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Correlation between Milk Urea Nitrogen (MUN) Levels with Metabolizable Energy (ME) and Crude Protein (CP) Provided with Ratio of Roughage Concentrate by Supplied Feed of Dairy Cattle and its Effect on Milk Yield and Milk Composition

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ABSTRACT

Background: Proportion of roughage and concentrate supply to dairy animal is a major challenge in commercial dairying of Bangladesh that directly correlated with quality and quantity of milk. Several studies revealed that, level of milk urea nitrogen (MUN) is an indication of proper feed supply of the animals. Therefore, the study was the attempt to find, the relationship between the level of MUN with metabolic energy (ME) and crude protein (CP) by supplied ratio of roughage, concentrate and effect of altered level MUN with milk quality and quantity in commercial dairy farms of Chittagong, Bangladesh.

Methods: For the study, feed and milk samples were taken from randomly selected three different farm categories for the period of July 2014 to June 2015 monthly. The herd average milk production was taken from the recorded data. The feed and milk composition were analyzed in the dairy lab of Chittagong Veterinary and Animal Sciences University (CVASU). MUN was determined by spectrophotometric method in the Poultry Research and Tanning Laboratory (PRTC) of CVASU, Chittagong, Bangladesh. Observed data was analyzed by using Pearson's correlation methods to find out the correlation among MUN level, supplied source of ME and CP and milk production and composition.

Result: The results suggested that there was significant level difference of MUN ($P<0.05$) irrespective of seasons and farm categories with the sources of ME and CP of supplied feed. Milk yield relate significantly ($P<0.05$) with MUN level when there was lowest supply of roughage and maximum M.E comes from concentrate feed irrespective of farm categories. So, determination of MUN level will be helpful to commercial dairy farmers when the issues related to milk quality and quantity.

Key words: Farm, Genotype, Lactating cow, Milk urea nitrogen, Performance, Season.

INTRODUCTION

The cattle population of Bangladesh is 248.56 million and the milking cows produced 140.68 million metric tons (MMT) per year against the requirement of 158.50 MMT per year of the peoples (DLS, 2023). Chittagong is the second largest city of Bangladesh and total number of dairy farms is 1136 those are fulfilling only 15% demand of milk of the people of Chittagong (Tanni *et al.,* 2021). Whereas Bangladesh has around 15,00,000 cow farms across the country (Abdul Jabbar Sikder 2021). There is a wide range of factors shaping milk yield, genetic (racial, individual, inheritance) and environmental (*e.g*. nutrition, living conditions, climatic conditions) are the two most important factors. Genetic factors are assigned 30% of the influence and the remaining 70% is assigned to non-genetic factors. Moreover, these factors are closely related to and influence each other (Wilkanowska, 2017). An ill-balanced ration that is not adjusted to the current physiological state and season can have a significant impact on milk production (Tumanowicz, 2018). Cow's milk composition depends on plane of nutrition of the cow. Dairy cows produce less amount of milk than the expected is a common complaint of commercial dairy farmers in Chittagong, Bangladesh. In Bangladesh, buffalo has never been addressed and remained neglected species despite their important role in the national economy. Hamid *et al*. (2016). To find out the causes, it was seen that commercial dairy farms of Chittagong, Bangladesh suffering from great problem for feeding dairy cows resulting from the incorrect feeding management (Tanni *et al.,* 2021). Green grass is not

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available all-round the year in this locality. Supply of higher amount concentrate instead of forage/ roughage is a common feature here. This practice is responsible for increasing the feed cost and leads to unbalancing the energy and protein ratio in the supplied diet. MUN is an indication of roughage concentrate ratio of supplied feed to animal (Ranaweera *et al*., 2017). Basset *et al*. (2010) stated that MUN can be a useful material, when observing at a dairy ration and high MUN levels could result from the incorrect feeding management. Recent research suggests that the milk urea nitrogen content depends mainly on the nitrogen/energy ratio in the diet (Munyaneza *et al*., 2017).
The highest MUN levels indicate an imbalance between the degradable and non-degradable protein associated with an energy deficiency in the diet (Francesco *et al*., 2015). Without a formulated feeding standard, commercial dairy farmers in Chittagong are providing rations based on fodder availability and are mainly dependent on concentrates. The concentrate feed is their main source of energy supply especially when cow's in peak lactation. There is no previous study found in Chittagong, Bangladesh, regarding the problem with providing the proper amount/imbalance supply roughage concentrate to the dairy animal, which directly related to the quality and quantity of milk. As there is no standard way to find out the effect of unbalanced roughage concentrate supply on milk quality and quantity, determination of level of milk urea nitrogen (MUN) may be an indication regarding this. Therefore, the current study was conducted with the objectives to (i) find out the correlation between milk urea nitrogen (MUN) and its relation to metabolizable energy (ME) and crude protein(CP) by the supplied ratio of roughage concentrate to the animals; and (ii) ascertain the correlation between changed level of MUN and milk quality and quantity of different seasons of a year under different categories of commercial dairy farms.

MATERIALS AND METHODS

A total of 1178 cows from 28 farms [7 for category A (50+ dairy cows), 8 from category B (31 to 50 dairy cows, and 13 for category C (11 to 30 dairy cows)] was randomly selected from different areas of Chittagong, Bangladesh. A questionnaire was developed for data collection in relation to feeding. The milk production data was collected from the record book kept by the dairy farms from 2014 to 2015 by direct visiting the farm. The year was divided into three seasons, summer (March to June), the monsoon (July to September) and dry/winter (October to February) for conducting the study. The body weight of cows was determined by using Shaeffer's formula as described by Machila *et al*.(2008).The required metabolizable energy (ME) and crude protein (CP) for cows was calculated according to the method of AOAC(1980) Feeding systems of this study was total mixed rations (TMR) type provided in confinement system of rearing. Farmers provided fresh chopped mixed grasses: German grass (*Echinochloapolystachya*), Helencha (*Enhydrafluctuans*) and Water hyacinth (*Eichhornacramipes*) in addition to rice straw. During monsoon they cultivated plenty of German grasses, however, in the dry season they providedHelencha and Water hyacinth as green roughage and a more amount of rice straw. Along with green forages, they provided concentrate mixture consists of broken maize, broken rice, rice polish, wheat bran, mustard oil cake, till oil cake, lentil husk, mung (*Vignaradiata*)husk, common salt and vitamin mineral premix. A few numbers of farmers rarely supplied the urea treated straw to their cows.

Feed and milk samples were collected monthly from selected farms. Feed samples were analyzed for moisture, CP, crude fiber, nitrogen free extract, ether extract in Poultry Research and Training Centre Laboratory of Chattogram Veterinary and Animal Sciences University (CVASU) as per AOAC (1994). Milk samples were stored at 4°C until further analysis for fat, solids- non-fat, proteinby using Lactostar (model no. 3510, Funke Gerber, Germany). For each group, separate TMR samples were collected the day before the milk sampling and stored at -20°C until further analysis for proximate composition.

The level of MUN in this trial was determined by infrared spectrophotometry (Fos 120 Milko Scan, Fos Electric, Hillered. Denmark) for the analysis of ammonia and urea in biological fluids as reported by Weatherburn (1967) and Chaney and Marbach (1962), respectively, urea and modified indophenol with enzyme modification. method. For changes to this method, Milk was replaced with serum or plasma, incubation temperature and time and enzyme concentration were varied.

Reagents, solutions

Reagent 1 (A_1): 50 g phenol and 0.25 g sodium nitroprusside were dissolved with deionized water in a volumetric flask and diluted to 1000 ml.
Reagent 2 (A_2): 25 g sodium hydroxide and 40 ml sodium hypochlorite (5.25%) were put into a volumetric flask and diluted to 1000 ml with deionized water.
Enzyme solution: from the lyophilized urease enzyme preserved at+4°C (5 U/mg), 0.6 g was weighted and diluted to 100 ml (30 U/ml) with deionized water in volumetric flask.
Standard solution: From the dried urea in the drying chamber, 0.8576 g was taken into volumetric flask, dissolved with deionized water and diluted to 1000 ml (40 mg/dl). By taking varying amounts from the standard solution, solutions with varying nitrogen content were prepared.

Preparation of the standard curve

100 μl urease solutions was put into spectro cuvettes, standard solutions containing 10 μl increasing concentrations of urea were added, the mixture was shaken and kept 10 minutes at 40°C temperature. Later, the cuvettes were sealed and turned upside-down following the addition of 1 ml of A1 and A2. The preparation was kept 3 minutes at 55°C and the absorbance were read in spectrophotometer (Schimadzu UV 1240) against the blind sample that contained deionized water on 625 nm.

Preparation and analysis of milk

100 µl urease solution was put into spectro cuvettes, supplemented with 10 µl well mixed milk sample heated at 40°C, mixed and kept for 10 minutes at 40°C. 1 ml A_1 and 1 ml A_2 were added, then the cuvettes were sealed and turned upside-down. After keeping the preparations 3 minutes at 55°C, absorbance was read in the same way.

Data analysis

The observed data were calculated on the basis of the regression equation obtained through the standard solutions using the General Linear Model (GLM) of SAS (SAS, 2009).

RESULTS AND DISCUSSION

Variation of MUN level in different seasons under farm categories

Table 1, shows that the farm average MUN levels was the highest in winter (A: 25.36±5.566, B: 31.03±3.7378 and C: 31.87±4.008 mg/dl) followed by summer and monsoon season irrespective of the farm categories. The average MUN level differs significantly (P<0.05) in different seasons and under different farm categories (Table 1). Table 1, also shows that MUN level was comparatively higher in farms under category C and B (31.03±3.7378 and 31.87±4.008 mg/dl when compared to farms under category A (25.36±5.566).The lowest MUN level was always observed in farms under category A in all three seasons compared to category B and C. The findings of this current study were similar with the results of (Doska *et al.*, 2012) and (Kˇrížová *et al*. 2013) who stated that the concentration of nitrogen fractions decreased in summer, in winter it was higher. The increased level of urea in milk may be caused by excess of protein in feed, too small an amount of easily fermentable carbohydrates in rumen (Beltran *et al*., 2019) stated that if the dietary intake is low in energy or high in protein to energy ratio, rumen bacteria will have reduced efficiency in utilizing free ammonia to synthesize protein, which can result in increased MUN. These finding was similar to the results of this current study.

Correlations of MUN with ME and CP levels of supplied diet and MUN under different seasons and farm categories

Table 1 indicated that the highest farms average ME and CP was supplied in dry/winter and lowest in the rainy season irrespective of farm categories. The level of supplied and required ME and CP in different seasons was significantly (p<0.05) differed among farm categories and seasons. From the Table 1, is also an indication of higher supplied ME and CP to their cows against requirements irrespective of farm categories and seasons with few exceptions. The supply of higher ME and CP against requirements was the most common feature in the farms under category B and C when compared to A (Tanni *et al.,* 2021). The positive correlation was observed between

Table 1: Relationship between MUN and ME and CP of supplied feeds and quality and quantity of milk with this changed level of MUN of different seasons under different categories of farm.

Types of farm	Av. body weight (kg)	Season	Required ME (MJ/day/cow)	Supplied ME (MJ/day/cow)	Required CP (g/day/cow)	Supplied CP (g/day/cow)	MUN (mg/dl)	Milk fat (%)	Milk protein (%)	Lactose (%)	Milk ash (%)	Milk yield (liter/farm/day)
A	363.3	Summer	100.76±1.025	107.71±13.659	1232.196± 220.997	1208.57±174.649	20.82±3.433	3.9±0.233	3.32±0.115	4.48±0.005	0.72±0.0395	892±337.54
		Rainy	101.30±1.359	102.14±15.873	1220.88± 215.717	1245.57±162.990	18.81±3.393	3.8±0.405	3.38±0.117	4.42±0.096	0.73±0.056	911.75±320.28
		Dry/winter	103.68±0.989	108.43±14.861	1292.47±153.370	1301.14±185.989	25.36± 5.566	4.05±0.267	3.47±0.102	4.44±0.084	0.74±0.0227	887.65±343.35
		Average	101.91±1.124	106.093±14.798	1248.51±196.695	1251.76±174.546	21.673±4.131	3.92± 0.302	3.39±0.111	4.45±0.062	0.73±0.039	867.24±333.73
B	356.63	Summer	91.91±1.025	113.5±14.861	1101.00±101.504	1167.00±273.002	23.38±1.768	3.72±0.196	3.35±0.125	4.47±0.118	0.68±0.036	350±25.42
		Rainy	95.82±2.2035	105.13±14.157	1251.98±146.234	1338.25±277.441	21.78±3.835	3.76±0.278	3.39±0.099	4.5±0.157	0.71±0.030	367.56±31.11
		Dry/winter	91.91±1.025	102.88±11.594	1156.63±136.523	1436.75±297.166	31.03±3.7378	4.06±0.276	3.48±0.089	4.45±0.087	0.69±0.026	330.55±31.40
		Average	93.21±1.418	107.17±13.537	1169.87±174.547	1314±282.536	25.398±3.114	3.847±0.25	3.407±0.104	4.497±0.121	0.69±0.031	332.68±
C	315.83	Summer	88.16±2.614	107.08±9.124	1099.00± 174.060	1105.54±230.702	26.35±3.960	3.8±0.415	3.35±0.118	4.5±0.072	0.73±0.0325	151.28±29.84
		Rainy	96.75±2.779	97.46±8.685	1220.73±101.647	1183.15±232.740	20.69±3.960	3.87±0.388	3.26±0.138	4.52±0.097	0.77±0.0478	174.71±32.98
		Dry/winter	81.40±2	83.77±10.553	990.019±119.001	1283.39±284.008	31.87±4.008	4.05±0.415	3.37±0.088	4.48±0.0819	0.72±0.027	127±44.89
		Average	88.77±2.464	96.10±9.454	1103.25±131.57	1190.69±131.569	24.59±3.976	3.91±0.406	3.33±0.344	4.5±0.084	0.74±0.036	169.52±
Level of significance			**	**	**	**	NS	NS	NS	NS	**	**

Legends: MJ= Mega Joule, MUN= Milk urea nitrogen, CF= Crude fat, CP: Crude protein, g= gram, %= Percentage.

**Significant at (P<0.05).

supplied ME and CP with MUN in summer and dry season and relationship was significant (P<0.01) in dry season whereas negative correlation was observed between ME and CP with MUN in the rainy season (Table 2). This is an indication that excess supply of concentrate than roughage or source of ME or CP is responsible to increase the level of MUN. However, the correlation of supply ME and CP by feed is closely related to increase and decrease of milk yield and was significant (P<0.05) in the dry season when concentrate was the sole source of energy (Table 2). The reduction may be due to reduced DMI in energy supply to the animal and lead to the reduction in milk yield (Sefa Salo 2018). According to (Reed *et.al.* 2017) the excessive dietary CP had a negative impact in the energy metabolism and this could have impacted the milk production performance was an agreement to our results.

Correlations with MUN with different milk constituents under farm categories in different seasons

Most of the milk constituents showed a non-significant correlation with MUN. Among the milk components fat percentage was negatively correlated with MUN with the summer season (-0.03) and monsoon (-0.06). In winter season when there was scarcity of green grasses positive and significant correlation (0.23) shown between MUN and milk fat. Milk protein shows a positive but non-significant correlation between MUN in summer (0.10) and monsoon (0.23) and negative correlation observed in winter (-0.04). Milk components, lactose was negatively correlated with MUN in summer (-0.21) and winter season (-0.10) but positive in monsoon (0.250).Among the milk components, ash was negatively correlated with MUN in summer (0.05) and positive in monsoon (-0.39) and winter (-0.19). However, the correlation was not significant (P<0.05). Our findings were similar to the findings of (Hadi *et al*., 2021).

Correlations between MUN with milk production in different seasons under different farm categories

There was always a negative correlation between milk yield with MUN in different seasons irrespective of farm categories, The highest and significant (-0.63*) (P<005) negative correlation was observed in the winter /dry season, when the fodder supply was comparatively lowest and concentrate supply was highest followed by non-significant negative correlation in summer (-0.33). and lowest negative non-significant correlation was observed in the monsoon/

Table 2: Correlation regression between MUN with ME, CP, milk quality and quantity in different seasons.

	MUN	ME	CP	Fat	Protein	Lactose	Ash	Milk Yield
Summer season								
MUN	1							
ME	0.42^{NS}	1						
CP	0.32^{NS}	0.98**	1					
Fat	-0.03^{NS}	0.10^{NS}	0.13^{NS}	1				
Protein	0.10^{NS}	-0.08^{NS}	-0.09^{NS}	-0.001^{NS}	1			
Lactose	-0.21^{NS}	-0.09^{NS}	-0.07^{NS}	-0.02^{NS}	0.04^{NS}	1		
Ash	0.05^{NS}	-0.24^{NS}	-0.16^{NS}	0.12^{NS}	0.29^{NS}	-0.01*	1	
Milk yield	-0.33^{NS}	-0.83**	-0.86**	0.04^{NS}	0.06^{NS}	-0.02^{NS}	-0.04^{NS}	1
Monsoon								
MUN	1							
ME	-0.23^{NS}	1						
CP	-0.22^{NS}	0.99**	1					
Fat	-0.06^{NS}	-0.08^{NS}	-0.099^{NS}	1				
Protein	0.23^{NS}	0.39*	0.43^{NS}	-0.12^{NS}	1			
Lactose	0.25^{NS}	-0.33^{NS}	-0.323^{NS}	-0.08^{NS}	-0.22^{NS}	1		
Ash	-0.39^{NS}	-0.35^{NS}	-0.41^{NS}	0.14^{NS}	-0.28^{NS}	-0.09^{NS}	1	
Milk yield	-0.24^{NS}	0.84**	0.80**	-0.05^{NS}	0.30^{NS}	-0.07^{NS}	-0.16^{NS}	1
Dry/Winter season								
MUN	1							
ME	0.53*	1						
CP	0.57*	0.90**	1					
Fat	0.23^{NS}	0.001^{NS}	0.06^{NS}	1				
Protein	-0.04^{NS}	0.40^{NS}	0.56^{NS}	0.09^{NS}	1			
Lactose	-0.10^{NS}	-0.22^{NS}	-0.22^{NS}	-0.19^{NS}	-0.06^{NS}	1		
Ash	-0.19^{NS}	0.32^{NS}	0.25^{NS}	0.16^{NS}	0.10^{NS}	-0.11^{NS}	1	
Milk yield	-0.63*	-0.85**	-0.87**	-0.057^{NS}	0.35^{NS}	0.01^{NS}	0.41^{NS}	1

*= Significant at (P<0.001), **Significant at (P<0.05); NS= Non significant.

rainy season (-0.24) when the fodder supply was comparatively highest and concentrate supply was lowest.

It's also an indication that high amount of nitrogen substances in feed increase the formation of ammonia in the rumen. In its transformation to urea, liver uses up more energy which is also required for the synthesis of milk in the udder. This results in less milk and more MUN in milk than in a situation when the amount of nitrogen substances in feed (ration) on an optimum. (Aguilar *et. al*. 2012) stated that excessive protein consumption can be caused by greater than expected DMI, deviations in forage protein, an improperly balanced ration and improper mixing of the ration. Inadequate fermentable or total energy in the diet could fail to support the energy needs of the cow, causing lower than expected milk production was an agreement to our results. This might be the cause of negative correlation between milk yield and MUN in all seasons.

CONCLUSION

Most of the farms irrespective of categories of Chittagong don't supply enough roughage due to shortage of land in all seasons. As a result, most of the farms depend on large quantities of highly expansive concentrate feeds for balancing the ration, which was identified from their MUN level. This practice increased the cost of milk production in this area due to supplying with excess ME and CP to the dairy ration which also affecting the quality of milk. Feed cost of the herd can be minimized by monitoring MUN level. The optimum range of MUN concentration should be 12 mg/dl to 17 mg/dl whereas in this study this level was ranging from 18.6 mg/dl to 31.03 mg/dl in dairy herd of Chittagong, Bangladesh. It also varied from farms under different categories and in different seasons. Balance energy, protein intake of dairy feeding is the major management problems in the case of dairy operation in the commercial dairy farms. Under this circumstance when dairy farmers do not have information on the ingredients/ composition and feed formulation and cattle intake, taking a sample of MUN to monitor the protein and energy intake balances, may provide alternative information on farmers. MUN is very important to know the nutritional status of animals. MUN concentration can assist to make ration economic. For routine monitoring the level of MUN in the dairy herd and balancing ration can be minimized the feed cost and increase the farm profit.

Conflict of Interest

All author declare that they have no conflict of interest.

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