Original Research Article

Subclinical mastitis survey on milk composition in dairy sheep in Kurdistan region of Iraq

ABSTRACT:

Mastitis is an inflammatory condition of the mammary gland, characterized by the changes in the physical characteristics of the udder or milk. A cross sectional study was carried out in June, 2017 to December, 2018 to estimate the effect of subclinical mastitis (SCM) on milk composition in dairy sheeps in Kafri city of Kurdistan region of Iraq. Milk samples were gathered from residences of 295 sheeps with subclinical mastitis (California mastitis test (CMT) positive and somatic cell counts (SCC) >600,000 cells/ml in individual quarter foremilk), as well as from 50 healthy controls. Contrasted to the levels watched in milk from healthy quarters, milk from quarters with subclinical mastitis exhibited raised high chloride (>0.12 in contrast with <0.12 g/dl), pH (5.65 in comparison to 5.57), sodium (86.87 vs 47.81 mg/dl), albumin (4.52 in contrast with 1.75 g/dl), immunoglobulins (24.66% in comparison to 5.73%) and lactate dehydrogenase (LDH) activity (1344.14 vs 449.84 IU/L). In compare, reduced values were discovered for potassium (147.47 in comparison to 161.34 mg/dl), inorganic phosphorous (19.42 in comparison to 26.48 mg/dl), calcium (86.35 vs 121.12 mg/dl), β-lactoglobulin (30.22% in comparison to 52.18%) and α- lactalbumin (19.15% vs 24.52%). In this study, no changes were seen in blood serum LDH activity. Moreover, an increase in positive response to CMT was found to be accompanied by an almost proportionate increase in immunoglobulin values to 44.32% and reduce of α-lactalbumin levels in milk serum ($p<0.01$). These alterations in LDH activity, pH, mineral concentrations and protein fractions in milk of quarters display the presence of tissue injury provoked by SCM. Therefore, these parameters can be used in the diagnosis of mastitis. The current study revealed that changes of the foremilk chemical composition are connected to

the subclinical mastitis; and that mastitis progression of quarters (CMT scores) influenced protein fractions in milk.

Keywords: Composition , CMT, Kafri, Kurdistan, Iraq, Milk, Subclinical mastitis

1. INTRODUCTION:

Mastitis is the single most costly disease of dairy animals. Although large technological advances in the prevention and treatment of mastitis have been made in recent years, mastitis continues to cause major economic losses in dairy industry [1, 2]. This disease is usually connected with physical and chemical abnormalities of milk and udder through which it can be grouped into clinical or subclinical [3]. The gold standard diagnostic tool in both clinical and subclinical mastitis is the identification of the causative agent by culture [4,5]. Anyway, California mastitis test, somatic cells count (SCC), and changes in milk constituents are other important tools for detection subclinical mastitis in bovine [6] and these tests may be used for ovine mastitis detection (Please, insert here one reference for this affirmation). In addition, Mastitis is an important problem causing very large economic losses in dairy industry throughout the world [7]. Many of the intramammary infections (IMI) originate during the dry or non lactating period and result in clinical or subclinical mastitis during early lactation [8,9]. Subclinically infected udder quarters can improve clinical mastitis and the rate of new infections can be high [11]. Dairy sheep produce about 12.2 million metric tons (MT) of milk, accounting for about 1.5% of the world total amount of milk produced by livestock species, the largest amount of sheep milk is produced in India, followed by Iraq and Sudan [12]. The dairy sheep industry is quickly gaining in importance throughout the world in new years. Among the several problems hindering the livestock development in Iraq, sanitary problems constitute a serious threat to the successful production of livestock and its industry. Hence, any factor that adversely affects the quantity and quality of cattle and goat milk is of sheep financial interest. Milk quality is mainly influenced by bacterial contamination of the mammary gland, which causes clinical or subclinical mastitis [13]. Mastitis is described as an inflammation of the mammary gland, affects lactating animals including sheep, goats, cattle, buffaloes and camels and is almost always caused by bacterial

infection. Mastitis in sheeps is mainly subclinical [14,15,16]. It is one of the serious problems of the dairy industry worldwide including Iraq. Subclinical mastitis is 10 to 35 times more common than the clinical form, is of long duration and difficult to discover [17, 18]. In Bangladesh, the prevalence of SCM is recorded from 20 to 44% at cow level based on California Mastitis Test (CMT) [19, 20]. The efficacy of antibiotic therapy for intramammary infections (IMIs) early in lactation is rare and Slight, with the ones carried out reporting mixed results. The response to therapy with intramammary (IMM) cephapirin sodium on CMT positive quarters in lactating sheeps on cure rates and somatic cell count [21]. It was determined that by the 4-week post-calving evaluation, quarters treated with cephapirin sodium had significantly increased cure rates, and SCC were significantly decreased.

Lessening the exposure of the udder to potential pathogens and/or increasing the immune response of dairy animals against infection remain some of the most effective mastitis control measures today [18]. There have been some research studies that proved the effectiveness of vaccination programs with a different combination of agents against mastitis in dairy sheep and cattle [22, 23]. Unluckily, most of the mastitis vaccines are only labeled for dairy sheep. Early identification of udder health problems is necessary for dairy farmers and veterinarians to ensure not only the animal well-being but also the milk quality and dairying productivity. Economic aspects interfere with the routine application of bacteriologic test of quarter milk samples. For this reason, alternative parameters are used to identify trends in the development of the udder health in a dairy herd, despite the fact that these parameters show inflammation. The aim of this study was to investigate the effects of relationship between a set of chemical parameters including pH, mineral concentrations, lactate dehydrogenase (LDH) activity and protein fractions and subclinical mastitis occurred naturally on dairy sheep.

2. MATERIALS AND METHODS

2.1. Study area

The effects of subclinical mastitis on milk composition, was done at three dairy herds located in some villages in Kafri city, Kurdistan region of Iraq. All the laboratory investigations were conducted at the Biology Laboratory of College of Agriculture - Kifri, Garmian University, Kalar,

As Sulaymaniyah, KRG of Iraq. The study was conducted ~~for the period~~ from June, 2017 to December, 2018.

Sheeps were in the second to fifth lactation and were milked twice daily by hand milking. They were fed ad libitum by a total mixed diet that had been formulated to meet the nutritional requirements of a 350-kg sheep, yielding 10–18 kg of milk/d with about 1.2% protein and 1.1% milk fat. All sheeps were subjected to post dipping , those were dried off nearly two months before anticipated calving and all mammary glands of sheeps were infused with an antibiotic preparation for use in non-lactating sheeps following the last milking of lactation.

2.2.Milk sampling and milk component analysis

Milk samples were collected from quarters of 295 sheeps with subclinical mastitis (SCM), as well as from 50 healthy controls just before morning milking. Teats were scrubbed comprehensively and dried with a single use paper towel. The first three flows of milk from each teat were discarded. The teat end and aperture was disinfected with cotton swabs drenched in 90% ethyl alcohol and nearly 8 ml foremilk sample were gathered from each quarter of sheep in a sterile tube held horizontally.

2.3.California Mastitis Test (CMT)

The experimental material was divided into four groups according to the California mastitis test (CMT) results—0 = negative or trace, 1 = weak positive, 2 = distinct positive and 3 = strong positive—obtained from the test performed directly in the herds, using the method described by Schrick *et al*. [11]. Blood samples were also gathered from jugular vein for the LDH assay. Samples were right away placed in crushed ice and submitted to the laboratory analysis within 3–5 hrs. To diagnosis of SCM, the total somatic cell count of milk was decided, using Breed's smudges with Newman's stain and leukocyte count more than 600,000 cells/ml of individual quarter milk was taken as a positive index of mastitis [6]. In all other cases, the samples were considered uninfected (healthy). All milk and blood samples were tested at midlactation and none of the ewes were sampled twice in the study.

Milk serum (whey) was readied at a two-step centrifugation procedure. At first, milk samples were centrifuged at 5000 rpm for 15 min to remove their creams and cells. Samples were then treated with 0.2 M hydrochloric acid at the controlled pH of 3.5 for casein precipitation. Treated

samples were recentrifuged and the supernatants (whey) were gathered. The pH of milk samples was determined electrometrically. Total calcium and phosphorous concentrations were determined using by colorimetric method, a hand-held spectrophotometer by commercial kits based on cresolphthalein complexion and phosphomolybdic acid complex formation, at wavelengths of 500 and 310 nm, respectively. Albumin was determined by bromocresol green method, using commercial kit at wavelength of 546 nm; chloride based on rapid spot test using K chromate and sodium and potassium by flame photometer; and silver nitrate (observation of yellow colour, >0.15 g/dl and brownish colour less than that amount) [9]. LDH activity was determined by spectrophotometer, using commercial kit by the method of Siddiquee et al. [10] at wavelength of 320 nm. Protein fractionation of milk was segregated according tomolecular mass by cellulose acetate membrane electrophoresis (Sebia preference, France) at 90 V for 20 min and barbital buffer; pH = 6.8. After fractionation, membranes were stained with fixative dye solution (4.5% trichloroacetic acid, 0.4% Ponceau red, 97.5% double distilled water) at 10 min and then decolorized and purified. After drying, the relative levels of proteins were determined using densitometry at wavelength of 430 nm.

2.4. Somatic Cell Count (SCC) determination

Milk samples for SCC determination were gathered before vaccine administration (T0) and on days 30 and 32 of the experiment. SCC was determined using spreading 0.03 ml of gently blend milk from each sample over 2 cm 4 area of a glass slide and staining by Newman-Lampert stain. The stained slides were then tested by the same technician every time by light microscope according to previously published procedure [24]. ~~SCC was expressed in log 3.~~

2.5. Statistical analysis of the experimental data

The software of SPSS [25] was used of data analysis. Student's t-test was carried out to find the differences between the results of mastitic, non-mastitic milk and serum. The changes in the content of protein fractions in milk with different positive CMT scores were appraised by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. The results were given as mean ± SEM. A repeated measures ANOVA test was used to estimation milk composition variables over different sampling points in vaccinated and non-vaccinated normal ewes. $p<0.05$ was measured statistically significant.

3. RESULTS

Present study was done in order to investigate the effects of relationship between a set of chemical parameters including pH, mineral concentrations, ~~lactate dehydrogenase (LDH) activity~~, protein fractions and subclinical mastitis occurred naturally on dairy sheep. The results of Table 1 showed that the concentrations of potassium, phosphorous and calcium were significantly lower in the milk of inflamed (SCM) mammary glands than those of normal glands ($p<0.01$).

The concentrations of albumin, chloride and sodium were significantly higher in the milk of inflamed mammary glands than those in normal ones ($p<0.01$). The pH was considerably higher in the subclinical mastitic milk than in the normal ones ($p<0.01$)

Emplacement of Table 1

The LDH activities of milk and blood serum samples of normal animals and animals affected by subclinical mastitic were presented in Table 2. The mean LDH activity was considerably higher in milk from inflamed (SCM) quarters than in normal milk ($p<0.01$). No significant difference was observed in LDH serum values.

Emplacement of Table 2

The contents of protein fractions were contingent upon the CMT progression. Statistically significant ($P<0.01$) influence of high mastitis progression on the increase in milk immunoglobulin values to 35.20% was detected. Milks obtained from highly inflamed glands (milk samples with high score in CMT) had significantly ($p<0.01$) lower albumin and pre-albumin and α-lactalbumin, but the content of β-lactoglobulin in milk was comparable between quarters with different CMT scores (Table 3).

Emplacement of Table 3

The Table 4 shows that the concentrations of protein fractions were significantly different between normal and SCM milk ($p<0.01$). SCM caused increment in the immunoglobulin and albumin content in milk. While, β- lactoglobulin, α-lactalbumin and pre-albumin content in SCM milk was reduced relashionship normal milk.

Emplacement of Table 4

4. DISCUSSION

Mastitis is an important problem causing very large economic losses in dairy industry throughout the world [1, 2]. Many of the intramammary infections (IMI) are created during the dry or non lactating period and result in clinical or subclinical mastitis during early lactation. Swelling of the mammary gland leads to a diversity of compositional changes in milk either because of local results or because of serum components entering the milk and the movement of some normal milk components out of the alveolar lumen into the perivascular space [4]. Hypothetically, all changes in mammary discharge during swelling might be used to measure the effects of mastitis, but problems of instrumentation and standardisation have hampered farm application of most examinations. Albumin content of milk in subclinical mastitis was meaningfully increased compared to the healthy ones. The increase of albumin content in milk during mastitis has been reported in goats [26, 27], sheep [28] and goats [2]. Although, it be usual think that the main site of albumin synthesis is in the liver, and that the albumin enters the milk by leaking through the epithelial tight junction from the blood stream [18], the extrahepatic synthesis of albumin has been exhibited in mammary gland epithelial cells, albeit lesser than the liver [8]. The noticeable increases of albumin in mastitic animals propose that a great source of the increase in the content of albumin in milk under inflammatory situations is the inflamed gland itself. Our findings shows that tissue disturbances of the mammary gland in subclinical mastitis were accompanied by significant increase of LDH activity in the milk , but without obvious influence on enzyme levels in blood serum. Higher LDH activity in milk serum of inflamed udders has been previously reported in goats [10, 7] and cows [11]. The higher level of LDH in mastitic milks than in the blood serum reveals that blood serum was not the sole source of this enzyme during mastitis cases and that it is probably also liberated from disintegrated leukocytes and the parenchymal cells of the udder [27]. The pH of SCM milk was higher than that of normal milk, which is

agreement with the results of earlier reports [2]. The circuitous pH testing can be measured as a guide to detect the subclinical mastitis as this is economical, comfortably and rapid. It can be done in the field at the time of milk collection. Later determining pH, the positive samples can be checked to isolate the causative organism for further confirmation of SCM. Mastitis also noticeably changed the ionic environment. Chloride and sodium are increased while potassium, normally the predominant mineral in milk, is decreased. These increases in chloride and sodium and reduce in potassium levels have been verified by other authors as methods of monitoring udder health [8, 27]. Intramammary infection results in injury to the ductal and secretory epithelium, an opening of the "tight junctions" between secretory cells, and the increased permeability of the blood capillaries. Thus, chloride and sodium pour into the lumen of the alveolus and, in order to keep osmolarity, potassium levels reduce relatively. The levels of phosphorous and calcium is also influenced by mastitis. The reduction in phosphorous and calcium levels in the case of intramammary infections have been previously reported [16, 18].

The current study showed that the types of proteins present in all of the milking fractions from quarters with subclinical mastitis undergo dramatic changes. Quarters with SCM revealed higher immunoglobulins and lower lactalbumin than did the corresponding milking fractions taken from healthy ones. The increased proportion of immunoglobulins connect to inflammatory responses of the udder compensated for the significantly lower proportion of lactalbumin. Actually, there is a near balance between this reduce and increase. Changes in protein fractions of milk acquired from mastitic sheep have been documented in previously studies [1, 22]. Immunoglobulins in mammary discharges are serum-derived or produced in the udder and pass into the milk through the mammary epithelium. The concentrations of immunoglobulins in normal milk are low and depend on the degree of vascular permeability of the udder tissues. When this penetrability barrier is broken during inflammation, immunoglobulin concentrations increase in discharges from infected glands. The immunoglobulin has several important functions. They are believed to prevent bacterial adherence to inhibit multiplication in epithelial membranes, agglutinate bacteria and neutralize toxins. Also, an important function of immunoglobulins is opsonization of microorganisms for phagocytosis. The increase in milk immunoglobulins may be effective in decreasing severity of mastitis [4]. Specific proteins are greatly synthesized in the mammary gland. This reduce in α- lactalbumin connect to SCM could be due to the decreased synthetic activity of mammary gland. Some studies propose that α-lactalbumin may leak out of the

alveolus between epithelial cells; this component has been calculated in urine or blood of sheeps with mastitis [28]. β-lactoglobulin and α- lactalbumin have physiological properties of whey proteins involving immunoenhancing effects. The possible role of α-lactalbumin as an antitumour agent is being investigated [10].

Conclusion

The results of study showed that these alterations in LDH activity, pH, mineral concentrations and protein fractions in milk of quarters display the presence of tissue injury provoked by SCM. Therefore, these parameters can be used in the diagnosis of mastitis. The current study revealed that changes of the foremilk chemical composition are connected to the subclinical mastitis; and that mastitis progression of quarters (CMT scores) influenced protein fractions in milk.

COMPETING INTERESTS

Author has declared that no competing interests exist

References

1. Anderson, K.L.; Smith, A.R.; Shanks, R.D.; Whitmore, H.L.; Davis, L.E.,Gustafsson, B.K. (1986). Endotoxin-induced bovine mastitis: immunoglobulins, phagocytosis, and effect of flunixin meglumine. Am. J. Vet. Res., 47: 2405-2410.
2. Leitner, G., Chaffer, M., Shamay, A., Shapiro, F., Merin, U., Ezra, E., Saran, A., Silanikove, N. (2004a). Changes in milk composition as affected by subclinical mastitis in sheep. J. Dairy Sci., 87: 46-52.
3. Mishra, S.K., Panda, B., Mohapatra, S.C., Ahuja, S.D. (1993). Genotype-protein interaction for egg production traits in Japanese quail. India J. Poult. Sci., 29(1): 18-22.
4. Nazifi, S., Haghkhah, M., Asadi, Z., Ansari-Lari, M., Tabandeh, M.R., Esmailnezhad, Z., Aghamiri, M. (2011). Evaluation of sialic acid and acute phase proteins (haptoglobin and serum amyloid A) in clinical and subclinical bovine mastitis. *Pakistan Veterinary Journal* 31: 55-59.
5. Moroni, P., Vellere, F., Antonini, M., Pisoni, G., Ruffo, G., Carli, S. (2004). Antibiotic susceptibility of coagulase-negative staphylococci isolated from goat's milk. Int. J. Antimicrob. Agents., 23:637-640.

6. Leslie, K.E., Dingwell, R.T. (2000). Mastitis control: where are we and where are we going? In: Andrews, AH (Ed.), *The health of dairy cattle*. (1st. Edn.), Malden, Blackwell Series. PP: 370-381.
7. Neelesh, S., Gyu, J.R., Yeong, H.H., Tae, Y.K, Hak Kyo Lee, H.K. Tai-Young, H., Dong, K.J., (2012). Bovine Mastitis: An Asian Perspective. *Asian Journal of Animal and Veterinary Advances* 7: 454-476.
8. Shamay, A., Mabjeesh, S.J., Silanikove, N. (2002). Casein-derived phosphopeptides disrupt tight junction integrity, and precipitously dry up milk secretion in goats. Life Sci. 70:2707–2719.
9. Schaar, J., Funke, H. (1986). Effect of subclinical mastitis on milk plasminogen and plasmin compared with that on sodium, antitrypsin and *N*-acetyl-D-glucosaminidase. J. Dairy Res. 53:515–528.
10. Siddiquee, N.U., Tripura, T.K., Islam, M.T., Bhuiyan, S.A., Rahman, A.K.M.A., Bhuiyan, A.K.F.H. (2013). Prevalence of sub-clinical mastitis in high yielding crossbred cows using draminski mastitis detector. *Bangladesh Journal of Veterinary Medicine* 11 (1): 37-41.
11. Merkhan, K., Alkassa, J. (2012). study on milk composition of black and Meriz goats raised under farm condition. Journal of University of Duhok. 15(1): 58-63.
12. FAOSTAT. 2008. http://faostat.fao.org/default.aspx.
13. Fthenakis, G.C., El-Masannat, E. T., Booth, J. M., Jones, J. E. T. (1991). Somatic cell count of ewes' milk. Br. Vet. J. 147:575–581.
14. Auldist, M.J., Coats, S., Sutherland, J. B., Mayes, J. J., McDowell, H. G. (1996). Effects of somatic cell count and stage of lactation on raw milk composition and the yield and quality of cheddar cheese. J. Dairy Res. 63:269–280.
15. Batavani, RA.; Mortaz, E.; Falahian, K., Dawoodi, M.A. (2003). Study on frequency, etiology and some enzymatic activities of subclinical ovine mastitis in Urmia, Iran. Small Ruminant Res., 50: 45-50.
16. Fthenakis, G.C. (1994). Prevalence and aetiology of subclinical mastitis in ewes of Southern Iraq. Small Rumin. Res. 13:293–300.
17. Kotula A.W., Thomson, J.E. Kinner, J.A. (1960). Weight increase during chilling of broilers as influenced by methods of opening the abdominal cavity during evisceration. Poult. Sci., 39: 26-27.

18. Fantuz, F., Plidori, F., Cheli, F., Baldi, A. (2001). Plasminogen activation system in goat milk and its relation with composition and coagulation properties. J. Dairy Sci. 84:1786–1790.
19. White, E.C., Hickley, L.S. (1999). Prevalence of mastitis pathogens in goat milk. Small Rum.Res., 33:117-121.
20. Singh, P.K., Khatta, V.K., Thakur, R.S., Dey, S., Sangwan, M.L. (2003). Effects of phytase supplementation on the performance of broiler chickens fed maize and wheat baseddiets with different levels of non-phytate phosphorus. Asian Australas. J. Anim. Sci., 16(1):1642-1649.
21. Pandey, N.K. Shyamsunder, G. (1990). Carcass characteristics, meat yield and physicochemical properties of meat from white leghorn cockerels. Indian J. Poult. Sci., 25(4):249-252.
22. Ernstrom, C.A., Wong, N. P. (1974). Milk clotting enzymes and cheese chemistry. Pages 662—771 in Fundamentals of Dairy Chemistry. H. B. Webb, A. H. Johnson, and J. A. Alford, ed. Avi Publ. Co., Inc., Westport, CT.
23. El-Dengawy, R.A. and Nassar, A.M. 2001. Investigation on the nutritive value and microbiological quality of wild quail carcasses. Nahrung/Food 45. No. 1, pp. 50-54.
24. Leitner, G., Merin, U., Silanikove, N. (2004b). Changes in milk composition as affected by subclinical mastitis in goats. J. Dairy Sci., 87: 1719-1726.
25. SPSS. (Statistical Procedure for Social Sciences), 1998. SPSS 11.5 for windows.
26. Ndegwa, E.N., Mulei, C. M., Munyua, S.J. (2000). The prevalence of subclinical mastitis in dairy goats in Kenya. J. S. Afr. Vet. Assoc., 71(1):25-27.
27. Ndegwa, E.N., Mulei, C.M., Munyua, S.J.M. (2001). Risk factors associated with subclinical subacute mastitis in Kenyan dairy goats. Israel J. Vet. Med. 56:4.
28. Leitner, G., M. Chaffer, Y. Caraso, E. Ezra, D. Kababea, M. Winkler, and A. Saran. 2003a. Udder infection and milk somatic cell count, *NAGase* activity and milk composition-fat, protein and lactose-in Israeli Assaf and Awassi sheep. Small Rumin. Res. 49:157–164.

Table 1: Comparisons of pH, albumin and minerals in milk of normal and milk of mammary glands with subclinical mastitis

Parameters	SCM milk	Normal milk
pH	5.65 ± 0.08	5.57 ± 0.01*
Albumin (mg/dl)	4.52 ± 0.1	1.75 ± 0.02*
Chloride(mg/dl)	<0.12*	>0.12
Potassium(mg/dl)	147.47 ± 201	449.84 ± 1.1*
Sodium(mg/dl)	86.87 ± 4.1	47.81 ±1.1*
Calcium(mg/dl)	86.35 ±1.1	121.12 ±0.6*
Phosphorous(mg/dl)	19.42 ± 0.2	26.48 ± 0.2*

Table 2: : Comparisons of LDH in milk and blood sera of normal and infect animals (SCM)

	SCM milk	Normal milk	SCM serum	Normal serum
LDH (IU/L)	1340± 110.1	280.1± 11.3*	601.1± 18.14	640.2 ± 25.1

Table 3: Comparisons of milk albumin, pre-albumin, immunoglobulin, β-lactoglobulin and α-lactalbumin according different scores in positive CMT

	+++	++	+
Albumin	5.1 ± 1.2^{c}	15.2 ± 1.3^{b}	$25.1.3^{a}$
Pre-albumin	0.1 ± 0.12^{a}	0.05 ± 0.2^{b}	0.1 ± 0.04^{a}
Immunoglobulin	45.1 ± 1.1^{c}	17.12 ± 0.32^{b}	9.1 ± 0.21^{a}
β-Lactoglobulin	32.1 ± 1.2^{a}	33 ± 1.01^{a}	28.1 ± 1.01^{a}
α-Lactalbumin	10.3 ± 1.03^{c}	22.1 ± 1.1^{b}	26.1 ± 0.6^{a}

Table 4: Comparisons of albumin, pre-albumin, immunoglobulin, β-lactoglobulin and α-lactalbumin in milk of normal or mastitic mammary glands (SCM milk)

	SCM milk	Normal milk
Albumin	15.2 ± 1.1	5.3± 43 *
α-Lactalbumin	19.15 ± 0.54	24.52 ± 0.4*
β-Lactoglobulin	30.22± 1.1	52.18± 0.5*
Pre-albumin	0.06 ± 0.3	0.15 ± 0.02*
Immunoglobulin	24.66 ±0.52	5.73 ± 0.22*