

Variations of Raw Milk Components and Amino Acid Profiles in Different Dairy Buffalo Crossbreds

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Abstract: This study aimed to assess the relationship between milk components and amino acid (AA) profiles among different buffalo crossbreds in Bangladesh. A total of thirty-six (36) lactating buffaloes were selected from Murrah, Nili-Ravi, and Mehsana crossbreds, and they were assigned to 03 groups, each with 12 buffaloes. The total experimental period was 10 weeks, including the initial 10 days of diet adjustment. The results from the experiment revealed that milk protein, fat, and total solids contents of Murrah crossbred was significantly ($p < 0.05$) higher than Mehsana and Nili-Ravi crossbreds. In contrast, the lactose content of all buffalo crossbreds was statistically similar ($p > 0.05$). In the case of milk amino acid contents, all the milk samples entailed a higher concentration of Glutamic acid (0.9-1.00 g/100 g of milk), whereas Cysteine had the lowest concentration (0.02-0.05 g/100 g). The most prevalent essential amino acids were Leucine, Lysine, and Phenylalanine, whereas the most prevalent non-essential amino acids were Glutamic acid, Proline, Asparagine, and Serine. Lysine, Isoleucine, Leucine, Phenylalanine, Cysteine, and Histidine concentrations differed significantly ($p < 0.05$), and at the same time as the other 11 AAs concentrations were found non-significant ($p > 0.05$). The highest Lysine: Methionine ratio observed in Murrah buffalo was about 3.20%, while in Mehsana and Nili-Ravi buffalo was about 2.80 and 2.50%, respectively. Thus, it was evident from the study that the raw milk components and amino acid composition vary considerably in the different lactating buffalo crossbreds. Finally, the compositional data of raw milk may create the way of sustainable use of milk from dairy buffalo crossbreds and improve food and nutrition security, particularly in developing countries.

Keywords: Amino acid profile, dairy buffalo, milk composition, tropical region.

INTRODUCTION

Buffalo (*Bubalus bubalis*) is the second vital livestock species after dairy cows, contributing 15.14% of total milk production around the globe [1]. Buffaloes are traditionally raised under extensive husbandry practices in South-East Asia, which plays a vital role in their economy. The buffalo population of the world is dominated by Asian buffaloes, representing 96.79% of the global population of 208.1 million [1, 2]. Among the Asian countries, South Asia represents 79.74% of buffaloes, and the rest 20.26% in other countries [1, 3]. Particularly, Bangladesh has diverse indigenous domestic buffalo resources dominated by the riverine population except for some swamp types, especially in the eastern part of the country [4]. In addition, the Murrah, Nili-Ravi, Surti, and Jafarabadi crossbreds are scantily available, encompassing the Indian border of Bangladesh due to border passage from India [5, 6]. However, buffalo selection was insufficient for milk production and productivity control in preceding decades. Thus, milk production is lower than selected buffalo populations in some Asian countries [7].

Buffalo milk introduces more research interest and investment, owing largely to its handsome nutrient contents [8]. It can manufacture a wide variety of dairy products like cow milk [9]. From time immemorial, it has long been valued for its privileged chemical composition, making it suitable for producing many traditional and industrial dairy products [10]. The good comparison between buffalo milk and cow milk is not only regarding the attributes of physicochemical, compositional, and sensory but also its nutritional and health aspects [11]. Buffalo milk proteins are regarded as complete proteins with high biological value, containing nearly all the essential amino acids required by the human body [12]. Besides, amino acids perform significant physiological roles such as neurotransmission by aminobutyric acid (GABA) alongside protein synthesis [13]. Moreover, the sensorial quality of milk is largely influenced by amino acids, and it was reported that glutamic acid is responsible for the umami taste in cheese [14].

Furthermore, amino acid profiles correlate to milk's technological and nutritional qualities are very important for the dairy industry and animal feeding and breeding strategies. Consequently, a more detailed overview of milk composition, especially amino acid profiles, is needed to set a standard on technological

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properties for milk processing. However, the dairy industry faces challenges regarding breeding and nutritional strategy to produce the most favorable quality of milk for diverse purposes. Therefore, detailed information on buffalo milk composition, particularly amino acid profile, is essential. Recently, Zhou *et al.* [15] compared milk protein, fat, lactose, total solids, and amino acid profiles on different buffalo breeds. Ren *et al.* [16] also compared milk protein, amino acid, and fatty acid profiles between two riverine buffalo crossbreds viz. Murrah and Nili-Ravi and their crossbreds with local swamp buffalo. Besides, Islam *et al.* [17] investigated dietary CP level effects on milk yield, AA composition and blood metabolites of the indigenous buffaloes.

However, considerable knowledge of raw milk components and amino acid profiles of buffalo milk at the crossbred level is very scarce. Therefore, analysis of raw milk components and AA characterization of buffalo milk can contribute new information about milk composition and could be a valuable resource for the milk producers and processors to produce novel health-promoting dairy products for the consumers and purposefully selective breeding practices. Thus, the target of the current study was to ascertain the buffalo milk components and the milk AA profile of crossbred buffaloes.

MATERIALS AND METHODS

The experiment was conducted at Buffalo Research Farm under the Animal Production Research Division of Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka, for 10 weeks, including the first 10 days of the adjustment period. The chemical analysis of feed and forage (silage) was done in the Animal Nutrition Laboratory of BLRI. Milk Amino Acids (AAs) composition was analyzed in the Food Chemistry Lab, Chinese Academy of Agricultural Sciences. The experimental protocol was approved by the Animal Care and Use Committee of the BLRI.

Animal, Diet, and Management

Thirty-six (36) lactating buffalo crossbreds with an average daily milk yield of 3.33 to 4.00 Litre and a body weight of 350-420 kg were selected for this study. The buffaloes were divided into three groups according to Murrah, Nili-Ravi, and Mehsana crossbreds, having twelve (12) animals each. All buffaloes were supplied with Napier silage *ad libitum* while additional concentrate was fed at 3.5 g/kg of BW. During the

experimental period, buffaloes were fed their allotted diets (Table 1), while concentrate was supplied in two equal portions at 07:00 AM and 16:30 PM. Residuals of Napier silage were weighed daily prior to the morning feeding to determine daily DMI. The dried samples of the concentrate mixture and Napier silage (offered and leftover) were ground to pass through a 1-mm sieve in a MAC® WILLEY grinder and pooled, and samples were analyzed for proximate composition [18].

Table 1: Ingredients and Chemical Composition of the Diet

Items	Proportion, g/100g (dry basis)	
	Concentrate	Napier silage
Wheat bran	51.00	-
Soybean meal	1.00	-
Broken maize	45.50	-
Dicalcium Phosphate (DCP)	2.00	-
Salt	1.00	-
Total	100.00	-
DMI (kg/h/d)	3.00	8.25
Chemical composition of the diet		
DM (%)	88.03	17.12
Ash (%)	5.07	10.22
CP (%)	14.22	7.68
NDF (%)	24.40	85.61
ADF (%)	6.68	45.33
TDN (%)	69.68	51.03

DMI = dry matter intake; DM = dry matter; CP = crude protein; NDF = neutral detergent fibre; ADF = acid detergent fibre; TDN = total digestible nutrients.

Milk Sample Collection and Analysis

Individual milk samples were collected weekly from two successive milking to analyze milk constituents (total solids, fat, protein, and lactose content). In addition, milk compositional parameters were analyzed by an automated milk analyzer (Lactoscan SLP, MILKOTONIC Ltd., Bulgaria).

Milk Amino Acids Determination

The AA composition of buffalo milk samples was determined according to the method of Islam *et al.* [17]. The initial weight of milk for sample preparation was 1.5 ml. Subsequently, milk samples were thoroughly mixed and hydrolyzed using 10 ml of 6 mol/L H₂SO₄ in a sealed glass apparatus under a continuous nitrogen flow at 110°C for 24 h. Afterward, the mixture was centrifuged, and the supernatant was transferred to a 5

ml centrifuge tube and brought to a final volume of 5ml with 0.02 mol/L H₂SO₄. Finally, the mixture was filtered through a 0.22 mm syringe filter before AA analysis using an AA analyzer (L-8900 Amino Acid Analyzer, Hitachi, Japan).

Statistical Analysis

The data regarding various factors were presented as means and standard error of the means (SEM). The statistical program used in the present study was SPSS 16.0 software (IBM-SPSS Statistics, IBM Corp., Armonk, NY) [19], with Duncan's multiple comparisons. The effects of three buffalo crossbreds on milk components and AA profile were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Milk Components

Major milk components, i.e., milk protein, fat, lactose, and total solids (TS) contents of Murrah, Nili-Ravi, and Mehsana crossbred buffaloes, are shown in Table 2. The protein, fat, and TS contents of Murrah crossbred buffaloes were significantly higher than those of other crossbreds ($p < 0.05$). However, the lactose contents of all buffalo crossbreds did not show significant differences ($p > 0.05$) but followed only a trend. The results related to protein, lactose, and TS were consistent, and fat contents differed from the findings of Zhou *et al.* [15], Ren *et al.* [16], and Han *et al.* [20], who determined that Murrah buffaloes had higher milk protein contents than Nili-Ravi buffaloes. It was found that the mean milk protein content of Murrah was lower compared to that reported by Zhou *et al.* [15] and was found higher compared to the findings of Ren *et al.* [16], Sun *et al.* [21], and Han *et al.* [20]. It was also reported that the average milk protein content of Nili-Ravi was lower compared to those reported by Zhou *et al.* [15], Ren *et al.* [16], and Sun *et al.* [21] and was higher than the results of Han *et al.* [20].

Additionally, it was observed that the average lactose concentration of Murrah buffaloes was greater than those claimed by Zhou *et al.* [15], Sun *et al.* [21], and Han *et al.* [20], and a similar report was obtained by Ren *et al.* [16]. In the case of Nili-Ravi buffaloes, lactose content was observed lower than those observed by Zhou *et al.* [15] and Ren *et al.* [16] and was found higher than those reported by Sun *et al.* [21] and Han *et al.* [20]. A higher amount of average milk fat was observed in Murrah buffaloes than those reported by Zhou *et al.* [15], Ren *et al.* [16], Sun *et al.* [21], and Han *et al.* [20].

In Nili-Ravi buffaloes, fat levels were discovered to be greater than those noted by Zhou *et al.* [15], Ren *et al.* [16], and Han *et al.* [20] and were observed to be lower than those described by Sun *et al.* [21]. On the other hand, total solids contents of Murrah and Nili-Ravi buffaloes were found to be higher than those proclaimed by Zhou *et al.* [15], Ren *et al.* [16], and Han *et al.* [20]. However, the TS content of Nili-Ravi buffaloes was lower than that Sun *et al.* reported [21]. In the current study, it is evident that the milk protein content of Nili-Ravi buffalo crossbreds was lower than that of Murrah and Mehsana buffalo crossbreds, whereas the TS content of Murrah buffalo crossbreds was found to be the highest, followed by Mehsana and Nili-Ravi buffalo crossbreds.

Amino Acids Profile of Milk

Amino acids are the building block of protein and play an important role in the body. The quality of protein is largely determined by amino acid composition. The data regarding the milk amino acid composition of three buffalo crossbreds are presented in Table 3. It was found that milk from Murrah, Nili-Ravi, and Mehsana crossbred buffaloes were rich in Glutamic acid (0.90–1.00 g/100 g of milk) and poor in Cysteine (0.02–0.05 g/100 g of milk). These results were consistent with the findings of Zhou *et al.* [15], who reported that all tested samples of Murrah, Nili-

Table 2: Milk Components of Different Crossbred Buffaloes

Parameters	Murrah	Nili-Ravi	Mehsana	SEM	p-value
Milk Components, g/100 g of milk					
Protein	4.88 ^a	4.23 ^b	4.81 ^a	0.071	0.013
Fat	7.88 ^a	6.78 ^b	7.47 ^a	0.711	0.049
Lactose	5.51	5.11	5.20	0.111	0.410
Total Solids	21.52 ^a	18.11 ^c	20.00 ^b	1.125	0.022

Superscript letters in the row represent a significant difference ($p < 0.05$).

Table 3: Amino Acid Composition of Milk (g/100 g of Milk) from Different Buffalo Crossbreds

Amino acids	Murrah	Nili-Ravi	Mehsana	SEM	p-value
Essential amino acids					
Methionine	0.14	0.14	0.14	0.017	0.210
Valine	0.25	0.21	0.20	0.014	0.312
Lysine	0.45 ^a	0.35 ^c	0.40 ^b	0.001	0.010
Isoleucine	0.22 ^a	0.19 ^b	0.19 ^b	0.003	0.024
Phenylalanine	0.20 ^c	0.28 ^a	0.25 ^b	0.140	0.012
Leucine	0.45 ^a	0.40 ^b	0.39 ^b	0.005	0.033
Threonine	0.21	0.21	0.21	0.001	0.511
Non- essential amino acids					
Asparagine	0.36	0.36	0.36	0.002	0.310
Serine	0.24	0.24	0.25	0.041	0.490
Glutamic acid	1.00	0.98	0.90	0.014	0.310
Proline	0.51	0.51	0.51	0.011	0.394
Glycine	0.10	0.10	0.10	0.110	0.211
Alanine	0.16	0.16	0.16	0.120	0.110
Cysteine	0.05 ^a	0.02 ^b	0.02 ^b	0.075	0.001
Tyrosine	0.19	0.19	0.19	0.140	0.120
Histidine	0.15 ^a	0.11 ^b	0.12 ^b	0.120	0.033
Arginine	0.14	0.14	0.14	0.124	0.091
EAA	1.92 ^a	1.75 ^b	1.75 ^b	0.110	0.049
NEAR	2.88	2.69	2.72	0.441	0.054
TAA	4.80 ^a	4.44 ^b	4.47 ^b	0.112	0.044
EAA/NEAA (%)	67	65	64	0.412	0.510

EAA = essential amino acid, NEAA = non-essential amino acid, TAA = total amino acid. Superscript letters in row represent significant differences ($p < 0.05$).

Ravi, and Triple-Crossbred buffalo milk were rich in Glutamic acid and poor in Cysteine. Among all the essential amino acids, Leucine, Lysine, and Phenylalanine were the most abundant essential amino acids. On the other hand, Glutamic acid, Proline, Asparagine, and Serine were the most prominent non-essential amino acids. Notable distinctions ($p < 0.05$) were inspected for Lysine, Isoleucine, Leucine, Phenylalanine, Cysteine, and Histidine concentrations in the milk of three buffalo crossbreds, while the additional 11 AAs revealed no significant variations ($p > 0.05$). The concentration of Lysine, Isoleucine, Leucine, Cystine, and Histidine was determined to be significantly higher ($p < 0.05$) in the milk samples of Murrah buffalo crossbreds than those AAs in Nili-Ravi and Mehsana buffalo crossbreds milk. Contrarily, milk samples from Nili-Ravi buffalo crossbreds showed the highest level of Phenylalanine ($p < 0.05$), followed by milk samples from Mehsana and Murrah buffalo crossbreds. Zhou *et al.* [15] agreed that milk amino

acid composition is widely varied among different breeds.

The content of each AA in each milk specimen was represented by corresponding to the total AA concentrations in the milk samples so that milk AA profiles could be correlated beyond breeds. The AA pattern of Murrah, Nili-Ravi and Mehsana crossbred buffaloes recorded in the current study agrees with previously specified data [15, 16, 21, 22]. It was unambiguous that each buffalo milk specimen was rich in sources of essential amino acids and the dominant amino acids were Glutamic acid, followed by Leucine, Proline, Lysine, and Asparagine (Figure 1). Furthermore, we have calculated the Lysine: Methionine ratio, which indicated that the highest ratio was found in Murrah with about 3.20%, followed by Mehsana and Nili-Ravi each with 2.80 and 2.50%, respectively (Figure 2). The findings are in agreement with Medhammar *et al.* [22].

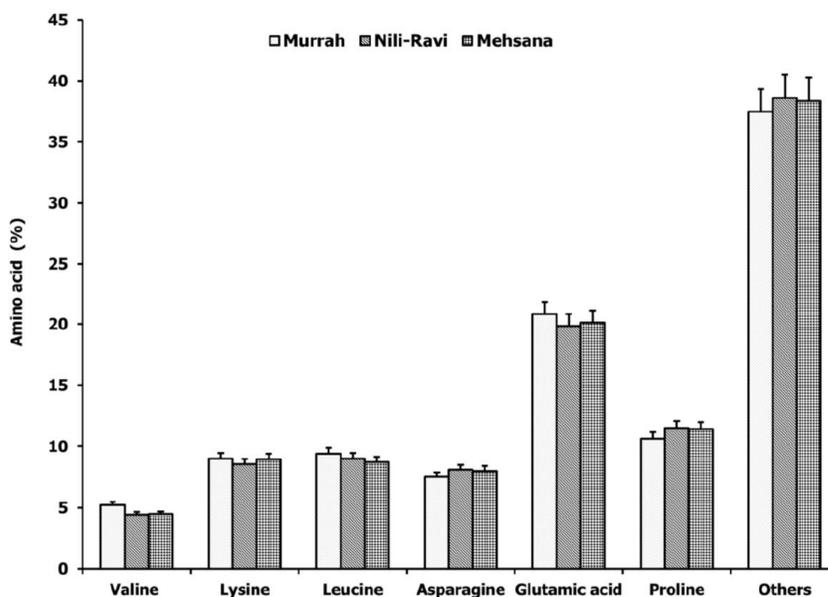


Figure 1: Amino acid (%) distribution of raw milk in three crossbred buffaloes.

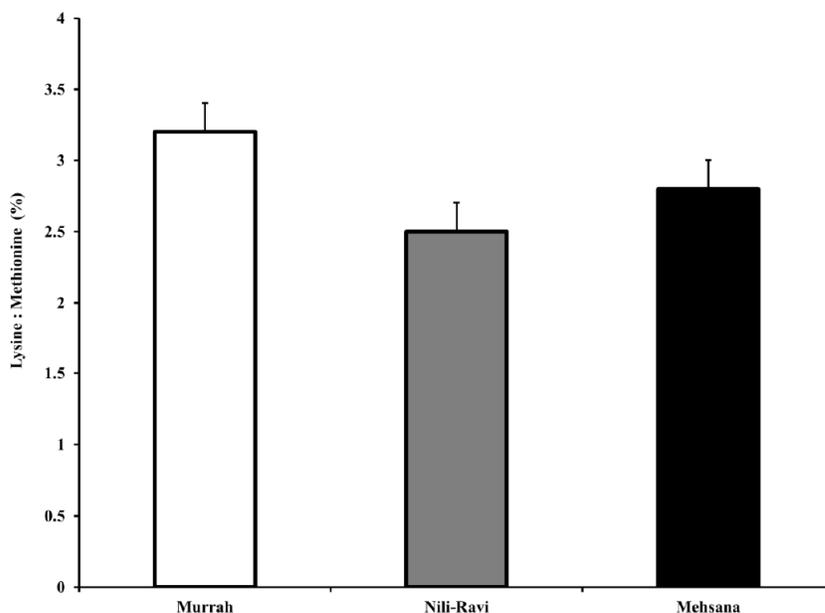


Figure 2: Lysine: Methionine ratio of raw milk in three crossbred buffaloes.

CONCLUSION

The analyzed raw buffalo milk components and amino acid profiles showed considerable variations among the different studied buffalo crossbreds. The milk of Murrah, Nili-Ravi, and Mehsana buffalo crossbreds was affluent in glutamic acid when traces in Cysteine contents. The results could be utilized for value-added dairy product promotion and making a variety of good quality dairy products. Consequently, they are essential for the dairy industry, particularly for milk processing. Besides, this information will help to improve the dairy industry economy, which could

dynamically contribute to regional development to ensure uniform and higher quality, nutritionally enriched buffalo milk. Furthermore, producers should be encouraged to access appropriate market linkage and information with initiatives in the dairy industry, such as the nutritional and health benefits of dairy products, to enhance sustainable buffalo production.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this research paper.

DATA AVAILABILITY

All the data and material of this research are available from the authors on request.

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