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RELATIONSHIP BETWEEN BLOOD METABOLIC PROFILES AND MILK YIELD ASSOCIAED WITH PARITY AND STAGE OF LACTATION IN CROSSBRED DAIRY COWS IN BANGLADESH

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ABSTRACT

Background: Cattle cross-breeding program has been launched throughout Bangladesh but this program has still focused mainly on biological rather than economic evaluation. Currently, 30000 dairy farms with mainly cross-bred cows are in operation throughout the country and this intensive dairy farming system with high milk yielding dairy cows is supposed to be associated with high incidence of subclinical nutritional and metabolic diseases.

Objectives: The objective of this study was to detect the relationship between major blood metabolic profiles and milk yield associated with parity and stage of lactation in cross-bred dairy cows.

Materials and Methods: Blood samples of 220 apparently healthy lactating cross-bred dairy cows (HF × L, n = 190; SH × L, n = 20 and JS × L, n = 10) of 10 dairy herds were collected for metabolic profile test (MPT) in Bangladesh during the period from July to November 2016. The major metabolic profiles which are associated with milk fever (calcium, phosphorus & magnesium) and ketosis (glucose) were considered in this study. These biochemical parameters were estimated to detect the influence of risk factors including herds, breeds, parities and lactation age of cross-bred lactating dairy cows by using the commercial kits in spectrophotometer method.

Results: The evaluation of the biochemical constituents of 220 cross-bred lactating dairy cows revealed that 30% (n = 66) had hypocalcaemia and 20.45% (n = 45) had hypoglycemia. Significantly lower levels of average calcium (7.93 ± 0.36 mg/dl; $p < 0.024$) and glucose (43.44 ± 3.63 mg/dl; $p < 0.0001$) values were recorded in HF × L cross-bred dairy herd of BAUDF, Mymensingh in comparison to other investigated nine dairy herds. The highest milk yield was recorded at 7th parity (16 ± 0.91 liter / day) and 1st week of lactation (17.33 ± 1.09 liter / day) were associated with low mean calcium (8.09 ± 0.85 mg / dl; 6.68 ± 0.13 mg/dl) and glucose (36.45 ± 7.67 mg/dl; 32.31 ± 3.90 mg / dl) levels in comparison to the respective values of different parity and lactation weeks.

Conclusions: A relationship between blood metabolic profiles and milk yield associated with herd, breed, parity and lactation stages was recorded in lactating crossbred dairy cows. The evaluation of blood metabolites at different stages of lactation cycle especially at transition period is required to detect the nutritional and metabolic health for optimum milk production and to achieve maximum reproductive potential of high yielding dairy cattle. The readily available milk samples could be used as a biological fluid to monitor the health and nutritional status of dairy cows by using mid-infrared (MIR) spectroscopy method to prevent sub-clinical metabolic disorders. Therefore, well developed laboratories with necessary equipment, test kits and reagent should be provided to perform MPT in both the blood and milk samples for practical uses.

Keywords: Compton metabolic profile test, Metabolic profile, Lactating cross-bred cows, Calcium, Phosphorus, Magnesium, Glucose, Parity, Lactation stages

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INTRODUCTION

Bangladesh is a tropical country with a total of 24.5 million cattle population which includes 22.05 million (90%) zebu and 2.45 million (10%) cross-bred cattle,[1] 3.53 million lactating and 2.61 million dry cows.[2] Approximately 30,000 dairy farms have established throughout the country[3] and the milk production per lactation is ranged from 300 to 400 liter in indigenous cows and 600 to 800 liter in cross-bred cows.[4] There is a huge gap between the milk production (9.4 MMT; 62.5%) and total requirement (15.04 MMT) with a deficit of 5.64 MMT; 37.5%) in Bangladesh.[5] High demand of fresh milk for consumption with extremely deficit status of milk production at national level in Bangladesh, government and BRAC (NGO) have extended the artificial insemination (AI) services throughout the country and many investors have established dairy farms. Quickly progressing intensification of the production without systemic control of the management and feeding system may frequently result in various types of metabolic disorders. The multi-factorial problems of dairy industry in Bangladesh have been described,[6-8] however, shortage of feeds without pasture lands, and lack of standard formulation of ration for dairy cows especially at dry and transition period have made more complex towards dairy industry development in Bangladesh. Under these circumstances, establishment of dairy farms with high yielding exotic and cross-bred cows without adequate knowledge of nutritional management during transition period under local condition, most of them make susceptible initially to subclinical metabolic disorders, followed by clinical diseases with consequences to several reproductive disorders.[9] The establishment of intensive dairy farming system with cross-bred cattle has introduced the importance of nutritional and metabolic diseases. The Compton metabolic profile testing (CMPT) was designed as a series of specific blood analytical tests used to diagnose metabolic problems in dairy herds.[10-13] Parallel advances in this direction have been made in both human and veterinary fields and progress has been helped by the development of auto-analyzers which can carry out several analyses simultaneously at relatively low costs.[14] Recently, some biochemical parameters have been compared between lactating and non-lactating dairy cows[15] and certain metabolic profiles have been used to monitor the nutritional status of high yielding crossbred cows around parturition[16] in Bangladesh. This paper describes the relationship between the blood metabolic profiles and milk yield associated with different stages of lactation and parity in cross-bred dairy cows.

MATERIALS AND METHODS

Most of the 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.[17] A cross-sectional study was carried out on randomly selected apparently healthy 220 cross-bred (190 Holstein Friesian × Local, 20 Shahiwal × Local and 10 Jersey × Local) lactating cows of Bangladesh Agricultural University Dairy Farm (BAUDF), Mymensingh, adjacent villages of BAU campus and eight different private dairy farms of Gazipur district during the period from July to November 2016 (Table 1). 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.

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. 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).

Data were entered in Microsoft Excel 2010 and transferred to SPSS 17.0 and one sample t-test was used for significance.

RESULTS

The farm and breed-wise blood metabolites of lactating cross-bred dairy cows of 10 investigated dairy herds are presented in Table 1. The overall recorded average blood biochemical values of calcium, inorganic phosphorus, magnesium and glucose were within the normal level but the ranged values varied greatly (Table 1). However, the overall highest mean calcium (10.29 ± 0.40 mg / dl), phosphorus (5.13 ± 0.25 mg / dl) and glucose (64.66 ± 4.17 mg / dl) levels were recorded in SH × L cross-bred dairy cows in comparison to the other two cross-bred of dairy cows (Table 1). Dairy herd-wise (farm-wise) results also showed that the highest blood calcium (10.48 ± 0.66 mg / dl) and glucose (70.99 ± 5.07 mg / dl) levels were recorded in SH × L cross-bred at the Dipti and Sons Farm House (DFH) in comparison to other nine dairy herds, however, significantly lowest mean values of calcium (7.93 ± 0.36 mg / dl; $p < 0.024$) and glucose (43.44 ± 3.63 mg / dl, $p < 0.0001$) were recorded in BAUDF, Mymensingh (Table 1).

Table 2 shows the effects of parity on milk production and blood biochemical constituents in lactating dairy cross-bred cows. It appears that the significantly ($p < 0.0001$) highest mean milk production (16 ± 0.91 liters / day) was recorded at 7th parity with comparatively lowest calcium (8.09 ± 0.85 mg / dl) and glucose (36.45 ± 7.67 mg / dl) levels (Table 2). It also evident that the

Table 1. Herd and breed-wise blood metabolic profile in lactating dairy cross-bed cows (Mean ± SE)

SN	Dairy herd/ Farm	Breed	No. of cows	Calcium (mg / dl)	Phosphorus (mg / dl)	Magnesium (mg / dl)	Glucose (mg / dl)
01	BAUDF	HF × L	28	6.15 - 11.68	2.71 – 6.93	1.65 - 3.98	20.83 - 77.25
				7.93 ± 0.36*	3.83 ± 0.22	3.09 ± 0.15	43.44 ± 3.63**
		SH × L	10	6.32 – 11.68	3.22 - 6.78	1.72 - 3.67	26.04 - 77.45
				10.26 ± 0.54	5.25 ± 0.37	2.19 ± 2.19	59.51 ± 5.82
		JS × L	07	6.13 – 11.36	3.23 - 5.98	1.72 - 3.86	41.66 - 83.67
				9.44 ± 0.71	4.58 ± 0.36	2.39 ± 0.31	61.15 ± 6.18
02	AVBAU	HF × L	16	6.54 - 11.68	3.23 - 6.99	1.71 - 3.89	20.83 - 86.54
				9.76 ± 0.55	4.98 ± 0.34	2.44 ± 0.22	67.90 ± 5.81
		SH × L	03	6.83 - 11.68	3.13 - 6.97	1.79 - 3.24	31.25 - 86.54
				9.96 ± 1.57	5.12 ± 1.11	2.27 ± 0.48	67.01 ± 19.90
		JS × L	01	9.13	4.27	2.54	46.87
03	DFH	HF × L	27	6.13 – 11.68	2.47 - 6.54	1.71 - 4.25	20.83 - 86.54
				9.66 ± 0.39	4.75 ± 0.26	2.52 ± 0.19	58.45 ± 3.94
		SH × L	07	7.13 - 11.68	3.54 - 5.87	1.71 - 3.46	52.08 - 86.54
				10.48 ± 0.66	4.96 ± 0.30	2.12 ± 0.25	70.99 ± 5.07
		JS × L	01	7.24	3.04	3.79	52.08
04	ZDF	HF × L	34	6.13 - 11.76	2.48 - 5.45	1.69 - 4.25	20.83 - 86.54
				9.38 ± 0.30	4.20 ± 0.14	2.36 ± 0.14	58.87 ± 2.88
		JS × L	01	11.36	5.27	1.79	76.56
05	ALDF	HF × L	25	6.09 - 11.98	2.97 - 6.98	1.67 - 4.25	20.83 - 76.56
				9.74 ± 0.32	4.56 ± 0.21	2.30 ± 0.13	55.77 ± 3.64
06	MADF	HF × L	15	6.05 - 9.93	2.89 - 5.89	1.43 - 3.95	20.83 - 69.27
				8.66 ± 0.35	4.21 ± 0.24	2.53 ± 0.22	55.62 ± 4.74
07	AZDF	HF × L	12	6.22 - 11.68	3.23 - 5.27	1.79 - 3.98	20.83 - 69.27
				8.99 ± 0.47	4.11 ± 0.16	2.66 ± 0.18	57.12 ± 5.00
08	MODF	HF × L	11	6.34 - 9.45	2.98 - 4.53	1.65 - 3.98	20.83 - 69.27
				8.11 ± 0.29	3.62 ± 0.16	2.86 ± 0.26	46.21 ± 6.14
09	JDF	HF × L	11	6.94 - 11.36	2.54 - 6.56	1.76 - 3.98	20.83 - 69.27
				9.93 ± 0.44	4.34 ± 0.36	2.67 ± 0.22	52.91 ± 5.71
10	APDF	HF × L	11	6.72 - 11.68	3.12 - 6.34	1.65 - 3.98	20.83 - 69.27
				8.72 ± 0.46	4.08 ± 0.33	2.74 ± 0.22	51.60 ± 4.87
	Overall	HF × L	190	6.05 - 11.98	2.47 - 6.99	1.43 - 4.25	20.83 - 86.54
				9.06 ± 0.13	4.30 ± 0.08	2.59 ± 0.06	55.01 ± 1.42
		SH × L	20	6.32 - 11.68	3.13 - 6.97	1.71 - 3.67	26.04 - 86.54
				10.29 ± 0.04	5.13 ± 0.25	2.18 ± 0.14	64.66 ± 4.17
		JS × L	10	6.13 - 11.36	3.04 - 5.98	1.72 - 3.86	41.66 - 83.87
				9.38 ± 0.58	4.46 ± 0.31	2.40 ± 0.26	60.35 ± 4.85

*Calcium level, t value = 2.705, p < 0.024) **Glucose level, t value 9.105, (p < 0.0001) significant

BAUDF = Bangladesh Agricultural University Dairy Farm
AVBAU = Adjacent Villages of BAU campus
DCH = Dipti and Sons Farm House, Valkartek, Gazipur
ZDF = Zahir Dairy Farm, Dhirashrom, Gazipur
ADF = Alim Dairy Farm, Bolodha, Gazipur
MADF = Masum Dairy Farm, Amuna, Gazipur
AZDF = Azafor Dairy Farm, Pragao, Gazipur
MODF = Mominul Dairy Farm, Aturi, Gazipur
JDF = Jaman Dairy Farm, Dakshin Khan, Gazipur
APDF = Apon Dairy Farm, Valkartek, Gazipur

blood calcium and glucose levels are maintained at standard levels up to 3rd parity and then the levels declined (Table 2).

Table 2. Influence of parity on milk production and blood biochemical constituents in lactating cross-bred dairy cows (Mean ± SE)

Parity No.	No. of cows	Milk yield (liter / day)	Calcium (mg / dl)	Phosphorus (mg / dl)	Magnesium (mg / dl)	Glucose (mg / dl)
1	36	02 – 17	6.76 – 11.68	2.86 – 6.82	1.67 – 3.77	36.45 – 86.54
		10.31 ± 0.87	9.75 ± 0.25	4.76 ± 0.18	2.3 ± 0.10	62.09 ± 2.16
2	75	02 – 18	6.15 – 11.98	2.84 – 6.99	1.56 – 3.98	20.83 – 86.54
		9.13 ± 0.59	9.82 ± 0.21	4.64 ± 0.13	2.31 ± 0.08	65.09 ± 1.65
3	40	02 – 25	6.09 – 11.68	2.47 - 6.98	1.43 – 4.25	20.43 – 86.54
		11.45 ± 0.93	9.05 ± 0.31	4.38 ± 0.21	2.65 ± 0.15	52.34 ± 3.52
4	30	05 – 25	6.13 – 11.68	2.54 – 6.34	1.65 – 4.25	20.83 – 69.27
		13.17 ± 0.68	8.24 ± 0.29	3.82 ± 0.15	2.96 ± 0.14	44.65 ± 3.75
5	20	03 – 18	6.05 – 11.36	3.11 – 5.87	1.65 – 3.98	20.83 – 76.56
		12.6 ± 0.79	8.41 ± 0.36	3.4 ± 0.19	2.79 ± 0.19	48.87 ± 4.41
6	11	07 – 17	6.21 – 10.77	2.84 – 5.27	1.72 – 3.67	26.04 – 76.56
		12.82 ± 1.12	8.09 ± 0.48	4.04 ± 0.25	2.69 ± 0.21	43.08 ± 5.04
7	04	14 – 18	6.36 – 9.93	2.71 – 4.32	2.13 – 3.98	20.83 – 52.08
		16 ± 0.91*	8.09 ± 0.85	3.5 ± 0.42	3.13 ± 0.47	36.45 ± 7.67
8	04	03 – 18	6.2 – 11.68	2.84 – 6.13	1.68 – 3.94	20.83 – 83.67
		9.5 ± 3.61	8.91 ± 1.51	4.37 ± 0.80	2.78 ± 0.62	50.65 ± 17.26
Overall	220	02 – 25	6.05 – 11.98	2.47 – 6.99	1.43 – 4.25	20.83 – 86.54
		11 ± 0.35	9.19 ± 0.12	4.38 ± 0.08	2.55 ± 0.06	56.14 ± 1.32

*t value 7.506 and Significant at ($p < 0.0001$)

The influence of lactation stage on milk production and blood biochemical profiles in lactating dairy cows are presented in Table 3. It appears that the mean highest milk production (17.33 ± 1.09 liters / day) was recorded at the 1st week of lactation and steadily decreases with the lowest 2 liters / day at the 13th weeks of lactation (Table 3). The highest level of milk production (17.33 ± 1.09 liter / day) during the 1st week of lactation caused lowered levels of both the blood calcium (6.68 ± 0.13 mg / dl) and glucose (32.31 ± 3.90 mg / dl) levels in comparison to the highest respective values of calcium (11.68 mg / dl) and glucose (76.56 mg / dl) with lowest milk production 2.00 liter / day during 13th weeks of lactation (Table 3). However, the similar trend of inorganic phosphorus level was recorded in relation to milk production, whereas the magnesium level was found a reverse higher trend (Table 3).

Table 3. Effects of lactation stage on milk production and serum biochemical profile in lactating cross-bred dairy cows (Mean ± SE)

Lactation Stage (wk)	No. of cows	Milk yield (liter / day)	Calcium (mg / dl)	Phosphorus (mg / dl)	Magnesium (mg / dl)	Glucose (mg / dl)
01	18	07 – 25	6.13 – 7.60	2.47 – 4.15	3.11 – 4.25	20.83 – 76.56
		17.33 ± 1.09*	6.68 ± 0.13	2.94 ± 0.10	3.87 ± 0.07	32.31± 3.90**
02	30	08 – 19	6.05 – 11.36	2.54 – 6.12	1.71 – 4.25	20.83 – 76.56
		14.97 ± 0.43	7.54 ± 0.25	3.63 ± 0.15	3.19 ± 0.13	35.36 ± 2.87
03	35	04 – 18	6.2 – 11.68	2.84 – 6.34	1.71 – 3.94	20.83 – 76.56
		12.46 ± 0.60	8.91 ± 0.25	4.2 ± 0.14	2.66 ± 0.12	50.74 ± 2.69
04	40	04 – 16	6.45 – 11.68	2.84 – 6.34	1.43 – 3.58	20.83 – 86.54
		12.3 ± 0.43	9.16 ± 0.22	4.27 ± 0.13	2.43 ± 0.1	56.9 ± 2.20
05	25	05 – 15	7.13 – 11.36	2.94 – 6.56	1.56 – 3.54	26.04 – 69.27
		11.88 ± 0.45	9.11 ± 0.21	11.25 ± 0.16	2.42 ± 0.12	61.27 ± 2.26
06	20	05 – 13	6.22 – 11.68	3.23 – 6.98	1.67 – 3.54	46.87 – 83.25
		10.3 ± 0.56	9.83 ± 0.32	4.61 ± 0.22	2.13 ± 0.12	65.62 ± 2.19
07	10	02 – 16	7.23 – 11.68	2.84 – 6.88	1.65 – 3.87	43.66 – 85.25
		5.7 ± 1.33	10.31 ± 0.61	5.24 ± 0.43	2.21 ± 0.26	71.02 ± 4.68
08	08	02 – 16	9.13 – 11.68	4.16 – 6.93	1.71 – 2.21	41.66 – 44.88
		5.88 ± 2.03	10.94 ± 0.38	5.77 ± 0.41	1.87 ± 0.07	74.37 ± 5.44
09	09	02 – 13	6.39 – 11.68	3.22 – 6.49	1.71 – 3.78	20.83 – 83.25
		4.44 ± 1.11	10.49 ± 0.65	5.29 ± 0.37	2.16 ± 0.25	68.74 ± 6.29
10	08	02 – 17	9.23 – 11.98	4.01 – 6.23	1.69 – 2.54	46.87 – 76.56
		5.38 ± 1.72	11.34 ± 0.31	5.23 ± 0.21	1.88 ± 0.10	70.12 ± 3.56
11	08	02 – 03	10.72 – 11.68	5.27 – 6.99	1.71 – 1.79	69.27 – 86.54
		2.25 ± 0.16	11.56 ± 0.12	5.77 ± 0.27	1.76 ± 0.01	80.23 ± 2.27
12	08	02 – 04	11.36 – 11.68	5.27 – 6.33	1.71 – 1.79	69.27 – 86.54
		2.63 ± 0.26	11.60 ± 0.05	5.65 ± 0.19	1.77 ± 0.01	75.99 ± 1.91
13	01	2.00	11.68	6.54	1.79	76.56
Overall	220	02 – 25	6.05 – 11.98	2.47 – 6.99	1.43 – 4.25	20.83 – 86.54
		11.0 ± 0.35	9.19 ± 0.12	4.38 ± 0.08	2.55 ± 0.06	56.14 ± 1.32

*Ca t value = 3.703, Significant at ($p < 0.003$) Glucose t value = 25.923, Significant at ($p < 0.0001$)

DISCUSSION

There are two main objectives of dairy farming, the first is to get optimum milk production and second is to achieve maximum reproductive potential that is a calf from a dairy cow per year. Cows must calve to produce milk and the lactation cycle is the period between one calving and the next. The lactation cycle is mainly divided into four phases, the early, mid and late lactation, each of about 120 days and the dry period, about 65 days. Dairy cows undergo several hormonal, metabolic and physiological changes but also changes in housing, daily routine and nutrition which often lead to an increased incidence in diseases. The most significant metabolic changes in dairy cows occur in the transition period which is 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. Transition period of a dairy cow is characterized by a metabolic stress that lead to a high incidence of metabolic, infectious and reproductive disorders associated with a severe negative energy balance (NEB). Imbalance under-feeding and poor management of dairy cows can lead to shorter, lower yielding lactations and increase calving interval.

The Compton Metabolic Profile Test (CMPT) was developed by Payne in Compton (England) as a diagnostic tool to study causes of production diseases in dairy cows.[10] The term 'metabolic profile test' (MPT) refers to the analysis of blood biochemical constituents that are useful to evaluate and prevent metabolic and nutritional problems in dairy herds.[11,18] The original objectives of the CMPT were: (a) to secure high yield at minimum costs, (b) to demonstrate the interrelationships of blood constituents with nutrition, productivity and fertility, (c) to monitor metabolic health of the herd, (d) to help diagnosis metabolic problems and production diseases, and (e) to identify metabolically superior cows.[10,19] The original CMPT measured 13 different blood constituents that included packed cell volume (PCV), haemoglobulin (Hb), glucose, blood urea nitrogen (BUN), total protein (TP), albumin (Ab), calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), sodium (Na), copper (Cu) and ferrous (Fe). The MPT is usually performed on blood samples at four time periods relative to calving (dry, puerperium, peak lactation and mid lactation) and measuring selected blood metabolites. However, the research reports show that the transition cow represents the target with the most utility for metabolic profiling tests.[9,20]

When the nutrient deficiency and imbalance is moderate in dairy animals, metabolism compensates by using their body reserves. If the nutrient deficiency and imbalance is severe, the animal exhausts its reserves and may develop metabolic disorders. However, subclinical metabolic disorders (SMD) are more common than clinical disorders in high yielding dairy cows, especially in early lactation. It is more difficult to diagnose SMD and frequently underestimated. Undetected or delayed detection makes them a risk factor for further disorders and more severe disease progressions and leads to reduce chances of recovery and often results in higher treatment costs. The MPT has been used as a practical tool in dairy herds to improve feeding management, detect subclinical metabolic disorders and prevent production diseases.[21-25]

Nutritional imbalances, deficiencies or erratic management of feeding programs for dairy cows can create large numbers and various types of health problems generally categorized as metabolic diseases.[26] The main metabolic disorders (milk fever, abomasal displacement, fatty liver syndrome, ketosis), mammary gland infections (mastitis and udder edema) and reproductive disorders (dystocia, retained placenta and uterine infections) have been reported to be associated with the health and production in dairy cows. The most commonly described metabolic disorders are ketosis, hypocalcaemia and hypomagnesaemia. Sub-clinical metabolic disorders which are not associated with obvious clinical signs are of particular interest due to their relatively high prevalence and significant effects on animal welfare and performance. Identification of sub-clinical disorders can also allow for timely management interventions to prevent the development of clinical disease.

Serum metabolic profile testing is usually used for monitoring the metabolic health and nutritional status of dairy cows. The metabolites evaluated in metabolic profile testing vary, but often include BHBA and fatty acids as indicators of energy balance, albumin and BUN as indicators of protein status, globulins as an indicator of chronic inflammatory disease, calcium and magnesium as indicators of macro-mineral status.[27]

The analysis of biochemical constituents in the form of metabolic profiles are used to diagnose the subclinical nutritional and metabolic diseases in dairy animals but acceptance has been limited as a result of high cost, selection of animals, the type of biochemical tests, the homeostatic mechanism of cows and the difficulty in the interpretation results.[28-30] However, metabolic profiles have more value when used appropriately in the diagnostic process or as part of a specific objective in herd monitoring programs for metabolic diseases.[28] Blood metabolites have been used in herd-level diagnostics of transition cow management for a number of years, mostly focus on identifying opportunities to decrease the incidence of metabolic disorders.[11,31] Recently, the meaning of MPT is extended which includes the measurement of any parameter in animal fluids that is able to reflect a dynamic response to genetic modification and physiological, pathophysiological and developmental stimuli.[32] The MPT is usually used in apparently healthy animals within certain well defined physiological process to evaluate disease risk in contrast to disease diagnosis. When used in association with animal, diet and management assessments, a metabolic profile can be a useful tool for prediction of peri-parturient problems and infertility, to diagnose metabolic diseases, to assess nutritional status,[11,33] stress conditions[34,35] and to determine welfare condition of the animals.[35]

Calcium and glucose are the major blood metabolites of transition and lactating cows associated with milk fever and ketosis respectively in dairy cows. The significantly lowered levels of mean values of serum calcium and glucose were found in HF × L cross-bred cows (Ca 7.93 ± 0.36 mg / dl; glucose 43.44 ± 3.63 mg / dl) in comparison to SH × L (Ca 10.26 ± 0.54 mg / dl ; glucose 59.51 ± 5.82 mg / dl) and JS × L (Ca 9.44 ± 0.71; glucose 61.15 ± 6.18 mg /dl) cross-bred lactating dairy cows in BAUDF in comparison to other nine investigated dairy herds. The low levels of calcium and glucose recorded in HF × L cross-bred in BAUF even in comparison with the same cross-bred cows reared at the adjacent villages of BAU campus have reflected the high milk yielding characteristic of the Holstein-Friesian cross-bred cows maintained at comparatively poor nutritional management system at BAUDF. The overall breed-wise blood metabolic profile in lactating dairy cross-bred cows shows comparatively lower levels of all the investigated biochemical constituents in HF × L dairy cows in comparison to SH × L and JS × L cross-bred with highest level in SH × L cross-bred dairy cows which might be associated with milk production status in these cross-bred dairy cows.

The significantly lowered level of mean serum calcium and glucose was recorded at the highest level of mean milk production (16 ± 0.91 liters / day) at the 7th parity in cross-bred dairy cows. The highest level of milk yield (17.33 ± 1.09 liters / day) recorded during the first week of lactation also associated with significantly lowered levels of both blood calcium and glucose in comparison to the lowest milk yield (2.0 liter / day) with highest level of both the serum calcium and glucose levels at 13th week of lactation. These findings support the earlier reports on the relationship between the levels of blood metabolites with increased milk yield in

lactating dairy cows.[36-38] However, the highest milk production during 7th parity and first week of lactation recorded in this study contradict the earlier reports[39,40] who reported highest milk yield in the 3rd lactation and early stage (55-90 days) of lactation. This difference might be due to feeding of imbalance and varied nutritional ration in different stages of lactation of these selected experimental dairy lactating cows. The serum calcium level was maintained at comparatively higher levels up to three parities (lactation) in comparison to the more than three parities in dairy lactating cross-bred cows. These findings support the earlier report who reported that the older cows had a tendency to lower calcium levels than younger cows.[41]

The NEB in 92% dairy cows at early lactation with decreased serum blood glucose level markedly 3 to 7 days post-partum and gradually increased blood glucose level at 30 and 56 to 60 days post-partum has been reported.[42] In addition, a markedly decreased blood calcium level after parturition in comparison to peak calcium level during 10-20 days before calving has also been reported.[42] The significantly ($p < 0.05$) lowered level of glucose concentration at the beginning of lactation (3.22 mmol/ l) in comparison with the middle lactation (3.69 mmol / l) and the dry period (3.74 mmol / l) have been reported.[36] The lower level of blood glucose recorded in high yielding lactating cows because the blood glucose is partly used for milk lactose synthesis, partly in other metabolic pathways in the mammary gland.[43]

Recently, the serum metabolites especially calcium, magnesium, phosphorus, glucose and total serum protein levels have been compared among the three stages which include stage 1 (1 month before parturition), stage 2 (within 7 days after parturition) and stage 3 (2 months after parturition) in 24 dairy cross-bred cows in Bangladesh.[16] A higher Ca, Mg and P levels have been reported at stage 1 in comparison to stage 2 and 3 whereas glucose level increased after two months of calving.[16] Similarly higher levels of some blood metabolites have also been reported in non-lactating than lactating daily cows in Bangladesh.[15] Moreover, the reference values for blood parameters in Holstein cows have been reported elsewhere.[44]

Evaluation of hypoglycemia

Dairy cows have a massive demand for glucose at the onset of lactation. A poor adaptation to this period leads to an excessive negative energy balance (NEB) with an increased risk for ketosis and impaired animal health and production.[45] Glucose concentrations measured in conjunction with other tests may provide some further insight into underlying mechanisms of hypoglycemia and ketosis.

Sub-clinical ketosis can cause economic losses through decreased milk production and association with peri-parturient diseases and 30% of tested cows suffered from sub-clinical ketosis in all the 2, 4 and 6 weeks of post-partum in Iran.[46] A cut-off point of glucose reported to be 35 mg/dl[47] and 0.26 mmol/l for NEFA concentrations[46] can be used during early lactation for diagnosis of sub-clinical ketosis in dairy cows. Glucose cannot be good criteria for diagnosis of SCK and it does not appear to be useful for monitoring SCK.[46] The onset of lactation in high yielding dairy cows is characterized by a NEB due to a drastic increase in energy requirements for milk yield and a simultaneous depression in dry matter intake around parturition. In case of NEB, the dam will mobilize fat and protein reserves in order to safeguard milk yield. The main problem of SCK is that it all too frequently remains undiagnosed and/or

incorrectly diagnosed, which prevents the implementation of appropriate corrective measures.[48] Significant level of hypoglycemia recorded in lactating cross-bred cows in this study correlated with the published reports.[29,30] Hypoglycemia (< 2.5 mmol /l) was more pronounced at early lactation (66.66%) in comparison to late lactation (33.33%) period and these metabolic characteristics were correlated with day in milk (DIM) and energy balance.[30] Although concentration of NEFA is more sensitive to energy balance changes in transition cows, concentrations of BHBA is most commonly used.[49] The reasons are of the high cost of reagents for NEFA determination and instability of NEFA in frozen serum samples.

Evaluation of hypocalcemia

Hypocalcemia is one of the most common peri-parturient abnormalities afflicting the high yielding dairy cows. It usually occurs as clinical and sub-clinical forms of milk fever. The sub-clinical hypocalcemia (SCH) may be defined as low blood calcium concentrations that are associated with peri-parturient health disorders, poorer production and reproduction outcomes or both without associated signs of postpartum paresis.[50] The overall 1.0% prevalence of hypocalcemia during first parity, 4.0% during second parity, 7.0% during third parity and 10% during 4th parity have been reported in Holstein dairy cows in USA.[51]

Dairy cows suffer blood calcium losses at transition period and early lactation and might be affected by hypocalcemia in its clinical <1.5 mmol/l,[52] and sub-clinical ≤ 2.14 mmol/l,[52] ≤ 8.0 mg /dl (2.0 mmol/l)[51,53] forms. However, some authors suggested that the cut-off point of blood calcium should be raised to 8.5 mg/dl (2.1mmol/l) because cows below this concentration were more likely to develop metritis or metabolic disorders.[54] Research suggests that the subclinical hypocalcemia may be directly associated with other metabolic disorders and may be the primary or secondary cause of decrease performance.

Examination of metabolic profiles by using blood samples has some advantages but its collection is somewhat difficult and costly. Recently, the readily available milk samples have been widely used to monitor the health and nutritional status of dairy cows.[55,56] It has been reported that the milk-fat-to-protein ratio of greater than 1.4,[57] and 2.0[58] in early lactation in dairy cows as indicators of NEB and sub-clinical ketosis, respectively. The changes in milk fat-to-lactose and milk fat-to protein ratios in early lactation have been suggested as early indicators of disease.[59] Milk urea nitrogen is routinely used to monitor and optimize protein nutrition.[55,56,60] More recently, mid-infrared (MIR) spectroscopy of milk has shown promise for assessing more complex animal health traits.[27,61-63] It is established that MIR spectral data can be used to screen for subclinical ketosis through identification of ketone bodies in milk[64] and to estimate energy balance in early lactation.[65]

CONCLUSIONS

The metabolic profile refers to the analysis of blood biochemical constituents that are useful for the assessment and prevention of metabolic and nutritional disorders in dairy animals. The significant relationships between blood metabolite concentrations and milk yield at different parity and lactation stages were recorded in this study. The blood metabolite concentrations, body condition score (BCS), feeding (nutrition) and milk production are closely related. Therefore, the cut off values of the blood metabolites could assess the milk production and

metabolic health of dairy cows. More research would be needed to compare the metabolic profiles between blood and milk (by using mid-infrared (MIR) spectroscopy) samples for the evaluation of the transition period, different parities and lactation stages for the early diagnosis of nutritional status and metabolic disorders in dairy animals.

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CONFLICTING INTEREST

The authors declare that there is no conflict of interest.

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