Agr. Nat. Resour. 55 (2021) 935–944

AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Effects of protein nutrition levels on milk yield, composition, amino acid profiles and plasma metabolites of indigenous lactating buffaloes

Md. Zakirul Islam[a,†], S.M. Rajiur Rahman[a,†,*], Md. Nurul Islam[a], Md. Mehedi Hasan Khandakar[a], Mohammad Shohel Rana Siddiki[a], Nathu Ram Sarker[b], Watcharawit Meenongyai[c], Md. Harun-ur-Rashid[a]

[a] *Department of Dairy Science, Bangladesh Agricultural University, Mymensingh- 2202, Bangladesh*

[b] *Bangladesh Livestock Research Institute, Savar, Dhaka-1341, Bangladesh*

[c] *Faculty of Natural Resources and Agro-Industry, Kasetsart University, Chalermphrakiat Sakon Nakhon Province Campus, Sakon Nakhon- 47000, Thailand*

Article Info

Article history:
Received 29 March 2021
Revised 30 October 2021
Accepted 4 November 2021
Available online 20 December 2021

Keywords:
Amino acid profile,
Blood metabolites,
Crude protein,
Dairy buffaloes,
Milk production and quality

Abstract

The dietary crude protein (CP) supply was optimized for indigenous lactating buffaloes in Bangladesh. Twelve buffaloes (age 4 yr) with an average daily milk yield of 2.33–3.03 kg/d and average ± SD body weight of 390 ± 10 kg were divided into three groups (each with four animals) that were randomly assigned to three levels of CP in the concentrate feed mixture: 14.22%, 15.62%, and 17.81% (dry matter basis) in a completely randomized design. The total duration of the study was 100 d with an initial 10 d of adjustment. The diets were formulated to ensure the same energy level. The dietary protein level showed no effect on dry matter intake ($p > 0.05$). The nitrogen intake of buffaloes increased linearly ($p = 0.01$) with increasing CP levels. The milk yield tended to increase in a quadratic fashion. No effect was observed ($p > 0.05$) on the milk protein, fat and lactose contents. The level of plasma urea nitrogen increased ($p < 0.05$) when the dietary CP levels were raised, whereas the glucose, protein, albumin, globulin, calcium and triacylglycerol levels remained unchanged ($p > 0.05$). The nitrogen efficiency of lactating buffaloes increased ($p < 0.05$) by optimizing dietary protein nutrition accurately to each animal's requirements. The milk amino acid composition was unaffected ($p > 0.05$) by the dietary CP supplies. In conclusion, the 15.62% CP level resulted in higher milk production ($p < 0.05$) and high nitrogen efficiency ($p < 0.05$) in the indigenous lactating buffaloes under the study conditions.

† Equal contribution.

* Corresponding author.
E-mail address: smrajiurrahman@yahoo.com (S.M.R. Rahman)

https://doi.org/10.34044/j.anres.2021.55.6.04

Introduction

Buffaloes inhabit and breed throughout Asia, Europe and South America over a wide range of geographical, environmental and agronomic settings and they are second only to cows in their milk production globally (Eldahshan et al., 2020). The greatest expense in dairy farming is feed, which accounts for more than 70% of the total cost in developing countries (Singh et al., 2003). It is more common for indigenous buffaloes to grow slowly, delay puberty and produce less milk due to inadequate protein consumption (Pasha, 2013; Habib et al., 2020; Mohd Azmi et al., 2021). Nitrogen is one of the main elements in the formulation of rations for ruminants, resulting in greater attention being paid to excretion by ruminants due to growing environmental concerns regarding the nitrogen and phosphorus released (Islam et al., 2002). Generally, excess dietary protein is not needed by ruminants and it is converted into ammonia (Abbasi et al., 2018). It has been established that the feed and feeding strategies in ruminant animals may further significantly influence the microbial population of the rumen (Huws et al., 2018) and the composition of the ruminal gut microbiota is significantly associated with feed efficiency (Guan et al., 2008; Carberry et al., 2012). Bevans et al. (2005) reported that alterations in the structure of the ruminant gut microbiota could substantially improve the feed intake, efficiency and body weight gain of ruminants. In addition, buffaloes can utilize feed more proficiently where the feed is supplied in lower quantity or quality or both (Chanthakhoun et al., 2012). Such feed efficiency can be explained by the buffaloes having a different rumen microbial ecology with a higher population of cellulolytic bacteria and fungal zoospores, a lower protozoal population and a greater capacity to recycle nitrogen to the rumen (Wanapat, 2000; Wanapat and Cherdthong, 2009; Cherdthong et al., 2010; Chanthakhoun et al., 2012). However, a good understanding of the protein requirement for dairy buffaloes is essential to ensure a proper supply of the assorted amino acid required for milk production and cow maintenance while minimizing the costs, and importantly, reducing unnecessary excretion of surplus nitrogen by circumventing any overfeeding of protein (Mohd Azmi et al., 2021). Better feeding and management could improve buffalo fertility and ensure a high milk yield of good quality (Qureshi et al., 2007; Delfino et al., 2021) and, likewise, in dairy cows (Islam et al., 2020). The higher supply of crude protein for dairy buffaloes in pasture over a sufficient period improved the nutritional status of the milk yield (Hayashi et al., 2005). The amount of milk protein could be increased from 0.05% to 0.15% by manipulating the diet based on the energy and protein contents (Santos, 2002). Increasing the amount of dietary protein for a constant dietary energy level had little effect on milk protein synthesis (Broderick, 2003). However, the abundance and effective use of nutrients in the rumen is crucial for their effective use by the animal host (Franzolin and Alves, 2010). Therefore, the promotion of a suitable level of efficiency of nitrogen metabolism by the animal via a healthy diet and the utilization of protein and amino acids is an effective way to minimize nitrogen losses while ensuring adequate levels to meet the actual physiological requirements of the animal (Steinfeld and Wassenaar, 2007).

Most small-scale buffalo farmers in tropical countries like Bangladesh are not aware of the benefits of better animal feeding; in general, farmers feed their animals with locally available crop wastes, roughage and a tiny quantity of concentrates (Rahman et al., 2019a, 2019b). Therefore, most of the buffaloes typically yield less milk and farmers are not aware of the potential from providing concentrate in the diet. Despite this, a few farmers fed their animals a small amount of concentrate feed without being aware of the specific crude protein (CP) requirements. Accordingly, the current experiment was designed to optimize the protein nutrition levels with the least quantity of concentrate supply possible by considering these factors. In addition, information is scanty regarding the effect of feeding the buffaloes various dietary protein levels particularly on the milk amino acid profiles. Therefore, dose-response experiments, which have not been done previously to the authors' knowledge, are required to determine the optimum protein supply of indigenous lactating buffaloes. Thus, this study was undertaken to investigate the effect of different protein levels in the diet of lactating buffaloes and to evaluate the milk yield, components, amino acid composition and blood metabolites.

Materials and Methods

Animals, feed types and management

The Buffalo Research Farm, Animal Production Research Division, Bangladesh Livestock Research Institute, Savar, Dhaka, Bangladesh, was the site of the experiment. Twelve, lactating indigenous buffaloes aged 4 yr and having average daily milk yields from 2.33 kg/d to 3.03 kg/d, and mean ± SD body weight (BW) of 390 ± 10 kg were randomly assigned to receive one of three CP levels (14.22%, 15.62% or 17.81%)

in the concentrate mixture (Table 1) in a completely randomized design experiment. The duration of the study was 100 d and the first 10 d were assigned for adjustment. There were three groups with four buffaloes in each. The buffaloes were kept in individual pens where water and mineral blocks were provided *ad libitum*. All buffaloes were supplied *ad libitum* with Napier silage (fodder cut at age 35 d, chopped into 2–3 cm lengths, compacted in a concrete silo pit and stored for 60 d), while additional concentrate was fed at 3.5 g/kg of BW. All buffaloes were fed their allocated diets during the period of the experiment, and concentrate was fed to them in two equal amounts at 0700 hours and 1630 hours. The daily dry matter intake (DMI) was determined by weighing any residual Napier silage feed before the morning feeding. The BW of each buffalo was measured at the beginning and the end of each period of 30 d. The dried concentrate mixture and Napier silage samples (offered and leftover) were ground in a MAC® WILLEY grinder to pass through a 1-mm sieve and pooled, with the proximate composition of the samples determined using the method of Association of Official Analytical Chemists (2005). All animals were cared for during milk and blood sampling according to the protocol of Animal Welfare and Experimentation Ethics Committee, Bangladesh Livestock Research Institute, Savar, Dhaka.

Feed TDN, ME, FCM and N efficiency calculation

Total digestible nutrient (TDN) was estimated according to the following equations: TDN for silage (% dry matter (DM)) = − 17.2649 + (1.2120 × %CP) + (0.8352 × %NFE) + (2.4637 × %EE) + (0.4475 × %CF); TDN for concentrate (%DM) = 40.3227 + (0.5398 × %CP) + (0.4448 × %NFE) + (1.4218 × %EE) − (0.7007 × %CF) as well as metabolizable energy (ME) values were estimated according to Kearl (1982); ME (MJ/kg DM) = [− 0.45 + (0.04453 × %TDN)] × 4.184. Fat corrected milk (FCM) was measured using software of Progressive Dairy Solutions. Inc. 120 S Sierra Ave, Oakdale, CA 95361. N efficiency was calculated as nitrogen in milk/nitrogen in CP according to Naveed-ul-Haque et al. (2018).

Milk and blood analysis

Once a week, individual samples of milk were collected from two successive milking events and used to analyze milk components (total solids, solids-not-fat, fat, protein, lactose, ash content). The milk compositional parameters were analyzed using an automated milk analyzer (Lactoscan SP, MILKOTONIC Ltd., Bulgaria). At intervals of 30 d, blood samples (about 10 mL) were taken from the jugular

Table 1 Ingredients and chemical composition of concentrates and Napier silage (NS) by treatment

Item	Treatment			
	14.22% CP	15.62% CP	17.81% CP	NS
Wheat bran (kg)	50.50	48.00	47.00	–
Soybean meal (kg)	1.00	6.00	10.00	–
Broken maize (kg)	45.50	43.00	40.00	–
DCP (kg)	2.00	2.00	2.00	–
Salt (kg)	1.00	1.00	1.00	–
Total (kg)	100.00	100.00	100.00	–
Chemical composition of the diet (%)				
TDN	69.68	69.74	69.70	51.03
DM	88.03	87.92	88.05	17.12
CP	14.22	15.62	17.81	7.68
NDF	24.40	24.28	24.36	85.61
ADF	6.68	9.72	10.18	45.33
Ash	5.07	4.73	5.79	10.22
‡ME (MJ/kg DM)	11.00	11.01	11.00	8.37

CP = crude protein; NS = Napier silage; DCP = di-calcium phosphate; TDN = total digestible nutrient; DM = dry matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; ME = metabolizable energy vein throughout the 90 d experimental period at 3 hr post offering of the concentrate mixture in the morning. Blood was collected into tubes containing 12 mg of ethylenediaminetetraacetic acid and the plasma was separated using centrifugation at 500×*g* at 4°C for 10 min and stored at -20°C until analysis. Various blood biochemical parameters were assessed using diagnostic kits (Span Diagnostics Ltd., Surat, India). The GOD–POD method was followed for glucose (Ambade et al., 1998), the cresolphthalein complexone method (Ranjan et al., 2012) for calcium, the modified Biuret and BCG dye-binding method (Ranjan et al., 2012) for total protein, albumin, globulin and the CHOD–PAP method for total cholesterol (Deeg and Ziegenhorn, 1983).

Determination of milk amino acids

For the buffalo milk samples, the composition of the amino acid (AA) was determined using the method of Rafiq et al. (2016). A sample (1.5 mL) of milk was used as the starting point for sample preparation. Following that, using 10 mL of H_2SO_4 (6 mol/L), milk samples were mixed thoroughly and hydrolyzed in a sealed glass apparatus at 110°C for 24 hr under a constant nitrogen flow. After centrifugation, the supernatant was moved to a 5 mL centrifuge tube and diluted with 0.02 mol/L H_2SO_4 to a final volume of 5 mL. Before operating the amino acid analyzer (Model: L-8900 Amino Acid Analyzer, Hitachi, Japan) for AA analysis, a 0.22 mm syringe filter was used to filter the previous mixture.

Statistical analysis

The data were analyzed using one-way analysis of variance. A general linear model for repeated measures procedure was used to analyze the blood profile data, considering treatment as the between-subjects main effect and sampling period as the within-subject factor. The significance level was set at $p < 0.05$. Duncan's multiple range test was used to ascertain the differences between means. All analyses were facilitated by the SPSS statistical program version 17.0 (SPSS Inc.; Chicago, IL, USA).

Result and Discussion

Feed intake, milk yield, and quality

Milk production and composition, dry matter intake (DMI), feed and nitrogen efficiencies and body weight data are provided in Table 2. The average DMI levels during the 90 d experimental period were not significantly different. This was supported by experiments on lactating buffaloes where differing protein supplies had no impact on DMI (Bovera et al., 2002; Bartocci et al., 2006; Terramoccia et al., 2012). Actual herd management records have shown that the variation in DMI of dairy cows in response to altered dietary protein or AA supply varies with the experimental period (Naveed-ul-Haque et al., 2018). It was possible that the length of treatment in the current study was sufficient to expect complete DMI

Table 2 Effects of crude protein supplies on dry matter intake, milk yield, milk composition, feed and nitrogen efficiencies and body weight of the lactating buffaloes

Item	14.22% CP	15.62% CP	17.81% CP	*p*-value
DMI (kg/d)	12.0±0.33	12.2±0.56	12.1±0.68	0.44
N intake (g/d)	200±4.23[c]	209±5.33[b]	216±6.87[a]	0.01
Milk yield (kg/d)	3.47±1.02[b]	3.82±0.74[a]	3.27±0.48[b]	0.02
FCM (kg/d)	5.66±1.05[b]	6.36±1.12[a]	5.40±1.22[b]	0.03
Protein (%)	3.64±0.45	3.65±0.53	3.66±0.26	0.43
Fat (%)	7.34±1.87	7.55±0.92	7.46±0.95	0.30
Lactose (%)	5.20±0.73	5.22±0.41	5.23±1.00	0.57
MY:DMI	0.29±0.01	0.31±0.03	0.27±0.03	0.41
FCM:DMI	0.47±0.02	0.52±0.02	0.45±0.11	0.11
[†]N efficiency	24.0±1.67[a]	20.1±1.83[b]	19.0±2.08[c]	0.01
Body weight (kg)	398±3.45[c]	400±3.98[b]	405±2.49[a]	0.02

CP = crude protein; DMI = dry matter intake; MY = milk yield; FCM = fat corrected milk

Mean ± SD values in a row superscripted with different lowercase letters differ significantly ($p < 0.05$).

responses. The total N intake increased linearly ($p < 0.05$) which was in agreement with Naveed-ul-Haque et al. (2018). The average daily milk production was 9.2% higher for the 15.62% CP (3.82 kg) than for the 14.22% CP group (3.47 kg) and 14.4% higher than for the 17.81% CP group (3.27 kg) ($p < 0.05$). Milk yield increased in a quadratic fashion with increasing CP supplies ($p < 0.05$). Increased dietary CP supplies were predicted to increase milk production in the current research. However, the results showed that the protein nutrition increased milk yields quadratically, which was in agreement with Naveed-ul-Haque et al. (2018). Additionally, in a study on lactating buffaloes, increasing the dietary CP levels from 9.3% to 12.2% (DM basis) increased the energy-corrected milk yield by 7% (Campanile et al., 1998). Likewise, several studies on lactating dairy cows have shown an increase in milk yield by increasing the dietary protein supplies (Vérité and Delaby, 2000; Brun-Lafleur et al., 2010). However, when coupled with the high-energy diets, increasing protein supplies had a higher impact on milk production (Brun-Lafleur et al., 2010), suggesting that supplies of protein and energy interact with each other. This effect has also been shown with lactating buffaloes, whereas increasing the amount of dietary CP to 17.90% from 15.50% enhanced milk production by 9% (Bartocci and Terramoccia, 2010). However, there was no effect of the treatments on the protein, fat or lactose contents of the milk ($p > 0.05$). In this regard, Santillo et al. (2016) reported that different dietary protein levels in dairy buffalo had no effect on fat and lactose but did affect the protein content of milk. Similarly, in the current study, there was no significant effect on feed efficiency (fat-corrected milk/DMI). Likewise, the dietary protein levels in the diets of growing buffaloes had no significant effect on feed efficiency (Singh et al., 2015). Increasing the dietary CP level linearly decreased the dietary N efficiency for milk ($p < 0.01$). According to the findings for lactating buffalo studies, the nitrogen efficiency rate ranges from 18 to 20%, accordingly in our investigation the rate averaged 21%, which is compatible with the findings of Bartocci et al. (2006), Terramoccia et al. (2012), and Naveed-ul-Haque et al. (2018). Nonetheless, in lactation, the N responses of milking buffaloes were lower than those of Holstein cows, which ranged from 26% to 28% (Recktenwald et al., 2014). In contrast, there was an effect on the body weight gain of buffaloes ($p < 0.05$) by changing the dietary protein levels. A higher protein level was associated with a small increase in body weight gain ($p < 0.05$), which was consistent with Dung et al. (2013) and Naveed-ul-Haque et al. (2018).

Blood biochemical profile

The plasma urea N (PUN), glucose, protein, albumin, globulin, calcium, and triglycerides levels increased progressively with advancement of the lactation stage ($p < 0.05$) but there was no significant difference in the dietary crude protein levels except for PUN ($p < 0.05$), as shown in Table 3. The PUN level increased linearly ($p < 0.05$) along with the rise in various periodic intervals by feeding dietary crude protein. Naveed-ul-Haque et al. (2018) and Singh et al. (2015) reported more or less similar findings to the current investigation regarding dietary protein supply. Additionally, Naveed-ul-Haque et al. (2018) found a significant effect of increasing CP levels on PUN but Singh et al. (2015) reported dietary protein levels did not affect plasma metabolites. However, a higher PUN level was observed following increased dietary CP, perhaps because of the increased dietary N contents. Jordan et al. (1983) used 23% and 12% CP in the diet of high-production dairy cows and reported a 3.5 times higher PUN for a diet containing 23% CP than for the diet having 12% CP. According to Neglia et al. (2014) and Patra et al. (2020), ruminal NH_3-N concentrations increased from 0.8 mg/100 mL to 56.1 mg/100 mL when the dietary CP was elevated from 8% to 24%. The surplus ammonia that forms in the reticulo-rumen is absorbed either in the reticulo-rumen or the lower gastrointestinal tract, and then transported to the liver, where it is converted into urea. However, a higher level of blood biochemical profiles with the advancement of lactation trial was explained by Ranjan et al. (2012) as a result of a positive energy balance on account of better nourishment of the animals with advancing stage of lactation. The lower N efficiencies in lactating buffaloes compared to cows could be explained by the higher PUN observed in the current investigation (22.22 mg/dL) and supported by the investigation by Bartocci et al. (2006), who reported 39 mg/dL compared to values in lactating cows of 15.6 mg/dL (Roseler et al., 1993) and 24 mg/dL (Colmenero and Broderick, 2006). In addition, with the N intake, PUN increased linearly as was reported in experiments with lactating dairy cows (Colmenero and Broderick, 2006), demonstrating the ineffective use of dietary protein. Furthermore, Rafsanjanny et al. (2019) reported a higher level (28 mg/dL) of blood urea concentration in crossbred lactating cows. A similar pattern was observed with the albumin concentration. Remarkably, with the PUN observed in the current study (17.52–22.22 mg/dL), optimizing the protein nutrition for lactating buffaloes provided useful information to improve N efficiency and to formulate economic diets.

Table 3 Blood biochemical profile of lactating buffaloes fed different level of crude protein

Item	14.22% CP	15.62% CP	17.81% CP	*p*-value	
				Treatment	Period
PUN (mg/dL)					
0 d	14.0±2.56	15.6±2.78	18.2±3.11	0.03	0.01
30 d	16.0±2.77	18.1±2.98	20.0±2.12		
60 d	19.0±3.00	21.3±3.13	24.1±3.39		
90 d	21.1±2.56	24.8±2.53	26.6±2.50		
Overall mean	17.52±3.14[c]	19.95±3.98[b]	22.22±3.82[a]		
Glucose (mg/dL)					
0 d	49.01±1.15	49.32±1.12	50.93±1.79	0.22	0.02
30 d	51.20±1.21	52.96±1.80	52.60±1.24		
60 d	52.11±1.32	53.55±1.59	53.11±1.55		
90 d	52.29±1.50	54.18±2.00	53.97±2.06		
Overall mean	51.15±1.25	52.50±1.63	52.65±1.52		
Protein (g/dL)					
0 d	7.55±0.031	8.92±0.11	8.63±0.64	0.42	0.00
30 d	7.96±0.051	8.96±0.32	8.91±0.91		
60 d	8.51±0.60	9.32±0.30	9.79±0.84		
90 d	9.10±0.42	9.76±0.45	10.12±0.27		
Overall mean	8.28±0.67	9.24±0.40	9.36±0.70		
Albumin (g/dL)					
0 d	2.79±0.21	2.60±0.45	2.60±0.20	0.13	0.01
30 d	3.12±0.49	2.90±0.35	2.97±0.46		
60 d	3.70±0.28	3.21±0.32	3.18±0.52		
90 d	3.90±0.47	3.69±0.32	3.56±0.24		
Overall mean	3.37±0.52	3.10±0.46	3.07±0.40		
Globulin (g/dL)					
0 d	4.97±0.44	5.19±0.54	4.93±0.72	0.10	0.02
30 d	5.22±0.44	5.93±0.48	5.33±0.48		
60 d	5.91±0.51	6.13±0.64	6.00±0.34		
90 d	6.21±0.47	6.50±0.12	6.62±0.24		
Overall mean	5.57±0.58	5.93±0.55	5.72±0.75		
Calcium (mg/dL)					
0 d	5.00±0.68	5.00±0.46	4.96±0.98	0.08	0.04
30 d	5.65±0.52	5.13±0.99	5.00±0.85		
60 d	5.95±0.52	6.50±0.19	6.55±0.52		
90 d	6.23±0.62	7.00±0.92	7.26±0.71		
Overall mean	5.70±0.53	5.92±1.00	5.94±1.15		
TG (mg/dL)					
0 d	175±3.21	176±1.89	178±1.93	0.89	0.56
30 d	176±2.54	180±2.22	181±1.99		
60 d	177±0.98	180±2.30	182±1.48		
90 d	181±1.25	182±1.79	184±1.27		
Overall mean	177.25±2.63	179.5±2.52	181.25±2.50		

CP = crude protein; PUN = plasma urea nitrogen; TG = triglycerides. Mean ± SD values in a row superscripted with different lowercase letters differ significantly ($p < 0.05$).

Milk amino acid profile

The quality of protein is largely determined by the amino acid composition. Amino acids are the building blocks of proteins and have a vital role in the human body (Wolfe et al., 2016). The milk amino acid composition data are presented in Table 4. There were no significant differences for the milk amino acid composition in the three dietary groups of buffaloes. Haque et al. (2012) stated that the dietary crude protein levels did not affect the amino acid composition of buffalo milk. In the current study, it was clear that each buffalo milk sample was rich in essential amino acids and the dominant amino acids were glutamic acid (0.98 g/100 g milk), followed by proline, lysine, leucine and asparagine (Table 4). Rafiq et al. (2016) studied amino acids profiling of milk from different animal species and stated that among the non-essential amino acids, glutamic acid was in the highest concentrations in both casein and whey proteins. However, in the current investigation, the ratios of essential and non-essential amino acids in response to the shift in the dietary protein levels were a bit higher numerically in the 17.81% CP group. Lysine and threonine are the scarcest amino acids in a wide range of protein resources; however, essential being catabolic-sensitive and necessary for protein synthesis (Rafiq et al., 2016). Notably, differences in amino acid profiles in dietary proteins influence their utilization in the body. For example, compared to soy protein, milk proteins elicited a greater increase in branched-chain amino acids (BCAAs) concentrations (26%) in peripheral tissues (Bos et al., 2000; Fouillet et al., 2002). Furthermore, BCAAs play a significant role in weight control by regulating glucose homeostasis and lipid metabolism (Rafiq et al., 2016). Furthermore, sulfur-containing amino acids, such as methionine and cysteine, boost the immune system via intracellular glutathione conversion, functioning as antioxidants (Hall et al., 2003). However, among different buffalo breeds, the milk amino acid composition was significantly different Zhou et al. (2018), though there has been a lack of scientific evidence regarding the amino acid composition of buffalo milk resulting from changing dietary protein levels.

Table 4 Amino acid composition of milk (g/100 g of milk) from indigenous lactating buffaloes

Amino acid type	14.22% CP	15.62% CP	17.81% CP	*p*-value
Essential amino acid				
Methionine	0.12±0.01	0.13±0.02	0.13±0.01	0.21
Valine	0.22±0.03	0.23±0.03	0.23±0.04	0.31
Lysine	0.45±0.04	0.45±0.02	0.45±0.02	0.50
Isoleucine	0.19±0.04	0.19±0.04	0.20±0.03	0.32
Phenylalanine	0.28±0.02	0.28±0.12	0.28±0.01	0.52
Leucine	0.39±0.10	0.40±0.05	0.40±0.02	0.13
Threonine	0.22±0.02	0.22±0.10	0.22±0.03	0.50
Non- Essential amino acid				
Asparagine	0.36±0.03	0.36±0.01	0.36±0.01	0.31
Serine	0.25±0.05	0.24±0.05	0.24±0.05	0.49
Glutamic acid	0.96±0.12	0.98±0.11	0.99±0.12	0.31
Proline	0.51±0.01	0.51±0.03	0.51±0.11	0.39
Glycine	0.10±0.01	0.10±0.00	0.10±0.01	0.21
Alanine	0.16±0.04	0.16±0.03	0.16±0.03	0.11
Cysteine	0.02±0.01	0.02±0.00	0.02±0.00	0.17
Tyrosine	0.18±0.01	0.18±0.01	0.18±0.04	0.12
Histidine	0.12±0.03	0.12±0.01	0.12±0.03	0.15
Arginine	0.13±0.02	0.13±0.04	0.13±0.01	0.08
EAA	1.87±0.12	1.9±0.25	1.98±0.14	0.05
NEAA	2.79±0.01	2.8±0.17	2.81±0.23	0.05
TAA	4.66±0.55	4.7±0.52	4.79±0.48	0.05
EAA/NEAA (%)	67±1.56	68±1.12	70±1.83	0.51

CP = crude protein; EAA = essential amino acid; NEAA = non- essential amino acid; TAA = total amino acid.
Mean ± SD values in a row superscripted with different lowercase letters differ significantly ($p < 0.05$).

The current study inferred that supplementing concentrate with 15.62% CP enhanced milk production and nitrogen efficiency in indigenous lactating buffaloes, while having no negative impact on DMI. The PUN also progressively increased with the level of dietary CP, whereas other blood metabolites and the amino acid composition of milk were not significantly influenced. In this regard, a strategic supply of CP can be recommended, especially for protein nutrition in the diet of indigenous lactating buffaloes while incorporating a limited supply of concentrate into the diet formulation. The current study would be beneficial for dairy processing industries to develop nutritional and functional milk-based innovative products for vulnerable parts of the population based on the buffalo milk amino acid profile. However, more research is needed to determine the relationship between the energy and protein levels in indigenous lactating buffaloes in Bangladesh and elsewhere in the world.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The authors are grateful to the Bangladesh Academy of Science, Dhaka, Bangladesh for financial support for this project. The Department of Dairy Science, Bangladesh Agricultural University assisted with samples analysis and the Bangladesh Livestock Research Institute conducted the trial at the Buffalo Research Farm, Animal Production Research Division.

References

Abbasi, I.H.R., Abbasi, F., Abd El-Hack, M.E., Abdel-Latif, M.A., Soomro, R.N., Hayat, K., Cao, Y. 2018. Critical analysis of excessive utilization of crude protein in ruminants ration: impact on environmental ecosystem and opportunities of supplementation of limiting amino acids—a review. Environ. Sci. Pollut. Res. 25: 181–190. doi.org/10.1007/s11356-017-0555-4

Ambade, V.N., Sharma, Y.V., Somani, B.L. 1998. Methods for estimation of blood glucose: A comparative evaluation. Med. J. Armed Forces India. 54: 131–133. doi.org/10.1016/S0377-1237(17)30502-6

Association of Official Analytical Chemists. 2005. Official Methods of Analysis of AOAC International, 18th ed. Association of Official Analytical Chemists, Washington, DC, USA.

Bartocci, S., Terramoccia, S. 2010. Variations in the production, qualitative characteristics and coagulation parameters of the milk of the riverine buffalo determined by the energy/protein content of the diet. Asian-Australas. J. Anim. Sci. 23: 1166–1173. doi.org/10.5713/ajas.2010.80578

Bartocci, S., Terramoccia, S., Tripaldi, C. 2006. The utilisation of a high level energy/protein diet for lactating Mediterranean buffaloes: Intake capacity and effects on quanti-qualitative milk parameters. Livest. Sci. 99: 211–219. doi.org/10.1016/j.livprodsci.2005.06.015

Bevans, D.W., Beauchemin, K.A., Schwartzkopf-Genswein, K.S., McKinnon, J.J., McAllister, T.A. 2005. Effect of rapid or gradual grain adaptation on subacute acidosis and feed intake by feedlot cattle. J. Anim. Sci. 83: 1116–1132. doi.org/10.2527/2005.8351116x

Bos, C., Gaudichon, C., Tome, D. 2000. Nutritional and physiological criteria in the assessment of milk protein quality for humans. J. Am. Coll. Nutr. 19: 191–205. doi.org/10.1080/07315724.2000.10718068

Bovera, F., Calabrò, S., Cutrignelli, M.I., Di Lella, T. 2002. Effect of dietary energy and protein contents on buffalo milk yield and quality during advanced lactation period. Asian-Australas. J. Anim. Sci. 15: 675–681. doi.org/10.5713/ajas.2002.675

Broderick, G.A. 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. J. Dairy Sci. 86: 1370–1381. doi.org/10.3168/jds.S0022-0302(03)73721-7

Brun-Lafleur, L., Delaby, L., Husson, F., Faverdin, P. 2010. Predicting energy × protein interaction on milk yield and milk composition in dairy cows. J. Dairy Sci. 93: 4128–4143. doi.org/10.3168/jds.2009-2669

Campanile, G., de Filippo, C., Di Palo, R., Taccone, W., Zicarelli, L. 1998. Influence of dietary protein on urea levels in blood and milk of buffalo cows. Livest. Prod. Sci. 55: 135–143. doi.org/10.1016/S0301-6226(98)00123-7

Carberry, C.A., Kenny, D.A., Han, S., McCabe, M.S., Waters, S.M. 2012. Effect of phenotypic residual feed intake and dietary forage content on the rumen microbial community of beef cattle. Appl. Environ. Microbiol. 78: 4949–4958. doi.org/10.1128/AEM.07759-11

Chanthakhoun, V., Wanapat, M., Kongmun, P., Cherdthong, A. 2012. Comparison of ruminal fermentation characteristics and microbial population in swamp buffalo and cattle. Livest. Sci. 143: 172–176. doi.org/10.1016/j.livsci.2011.09.009

Cherdthong, A., Wanapat, M., Kongmun, P., Pilajun, R., Khejornsart, P. 2010. Rumen fermentation, microbial protein synthesis and cellulolytic bacterial population of swamp buffaloes as affected by roughage to concentrate ratio. J. Anim. Vet. Adv. 9: 1667–1675. doi.org/10.3923/javaa.2010.1667.1675

Colmenero, J.J.O., Broderick, G.A. 2006. Effect of dietary crude protein concentration on milk production and nitrogen utilization in lactating dairy cows. J. Dairy Sci. 89: 1704–1712. doi.org/10.3168/jds.S0022-0302(06)72238-X

Deeg, R., Ziegenhorn, J. 1983. Kinetic enzymic method for automated determination of total cholesterol in serum. Clin. Chem. 29: 1798–1802. doi.org/10.1093/clinchem/29.10.1798

Delfino, N.C., Silva, R.D.G.E., Alba, H.D.R., Oliveira, M.X.D.S., Carvalho, G.G.P.D., Araújo, M.L.G.M.L.D., Freitas Júnior, J.E.D. 2021. Milk yield and composition, blood, and urinary parameters of Murrah buffaloes in different maturity stages during the transition period and early lactation. J. Appl. Anim. Res. 49: 247–256. doi.org/10.1080/09712119.2021.1940188

Dung, D.V., Ba, N.X., Van, N.H., Cuong, V.C., Yao, W. 2013. Practice on improving fattening local cattle production in Vietnam by increasing crude protein level in concentrate and concentrate level. Trop. Anim. Health Prod. 45: 1619–1626. doi.org/10.1007/s11250-013-0407-2

Eldahshan, E., Saddick, E., Selim, S. 2020. Lactation performance, milk fatty acid composition, and blood lipid profile of lactating buffaloes in response to dietary soybean and linseed oils. Trop. Anim. Sci. J. 43: 254–262. doi.org/10.5398/tasj.2020.43.3.254

Fouillet, H., Mariotti, F., Gaudichon, C., Bos, C., Tome, D. 2002. Peripheral and splanchnic metabolism of dietary nitrogen are differently affected by the protein source in humans as assessed by compartmental modeling. J. Nutr. 132: 125–133. doi.org/10.1093/jn/132.1.125

Franzolin, R., Alves, T.C. 2010. The ruminal physiology in buffalo compared with cattle. In: Proceedings of the 9th World Buffalo Congress. Buenos Aires, Argentina, pp. 104–111.

Guan, L.L., Nkrumah, J.D., Basarab, J.A., Moore, S.S. 2008. Linkage of microbial ecology to phenotype: Correlation of rumen microbial ecology to cattle's feed efficiency. FEMS Microbiol. Lett. 288: 85–91.

Habib, M.R., Islam, M.Z., Bari, M.S., Islam, M.Z., Sarker, M.A.H., Rashid, M.H., Islam, M.A. 2020. Effect of concentrate supplementation during transition period on production and reproduction of indigenous buffalo. Bangladesh J. Anim. Sci. 49: 83–90. doi.org/10.3329/bjas.v49i1.49385

Hall, W.L., Millward, D.J., Long, S.J., Morgan, L.M. 2003. Casein and whey exert different effects on plasma amino acid profiles, gastrointestinal hormone secretion and appetite. Br. J. Nutr. 89: 239–248. doi.org/10.1079/BJN2002760

Haque, M.N., Rulquin, H., Andrade, A., Faverdin, P., Peyraud, J.L., Lemosquet, S. 2012. Milk protein synthesis in response to the provision of an "ideal" amino acid profile at 2 levels of metabolizable protein supply in dairy cows. J. Dairy Sci. 95: 5876–5887. doi.org/10.3168/jds.2011-5230

Hayashi, Y., Maharjan, K.L., Kumagai, H. 2005. Feeding traits, nutritional status and milk production of dairy cattle and buffalo in small-scale farms in Terai, Nepal. Asian-Australas. J. Anim. Sci. 19: 189–197. doi.org/10.5713/ajas.2006.189

Huws, S.A., Creevey, C.J., Oyama, L.B., Mizrahi, I., Denman, S.E., Popova, M., Morgavi, D.P. 2018. Addressing global ruminant agricultural challenges through understanding the rumen microbiome: past, present, and future. Front. Microbiol. 9: 2161. doi.org/10.3389/fmicb.2018.02161

Islam, M.R., Ishida, M., Ando, S., Nishida, T. 2002. *In situ* dry matter, nitrogen and phosphorous disappearance of different feeds for ruminants. Asian-Australas. J. Anim. Sci. 15: 793–799. doi.org/10.5713/ajas.2002.793

Islam, M.Z., Islam, M.Z., Barman, K.K., Bari, M.S., Habib, M.R., Rashid, M.H., Islam, M.A. 2020. Concentrate supplementation impact on the performances of extended transitional crossbred Zebu cow. J. Bangladesh Agril. Univ. 18: 117–123. doi.org/10.5455/JBAU.94751

Jordan, E.R., Chapman, T.E., Holtan, D.W., Swanson, L.V. 1983. Relationship of dietary crude protein to composition of uterine secretions and blood in high-producing postpartum dairy cows. J. Dairy Sci. 66: 1854–1862. doi.org/10.3168/jds.S0022-0302(83)82023-2

Kearl, L. 1982. Nutrient Requirement of Ruminant in Developing Countries. Ph.D. thesis, Utah State University. Logan, UT, USA.

Mohd Azmi, A.F., Ahmad, H., Mohd Nor, N., et al. 2021. the impact of feed supplementations on Asian buffaloes: A review. Animals 11: 2033. doi.org/10.3390/ani11072033

Naveed-ul-Haque, M., Akhtar, M.U., Munnawar, R., Anwar, S., Khalique, A., Tipu, M.A., Ahmad, F., Shahid, M.Q. 2018. Effects of increasing dietary protein supplies on milk yield, milk composition, and nitrogen use efficiency in lactating buffalo. Trop. Anim. Health Prod. 50: 1125–1130. doi.org/10.1007/s11250-018-1539-1

Neglia, G., Balestrieri, A., Gasparrini, B., et al. 2014. Nitrogen and phosphorus utilisation and excretion in dairy buffalo intensive breeding. Ital. J. Anim. Sci. 13: 3362. doi.org/10.4081/ijas.2014.3362

Pasha, T.N. 2013. Prospect of nutrition and feeding for sustainable buffalo production. Buffalo Bull. 32: 91–110.

Patra, A.K., Pal, K., Lalhriatpuii, M. 2020. Prediction of nitrogen excretion in buffalo production systems using dietary and animal variables. Agric. Syst. 182: 102845. doi.org/10.1016/j.agsy.2020.102845

Qureshi, M.S., Khan, S., Ahmad, N. 2007. Pregnancy depresses milk yield in dairy buffaloes. Ital. J. Anim. Sci. 6: 1290–1293. doi.org/10.4081/ijas.2007.s2.1290

Rafiq, S., Huma, N., Pasha, I., Sameen, A., Mukhtar, O., Khan, M.I. 2016. Chemical composition, nitrogen fractions and amino acids profile of milk from different animal species. Asian-Australas. J. Anim. Sci. 29: 1022–1028. doi.org/10.5713/ajas.15.0452

Rafsanjanny, H., Sarker, M.A.H., Islam, M.Z., Islam, K.M.S., Haque, M.R., Islam, M.N., Siddiki, M.S.R. 2019. Blood profile of lactating crossbred cows rearing at Bangladesh Agricultural University Dairy Farm. Bangladesh J. Anim. Sci. 48: 139–144. doi.org/10.3329/bjas.v48i2.46768

Rahman, S.R., Islam, M.N., Harun-ur-Rashid, M., Sarker, N.R., Siddiki, M.S.R., Islam, M.Z., Islam, M.A. 2019a. Buffalo milk yield, quality, and marketing in different agro-climatic districts of Bangladesh. J. Buffalo Sci. 8: 62–67. doi.org/10.6000/1927-520X.2019.08.03.2

Rahman, S.R., Islam, M.N., Harun-ur-Rashid, M., Siddiki, M.S.R., Islam, M.A. 2019b. Dairy buffalo production under intensive system in semi-arid area of Bangladesh. Buffalo Bull. 38: 83–98.

Ranjan, A., Sahoo, B., Singh, V.K., Srivastava, S., Singh, S.P., Pattanaik, A.K. 2012. Effect of bypass fat supplementation on productive performance and blood biochemical profile in lactating Murrah (*Bubalus bubalis*) buffaloes. Trop. Anim. Health Prod. 44: 1615–1621. doi.org/10.1007/s11250-012-0115-3

Recktenwald, E., Ross, D., Fessenden, S., Wall, C., van Amburgh, M. 2014. Urea-N recycling in lactating dairy cows fed diets with 2 different levels of dietary crude protein and starch with or without monensin. J. Dairy Sci. 97: 1611–1622. doi.org/10.3168/jds.2013-7162

Roseler, D.K., Ferguson, J.D., Sniffen, C.J., Herrema, J. 1993. Dietary protein degradability effects on plasma and milk urea nitrogen and milk nonprotein nitrogen in Holstein cows. J. Dairy Sci. 76: 525–534. doi.org/10.3168/jds.S0022-0302(93)77372-5

Santillo, A., Caroprese, M., Marino, R.O., Sevi, A., Albenzio, M. 2016. Quality of buffalo milk as affected by dietary protein level and flaxseed supplementation. J. Dairy Sci. 99: 7725–7732. doi.org/10.3168/jds.2016-11209

Santos, J.E.P. 2002. Feeding for milk composition. In: Proceedings of the 6th International Congress on Bovine Medicine. Spanish Association of Specialists in Bovine Medicine, Santiago de Compostela, Spain, pp. 163–172.

Singh, S., Kushwaha, B.P., Maity, S.B., Singh, K.K., Das, N. 2015. Effect of dietary protein on intake, nutrients utilization, nitrogen balance, blood metabolites, growth and puberty in growing Bhadawari buffalo (*Bubalus bubalis*) heifers. Trop. Anim. Health Prod. 47: 213–220. doi.org/10.1007/s11250-014-0713-3

Singh, J., Singh, B., Wadhwa, M., Bakshi, M.P.S. 2003. Effect of level of feeding on the performance of crossbred cows during pre-and post-partum periods. Asian-Australas. J. Anim. Sci. 16: 1749–1754. doi.org/10.5713/ajas.2003.1749

Steinfeld, H., Wassenaar, T. 2007. The role of livestock production in carbon and nitrogen cycles. Annu. Rev. Environ. Resour. 32: 271–294. doi.org/10.1146/annurev.energy.32.041806.143508

Terramoccia, S., Bartocci, A., Giovanni, S.D., Bartocci, S. 2012. The influence of dietary characteristics on the milk quantity and quality of riverine buffaloes: Estimate of the energy/protein requirements, for a medium-high production, in the first ninety days of lactation. Asian-Australas. J. Anim. Sci. 25: 335–340. doi.org/10.5713/ajas.2011.11220

Vérité, R., Delaby, L. 2000. Relation between nutrition, performances and nitrogen excretion in dairy cows. Ann. Zootech. 49: 217–230. doi.org/10.1051/animres:2000101

Wanapat, M. 2000. Rumen manipulation to increase the efficient use local feed resources and productivity of ruminants in the tropics. Asian-Australas. J. Anim. Sci. 13: 59–67.

Wanapat, M., Cherdthong, A. 2009. Use of real-time PCR technique in studying rumen cellulolytic bacteria population as affected by level of roughage in swamp buffalo. Curr. Microbiol. 58: 294–299. doi.org/10.1007/s00284-008-9322-6

Wolfe, R.R., Rutherfurd, S.M., Kim, I.Y., Moughan, P.J. 2016. Protein quality as determined by the digestible indispensable amino acid score: Evaluation of factors underlying the calculation. Nutr. Rev. 74: 584–599. doi.org/10.1093/nutrit/nuw022

Zhou, L., Tang, Q., Iqbal, M.W., et al. 2018. A comparison of milk protein, fat, lactose, total solids and amino acid profiles of three different buffalo breeds in Guangxi, China. Ital. J. Anim. Sci. 17: 873–878. doi.org/10.1080/1828051X.2018.1443288