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Effects of protein concentrate supplementation-based diet on growth and nutritional status in dairy heifers

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Abstract

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This experiment was conducted to study the effect of protein rich ingredients supplementation into concentrate diet on body growth and some biochemical blood parameters of crossbred heifers. Six heifers aged above two years were divided into two equal groups: (a) control (farm feeding practices) and (b) protein supplemented. Soybean meal and mustard oil cake were added in concentrate mixture as protein supplement in supplementary group. The heifers of both dietary groups had access to required amount of para and german grass as basal roughage. Growth measurement parameters were studied at fortnightly; blood collection and its analysis for various blood metabolites were assayed at thirty days intervals. It was noticed that heifer dry matter intake was significantly ($P=0.001$) affected by the diet and found higher in supplemented group than control group. About 16% higher ($P=0.149$) body weight gain was found in supplemented group compared to control group. Protein supplemented heifer showed 0.12, 0.74 and 0.26 cm higher gains for wither height ($P=0.01$), body length ($P=0.002$) and heart girth ($P=0.016$), respectively. The blood biochemical profile revealed that serum urea and blood urea nitrogen concentrations were higher ($P=0.015$) in protein supplemented group compared to that of the control group. In conclusion, supplementation of protein rich ingredients in heifer diets might increase the growth measurements and blood serum protein status which would be more advantageous for the development of dairy herd replacement stock.

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Introduction

Heifer rearing is a major portion of dairy herd turnover and farmers want to rear heifers for entering into the herd at optimum weight and age to acquire the best production and fertility achievement (Tegasc, 2014). Grazing forage or green grass may be the source of heifer feed but it is not well balanced until supply of concentrate mixture and minerals along these. Concentrate mixture should have high density of energy and nitrogen which will be the best useful way to gain maximum output from heifers (Wanapat, 1999). Addition of nutrient rich ingredients in the concentrate mixture depends on types of basal diet (Doyle *et al.*, 2008). Soybean meal (*Glycine max*) and mustard oil cake (*Brassica napus*) are well known protein source ingredients for ruminant feeding. Attributes of soybean meal makes it more acceptable to the animal as feed (Baker, 2000). Again, crude protein content of mustard oil cake (MOC) varies from 33-40%; of which 80-83% is true protein with appreciable proportion of albumin, glutelin and globulin. Also, MOC is rich in lysine and sulphur containing amino acids (Klockeman, 1997). It has been scientifically proved that addition of fish meal in the concentrate mixture improve the reproductive performance of heifer (Alam *et al.*, 2012). Monitoring of nutritional status of the animal are vital because these are the indicators of health status (Ate *et al.*, 2009); and

used for measuring energy and protein status. These indicators also used as an adjunct to other measures such as body weight and body condition score that reflect the integrated effects of nutrition over time (Hammond, 1997).

Again, farmers of tropical countries provide large amount of lower grade roughage as basal feed to their livestock (Seresinhe and Pathirana, 2008) but heifer don't get enough nutrients through this type of roughage to improve her reproduction (Peripolli *et al.*, 2016). Along with concentrate mixture and non-leguminous fodder in diet partial legume hay could be a good source of nitrogen for improving reproductive performance (Habib *et al.*, 2018). It has been assumed that supply of protein rich ingredients to the heifer of tropical areas may have important potential because it improves forage digestibility and energy utilization through increasing the amino acid supply for the animal (Gilbery *et al.*, 2006). Very recently Prima *et al.* (2018) carried out a research work on pre- and post-partum concentrate supplementation to crossbred cow and found significant changes in milk yield, milk protein and post-partum heat period. In our countries, heifers supplied with small amount of or almost no concentrate feed and if supplied but not nutritionally balanced. Thus, animals are being physiologically challenged which interfere with

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reproductive functions ultimately affects the profitability of the dairy farm. Hence, supplementation of protein rich ingredients in the diet of heifer is essential along with the supply of adequate amount of green grass. Owing to the aforesaid circumstances, it is essential to monitor the changes of dry matter intake, growth performances as well as blood serum nutrients of heifers in response to protein supplementation. The objective of this research was to observe the effect of feeding protein rich ingredients along with concentrate mixture on the growth and nutritional status of the dairy heifers.

Materials and Methods

Animal management and diets

Animal procedures were conducted at the Dairy Farm, Department of Dairy Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh. Six (06) crossbred heifers (50% Sahiwal, ShX; and 50% Holstein Friesian, HFX) whose aged above 2 years were divided into two groups like control and supplemented; and the average live weight of each group was 150±5 kg. The study was conducted for a period of 100 days of which first 10 days were for diet adjustment. Each heifer was kept in a face-out stanchion barn with individual manger and water trough. The basal diet consists of para grass (*Brachiaria mutica*) and german grass (*Echinochloa crus-galli*) which were supplied at 50:50 (18 kg/hd/d). Mixed concentrate (1.9kg/hd/d) was offered to the heifers twice daily. Concentrate ingredients in control diet included rice polish, wheat bran, DCP and common salt. In addition to these, mustard oil cake and soybean meal were supplied to the supplemented group. Dry matter was offered @ 3.5% of the live weight. Concentrate was fed prior to the green grass at 08:00 am and at 13:00 pm. Proximate composition of each ingredients were determined as described by AOAC (2000). Feed ingredients and chemical constituents of both control and protein supplemented diets are given in Table 1.

Estimation of DMI and growth measurement

DM intake (kg) calculated from supplied feed and daily leftover. Body weight, body length, heart-girth and wither height of the experimental heifers were measured through weighing balance, measuring tape and wooden scale, respectively. Data for these variables were recorded on each heifer at fifteen days interval.

Collection of blood and serum analysis

About 10 mL blood was collected at 30 days intervals from jugular vein of each experimental heifer and kept in a tube (without anticoagulant). Then collected blood was allowed to clot at room temperature for a period of time (40-45 minutes). Thereafter, centrifugation was done using centrifuge (EBA 21 Hettich Zentrifugen, Germany) at 3000 rpm for 15 min. Following these, serum was harvested and kept in kitchen freezer until assayed.

Table 1. Ingredient and nutrient composition of the diet

	Dietary groups	
	Control	Supplemented
Ingredient composition (g kg ⁻¹ DM)		
Green grass		
Para grass	280.00	280.00
German grass	380.00	380.00
Concentrate mixture		
Rice polish	180.00	150.00
Wheat bran	152.00	100.00
Mustard oil cake	-	50.00
Soybean meal	-	32.00
DCP	4.00	4.00
Common salt	4.00	4.00
Chemical composition (g kg ⁻¹ DM)		
Dry matter	435.20	432.60
Crude protein	102.57	120.45
Crude fibre	229.50	228.90
Ether extract	45.28	47.50
Nitrogen-free-extract	400.70	487.60
Ash	99.86	98.66
ME (MJ kg ⁻¹ DM)	9.86	10.18

†Metabolizable energy (ME) values estimated according to the equation of Kearn (1982).

ME (MJ/kg DM) = [- 0.45 + (0.04453×% TDN)] × 4.184

TDN is estimated according to the following equations:

TDN for roughages (% of DM) = -17.2649+1.2120 (%CP) +0.8352 (%NFE) +2.4637 (%EE) +0.4475 (%CF)

TDN for concentrate (% of DM) = 40.3227+0.5398 (%CP) +0.4448 (%NFE) +1.4218 (%EE)-0.7007 (%CF)

Glucose level in blood serum was measured through glucose oxidase and peroxidase method using enzymatic quantitative colorimetric kit (Vitro Scient, Egypt). Quantitative colorimetric kits albumin (Vivia-Biotech., Spain) and urea-LQ (Chemelex, Spain) were used to measure blood serum albumin and urea concentrations, respectively. The factor 2.14 was used to obtain the concentration of blood urea. URIT-810 chemistry analyser (URIT Medical Electronic Company Limited, China) was used as at 550, 630 and 340 nm wavelengths to measure the serum glucose, albumin and urea, respectively.

Statistical analysis

Repeated Measures Analysis of Variance (RMANOVA) in Completely Randomized Design was executed to scrutinize the impacts of protein rich ingredients feeding to the dairy heifers on DMI, growth performance and blood serum nutrients. In this analysis, experimental heifer's initial body weight was not thought as a covariate because of non-significant influence on the dependent variables. Again, period of time was treated in the within-subjects factor to measure the parameters recurrently on the experimental units; whereas, dietary groups (independent variable) were subjected in between-subjects factor.

Results and Discussion

Dry matter intake and growth measurements

Relevant data on between subjects and within subject effects are presented in Table 2 and 3, respectively. About 23% higher ($P=0.001$) DMI was found in protein supplemented group than that of the control group. It has been noticeable that time period has significant ($P=0.01$) impact on DMI whereas, interaction effect of both time and diet also significantly ($P=0.01$) influences on DMI of heifer. This trend of raising DMI are similar to Khandaker *et al.* (2012) who explained that addition of mustard oil cake makes the rice straw softer by disrupting the plant cell wall components. Similarly, Lanyi (1988) revealed that DMI of heifers increased by consumption of protein rich ingredients in diet. Again, DMI may increase significantly by the animal when protein levels in diet are raised from below the requirement to the optimum levels.

Table 2. Dietary effects on DM intake, growth performance and nutritional status of heifers

Parameters	Dietary groups		SEM (±)	P-value
	Control (n=3)	Supplemente d (n=3)		
DM intake and growth performance				
DMI (kg^{-1}d)	4.423	5.433	0.04	0.001
BWG ($\text{kg}/15\text{d}$)	4.533	5.267	0.29	0.149
WHG ($\text{cm}/15\text{d}$)	0.519	0.643	0.02	0.01
BLG ($\text{cm}/15\text{d}$)	1.234	1.977	0.07	0.002
HGG ($\text{cm}/15\text{d}$)	1.133	1.400	0.05	0.016
Nutritional status parameters				
Glucose (mmol/L)	2.895	2.825	0.06	0.493
Albumin (g/dL)	3.676	4.117	0.40	0.479
Urea (mg/dL)	19.701	28.050	1.45	0.015
Blood urea nitrogen (mg/dL)	9.20	13.10	0.67	0.015

SEM, standard error of the mean; DMI, dry matter intake; BWG, body weight gain; WHG, wither height gain; BLG, body length gain; HGG, heart-girth gain.

Body weight gain of the heifers non-significantly ($P=0.149$) influenced by the control and protein supplemented diet (Table 2). Probable causes of this insignificant difference might be increasing little percentages of protein rich ingredients in diet and another cause may be the heifers reached maximum growth stage of their life before starting the research trial. It has been literally proved by Wiltbank *et al.* (1966) that growth of the animals after reaching certain age did not increase in significant trend. By the way, it can be found from Table 3 that there existed a significant ($P=0.01$) time influence on BWG of heifers and interaction impact of both time and diet has also significant ($P=0.05$) on BWG. However, Divya and Anil-kumar (2009) concluded that significantly higher BWG found in those heifers received good plane of nutrition compared to the grass based diets. Live weight gain of heifers improved by concentrate and green grass feeding along with hay supplementation (Al-Shami, 2007).

Wither height gain (WHG), body length gain (BLG) and heart-girth gain (HGG) of the heifers obtained significantly ($P=0.01$, 0.002 and 0.016, respectively) higher in protein supplemented group compared to the control group (Table 2). This higher body growth in heifer could be narrated due to addition of protein rich ingredients in the concentrate mixer which supported by Strzetelski *et al.* (2007) who mentioned that increasing dietary energy and protein in the ration of heifer improve WH. Again, highest HG recorded in crossbred heifer by Singh *et al.* (2015) through feeding concentrate based diet. Time greatly influences on BWG, BLG and HGG of the heifers but the interaction effect of time and diet showed non-significant response on WHG and HGG (Table 3). These finding are supported by Habib *et al.* (2018) who described that heifers fed green fodder, soybean hay along with mixed concentrate had non-significant interaction effect of time and diet on these parameters.

Table 3. Tests of within-subjects effects on DM intake, growth performance and nutritional status parameters

Parameters	Source	F-values
DMI	Time	7.54**
	Time × diet	5.66**
BWG	Time	16.69**
	Time × diet	4.43*
WHG	Time	11.59**
	Time × diet	2.78 ^{NS}
BLG	Time	72.45**
	Time × diet	13.43**
HGG	Time	4.69*
	Time × diet	0.82 ^{NS}
Glucose	Time	36.62**
	Time × diet	1.29 ^{NS}
Albumin	Time	18.7**
	Time × diet	3.21 ^{NS}
Urea	Time	9.59**
	Time × diet	0.76 ^{NS}
Blood urea nitrogen	Time	9.54**
	Time × diet	0.75 ^{NS}

DMI, dry matter intake; BWG, body weight gain; WHG, wither height gain; BLG, body length gain; HGG, heart-girth gain. **Significant at $P<0.01$, *significant at $P<0.05$ and NS, non-significant.

Nutritional status

The data on blood biochemical profile are mentioned in Table 2 and results revealed that blood serum glucose and albumin shows non-significant ($P=0.493$ and 0.479, respectively) response through feeding protein supplemented and control diets. Again, tests of within-subjects indicated that there existed significant ($P=0.01$) time effect on serum glucose and albumin of heifers; but the interaction effect of diet and time was non-significant ($P>0.05$; Table 3). However, the blood glucose and albumin concentrations were within the normal levels 2.5-4.16 mmol/L and 3.0-5.0 g/dL , respectively as reported by Kaneko *et al.* (1997). This finding are closely related to Melvin (1982) who stated that blood glucose and albumin remain same among the dietary groups through supplying energy rich ingredients to the cattle. Albumin is the protein precursor which plays vital role for growth and other physiological purposes; that's the reason to increase protein level in diet for increasing blood serum in animals (Shetaewi and

Ross, 1991). Albumin level in blood serum reflects the protein availability in the diet and vice-versa which potentially beneficial to the mammals through increasing the available amino acids for absorption (Wada *et al.*, 2017).

Blood urea nitrogen (BUN) and serum urea concentrations of the heifers noticeably significant ($P=0.015$) which are presented in Table 2. With the advancement of feeding time, urea and BUN concentrations increased significantly ($P=0.01$). BUN is a metabolic indicator that is being used for assessing the short-term or real-time changes in nutritional profile of animals. Higher urea and BUN found in protein rich ingredients supplemented group that might be increased of ME and CP content in the supplied diet. In this study, serum levels of urea and BUN of supplemented groups were 28.05 and 13.10 mg/dL, respectively which are supported by Kaneko *et al.* (1997). However, Moss and Murray (1992) found higher plasma urea concentration due to supplementary protein intake in heifers. Similarly, Sun and Christopherson (2005) obtained positive relationship between BUN and dietary CP in ruminants.

Conclusion

Supplementation of protein rich ingredients (soybean meal and mustard oil cake) in the concentrate mixture of heifers influences dry matter intake and nutritional status. During pre-pubertal period this nutritional status of heifers indicates the impact of such feeding programs for initiating heifer development and replacement. For improving heifer ration quality this findings might have implication in the tropical countries where cattle are often fed rations deficient in protein. It could be concluded that feeding protein rich ingredients with concentrate mixture would effectively improve the growth and nutritional status of the dairy heifers.

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