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GENETIC VARIATIONS IN MILK COMPONENTS AND THE EFFECT OF MILK PROTEIN GENETIC VARIANT ON HEAT STABILITY

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ABSTRACT

The composition and physical nature of milk is very complex. The stability of milk depends upon the different constituent of milk. Determination of heat stability of milk is one of the factors that assess the stability of milk. In this study an attempt was made to measure the heat stability of milk obtained from different varieties of cows and also made an attempt to identify the different components on heat stability. The genetic variants and protein concentration of casein in the milk of six different varieties of cows were evaluated. The protein concentration in inbreed varieties are more than that of others followed by Holstein and Holstein-Friesian was found least. We also found that the concentration of casein was highest in inbreed varieties followed by Holstein and was least in Holstein-Friesian. The heat stability was maximum in Swiss Brown followed by inbreed and was least in Holstein-Jersey and Holstein. Lactose concentration was maximum in inbreed varieties followed by Sindhi cross and was least in Swiss Brown. Calcium concentration in inbreed variety was found to be maximum followed by Holstein, however, least was found in Swiss Brown. In Holstein varieties, the potassium concentration was found to be maximum, however the concentration was least in inbreeds. We did not find much difference in the pH and specific gravities of milk in various varieties.

INTRODUCTION

The economic and social factors have recently focused attention on the possibility of genetically altering milk composition. Milk is a complex mixture of proteins, carbohydrates, vitamins and fat. The composition of milk is well known and many studies have shown the importance of each constituent of the milk in making the milk an important article of commerce and its nutritive value is important in public health as it is free from colostrums, contains not less than 8.5% milk solid -not fat and not less than 3.5% milk fat (1). The composition of the milk varies widely among the species. In addition to the species difference the composition of mature milk varies with breed individual stage of lactation, state of nutrition, diet, season and climate. The quantity and genotypic variation is more predominant in milk protein, which varies in different varieties of cows (18). Genetic aspect of milk protein polymorphism and their frequency distribution in the population of few Indian breeds of cattle (Sahiwal, Tarparkar, Red Sindhi) and cross breed (Brown swiss X Sahiwal) were studied previously (27). The present work was proposed to estimate the quantity and quality of different components in the milk, such as lactose, calcium, potassium, protein and heat stability of milk in different varieties of cow (cross breed type and indigenous inbreed types) and also evaluate the heat stability of different milk proteins in various varieties of cows, which will help to analyze whether the changes produced by the cross breeding are all beneficial or not.

MATERIALS AND METHODS

Selection of samples

The present study was carried out on thirty-six cows belonging to five crosses and one indigenous inbreed variety, viz. 6 Swiss Brown, 6 Holstein, 6 Holstein Jersey, 6 Holstein Friesian, 6 Sindhi cross and 6 Inbreed cows. Milk samples were taken from cows in the experimental herd during October-November 2007 and July-August 2008. The samples were collected directly from the teat and milk sample of all the four teats were mixed together and transported to the lab immediately.

Preparation of casein

Fractionation of casein was started within an hour of sample collection by precipitation with acid (18) and the procedure is given elsewhere. Excess of moisture was removed from the precipitate of casein by a vaccum pump. Enough 95% alcohol was added so as to cover the precipitate and stirred for a few minutes. The alcohol was filter and removed. The procedure was repeated for three or four times, then added enough amount of ether (Di-ethyl ether) and stirred continuously for a few minutes. Filtered and repeated the extraction with ether for complete removal of lipid. Filtered the precipitate and allowed the ether to evaporate spontaneously and added glass distilled water for cleaning the casein and stirred for one to two minutes. Finally filter the precipitate (Casein) and transferred the casein into Petri plates for drying, which was used for later experiments.

Separation of genetic variants of casein

The variant of casein were separated by vertical polyacrylamide gel electrophoresis by the method of Medrano and Sharrow (42), with slight modification. The genetic variants were identified by their electrophoretic mobility as per the nomenclature of major and minor milk proteins of cow's milk as described by Wake and Baldwin (43) and Eigel *et al* (21). 10mg casein of each cow's milk was dissolved in 5 ml of 6 M urea solution taken in boiling tube for the application for the sample for electrophoresis.

Heat stability of milk

Heat stability of milk is determined by determining heat coagulation time. A thermostatically controlled paraffin oil bath maintaining temperature within ±0.1 to 0.5°C fitted with a stirrer to maintain uniform temperature. The sample is placed in a heat stable corning glass culture tubes fitted with high temperature resistant screw cap provided with tygon washers to make those leak proof. Bath temperatures were measured using mercury thermometer and stop watch was used to measure the time for sample to clot.

Estimation of total milk protein and Casein

Total protein and amount of casein was estimated by the method of Lowry *et al* (44).

Estimation of lactose, Calcium and Potassium

Lactose is determined by the method of Folin and Wu (44). The amount of milk calcium is determined by the method of Collip and Clark (44). The amount of potassium is estimated by flame photometer as described elsewhere (45).

Determination of specific gravity and pH

Specific gravity of milk is determined by lactometer method (18). pH of the milk is determined by using a pH meter.

RESULTS

The genetic variants of casein in the milk of six different varieties of cows are given in table1. Table -2 Shows the total protein content in the milk of six different varieties of cows (g/100ml). The protein concentration in inbreed varieties are more than that of others followed by Holstein. However, Holstein-Friesian, the concentration was found least, when compared with others. We also found that the concentration of casein was highest in In breed varieties followed by Holstein. The casein concentration was found to be least in Holstein-Friesian (Table 2). Table 2 shows the lactose concentration in the milk of six different varieties of cows. Lactose concentration was maximum in inbreed varieties followed by Sindhi cross and was least in Swiss Brown. The heat stability was maximum in Swiss Brown followed by inbreed, However, we found that the heat stability was least in Holstein-Jersey and Holstein (Table 3). Calcium concentration in the milk of 6 different varieties of cows are given in table 3. Calcium concentration in inbreed variety was found to be maximum followed by Holstein. However, least was found in Swiss Brown. Table - 3 shows the concentration of potassium in mg% of six different varieties of cows' milk. In Holstein varieties, the potassium concentration was found to be maximum, however the concentration was least in inbreeds. We did not find much difference in the pH and specific gravities of milk in various varieties.

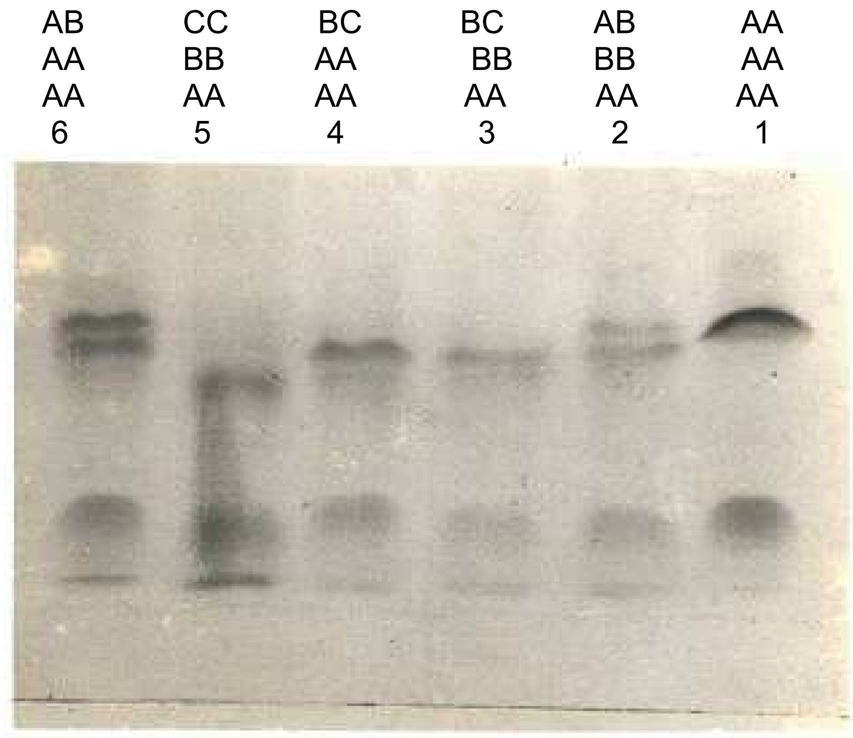

Figure -1

Polyacrylamide gel electrophoresis patterns of caseins from the milk of six different varieties of cows. All of the six possible types are shown with corresponding genotype designation.

1. Inbreed
2. Swiss Brown
3. Holstein Jersey
4. Holstein
5. Holstein Friesian
6. Sindhi cross

Table 1

Genetic variants of casein in the milk of six different varieties of cows.

Types of breeds	α- S1 Casein	β-casein	κ-casein
Inbreed	AA	AA	AA
Swiss Brown	AB	BB	AA
Holstein Jersey	BC	BB	AA
Holstein	BC	AA	AA
Holstein Friesian	CC	BB	AA
Sindhi cross	AB	AA	AA

Table 2

Shows the total protein content in the milk of six different varieties of cows (g/100ml)

Types of breeds (n=6)	Protein conc. (g/100mL)	Conc. Casein (g/100mL)	Conc. of Lactose (g/100mL)
Inbreed	3.450 ± 0.013	2.486 ± 0.026	5.17 ± 0.010
Swiss Brown	2.779 ± 0.021	1.891 ± 0.006	4.19 ± 0.189
Holstein-Jersey	3.121 ± 0.018	1.996 ± 0.037	4.42 ± 0.011
Holstein	3.295 ± 0.028	2.229 ± 0.021	4.83 ± 0.011
Holstein-Friesian	2.530 ± 0.034	1.827 ± 0.171	4.65 ± 0.010
Sindhi cross	2.634 ± 0.054	2.306 ± 0.027	4.90 ± 0.006

Table 3

Shows the level of calcium, potassium, heat stability, pH and specific gravity in the milk of six different varieties of cows.

Type breeds (n=6)	Heat stability	Conc. of Calcium (mg/100mL)	Conc. of Potassium (mg/100mL)	pH	specific gravity
Inbreed	62.00 ± 3.20	133.00 ± 8.00	120.31 ± 42.35	6.64 ± 0.004	1.028 ± 2.96E-6
Swiss Brown	66.00 ± 3.20	108.00 ± 27.20	133.90 ± 64.89	6.64 ± 0.006	1.022 ± 9.66E-7
Holstein -Jersey	41.66 ± 2.66	125.66 ± 8.66	137.46 ± 72.37	6.58 ± 0.002	1.021 ± 1.86E-6
Holstein	42.83 ± 3.76	130.66 ± 4.66	146.47 ± 38.75	6.59 ± 0.011	1.017 ± 1.36E-6
Holstein - Friesian	58.83 ± 5.36	112.08 ± 9.44	141.56 ± 59.77	6.68 ± 0.001	1.025 ± 1.20E-6
Sindhi-cross	48.33 ± 3.46	114.33 ± 22.56	143.03 ± 30.10	6.66 ± 0.001	1.023 ± 5.46E-6

DISCUSSION

The composition and physical nature of milk is very complex. But it is very stable in normal favourable condition. There are certain factors which can make the milk unstable. High temperature is one such factor. Normal milk is stable at boiling point but AS time elapses after milking, it may get clogged even at normal boiling point. There are variations in the stability of milk, which depend upon the different constituents of milk. By measuring the heat stability of milk we can have an idea about long storage potential of a milk sample.

Here in this study an attempt was made to measure the heat stability of milk obtained from different varieties of cows. We observed a significant in the variation heat stability of milk between species. The milk of Swiss Brown and local varieties were found to have high heat stability. In the local variety the amount of casein was higher than that of the cross breed ones. This increased casein number might have resulted in the high heat stability. But in the case of Swiss Brown milk the quantity of casein protein is not high but they produced milk of high heat stability. This may be due to the variation in the ratio of different types of casein proteins. Concentration of κ-casein, calcium etc, were found to affect the heat stability of milk. More detailed investigation is required to get a clear picture about this. Heat stability can vary with multiplicity of factors, sum of which may be effective in some milk.

As a next step in this regard we have electrophoretically separated the casein protein of different varieties of cows. The result showed that the genotypes of the casein AA/AA/AA (inbreed), AB/BB/AA (Swiss Brown), BC/BB/AA(Holstein Jersey), BC/AA/AA(Holstein), CC/BB/AA(Holstein Friesian) and AB/AA/AA(Sindhi cross). Here we were able to see a higher homozygosity in the different milk protein gene. This might have resulted in the more or less identical composition of milk in the different varieties of cows. Heterozygosity was higher in the case of α- S1 protein gene. Whereas in the case of κ-casein all the cows showed homozygosity. The obtained result make it's difficult to explain the high heat stability nature of milk of inbreeds and Swiss Brown cows. The result of this study warrants the requirement of thorough study regarding the gene expression and also the ratio of different type's casein protein in the milk of these varieties.

Perspective of the fact that we were practicing artificial insemination in cows for the last few decades, there observed a higher homozygosity in the individual milk protein genes. This is beneficial to certain extent as animals homozygous for individual milk proteins are reported to show high milk yield (28). Historically milk pricing has focused on milk volume and fat yield, however, with change in consumer attitude and the result in the change in demand for different products, the non-fat solid milk have become important. There is strong positive correlation between fat and protein percent. This indicates that it will be very difficult in opposite direction of these two traits (47). This indicates that if one selects for proteins, the yield of fat also increases.

Mityuko and Ukolov (28) have reported that groups having α- S1 casein BB, β-casein AA and κ-casein AA show high milk yield. Here none of the cows showed these genotypes. This may be the reason for the medium level of milk yield in these varieties. The inbreed varieties are having a genetic composition α- S1 AA, β-casein AA and κ-casein AA. We can think of a genetic manipulation in this variety which may help to change α- S1 casein AA genotype to α- S1 casein BB genotype and look for the yield of milk. Holstein and Sindhi cross also showed a closer similarity in the genotypes. It should be noted that in spite of the continued practice of artificial insemination for decades, we were not able to achieve the desired genetic composition at least for increased milk yield.

There is a significant difference in the amount of casein in different varieties of cows. The maximum amount of casein obtained in the varieties of inbreeds and Sindhi cross. The amount decreased in the following order in the six varieties of cows. Inbreed > Sindhi cross > Holstein > Holstein Jersey > Swiss Brown > Holstein Friesian. In all these varieties three electrophoretic bands of casein have been distinguished. The quality of milk is determined by its casein content and its genetic variant. The κ-casein of milk promote curd firmness and lesser extent to RCT. B allele of κ-casein is responsible for high heat stability. In this study we have observed that all the varieties of cows made under study were of κ-casein AA type. That shows that in this also we were not able to achieve the desired genetic trait.

The concentration of calcium in the different varieties of cows is significantly different. The result showed that the maximum amount of calcium present in the inbreed variety. The amount decreased in six varieties of cows in the following Order. Inbreed, Holstein, Holstein Jersey, Sindhi cross, Holstein Friesian, and Swiss Brown. Calcium helps in bone formation. The calcium content of the inbreed cows were higher than the other varieties. The enhanced calcium level might have some role in increasing the heat stability. But it may be noted that the milk of Holstein is also having high calcium content. Irrespective of this the milk showed very low heat stability. This shows that rather than calcium there are some other factors which affect heat stability of milk.

The concentration of the milk sugar varies significantly in different varieties of cows. The result showed the maximum amount of lactose in Swiss Brown and inbreeds cows. The decrease in the amount of lactose decreases in six different varieties of cows is in the following order. Swiss Brown>Inbreed>Sindhi cross >Holstein >Holstein Friesian > Holstein Jersey. Compared with the other sugar lactose is less soluble and less sweet. The lack of sweetness is considered to be advantageous in the feeding of the infant and invalids. Since there is less tendency to cloy the appetite. When the diet contains lactose, calcium retention is improved in children. Tetany is relieved in parathyroidectomised animal and there is greater deposit of bone ash (5). The absorption of calcium is increased when lactose is fed to infants. There fore high lactose containing cow's milk is better to bottle feeding babies. The main difference between cows milk and human milk is the amount of lactose and fat content. Human milk has high lactose content. In this respect the milk of Swiss Brown and inbreed cows are some what related to human milk.

The lactose content of Swiss Brown and Inbreed are higher than that of other varieties .The lactose concentration have an affect on heat stability Enzymatic hydrolysis of lactose by β-galactosidase increased heat stability of milk(39).This may be the reason for high heat stability of these cows milk.

Specific gravity of milk is significantly different in different varieties of cows. The result showed that the inbreed varieties of milk have high specific gravity. This may be due to the increased level of solids in the milk. There are no significant variations in the pH of different varieties of milk. Calcium content was also higher in the milk of inbreed variety. This is an additional advantage as calcium absorption and retention increases in the presence of lactose.

Previous studies show breed to breed variation in the expression of gene Cows of same genotype belonging to different breeds showed different in milk yield, milk quality etc.(27). Our study also support this In our we observed a close similarity in genotype between the indigenous breed and Sindhi cross. But there is marked difference in the milk yield and milk composition of these two varieties. So it clears that a separate factor there influences these characters.

Milk composition can be changed through feeding, environmental conditions or genetics, climate, stage of lactation and succulent forages have been shown to impact milk composition .With feeding environmental effects the results are immediate but will stop as soon as the treatment is discontinued. Genetic change is much slower, but the results are cumulative and continue in the absence further selection.

Selection programs depend upon the heritability of traits, amount of variation among animals for the traits, genetic correlation among the traits and relative net economic importance for the traits. One of the major problems of genetic improvement in dairy cattle is the long delay between initial selection of the bull and majority of the offspring entering the milking herd. It will be six years before anyone will know how good he is genetically.

Another important factor which should be taken into consideration is the consumer attitude. A larger percentage of our milk supply goes into manufactured products each year and the consumption of low fat milk products continues to increase. This is causing increased selection pressure on milk components. As we plan breeding programs for the future, we will need to move more of our emphasis to the non fat solids.

Genotypes with favourable attributes should be considered as important criteria in the final selection of bulls and cows. Had we had an idea about the most favourable genetic combination in respect to all desired characters, we can utilise the knowledge in selection. We can apply the techniques of the molecular genetics and find out the genetic combination of bulls and cows. Using this information we can manipulate our breeding practice.

By using the conventional selection based on phenotypic expression is much time consuming and it may not be cent percent correct as phenotypic expression, is much controlled by the environmental factors. On the other hand if we had an idea about favorable genetic combination we can make use of this in selection. Immediately after birth, the offspring's can be genetically typed. It is not necessary to wait until the new ones start milk yield. Based on the information so obtained we allow it to grow and reproduce or can be culled. By such continued breeding practice, by a short time we can have a better generation of cattle.

CONCLUSION

The study was conducted among a small cross section of our cattle herd and have obtained a few striking result. The milk of local indigenous breed and that of Swiss Brown showed high heat stability. Casein and calcium concentration were also higher in local variety. The lactose content also was higher in local variety. This makes it more suitable for infant and invalids. There is a high degree of homozygosity in the case of κ casein gene among the different varieties studied. The above mentioned findings show that we are not achieved much advancement in the desirable traits of our cattle herd. The present practice of artificial insemination is not much successful, though it produced some favourable effect. It is high time to think of an alternative. It is highly essential to have a clear picture of the genetic combination of our cattle herd and should realise which of the combination is much favorable in economical and quality wise. The scope of applying molecular techniques is much high. Having a fixed idea about nature of traits which should be inherited, we can plan a breeding strategy by selecting cows and bulls having desired characteristics. The recent advancement in induced ovulation, multiple ovulation and embryo transfer (MOET) etc. can effectively be utilized in this respect. This warrants extensive scientific and well designed study in this area.

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