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RESEARCH ARTICLE

Comparative Protein Profiling of Milk of Nili-Ravi Buffaloes, Sahiwal and Cross Bred Cows by SDS-PAGE

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ABSTRACT

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approach for high-resolution analyses of the complex mixture of milk proteins by SDS polyacrylamide gel electrophoresis (PAGE) is of great importance due to its exceptional resolving capacity. Degreased milk samples (non-infected) of Nili-Ravi buffaloes, Sahiwal and cross-bred cows were used for comparative protein profiling using 12% SDS polyacrylamide gels. Protein profiling clearly resolved the major milk peptides. Qualitative differences were observed in high, medium and low molecular weight (MW) zones. In high molecular weight (M. wt.) zone, proteins of ~208 kDa and ~190 kDa were detected in all tested samples. In medium M. wt. zone, three peptides *i.e.* lactoferrin (78.2kDa), serum albumen (66.2kDa) and heavy chain of immunoglobulin (IgG) (54 kDa) were detected in all samples while a prominent band of ovalbumen (45kDa) was also detected mainly in cow milk samples. In low M. wt. zone, clear bands of milk caseins were detected. All four casein (CN) bands *i.e.* $\alpha S_2 - CN$ (29 kDa), $\alpha S_1 - CN$ (27 kDa), β - CN (24 kDa) and κ- CN (22 kDa) were detected in cross-bred and Sahiwal cows. However, in milk of Nili-Ravi buffaloes, three casein protein *i.e.* $\alpha S_2 - CN$ (29 kDa), β - CN (25 kDa) and κ - CN (22 kDa) were detected. In milk of Nili-Ravi buffaloes, αS_1 – CN (27 kDa) was not detected. Moreover, a band of β-lactoglobulin (~18 kDa) was detected in milk of cross-bred cows and not in other samples especially Nili-Ravi buffaloes. As the αS_1 -case and β -lactoglobulin are the major allergens, milk of Nili-Ravi buffaloes that lacks these peptides can be used for development of non-allergic or hypoallergenic dairy products. Deferential peptides may also help to distinguish the milk from different tested breeds.

Milk of different species may vary in protein quality and quantity. A proteomic

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INTRODUCTION

The milk proteins have been analyzed continuously and information about this very important and complex system has increased incrementally during recent years, mainly because of technological advances (Suharoschi *et al.*, 2010; Shahid *et al.*, 2016). Moreover, due to rising awareness about the bioactive proteins present in milk, the extent of research work on analysis of proteins in bovine milk has considerably increased (Tacoma *et al.*, 2016). Milk of different species may vary in protein quality and quantity. A proteomic approach for high resolution investigation of the complex combination of milk proteins is of great importance due to its exceptional resolving capacity (O'donnell *et al.*, 2004). A total of 269 proteins in bovine milk have been identified using the 1D-gels, followed by mass-spectrometry (Hettinga *et al.*, 2011). Analyses of milk proteins have been reported for some mammalian species like goat (Roncada *et al.*, 2002), sheep (Pelmus *et al.*, 2012) and cow (Holland *et al.*, 2004).

The milk proteins can be divided in two major categories: soluble proteins (whey proteins), present in milk lactoserum and insoluble proteins (the casein family). The second family of proteins contains of many types of caseins i.e. α s1-, α s2-, β -, K- and γ -, while the whey or lactoserum proteins are β -lactoglobulin and α -lactalbumin (Cozma *et al.*, 2011). Major milk proteins

including casein proteins (β -casein, α s₁-casein, α s₂-casein, and K- casein), β -lactoglobulin and α -lactalbumin have been detected in cow (Tay and Gam, 2011) and buffalo (Giacinti *et al.*, 2013) milk.

Genetic variations in chief milk proteins i.e. β-CN and ĸ-CN has been reported in Danish Holstein-Friesian and Jersey cows representing rennet milk coagulation extremes (Jensen et al., 2012). Genetic polymorphism in milk proteins has been reported for Maiwa and Jiulong Chinese vaks (Mao et al., 2004). The genetic variants of different milk proteins are considered to be prospective selection standards in cattle husbandry. The documentation of the genetic variant of milk protein also permits the detection of adulteration of milk with different types of animal milk (Borkova and Snaselova, 2005).

In most of developed countries, around 2 to 3% of infants have allergy to proteins in cow milk during their first year of life (Host, 2002). In fact, the cows' milk causes allergic reactions due to variation in the protein composition of cow milk and human milk. β-lactoglobulin is a major cow milk protein that is allergenic in humans (D'auria *et al.*, 2005). Moreover, α S₁-casein is also a main allergen (Elsayed et al., 2004). According to a previous report, Chinese river buffalo (Murrah) milk lacks one kind of α S-casein and further studies were suggested which development of non-allergic may help in or hypoallergenic products from milk of Chinese buffalo (Yang et al., 2013). Absence of αS_1 case in the milk protein profile of two Brazilian goat breeds has also been reported (Da-Costa et al., 2014). Similar studies providing information about presence or absence of allergen proteins in milk of Pakistani cattle breeds are lacking.

Sahiwal cow is famous Pakistani cattle breed that is best for milk production under tropical environments. On the other hand, the most reputable and high milk producing breed of buffalo in Pakistan is Nili-Ravi buffalo (Afzal *et al.*, 2007). No information is available regarding milk protein profiles of these cattle species. In this view, the current study was carried out to compare the milk protein profiles of Nili-Ravi buffaloes, Cross bred and Sahiwal cows by SDS-PAGE.

MATERIALS AND METHODS

The study was conducted on lactating cattle and buffaloes managed at Livestock Experimental Station, University of Agriculture, Faisalabad and commercial dairy farms. Sick and clinically mastitic animals were excluded from the panel of experimental animals. The detection and exclusion of animals with subclinical mastitis and grouping of experimental animals has been reported earlier (Kausar *et al.*, 2015).

Sample preparation: As a first step, degreasing of milk was performed. For degreasing the milk, samples (10mL) were centrifuged at 5000rpm, for 20min, and then kept for 30min at 4°C. 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 preparation of sample for electrophoresis, 100 μ l degreased milk samples were taken in the 1.5mL tubes and 25 μ l of cracking dye solution was added.

Samples were then boiled for 2-3min at 100°C in water bath for complete denaturation of proteins. Finally, the samples were centrifuged at 10,000rpm for 10min and subsequently used for electrophoresis.

Protein profiling using SDS-PAGE: Degreased milk samples were used for comparative protein profiling using sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis (PAGE) as described earlier (Laemmli, 1970). Briefly, 30 µl of each sample along with 15 µl of the protein marker (Bio Basic Int.: RM-0011) was loaded in 12% gels. Electrophoresis was performed at a constant voltage of 170 Volts. On completion of gel electrophoresis, gels were fixed in solution containing 10% acetic acid and 40% ethanol for 15 minutes with agitation. After fixing, gels were washed in distilled water for 1 hour with water changing after every 12 min. gels were then stained with Coomassie brilliant blue G-250 till all bands got clear. After that gel was washed with distilled water to remove the excessive stain. Gel could be documented at this stage with clear bands. However, for more clear background, gel was placed in distilled water for overnight. Finally, gels were photographed using UVIproplatinum gel documentation system (UVITec UK). Computerized gel analysis was performed using UVI pro Platinum 1.1 Version 12.9 for windows (copyright® 2004-2006). Molecular weight determination was performed by comparison of peptides with protein molecular weight marker (Bio Basic Int.: RM-0011) using software UVI BANDMAP version 11.3 by UVITec UK.

RESULTS

Degreased milk samples were used for comparative protein profiling using SDS-PAGE. Milk proteins were resolved using 12% SDS polyacrylamide gels. SDSpolyacrylamide gel showing the comparative milk protein profiles of different cattle breeds is presented as Figure 1. The approximate molecular weight (kDa) of detected peptides in milk samples are presented in Table 1. Clear quantitative as well as qualitative deference in the milk proteins were detected among the tested breeds. Qualitative differences in the milk proteins were observed among the breeds in high, medium and low molecular weight zones.

In high molecular weight zone, proteins with approximate molecular weight of ~208 kDa and ~190 kDa were detected in all tested samples with slight variation in molecular weight. In medium molecular weight zone, four prominent peptides were detected. In this zone, lactoferrin (78.2kDa) with slowest mobility and lighter intensity was detected in all samples. Moreover, a more prominent band of serum albumen (66.2kDa) was also detected in all samples. Similarly, next to serum albumen, a protein peptide of 54kDa was detected in all samples that may be the heavy chain of immunoglobulin (IgG). In this zone, a prominent band of (45kDa) was also detected mainly in cow milk samples. A faint protein peptide of 45kDa was also detected in one of Nili-Ravi milk samples. This protein may the ovalbumen. A protein with molecular weight of 38 kDa was detected in one sample of each tested species/cattle breeds and seems to be κ - CN.

In low molecular weight or high mobility zone, clear bands of milk caseins were detected. All four casein (CN) proteins i.e. $\alpha S_2 - CN$ (29kDa), $\alpha S_1 - CN$ (27kDa), β - CN (24 kDa) and κ - CN (22kDa) were detected in cross-bred and Sahiwal cows. However, in milk of Nili-Ravi buffaloes, three casein protein i.e. αS_2 – CN (29kDa), β - CN (25kDa) and $\kappa\text{-}$ CN (22kDa) were detected. In milk of Nili-Ravi buffaloes, αS_1 - CN (27kDa) was not detected. Moreover, the molecular weight of β - CN in Nili-Ravi buffaloes was slightly higher (25kDa) as compared to that in Sahiwal and cross-bred cows (24kDa). A protein band with molecular weight of ~18kDa that may be β -lactoglobulin was detected in milk of cross-bred cows while not detected in other samples. Similarly, a low molecular weight band of ~14kDa was detected in all tested samples and it seems to be α -lactalbumin.

DISCUSSION

During last few years, owing to increasing attention in the bioactive proteins of milk, the amount of research work on analysis of bovine milk proteins has increased (Tacoma et al., 2016). The milk proteins have been analyzed continuously and information about this very and important complex system has increased incrementally during recent years, mainly because of technological advances (Suharoschi et al., 2010). Milk of different species may vary in protein quality and quantity. A total of 269 milk proteins in bovine have been identified using the 1D-gels, followed by massspectrometry (Hettinga et al., 2011). Milk Proteomic analyses of milk proteins have been reported for some mammalian species like goat (Roncada et al., 2002),

sheep (Pelmus *et al.*, 2012) and cow (Holland *et al.*, 2004). However, comparative interspecies proteomic analysis of milk from Nili-Ravi buffaloes, Sahiwal and cross-bred cows has not yet been investigated. In this study, in high molecular weight or low mobility zone, proteins with approximate molecular weight of ~208kDa and ~190kDa were detected in all tested samples with slight variation in molecular weight. These high molecular weight peptides seem to be co-aggregates of other milk proteins like caseins.

Lactoferrin (Lfe) plays an important role in mammary gland protection mechanisms and contribute in prevention of microbiological infection. In the present study, a protein band of 78.2kDa (lactoferrin) with lighter intensity was detected in all samples. This 78.2kDa peptide was designated as lactoferrin. The milk whey proteins include serum albumin of 66.2kDa (Madureira *et al.*, 2007; Buffoni *et al.*, 2011) with several biological functions. In the present study, a prominent band of serum albumen (66.2kDa) was also detected in all samples. Previously, with similar molecular mass, serum albumin (66.397kDa) has been identified in Mediterranean water buffalo milk (*Bubalus bubalis*) using ESI-MS and RP-HPLC protocols (Buffoni *et al.*, 2011).

In the present study, next to serum albumen, a protein peptide of 54 kDa was detected in all samples that may be the heavy chain of immunoglobulin (IgG) (Madureira *et al.*, 2007). In this zone, a prominent band of (45kDa) was also detected mainly in cow milk samples. A faint protein peptide of 45 kDa was also detected in one of Nili-Ravi milk samples. This protein may the ovalbumen. A protein with molecular weight of 38kDa was detected in one sample of each tested species/cattle breeds and seems to be κ - CN.



- 3. Immunoglobulin (54 kDa)
- 4. Ovalbumen (45 kDa)

Fig. 1: Protein profiles of milk from uninfected mammary glands of Nili-Ravi bafaloes, Sahiwal and cross-bred cows using 12% SDS-PAGE. M: Prestained protein marker Bio Basic Inc. Cat. No. RM0011 (Recombinant protein: 163kDa, Phosphorylase B: 108kDa, Bovine Serum Albumin: 78.6kDa, Ovalbumin: 50.6kDa, Carbonic anhydrase: 35.9kDa, Soybean trypsin inhibitor: 27.1kDa and Lysozyme: 19.2kDa).

kDa	Sw4	Nr5	Sw7	Nr8	Cb18	Sw20	Nr21	Cb26	Cb30
~208	~208	~208	~208	~208	~208	~208	~208	~208	~208
~190	~190	~190	~190	~190	~190	~190	~190	~190	~190
78.2	78.2	78.2	78.2	78.2	78.2	78.2	78.2	78.2	78.2
66.2	66.2	66.2	66.2	66.2	66.2	66.2	66.2	66.2	66.2
54	54	54	54	54	54	54	54	54	54
45	45		45	45	45	45		45	45
38.8		38.8	38.8					38.8	
29	29	29	29	29	29	29	29	29	29
27	27		27				27	27	27
26		25		25			25		
24	24	24	24	24	24	24	24	24	24
22	22	22	22	22	22	22	22	22	22
~18								~18	~18
~14								~14	~14

Table 1: Approximate molecular weight (kDa) of detected peptides in milk samples obtained from uninfected mammary glands of Nili-Ravi bafaloes, Sahiwal and cross-bred cows

Sw: Sahiwal cows (animal no); Nr: Nilli-Ravi buffaloes (animal no); cb: cross-bred cows (animal no)

The milk proteins can be divided in two major categories: soluble proteins (whey proteins), present in milk lactoserum and insoluble proteins (the casein family) (Cozma et al., 2011). Milk also contains minor proteins, like serum albumin, lactoferrin and immunoglobulins (Park et al., 2007). Major milk proteins including casein proteins (β -casein, α s₁-casein, α s₂-casein and K- casein), α -lactalbumin and β -lactoglobulin have been detected in cow (Tay and Gam, 2011) and buffalo (Giacinti et al., 2013) milk. Moreover, cow's UHT milk whey proteins (βlg=18kDa, α-la=14kDa) and casein fractions of about 22 to 26kDa have been characterized by SDS-PAGE (Wroblewska and Kaliszewska, 2012). Similarly, βcasein, as1-casein, as2-casein and K- casein have been visualized as sharp bands after Tricine-SDS-PAGE analysis of bovine milk caseins (Pardo and Natalucci, 2002). In present study, clear bands of milk caseins were detected in similar molecular weight range. All four casein (CN) proteins i.e. $\alpha S_2 - CN$ (29 kDa), $\alpha S_1 - CN$ (27 kDa), β - CN (24 kDa) and κ- CN (22 kDa) were detected in milk of Sahiwal and cross-bred cows. However, in milk of Nili-Ravi buffaloes, three casein protein i.e. $\alpha S_2 - CN$ (29 kDa), β - CN (2kDa) and κ - CN (22kDa) were detected. In milk of Nili-Ravi buffaloes, $\alpha S_1 - CN$ (27 kDa) was not detected. Moreover, the molecular weight of β - CN in Nili-Ravi buffaloes was slightly higher (25kDa) as compared to that in cross-bred and Sahiwal cows (24kDa). Previously, in Chinese river buffalo (Murrah), SDS-PAGE analysis revealed a somewhat faster movement of α_s -case than standard α_s -case ; while a somewhat slower movement of buffalo B-casein than standard β-casein (Yang et al., 2013). Genetic variations in main milk proteins i.e. β – CN and κ - CN has been reported in Danish Holstein-Friesian and Jersey cows representing rennet milk coagulation extremes (Jensen et al., 2012). Genetic polymorphisms in milk proteins has been reported for Maiwa and Jiulong Chinese yaks being κ -CN, α -La and β -CN as monomorphic, and β -Lg and α s1-CN as polymorphic, with β -Lg E and α s1-CN D as dominant genes (Mao et al., 2004). Actually, the substitution or deletion of amino acids caused the polymorphism in milk proteins. That's why, the genetic variants of different milk proteins are considered to be prospective selection standards in cattle husbandry. The documentation of the genetic variant of milk protein also permits the detection of adulteration of milk with different types of animal milk (Borkova and Snaselova, 2005).

One of the important findings of the present study was absence of αS_1 casein (27kDa) in milk of Nili-Ravi buffaloes. According to a previous report, Chinese river buffalo (Murrah) milk lacks one kind of αS-casein. (Yang et al., 2013). As the αS_1 -case in is the major allergen (Elsayed et al., 2004), they suggested further analysis on it which may help in development of non-allergic or hypoallergenic dairy products from milk of Chinese buffalo (Yang et al., 2013). Similarly, milk of Nili-Ravi buffaloes that lacks αS_1 casein can be used for development of non-allergic or hypoallergenic dairy products, which is worth of further investigations. Recently, absence of αS_1 case in the milk protein profile of two Brazilian goat breeds has also been reported (Da-Costa et al., 2014). In another report, absence of aS1caseins was reported in caprine milk (Kim and Jimenez-Flores, 1994).

The most common food allergy in childhood is cows' milk allergy, which has a reported prevalence of 2 to 7.5%. In most of developed countries, around 2 to 3% of infants have allergy to proteins in cow milk during their first year of life (Host, 2002). In fact, the cows' milk causes allergic reactions due to change in the protein composition of cow milk and human milk. The β -lactoglobulin (BLG), a milk whey protein is formed in cows and other ruminants however, it is absent in human milk. Therefore, it is very simple to understand that β -lactoglobulin is a major cow milk protein that is allergenic in humans (D'auria *et al.*, 2005).

The dairy industry has developed some hypoallergenic formulas to reduce the allergenic reaction of milk that are mostly based on enzymatic hydrolysis of milk whey proteins (Jabed et al., 2012). In fact, hydrolysis of whey proteins is not a perfect solution to this problem as the resulting hydrolyzed peptides may still show residual antigenicity. Another issue with this approach is processing technique which is very costly (Jabed et al., 2012). The other alternate options to postharvest processing are more straight approaches to decrease BLG, such as knockdown of gene by RNAi or KO of the BLG gene (LGB) through homologous recombination (Jabed et al., 2012). However, gene KOs have proven to be disreputably difficult to accomplish in livestock species (Jabed et al., 2012). In spite of being an attractive research target, so far no BLG KO animals have yet been reported to our knowledge. The molecular weight of β lactoglobulin from cow milk has been reported to be

18.277kDa (Madureira et al., 2007). In the present study, a protein band of β -lactoglobulin with molecular weight ~18kDa was detected in milk of cross-bred cows while not detected in other samples especially Nili-Ravi buffaloes. This is very important finding as Nili-Ravi buffalo milk was β -lactoglobulin free. It is important to mention here that buffalo milk is not always β lactoglobulin free. Recently, beta-lactoglobulin (whey protein) with molecular weight of 18kDa has been detected in both Indian cow and buffalo milk (Chitra et al., 2013). Similarly, beta-lactoglobulin has been detected and purified from milk of Murrah breed of buffalo from West Bengal India (Aich et al., 2013). Moreover, with similar molecular mass, β-lactoglobulin (18.26kDa) has been identified in Mediterranean water buffalo milk (Bubalus bubalis) using ESI-MS and RP-HPLC methodologies (Buffoni et al., 2011).

The molecular weight of cow milk α -lactalbumin has been reported to be 14.177kDa (Madureira *et al.*, 2007). Similarly, a low molecular weight band of ~14kDa was detected in all tested samples and it seems to be α lactalbumin. Previously, with similar molecular mass, α lactoalbumin (14.2kDa) has been identified in Mediterranean water buffalo milk (*Bubalus bubalis*) using RP-HPLC and ESI-MS (Buffoni *et al.*, 2011).

Conclusions: SDS-PAGE resolved 14 peptides in different tested species/cattle types. In milk of Nili-Ravi buffaloes, $\alpha S_1 - CN$ (27 kDa) and β -lactoglobulin (~18 kDa) were not detected which are the major allergens and its milk can be used for production of non-allergic or hypoallergenic dairy products. Deferentially detected peptides in milk of tested species/cattle types may help to distinguish the milk samples and to check the milk adulterations.

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Author's contribution: RK, GM, MZQ and AH conceived and designed the project. RK and AH executed the experiment and analyzed the milk samples and resulting data. All authors critically revised the manuscript for important intellectual contents and approved the final version.

REFERENCES

- Afzal M, Anwar M and Mirza MA, 2007. Some factors affecting milk yield and lactation length in Nili Ravi buffaloes. PakVet J 27:113-7.
- Aich R, Batabyal S and Joardar SN, 2013. Simple purification method for beta-lactoglobulin from buffalo milk. Adv Anim Vet Sci 2:78-80.
- Borkova M and Snaselova J, 2005. Possibilities of different animal milk detection in milk and dairy products-a review. Czech J Food Sci 23:41-50.

- Buffoni JN, Bonizzi I, Pauciullo A, et al., 2011. Characterization of the major whey proteins from milk of mediterranean water buffalo (Bubalus bubalis). Food Chem 127:1515-20.
- Chitra DT, Manomani E and Ravikumar R, 2013. Isolation and purification of proteins from cow and buffalo milk. Int J Engg Res Indu Appls 6:45-55.
- Cozma A, Andrei S, Miere D, et al., 2011. Proteins profile in milk from three species of ruminants. Not Sci Biol 3:26-9.
- D'auria E, Agostoni C, Giovannini M, et al., 2005. Proteomic evaluation of milk from different mammalian species as a substitute for breast milk. Acta Paedia 94:1708-13.
- Da-Costa WKA, De-Souza EL, Beltrao-Filho EM, et al., 2014. Comparative protein composition analysis of goat milk produced by the alpine and saanen breeds in Northeastern Brazil and related antibacterial activities. PLoS One 9:e93361.
- Elsayed S, Hill D and Do T, 2004. Evaluation of the allergenicity and antigenicity of bovine milk αs1 casein using extensively purified synthetic peptides. Scand J Immunol 60:486-93.
- Giacinti G, Basiricò L, Ronchi B and Bernabucci U, 2013. Lactoferrin concentration in buffalo milk. Ital J Anim Sci 12:e23.
- Hettinga K, Van Valenberg H, De Vries S, et al., 2011. The host defense proteome of human and bovine milk. PLoS One 6:e19433.
- Holland JW, Deeth HC and Alewood PF, 2004. Proteomic analysis of κ-casein micro-heterogeneity. Proteomics 4:743-52.
- Host A, 2002. Frequency of cow's milk allergy in childhood. Ann Allergy Asthma Immunol, 89: 33-7.
- Jabed A, Wagner S, Mccracken J, et al., 2012. Targeted microrna expression in dairy cattle directs production of β-lactoglobulinfree, high-casein milk. Proc Nat Acad Sci, USA 109:16811-6.
- Jensen HB, Holland J, Poulsen NA and Larsen LB, 2012. Milk protein genetic variants and isoforms identified in bovine milk representing extremes in coagulation properties. J Dairy Sci 95:2891-903.
- Kausar R, Muhammad G, Qureshi Z and Mahmood F, 2015. Comparative protein profiles of milk of Nili-Ravi buffaloes, Sahiwal and crossbred cows. J Anim Plant Sci 25:645-9.
- Kim H-H and Jimenez-Flores R, 1994. Comparison of milk proteins using preparative isoelectric focusing followed by polyacrylamide gel electrophoresis. | Dairy Sci 77:2177-90.
- Laemmli UK, 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage t4. Nature 227:680-85.
- Madureira AR, Pereira CI, Gomes AM, et al., 2007. Bovine whey proteins—overview on their main biological properties. Food Res Int 40:1197-211.
- Mao Y, Zhong G, Zheng Y, et al., 2004. Genetic polymorphism of milk protein and their relationships with milking traits in chinese yak. Asian-Australas J Anim Sci 17:1479-83.
- O'donnell R, Holland JW, Deeth HC and Alewood P, 2004. Milk proteomics. Int Dairy J 14:13-23.
- Pardo MF and Natalucci CL, 2002. Electrophoretic analysis (tricine-sdspage) of bovine caseins. Acta Farmacéut Bonaerense, 21: 57-60.
- Park Y, Juárez M, Ramos M and Haenlein G, 2007. Physico-chemical characteristics of goat and sheep milk. Small Rum Res 68:88-113.
- Pelmus RS, Pistol GC, Lazar C, et al., 2012. Preliminary study on milk composition and milk protein polymorphism in the romanian local sheep breed teleorman black head tsigai. Rom Biotech Lett 17:7583.
- Shahid R, Qureshi ZI, Lodhi LA, et al., 2016. Effect of recombinant bovine somatotropin (rbst) and oxytocin on health biomarkers, reproductive performance and milk composition of Nili-Ravi buffaloes (Bubalus bubalis) during spring and summer seasons. Pak J Agri Sci 53:985-90.
- Roncada P, Gaviraghi A, Liberatori S, et al., 2002. Identification of caseins in goat milk. Proteomics 2:723-6.
- Suharoschi R, Corina-Irina B, Raul P, et al., 2010. Two-dimensional gel electrophoresis of peptides as lap in bovine milk. J Agroaliment Processes Technol 16:169-176.
- Tacoma R, Fields J, Ebenstein DB, *et al.*, 2016. Characterization of the bovine milk proteome in early-lactation Holstein and Jersey breeds of dairy cows. J Proteomics 130:200-210.
- Tay E-P and Gam L-H, 2011. Proteomics of human and the domestic bovine and caprine milk. Asia-Pac JMol Bio Biotech 19:45-53.
- Wroblewska B and Kaliszewska A, 2012. Cow's milk proteins immunoreactivity and allergenicity in processed food. Czech J Food Sci 30:211-9.
- Yang T, Li H, Wang F, et al., 2013. Effect of cattle breeds on milk composition and technological characteristics in china. Asian-Australas J Anim Sci 26:896-904.