feeding system, 30% by stall cum open feeding system and the rest 5% by open feeding system.[6] The milk production per lactation is ranged from 300 to 400 liter in indigenous cows and 600 to 800 liter in cross-bred cows.[7] The dairy cattle in Bangladesh generally consist of indigenous[8] and crossbreds including mostly Sindhi, Sahiwal, Holstein Friesian and Jersey cross cows.[9,10] An indigenous cow has been reported to produce an average of 1.91 liter milk whereas cross-bred cow produces 7.80 liter milk per day at the smallholder farmers' management system in Bangladesh.[1] An overall 2,84,500 smallholder milk suppliers are being supplying milk to the 14 milk processing and marketing organizations in Bangladesh.[11] The dairy industry in Bangladesh has made tremendous strides in improving the average milk production per cow during the last two decades, mainly by the improvement of the genetic pool of indigenous cattle through cross-breeding program by using AI. The genetic progressed of cows have been made but the availability of feeds and fodder with feed efficiency has not yet been progressed at the same rate as milk production in Bangladesh. Nutritional deficiencies, imbalances ration or erratic management of feeding programs for dairy cows might have already created various types of health problems especially metabolic diseases.[12] Prevalence of metabolic diseases is closely related to ration, dairy farm management and some extent to animal's genetics. Imbalance and inadequate feeding of high milk yielding dairy cattle at pregnancy and pre-partum are usually associated with marked metabolic abnormalities at transition period (3 weeks around parturition) that makes them more susceptible to metabolic and even infectious diseases.[13,14] The occurrence of metabolic disorders in dairy cows depend on the ability of the cows to cope with the metabolic demands of high milk production and its etiology can be traced back to insults that occur during transition period. An increased energy and calcium demands for colostrum and milk production, combined with a decline of dry matter intake (DMI) around parturition, can result NEB, increased lipid mobilization[15,16] and a reduction in blood concentrations of calcium.[17,18] These changes increase the risk of metabolic and infectious diseases with animal welfare concern and an important cause of production and economic losses to the dairy industry.[19] Susceptibility to infectious diseases at transitional period occurs due to immune suppression during the peri-parturient period.[20-22] The metabolic disorders are usually occur in the high milk yielding pure exotic and crossbred dairy animals and such population was very limited in the then East Pakistan and early Bangladesh and accordingly there is a dearth of research reports on the occurrence of these diseases in inland literature. However, an overall 2.97% Milk fever and 3.75% Ketosis cases in cows have been reported based on analysis of hospital clinical cases from Bangladesh.[23] Recently, an overall

25% prevalence of SCK[24] and application of metabolic profiles tests to detect the metabolic profiles in lactating cross-bred dairy cows have been evaluated in Bangladesh.[10] This paper describes the prevalence of sub-clinical milk fever (SCMF) and sub-clinical ketosis (SCK) with their associated risk factors and therapeutic management of these sub-clinical hypocalcemia and SCK in lactating dairy cross-bred cows of Bangladesh.

MATERIALS AND METHODS

A cross-sectional study was conducted on randomly selected apparently healthy 220 cross-bred (190 Holstein Friesian = HF × Local = L, 20 Sahiwal = SH × Local = L and 10 Jersey = JS × Local = L) lactating cows of nine dairy farms and one smallholders' farm, of which two located in the district of Mymensingh and eight in the district of Gazipur during the period from July to November 2016. Two farms of Mymensingh district include ① Bangladesh Agricultural University (BAU) Dairy Farm (n = 45) and ② Smallholder dairy farm of adjacent villages of BAU Campus (n = 20). Randomly selected eight dairy farms in the district of Gazipur include ① Dipti and Sons Farm House (DFH), Valkartek (n = 35), ② Zahir Dairy Farm (ZDF), Dhirashrom (n = 35), ③Alim Dairy Farm (ALDF), Bolodha (n = 25), ④ Masum Dairy Farm (MDF), Amuna (n = 15), ⑤ Azafor Dairy Farm (AZDF), Pragao (n = 12), ⑥ Mominul Dairy Farm (MDF), Aturi (n = 11), ⑦ Jaman Dairy Farm (JDF), Dakshin Khan (n = 11) and ⑧ Apon Dairy Farm (APDF), Valkartek (n = 11). These randomly selected lactating cows aged between 3.5 to 14 years, at different lactation stages, parity and level of milk production. The animals of the selected dairy farms are reared under semi-intensive management system with raised floor. They are often provided with water hyacinth, maloncha, Jumbo grass, green grass in addition to concentrate diet and feeding two times daily. These dairy cattle are kept together in common shed but they are maintained in separate shed at transition period. The cross-breed dairy cows selected at the adjacent villages of BAU campus, Mymensingh are maintained under traditional rural husbandry practices.

A structured questionnaire was used to collect animal and farm level data on age, breed, parity, body condition score (BCS), previous milk production record, present milk production, lactation stages (weeks), number of lactating cows in the herd, status of calves, feeds and feedings including grazing, milking system, disease and treatment history of all the selected dairy farms. These data were collected by interviewing the farm owners and in some cases abstracting the farm records.

Collection and testing of urine samples

Fresh urine samples from each of 220 randomly selected lactating dairy cows were collected conveniently with the help of farm attendants or owners in plastic sample containers and tested directly at cow-side level or taken to the laboratory for the determination of ketone body in the urine. Each of the collected urine samples was tested for the presence of ketone body in the urine by using urinalysis reagent strips Uric 10 CF (Atena Medical Instrument Co., Guangdong, China). The urinalysis reagent strips are plastic strips to which chemically specific reagent pads are affixed. The reagent pads react with the sample urine to provide a standardized visible color reaction within 30 seconds to 1 minute depending on the specific panel screen. The color is

then visually compared to the included color chart to determine the level of each chemical factor. Each of the 10 reactive reagent pads on the test strip was compared to the corresponding line of color blocks on the chart. The closet color match indicated the tests result. The test procedure was used as per instruction of the kit manufacturer company. In briefly, after collection of the urine samples in a clean dry plastic container, one reagent strip was removed from the bottle and immediately the container cap was replaced minimizing the exposure of the remaining test strips to light and air. The reagent pads of the strip were immersed completely in the urine sample and then removed immediately to avoid dissolving out the reagent pads. While removing the reagent strip, the edge of the strip was run against the rim of the specimen container to remove excess urine. The strip was then held in a horizontal position pads. The color change of the reagent pads was compared to the corresponding color chart on the bottle label. According to the chart's timeframe (i.e. 45 seconds for ketone body) the reading was taken. The colors range from beige or buff-pink color for a 'negative' reading to pink and pink-purple for a 'positive' reading.

Collection and testing of serum samples

About 10 ml of blood samples of each of the 220 lactating dairy cows were collected by using sterile disposable syringe and transferred into falcon tube without adding any anticoagulant and kept at room temperature for three hour. The blood samples were then kept in the refrigerator overnight at 4 ^{0}C. Then the blood samples were centrifuged at 3000 rpm for 15 minutes and serum was collected in Eppendorf tube by using pasture pipette and stored at - 20 ^{0}C until analysis.

The selective biochemical parameters e.g. serum calcium, phosphorus, magnesium and glucose levels were determined by using commercial test kits as per instruction of the kit manufacturing companies at the Central Laboratory of the BAU, Mymensingh.[10] The serum calcium concentration was determined with quantitative colorimetric Kit Calcium Arsenazo III (Reactivous GPL, Barcelona, Spain), inorganic phosphorus by using quantitative colorimetric Kit Vitro Inorganic phosphorus reagent (In vitro Diagnostics, Vitro Scient, Egypt) and the magnesium concentration by using quantitative colorimetric Kit (Magnesium Xylidyl Blue, Prestige Diagnostics, UK). The serum glucose concentration was determined by glucose oxidase (GOD) and peroxidase (POD) method using enzymatic qualitative colorimetric kit LABKIT reagents (Glucose GOD-POD Liquid, Barcelona, Spain).

Therapeutic management of SCHC and SCK affected cows

A total of 66 SCHC and 55 SCK affected lactating cross-bred cows were selected for treatment trials. Each of the SCHC affected cow was treated with calcium bolus (CP-Vet Plus® bolus, The Acme Laboratories Ltd.) @ 4 bolus / animal orally once daily for 5 days. Each CP-Vet Bolus contains calcium 830 mg, phosphorus 500 mg, Magnesium 375 mg, Potassium 250 mg, Sodium 125 mg, Vitamin D_3 12500 iu, Vitamin E 75 iu and Vitamin B_{12} 25 mcg. Each of the SCK affected cows received propylene glycol (Vita-D Plus® The Acme Laboratories Ltd.) @ 200 ml / animal orally, administered after mixing with equal volume of water twice daily for first two days and then half of the dosage for next two day. Each 100 ml of Vita-D Plus

contains Vitamin D_3 500000 iu and Propylene glycol q.s to 100 ml. The serum calcium and glucose levels were estimated at the pre-treatment and post-treatment of all the treated cows after 7 days to detect the effectiveness of the administered drugs.

Statistical analysis

Data were entered in Microsoft Excel 2010 and transferred to IBM SPSS (Statistical Package for Social Science) statistics 20.0 software. Z test for comparison of proportion, Chi-square test, Paired 't' test, Odd ratio, 95% Confidence interval and p-value calculation were done to find out the significant differences in the prevalence of bovine SCMF and SCK in terms of breed, age, parity, BCS, lactation stage, milk yield and blood glucose level of cows.

RESULTS

A total of 220 cross-bred (190 HF × L, 20 SH × L and 10 JS × L) lactating dairy cows of 10 dairy herds between 1 to 13 weeks of lactation with high producing records were tested for SCMF and SCK. The SCMF was diagnosed based on the hypocalcemia (≤ 8 mg/dl) and SCK by the detection of positive level of urinary ketone bodies using 10 CF urinalysis reagent strip and hypoglycemia (≤ 44 mg/dl).

Prevalence and risk factors of SCMF

The overall 30.0% prevalence of SCMF (sub-clinical hypocalcemia = SCHC) was recorded in lactating dairy cows, of which highest prevalence was found in HF × L (32.11%), followed by JS × L (20.0%) and lowest in SH × L (15.0%) cross-bred cows (Table 1 & Fig 1). Serum calcium, inorganic phosphorus and magnesium concentrations were estimated in all the 220 lactating cows, of which 66 (30.0%) had both the hypocalcemia (≤ 8.0 mg/dl) and hypophosphatemia (< 4.0 mg/dl) but all these 66 (30.0%) hypocalcemic cows had hyper-magnesemia (>3 mg/dl). These hypocalcemia and hypophosphatemia with hypermagnesemia findings were higher in HF × L, followed by JS × L and HS × L cross-bred of lactating cows (Table 1 & Fig 1). The overall hypocalcemia and hypophosphatemia with hypermanesemia were found significantly ($p < 0.05$) higher at > 5 years of age in comparison to < 4 years and 4 to 5 years of age groups in lactating dairy cows (Table 1 & Fig. 2).

The hypocalcemia and hypophosphatemia with hypermagnesemia status were also observed in all the parity from 1^{st} to 8^{th} but significantly ($p < 0.05$) lower (16.67%) at 1^{st} parity in comparison to other parity (Table 2 & Fig. 3).

Table 3 shows significantly ($p < 0.01$) higher prevalence of SCMF (hypocalcemia) with hypophosphatemia and hypermagnesemia at the 1^{st} week of lactation in comparison to 2^{nd} to 13^{th} weeks of lactation stages (Fig. 4). Table 4 presents the effects of milk production and BCS on the prevalence of SCHC and its relationship with blood phosphorus and magnesium concentrations in lactating dairy cross-bred cows. A strong relationship was observed between high milk production and the prevalence of SCHC in the lactating dairy cows. The lactating cows producing milk > 15 liter / day had significantly higher ($p < 0.05$) and 2.5% cows producing < 5 liter milk / day had a significantly lower ($p < 0.05$) prevalence of SCHC (Table 4 & Fig. 5).

Table 1. Breeds and age factors associated with the prevalence of sub-clinical hypocalcemia and its relationship with phosphorus and magnesium concentrations in lactating dairy crossbred cows

S/N	Variable	No. of cows tested	Serum macro-mineral levels (mg/dl)					
			Calcium Range Mean ± SE	SCHC (≤ 8 mg/dl)[1] Positive No. (%)	Phosphorus Range Mean ± SE	HP (< 4mg/dl)[1] Positive No. (%)	Magnesium Range Mean ± SE	HM (>3 mg /dl)[1] Positive No. (%)
A. Breeds								
①	HF × L	190	06.05-11.98 09.06 ± 0.13	61 (32.11)	2.47 – 6.99 3.30 ± 0.08	61 (32.11)	1.43-4.25 2.59 ± 0.06	61 (32.11)
②	SH × L	020	06.32 - 11.68 10.29 ± 0.40	03 (15.00)	3.13 - 6.97 5.13 ± 0.25	03 (15.00)	1.71-3.67 2.18 ± 0.14	03 (15.00)
③	JS × L	010	06.13 - 11.36 09.38 ± 0.58	02 (20.00)	3.04 – 5.98 4.46 ± 0.31	02 (20.00)	1.72-3.86 2.4 ± 0.26	02 (20.00)
	Overall	220	06.05 -11.98 09.19 ± 0.12	66 (30.00)	2.47 – 6.99 4.38 ± 0.08	66 (30.00)	1.43-4.25 2.55 ± 0.06	66 (30.00)
B. Age (years)								
①	< 4.0	036	06.67 - 11.68 09.75 ± 0.25	06 (16.67)	2.86-6.82 4.76 ± 0.18	06 (16.67)	1.67-3.77 2.3 ± 0.09	06 (16.67)
②	4 – 5	075	06.13 - 11.98 09.74 ± 0.21	19 (25.33)	2.84-6.99 4.60 ± 0.13	19 (25.33)	1.56-3.98 2.34 ± 0.09	19 (25.33)
③	> 5	109	06.05 - 11.68 08.62 ± 0.17	41 (37.16)*	2.47-6.98 4.10 ± 0.10	41 (37.16)	1.43-4.25 2.77 ± 0.08	41 (37.16)
Overall		220	06.05 - 11.98 09.19 ± 0.12	66 (30.00)	2.47 - 6.99 4.38 ± 0.08	66 (30.00)	1.43-4.25 2.55 ± 0.06	66 (30.00)

SCHC = Sub-clinical hypocalcemia HP = Hypophosphatemia HM = Hypermagnesemia
*Significant at ($p < 0.05$) [1]Cut off points
HF = Holstein Friesian SH = Sahiwal JS = Jersey L = Local

The higher prevalence of SCHC was recorded in lactating cows with higher BCS (> 3.5) at 36% in comparison to lower BCS (3 to 3.25) at 25% (Table 4 & Fig. 6).

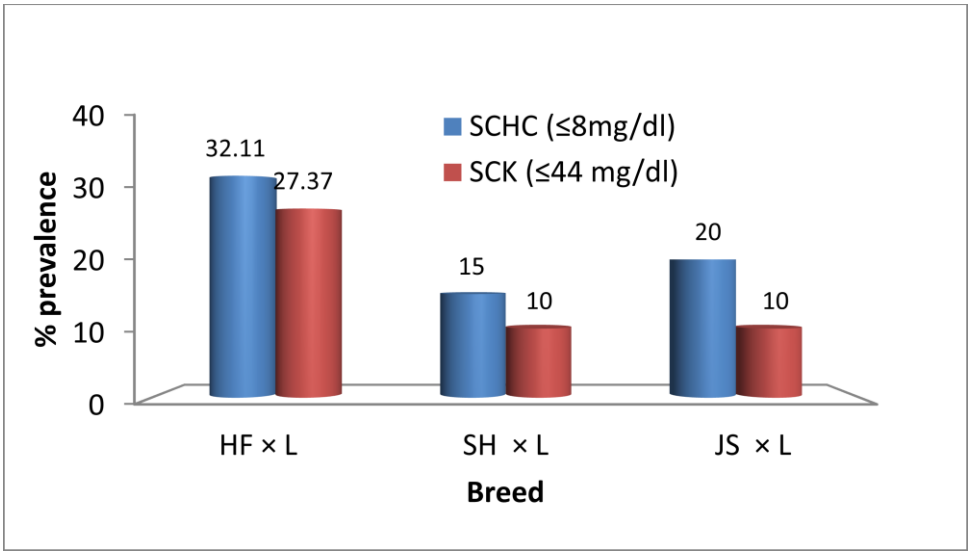

Fig.1. Breed-wise prevalence of subclinical hypocalcemia (SCHC) and subclinical Ketosis (SCK) in lactating cross-bred cows

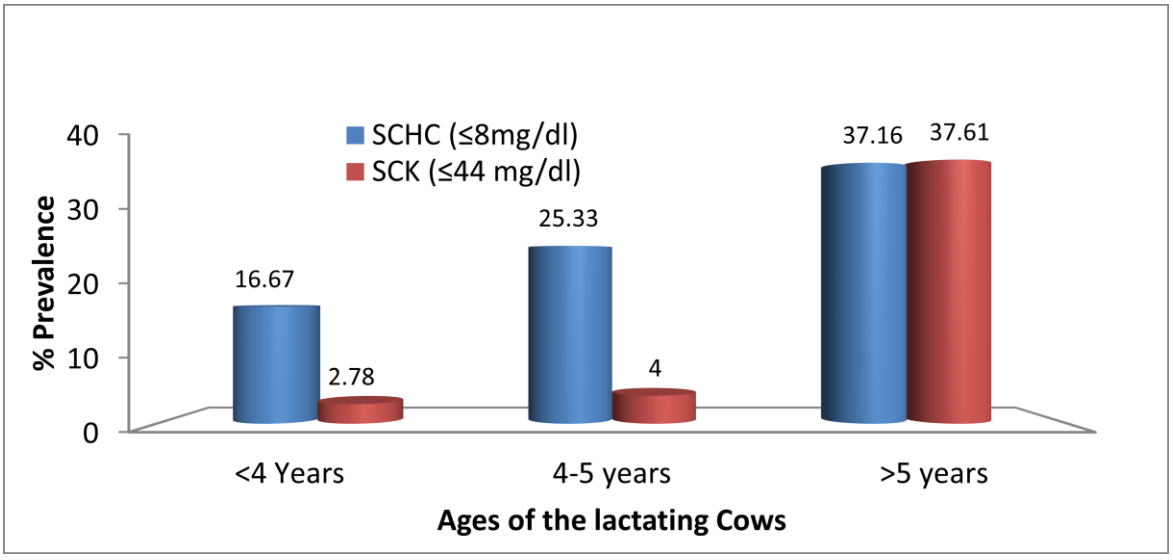

Fig.2. Age-wise prevalence of SCHC and SCK in lactating cross-bred cows

Table 2. Influence of parity on the prevalence of sub-clinical hypocalcemia and its relationship with phosphorus and magnesium in lactating dairy cross-bred cows

Variable (Parity No.)	No. of cows tested	Serum macro-mineral levels (mg/dl)					
		Calcium Range Mean ± SE	SCHC (≤ 8 mg/dl)[1] Positive No. (%)	Phosphorus Range Mean ± SE	HP (< 4mg/dl)[1] Positive No. (%)	Magnesium Range Mean ± SE	HM (>3 mg /dl)[1] Positive No. (%)
1	36	6.67-11.68 9.75 ± 0.25	06 (16.67)	2.86-6.82 4.76 ± 0.18	06 (16.67)	1.67-3.77 2.3 ± 0.10	06 (16.67)
2	75	6.15-11.98 9.81 ± 0.21	19 (25.33)	2.84-6.99 4.63 ± 0.13	19 (25.33)	1.56-3.98 2.31 ± 0.84	19 (25.33)
3	40	6.09-11.68 9.05 ± 0.31	14 (35.00)	2.47-6.98 4.37 ± 0.21	14 (35.00)	1.43-4.25 2.64 ± 0.15	14 (35.00)
4	30	6.13-11.68 8.24 ± 0.29	14 (46.67)*	2.54-6.34 3.82 ± 0.15	14 (46.67)	1.65-4.25 2.96 ± 0.14	14 (46.67)
5	20	6.05-11.36 8.42 ± 0.37	07 (35.00)	3.11-5.87 3.96 ± 0.19	07 (35.00)	1.65-3.98 2.79 ± 0.19	07 (35.00)
6	11	6.21-10.77 8.09 ± 0.48	04 (36.36)	2.84-5.27 4.03 ± 0.25	04 (36.36)	1.72-3.67 2.69 ± 0.21	04 (36.36)
7	04	6.36-9.93 8.09 ± 0.86	01 (25.00)	2.71-4.32 3.5 ± 0.42	01 (25.00)	2.13-3.89 3.13 ± 0.47	01 (25.00)
8	04	6.0-11.68 8.91 ± 1.51	02 (25.00)	2.84-6.13 4.36 ± 0.79	01 (25.00)	1.68-3.94 2.78 ± 0.62	01 (25.00)
Overall	220	6.05-11.98 9.19 ± 0.12	66 (30.0)	2.47-6.99 4.38 ± 0.08	66 (30.00)	1.43-4.25 2.55 ± 0.06	66 (30.00)

*Significant at ($p < 0.05$) [1]Cut off points

Table 3. Influence of lactation stages on the prevalence of sub-clinical hypocalcemia and its relationship with phosphorus and magnesium in lactating cross-bred cows

Variable (Lactation stages: weeks)	No. of cows tested	Serum macro-mineral levels (mg/dl)					
		Calcium Range Mean ± SE	SCHC (≤ 8 mg/dl)[1] Positive No. (%)	Phosphorus Range Mean ± SE	HP (< 4mg/dl)[1] Positive No. (%)	Magnesium Range Mean ± SE	HM (>3 mg/dl) [1] Positive No. (%)
1	18	06.13-07.6 06.68 ± 0.13	17 (94.44)**	2.47-4.15 2.94 ± 0.10	17 (94.44)	3.11-4.25 3.87 ± 0.07	17 (94.44)
2	30	06.05-11.36 07.54 ± 0.25	20 (66.67)	2.54-6.12 3.63 ± 0.15	20 (66.67)	1.71-4.25 3.19 ± 0.13	20 (66.67)
3	35	06.20-11.68 08.91 ± 0.25	10 (28.57)	2.84-6.34 4.2 ± 0.14	10 (28.57)	1.71-3.94 2.66 ± 0.12	10 (28.57)
4	40	06.45-11.68 09.16 ± 0.22	09 (22.50)	2.84-6.34 4.27 ± 0.13	09 (22.50)	1.43-3.58 2.43 ± 0.10	09 (22.50)
5	25	07.13-11.36 09.11 ± 0.21	04 (16.00)	2.94-6.56 4.25 ± 0.16	04 (16.00)	1.56-3.54 2.42 ± 0.12	04 (16.00)
6	20	06.22-11.68 09.83 ± 0.32	03 (15.00)	3.23-6.98 4.61 ± 0.22	03 (15.00)	1.67-3.54 2.13 ± 0.12	03 (15.00)
7	10	07.23-11.68 10.31 ± 0.61	02 (20.00)	2.84-6.88 5.24 ± 0.43	02 (20.00)	1.65-3.87 2.21 ± 0.26	02 (20.00)
8	08	09.13-11.68 10.94 ± 0.38	0	4.16-6.93 5.77 ± 0.41	0	1.71-2.21 1.87 ± 0.07	0
9	09	06.39-11.68 10.49 ± 0.65	01 (11.11)	3.22-6.49 5.29 ± 0.37	01 (11.11)	1.71-3.78 2.16 ± 0.25	01 (11.11)
10	08	09.23-11.98 11.34 ± 0.31	0	4.01-6.23 5.23 ± 0.21	0	1.69-2.54 1.88 ± 0.10	0
11	08	10.72-11.68 11.56 ± 0.12	0	5.27-6.99 5.77 ± 0.27	0	1.71-1.79 1.76 ± 0.01	0
12	08	11.36-11.68 11.60 ± 0.05	0	5.27-6.33 5.65 ± 0.19	0	1.71-1.79 1.77 ± 0.01	0
13	01	11.68	0	6.54	0	1.79	0

**Significant at ($p < 0.01$)

[1]Cut off values

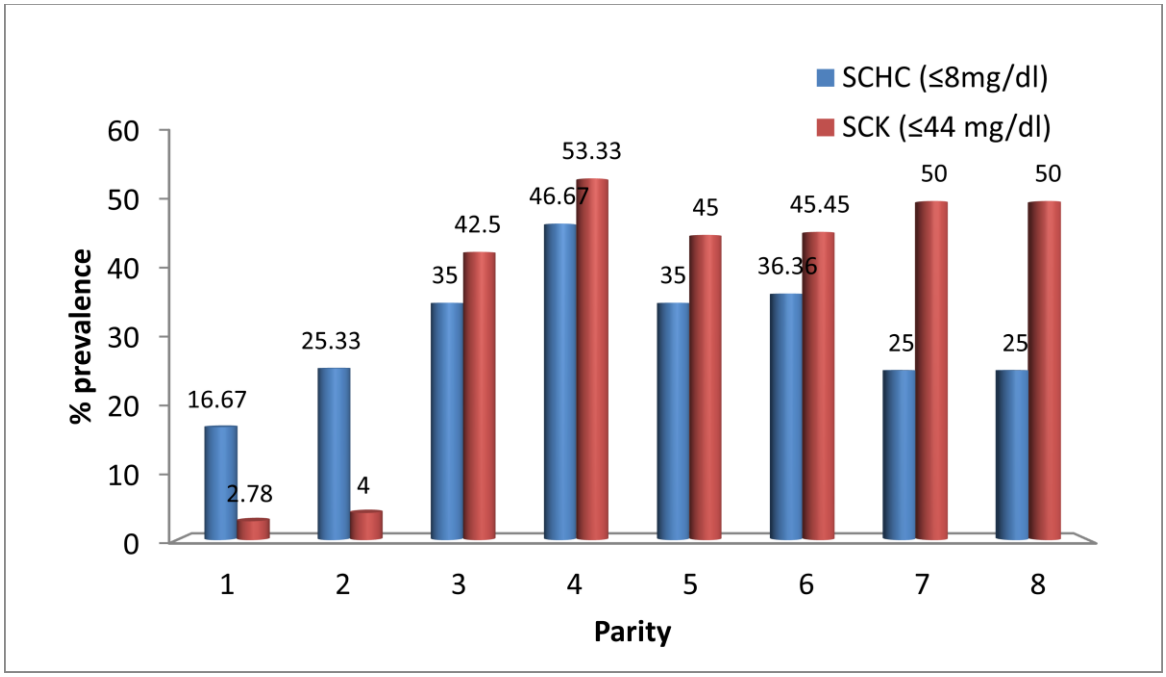

Fig. 3. Parity-wise prevalence of SCHC and SCK in lactating cross-bred dairy cows

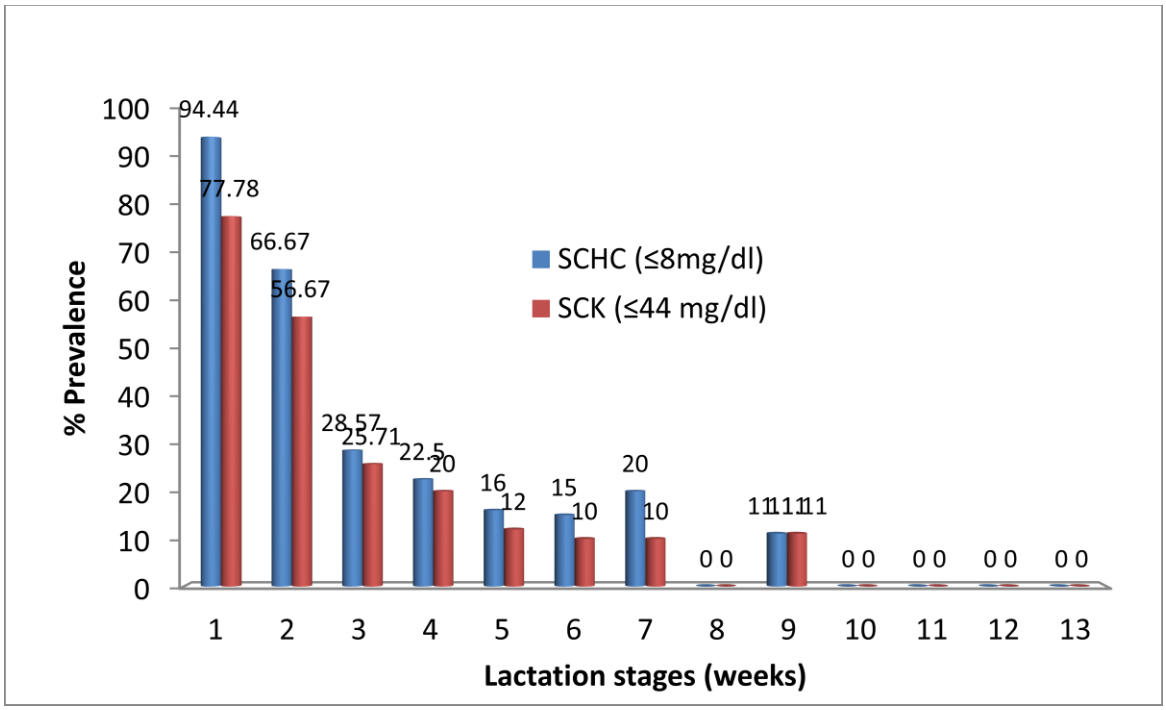

Fig. 4. Lactation stage-wise prevalence of SCHC and SCK in lactating dairy cows

Table 4. Effects of milk yield (liter / day) and body condition score (BCS) on the prevalence of subclinical hypocalcemia and its relationship with phosphorus and magnesium in lactating cross-bred cows

S/N	Variables	No. of cows tested	Serum macro-mineral levels (mg/dl)					
			Calcium Range Mean ± SE	SCHC (≤ 8 mg/dl)[1] Positive No. (%)	Phosphorus Range Mean ± SE	HP (< 4mg/dl)[1] Positive No. (%)	Magnesium Range Mean ± SE	HM (>3 mg /dl)[1] Positive No. (%)
①	Milk yield (liter/day)							
	< 05.0	040	07.89-11.68 11.38 ± 0.13	01 (2.50)**	3.81-6.99 5.7 ± 0.12	01 (2.50)	1.65-3.10 1.83 ± 0.05	1 (2.50)
	05.0-10.0	030	07.23-11.98 10.28 ± 0.31	06 (20.00)	2.94-6.98 5.20 ± 0.21	06 (20.00)	1.65-3.23 2.17 ± 0.11	06 (20.00)
	11.0-15.0	115	06.13-11.68 08.66 ± 0.13	35 (30.43)	2.84-6.34 4.03 ± 0.07	35 (30.43)	1.43-3.98 2.63 ± 0.07	35 (30.43)
	> 15.0	035	06.05-09.93 07.47 ± 0.23	24 (68.57)*	2.47-4.76 3.32 ± 0.11	24 (68.57)	2.13-4.25 3.43 ± 0.13	24 (68.57)
	Overall	220	06.05-11.98 09.19 ± 0.12	66 (30.00)	2.47-6.99 4.38 ± 0.08	66 (30.0)	1.43-4.25 2.55 ± 0.06	66 (30.00)
②	Body condition score (BCS)							
	3.0-3.25	120	06.13-11.98 09.41 ± 0.16	30 (25.00)	2.84-6.99 4.54 ± 0.09	30 (25.00)	1.56-3.98 2.46 ± 0.07	30 (25.00)
	> 3.5	100	06.05-11.68 08.92 ± 0.19	36 (36.00)	2.47-6.98 4.20 ± 0.11	36 (36.00)	1.43-4.25 2.66 ± 0.09	36 (36.00)
	Overall	220	06.05-11.98 09.19 ± 0.12	66 (30.00)	2.47-6.99 4.38 ± 0.08	66 (30.00)	1.43-4.25 2.55 ± 0.06	66

*Significantly higher at ($p < 0.05$) **Significantly lower at ($p < 0.05$) [1]Cut off points

The potential risk factors for SCHC in lactating dairy cows associated with breed, ages, parity, lactation stages, milk yield and BCS have been analyzed and evaluated (Table 5).

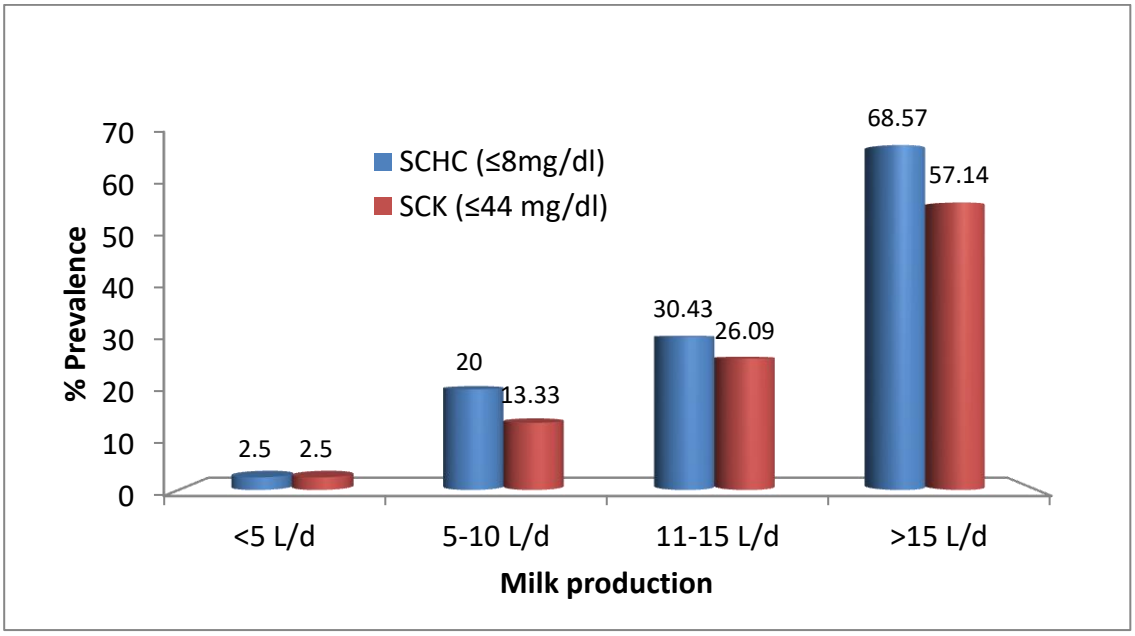

Fig. 5. Milk production-wise prevalence of SCHC and SCK in lactating dairy cows

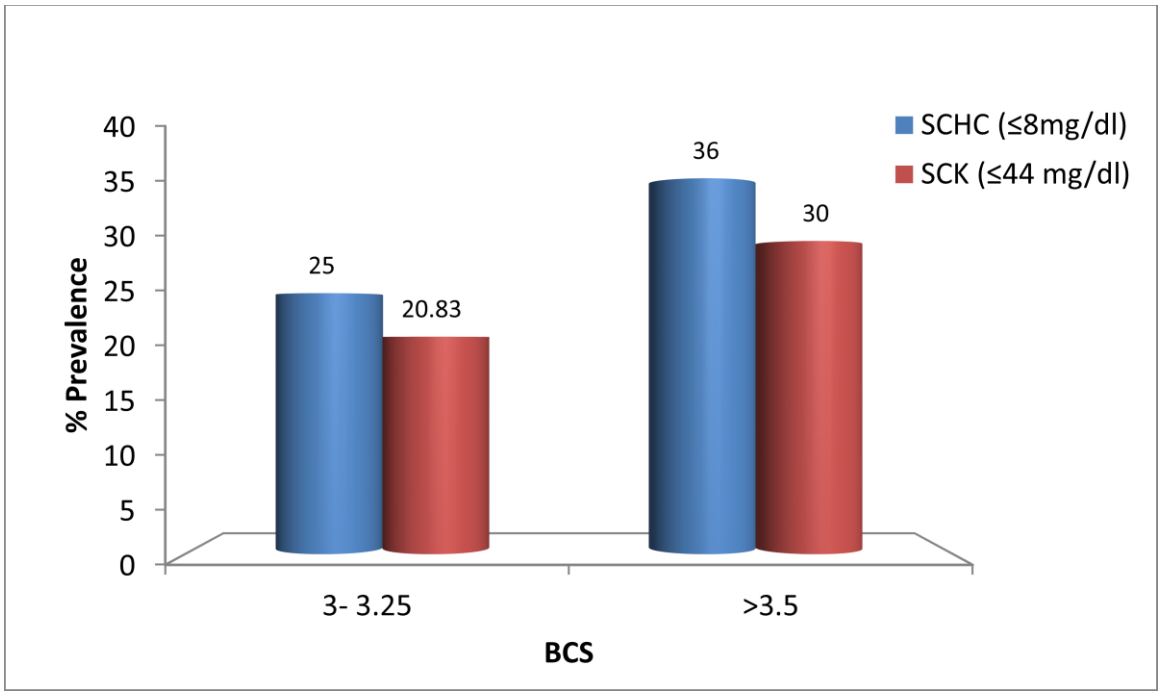

Fig. 6. Body condition score (BCS)-wise prevalence of SCHC and SCK in lactating cows

Table 5. Risk factors analysis of subclinical hypocalcemia (SCHC) in lactating crossbred dairy cows

S/N	Risk factors	Categories	SCHC				
			Positive (n = 66)	Negative (n = 154)	Odd ratio	95% CI	p-value
1.	Breed	HF × L	61	129	02.68	00.76 - 09.49	0.13
		SH × L	03	017	Reference		
		JS × L	02	008	01.42	00.20 - 10.23	0.73
2.	Ages (years)	< 4.0	06	030	Reference		
		4.0-50	19	056	01.70	00.61 - 04.70	0.31
		> 5.0	41	068	03.02	01.16 - 07.86	0.02
3.	Parity	01	06	030	Reference		
		02	19	056	01.70	00.61 - 04.70	0.31
		03	14	026	02.69	00.90 - 08.02	0.08
		04	14	016	04.38	01.41 - 13.58	0.01
		05	07	013	02.69	00.76 - 09.59	0.13
		06	04	007	02.86	00.63 - 12.92	0.17
		07	01	003	01.67	00.15 - 18.88	0.68
		08	01	003	01.67	00.15 - 18.88	0.68
4.	Lactation stages (week)	01	17	001	136.00	07.51 - 2463	0.0009
		02	20	010	16.00	01.75 - 146.3	0.01
		03	10	025	03.20	00.35 - 29.01	0.30
		04	09	031	02.32	00.26 - 21.12	0.45
		05	04	021	01.52	00.15 - 15.78	0.72
		06	03	017	01.41	00.13 - 15.78	0.78
		07	02	008	02.00	00.15 - 26.74	0.60
		08	00	008	-	-	-
		09	01	008	Reference		
		10	00	008	-	-	-
		11	00	008	-	-	-
		12	00	008	-	-	-
		13	00	001	-	-	-
5.	Milk yield (liter/day)	< 5.0	01	039	Reference		
		05-10	06	024	09.75	01.11 - 86.02	0.04
		10-15	35	080	17.06	02.25 - 129.2	0.006
		> 15.0	24	011	85.09	10.30 - 701.4	< 0.0001
6.	Body condition score (BCS)	03-3.25	30	090	Reference		
		> 3.5	36	064	01.69	00.94 - 03.02	0.08

95% CI = 95% Confidence interval

Prevalence and risk factors of SCK

Out of 220 lactating dairy cows tested, of which 55 (25.0%) animals had SCK detected by the both hypoglycemia and urinary ketone bodies tests (Table 6). The highest prevalence of SCK was recorded in HF x L (27.37%) in comparison to SH x L (10.0%) and JS x L (10.0%) and > 5 years of age had significantly ($p < 0.01$) higher prevalence (46.78%) in comparison to < 4 years (2.78%) and 4 to 5 years (4.0%) age groups in lactating cows (Table 6 & Fig. 1-2).

Table 6. Breeds and age-wise prevalence of sub-clinical ketosis detected by blood glucose and urinary ketone bodies in lactating dairy crossbred cows

S/ N	Variables	No. of cows tested	Serum glucose level (mg/dl)				Urinary ketone bodies test
			Range Mean ± SE	Sub-clinical ketosis ≤ 35 mg[1] Positive No. (%)	 36-44 mg[1] Positive No. (%)	 Total	 Positive No. (%)
A. Breed							
①	HF × L	190	20.83-86.54 55.02 ± 1.42	43 (22.63)	09 (04.74)	52 (27.37)	52 (27.37)
②	SH × L	020	26.04-86.54 64.66 ± 4.17	02 (10.00)	0	02 (10.00)	02 (10.00)
③	JS × L	010	41.66-83.67 60.35 ± 4.85	0	01 (10.00)	01 (10.00)	01 (10.00)
Overall		220	20.83-86.54 56.14 ± 1.32	45 (20.45)	10 (04.55)	55 (25.00)	55 (25.00)
B. Age (years)							
①	< 4.0	036	36.45-86.54 62.09 ± 2.16	01 (02.78)	0	01 (02.78)	01 (02.78)
②	4.0-5.0	075	20.83-86.54	03 (04.00)	0	03 (04.00)	03 (04.00)
③	> 5.0	109	20.83-86.54 48.21 ± 2.03	41 (37.61)	10 (09.17)	51 (46.78)**	51 (46.78)**
Overall		220	20.83-86.54 56.14 ± 1.32	45 (20.45)	10 (04.55)	55 (25.00)	55 (25.00)

**Significant at $p < 0.01$) [1]Cut off points HF = Holstein Friesian SH = Sahiwal JS = Jersey
≤ 35 mg/dl considered CK[108]

The influence of parity on the prevalence of SCK in lactating crossbred cows was recorded through the significantly ($p < 0.01$) highest prevalence of SCK occurred during the 4th (53.33%) parity and significantly ($p < 0.01$) lower prevalence at the 1st (2.78% and 2nd (4.0%) parity (Table 7 & Fig. 3).

Table 7. Influence of parity on the prevalence of sub-clinical ketosis detected by blood glucose and urinary ketone bodies in lactating dairy crossbred cows

Variables (Parity No.)	No. of cows tested	Serum glucose level (mg/dl)				Urinary ketone bodies test
		Range (Mean ± SE)	Sub-clinical ketosis			
			≤ 35 mg Positive No. (%)	36-44 mg Positive No. (%)	Total	Positive No. (%)
1	36	36.45-86.54 (62.09 ± 2.16)	01 (02.78)	0	01 (02.78)**	01 (02.78)
2	75	20.83-86.54 (65.09 ± 1.65)	03 (04.00)	0	03 (04.00)**	03 (04.00)
3	40	20.83-86.54 (52.34 ± 3.52)	13 (32.50)	04 (10.00)	17 (42.50)	17 (42.50)
4	30	20.83-69.27 (44.65 ± 3.75)	13 (43.33)	03 (10.00)	16 (53.33)*	16 (53.33)*
5	20	20.83-76.56 (48.87 ± 4.41)	06 (30.00)	03 (15.00)	09 (45.00)	09 (45.00)
6	11	26.04-76.56 (43.08 ± 5.04)	05 (45.45)	0	05 (45.45)	05 (45.45)
7	04	20.83-52.08 (36.45 ± 7.67)	02 (50.00)	0	02 (50.00)	02 (50.00)
8	04	20.83-83.67 (50.65 ± 17.26)	02 (50.00)	0	02 (50.00)	02 (50.00)
Overall	220	20.83-86.54 (56.14 ± 1.32)	45 (20.45)	10 (04.55)	55 (25.00)	55 (25.00)

*Significantly higher at ($p < 0.01$) ** Significantly lower at ($p < 0.01$) [1]Cut off point

The prevalence of SCK was recorded from 1st to 9th weeks of lactation period but significantly ($p < 0.01$) highest prevalence was recorded at the 1st (77.778%) and 2nd (56.67%) weeks of lactation period than the higher lactation weeks (Table 8 & Fig. 4). Table 9 shows the effects of milk production and BCS on the prevalence of SCK in dairy lactating cows. It appears that the higher milk production is associated with the higher prevalence of SCK and a significantly ($p < 0.01$) higher prevalence of SCK (57.14%) was recorded in the cows producing > 15 liter milk / day (Table 9 & Fig.5). The higher prevalence of SCK (30.0%) was observed with higher BCS (>3.5) than 20.83% with lower BCS (3.0 - 3.25) in lactating dairy crossbred cows (Table 9 & Fig. 6).

The potential risk factors for sub-clinical ketosis in lactating dairy cows associated with breed, ages, parity, lactation stages, milk yield and BCS have been analyzed and evaluated (Table 10).

Table 8. Influence of lactation stages on the prevalence of sub-clinical ketosis detected by blood glucose and urinary ketone bodies in lactating dairy crossbred cows

Variables (Lactation stages (weeks)	No. of cows tested	Serum glucose level (mg/dl)				Urinary ketone bodies test
		Range (Mean ± SE)	Sub-clinical ketosis ≤ 35 mg[1] Positive No. (%)	36-44 mg[1] Positive No. (%)	Total Positive No. (%)	Positive No. (%)
01	18	20.83-76.56 (32.31 ± 3.90)	13 (72.22)	01 (05.56)	14 (77.78)*	14 (77.78)
02	30	20.83-76.56 (35.36 ± 2.87)	17 (56.67)	0	17 (56.67)*	17 (56.67)
03	35	20.83-76.56 (50.74 ± 2.69)	08 (22.85)	01 (02.86)	09 (25.71)	09 (25.71)
04	40	20.83-86.54 (56.90 ± 2.20)	05 (12.50)	03 (07.50)	08 (20.00)	08 (20.00)
05	25	26.04-69.27 (61.27 ± 2.26)	01 (04.00)	02 (08.00)	03 (12.00)	03 (12.00)
06	20	46.87-83.25 (65.62 ± 2.19)	0	02 (10.00)	02 (10.00)	02 (10.00)
07	10	43.66-85.25 (71.02 ± 4.68)	0	01 (10.00)	01 (10.00)	01 (10.00)
08	08	41.66-44.88 (74.37 ± 5.44)	0	0	0	0
09	09	20.83-83.25 (68.74 ± 6.29)	01 (11.11)	01 (11.11)	01 (11.11)	01 (11.11)
10	08	46.87-76.56 (70.12 ± 3.56)	0	0	0	0
11	08	69.27-86.54 (80.23 ± 2.27)	0	0	0	0
12	08	69.27-86.54 (75.99 ± 1.91)	0	0	0	0
13	01	76.56	0	0	0	0
Overall	220	20.83-86.54 (56.14 ± 1.32)	45 (20.45)	10 (04.55)	55 (25.00)	55 (25.00)

*Significantly higher at ($p < 0.01$) [1]Cut off points ≤ 35 mg /dl considered CK[108]

Concurrent prevalence of sub-clinical hypocalcaemia and sub-clinical ketosis

Of the 220 lactating cross-bred dairy cows tested, of which 46 (20.91%) were affected with both the SCHC and SCK concurrently and only 09 (4.09%) and 20 (9.09%) affected alone with SCK and SCHC, respectively (Table 11). The 20.19% concurrent prevalence of SCHC and SCK was found significantly ($p<0.001$) higher in comparison to single occurrence of SCK (4.09%) and SCHC (9.09%) in lactating cross-bred cows (Table 11).

Table 9. Milk yield (liter/day) and body condition score (BCS) associated with the prevalence of sub-clinical ketosis in lactating dairy crossbred cows

Variables	No. of cows tested	Serum glucose level (mg/dl)				Urinary ketone bodies test
		Range (Mean ± SE)	Sub-clinical ketosis			
			≤ 35 mg[1] Positive No. (%)	36-44 mg[1] Positive No. (%)	Total Positive No. (%)	Positive No. (%)
① Milk yield (liter/day)						
< 5.0	040	46.87-86.54 (76.88 ± 1.22)	01 (02.50)	0	01 (02.50)	01 (02.50)
5.0-10	030	36.45-86.54 (68.23 ± 2.50)	03 (10.00)	01 (03.33)	04 (13.33)	04 (13.33)
11-15	115	20.83-76.56 (52.25 ± 1.49)	22 (19.13)	08 (06.67)	30 (26.09)	30 (26.09)
>15.0	035	20.83-63.13 (34.83 ± 2.46)	20 (57.14)	0	20 (57.14)**	20 (57.14)
Overall	220	20.83-86.54 (56.14 ± 1.32)	45 (20.45)	10 (04.55)	55 (25.00)	55 (25.00)
② BCS						
3.0-3.25	120	20.83-86.54 (60.32 ± 1.56)	15 (12.50)	10 (08.33)	25 (20.83)	25 (20.83)
> 3.5	100	20.83-86.54 (51.10 ± 2.11)	30 (30.00)	0	30 (30.00)	30 (30.00)
Overall	220	20.83-86.54 (56.14 ± 1.32)	45 (20.45)	10 (04.55)	55 (25.00)	55 (25.00)

**Significantly higher at ($p < 0.01$) [1]Cut off points ≤ 35 mg/dl considered CK[108]

Therapeutic management of SCHC and SCK affected cows

An overall blood calcium and glucose levels of both the SCHC and SCK affected lactating cows treated with CP-Vet bolus® (Calcium contained mineral and vitamin preparation bolus) and Vita-D Plus® (contained propylene glycol with vitamin D) increased significantly ($p < 0.001$) after seven days of post-treatment in comparison to pre-treatment values (Table 12) and Vita-D Plus® (contained propylene glycol with vitamin D) increased significantly ($p < 0.001$) after seven days of post-treatment in comparison to pre-treatment values (Table 12).

Table 10. Risk factors analysis of subclinical ketosis (SCK) in lactating crossbred cows

S/N	Risk factors	Categories	SCK		Odd ratio	95% CI	p-value
			Negative (n = 55)	Positive (n = 165)			
1.	Breed	HF × L	52	138	03.39	00.42 - 27.43	0.25
		SH × L	02	018	01.00	00.08 - 12.56	1.00
		JS × L	01	009	Reference		
2.	Ages (years)	< 4.0	01	035	Reference		
		4.0-50	03	072	01.46	00.15 - 14.53	0.75
		> 5.0	51	058	30.78	04.07 - 232.7	0.0009
3.	Parity	01	01	035	Reference		
		02	03	072	01.46	00.15 - 14.53	0.75
		03	17	023	25.87	03.22 - 207.9	0.002
		04	16	014	40.00	04.83 - 332.1	0.0006
		05	09	011	28.64	02.26 - 251.9	0.002
		06	05	006	29.17	02.88 - 295.4	0.004
		07	02	002	35.00	02.15 - 570.8	0.01
		08	02	002	35.00	02.15 - 570.8	0.01
4.	Lactation stages (week)	01	14	004	31.50	03.02 - 328.9	0.004
		02	17	013	11.77	01.32 - 105	0.027
		03	09	026	03.12	00.35 - 28.14	0.31
		04	08	032	02.25	00.25 - 20.44	0.47
		05	03	022	01.23	00.11 - 13.43	0.87
		06	02	018	01.00	00.08 - 12.56	1.00
		07	01	009	Reference		
		08	00	008	-	-	-
		09	01	008	01.125	00.06 - 21.09	0.94
		10	00	008	-	-	-
		11	00	008	-	-	-
		12	00	008	-	-	-
		13	00	008	-	-	-
5.	Milk yield (liter/day)	< 5.0	01	039	Reference		
		05-10	04	026	06.00	00.63 - 56.75	0.12
		10-15	30	085	13.76	01.81 - 104.6	0.01
		> 15.0	20	015	52.00	06.40 - 422.4	0.0002
6.	Body condition score (BCS)	03-3.25	25	095	Reference		
		> 3.5	30	070	01.63	00.88 - 03.01	0.12

95% CI = 95% Confidence interval

Table 11. Concurrent occurrence of sub-clinical ketosis (SCK) and sub-clinical hypocalcaemia (SCHC) in lactating dairy cows

SN	Cross-bred cows	No. of cows tested	SCK + SCHC Positive No. (%)	SCK Positive No. (%)	SCHC Positive No. (%)	Total Positive No. (%)
①	Holstein-Friesian × Local	190	44 (23.16)	08 (04.22)	17 (08.95)	69 (36.32)
②	Sahiwal × Local	020	02 (10.00)	0	01 (05.00)	03 (15.00)
③	Jersey × Local	010	0	01 (10.00)	02 (20.00)	03 (30.00)
	Overall	220	46 (20.91)**	09 (04.09)	20 (09.09)	75 (34.09)

**Significantly higher at ($p < 0.001$)

Table 12. Comparison of blood calcium and glucose levels between pre and post-treatment of sub-clinical hypocalcemia and sub-clinical ketosis affected lactating dairy cows [Range, Mean ± SE]

SN	Cross-bred cows	Serum calcium levels (mg/dl)			Serum glucose levels (mg/dl)		
		No. of positive cows treated	Pre-treatment Range Mean ± SE	Post-treatment Range Mean ± SE	No. of positive cows treated	Pre-treatment Range Mean ± SE	Post-treatment Range Mean ± SE
①	HF × Local	61	6.05-7.96 6.93 ± 0.07	09.56-12.87 11.58 ± 0.14	52	20.83-44.00 27.75 ± 1.13	45.27-59.45 53.08 ± 0.66
②	SH × Local	03	6.32-7.13 6.76 ± 0.24	10.34-11.23 10.93 ± 0.30	02	26.04-31.25 28.65 ± 2.61	45.27-58.34 51.81 ± 6.54
③	JS × Local	02	6.13-7.24 6.69 ± 0.56	12.21-12.87 12.54 ± 0.33	01	42.01	58.12
Overall		66	6.05-7.96 6.92 ± 0.07	09.56-12.87 11.58 ± 0.13**	55	20.83-44.00 28.04 ± 1.10	45.27-59.45 53.12 ± 0.65**

**Significantly higher at ($p < 0.01$) HF = Holstein-Friesian SH = Sahiwal JS = Jersey

DISCUSSION

Metabolic diseases are the multifactorial disorders of high milk yielding dairy cattle, primarily caused by imbalance and inadequate feeding, and erratic management of animals associated with heavy economic losses in dairy industry worldwide.[12,19,25-27] Approximately 75% of diseases in dairy cattle occur in the first month post-partum and 50% of dairy cattle suffer from metabolic and infectious diseases in the transition period.[28] The transition period which is the beginning at the last three weeks of pregnancy and extending into the third week of lactation that is around 3 weeks peri-parturition in cows.[10,20,21] The transition period during parturition and the initiation of lactation is very critical for the dairy cow production cycle because there are increased hormonal and metabolic changes.[29] There is an increased energy and calcium demands for colostrum and milk production, combined with a decline of dry matter intake (DMI) around parturition, can result NEB, increased lipid mobilization[16,30] and a reduction in blood concentrations of calcium.[17,18] This metabolic stress of the transition period of a dairy cow lead to a high incidence of metabolic, infectious and reproductive disorders associated with a severe negative energy balance (NEB). This period of NEB lasts approximately five weeks after calving.[31] Among the metabolic diseases, milk fever (MF) and ketosis occur commonly associated with transition period and peak milk production in dairy cattle.[32] Both the clinical and sub-clinical forms of metabolic diseases occur in daily animals. However, the severity of the disease is higher in the clinical cases but subclinical cases are more important because (a) they are far more frequent, (b) they cannot be easily diagnosed, and (c) may impair the longevity and production of the cow.[17,33]

Dairy cows affected with clinical MF and clinical ketosis (CK) could be diagnosed in the field on the basis of disease history, clinical findings and response to therapy. However, dairy cows with SCMF and SCK do not show any clinical symptoms but have a SCHC and sub-clinical hypoglycemia (SCHG) with the positive level of ketone bodies regarded as SCK, respectively. Thus the only way to know whether dairy cows are experiencing SCHC and SCK are to analyze blood for the concentrations of specific biochemical constituents. In addition, blood, milk and urine samples could also be used to detect the ketone bodies for the diagnosis of SCK in dairy cows.

Hypocalcemia

Hypocalcemia is one of the most common metabolic disorders in dairy cattle, classified into two forms, clinical hypocalcemia (parturient paresis (PP) / MF) and SCHC. Clinical hypocalcemia (MF) is easily identified, treated and readily responds to therapy when treatments are initiated promptly, whereas the SCHC cases cannot be treated easily due to the absence of obvious clinical signs required for diagnosis. The SCHC has been defined as low calcium concentrations (serum Ca $\leq$ 8.0 mg/dl) without any clinical symptoms of MF in dairy animals. Hypocalcaemia in dairy cows can also be called the ‘gateway disease’ as it increases the risk of other diseases and disorders like decreased milk production, decrease immune function, slower uterine involution, delayed first ovulation after calving, reduced gastro-intestinal motility, increased risk of ketosis, decreased reproductive performance, retained placenta, mastitis, metritis, endometritis, displaced abomasum and increased risk of early removal from the herd.[17,32,34-39]

Approximately 82% of the cows and 5.1 times greater incidence of displaced abomasum had serum calcium values ≤ 2.0 mmol/L (≤ 8.0 mg/dl) in the first week after calving.[35,40] Hypocalcemia associated with decrease or loss of muscle tone in the uterus increased the incidence of dystocia, uterine prolapse and retained placenta in post-partum dairy animals.[32,41,42] MF affected cows have been reported to be 3 to 6 times more susceptible to dystocia than that of normal cows.[34,41-43]

Hypocalcaemia is associated with impaired immune function and diminished muscle contraction that lead to develop metritis in post-parturient dairy cows.[33,34,44] It has also been reported that SCHC had 4.85 greater odds of having metritis[45] and higher incidence of endometritis in MF cases.[46]

Hypocalcaemia reduces contraction of smooth and skeletal muscles[33] and thereby reduces the teat sphincter muscle and myometrium contraction[47] that may prevent efficient teat closure along with impaired immune function leading to milk leakage which invites environmental pathogens to enter the udder and entrance of bacteria causing mastitis.[17,34,44] In addition, hypocalcemic cows tend to spend more time lying down which could increase teat end exposure to environmental pathogens.[17,41,48] The milk fever affected cows have been reported to be eight times more likely to develop mastitis than normal cows.[34] The dairy animals experience some degree of immune-suppression during the transition period[49-52] which might be due to decreased polymorphonuclear leukocytes, glycogen stores, decreased blood calcium level and increased non-esterified fatty acids (NEFA) and BHBA.[49] Hypocalcaemia reduces the ability of immune cells to respond to stimuli, thus contributing to infections such as mastitis.[53]

The SCHC has also been reported to be associated with the reduction of ruminal and abomasal motility that may cause a reduction of feed intake and weight loss especially in early lactation[54-58] and well exacerbate NEB in cows that are already underfed.[41]

Both the clinical and SCHC have been reported to be associated with decreased fertility especially increased number of services per conception, increased calving to conception interval, estrus cyclicity and pregnancy rate in dairy animals.[41,45,59-61] Hypocalcaemia results in reduced fertility in dairy cows due to its effect on uterine muscle function, slower uterine involution[41,59] and reduced blood flow to the ovaries.[62]

An increased risk of culling has been reported for cows with hypocalcaemia.[35,63] The serum calcium levels of 1.8 mmol/L, ≤ 2.2 mmol/L and ≤ 2.3 mmol/L resulted 3, 2.4 and 5.3 times more likely to be culled in the first 60 days, 1st and 2nd weeks of lactation, respectively.[35,50] Even the average productive life of a cow can be reduced 3.4 years with hypocalcemia.[25,64] The losses from SCHC have been estimated to be several times more than clinical cases in a herd.[65]

Cutoff point (hypocalcemia)

The cut-off points for blood calcium level as ≤ 8 mg/dl (≤ 2.0 mmol/L) for SCHC[17,18,26] and ≤ 6.0 mg/dl for clinical hypocalcemia[17,18] have been reported. However, there are other studies that apply different concentrations of the normal reference values for serum calcium of cattle have been defined as 8.5 to 10 mg / dl,[17] 2.0 to 2.5 mmol/L,[26] above 8.8 mg / dl,[36] 8.59 mg / dl[37] and 2.12 ± 0.50 mM[66] but different cutoff values for SCHC have also been used as ≤ 2.0 mmol/L,[18,26] ≤ 2.14 mmol/L[37] and 1.88 mmol/L (7.5 mg/dl).[67]

Several different blood calcium thresholds that have been reported in different problems including ≤ 1.93 mmol/L for ketosis, ≤ 2.05 mmol/L for retained placenta and metritis and ≤ 2.10 mmol/L for displaced abomasum.[45] Blood calcium threshold ≤ 1.97 mmol/L for 2nd parity animals at 2 day-in-milk (DIM) associated with the risk of metritis and/or abomasal displacement, whereas blood calcium levels of primiparous cows are not associated with lower milk production at any of the DIM assessed.[39] It has also been suggested that this cut off point should be raised to 8.5 mg / dl (2.1 mmol/L) because cows below this concentration have developed metritis or metabolic disorders.[68] The lowest serum calcium concentration typically occurs within the few days after calving[37] and returns to normal within 2 to 3 days.[69,70] The comparatively higher levels of serum calcium (12.97 ± 6.67 mg/dl) and phosphorus (7.26 ± 0.41 mg/dl) have been reported in non-lactating than lactating cows as calcium (10.05 ± 2.51 mg/dl) and phosphorus (6.52 ± 0.30 mg/dl) in an inland report.[71]

Prevalence of SCHC

The overall prevalence of SCHC was recorded in 30.0% lactating three types of cross-bred dairy cows with significantly ($p < 0.05$) highest prevalence in HF × L (32.11%) in comparison to SH × L (15.0%) and JS × L (20.0%) cross-bred cows. It appears that the prevalence of SCHC (30.0%) is at least 10 times more prevalence than clinical MF (2.97%) in dairy cattle in Bangladesh.[23] However, the overall lower prevalence of 30.0% SCHC recorded in cross-bred lactating dairy cows in Bangladesh in comparison to 50% in US dairy herds,[18] 40 to 50% in multiparous Iranian dairy herds,[72] 40.34% in lactating cows in Brazil[73] and 47.6% within 48 hours of parturition in Germany[74] and 30 to 50% on the day of calving elsewhere.[18,75,76]

Hypocalcaemia (MF/PP) affects high milk producing dairy animals in their transition period. Generally, pure exotic and their cross-bred are highly susceptible to hypocalcaemia due to high amount of colostrum and milk production. However, certain breeds of cattle have been reported to be more susceptible to hypocalcemia, particularly Channel Island, Swedish Red and White, and Jersey cattle.[77] The intestinal receptors for $1,25(OH)_2D_3$ are lower in Jersey than aged-matched Holsteins. Lower numbers of receptors would result in a loss of target tissue sensitivity to $1,25(OH)_2D_3$, resulting in increased susceptibility to hypocalcemia. The risk of developing MF is higher in Jerseys and also increases with increasing parity and higher levels of milk production and thus advocating for special considerations when dairy cows fit these criteria.[78] The high prevalence rate of SCHC in adult dairy cattle at transition period[79] and therefore, prevention of hypocalcemia in peri-parturient animals has significant importance in cow health, finance and welfare and accordingly the SCHC has become a major topic of recent research works in dairy animals.

Influence of age on prevalence of SCHC

The influence of age on the prevalence SCHC showed highest prevalence of SCHC in cows more than five years of age (37.16%) in comparison to lower aged cows less than four years (16.67%) and four to five years (25.33%). Age has a profound effect on susceptibility of dairy cows to hypocalcaemia and the risk of hypocalcaemia increases by approximately 9% per lactation. Older cows are affected by hypocalcaemia more common and more severe than

young cows. Older and over-conditioned cows have been reported to be at greater risk for experiencing MF,[26] whereas primiparous cows are much less likely to have low blood calcium levels near calving.[80] In addition to increased milk production from the 3rd lactation resulting high calcium demand, ageing also results in a diminished ability to mobilize calcium from bone stores and a decline in the active transport of calcium in the intestine as well as impaired production of $1,25(OH)_2D_3$ due to decrease number of $1,25(OH)_2D_3$ receptors. The hypocalcemia at calving is age related and most marked in cows from 3rd to 7th parity and it is infrequent at the first parturition.

Influence of parity on prevalence of SCHC

The influence of parity on the prevalence of SCHC showed significantly ($p < 0.05$) higher prevalence of SCHC at 4th parity (46.67%) and significantly ($p < 0.05$) lower at 1st parity (16.67%) in comparison to other parities in dairy cows. These observations support the earlier reports that the prevalence of SCHC increases as the parity increases.[18,81] Primiparous cows have a lower risk of suffering from SCHC while multiparous cows have an increased risk. Multiparous cows, especially after the third parturition have an increased risk of suffering from severe SCHC. Significantly ($p <0.05$) higher prevalence of MF has been reported in Jerseys cows beyond their 4th parity (24.85%) than 2nd (5.9%), 3rd (6.49%) and 4th (8.73%) parities. Similarly, Holstein cows had higher MF beyond 4th parity (8.29%) than 2nd (1.43%), 3rd (1.82%) and 4th (2.91%) parities.[18,81]

The age and parity-associated susceptibility might be related calcium homeostatic mechanisms. With increasing age and parity, the hemostasis process is impeded and results in moderate to severe hypocalcaemia. It has been assumed that the number of vitamin D receptors in intestines decline with increasing age.[82] In addition, as animals age increase, the number of receptors for PTH on target tissue decline.[83]

Influence on lactation stages on prevalence of SCHC

The prevalence of SCHC was found significantly ($p < 0.05$) higher (94.44%) at the 1st week of lactation in comparison to higher lactation stages. Blood calcium levels remarkably declines in dairy cows around calving, with the lowest levels occurring about 12 to 24 hours after calving.[17,53] Blood samples tested at this stage can reveal the extent of hypocalcaemia experienced by a dairy herd.[84] The SCHC increased with age and 41, 49%, 51%, 54% and 42% have been reported at 2nd to 6th lactation cows, respectively.[18,72]

Influence SCHC on milk yield

An association between the prevalence of SCHC and increased of milk yield was recorded with a significantly ($p < 0.05$) higher prevalence of SCHC in cows produced more than 15 liter milk / day (68.57%) than cows produced less than 5.0 liter /day (2.50%), 5 to 10 liter/ day (20.0%) and 11 to 15 liter/day (30.43%) of milk. These results are in conformity with the findings of cows with SCHC produced an average of 5.7 kg / day more milk during 2, 3 and 4 weeks compared with control cows.[85]

Influence of BCS on SCHC

This study also recorded higher BCS (> 3.5) had higher prevalence of SCHC than the lower BCS (3 - 3.25). This finding supports that the higher BCS (≥ 3.75 out of 5.0) at calving developed up to 4 times more hypocalcemia in dairy cows.[86] Higher BCS at calving have a higher calcium output in milk and also results in decreased feed intake during gestation period due to reduced appetite in critical period and around calving which predisposes them to hypocalcaemia.[87]

Relationship between macro-minerals and Hypocalcemia

The average reference values of serum micro-minerals of lactating dairy cows (calcium 2.12 ± 0.50 mmol/L, phosphorus 1.81 ± 0.48 mmol/L, magnesium 0.97 ± 0.52 mmol/L) have been reported.[66] The SCHC has been reported to be associated with hypophosphatemia and hypermagnesemia in association with all the investigated risk factors including breeds, age, parity, lactation stages, milk yield and BCS in lactating dairy cows. These results suggest that the magnesium and phosphorus play an important role in calcium homeostasis.[26]

Animals fed diets containing less phosphorus than necessary to meet physiologic needs suffer hypophosphatemia. The hypophosphatemia is usually complicated by concurrent hypocalcemia, hypomagnesemia and in some cases hypoglycemia. At the onset of lactation in the dairy cow, production of colostrum and milk draw large amounts of phosphorus out of extracellular phosphorus pools, depressing blood phosphorus concentrations. Within a day or two of calving it is typical to find blood phosphorus concentration between 3.2 and 4 mg/dl in healthy cows. However, cows that develop hypocalcemia have low blood phosphorus concentrations that are even further depressed. Plasma inorganic phosphorus concentrations in cows with hypocalcemia are often between 1 and 2 mg/dl. Plasma phosphorus concentrations usually increase rapidly following treatment of the hypocalcemic cow with intravenous calcium solutions. Restoring normo-calcemia decreases parathyroid hormone secretion, which reduces urinary and salivary loss of phosphorus and stimulates resumption of gastro-intestinal motility, which in turn allows absorption of dietary phosphorus and reabsorption of salivary phosphorus secretions. Protected hypophosphatemia in some cows appears to be an important factor in some nonresponsive hypocalcemic cases. Unlike typical cases of parturient paresis, plasma phosphorus levels in these cows remains low, despite successful treatment of the hypocalcemia. Instituting a program to control hypocalcemia and MF generally is an effective means of preventing the low phosphorus downer cow syndrome.[17] In dry cows, high dietary levels of phosphorus (> 0.5% DM intake) increase the serum level of inorganic phosphorus (IP) which has inhibitory effect on the renal enzyme (1α-hydroxylase) that catalyzes the conversion of vitamin D into its active form (1,25(OH)2D3) and thereby predisposes cows to hypocalcaemia.[88-90] High dietary phosphorus has also been reported to have a negative effect on intestinal magnesium absorption which further makes periparturient cows susceptible to hypocalcaemia.[91,92]

Hypomagnesemia affects calcium metabolism by reducing PTH secretion in response to hypocalcaemia and by reducing ability of PTH stimulated cells to produce cyclic AMP resulting in failure to activate the target tissues to PTH.[17,91] Low magnesium levels in the diet

reduced calcium absorption in the gut. On the contrary, serum calcium and magnesium concentrations are negatively associated. Cows suffering from hypocalcaemia have higher serum magnesium level.[74] In a period of low serum calcium level, PTH is secreted into the blood. PTH secretion raises the threshold for renal magnesium excretion resulting in a higher serum magnesium concentration.[17,93] Hypocalcemia associated with hypermagnesemia may be due to in part to the suppressive effects of hypermagnesemia on PTH.[94]

Hypoglycemia and SCK

Ketosis is a major metabolic disorder of dairy cows in early lactation which develops when dairy cows fall into a condition of excessive NEB caused by insufficient dietary intake and generous lactation and characterized by relatively high concentrations of the ketone bodies (acetoacetate, BHBA and acetone) and a concurrent low concentration of blood glucose.[95] The decreased DMI at pre-partum causes NEB and increases NEFA and BHBA concentrations that cause ketosis at early lactation.[28]

Bovine ketosis typically occurs in early lactation in both the clinical and sub-clinical forms. The CK is characterized by diminished appetite, decreased milk production, weight loss, hypoglycemia and hyperketonemia whereas SCK remain undetected clinically but have effects on productivity like clinical ketosis.[96] The SCK (more correctly called hyperketonemia) may be defined as increased concentrations of circulating ketone bodies without the presence of clinical signs of ketosis in lactating cows.[97,98] However, it can appear during the transition period, dry period or at calving or in early lactation where the highest incidence of SCK occurs within the first 2 to 3 weeks of lactation.[99]

The serum BHBA 1.2 to 1.4 mmol/L during the first and second weeks of calving have been associated with 1.5 to 2.4 kg milk loss daily.[36,99-102] The serum BHBA concentrations ≥ 1.2 mmol/L during the first week of calving have been reported to be associated with displacement of abomasum and metritis[99,103] and > 1.0 mmol/L associated with significantly less conception rate after first AI.[104] The increased ketone bodies in cows immediately after parturition has a negative impact on health of cows and associated with reduced milk production.[97,105] The total cost of SCK per case per year resulted for 36% from a prolonged calving interval, 24% from reduced milk production, 19% from treatment, 14% from discarded milk and 6% from removal.[27]

Diagnosis and Cutoff value for ketosis

Two major changes occur in the blood of ketosis affected dairy animals: (a) Hypoglycemia and (b) ketonemia. Ketosis occurs in early lactation because of the decrease in blood glucose levels, which leads to a high degree of fatty acid mobilization in the form for NEFA. The NEFAs are then oxidized by the liver, leading to ketone body (acetone, acetoacetate & BHBA) production. Diagnosis of ketosis is preferred by measuring acetoacetate and BHBA levels in the blood, urine or milk and blood glucose levels.

The average reference values of serum glucose (3.15 ± 0.67 mmol/L) with high milk yielding (3.01 ± 0.65 mmol/L), low milk yielding (3.18 ± 0.69 mmol/L) and dry cows (3.25 ± 0.64

mmol/L) in dairy cows have reported.[66] Significantly ($p < 0.01$) lower plasma concentrations of glucose (4.23 ± 0.58 & 2.74 ± 0.51 mmol/L) and calcium (2.18 ± 0.14 & 2.08 ± 0.15 mmol/L) have been reported in ketosis affected lactating cows in comparison to healthy cows.[106]

Hypoglycemia, hyperketonemia, ketonuria and ketolactia are the biochemical characteristics of ketosis, and blood glucose levels are reduced from the normal of 50 mg/dl to 20 to 40 mg/dl.[95] The blood glucose level has been reported to be consistently low in cows at the time of their first ketosis diagnosis, even if the cows in very early lactation.[107] The CK cases are usually associated with plasma glucose concentrations less than 35 mg/dl and NEFA concentrations more than 1000 μEq/L.[108]

Blood glucose levels of 44.8 ± 2.2 mg/dl in CK affected cows has been reported to be lower than SCK (51.6 ± 2.3) affected cows and blood glucose levels of both types of ketosis had lower than healthy (68.3 ± 1.8) cows[109] However, blood glucose level ≤ 44.0 mg/dl has been considered as SCK in dairy cows.[110]

The 'gold standard' test for ketosis is serum BHBA because it is more stable in blood than acetone or acetoacetate[80] and its threshold levels for SCK ranges from 1.0 to 1.4 (1.2) mmol/L [112-114] whereas CK (reduced milk yield, lethargy and loss of appetite) > 3.0 mmol/L.[28,115-118] However, some cows have been reported to be exposed with high levels of BHBA (> 3 mmol/L) without showing any clinical signs and some other cows develop CK at reduced BHBA (< 3 mmol/L) levels.[13,98,119]

The cow-level prevalence of hyperketonemia, hypoglycemia, and simultaneous hypoglycemia and hyperketonemia has been reported to be 20.0%, 13.8% and 6.2%, respectively. The herd level average prevalence of hypoglycemia within the subset of hyperketonemic cows only has reported as 30.6%.[120]

Comparative evaluation of three cow side tests for detection of SCK in early lactating cows showed that both the Ketostix and Keto-Test strips have provided acceptable results for screening individual cows on commercial dairies to detect SCK.[121] Urine dipstick test showed sensitivity and specificity of 86% and 100% respectively.[122] Using trace as a positive result for the Ketostick and blood BHBA ≥ 1.2 mmol/L as the gold standard for diagnosis of SCK resulted in sensitivity of 88% and specificity of 95%.[123] Non-availability of electronic cow side test (Precision Xtra), the ketone strips (Atena Medical Instrument Co., China) are a dipstick containing the salt nitroprusside which becomes pink in the presence of acetoacetate (AcAc) has been used in this study.

Overall prevalence of SCK

The overall 25.0% prevalence of SCK was recorded in this study in lactating dairy cows based on the hypoglycemia and urinary ketone bodies tests. It appears that the prevalence of SCK (25.0%) is at least six times higher than the prevalence of clinical ketosis (3.75%) in dairy cattle in Bangladesh.[23] Insignificantly ($p > 0.05$) higher prevalence of SCK was recorded in HF × L (27.37%) than SH × L (10.0%) and JS × L (10.0%) cross-bred lactating dairy cows. These findings are in conformity with earlier report of an overall 25% prevalence of SCK 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.[24] However, reports on both the higher and lower

prevalence of SCK have been reported in lactating dairy cows elsewhere. The prevalence of SCK has been reported to be 7.5 to 14% in Canada and France,[124,125] 13.9% in Iran,[107] 18.0% in Turkey,[109] 36.6% in Europe,[116] 34% in Denmark,[126] and 29.3% in Hungarian dairy cattle.[127]

Prevalence of SCK ranges from 10 to 40% within 3 weeks of first lactation in small herds,[80,99,100,101,114,116-118] 7 to 14% in the first 60 days of lactation[128] but it varies from 8.3 to 40.1% with an average of 24.1% in large population of 8,902 dairy cows kept at 541 dairy farms of different countries in the world at the second week of lactation (> 95%) in dairy cows.[129] The prevalence of SCK has also been reported as range from 9 to 43% in the first 2 months of lactation[100,101,112] with the highest risk occurring within the first 21 days of lactation.[80,99] The average prevalence of SCK from 3 to 16 DIM has been reported to be 43% with range from 26 to 56%[130] with peak at 5 DIM.[101] However, it has also been reported in wide range between 7 and 73% in dairy herds.[112,116,131,132] Approximately 40% of all cows have been reported to had SCK at least once and > 90% had SCK in the first and second months after calving.[133] In comparison to the prevalence clinical ketosis ranges from 2 to 15% higher prevalence of 10-60% SCK have been reported.[134]

The higher prevalence of ketosis is significantly associated with exotic pure/cross-bred than native/non-descriptive cattle.[95] The higher prevalence of ketosis in exotic pure animals of high genetic potential because these animals are unable to withstand the pressures arising from the high nutritional demands generated by the production of high milk yield, which ultimately results in development of hypoglycemia that can remain as a challenge to the successful dairy farming business, eventually ruining the life of dairy farmers.[95]

Influence of Age on the prevalence of SCK

Significantly ($p < 0.01$) higher prevalence of SCK was recorded in more than 5 years of age (46.78%) in comparison to 4 to 5 years (4.0%) and < 4 years (2.78%) of aged lactating cows. These findings can be compared with the higher (29.31%) prevalence of SCK which has been reported in 8 to 9 years of age and lower (9.52%) in more than 9 years of age group of cows.[135] However, the prevalence of SCK is more important based on parity than age.

Influence of parity on the prevalence of SCK

The significantly ($p < 0.05$) highest prevalence of SCK was recorded at 4th parity (53.33%) and significantly ($p < 0.01$) lower prevalence at the 1st (2.78%) and 2nd (4.00%) parities. A significant positive relationship between the parity and the probability of occurrence of ketosis has been reported. The probability of ketosis and positive cases has been reported to be peaked at the third and 4th lactations.[127] Therefore, hypoglycemia mainly occurred in multiparous cows with early-onset hyperketonemia whereas primiparous cows were at a lower risk for hypoglycemia.[136]

Influence of lactation stages on the prevalence of SCK

The significantly ($p < 0.01$) higher prevalence of SCK was recorded at 1st (77.78% and 2nd (56.67%) weeks of lactation stages in comparison to all recorded higher weeks of lactation stages (0 to 25.71%) in cross-bred lactating cows. The significantly higher prevalence of SCK

in the first two weeks of lactation recorded in this study supports the earlier reports.[100,116,132,137-140] However, comparatively higher rate of 90%[133] and lower rates of 47.2%[141] and 13.19%[107] SCK have been reported in lactating dairy cows elsewhere. The higher prevalence of ketosis has also been reported during 2nd (42.2%) and 3rd (24.8%) weeks post calving and accordingly it has been suggested that the 14 and 17 days after calving are the best time to detect CK and SCK in dairy cows.[107]

Influence of milk yield on the prevalence of SCK

The prevalence of SCK was found higher with increased milk production which was found significantly ($p < 0.01$) higher in cows produced milk more than 15 liter / day (57.14%) in comparison to < 5 liter (2.50%), 5 to 10 liter (13.33%) and 11 to 15 liter (26.09%) milk / day. These observations support the findings of average milk production for cows suffering from CK, SCK and healthy have been reported as 28, 35 and 45 kg per day, respectively.[107]

Influence of BCS on the prevalence of SCK

The BCS prior to calving is an important risk factor for subsequent development of SCK during lactation.[142,143] Statistically ($p > 0.05$) insignificant higher prevalence of SCK was recorded with > 3.5 BCS (30.0%) in comparison to BCS 3.0 - 3.25 (20.83%) in lactating cows. The higher BCS causes increased prevalence of SCK has been reported elsewhere.[114,118,139,141,144] The cows with BCS ≥ 3.75 out of 5.0 at calving have increased risk of ketosis and cows with excessive adipose stores (BCS ≥ 3.75 out of 5) at calving are at greater risk of developing CK.[145] A cow with a BCS ≥ 3.5 has been reported to be 2.5 times more likely to develop ketosis than cows with scores as lower than 3.25 at calving.[144] The change in BCS directly reflects the energy status of dairy cows[146-148] and has negative relationship between BCS to milk production in the middle lactation stage.[149]

Biochemical types of bovine ketosis

The physiological tendency of dairy cows to respond to NEB by catabolism and utilization of their body reserves generates subclinical ketosis with biosynthesis of ketone bodies like acetone, acetoacetate and BHBA.[99] Blood glucose, insulin, non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA) are the most important biochemical constituents associated with the diagnosis of ketosis. Within four days post-partum in dairy cows, demands for glucose, amino acids and fatty acids due to milk production are two to five times higher than pre-partum requirements.[30] Higher demand of energy and nutrients for the synthesis of colostrum and milk combined together with decreased feed intake force the transition cows to undergo negative energy balance (NEB) and micro-nutrient deficiencies. NEB is a result of an imbalance between energy input and output because the energy requirements for milk production and maintenance exceed the available energy from feed intake.[150,151] The NEB (hypoglycemia) stimulates cows to mobilize body fat (an increase in lipolysis) in the form of NEFA.[145,152] The decrease of insulin and increase of NEFA showed the difficulty of dairy cows to cope with the energy demand during transition period.[153] The liver convert 15 to 20% of NEFA in ketone bodies (acetone, acetoacetate and BHBA), in triglycerols (TAGs) and

packaged into very low-density lipoproteins for transport back to the adipose tissue or stored as TAGs.[153-155] Excessive fat accumulation in the liver impairs normal liver function which may lead to hyperketonemia.[128]

It has been explained the theory of Type I and Type II ketosis based on differing in their onset and pathophysiology.[128,156] Type I ketosis was described as spontaneous or underfeeding ketosis, occurs 3 to 6 weeks post-partum when milk secretion is so extensive that the demand for glucose exceeds the capacity for gluconeogenesis in the liver. The plasma levels of glucose (hypoglycemia) and insulin are low and the levels of ketone bodies are high. Type II generally occurs earlier in lactation within the first two week of post-partum as a result of insulin resistance and excessive body fat mobilization prior to or at calving especially in obese cows and is also known as 'fat cow syndrome.' Blood insulin and glucose concentration are high whereas blood ketone concentrations are lower in Type II ketosis than in Type I.[156] Several studies evaluated the effects of HYK on milk yield[36,99,1001,157,158] and reproductive performance[101,104,157] within 2 weeks after parturition. The first 14 to 16 days after calving have been described as the main risk period for HYK.[101,112] But no consistent results have been found for the evaluation of associations between elevated post-partum BHBA and reproductive measures.[159] Moreover, other report suggested that early onset of HYK seems to be primary an adaptation response to a high metabolic load caused by higher milk production in the beginning of lactation without the negative side effects on health.[136]

The comparatively higher serum glucose level in lactating cows (63.02 ± 6.67 mg/dl) than the non-lactating cows (58.47 ± 3.84 mg/dl) has been reported from Bangladesh[71] which not only contradicts the higher serum glucose level reported in dry cows (69.8 ± 3.7 mg/dl) than lactating cows 65.2 ± 1.7 mg/dl).[160] However, hyperglycemia in lactating cows could be explained probably due to stress, late pregnancy, insulin resistance Type II ketosis,[136] methodological error and other reasons which need to be investigated for further explanation.

Interaction of macro-minerals and glucose in metabolic diseases

Blood macro-minerals and glucose levels have very important roles to health, growth, production and reproduction, immune and endocrine system functions. Homeostasis of calcium, phosphorus and magnesium is primarily affected by the very same homeostatic mechanisms and as a result, the changes in their concentrations are in most cases mutually linked.[161] The MF of dairy herds is biochemically characterized by hypocalcemia, frequently accompanied by hypophosphatemia and/or hypoglycemia but magnesium concentration may be normal, reduced or increased. However, in the course of disease the concentration of calcium and phosphorus decreases while the magnesium concentration increases which might be due to imbalance of ratios of these minerals.[161] The decrease in calcium level occurring at calving may results in the increase in magnesium[162] which may be due to increased calcium renal threshold, when in a response to reduced blood calcium level the PTH is excreted resulting in an increase in the magnesium renal threshold.[17,163]

The concurrent prevalence of SCHC and SCK (20.91%) was found significantly ($p < 0.001$) higher in comparison to single occurrence of SCHC (8.64%) and SCK (5.05%) in lactating cross-bred cows. These results are in supports of findings that the hypocalcemic cows had

significantly higher association with ketosis on day 7 and day 35 post-partum.[18,47,164] Hypocalcaemia has been attributed to the occurrence of ketosis in dairy cows[34] and it has also reported that the hypocalcemic cows had 5.5 greater odds of having ketosis than normo-calcemic cows.[45] It appears that the hypocalcaemia has impact on feed intake (reduction of appetite) and resulting NEB that might be the factor in promoting ketosis. Hypocalcaemia had elevated concentrations of NEFA and BHBA as indicators of increased lipid metabolism.[18,37,68] Hypocalcaemia may also deplete adipocyte calcium stores resulting in increased lipolysis.

Therapeutic trials against SCHC and SCK

Treatment of clinical and subclinical MF and SCK are the main aimed at restoring calcium and glucose levels in the blood, respectively. Administration of intravenous glucose and/or propylene glycol orally and calcium salt orally is commonly used for restoring blood glucose and calcium levels in SCK and SCHC affected lactating cows. The blood calcium and glucose levels of both the SCHC and SCK affected cattle treated with the oral calcium salt and propylene glycol increased significantly ($p < 0.001$) at 7 days of post-treatment in comparison to pre-treatment values, respectively. These findings are conformity with earlier reports.[130,165,166] Treatment with four doses of an oral calcium supplement (providing 50 g calcium/dose, given before calving, at calving, 12 hours post-calving and 24-hours post-calving) reduces the risk of clinical and sub-clinical Milk fever in dairy cows by about half.[167,168] However, oral supplementation with calcium chloride and calcium sulfate has been shown to have significant effects on improving calcium status in the period following calving.[165,169] In addition, the administration of the oral calcium bolus to high-producing cows leads to higher milk production.[170]

Cows treated with oral propylene glycol @ 300 ml / cow daily produced 0.23 kg more milk per milking in the first 30 days of lactation, for a total difference of 0.69 kg / cow per day. Propylene glycol treated cows have reported to be 1.5 times more likely to resolve their SCK and 0.54 times less likely to develop clinical ketosis. In addition, oral propylene glycol improves milk yield during early lactation in cows diagnosed with SCK.[130] It is identified that propylene glycol most likely reduces fatty acid mobilization from adipose tissue and by this mechanism can be protective against ketosis and fatty liver syndrome.[171]

CONCLUSIONS

The sub-clinical metabolic disorders are the global issue and their prevalence can be varied in different herds and countries based on breeds, management and other risk factors. This study has recorded some reliable information on the prevalence of SCHC and SCK in lactating dairy crossbred cows in Bangladesh. These findings may be useful to set base-line data on levels of macro-minerals and glucose in dairy cows for different crossbred, ages, parity, BCS and lactation stages. The prevalence of SCHC was found 10 times and SCK six times greater than their clinical forms in lactating dairy cows. The high prevalence of SCHC and SCK should be viewed as a potential health risk to the transition cows. Culling, health problems, complications of parturition, loss of milk production, productive and reproductive problems are the common outcomes of these disorders. Measuring the calcium and glucose status of the fresh cow is the

first step in making intervention or management decisions in order to decrease the long-term consequences of SCHC and SCK in dairy cattle herds. Recently, an electronic BHBA handheld meter (Precision Xtra) has been validated for determination of BHBA from whole blood in dairy cows could be used in dairy herds in Bangladesh. Prevention of SCHC and SCK based on efficient diet, periodic screening for calcium, glucose and ketone bodies detection if different body fluids and BCS evaluation in dairy cattle are the best methods to early detection of these sub-clinical metabolic disorders in transition animals.

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CONFLICTING INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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