Raw Milk Components and Amino Acid Profiles Considerably Vary among Different Crossbred Dairy Buffaloes

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Abstract

This study aimed to assess the relationship between milk components and amino acid (AA) profiles among different crossbred buffaloes in Bangladesh. A total of thirty-six (36) lactating buffaloes were selected from Murrah, Nili-Ravi, and Mehsana crossbreds, and 03 groups were made, each with 12 buffaloes. The total experimental period was 10 weeks, including 10 days of diet adjustment. The results revealed that milk protein and total solids contents of Murrah, Mehsana, and Nili-Ravi crossbred buffaloes showed a higher to lower trend ($p < 0.05$). Considerable variations of milk protein, fat, lactose, and total solids contents were observed among Murrah, Nili-Ravi, and Mehsana crossbred buffalo. The highest amount of amino acid was Glutamic acid (0.9-1.00 g/100 g of milk), and the trace amount of amino acid was Cysteine (0.02–0.05 g/100 g of milk) among all the milk samples. 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 ($p < 0.05$) at the same time as the other 11 AA concentrations were found non-significant ($p > 0.05$). The highest Lysine: Methionine ratio observed in Murrah buffalo is about 3.20%, while in Mehsana and Nili-Ravi buffalo is about 2.80 and 2.50%, respectively. Thus, it is evident from the study that the raw milk components and amino acid composition vary considerably in the different crossbred lactating buffaloes. Finally, the compositional data of raw milk may create the way of sustainable use of milk from crossbred dairy buffaloes and improve food and nutrition security, particularly in developing countries.

Introduction

Buffalo (*Bubalus bubalis*) is the second vital livestock species after dairy cows, with 13% of total milk production and the highest annual growth rate [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 92.52% of the global population of 194.29 million [2]. Among the Asian countries, South Asia represents 79.74% of buffaloes, and the rest 20.26% in other countries [3]. Particularly, Bangladesh has diverse indigenous domestic buffalo resources dominated by the population of riverine except for some swamp types, especially in the eastern part of the country [4]. In addition, the crossbreds of Murrah, Nili-Ravi, Surti, and Jafarabadi are scantly 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 compared to 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 be used to 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 advantageous 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 and are very important for the dairy industry and animal feeding and breeding strategies. Consequently, a more detailed overview of milk composition is needed to set a standard on technological properties for milk processing, especially amino acid profiles. However, the dairy industry faces challenges regarding breeding and nutritional strategy to produce the most favourable quality 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 among two crossbreds of riverine buffalo viz. Murrah and Nili-Ravi and their crossbreds with local swamp buffalo. Besides, Rafiq et al. [17] correlated the AA profiles of buffalo, cow, sheep, goat, and camel milk.

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 innovative 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. The chemical analysis of feed and forage (silage) was done in the Animal Nutrition Laboratory of BLRI. Milk AA 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 crossbred buffaloes with an average daily milk yield of 3.33 to 4.00 L and 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 crossbred, 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, while concentrate was supplied to them 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].

Milk Sample Collection and Analysis

Individual milk samples were collected weekly from two successive milking for analyzer 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 Rafiq 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 H_2SO_4 in a sealed glass apparatus under a continuous nitrogen flow at 110^{o}C for 24 h. Afterwards, 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 H_2SO_4. Finally, the mixture was filtered through a 0.22 mm syringe filter before AA analysis using an amino acid 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 significant at $P < 0.05$.

Results And Discussion

Milk Components

Major milk components like 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 observed as significantly higher than those of other crossbreds ($p \leq 0.05$). However, the lactose contents of all buffalo crossbreds did not show any significant differences ($p > 0.05$) but were only a trend. However, the highest protein, fat, lactose and TS contents were observed at 4.88, 7.88, 5.51 and 21.52 g/100 g of milk, respectively, in Murrah, followed by 4.81, 7.47, 5.20, 20.00 g/100 g of milk, in Mehsana; whereas the lowest was observed 4.23, 6.78, 5.11 and 18.11 g/100 g of milk, respectively in Nili-Ravi. The results related to protein, lactose, and TS were found consistent, and fat contents were found different from the findings of Zhou et al. [15], Ren et al. [16], and Han et al. [20], who determined that Murrah buffaloes were higher milk protein contents than that of Nili-Ravi buffaloes. It was found that the mean milk protein contents of Murrah was lower compared to that reported by Zhou et al. [15] and was found higher by Ren et al. [16], Sun et al. [21] and Han et al. [20]. It was also reported that the average milk protein contents of Nili-Ravi were lower compared to those reported by Zhou et al.

[15], Ren et al. [16] and Sun et al. [21] and was obtained higher by Han et al. [20]. Furthermore, it was noted that the average lactose contents of Murrah buffaloes were higher than those stated 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].

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
PDF (%)	24.40	85.61
ADF (%)	6.68	45.33
TDN (%)	69.68	51.03

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 ($p \leq 0.05$).					

In Nili-Ravi buffaloes, fat contents were found to be higher 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], except lower was observed for Nili-Ravi buffaloes from those recorded by Sun et al. [21]. In the current study, it is evident that the milk protein and TS contents of Mehsana crossbred buffalo were higher than those of Nili-Ravi and lower than those of Murrah milk.

Amino Acids Profile of Milk

Amino acids are referred to as 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 crossbred buffaloes are presented in Table 3. It was found that milk from Murrah, Nili-Ravi and Mehsana crossbred buffaloes was rich in Glutamic acid (0.90–1.00 gm/100 gm of milk) and poor in Cysteine (0.02–0.05 gm/100 gm of milk). These results were found consistent with the findings of Zhou et al. [15] (2018), who reported that all tested samples of Murrah, Nili-Ravi and Triple-Crossbred buffalo milk is rich in Glutamic acid and poor in Cysteine. Among all the essential amino acids, Leucine, Lycine, 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 AA revealed no significant variation ($p > 0.05$). Combinations of Lysine, Glutamic acid and Histidine in Murrah and Mehsana buffalo milk were higher than that AA in Nili-Ravi buffalo milk. Zhou et al. [15] agreed that sort of milk amino acids composition is widely varied among different breeds.

Table 3
Amino acid composition of milk (gm/100 gm of milk) from different crossbreds of buffalo.

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	0.050
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 ($p \leq 0.05$).

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 (Fig. 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 (Fig. 2). The findings are in agreement with Medhammar et al. [22].

Conclusion

It may conclude that the raw buffalo milk components and amino acid profiles varied considerably among different crossbred buffaloes studied. The milk protein and fat were higher in Murrah than in Nili-Ravi and Mehsana crossbred buffaloes. The milk of Murrah, Nili-Ravi, and Mehsana crossbred buffaloes was affluent in glutamic acid when traces in Cysteine contents. These results from the study can help to promote dairy products of buffalo milk with the new value-addition and make the production systems sustainable. Specifically, more attention needs to be paid to the uniform and higher quality of the raw buffalo milk with selective breeding and production performance of the animals. Finally, country-specific data on milk composition and amino acid profiles are necessary to fulfil the growing demand of the dairy industry and meet the public need for nutrition.

Declarations

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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Figures

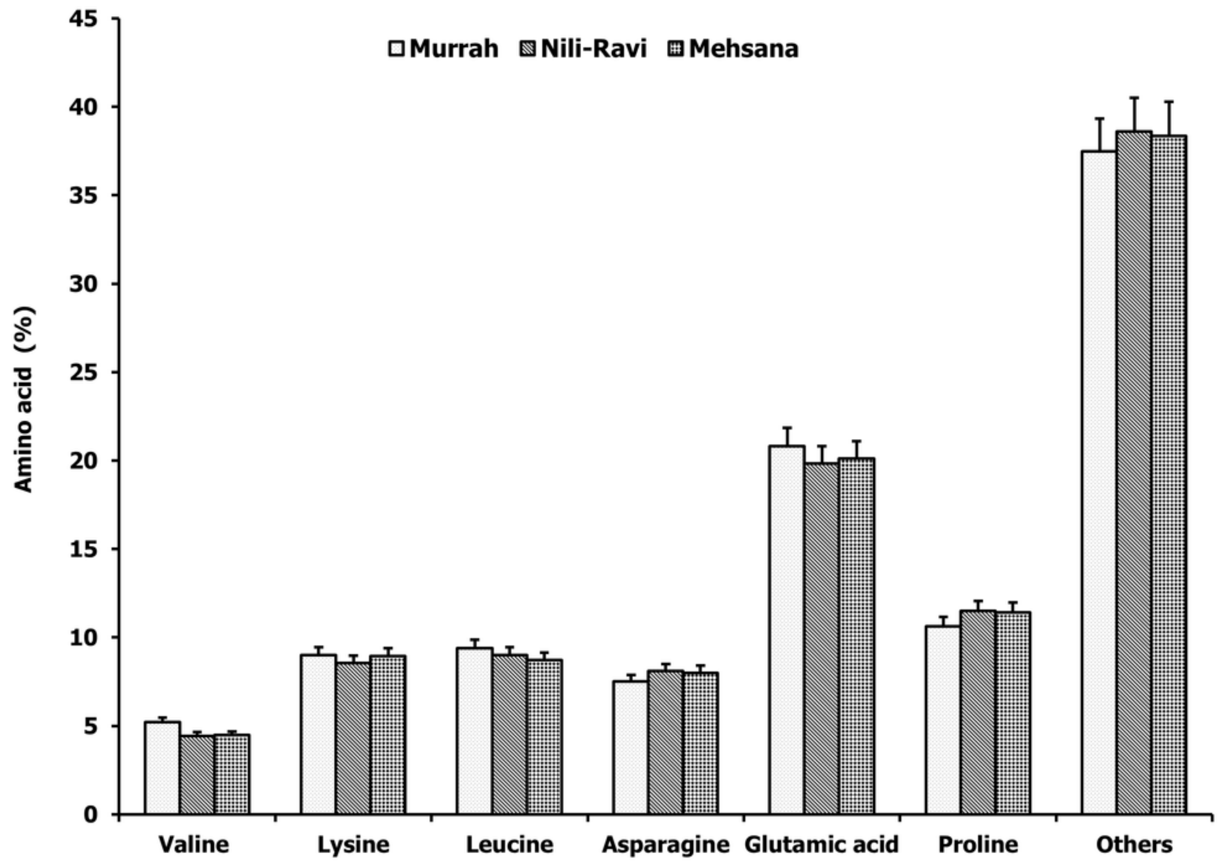

Figure 1

Amino acid (%) distribution of raw milk in three crossbred buffaloes

Figure 2

Lysine: Methionine ratio of raw milk in three crossbred buffaloes