ISSN: 2279 - 0594

Journal of Biomedical and Pharmaceutical Research

Available Online at www.jbpr.in

CODEN: - JBPRAU (Source: - American Chemical Society)

Index Copernicus Value: 63.24

PubMed (National Library of Medicine): ID: (101671502)

Volume 6, Issue 2: March-April: 2017, 72-77

Research Article

Comparative studies of healthy and infected milk samples of bovine animals for determining its immunological activity

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Received01March 2017; Accepted 08April 2017

ABSTRACT

Interest in bovine milk samples and their effect on human health is an ongoing concern. Several questions were raised related to milk that was unsafe and is responsible for spreading the disease like tuberculosis and other diseases. After the introduction of pasteurization, milk is considered to be one of the healthy drinks that will save millions of people from malnourishment. Recently, lot of questions was raised related to milk samples of bovine animals especially healthy and infected ones. In this paper, we analyzed some amino acids, lactose, casein, cells population in terms of lymphocytes, monocytes and granulocytes count and also measured lactoferrin content in healthy and infected milk samples of bovine animals especially cow. The results of these studies showed that infected bovine (cow) milk samples showed drastic decline in lactose and casein content as compared to healthy ones. In addition, amino acids especially glutamic acid and tyrosine showed some enhancement but there is decline in histidine including enhancement of granulocytes count and decline in lymphocytes count in case of infected ones as compared to healthy ones. Similarly, lactoferrin also got similar results there is some enhancement in infected as compared to healthy bovine animals. Overall, there is clear cut demarcation between infected and healthy bovine milk samples.

Keywords: Bovine; milk samples; lactose; casein; amino acids; lactoferrin

INTRODUCTION:

Milk, white liquid that is produced by the mammary glands of mammals including humans. All these mammals will produce abundant source of milk in order to feed their offspring [1]. Generally, milk contained more valuable nutrients that can offer a wide range of health benefits e.g. calcium (prevent osteoporosis). In some circumstances, people cannot be able to digest lactose (sugar content in milk) because of absence of enzyme i.e. lactase.Lactose is one of the important component in milk samples of bovine animals and it is broken down into two simplex forms i.e. glucose and galactose using enzyme (lactase) that are present abundantly in the saliva and stomach [2, 3]. In some circumstances, body cannot be able to digest lactose because of little or complete absence of lactase enzyme [4]. Those individuals are referred as lactose intolerant [2-4]. However, lot of variations exists in the amount of lactose content that are reported in colostrum at different time intervals e.g. first milking colostrum sample of bovine animals that are collected within 6 h showed higher content of lactose as compared to 24 h and 48 h colostrum sample [5]. The graphical representation of bovine animals in the form of **Table 1**with respect to total protein, casein and total whey protein including fat and carbohydrates content especially lactose i.e. glucose and galactose content in different animals (information collected from the literature)**.**

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Table 1: Components of bovine animals in healthy bovine milk samples

Milk components	Cow, (%, n =10; 100 ml)	Buffalo, (%, n =10; 100 ml)	Sheep, (%, n =10; 100 ml)	Goat, (%, n =10; 100 ml)
Protein	3.2 - 3.6	3.8 - 4.2	5.6 - 6.2	3.4 - 3.8
Casein	2.4 – 3.0	3.4 – 4.2	4.5- 5.3	2.2 - 2.8
Whey protein	0.6 - 0.7	0.5 – 0.6	0.8 - 1.2	0.8 - 1.2
Fat	3.4 – 3.8	7.1- 7.8	7.0 – 8.2	3.6 – 4.4
Carbohydrates	4.4 - 4.8	4.4 - 4.8	4.3 - 4.5	4.5 - 4.7

In milk samples of different bovine animals including mammals, one of the most essential components are reported i.e. amino acids that are required for infants, mainly in the form of proteins [6, 7]. The chain of amino acids i.e. proteins are one of the crucial nutrients for infant growth and development and also be able to increase the uptake of other nutrients and enhancing the infant's immune function against various pathogenic bacteria, virus including yeasts [8]. In this study,milk samples (n =10) were collected in sterilized test tubes (autoclaved at 15 lbs. for 20 min) from Baramati region. For these studies, we worked on amino acids content in different milk samples of bovine animals and results of these studies as shown in **Fig.1**. The results showed that healthy bovine animals especially goat showed higher amount of amino acid content followed by sheep, buffalo and cow. Similar results also reported in case of infected bovine animals (data not shown). Lot of research work related to milk samples that have already been done in other countries including Thailand, Mexico and America. In this regard, lot of variations related to amino acid content in milk samples that are reported and may differ between countries [9, 10].

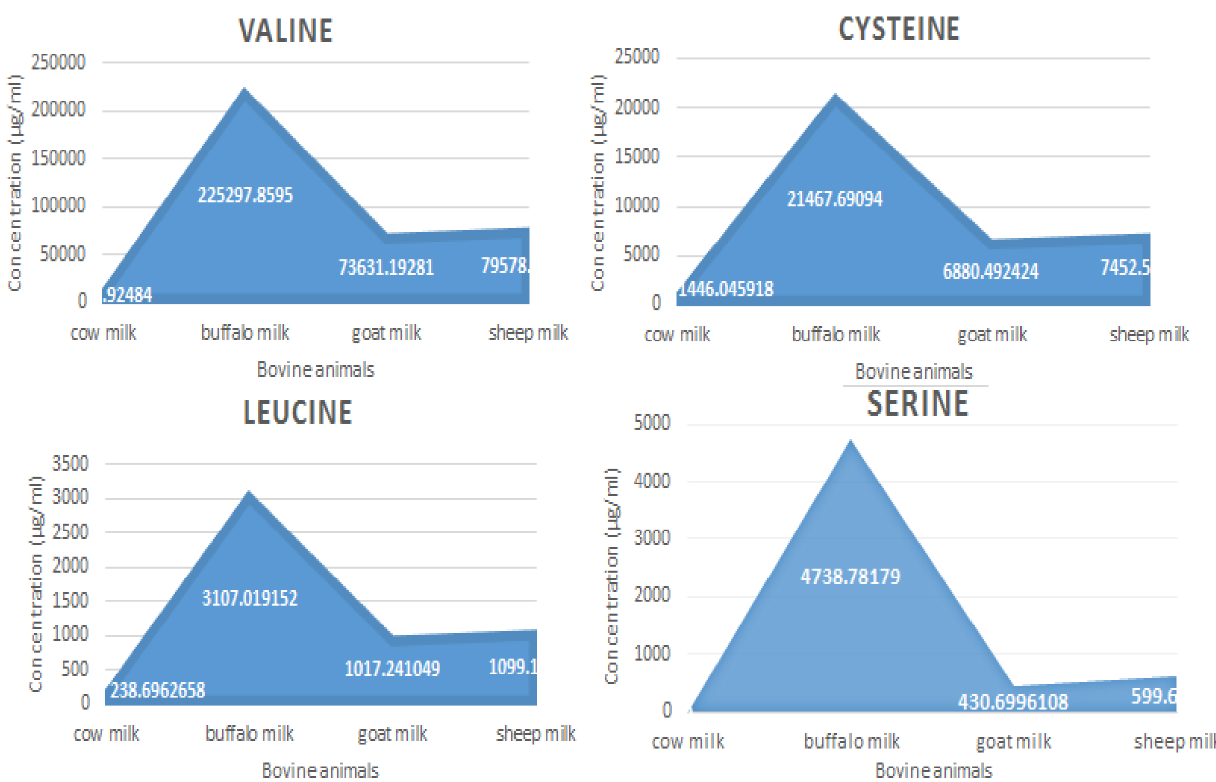

Fig.1. Amino acid content in healthy bovine milk samples especially cow. Values are represented as Mean ±S.E. In this study, buffalo milk sample showed higher amount of amino acid content especially valine, cysteine, leucine and serine as compared to cow, goat and sheep milk samples.

In general, milk becomes contaminated during milking time or through cooling and storage. Milk is considered to be one of the excellent medium for the growth of bacteria, yeasts including moulds that are included under the group of common contaminants [11]. Their rapid growth of these contaminants, particularly reported at very high temperatures can cause marked deterioration, spoiling the milk for liquid consumption or manufacture into dairy products. In an effort to reduce the burden of infection rate in milk samples, following precautions should be taken for further consideration i.e. udders and teats should be clean; minimizes bacterial contamination during milking time; store milk in clean containers [12]etc. For these studies, we collect milk samples of normal and infected bovine animals especially cow and determined their amino acid content in whey protein concentration through Nanodrop method as shown in **Fig.2.** The results showed that infected cow showed drastic increase in amino acid content especially glutamic acid and tyrosine but decline in histidine in case of infected bovine animals as compared to control healthy ones.

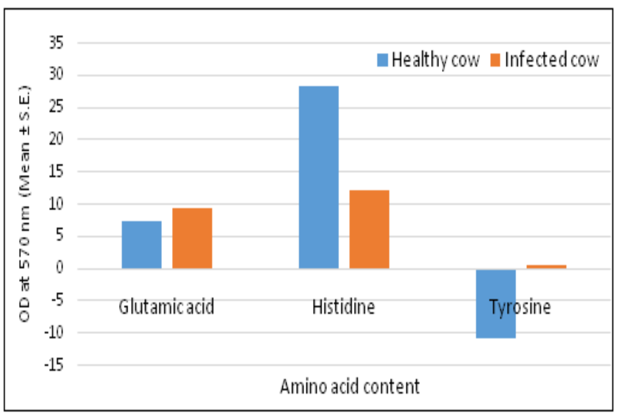

Figure 2: Estimation of amino acid content in infected bovine animals especially cow.

One of the most severe infection reported in bovine animals i.e. mastitis (inflammation of the udder) that can be distinguished by the number of lymphocytes, monocytes and granulocytes count in bovine animals as compared to normal or uninfected bovine animals [13]. Although, lot of microbial agents i.e. bacteria, fungi including virus can cause udder infection and the most common agents that are reported in mastitis i.e. bacteria. Most of the bacterial pathogens in the form of gram positive and gram negative that are reported in mastitis disease i.e. *Staphylococcus aureus, Streptococcus agalactiae, Str. dysgalactiae, Str. uberis* and *Escherichia coli* though other pathogens can cause occasional herd outbreaks [13, 14].

Mastitis microorganisms especially bacteria that originates in various sites on bovine animals especially cow. These bacteria are multiplied in various ways and spread from cow to cow. The most common observable types of mastitis bacteria that are generally originated from the udders of infected cows including sores on teats. These pathogens multiply in teat sores and are spread during milking. One of the most devastating diseases that are reported in bovine animals i.e. mastitis showed huge amount of economic losses especially farmers with respect to less production of milk and somehow contaminated with antibiotics [13-16]. Most of these milk processing industry also showed some losses because of the presence of antibiotic in milk and reduced chemical and bacterial quality of mastitic milk. In other words, during infection rate of mastitis, granulocytes count number will increased and decreased in lymphocytes count as compared to control ones (**Fig.3**). Normally, bacterial count number will increased above 50,000 per ml during subclinical stage whereas its count number will suddenly increase in terms of millions/ml at clinical stage.When clinical mastitis occurs, effective therapy is a course of antibiotic infusions through the teat duct. These therapies always remedy some of the clinical diseases and eliminated bacterial infection. Some of bacterial Infections may spontaneously recover but most of them persists and eliminated eventually through antibiotic therapy.

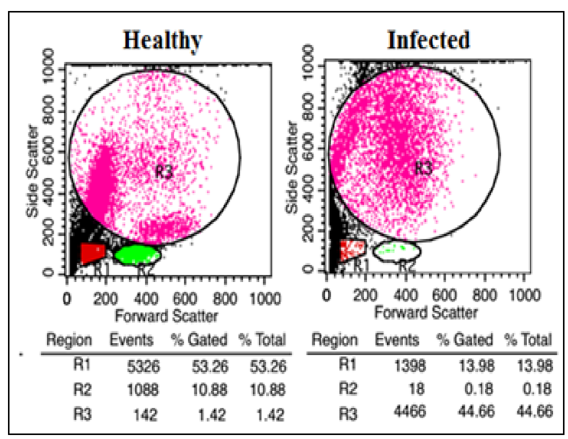

Figure 3: Comparative investigation of healthy and infected (mastitis) bovine milk samples in order to analyzed lymphocytes, monocytes and granulocytes count.

In short, milk is considered as one of the most complex food material that is made up of different components, which may have some negative or positive health effects, respectively. In this paper, we discussed about some amino acids including determination of whey protein concentration in different bovine animals. In other words, bovine animals milk contained some nutrients that are required for the growth and development of calf and is considered to be one of the useful resource of proteins, lipids, vitamins, amino acids, nucleotides, peptides, enzymes, minerals, immunoglobulins, cytokines, bioactive peptides [1-4] etc. In bovine milk samples, lipids are generally in the form of emulsified globules that are coated with membranes whereas proteins are in colloidal dispersions as micelles and finally casein micelles that occur as colloidal complexes of protein and salts, primarily calcium. Among these, lactose and most minerals are in solution. In bovine mastitis, number of bacterial pathogens [15, 16] that are reported and showed decline in lactose content as compared to healthy bovine animals especially cow.

In this study, we collect milk samples from infected cow and showed higher protein content including whey protein concentration but decline in casein and lactose content when compared to milk samples from healthy bovine animals especially cow as shown in **Fig.4.**Lactose (disaccharide sugar; 4.5~5% by weight) is found in milk samples and is generally formed from galactose and glucose. The most significant enzyme lactase that is important for digestive hydrolysis of lactose in milk. If there is some deficiency related to this enzyme i.e. lactase causes lactose intolerance. In short, lactose is split into glucose and galactose. For determination of lactose content in milk samples (using standard lactose, himedia), glucose reacts with phenolic compound through an enzymatic reaction, with peroxidase and forms a pink colored complex. The absorbance or optical density of the complex is read at 505 nm and its value is directly proportional to the concentration of lactose in the milk sample. In addition, casein is also measured using milk samples of healthy and infected cow. First of all, milk samples were diluted in PBS and then centrifuged at high speed in order to separate the casein, whey protein and fat molecules. The results of these studies showed that infected cow milk sample showed less casein content as compared to healthy ones. In mastitis, there is increase or enhancement in the permeability of blood-milk barrier and also increased influx of serum proteins including enzymes from the blood, which may lead to an increase in proteolysis.

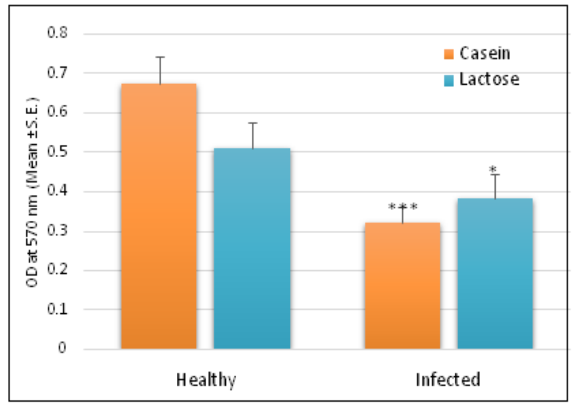

Figure 4: Effect of lactose andcasein content in healthy and infected milk samples of bovine animals especially cow.

Healthy and fresh milk samples were collected (n=5, each group) and then diluted in PBS. Firstly, determination of lactose content in milk samples using variable dilution of standard lactose and then prepare the standard curve in order to determining lactose content in infected and healthy milk samples of cow. Similarly, casein content also measured in the same way using standard casein. Values are expressed as Mean ±S.E. The difference between healthy and infected is determined by one way ANOVA test (*P < 0.05; **P < 0.01 and ***P < 0.001).

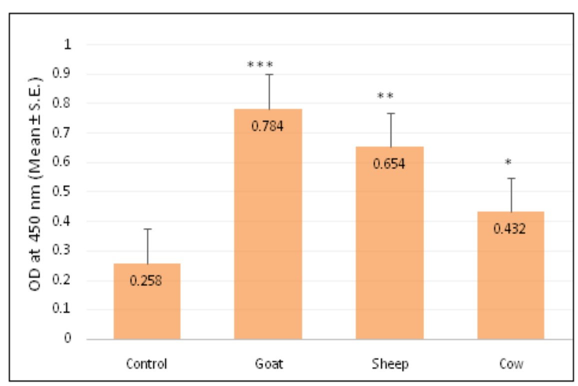

Figure 6: Determination of lactoferrin content in bovine animals.

Values are expressed as Mean ±S.E. The difference between healthy and infected is determined by one way ANOVA test (*P < 0.05; **P < 0.01 and ***P < 0.001).

Generally the composition of milk nutrients especially observed in bovine animals that are totally varied according to the stage of lactation, age, breed, nutrition, energy balance and health status of the udder. The most significant feature observed in bovine animals i.e. concentration of milk proteins is totally doubled in colostrum as compared to later stage in lactation period. Some of the milk proteins are involved in early development of immune response whereas other proteins take part in non-immunological defense e.g. lactoferrin.

Lactoferrin is one of the major component of whey protein and it generally consists of 689 amino acid residues approximately where as in case of human lactoferrin consists of 691 residues. According to the literature, the concentration of lactoferrin content in human milk (2 mg/ml) and colostrum (7 mg/ml) sample is reported while in case of bovine (0.2 mg/ml) milk and colostrums (1.5 mg/ml) samples are observed [17, 18]. In this study, we collect milk samples of healthy and infected cow in order to determine the lactoferrin content using ELISA. For these studies, plates were coated with lactoferrin capsule (1 mg/ml; 200 µl). Incubate the plate for 24 h at 4 ºC and then plates were blocked with 1% BSA (bovine serum albumin). After coating and blocking the plates, add milk samples of healthy and infected in order to analyze the lactoferrin content. For these studies, horse anti-serum used as secondary antibody. The optical density of milk samples for determining lactoferrin content at 450 nm. The results of these studies showed that goat milk sample showed higher lactoferrin content followed by sheep and then cow as shown in **Fig.5**.In short, Lactoferrin played a vital role in cell metabolism and also helpful in transporting oxygen. In general, sports person actually use lot of iron based supplements (reduce iron related sickness) that are required for health nutrition in order to boost oxygenation and also improved its performance rate. In contrast, lactoferrin is considered as cell-secreted mediator that bridges the innate and adaptive immune responses.

CONCLUSION

The consumption of bovine milk samples has continuously decreased for the last 10 years. This trend may be due to decline in health effects or disease burden that have been attributed to milk and milk products. The major objective of thus paper is to discuss or describe some effects of milk components especially lactose, casein, cells population, lactoferrin etc. that are of particular interest for human health and tried to give an overview of infected and healthy bovine milk samples. In short, it gives some information about nutritional composition of the milk for human consumption.

AUTHORS CONTRIBUTION

This work was carried out in collaboration between four authors. AG and SSS designed the study, wrote the protocol and interpreted the data where SS and VP anchored the field study, gathered the initial data related to M.Sc Microbiology dissertation work under AG guidance and performed preliminary data analysis. AG, SSS, VP and BS managed the literature searches whereas AG and SSS produced the initial draft. The final manuscript has been read and approved by all authors.

ACKNOWLEDGEMENT

All authors are highly acknowledged to Vidya Pratishthan's Arts, Science and Commerce College, Baramati, District Pune, Maharashtra, India.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1. Uruakpa FO, Ismond MAH, Akobundu ENT. Colostrum and its benefits: A review. Nutrition Research 2002; 22 (6): 755–767.
2. Heyman MB. Lactose Intolerance in Infants, Children, and Adolescents. Pediatrics Review 2006; 118 (3): 1279–1286.
3. Deng Y, Misselwitz B, Dai N, Fox M. Lactose Intolerance in Adults: Biological Mechanism and Dietary Management. Nutrients Review 2015; 7 (9): 8020–8035.
4. BerniCanani R, Pezzella V, Amoroso A, Cozzolino T, Di Scala C, Passariello A. Diagnosing and treating Intolerance to

carbohydrates in Children. Nutrients Review 2016; 8 (3): E157.

5. Uruakpa FO, Ismond MAH, Akobundu ENT. Colostrum and its benefits: A review. *Nutrition Research 2002; 22 (6): 755–767.*

6. Jensen RG, Newburg DS. Bovine milk lipids. In: Jensen RG, editor. Handbook of milk composition. Academic Press USA 1995; 543–575.

7. Gupta A, Jadhav JB, Gunaware KD, Shinde B. Whey proteins and its impact on human health nutrition: review. Journal of analytical Pharmaceutical research 2016; 3(7): 00083

8. Jensen RG, Newburg DS. Bovine milk lipids. In: Jensen RG, editor. Handbook of milk composition. Academic Press USA 1995; 543–575.

9. Baumrucker CR. Amino acid transport systems in bovine mammary tissue. Journal of Dairy Science 1985; 68:2436.

10. Coppock CE. Energy nutrition and metabolism of the lactating cow. Journal of Dairy Science 1985; 68:3403.

11. Barbano DM, Ma Y, Santos MV. Influence of raw milk quality on fluid milk shelf life. Journal of Dairy Science 2005; 88(1): 77.

12. Bergere JL, Sivela S. Detection and enumeration of clostridial spores related to cheese quality – classical and new methods. Bulletin IDF 1990; 251: 18–23.

13. Chopade D,Gupta A, KaleP,Chaphalkar SR. Prevalence of bacteria from mastitis disease in dairy animals: Antibiotic sensitivity. Asian Journal of clinical research 2016; 3: 24-28.

14. Gupta A, Chopade D,Chaphalkar SR. Prevalence of bacterial pathogens in mastitis disease: Review. Asian Journal of clinical research 2016; 3: 8-11.

15. *Barbosa-Cesnik C, Schwartz K and Foxman B. Lactation mastitis. JAMA 2003; 289 (13): 1609–1612.*

16. BlumS, HellerED, KrifucksO, et al.Identification of a bovine mastitis *Escherichia coli*subset. Vet Microbiol 2008;132:135–148.

17. Patil VS, Gupta A, Kamble S, Shinde B. Surveillance of bovine milk samples and determined its immunological activity of lactoferrin content. Indo American Journal of Pharmaceutical Research 2017; 7(2): 7640-7645.

18. Gupta A, Chaphalkar SR. Quantitative estimation of lactoferrin and leukocytes contents in bovine, sheep and goat samples. Journal of Advances in Food Science and Technology 2016; 3(1):21 – 24.