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Short Communication

Characterization of Milk Proteins from Different Animal Species through Gel Electrophoresis

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FG, NH and SR planned and executed the research work and wrote the article. IP, AS, I Hayat and I Hussain helped in data analysis and results interpretation.

Key words
Milk protein, Casein, Whey protein.

ABSTRACT

Present investigation covers the chemical composition, nitrogenous compounds and protein fractionations of casein and whey proteins of milk from different dairy animals like buffalo, cow, sheep, goat and camel. The buffalo and sheep milks have comparatively higher fat, solid-not-fat and total solid contents than other milks. Maximum whey proteins were found in the sheep milk (0.78%) whereas cow milk had lowest contents (0.54%). Non-casein-nitrogen (NCN) contents in sheep milk were higher followed by camel and buffalo milks. Electrophoresis study of caseins on Urea-PAGE showed darker bands of αS_1-CN and β-CN in sheep, buffalo, goat and camel while *k*-CN band only appeared in buffalo's milk. The αS_2-CN was found to be absent in camel milk whereas merged in buffalo and sheep with αS_1-CN. Electophoretic mobility of αS_1-CN in goat and sheep milk was higher than buffalo milk. Examining the SDS-PAGE of whey proteins of these species, β-Lg was found to be the major protein in sheep and buffalo milks, while in goat it was the second predominant protein and it was absent in the camel milk. The concentration of immunoglobin was found higher in camel and goat milks as compared to other species. Moreover, the strength and mobility of bovine serum albumin (BSA) was different in all milk species.

Milk is a biologically complex fluid, constituted mainly of water, proteins, lactose, fat and inorganic compounds. Caseins and whey proteins are the main proteins present in various milk species in different proportion. The ratio of casein and whey proteins is 40:60 in human milk, 50:50 in equine milk, while in milk of cow, sheep, goat and buffalo; it is 80:20 (Lara-Villoslada *et al*., 2005). Caseins in milk are present in the form of micelles which are composed of αS_1-casein (αS_1-CN), αS_2-casein (αS_2-CN), β-casein and *k*-casein. Whey proteins contain four major proteins *i.e.* α-lactalbumin (α-lac), β- lactoglobulin (β-lg), blood serum albumin (BSA) and immunoglobulins (Ig). Besides these, the whey fraction contains proteoses and peptones (PP), lactotransferin, serotransferin, osteopontins, vitamin binding proteins, lactoferrin and about 60 indigenous enzymes (Fox, 2003).

Proteins and peptides present in milk have important nutritional, functional, biological and technological properties (Kappeler *et al*., 2003). Caseins are highly digestible than whey proteins and are important for growth and development of infants. When substantial whey protein is not digested fully in the intestine, some of the intact protein may stimulate a localized intestinal or a systemic immune response. This is sometimes referred to as milk protein allergy and is most often thought to be caused by β-Lg. Milk protein allergy is only one type of food protein allergy. Since camel milk is devoid of β-Lg (Laleye *et al*., 2008) or contains a very small amount of it, and could be interesting as a new raw material for nutritional infant formula in countries where these animals thrive.

The physicochemical characteristics of many dairy products depend on properties of milk proteins. During the classical cheese making process, it is the casein fraction which constitutes cheese curd after enzyme-triggered milk coagulation step (McSweeney, 2004). Milk proteins improve the whipping properties and other eating characteristics of ice cream. In yogurt, proteins improve the body, increase its thickness and also affect the flavor. Several techniques have been developed for the separation and characterization of milk proteins. These include mainly gel electrophoretic methods, such as polyacrylamide gel (PAGE) (Basch *et al*., 1985) with urea (Urea-PAGE) or SDS-PAGE. The PAGE separates the proteins by molecular mass.

Pakistan is ranked at the top for buffalo's milk (67.04%) production followed by cow (31.56%) milk (Tahira *et al*., 2014). The share of goat, sheep and camel milk is 1.65%, 0.08% and 1.81%, respectively of total milk production (47.951 million tons) in the country (GoP,

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2013-14). Therefore, an attempt is being carried out for profiling milk proteins from buffalo, cow, sheep, goat and camel milk species found in Pakistan.

Materials and methods

The samples of buffalo, cow, sheep, goat and camel milks were collected from the Dairy Farm, Department of Livestock Management, University of Agriculture, Faisalabad, Pakistan. All milk samples were then placed in the refrigerator immediately at 4°C for further analysis.

The buffalo and cow milk samples were analysed for pH using pH meter (Hanna, HI-99161), acidity (AOAC, 2000), protein (Kjeldahl's method; AOAC, 2000), fat (Marshall, 1993), solid-not-fat (SNF) contents (David, 1977), total solids (AOAC, 2000) and ash contents (AOAC, 2000).

The main fractions of protein such as crude protein (CP), true protein (TP), casein, non-casein-nitrogen (NCN), whey proteins and non-protein-nitrogen (NPN) contents were determined according to standard protocol of IDF (1993). After calculating the total amount of nitrogen (%), it was multiplied with a factor 6.38 to get crude protein. True proteins in the milk sample were determined by treating with 12% TCA. The nitrogen (%) was converted to NPN and NCN contents by using the conversion factor 3.60 and 6.25, respectively (Karman and van Boekel, 1986).

For the separation of caseins and whey proteins, milk samples were defatted by centrifugation at 5000 ×*g* for 15 min at 4°C. The skim milk heated to 37°C, was separated into whole casein and whey proteins by isoelectric precipitation at pH 4.6 with 1N HCl. After centrifugation (5000×g, 15 min, 30°C), the supernatant (whey proteins) was collected and dialyzed at 4°C against several changes of distilled water while the precipitated caseins were washed with acidified distilled water (pH 4.6). Dialysis tubing with a molecular weight cut off of 12000-14000 Daltons was used. Both fractions were then freeze-dried for further use for electrophoresis.

The Urea- and SDS-PAGE were carried out for characterization of casein and whey proteins according to the method of Basch *et al*. (1985). The samples (10 µL) were run on volume basis, on assumption that protein content is 10 mg/mL. 5µL of protein marker (fermantas 11-170 kDa) was loaded for whey samples while sodium caseinate was used as standard for casein samples.

Statistical significance of experimental data was performed by applying completely randomized design (CRD) at 5% level of significance while significant differences between means were compared using Tukey's HSD test. In the study, dairy animal species were only the source of variation while keeping other factors (diet, farm, age etc.) constant.

Results and discussion

The physico-chemical composition (Table I) of milk indicated that sheep milk contains comparatively higher fat, solid-not-fat and total solids contents ($p<0.05$) than other milk species. Several factors such as breed and health of animal, stage of lactation, feeding systems, seasonal changes, milking frequency and milking systems, nutrition and genetics can cause variation in relative proportion of milk constituents. The Murrah and Nili-Ravi breeds of buffalo milk had 6.57% and 6.53% fat contents, respectively (Han *et al*., 2007). The lower pH of fresh milk may be due to bacterial action and higher one indicates the udder infection or mastitis (Ullah *et al*., 2005).

The substantial variation among different milk species was observed regarding major protein fractions like crude protein (CP), true proteins (TP), caseins and whey proteins; and the nitrogen components such as NCN and NPN contents. Relatively higher contents of caseins, CP, TP and NPN were present in sheep milk while camel milk had lower as compared to other species. Although, the animals

Table I.- Physico-chemical composition of milk of different species.

	Cow	Buffalo	Sheep	Goat	Camel
pH	6.65±0.01^{a}	6.64±0.01^{a}	6.66±0.02^{a}	6.66±0.01^{a}	6.66±0.01^{a}
Acidity (%)	0.11±0.02^{bc}	0.12±0.01^{ab}	0.13±0.01^{a}	0.11±0.01^{bc}	0.11±0.02^{c}
Ash (%)	0.73±0.01^{b}	0.83±0.01^{ab}	0.86±0.01^{a}	0.83±0.02^{ab}	0.76±0.01^{ab}
Fat (%)	3.56±0.01^{d}	7.13±0.03^{b}	7.26±0.02^{a}	4.13±0.02^{c}	1.90±0.03^{e}
SNF (%)	9.78±0.01^{c}	11.23±0.01^{b}	12.46±0.02^{a}	9.81±0.03^{c}	8.53±0.02^{d}
TS (%)	13.35±0.03^{d}	18.36±0.02^{b}	19.73±0.02^{a}	13.91±0.03^{c}	10.42±0.02^{e}
CP (%)	3.60±0.02^{c}	4.20±0.02^{b}	5.76±0.01^{a}	3.45±0.02^{d}	3.42±0.02^{d}
TP (%)	3.12±0.01^{c}	3.67±0.02^{b}	5.1±0.026^{a}	3.12±0.02^{c}	3.11±0.01^{c}
Casein (%)	2.58±0.02^{c}	2.93±0.01^{b}	4.38±0.02^{a}	2.57±0.01^{c}	2.46±0.02^{c}
WP (%)	0.54±0.02^{b}	0.73±0.01^{a}	0.78±0.02^{a}	0.55±0.02^{b}	0.61±0.01^{b}
NCN (%)	1.01±0.02^{c}	1.27±0.02^{b}	1.38±0.02^{a}	0.94±0.01^{c}	0.94±0.01^{c}
NPN (%)	0.48±0.02^{b}	0.54±0.01^{ab}	0.60±0.02^{a}	0.39±0.01^{c}	0.33±0.02^{c}

Analysis were performed in triplicate and results are expressed as means ± standard deviation; Means sharing similar letter (superscript) in a row or in a column are statistically non-significant (P> 0.05). SNF, solid-not-fat; TS, total solids; CP, crude protein; TP, true protein; WP, whey proteins; NCN, non-casein nitrogen; NPN, non-protein nitrogen.

were from same farm and under similar feeding pattern but the difference in protein contents may be due to genetics of these animal species (Pavic *et al*., 2002; Mal *et al*., 2007). Whey proteins were highest in the sheep (0.78%) milk; whereas, cow milk represented lowest content (0.54%). The NCN contents in sheep milk were higher (1.38%) followed by buffalo (1.27%) milk species. Regarding the NPN fraction, no significant difference was observed between the camel and goat milks. However, the NPN content was the highest (0.60%) in sheep milk (Table I).

The previous findings on cow and goat milk proteins by Ozrenk and Inci (2008) and Strzalkowska *et al*. (2009) are in agreement with present research work. Shamsia (2009) reported that camel milk constitutes 2.15% to 4.90% protein. The casein contents of sheep and goat milks are in accordance with the study of Borkova and Snaselova (2005). Casein and whey proteins in camel milk are also comparable with those reported by many investigations (Khaskheli *et al*., 2005; Stancheva *et al*., 2011).

The profiling of casein and whey proteins has been conducted using Urea-PAGE and SDS-PAGE. However, the best results for casein's fractionation were obtained on Urea-PAGE while whey proteins were well characterized on SDS-PAGE.

Electrophoretic pattern of cow, buffalo, sheep, goat and camel (Fig. 1) indicates that there is distinct difference among protein profiles of these species. Na- caseinate which was run as standard, has four major bands of αS_1, αS_2, β and κ-CN. The profile of cow milk indicates that the αS_1 and β-CN have more concentrations and band thickness as compare to αS_2 and κ-CN. There is lack of αS_2-CN in buffalo milk and only αS_1, β and κ-CN bands exist. Among these three bands, β-CN has more concentration followed by αS_1 and κ-CN. There was slight difference found in electrophoretic mobility of cow and buffalo milks. In sheep milk, more visible bands of αS_1 and β-CN were observed. The electrophoretic mobility of sheep, buffalo and cow αS_1 was almost same. However, the bands in the sheep milk were denser than others, which might be due to higher protein content or difference in casein to whey proteins ratio. Electrophoretic profile of goat milk revealed that there were three dominant bands of αS_1, αS_2 and β-CN while κ-CN was absent in it. Camel milk also showed presence of three distinct bands of αS_1, β, *k*-CN during electrophoresis. However, these bands were thinner and also have low mobility as compared to other animal species.

Tomotake *et al*. (2006) observed that the caprine casein had two major bands corresponding to αS_2 and β-CN, while profile for bovine casein showed the presence of two major bands corresponding to αS1 and β-CN. The present findings regarding buffalo casein are in agreement with Nagassawa *et al*. (1993) who also concluded that there was low mobility of buffalo αS1-CN than bovine αS1-CN. However, buffalo and cow β-CN has shown same mobility. Previous studies suggested that although goat milk contains the same proteins (including β-lactoglobulin) as cow milk, some goat milk proteins differ in their genetic polymorphisms, resulting in lower allergenicity (Ribeiro and Ribeiro, 2010). The major fraction in goat and camel milk casein is β-casein, which makes it similar to human milk (Al Haj and Al Kanhal, 2010).

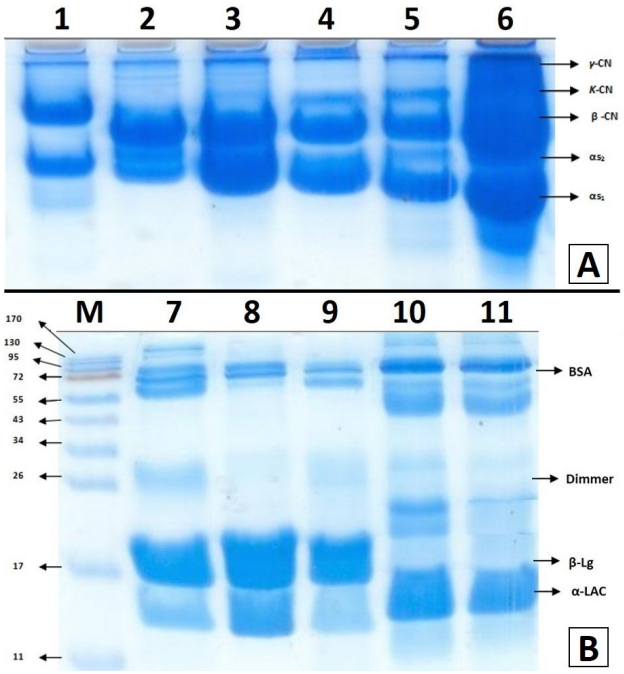

Fig. 1. Electrophoretic pattern of cow, buffalo, sheep, goat and camel milk casein (A) and whey protein (B) on SDS-PAGE representing differences in band pattern. The Marker (Fermentas 11-170KDa) was used to identify molecular weight of whey proteins. Lane M, marker; Lane 1, camel casein; Lane 2, goat casein; Lane 3, sheep casein; Lane 4, buffalo casein; Lane 5, cow casein; Lane 6, sodium casein (standard used to identify bands of different milk species); Lane 7, cow whey; Lane 8, buffalo whey; Lane 9, sheep whey; Lane 10, goat whey; Lane 11, camel whey.

Electrophoretic pattern of whey proteins on SDS-PAGE has revealed substantial differences among different corresponding fractions of milk species under examination. Prestained protein ladder (Fermentas) ranging from 11-170 KDa was used for purpose of comparing the molecular weights of whey proteins of these species. In cow milk, three major bands of α-LAC, β-Lg and BSA with their corresponding molecular weights 14 KDa, 17 KDa, 70 KDa were noticed, respectively. However, the concentration and band thickness of β-Lg was more in comparison to α-LAC.

In buffalo milk, four bands of whey protein fractions were observed. The lowest molecular band of α-LAC at 14KDa was found. The other band was at 17 KDa could

be β-Lg because it had electrophoretic mobility similar to bovine β-Lg. However, the concentration of β-Lg was more in buffalo milk whey proteins. The third band was observed at 70 KDa could be of buffalo serum albumin. Three major bands of α-LAC, β-Lg and serum albumin were found in sheep milk. The first band at 14 KDa would be of α-LAC and second band of β-Lg was at 18 KDa. Another band at 26 KDa might be the dimmer of α-LAC. The sheep serum albumin has also been noticed almost nearer to 70 KDa.

In goat milk, several bands were observed, the band before α-LAC was of low molecular weight proteins and might be proteose and peptones. The α-LAC was noted at 14 KDa, β-Lg was at 20 KDa and serum albumin was at 51 KDa. In camel milk, α-LAC was at 13 KDa and β-Lg was missing in camel milk. The camel serum albumin was at 51 KDa and last band at 108 KDa might be Ig.

The findings regarding to the fractions of whey proteins of bovine and camel milk are concordant to the study of Merin *et al*. (2001). Similar results have also been evaluated by Farah (1986) and Farah and Farah-Riesen, (1985). SDS-PAGE was performed to observe the difference in protein pattern of Holstein cow's milk and Japanese-Saanen goat's milk (Tomotake *et al*., 2006). It was observed that α-LAC and β-Lg were the major bands in both kinds of milk and content of whey proteins were almost same in the two kinds of milk. Camel and goat milks do not contain measurable amounts of β-Lg as human milk (Li *et al*., 2010). Therefore, the main whey protein is α-LAC in both milks (Al Haj and Al Kanhal, 2010). These characteristics could contribute to higher digestibility rate and lower incidence of allergy than cow milk (El-Agamy *et al*., 2009).

Statement of conflict of interest

The authors have no conflict of interest to declare.

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