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COMPARATIVE PROTEIN PROFILES OF MILK OF NILI-RAVI BUFFALOES, SAHIWAL AND CROSSBRED COWS

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ABSTRACT

Quantitative protein profiling of milk of Nili-Ravi buffaloes, Sahiwal and cross-bred cows were performed. Total proteins and caseins were the highest in milk of cross-bred cows while, whey proteins were the maximum in Sahiwal cow's milk. The mean values of milk total proteins were 78.103±1.878, 59.333±0.611 and 81.594±2.026 mg/mL for Nili-Ravi buffaloes, Sahiwal cows and cross-bred cows, respectively. The milk lactoserum proteins were comparatively higher in milk of Sahiwal cows (28.842±1.25mg/mL) followed by Nili-Ravi buffaloes (20.026±0.665mg/mL), and the lowest in cross-bred cow's milk (10.176±0.568mg/mL). The mean value of caseins in milk of Nili-Ravi buffaloes was58.077±0.90mg/mL, while the least (30.491±1.15mg/mL) in Sahiwal cow's milk and the highest (71.418±1.20mg/mL) in milk of cross-bred cows. Effect of different quarters on milk protein profiles was non-significant in all three species/cattle breeds. Bulk milk contained 73.555±0.887 mg/mL total proteins, 19.586±0.767 mg/mL lactoserum proteins and 53.969±1.464 mg/mL caseins.

Keywords: Bulk milk; caseins; lactoserum; mammary gland; Pakistan; quarters; total proteins; whey.

INTRODUCTION

Pakistan is at 4th place among largest milk producing countries of the world and it produces 49.5 million tons(gross production) of milk annually. Dairy industry of Pakistan mainly consists of buffaloes and cows. The share in annual milk production of country by cow is 17.37 million tons and by buffalo is 30.46 million tons. Milk proteins especially caseins and lactoserum (whey) proteins are nutritionally very important because these proteins contain all essential aminoacids in optimal amounts and are the most vital source of bioactive peptides(Cozma *et al.*, 2011).Whey, a by-product from the curd and cheese manufacturing, was once considered a waste product(Pelmus *et al.*, 2012). Later, more consideration was given to its benefits and now whey has become a new source of many functional ingredients (Marshall, 2004). Whey proteins actually formed about 18-20% of the total proteins present in milk while its major components are α-Lactalbumin (α-La), β-Lactoglobulin (β-Lg), bovine serum albumin (BSA) and immunoglobulin (Ig), representing, 20%, 50%, 10% and 10% of the whey fraction, respectively (Jovanovic *et al.*, 2007).

A well-known Pakistani cattle breed Sahiwal cow is the best breed of cattle for milk production under tropical environments (Maule, 1990). Sahiwal cow is one of the most productive of the *Bos indicus* species and has been suggested as model for developing breeding programs for developing countries like Pakistan. Nili-Ravi breed is one of the most reputable and high milk producing breeds of buffalo in Pakistan (Khan *et al.*, 1997; Afzal *et al.*, 2007). It was originated in the valleys of Ravi and Sutlej rivers and is the most dominant breed of buffalo in the Punjab province (Afzal *et al.*, 2007).Performance of Nili-Ravi buffaloes much better than crossbred and local cattle breeds in Muzaffarabad, Azad Jammu and Kashmir, that indicated its wider adaptation to different environmental conditions in the country (Afzal *et al.*, 2007). Nili-Ravi buffaloes are mostly owned by the small poor farmers and contribute around 70% of the total milk produced and 25 % of the total meat produced in the country (Khan *et al.*, 1997).

The quantitative amount of different proteins in milk may differ in different dairy species or cattle types and can have impact on overall uptake of bioactive peptides and essential amino acids coming from the milk. It is therefore very essential to have knowledge about the comparative protein profiles of milk from different dairy species or cattle types that may help in selection of superior milk source. So far, the comparative values of proteins in the milk from uninfected mammary glands of Nili-Ravi buffaloes, Sahiwal and cross-bred cows have not been measured. Therefore, the present study was designed to measure and compare the total proteins, lactoserum proteins and caseins in milk of these dairy species/cow types and in the bulk milk. In parallel the effect of different quarters on milk protein profiles was also investigated.

MATERIALS AND METHODS

The present study was conducted to measure the comparative values of total proteins, lactoserum protein and caseins in the milk from uninfected mammary glands of Nili-Ravi buffaloes, Sahiwal and cross-bred cows. The study was conducted on lactating cattle and buffaloes managed at Livestock Experimental Station, University of Agriculture, Faisalabad and commercial dairy farms. Only the animals in the first two months of their 1st to 5th lactation were sampled. Sick and clinically mastitic animals were excluded from the panel of experimental animals. As a first step, Surf Field Mastitis Test (SFMT), (Muhammad *et al.*, 2010) and California Mastitis Test (CMT) (Schalm *et al.*, 1971) were performed for the detection of subclinical mastitis. Secondly, microbiological examination of duplicate quarter foremilk samples was performed (Pantoja *et al.*, 2009) to further confirm and determine the intramammary infection status (http://nmconline.org);(Hogan *et al.*, 1999). Out of 270 dairy animals tested using SFMT, CMT and microbiological examination, all four quarters of 90 animals (30 each from Nili-Ravi buffaloes, Sahiwal and cross-bred cows) were found to be negative in these tests and included in the study. Table 1 summarizes the grouping of experimental animals, types and number of animals sampled for analysis.

Contents of milk total proteins, milk caseins and lactoserum (whey) proteins were estimated. As a first step, degreasing of milk was performed. For degreasing the milk, samples (10 mL) were centrifuged at 5000 rpm, for 20 minutes, and then kept for 30 minutes at 4 ºC. The upper fat layer was removed using a spatula, and the lower aqueous phase (the degreased milk) was transferred to another tube and used for protein profiling. For precipitation of casein and estimation of lactoserum (whey) proteins, first, the pH of the degreased milk was determined using a pH meter. The precipitation of casein was performed by treating 5 ml of degreased milk with 1M HCl and brought to a pH of 4.6, and then the samples were centrifuged for 20 minutes at 5000 rpm. The casein precipitate formed a pellet, while the upper aqueous phase being the lactoserum was separated and used for analysis (Cozma *et al.*, 2011).

Quantitative protein estimation was performed by dye binding assay using bovine serum albumin (BSA) as standard (Bradford, 1976). A standard curve was prepared by plotting the concentration on x-axis against their absorbance at 595 nm on y-axis. A simple linear regression equation was also calculated. For protein estimation, 5 µl of degreased milk sample or lactoserum and 95 µl 150 mM NaCl were mixed with 1.0 mL of dye reagent (100 mg Coomassie Brilliant Blue G-250 dye was dissolved in 50 mL 95 % ethanol and 100 mL 58 % (w/v) phosphoric acid and diluted to one litter). The mixture was left for 5 min to form a protein dye complex. Then, the absorbance was measured at 595nm by using spectrophotometer (HITACHI, U2800). The difference between the milk total proteins and the lactoserum (whey) proteins was considered as caseins of milk samples. The protein contents were expressed as mg mL^{-1} of milk.

Statistical analysis: All samples were analyzed in triplicates and descriptive statistics was applied to analyze and organize the resulting data. Significance of data was tested by analysis of variance and Tukey's (HSD) Test at $p<0.05$ confidence level (95%) using XL-STAT software Version 2012.1.02, Copyright Addin soft 1995-2012 (http://www.xlstat.com).

RESULTS AND DISCUSSION

Milk proteins especially caseins and lactoserum (whey) proteins are nutritionally very important because these proteins contain all essential amino acids in optimal amounts and are the most vital source of bioactive peptides (Cozma *et al.*, 2011). In the present study, quantitative levels of milk total protein, milk casein and milk lactoserum proteins were measured and compared among Nili-Ravi buffaloes, Sahiwal and cross-bred cows (Table 2). The value of total proteins in milk was the highest for cross-bred cows as compared to other species/cattle breeds. The mean values for milk total proteins were found to be 78.103±1.878, 59.333±0.611 and 81.594±2.026 mg/mL for Nili-Ravi buffaloes, Sahiwal cows and cross-bred cows, respectively (Table 2). These values can potentially be used as reference values of milk total proteins for Nili-Ravi buffaloes, Sahiwal and cross-bred cows. In a previous study, the highest total protein content was detected in sheep milk (65.92 mg/ml) while the lowest in cow milk (40.03 mg/ml) and intermediate value in goat milk (46.79 mg/ml) (Cozma *et al.*, 2011). In cow, milk total proteins ranged up to 54.71 mg/mL (Cozma *et al.*, 2011). Comparing this reported cow's milk value with the values observed in the present study, later were found to be higher. Therefore, it can be stated that milk of tested Pakistani species/cattle breeds has higher total protein content as compared to that reported earlier in the cow milk.

Whey, a by-product from the curd and cheese manufacturing, was once considered a waste product (Pelmus *et al.*, 2012). Later, more consideration was given to its benefits and now whey has become a new source of many functional ingredients (Marshall, 2004). Whey proteins actually formed about 18-20 % of the total proteins present in milk while its major components are α-Lactalbumin (α-La), β-Lactoglobulin (β-Lg), bovine serum albumin (BSA) and immunoglobulin (Ig), representing, 20 %, 50 %, 10 % and 10 % of the whey

fraction, respectively (Jovanovic *et al.*, 2007). The amount of milk lactoserum proteins was measured and compared among Nili-Ravi buffaloes, Sahiwal and cross-bred cows. The values for milk lactoserum proteins differed significantly ($p<0.05$) among these tested species/cattle breeds (Table 2). The mean values of milk lactoserum proteins were 20.026±0.665, 28.842±1.25 and 10.176± 0.568 mg/mL for Nili-Ravi buffaloes, Sahiwal and cross-bred cows, respectively. These values can potentially be used as reference values of milk lactoserum proteins for Nili-Ravi buffaloes, Sahiwal and cross-bred cows. Previously, for whey proteins, the highest value was detected in sheep milk (23.36 mg/mL) and the lowest in cow milk (11.79 mg/mL) with intermediate value (17.7 mg/mL) for goat milk (Cozma *et al.*, 2011). The reported value for cow milk was comparable with the value for cross-bred cows in the present study while it was lower compared to the values recorded in milk of Nili-Ravi buffaloes, Sahiwal cows.

The caseins are insoluble proteins present in the and are of many types i.e. αs1-casein, αs2-casein, β-casein, K-casein and γ- casein (Cozma *et al.*, 2011). The detected level of milk caseins was the highest for cross-bred cows and lowest for Sahiwal cows (Table 2). The mean values of milk casein were 58.077±0.90, 30.491±1.15 and 71.418±1.20 mg/mL for Nili-Ravi buffaloes, Sahiwal and cross-bred cows, respectively. These values can be used as reference values of milk caseins for Nili-Ravi buffaloes, Sahiwal and cross-bred cows. Previously, casein content in the range of 68 to 72% and level of whey proteins in the range of 21.89 to 28.14 % have been reported for buffalo milk (Pasquini *et al.*, 2011). In sheep, the percentage analysis of the protein fractions of milk revealed that caseins represented the 74.1 % of total protein followed by whey protein (25.84 %) (Pelmus *et al.*, 2012). In a previous study, the lowest quantity of caseins was detected in cow milk (28.26 mg/mL), followed by sheep (42.55 mg/mL) and goat milk (44.03 mg/mL)(Cozma *et al.*, 2011). These reported values are lower as compared to those observed in the present study indicating comparatively higher milk casein content in tested Pakistani species/cattle types.

Table 1. Scheme of milk sample collection for the study

Groups	Dairy Farms	Species / cattle types	Total animals (No)	Uninfected animals (No)*	Infected animals (No)**	Animals with one quarter infected (No)**
A	Livestock Experimental Station, UAF	Nili-Ravi Buffalo	90	**30**	45	15
B	SB Dairy Farm, Faisalabad	Cross-bred Cow (Sahiwal x Holstein Frisian)	90	**30**	40	20
C	SB Dairy Farm, Faisalabad	Sahiwal Cow	90	**30**	40	20
Total	02	03	270	**90**	125	55

No = number of animals; UAF = University of Agriculture Faisalabad, Pakistan
* = SFMT, CMT and culture negative and selected for analysis
** = SFMT, CMT and culture positive

Table 2. Quantitative values of total proteins, lactoserum (whey) proteins and caseins in degreased milk of Nili-Ravi buffaloes, Sahiwal and cross-bred cows.

	Total milk proteins (mg/mL)		
Species/Cattle breeds	Minimum	Maximum	Mean±SEM
Nili-Ravi buffaloes	58.267	84.667	78.103±1.878[b]
Sahiwal cows	51.200	62.333	59.333±0.611[c]
Cross-bred cows	57.267	93.733	81.594±2.026[a]
	Milk lactoserum proteins (mg/mL)		
Nili-Ravi buffaloes	11.733	34.133	20.026±0.665[b]
Sahiwal cows	15.000	51.600	28.842±1.251[a]
Cross-bred cows	3.400	20.733	10.176±0.568[c]
	Milk caseins (mg/mL)		
Nili-Ravi buffaloes	39.067	71.800	58.077±0.90[b]
Sahiwal cows	10.733	42.800	30.491±1.15[c]
Cross-bred cows	45.400	86.400	71.418±1.20[a]

Values followed by different superscript letter differ significantly ($p<0.05$). SEM = standard error of mean

Possible effect of different quarters i.e. left front, left rear, right front and right rear on milk protein profiles was also investigated and found to be generally non-significant in all three species/cattle breeds. Milk proteins contents including milk total protein, lactoserum proteins and milk casein contents were statistically similar for all quarters (Figure 1).

The overall mean values of different milk parameters across the all tested species/cattle breeds were also calculated. These overall reference values across the species/cattle breeds are presented in the Table 3. The mean values for each parameter were calculated by combining the data of Nili-Ravi buffaloes, Sahiwal and cross-bred cows. These values can be considered as mean values of parameters for bulk milk. The mean value for milk total protein was 73.555 ± 0.887 mg mL^{-1} while it ranged from a minimum value of 51.2 mg mL^{-1} to a maximum value of 93.733 mg mL^{-1}. The value for lactoserum proteins ranged from a minimum of 3.4 mg mL^{-1} to a maximum of 51.6 mg mL^{-1} with a mean value of 19.586 ± 0.767 mg mL^{-1}. The mean value for milk casein was 53.969± 1.464 mg mL^{-1} while it ranged from a minimum value of 10.733 mg mL^{-1} to a maximum value of 86.4 mg mL^{-1}.

Table 3. Quantitative values of total proteins, lactoserum (whey) proteins and caseins in degreased milk across the species/cattle breeds (bulk milk).

Parameters	Minimum	Maximum	Mean	SEM
Total milk proteins(mg/mL)	51.200	93.733	73.555	0.887
Milk lactoserum proteins(mg/mL)	3.400	51.600	19.586	0.767
Milk c**aseins**(mg/mL)	10.733	86.400	53.969	1.464

SEM = standard error of mean

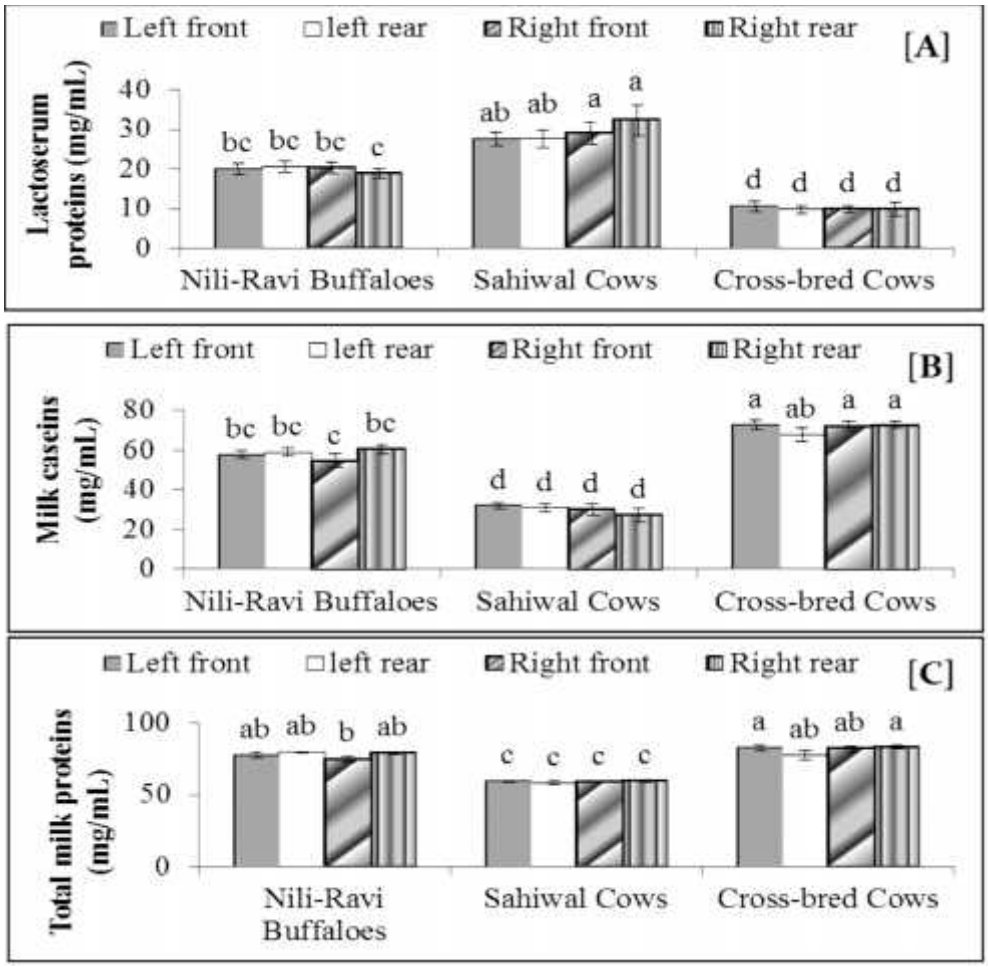

Figure 1: Comparison of total proteins, lactoserum (whey) proteins and caseins in milk from different quarters of Nili-Ravi buffaloes, Sahiwal and cross-bred cows.

Bars with different letter differ significantly ($p<0.05$).

Conclusions: Total proteins and caseins were the highest in milk of cross-bred cows while, whey proteins were the highest in Sahiwal cow's milk. Cow milk has comparatively higher protein contents as in the buffalo milk. Effect of different quarters on milk protein profiles was non-significant in all three species/cattle breeds.

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