A comparison of milk protein, fat, lactose, total solids and amino acid profiles of three different buffalo breeds in Guangxi, China

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ABSTRACT
This study compared the milk protein, fat, lactose, total solids and 17 AA profiles of Murrah buffalo, Nili-Ravi buffalo and crossbreed buffalo. Buffalo milk samples of Murrah (n=25), Nili-Ravi (n=20) and Triple-crossbred (n=23) were collected and milk protein, fat, lactose, total solids and 17 AA profiles were determined. Milk protein and total solids contents of Murrah buffalo were higher than those of Nili-Ravi buffalo (p<0.05). The average milk protein, fat, lactose and total solids contents of all buffalo samples were 4.76, 7.31, 5.19 and 18.40 g/100 g of milk, respectively. The AA composition of all three kinds of milk samples was rich in glutamic acid (0.89–0.96 g/100 g of milk) and poor in cysteine (0.01–0.02 g/100 g of milk). Leucine, lysine, valine and isoleucine were the most prevalent essential amino acids; while glutamic acid, proline, aspartic acid and serine were the most prevalent non-essential amino acids in the three kinds of milk samples. Significant differences (p<0.05) were observed for concentrations of Lys, Ile, Leu, Phe, Cys and His, while the other 11 amino acids had no significant differences (p>0.05). Conclusively, the milk of Murrah buffalo, Nili-Ravi buffalo and crossbreed buffalo was all rich in glutamic acid while poor in cysteine contents. After crossbreeding, the milk production of Triple-crossbred buffalo was higher than the local Chinese buffalo and its milk composition was in between those of Murrah and Nili-Ravi.

Introduction

Buffaloes (Bubalus bubalis) are important livestock species for milk and meat production worldwide, and are second in milk production after dairy cows with 13% of total milk production and highest annual growth rate (IDF 2009). Buffaloes are adapted to hot climates and high roughage feeding, and they are mostly raised in tropical and subtropical regions. China ranks third in the world regarding the number of dairy buffalo herds and production of buffalo milk. Buffalo milk is rich in protein and fat, and buffalo plays a significant role in milk production in Guangxi, China. Buffalo milk receives more research interest and investment, owing mainly to its attractive nutrient contents (Amarjit and Toshihiko 2003). Buffalo milk is more suitable for processing of milk products such as, cheese, butter, ice cream and yoghurt than cow’s milk (Fundora et al. 2001; Wedholm et al. 2006). In Guangxi, China, the average daily milk yield of local swamp buffalo is low. To increase milk production, the local government of the Guangxi Autonomous Region imported Murrah and Nili-Ravi buffalo breeds from India and Pakistan in 1957 and 1974, respectively. Animal breeding researchers worked and achieved excellent buffalo offsprings by crossbreeding the local Guangxi swamp buffalo with riverine buffalo breeds, for example, Murrah and Nili-Ravi.

Recently, Ren et al. (2015) compared milk protein and amino acid (AA) composition among two breeds of riverine buffalo (Murrah and Nili-Ravi) and their hybrids F1 and F2 (crossbred with local swamp buffalo). Saima et al. (2016) compared the AA profiles of buffalo, cow, sheep, goat and camel milk. However, little information is available on fat, protein, lactose and total solids contents and AA composition of buffalo milk from Guangxi, China, especially Triple-crossbred buffalo (Murrah buffalo, Nili-Ravi buffalo and local Guangxi swamp buffalo). Evaluation of protein, fat,
lactose, total solids contents and amino acid profile of buffalo milk can provide milk composition knowledge to milk producers, processors and consumers. Thus, the objective of this study was not only to determine the buffalo milk composition but also to compare the milk AA profile and protein, fat, lactose and total solids contents of Murrah buffalo, Nili-Ravi and Triple-crossbred buffalo in Guangxi, China.

Materials and methods

Animal care and ethics statement

Experimental protocol was approved by the Animal Care and Use Committee of the Buffalo Research Institute, Chinese Academy of Agricultural Sciences, and experimental buffalos were kept in accordance with Chinese standards. This study was conducted on an experimental farm, called Buffalo Breeding Farm in Buffalo Research Institute, Chinese Academy of Agricultural Sciences, Nanning, Guangxi, China.

General description of the experiment and experimental buffalo herds

Milk samples were collected from buffalos which housed at Buffalo Breeding Farm of the Buffalo Research Institute, Nanning, Guangxi, China. Buffalos were typically fed on both fresh and preserved fodder crops, such as elephant grass, corn and cassava, and they were offered varying amounts of grains and waste products from processed plant material. All experimental animals were selected from parity 2 or 3 with 30–40 days of milking period, and fed with the same diets (Table 1). Nutritive profile of diet was determined following the AOAC methods (AOAC 1997): dry matter, 930.15; crude protein, 928.08; neutral detergent fibre and acid detergent fibre, 973.18. In order to obtain a large sample set, experiment was conducted on two periods: 28 September 2015 (15 Murrah buffalo, six Nili-Ravi buffalo, 13 Triple-crossbred buffalo) and 15 January 2016 (10 Murrah buffalo, 14 Nili-Ravi buffalo, 10 Triple-crossbred buffalo). The term ‘Triple-crossbred buffalo’ in this article refers to crossbred of three breeds (Murrah, Nili-Ravi and local Guangxi swamp buffalo).

Milk sample collection and analysis

Milk samples were collected at 8:30 h and 16:30 h during experimental time period, and approximately 500 mL was collected per head per time. Milk samples collected in the morning and afternoon were separated into two parts; one part was used for immediate testing of milk protein, fat, lactose and total solids contents using multifunction analyser for dairy products (MilkoScan FT-120, FOSS Electric A/S, Hillerod, Denmark), and the second part was stored at 4°C until further used for AA testing. Morning milk samples and afternoon milk samples were mixed thoroughly in a fixed proportion of 1:1. The mixed sample was stored at −20°C until analysed for AA profile. Average milk protein, fat, lactose and total solids contents for morning and afternoon collected milk samples are given in Table 2.

Milk amino acids determination

The AA profiles of three kinds of buffalo milk samples were determined according to the Chinese standard method GB/T 5009.124-2003. The initial weight of animal milk for samples preparation was 1.5 g (accurate to 0.1 mg). Subsequently, animal milk samples were thoroughly mixed and hydrolysed using 10 mL of 6 mol/L hydrochloric acid in sealed glass ampules under continuous flow of nitrogen at 110°C for 24 h. The mixture was centrifuged, and the supernatant was transferred to a 5 mL centrifuge tube and brought to a final volume of 5 mL with 0.02 mol/L hydrochloric acid. The mixture was filtered through a 0.22μm syringe filter prior to AA analysis by using an amino acid analyser (L-8900 Amino Acid Analyzer, Hitachi, Tokyo, Japan). All the AA were analysed in the Research Center for Test and Analysis, Guangxi Zhuang Autonomous Region.

Table 1. Feed intake of experimental buffalos.

| Proportion, g/kg (air dry basis) |
|---------------------------------|
| **Concentrate composition**     |
| Maize grain                     | 540.00 |
| Wheat bran                      | 185.00 |
| Soy bean meal                   | 80.00  |
| Cottonseed meal                 | 150.00 |
| Stone powder                    | 20.00  |
| CaHPO₄·2H₂O                     | 15.00  |
| NaCl                            | 20.00  |
| Premix                          | 10.00  |
| Total concentrate intake, kg DM/d| 3.00   |
| **Forage composition**          |
| Corn silage                     | 600.00 |
| Cassava residuals               | 400.00 |
| Total intake, kg DM/d           | 11.92  |
| **Nutritional level of diet**   |
| Crude protein                   | 116.70 |
| Acid detergent fibre            | 469.50 |
| Neutral detergent fibre         | 324.40 |

Statistical analysis

All data were presented as means and standard error of the means (SEM). Variations in milk protein, fat,
lactose, total solids and amino acid profiles of three kinds of buffalo milk were analysed using restricted maximum likelihood variance components analysis and linear mixed models with removal of sample collection period, milk periods and parity factors. The statistical programme used in the present study was SPSS 17.0 software (IBM-SPSS Statistics, IBM Corp., Armonk, NY), with Duncan’s multiple comparison. The effects of three buffalo breeds on milk protein, fat, lactose, total solids and AA profile were declared significant at \( p < .05 \), unless otherwise noted, and trends were discussed at the \( p < .10 \) significance level.

### Results and discussion

#### Milk protein, fat, lactose and total solids contents

Milk protein, fat, lactose and total solids contents of Murrah buffalo, Nili-Ravi buffalo and Triple-crossbred buffalo are presented in Table 2. Lactose content in the milk from all buffalo breeds got no significance (\( p > .05 \)). However, milk protein, fat and total solids contents of Murrah buffalo were significantly higher than those of Nili-Ravi buffalo (\( p < .05 \)). These results were found consistent with the findings of Ren et al. (2015) and Han et al. (2007), who determined that milk protein content of Murrah was higher than that of Nili-Ravi. The average milk protein, fat and total solids contents of Murrah and Nili-Ravi were higher than those reported by Ren et al. (2015) and Han et al. (2007) and different from that reported by Sun et al. (2014). Sun et al. (2014), Ren et al. (2015) and Han et al. (2007) found the first (F1) and second (F2) generations of Murrah or Nili-Ravi × Guangxi swamp crossbred buffalo had higher protein and fat contents than those of Murrah or Nili-Ravi. Whereas, in the current study, the milk protein and fat contents of Triple-crossbred buffalo were higher than those of Nili-Ravi milk and lower than those of Murrah milk.

Milk protein, fat, lactose and total solids contents of buffalo milk published in some previous studies (g/100 g of milk) are presented in Table 3. In the current study, the average milk protein, fat, lactose and total solids contents of three kinds of milk samples were similar to those reported by Medhammar et al. (2012). In this study, the average fat corrected daily milk yield for Murrah and Nili-Ravi were 12.62 and 12.00 kg, respectively. Whereas, Triple-crossbred buffalo produced 12.34 kg average fat corrected daily milk yield. The milk protein, fat, lactose and total solids contents of Triple-crossbred in the current study were in between those of pure riverine buffalo breeds, Murrah and Nili-Ravi. It is well known that there is a negative correlation between milk yield and milk composition. This explains why the Triple-crossbred buffalo contained higher levels of these components than Nili-Ravi buffalo and lower levels than Murrah buffalo. Overall, the fat content of buffalo milk was double than that of milk from Holstein cows reared in China. The total solids content of buffalo milk were 1.5 times that of Holstein cow milk (Table 3).

#### Amino acids composition of milk

Proteins in milk play a crucial role in nutrition, especially in developing countries where diets are mostly cereal based. Data on milk AA profiling at breed level for buffalo are very limited. The concentrations of 17 AA (g of AA/100 g of milk) in buffalo milk are presented in Table 4. The AA composition of Triple-crossbred buffalo milk was similar to the AA composition of Murrah and Nili-Ravi buffalo milk. All samples tested were rich in Glu (0.89–0.96 g/100 g of milk) and poor in Cys (0.01–0.02 g/100 g of milk). Leu, Lys, Val and Ile were the most abundant essential amino acids. Leu plays a distinct role in protein metabolism and the translation initiation pathway of muscle protein synthesis. Glu, Pro, Asp and Ser were the most abundant non-essential

### Table 2. Milk protein, fat, lactose and total solids contents and milk yield of buffalo milk from different breeds of buffalo.

| Breeds              | Murrah | Nili-Ravi | Triple-crossbred | SEM | \( p \) Value |
|---------------------|--------|-----------|------------------|-----|----------------|
| n                   | 25     | 20        | 23               |     |                |
| Milk yield, kg/d    |        |           |                  |     |                |
| Daily Milk yield    | 8.09   | 8.70      | 8.10             | 0.725 | .862           |
| Daily 4% fat correct milk yield | 12.62 | 12.00 | 12.34 | 0.508 | .884 |
| Daily milk protein yield | 395.51 | 392.81 | 394.37 | 34.141 | .900 |
| Daily milk fat yield | 625.61 | 567.91 | 605.41 | 59.878 | .607 |
| Milk composition, g/100 g of milk |        |           |                  |     |                |
| Protein             | 4.92a  | 4.54b     | 4.82a            | 0.089 | .015           |
| Fat                 | 7.82a  | 6.77b     | 7.34ab           | 0.811 | .078           |
| Lactose             | 5.18   | 5.28      | 5.13             | 0.091 | .332           |
| Solids              | 19.03a | 17.74b    | 18.44ab          | 0.925 | .024           |

Means in the same row with different superscripts are different at \( p < .05 \). SEM: standard error of mean.

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amino acid. Significant differences ($p < .05$) were observed for Lys, Ile, Leu, Phe, Cys and His concentrations in the milk of three buffalo breeds, while other 11 AA showed no significant difference ($p > .05$, Table 4).

Concentrations of Lys, Ile, and Cys in Murrah and Triple-crossbred buffalo milk were higher than those amino acids in Nili-Ravi buffalo milk ($p < .05$). Concentrations of Leu and His in Murrah buffalo milk were higher than those in Nili-Ravi buffalo milk ($p < .05$). Because the total AA composition of milk varied so widely among different breeds, the content of every individual AA in every milk sample was expressed relative to the total amino acid concentrations in the milk samples so that milk AA patterns could be compared across breeds. When the absolute concentrations of the individual amino acids were normalised to percentage of total AA, the variation in the data decreased considerably, indicating high consistency in the AA profile of buffalo milk (Figure 1). The AA profile of water buffalo reported in the current study is in agreement with previously reported data (Sun et al. 2014; Medhammar et al. 2012; Ren et al. 2015).

### Table 3. Milk protein, fat, lactose and total solids contents of buffalo and cow milk published in the literature (g/100 g of milk).

| Breeds      | Feeding area | Samples | Protein | Fat | Lactose | Total solids | References                  |
|-------------|--------------|---------|---------|-----|---------|--------------|------------------------------|
| Buffalo     | Pakistan     | 6       | 4.25    | 6.58| –       | –            | Rafiq et al. (2016)          |
| Murrah      | China        | 14      | 4.52    | 6.53| 5.51    | 16.53        | Ren et al. (2015)            |
| Nili-Ravi   | China        | 14      | 4.27    | 6.43| 5.68    | 16.47        | Ren et al. (2015)            |
| Buffalo F1  | China        | 10      | 5.15    | 7.38| 5.7     | 18.38        | Ren et al. (2015)            |
| Buffalo F2  | China        | 10      | 4.89    | 6.96| 5.61    | 17.46        | Ren et al. (2015)            |
| Murrah      | China        | 16      | 4.27    | 6.57| 5.07    | 16.69        | Han et al. (2007)            |
| Nili-Ravi   | China        | 16      | 4.16    | 6.53| 4.56    | 17.14        | Han et al. (2007)            |
| Buffalo F1  | China        | 32      | 5.23    | 8.81| 4.8     | 19.75        | Han et al. (2007)            |
| Buffalo F2  | China        | 32      | 5.1     | 7.9 | 4.64    | 19.21        | Han et al. (2007)            |
| Buffalo F1  | China        | 16      | 4.75    | 7.56| 4.61    | 18.22        | Han et al. (2007)            |
| Murrah      | China        | 18      | 4.75    | 6.86| 4.6     | 17.07        | Sun et al. (2014)            |
| Nili-Ravi   | China        | 18      | 5.14    | 7.99| 4.74    | 19.8         | Sun et al. (2014)            |
| Buffalo F1  | China        | 18      | 5.78    | 8.35| 4.59    | 20.05        | Sun et al. (2014)            |
| Buffalo F2  | China        | 18      | 5.51    | 8.69| 4.45    | 20.22        | Sun et al. (2014)            |
| Buffalo     | Multi-area   | 4       | 4       | 7.4 | 4.4     | 16.8         | Medhammar et al. (2012)      |
| Holstein cow| China        | 5       | 3.05    | 3.35| 5.09    | 11.80        | Wang et al. (2017)           |
| Holstein cow| America      | 170     | 3.25    | 2.54| 4.83    | 11.54        | Faisao et al. (2017)         |

F1, F2: 1st and 2nd generation of crossbreed buffalo (Murrah or Nili-Ravi buffalo × Guangxi local swamp buffalo). FH: crossbreed buffalo (multi-cross breeds); n: number of samples.

### Table 4. Amino acid composition (g of AA/100 g of milk) of milk from different breeds of buffalo.

| Amino acids | Murrah (n = 25) | Nili-Ravi (n = 20) | Triple-crossbred (n = 23) | SEM | p Value |
|-------------|-----------------|-------------------|---------------------------|-----|---------|
| EAA         |                 |                   |                           |     |         |
| Met         | 0.11            | 0.11              | 0.10                      | 0.006 | .102 |
| Val         | 0.27            | 0.24              | 0.26                      | 0.019 | .256 |
| Lys         | 0.40a           | 0.35b             | 0.39a                     | 0.027 | .020 |
| Ile         | 0.25a           | 0.21b             | 0.24a                     | 0.017 | .024 |
| Phe         | 0.20a           | 0.18b             | 0.19b                     | 0.005 | .021 |
| Leu         | 0.44a           | 0.40b             | 0.42ab                    | 0.023 | .029 |
| Thr         | 0.19            | 0.19              | 0.19                      | 0.007 | .312 |
| NEAA        |                 |                   |                           |     |         |
| Asp         | 0.35            | 0.32              | 0.34                      | 0.011 | .110 |
| Ser         | 0.22            | 0.22              | 0.22                      | 0.011 | .424 |
| Glu         | 0.95            | 0.89              | 0.96                      | 0.040 | .248 |
| Pro         | 0.46            | 0.42              | 0.43                      | 0.011 | .255 |
| Gly         | 0.08            | 0.08              | 0.08                      | 0.002 | .149 |
| Ala         | 0.14            | 0.13              | 0.14                      | 0.005 | .097 |
| Cys         | 0.02a           | 0.01b             | 0.02a                     | 0.004 | .003 |
| Tyr         | 0.17            | 0.18              | 0.16                      | 0.018 | .103 |
| His         | 0.11a           | 0.10b             | 0.11a                     | 0.006 | .037 |
| Arg         | 0.12            | 0.11              | 0.12                      | 0.004 | .067 |
| EAA         | 1.94            | 1.76              | 1.87                      | 0.112 | .058 |
| NEAA        | 2.63            | 2.46              | 2.58                      | 0.075 | .074 |
| TAA         | 4.57b           | 4.22b             | 4.45b                     | 0.111 | .048 |
| EAA/TAA%    | 42.48           | 41.75             | 41.93                     | 0.329 | .277 |
| Lys/Met     | 3.84            | 3.16              | 3.76                      | 0.152 | .000 |

AA: amino acid; EAA: essential AA; NEAA: non-essential AA; TAA: total amino acid; EAA/TAA: the ratio of EAA to TAA; SEM: standard error of mean.

Means in the same row with different superscripts are different at $p < .05$. 

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Concentrations of amino acids (g of AA/100g of milk protein) of Murrah buffalo milk, Nili-Ravi buffalo milk and crossbred buffalo milk were approximately the same ($p > .05$), except for Met and Cys (Table 5).

It was evident that all buffalo milk samples were rich source of essential amino acids and the predominant amino acids were Glu, followed by Leu, Pro, Lys and Asp.
Conclusions

Milk protein, fat and total solids contents of Murrah buffalo were higher than those of Nili-Ravi and crossbred buffalo. The milk of Murrah buffalo, Nili-Ravi buffalo and crossbreed buffalo were all rich in glutamic acid while poor in cysteine contents. After crossbreeding, the milk production of Triple-crossbred buffalo was higher than local Chinese buffalo and its milk composition was in between those of Murrah and Nili-Ravi.

Disclosure statement

We certify that there is no conflict of interest with any financial organisation regarding the material discussed in the manuscript.

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