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Protein and fat examination from the raw milk of different mammalian species (Cow, buffalo, goat, and human) with successive lactation days

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Abstract
Milk is an important food because of their high nutritional value for public health. Biochemical methods were used for the examination of the quality of milk but lack the study in lactation stages. In the present study, raw milk samples (n=3) from mammalian species i.e Cow, Buffalo, Goat, and Human were collected and analyzed for protein and fat determination during successive lactation days. The presence of fat and protein percentage determination was evaluated by the Gerber method and formal titration method respectively. The percentage of protein and fat ratio in each mammal were considered during their lactation days. The study shows the protein content of cow milk (5.85%) is higher at 11th day of lactation than in Buffalo, goat and human milk, but it is low in human milk (1.0%) at 150th day as compared with buffalo, goat, and cow milk. Among these samples, Cow milk had the highest protein content during the early stage. The fat content (6.5%) in buffalo milk at the 3rd day of lactation is higher than other milk, while fat content (2.5%) is lower in cow milk at 150th day of lactation than in human, goat, and buffalo milk. The result indicated that analyzed milk through entire lactation states could contribute a marker for highly nutritional value based on their milk profile. This study put forward that milk composition is largely a function related to maternal nutrient intake by infants and duration of milk production. Conclusions were made possible by including the markable variation in biochemical components mainly fat and protein among species at different lactation stages.

Keywords: Mammalian species, lactation, biochemical components

Introduction
Milk is the characteristic secretion of the mammary gland of all mammals. Because of its function of the nourishment of the young, it is necessarily complex; it must supply nutrients, minerals, and vitamins in proper form, kind and amount [Anonymous, 1973] [3, 4]. It is a complex mixture of proteins, carbohydrates, vitamins, minerals and other constituents dispersed in water [Harding, F, 1999] [16]. On the basis of the protein content of milk, it is generally regarded as "nature's most nearly perfect food" owing to its rich protein profile containing more essential amino acids than any other natural food [Foley *et al*., 1972] [12].
Milk composition varies greatly among the different species, which is partly attributed to the inherited capabilities. It has been reported that breeds producing milk with high-fat content produce less milk than those with lower fat % age [Campbell *et al*., 1957] [8]. According to Malcolm and Paul [Athar *et al*. 1986] [6] cow milk contains 12.5% total solids, 3.8% fat, 8.7% solid non fat, 4.6% lactose, 0.8% ash, 0.2% NPN (Non Protein Nitrogen), 3.1% protein and 87.5% water. Due to its high nutritive value, cow milk is widely consumed by infants and adults alike to meet their basic nutritional needs. Cow milk is the most universal raw material for processing dairy products resulting in the broadest spectrum of manufactured dairy products. More than 5% of the world's milk comes from buffaloes. Over 95% of the world buffalo milk is produced in Asia [Charan, 1994] [10]. Buffalo milk is used in much the same way as cow's milk. It is high in fat and total solids, which gives it a rich flavor. Many people prefer it than cow's milk. The value of goat milk in human nutrition has so far received very little factual and academic attention [Haenlein 1992; Park, 1991] [15, 21] despite its medical need for some people especially infants afflicted with various ailments, including cow milk protein sensitivities [Lothe *et al*., 1982 and Host *et al*., 1988] [20, 17]. Goat milk proteins and fats have many significant differences in their compositions from the milk of other mammalian species, especially in relative proportions of the various milk proteins and fats and in their genetic polymorphisms [Ambrosoli *et al*., 1988] [2]. In humans, breast milk provides all the energy and nearly all nutrients required for infant growth and development during the first 4 to 6 months

of life, as well as various immunological factors and bioactive components [Titi *et al*., 2014] [24].

Lactation stage is one of the major factors influencing the yield and composition of milk in cattle [Ibeawuchi and Dangdut 1996] [18], Buffalo [Şekerden 1999] [23] and goat [Akingbade *et al* 2003] [1]. The concentration of total protein, fat, casein and whey protein increases during the advanced lactation stage in pasture-fed dairy cows [Auldist *et al* 1998] [7]. Within a given species, genetic factors and environmental conditions such as the climate and the stage of lactation influence the composition [Anonymous, 1973] [3, 4]. Persistency of lactation is the ability of a milk animal to maintain milk production at a high level after reaching the peak yield. High persistence is associated with a slow rate of decline in yield following peak milk yield and low persistence is associated with a rapid rate of decline [Appuhamy *et al* 2007] [5]. It is important to determine the characteristics of the lactation curve of a milk animal in order to analyze the milk production potential improve milk yield and obtain a more desired lactation curve [Keskin and Dag 2006] [19]. Moreover, the lactation curve is also useful for assessing the nutritional and health status of milking animals [Dudouet 1982] [11] and it helps to determine the suitable time to end milking [Chang *et al* 2001] [9]. According to Neville MC and Jensen RG (1995), there are limited data about biochemical composition study at different lactation stages.

There are several studies dealing with the chemical composition of milk, but they are foreign. The aim of this work is to determine and compare the biochemical composition (protein and fat) of human, cow, buffalo and goat milk during lactation stages since such comparisons are rare in the study. It is known that lactation has a significant effect on the natural physiological function of both human and animals, so it was very important to make such study, hoping to give understanding the two main important biochemical component protein and fat from human, goat, cow and buffalo milk samples at stages of lactation and comparing the obtained value with each other in terms of which milk is good for consumption and other economic importance in dairy.

Materials and Methods

Milk sample collection

Milk was obtained from four species: Human (Hominine), cow (bovine), Buffalo (bubaline), and goat (caprine). Human milk was obtained from Shraddha Hospital; un-sterilization milk of a cow, buffalo were collected from nearby dairy sheds and goat milk was obtained from loafing shed. All the samples are collected during the morning in sterilization labeled falcon tubes and kept in the refrigerator at 4°C until taken to the research lab. Transport of samples was done in the icebox. A number of samples for each lactating mammals were taken into three sets i.e. human (n=3), cow (n=3), Buffalo (n=3), and goat (n=3).

Milk collection at a time interval based on lactation stages: To carry out this study, milk samples were collected at a certain interval of lactation stages. The collection was done on the 3rd day of lactation stage that is colostrum stage, on the 6th and 11th day of Transition stage and on 18th, 23rd, 30th, 44th, 58th, 72nd, 79th, 107th, 122nd, 143rd & 150th days of Mature milk. Protein and fat percentage from the milk sample was determined by standard methods mentioned below.

Determination of protein from the milk samples

Protein determination from milk samples at each lactation stages was done by formal titration method [Pyne, 1933] [22]

Requirements

- NaOH
- Potassium hydrogen phthalate (KHP)
- Phenolphthalein, Distilled water
- Titration set
- Milk samples

Determination of Fat from the milk samples

Fat determination from milk samples at each lactation stages was done by Gerber Butterfat test. [Niklaus Gerber, 1891] [13]

Requirements

- Gerber milk butyrometer
- Automatic tilt measure or pipettes for acid (10ml) and amyl alcohol (1 ml).
- Pipettes 10.75 ml for milk
- Butyrometer, stands, stoppers /Lock stopper.
- Water bath.
- Gerber centrifuge, 1100 rpm
- Sulphuric acid and Amyl alcohol and milk samples.

Results and Discussion

Milk composition of mammalian species varies widely with reference to genetic, physiological, nutritional factors and environmental conditions. The use of milk proteins to give food desirable organoleptic or textural properties are strongly influenced by their functional properties. Moreover, the lactation curve is also useful for assessing the nutritional and health status of milking animals (Dudouet 1982) [11] and it helps to determine the suitable time to end milking (Chang *et al* 2001) [9]. Milk composition varies greatly among the different species, which is partly attributed to the inherited capabilities

Milk protein is the protein present in the milk. The present study shows the difference in protein% is shown in Table 1 in successive lactation stages of all samples and taking the average value of three sets of each species. Lactation stage is one of the major factors influencing the yield and composition of milk in cattle (Ibeawuchi and Dangut 1996) [18], Buffalo (Şekerden 1999) [23] and goat (Akingbade *et al* 2003) [1]. Human milk sample had a protein% range from 1.9 to 1.0 that showed a decrease in content during the last days. Cow milk samples had protein%range from 5.85 to 3.48, in transition stage it increased and then gradually decreases to 3.48 in the mature stage. Buffalo milk samples had a protein% range from 4.10 to 3.01, in the first and second month it increased, that decreased in late days to approximately 3.0. Goat milk samples had a protein% range from 3.18 to 1.10, it decreased consistently during the successive lactation. The study shows the protein content of cow milk (5.85%) is higher at 11th day of lactation than in Buffalo, goat and human milk, but it is low in human milk (1.0%) at 150th day as compared with buffalo, goat, and cow milk. Among these samples, Cow milk had the highest protein content during the early stage.

Table 1: Protein% of milk samples at days of Lactation

Lactation Stages	Days	Human (Hominine)H	Cow (Bovine)C	Buffalo (Bubaline)B	Goat (Caprine)G
Early	3rd day	1.90	5.63	4.02	3.18
Transition	11th day	1.50	5.85	4.10	2.94
Mature	23rd day	1.20	5.23	3.45	2.01
	44th day	1.23	5.16	3.32	1.68
	72nd day	1.10	4.62	3.11	1.35
	150th day	1.00	3.48	3.01	1.10

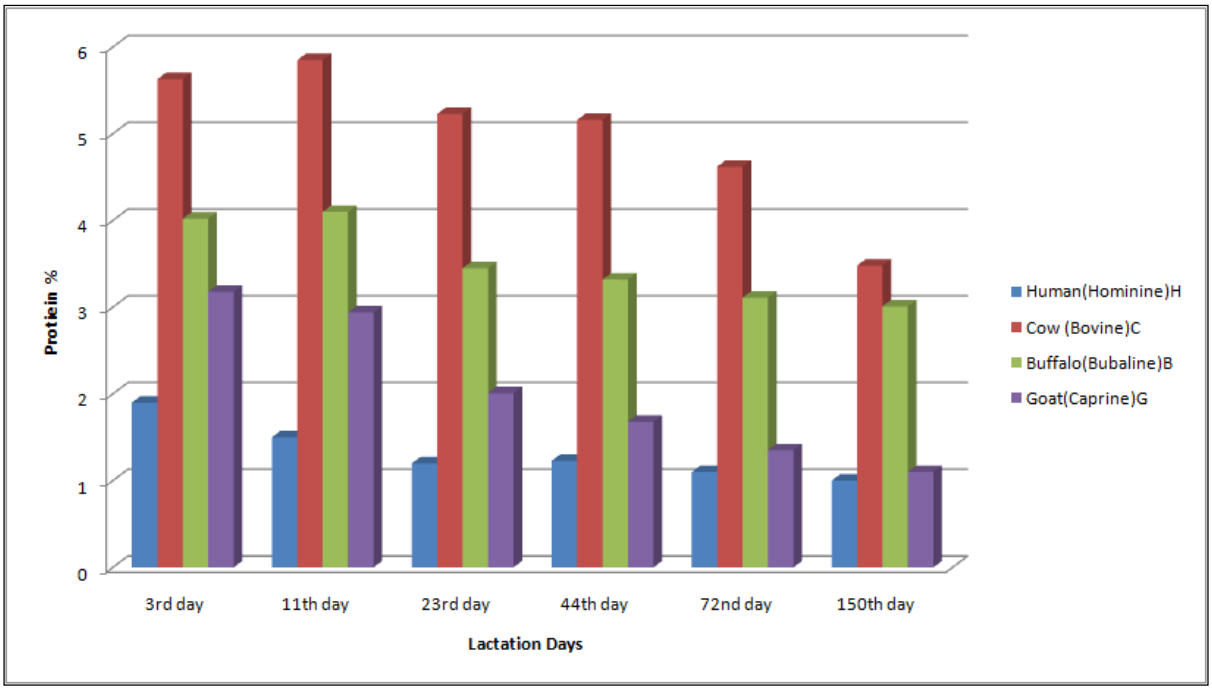

Fig 1: Protein% of milk samples at lactation days

It is important to determine the characteristics of the lactation curve of a milk animal in order to analyze the milk production potential improve milk yield and obtain a more desired lactation curve (Keskin and Dag 2006) [19]. These findings support those results given by Siddig (2002) but with variation in protein content in entire lactation stages.

The current research shows the variations in Fat% as shown in Table 2 in successive lactation stages of all samples and taking the average value of three sets of each species. Also, in order to calculate the correct amount of feed ration for high yielding dairy cows, it is important to know the butterfat percentage as well as well as the yield of the milk produced Human milk sample had fat % range from 4.2 to 3.0 that gradually decreases in late days. Cow milk samples had fat% range from 4.5 to 2.0 that shows the decrease in % as successive lactations. Buffalo milk samples had fat% range from 6.5 to 5.2 shows the high-fat % and decreases gradually by the late days. Goat milk samples had fat% range from 5.7 to 3.9 also showed a decrease in fat % by the late lactation stage. Among these, The fat content (6.5%) in buffalo milk at the 3rd day of lactation is higher than other milk, while fat content (2.5%) is lower in cow milk at 150th day of lactation than in human, goat, and buffalo milk. These results were related to the findings of Kanwal R *et al*. (2004) but this study showed variation in fat content during lactation stages. The fat content of milk and cream is the most important single factor in determining the price to be paid for milk supplied by farmers in many countries.

Table 2: Fat% of milk samples at days of Lactation

Lactation Stages	Days	Human (Hominine) H	Cow (Bovine) C	Buffalo (Bubaline) B	Goat (Caprine) G
Early	3rd day	4.2	4	6.5	5.7
Transition	11th day	4	3.9	6.1	5.5
Mature	23rd day	3.9	3.4	6	5.2
	44th day	3.8	3.1	5.8	4.9
	72nd day	3.6	2.8	5.5	4.1
	150th day	3	2.5	5.2	3.9

The content of fat and protein during all lactation stages in goat (3.9 to 5.7%, 1.10 to 3.18%), cow (4.0 to 5.0%, 4.46 to 5.75%) and Buffalo (4.0 to 6.5%, 3.12 to 4.12%) respectively (Hadjipanayiotou 1995) [14]. The concentration of total protein, fat, casein and whey protein increases during the advanced lactation stage in pasture-fed dairy cows (Auldist *et al* 1998) [7]. The species plays an important role in the socio-economic life of its rearers.

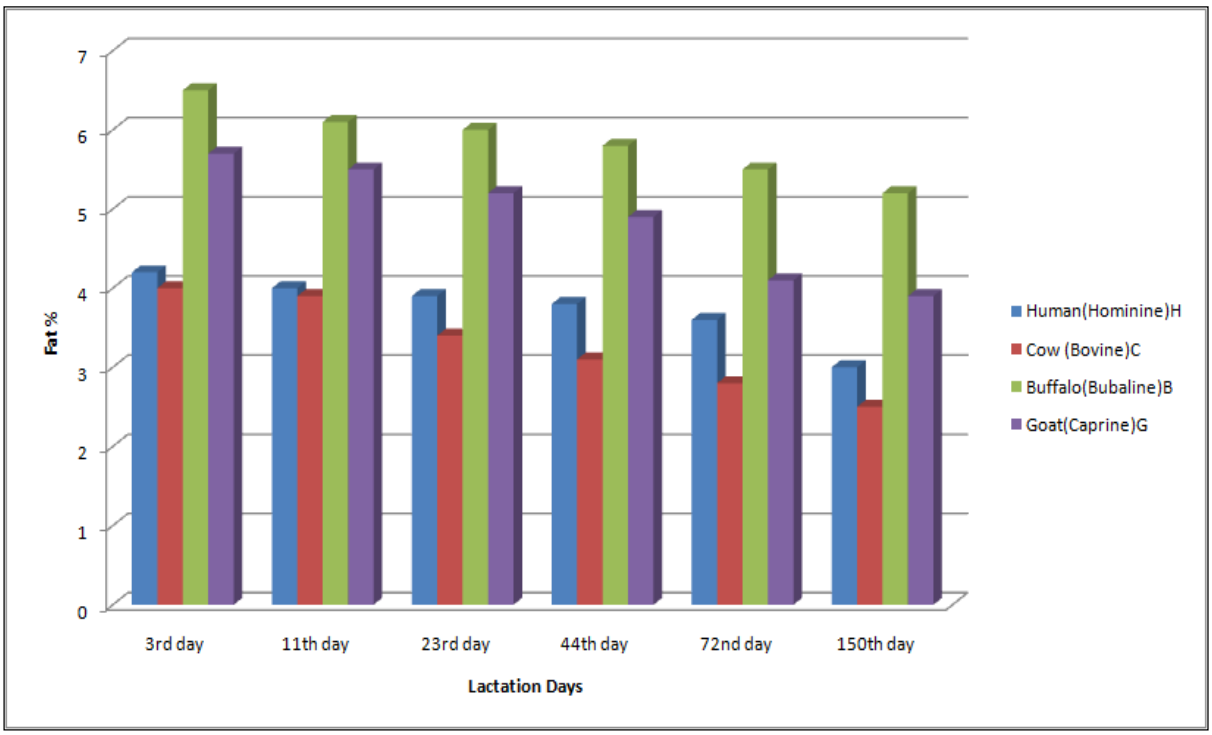

Fig 2: Fat% of milk samples at lactation days

Furthermore, the butterfat percentage in the milk of individual animals must be known in many breeding programmes. Butterfat tests are also done on milk and milk products in order to make accurate adjustments of the butterfat percentage in standardized milk and milk products.

Conclusion

The biochemical composition of milk determined was broadly from different types of mammals and analyzed for the improvement of nutritional outcome. Milk production is not regular, the main causes of variations are related to race, species, and environment, but also depend on individual factors related to the health, nutrition, age and lactation stages of the animal. Overall this study shows the variations in milk protein and fat, which are an important biochemical component in milk, due to changes in phases of lactation. The different stages of lactation affected significantly on the many components of milk. However, this study provides an important foundation for future biochemical analysis of protein and fat-related studies. It is noteworthy that the milk of each species has a particular individual pattern of biochemical components, which may be a marker of relative nutritional importance. Milk composition may be improved on-farm through breed selection in order to improve the overall concentration available for human consumption. Finally, an improvement of high-quality milk and feed ration should be obtained from nourished healthy lactating animals. Such variations in milk profiles can be used in future to come up with more improved milk for the benefit of human health and nutrition.

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