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A Comparison of Milk Protein, Amino Acid and Fatty Acid Profiles of River Buffalo and Their F1 and F2 Hybrids with Swamp Buffalo in China

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Abstract. The objective of this study was to compare milk protein, amino acid and fatty acid content and composition among two breeds of river buffalo (Murrah and Nili-Ravi), and their hybrid F1 and F2 (crossbreeding with local swamp buffalo). The protein and fat content in milk from crossbreed F1 and F2 were significant higher than that of river buffalo, but the milk yield was still lower than Nili-Ravi and Murrah. The protein composition in crossbreed milk was similar to Nili-Ravi, except κ-casein. Most of amino acid profile of crossbreed milk was similar with river water buffalo, however, significant difference was found in three essential amino acids (Met, Leu and Phe) and four nonessential amino acids (Ser, Glu, Ala and Cys). The fatty acid profile of crossbreed milk was also similar with Nili-Ravi milk, but the content of SFA was lower and UFA content was higher than Murrah milk. However, PUFA content in crossbreed's milk was lower than that of river buffalo. In brief, crossbreeding improved the milk quality and yield of Chinese swamp buffalo, but the crossbreed's milk has similar nutrition to the river buffalo such as Murrah and Nili-Ravi.

Key words: Water buffalo, crossbreed, milk protein, fatty acid profiles, amino acid.

INTRODUCTION

Milk production from buffaloes is ranked the second after cow, being around 13% of the total milk production (87.5 million tonnes per year) in the world, with highest annual growth rates (IDF, 2009). Buffalo milk receives increasing research interest and investment in many countries, owing mainly to its attractive nutrient content, flavour and taste (D'Ambrosio *et al.*, 2008; Nanda and Nakao, 2003; Tahira *et al*., 2014). Because of its high contents of nutrients, buffalo's milk is more suitable for processing of milk products, such as cheese, butter fat, ice cream and yoghurt, than cow's milk (Fundora *et al.*, 2001; Wedholm *et al.*, 2006).

China is one of the largest producers of buffalo milk. Both buffalo herds (more than 23 million) and buffalo milk production listed third all over the world, after India and Pakistan. Most of the local buffalo belong to swamp type, with excellent animal labor but poor milk producer. Therefore, river type of buffalo breeds such as Murrah and Nili-Ravi has been introduced from India and Pakistan, and crossbreed with the native swamp buffalo to improve the milk performance during the last few years. The microbiological and chemical composition of different types of buffalo milk have been reported in China (Han *et al.*, 2007), but little information is available on their milk quality and nutritive value, especially amino acid and fatty acid composition.

Buffalo milk is rich with nutrient content from a compositional point of view. Particularly, fat and protein constitute the main fraction of buffalo milk and are responsible for its high energetic and nutritive value. The objective of this study is to compare the milk performance, amino acid and fatty acid composition among different types of buffalo. The physicochemical parameters of protein and fat were analyzed to reveal the quality and nutritional value of buffalo milk.

MATERIALS AND METHODS

Sampling

Buffalo milk samples were obtained from Buffalo Research Institute located in Nanning, Guangxi province of China. A total of 48 buffalo

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milk samples were collected from four types of buffalo representing the typical breeds in Guangxi province. These samples included crossbreeds of river buffalo × swamp buffalo (F1 and F2, the 1st and 2nd generations, 10 samples each), and pure river buffalo (Murrah and Nili-Ravi, 14 samples each). All of the animals from which milk samples were taken at their mid-lactation, were fed on the same diets. About 500 ml of milk sample was taken for each type from the milk container into sterile glass bottles. The milk sample was collected less than 15 min during milking at ambient temperatures and stored at 4°C before analysis. Milk protein and fat content were analyzed by infrared using the Milko Scan FT (Foss Electric, Denmark). The milk yield data also collected, included total milk yield, and total milk yield adjusted for 305 days.

Milk protein profiles

Milk samples were prepared following the method by Bobe *et al*. (1998). No preliminary separation or precipitation procedures of the casein fraction were required. The concentration of milk protein in the final diluted solution was approximately 4 mg/mL, detected by RP-HPLC. The gradient elution was carried out following the method of Bonfatti *et al*. (2008). The flow rate was 0.5 ml/min, the column temperature was kept at 45 °C and the detection was made at a wavelength of 214 nm. The injection volume was 10 µl.

Amino acid profiles

Thawed milk samples were thoroughly mixed and hydrolyzed using 6 M HCl in sealed glass ampullae for 22 h at 110°C. The hydrolyzate was centrifuged and the supernatant was used for AA analysis after filtering through a 0.22 µm syringe filter. The amino acid composition of the hydrolysate was determined using a 12-cm high-performance sodium column with sodium buffers by an amino acid analyzer (Beckman 6300 amino acid analyzer, Beckman Instruments, Palo Alto, California). The amino acids standards were purchased from Sigma (St Louis, MO, USA). Amino acids include free amino acids and amino acids in proteins were determined. The results were expressed as percentages of each amino acid to total amino acids.

Milk fatty acid profiles

The total lipids of milk samples were extracted using the method described by Bligh and Dyer (1959). Transmethylation was performed using sodium methylate and methanolic BF_3 and dissolved in hexane (Bligh and Dyer, 1959; Lopez-Lopez *et al.*, 2001). Fatty acids were separated and quantified using a gas chromatograph (Agilent 6890). An aliquot µl of the hexane phase were injected in split-mode (100:1) onto a fused 5% phenyl methyl polysiloxane column (DB-23; 30 m × 0.32 mm id and a film diameter of 0.25µm; Santa Clara, CA, USA). Fatty acid quantification was achieved by utilizing the internal standard method, using erucic acid (C22:1, F-45629, Sigma-Aldrich, USA) as standard. Data acquisition and processing were performed with Agilent-Chemstation software for gas chromatographic systems.

Statistical analysis

All data are expressed as mean and SEM. Difference among milks from different buffalo types was studied by variance of analysis. One-way ANOVA was applied to the different parameters in accordance with the GLM. All analyses were completed using SPSS (version 17.0) for Windows. Least significant differences were used to separate means at $P<0.05$.

RESULTS AND DISCUSSION

Chemical analysis of milk

Milk yield and chemical composition of buffalo milk are presented in Table I. The milk yield of crossbreeds (F1 and F2) was similar with Murrah, but significantly lower than Nili-Ravi ($P<0.05$). In this study, the milk yield of crossbreed was significant higher than local swamp buffalo (less than 2 000 kg) and similar with the Murrah, but still significantly lower than Nili-Ravi. The 305 day milk yield of buffalo was slightly lower compared with other research (Campanile *et al.*, 2008).

Protein, fat and total solids content in crossbreed milk, especially for F1 were significantly higher than those in the river buffalo, Murrah and Nili-Ravi. Compared with cow milk and Jersey milk, buffalo milk had higher protein, fat, lactose and total solid contents (Lindmark-Mansson *et al.*, 2003).

Table I.- Chemical analysis of milk from river buffalo and their F1 and F2 hybrid with swamp buffalo.

	Buffalo breed⁺				SEM⁻
	M (n=14)	N (n=14)	F1 (n=10)	F2 (n=10)	
Milk yield (kg)	2230[a]	2670[b]	2033[a]	2113[a]	448.91
Protein (%)	4.52[a]	4.27[a]	5.15[b]	4.89[ab]	0.91
Fat (%)	6.53[a]	6.43[a]	7.38[b]	6.96[ab]	0.81
Lactose (%)	5.51	5.68	5.70	5.61	0.22
Total solids (%)	16.53[a]	16.47[a]	18.38[b]	17.46[ab]	1.09

⁺M, N: river buffalo Murrah and Nili-Ravi; F1, F2: 1st and 2nd generations of crossbreed buffalo (river × swamp); ⁻SEM = Standard error of least squares means; [ab]Means bearing different superscripts in the same column differ significantly ($P < 0.05$).

Table II.- Protein profiles (%) of milk from river buffalo and their F1 and F2 hybrid with swamp buffalo.

	Buffalo breed⁺				SEM⁻
	M (n=14)	N (n=14)	F1 (n=10)	F2 (n=10)	
Total casein	75.17	75.97	75.83	74.23	3.06
α_{s1}-casein	26.09	27.19	27.80	27.03	2.44
α_{s2}-casein	12.37	12.02	11.33	12.19	2.05
β-casein	24.56	23.02	25.39	25.32	3.15
κ-casein	10.96[a]	13.75[b]	12.16[ab]	12.83[b]	2.02
β-lactoglobulin	12.29	11.33	11.27	11.02	1.64
α-lactalbumin	7.10	6.37	6.78	7.24	1.43
Others	6.82[b]	6.16[ab]	5.09[a]	5.05[a]	0.68

⁺M, N: river buffalo Murrah and Nili-Ravi; F1, F2: 1st and 2nd generations of crossbreed buffalo (river × swamp); ⁻SEM = Standard error of least squares means; [ab]Means bearing different superscripts in the same column differ significantly ($P < 0.05$).

The average of total solids, fat and protein contents were slightly lower than those reported by Han *et al*. (2007) and Zeng *et al*. (2007). Our findings are consistent with other reports that total solids, protein and fat contents in milk of crossbreed were higher than those of pure river buffalo, but lower than the local swamp buffalo (Han *et al.*, 2007).

Protein composition comparison

The protein composition of buffalo milk is presented in Table II. The content of total casein in crossbreed milk was similar with the river buffalo, but the κ-casein content was the highest in Nili-Ravi milk, and the lowest in Murrah milk. No significant differences were observed for the average counts of other caseins. However, the milk of Murrah contained more whey protein except β-lactoglobulin and α-lactalbumin than Nili-Ravi, F1 and F2 milk, suggesting that the milk of Murrah may contain more functional protein, such as lactoferrin, bovine serum albumin and immunoglobulin than other breeds. No significant difference was found in the main whey protein, β-lactoglobulin and α-lactalbumin among different types of milk.

To a large extent, the protein composition determines the nutritional value and the technological properties of milk. Especially, cheese yield increases with casein concentration, and cheese properties like milk coagulation time and curd firmness depend on the casein composition (Wedholm *et al.*, 2006). Milk with high concentrations of κ-casein and has high ratio of κ-casein to total casein is superior for cheese making (Wedholm *et al.*, 2006). Data from Tables I and II showed that the milk of F1 and F2 may be suitable for cheese-making more than pure river buffalo, because of its high concentration of κ-casein and high ratio of κ-casein to the total casein in milk.

Amino acid profile

Amino acid composition of four types of water buffalo milk is showed in Table III. For the essential amino acids (EAA), significant difference is found in Met, Leu and Phe (P<0.05). After hybrid,

Table III.- Amino acid profiles (%) of milk from river buffalo and their F1 and F2 hybrid with swamp buffalo.

	Buffalo breed⁺				SEM⁻
	M (n=14)	**N (n=14)**	**F1 (n=10)**	**F2 (n=10)**	
EAA					
THR	3.44	3.76	3.37	3.46	0.07
VAL	6.29	6.11	6.24	6.03	0.06
MET	2.65^{b}	2.31^{a}	2.61^{ab}	2.54^{ab}	0.05
ILE	5.70	5.43	5.71	5.61	0.05
LEU	10.35^{b}	9.82^{a}	10.41^{b}	10.12^{ab}	0.09
PHE	4.97^{b}	4.88^{b}	4.95^{b}	5.29^{a}	0.21
LYS	8.14	7.85	8.20	8.38	0.16
Total	41.54^{ab}	40.17^{a}	41.49^{ab}	41.92^{b}	0.27
NEAA					
ASP	6.74	6.64	6.80	6.51	0.06
SER	3.74^{a}	4.57^{b}	3.75^{a}	4.30^{ab}	0.14
GLU	19.65^{a}	20.88^{b}	19.59^{a}	19.71^{a}	0.18
GLY	1.85	1.83	1.92	1.90	0.02
ALA	3.28^{b}	3.09^{a}	3.29^{b}	3.28^{b}	0.03
TYR	5.08	4.94	5.09	4.98	0.03
CYS	1.95^{ab}	2.13^{b}	1.89^{ab}	1.63^{a}	0.07
HIS	2.61	2.53	2.61	2.60	0.03
ARG	2.89	2.80	2.91	2.75	0.04
PRO	10.68	10.41	10.65	10.41	0.10
Total	58.46^{ab}	59.83^{b}	58.51^{ab}	58.08^{a}	0.27

⁺M, N: river buffalo Murrah and Nili-Ravi; F1, F2: 1st and 2nd generations of crossbreed buffalo (river × swamp); ⁻SEM = Standard error of least squares means; [ab]Means bearing different superscripts in the same column differ significantly (P < 0.05).

the Phe content in F2 is significantly higher than Murrah, Nili-Ravi and F1. The top content of EAA was Leu and Lys, about 10% and 8% respectively, the lowest EAA is Thr (3.37~3.76%) and Met (2.31~2.65%). Compared with Nili-Ravi, the total content of EAA in F2 was higher (P< 0.05). The ten kinds of nonessential amino acids (NEAA) of four type milk are showed in Table III. Significantly difference (P< 0.05) is found in four amino acids (Ser, Glu, Ala and Cys). Different with EAA, the Ser, Glu and Cys was highest, and Ala is lowest in F2. For the ten kinds of NEAA, the Glu content was highest, nearly 20%, then is Pro, about 10%, the Gly is lowest, less than 2%.

The content of three kinds of EAA and four type of NEAA was found significant difference among the Murrah, Nili-Ravi, F1 and F2 milk. Except that, the total EAA was also different among them. This difference could be attributed to the different content of protein in milk, such as the κ-casein and whey protein as showed in Table II. In accordance with our study, other researchers also found crossbred could effect the milk composition, such as Nguni and local crossbred cows in South Africa (Mapekula *et al.*, 2011). Different with this study, their results showed that the EAA in crossbred cows is lower than Nguni, although crossbred significantly improved the milk yield. This difference may due to the different cow breed we used. Trp is another important EAA that need further study. The amino acids and protein composition could be used to breed selection from the view of nutrition in future.

Fatty acid profiles comparison

Table IV shows the fatty acid compositions in the four types of buffalo milk. Fatty acids were grouped into the saturated fatty acids (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA). Buffalo milk contains at least eleven fatty acids in amounts over 1%: C6:0, C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C18:0, C16:1. C18:1 and C18:2*n*-6.

Table IV.- Fatty acid profiles (%) in the milk from river buffalo and their F1 and F2 hybrid with swamp buffalo.

	Buffalo breed+				SEM-
	M (n=14)	N (n=14)	F1 (n=10)	F2 (n=10)	
Saturated					
C6	3.19	2.23	2.62	2.44	1.27
C8	1.90[b]	1.23[a]	1.37[ab]	1.29[ab]	1.43
C10	3.18[b]	2.21[a]	2.33[a]	2.24[a]	2.48
C11	0.05	0.22	0.32	0.04	0.16
C12	3.28[b]	2.40[ab]	2.30[a]	2.48[ab]	0.66
C13	0.11	0.11	0.10	0.10	0.10
C14	11.82	11.38	10.76	10.59	1.62
C15	1.13	1.25	1.23	1.19	0.18
C16	31.87	29.59	31.11	30.71	5.75
C17	0.58	0.66	0.67	0.67	0.11
C18	11.09	12.34	11.95	13.07	2.09
C20	0.29	0.25	0.29	0.32	0.15
C24	0.15	0.13	0.04	0.07	0.11
Total	68.41	63.85	64.96	65.01	5.24
Monounsaturated					
C14:1	0.63	0.64	0.70	0.57	0.21
C15:1	0.09	0.18	0.21	0.15	0.19
C16:1	1.58	1.70	1.75	1.45	0.43
C17:1	0.25	0.27	0.27	0.27	0.07
C18:1	21.14	24.42	24.19	24.42	3.68
C20:1	0.23	0.26	0.27	0.28	0.04
Total	23.85[a]	27.32[b]	27.12[b]	26.85[b]	3.93
Polyunsaturated					
C18:2*n*6	1.69	1.83	1.80	1.75	0.32
C18:2(CLA)	0.21	0.21	0.17	0.18	0.06
C18:3*n*6	0.06	0.05	0.05	0.08	0.02
C18:3*n*3	0.22[a]	0.29[b]	0.31[b]	0.34[b]	0.12
C20:3n6	0.07	0.07	0.11	0.09	0.01
C20:4*n*6	0.19[b]	0.16[ab]	0.10[a]	0.16[ab]	0.07
C22:6*n*3	0.12	0.10	0.12	0.14	0.04
Total	2.38	2.49	2.27	2.31	0.45
n-6	1.85	1.94	1.80	1.82	0.35
n-3	0.32	0.38	0.37	0.39	0.12
n-6/*n*-3	6.41[b]	5.62[ab]	5.11[a]	5.13[a]	1.95

+M, N: river buffalo Murrah and Nili-Ravi; F1, F2: 1st and 2nd generations of crossbreed buffalo (river × swamp); -SEM = Standard error of least squares means; [ab]Means bearing different superscripts in the same column differ significantly ($P < 0.05$).

The SFA accounted for 63.9-68.4% of the total fatty acids in buffalo milk lipid (Table II), similar with the Indian, Italian and Pakistan buffalo milk (69.5, 67.7, and 63.9%, respectively) (Achaya and Banerjee, 1946; Polidori *et al.*, 1997; Talpur *et al.*, 2008). The SFA were higher in Murrah milk (68.4%), followed by F1 (65.0%) and F2 milk (65.0%), and the lowest (63.8%) in the Nili-Ravi milk. Compared with other three types, Murrah milk contained higher content of C8, C10 and C12 ($P<0.05$). Short chain fatty acids are important energy sources for colonocytes and are assumed to play a key role in gut health (Bloemen *et al.*, 2009, 2010). Therefore, Murrah milk may be suitable for the patients with diminished liver or gut function. No significant difference was found in the medium- and long-chain saturated fatty acids among the four types of milk.

Milk from Murrah contained 23.9% of MUFA, significant lower than other three: 27.3% for Nili-Ravi, 27.1% for F1 and 26.9% for F2 ($P<0.05$). Oleic acid (C18:1) in Murrah milk (21.1%) was lower than Nili-Ravi (24.4%), F1 (24.2%) and F2 (24.4%). Oleic acid is considered an important energy source, accounting for more than 80% of the MUFA. Milk from Italian buffalo, contained 29.3% of total MUFA (Polidori *et al*., 1997), and the content of MUFA in Pakistan buffalo was 27.2% (Talpur *et al*., 2008).

Content of PUFA were 2.27-2.49% of total fatty acids (Table III) in milk from Nili-Ravi, slightly higher than other three breeds. The milk of Murrah had significantly lower content of C18:3*n*3, but significantly higher content of C20:4*n*6 than Nili-Ravi, F1 and F2 ($P<0.05$). No significant difference was detected among them in C18:2*cis*, C18:2*tran*, C18:3*n*6, C20:3*n*6 and C22:3*n*6. The essential fatty acid, linoleic acid, was 1.69-1.83% in Chinese buffalo milk, a little higher than that of Italian buffalo (1.18%) and Pakistan buffalo (1.17%) (Polidori *et al*., 1997; Talpur *et al*., 2008). This may attribute to that the Chinese buffalo consume more vegetable oil.

The PUFA, such as linoleic acid and conjugated linoleic acid (CLA) has been shown to have potential health effects on experimental animals and human, including prevention of the mammary gland and skin tumors, interaction with ion channels or nuclear receptors (Barcelo-Coblijn and Murphy, 2009; Guizy *et al*., 2008). Furthermore, naturally CLA enriched butter has been shown to reduce the number of incidence of mammary tumors in the rat (Ip *et al*., 1999). Total CLA content in milk of Chinese buffalo was from 0.17 to 0.21%, lower than Pakistan buffalo (0.48-0.66%) and other buffalo (Van Nieuwenhove *et al*., 2007). The level of C20:4*n*6 observed in this study was from 0.1 to 0.16%, with significantly higher in milk of Murrah. The results were similar to those found in the milk lipid of Pakistan buffalo (Talpur *et al*., 2008).

No significant difference was found in *n*-6 and *n*-3 PUFA among the four types, but the *n*-6/*n*-3 ratio in Murrah milk was significantly higher than that in F1 and F2. However, for the indeterminacy fatty acids, Murrah milk was significantly lower than Nili-Ravi. The *n*-6/*n*-3 ratio in the milk of Chinese buffalo was 5.11-6.41, within the desirable range (5-15:1) good for human health (Silva *et al*., 2005).

CONCLUSION

In conclusion, our results suggest that the crossbreeding has some favorable effect on the milk quality. The milk from the crossbreed between Nili-Ravi, Murrah and local swamp buffalo (F1 and F2) had higher content of protein , fat and total solids than pure river buffalo (Nili-Ravi and Murrah), but the milk yield was still less than river buffalo, especially for Nili-Ravi. Crossbreeding could improve the milk quality of Chinese buffalo, such as the κ-casein content, in terms of milk processing and human nutrition. The nutritive value in crossbreed milk (F1 and F2), such as compositions of amino acid and fatty acid, was close to the Nili-Ravi and Murrah.

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