MILK PROTEIN GENETIC VARIANTS IN CROSSBRED DAIRY CATTLE

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree

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Department of Animal Breeding and Genetics
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DECLARATION

I hereby declare that the thesis entitled "MILK PROTEIN GENETIC VARIANTS IN CROSSBRED DAIRY CATTLE" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Mannuthy,
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CERTIFICATE

Certified that this thesis entitled, "MILK PROTEIN GENETIC VARIANTS IN CROSSBRED DAIRY CATTLE" is a record of research work done independently by Dr. K. Madhavan, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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INTRODUCTION

Dairy cattle breeder has selection and breeding as two tools for the improvement of dairy cattle pertaining to quantitative traits such as milk production. The success in dairy cattle improvement depends on how efficiently he selects breeding sire and dam to form the parental generation. Generally selection is based on individuality, pedigree, progeny, or in combination of any two or three of these. The above mentioned selection has been based exclusively on quantitative traits which are controlled by multiple loci. Genetic improvement of quantitative traits is relatively slow as they are affected by numerous polygenes and highly influenced by environmental factors. This undoubtedly lowers the accuracy of genetic evaluation of sires and dams. To accelerate the process of selection, genetic markers will go along with in identifying superior breeding stock even during its early life. Various genetic markers have been attempted and they include blood groups, enzymes and blood serum proteins. Both quantitative and qualitative differences occur in the milk of different species.

The milk protein of all mammals can be classified into two classes; the caseins and wheyproteins. The caseins which

make up nearly 80 per cent of the proteins in cow milk, consist of κ-casein, and the so called calcium sensitive caseins α-S_1, αS_2 and β-caseins. The major whey proteins are α-lactalbumin, β-lactoglobulin and whey acidic protein which is not found in cattle, goat and sheep. κ-casein is important for the precipitation of other caseins by calcium through micelle formation. The calcium sensitive caseins are phosphorylated and together with κ-casein are the primary source of amino acids, phosphate and calcium for the young suckling animals. Furthermore, they are the raw materials for the cheese making industry. The whey protein, β-lactoglobulin is a possible carrier of small hydrophobic molecule.

Recent studies have revealed existence of polymorphism of milk proteins and that the variants are universal, though some are found to be breed specific.

Cheese yield is an important indicator of the profit of the cheese maker, the milk producer and for the dairy industry in general as it reflects the amount of cheese made from a given amount of milk. Many environmental factors may influence the composition of milk and its manufacturing properties. The presence of certain genetic variants of casein may influence the cheese yielding capacity of milk because it is known that coagulating properties of milk and firmness of curd during cheese making is influenced by the

casein content. In this context breeding for specific milk protein variant becomes increasingly important from an economic and nutritional stand point.

With the view the present study was undertaken with the following objectives.

1. To separate and identify various milk proteins viz. α-S_1-casein, β-casein, κ-casein and β-lactoglobulin in crossbred cattle.

2. To study their inheritance pattern such as gene frequencies, genetic group variations and linkage relationship between protein variants utilising their pedigree data.

Review of Literature

REVIEW OF LITERATURE

Milk protein studies have gained tremendous importance for many years due to its many fold utilities. As a dietary constituent they are one of the richest sources of animal protein for both man and animals. Milk proteins have been subjected to study for their chemical nature, proportion, rate of synthesis and interaction with other components. Results of many of these studies have contributed substantially to the product formulation and development in dairy industry. The newer findings and conceptions on the genetic variation of milk proteins have opened up newer vistas in the research of biochemical genetics of milk proteins.

Milk proteins

The two major protein components in milk are colloidal caseins and soluble whey proteins. Casein colloid comprise of α-s_1, β-and κ-caseins along with a divalent ion to form a stable micelle (Waugh, 1958). The techniques for separation of casein from milk by acidification process were devised much earlier. Casein exists as large colloidal particles containing calcium and phosphorus as inorganic ions. Casein particle size varies from 30 to 300 mμ (Payens et al., 1963). Casein micelle consists of about 94 per cent protein and

6 per cent minerals (Jenness, 1959). Casein may be considered both as a phosphoprotein and a glycoprotein. α-casein contains more phosphorus and the nitrogen content of α and β components of casein is almost the same as in whole casein. Casein has the highest concentration of glutamic acid among the amino acids. Amino acids proline, aspartic acid, leucine, lysine and valine are also present at relatively higher level in casein.

The important soluble proteins present in 'milk serum' or whey are α-lactalbumin and β-lactoglobulin. Small amounts of blood serum proteins and immunoglobulins are also present in the whey. α-lactalbumin constitutes about 20 per cent of the whey proteins. This protein has essential role in the biosynthesis of lactose being a component in the enzyme lactose synthetase. The molecular weight of bovine α-lactalbumin was found to be between 15,000 and 16,000 as evaluated by different procedures (Mc Kenzie, 1967). α-lactalbumin has a single peptide chain with glutamic acid and leucine as the N and C-terminal amino acid respectively. α-lactalbumin has disulphide bonds and is rich in tryptophan.

Genetic polymorphism of milk proteins

Starch gel electrophoretic method for the revelation of casein variants was first reported by Wake and Baldwin

(1961). Application of polyacrylamide gel for high resolution of milk proteins was first achieved by Peterson (1963).

Caseins

α-S_1-Casein

The most remarkable observation with heterogeneity of α-S_1-casein was made by Thompson et al. (1962) using starch gel electrophoresis. The three genetic variants viz., A, B and C differed slightly in their mobility and occurred singly (A, B or C) or in pairs (AB, AC or BC). They were all observed in a straight forward Mendelian manner.

By starch-urea gel electrophoretic studies of caseins from milk of individual cows Kiddy et al. (1964) reported that αS_1-casein exists in three major forms viz., A, B and C, in the order of decreasing mobility towards anode. Family studies indicated that the variation was controlled by three autosomal allelic genes with no dominance. Six possible genotypes viz. A (A/A), B (B/B), C (C/C), AB (A/B), AC (A/C) and BC (B/C) were observed.

The committee for the nomenclature of proteins of cow's milk (Thompson et al., 1964) during second revision recommended the term α-S_1-casein referring to those components of α-casein complex that were precipitated by calcium and

stabilized by κ-casein against precipitation by calcium. They contributed the major proportion of the αS-casein and have been reported to exist in three genetic forms A, B and C. The genetic forms of αS_1-casein can be conveniently identified by their relative mobilities by zone electrophoresis. The committee recommended that the three genetic variants (A, B and C) of the αS_1-casein can be referred to as αS_1-A, αS_1-B and αS_1-C. A statement of the relative electrophoretic mobility value for each variant should accompany the genetic designations for purposes of identification αS_1-A (1.18), αS_1-B (1.10), αS_1-C (1.07). αS_1-B and αS_1-C are virtually identical in composition with only significant difference on that αS_1-C has one more glycine residue per molecule than αS_1-B (Gordon et al., 1965) which is replaced by glutamic acid. The molecular weight of the variants was observed to be identical and about 30,000.

Mercier et al. (1971) reported that the αS_1 - casein (αS_1-CN) consisted of one major and one minor component with the same amino acid sequence.

The committee for the nomenclature of milk proteins during fifth revision (Eigel et al., 1984) established the occurrence of five genetic variants of αS_1-casein viz. A, D, B, C and E in the order of decreasing relative electrophoretic mobilities in alkaline gels containing urea.

β-Casein

Genetic variants of β-casein was first reported by Aschaffenburg (1961) by using paper electrophoretic method. The three variants β-casein A, B and C with different electrophoretic mobilities were detected in British breeds either singly (A, B or C) or in pairs (AD, BC, and AC). The author assigned the locus symbol β-casein.

Thompson et al. (1964) confirmed the findings of Aschaffenburg (1961) and reported that the occurrence of genetic forms of β-casein was breed specific. On the basis of the examination of 1349 individual cow of 5 major dairy breeds, these authors reported that A and B alleles of β-casein occur is Jersey and Holstein, A, B and C alleles in Guernsey and Brown Swiss breeds, and only A allele in Ayrshire breed.

Thompson and Pepper (1964) developed a convenient method for obtaining the genetic variants of β-casein using DEAE-cellulose chromatography in the presence of 3.3 M urea as dissociating agent. Separation of β-casein variants were accomplished with column chromatography and found that β-casein A, B and C contained 15.18, 15.33 and 15.45 per cent nitrogen and 0.59, 0.57 and 0.50 per cent phosphorus respectively.

The nomenclature committee for proteins of cows' milk in its second revision (Thompson et al., 1964) defined casein as that fraction of casein soluble in 3.3 M urea, but insoluble in 1.7 M urea at pH 4.6. Furthermore that casein which undergoes temperature dependent molecular dissociation at 4°C. β-casein is monomeric, at 8.5°C. Concentration-dependent aggregation is observed and at 20°C it is completely polymeric. β-casein was insensitive to calcium ions at low temperature but aggregate at 35°C. The committee reported that the variants could be correctly identified by starch gel electrophoresis (SGE), Polyacrylamide gel electrophoresis (PAE) or paper electrophoresis. It was suggested that the caseins were termed β-casein A, β-casein B and β-casein C in the order of decreasing electrophoretic mobility. For the identification on SGE or PE the genetic form was followed by appropriate relative position on the gel as β-casein A (0.80) by SGE or β-casein A (0.65) by PAE. The authors suggested that the system has to be flexible to allow for the inclusion of additional casein differing in net negative charge.

Peterson and Kopfler (1966) could resolve β-casein A into three variants viz. A^1, A^2 and A^3 in acid pH medium using polyacrylamide electrophoresis. Acid pH of the system enabled the authors to reveal these new species in the β-casein A variants. These authors suggested the renaming of the

casein variants as A, B, C, D and E from their mobility order in acid-gel electrophoresis, where A has the greatest mobility and the others follow in decreasing order. β-casein typed C in alkaline electrophoresis became A and B remained as B, C, D and E were the newly resolved variants from β-casein A.

Aschaffenburg et al. (1968) reported a new variant β-casein D, in Indian and African Zebu Cattle. The β-casein D migrates between β-casein B and C in alkaline gel electrophoresis but migrates identical to β-casein B in acid medium. β-casein D differs from β-casein B in the basic amino acid composition. In β-casein B basic amino acid composition of lysine, arginine and histidine were 11, 6 and 5 residues respectively and in the β-casein B 12, 5 and 4 respectively.

Eigel et al. (1984) during the fifth revision of proteins of cow milk established the presence of seven genetic variants of β-casein viz. A^1, A^2, A^3, B, D, E and C. In alkaline gels containing urea they migrated in the order $A^1 = A^2 = A^3 > B > E > C$ and in acidic condition their order were $C > B = D > A^1 = E > A^2 > A^3$.

κ-Casein

Methods were developed by Zittle and Custer (1963) to obtain αS-casein and κ-casein free of contaminant and judged

by starch urea gel electrophoresis. κ-casein was prepared by extraction of whole casein with urea-sulfuric acid and supplemented with ethanol precipitation.

Neelin (1964) modified electrophoretic method (with the addition of cysteine or mercapto-ethanol to the concentrated urea solution) in starch gel and the improved resolution obtained demonstrated the variants of κ-casein in individual milk. The zones given interim designations as 'a' and 'b' were found to occur singly or in combination.

Schimidt (1964) reported κ-casein variants in Friesian MRIJ cows. He reduced the κ-casein or whole casein preparation with 2 mercaptoethanol and observed the appearance of two κ-casein bands by urea starch gel electrophoresis. On the basis of the study on 48 Friesian and 22 MRIJ cows, he reported the presence of two genetic variants of the κ-casein.

Mackinlay and Wake (1965) made an extensive study on the κ-casein variants and observed the variation with respect to motility and named them as κ-casein A and B in the order of decreasing mobility.

Woychik et al. (1966) reported the detailed amino acid analysis of κ-casein A and B. The composition of both the variants appeared to be very much alike. κ-casein A contained

one more residue of aspartic acid and threonine than κ-casein B, whereas κ-casein B had one additional residue of alanine and isoleucine than κ-casein A.

Eigel et al. (1984) declared the existence of two variants of κ-casein namely A and B and stated that the nomenclature of κ-casein could not be precise since the exact nature of minor components of casein were not known and more conclusive results were needed before the nomenclature be finalised.

Whey protein polymorphism

The term 'whey protein' has been used to describe the group of milk proteins that remain soluble in 'milk serum' or 'whey' after precipitation of casein at pH 4.6 at 20°C. β-lactoglobulin, α-lactalbumin, serum albumin and immunoglobulin have been considered to be the major components of this fraction.

β-lactoglobulin

Aschaffenburg and Drewry (1955) observed two electrophoretically distinct β-lactoglobulins in the milk of individual cows belonging to four breeds viz. Shorthorn , Friesian, Ayreshire and Guernsey. Type of β-lactoglobulin appeared to be characteristic for a given cow. Using paper

electrophoresis the authors observed that the fastest moving type Lg A existed by itself or with a slower moving type Lg B and it was proved that a pair of autosomal alleles without dominance was responsible for such variation.

The 'C' variant of β-lactoglobulin was discovered by Bell (1962) in Australian Jersey cattle, using an improved method of starch gel electrophoresis. By zonal electrophoresis at alkaline pH β-lactoglobulin 'C' migrated slower than β-lactoglobulin B. The author observed the 'C' variant of β-lactoglobulin only in Jersey breed. The only aminoacid difference between β-lactoglobulin C and B is the replacement of glutamine residue in the former by histidine in the latter.

Grosclaude et al. (1966) first reported the D variant of β-lactoglobulin in low frequency in Montbeliarde cattle. It was also observed in 2 German Mountain breeds and in Polish Simmental cows. The D variant was appeared to be characteristic of Simmental cattle.

Three additional genetic variants of β-lactoglobulin E, F and G have been observed in the milk of Bali (Banteg) cattle by Bell et al. (1981). Through a combination of aminoacid analysis of different peptides the authors showed that β-lactoglobulin E in Bali milk appeared to possess the same glycine/glutamic acid substitution at position 158 as

β-lactoglobulin Dyak. The lactoglobulin F appeared to differ from the bovine B variant by the same substitution of 158 as well as substitution of serine or proline at 50 and tyrosine for aspartic acid at either 129 or 130. The β-lactoglobulin G also appeared to differ from 'B' variant by substitution of glycine for glutamic acid at position 158 as well as by methionine for isolencine at position 78.

In the report of the nomenclature committee for milk proteins of cow's milk in its fifth revision (Eigel et al., 1984) it was declared that seven genetic variants of β-lactoglobulins are known (A, B, C, D, E, F, and G) and positions of amino acid substitutions have been determined for five of those variants viz., A, B, C, D and E.

Major proteins of bovine milk and some of their properties Eigel et al. (1984)

Protein	Molecular weight	Amino acid residue/ mole	Content (g/litre)	Genetic variants
Casein (CN)				
α-S_1-CN	23.6×10^3	199	10.0	A,B,C,D,E
α-S_2-CN	25.2×10^3	207	2.6	A,B,C,D
β-CN	23.9×10^3	209	9.3	A^1,A^2,A^3 B,C,D,E
κ-CN	19.0×10^3	169	3.3	A,B
Whey proteins				
α-lactalbumin	14.1×10^3	123	1.2	A,B
β-lactoglobulin	18.3×10^3	162	3.2	A,B,C,D, E,F,G
Serum albumin	66.2×10^3	582	0.4	A

Breed distribution and gene frequencies of milk protein variants

αS_1-casein

Kiddy et al. (1964) observed six αS_1-casein phenotypes among 1378 cows. The distribution of phenotypes were 98 Ayreshires (all B) 203 Brownswiss (192 B and 11 BC) 400 Guernseys (188 B, 32 C and 180 BC) 542 Holsteins (2 A, 410 B, 5 AC and 44 BC) 67 Jerseys (44 B, 2 C and 21 BC) 68 crossbreds (67 B and 1 BC). The reason for the predominance was not apparent.

Aschaffenburg (1968) reported that the A variant of αS_1-casein was very rare and appeared to be confined to single blood line of Holstein originating in the state of Michigan. 'B' variant of αS_1-CN predominated in Western breeds of cattle and frequency was lower in some of the African Zebu breeds and very low in some Indian breeds. Frequency of 'C' variant was high in Indian breeds and very low in Western breeds. The gene frequency of αS_1-CN B variant was exceeding 0.90 in many breeds and reached 1.00 in some breeds like Ayreshire.

By starch urea gel electrophoresis from the milk of 65 Sahiwal, 41 Rathi, and 27 Jersey x Sahiwal crossbred cows Juneja and Chaudhary (1973) observed that the gene frequency of αS_1- Cn 'C' was 0.9 in Zebu cattle. Jersey crossbred cattle had an equal proportion of B and C alleles of

αS_1-Casein. The authors suggested that due to large difference in gene frequency at the αS_1 locus, this locus could be more effectively used to study the impact of crossbreeding of Zebu with Jersey breeds.

Zhebrovski et al. (1978) tabulated the gene frequencies for Russian Black pied cattle at three farms, the frequencies of αS_1, β and κ-casein and β-lactoglobulin types in milk and levels of homozygosity and heterozygosity for loci controlling them in relation to breeding method. It was observed that inbreeding increased homozygosity at the αS_1-casein.

Genetic aspects of milk protein polymorphism and their frequency distribution in the population of some of the Indian breeds of cattle (Sahiwal, Tharparkar, Red Sindhi) and crossbred (Brown swiss x Sahiwal) were studied by Jairam and Nair (1983b). Starch gel electrophoresis was employed separately using tris glycine buffer (pH 8.6). The frequencies of αS_1-B allele in Sahiwal, Tharparkar and Red Sindhi were 0.09, 0.09 and 0.03 respectively. Crossbreds of Brownswiss x Sahiwal cows had a frequency of 0.51 and found that inheritance of B allele in crossbred population was significantly different from that in Zebu population.

Ng-Kwai-Hang et al. (1984) phenotyped casein for 2045 cows and whey proteins for 3870 cows distributed in 63 Qubec dairy herds by polyacrylamide gel electrophoresis. Gene frequencies observed were αS_1 casein A 0.003, αS_1 casein B

0.970 and αS_1 casein C 0.027. The predominant variant of αS_1-Cn in Holstein population was αS_1-Cn B (97%). 94 per cent of the animals were homozygous for αS_1-casein B The A and C variants of αS_1-casein were rare and appeared only as heterozygous AB and BC.

McLean et al. (1984) reported genetic variants at the αS_1-casein locus in Jersey and Holstein breeds. The gene frequency observed for αS_1-casein variants in Jersey breed were αS_1-casein B - 0.628 and αS_1-casein C - 0.372 and Friesian cows 0.963 and 0.037 for αS_1-B and C variant respectively. No deviation from Hardy- Weinberg expectation was obtained in the breed genotype distribution of the milk proteins.

Lin et al. (1986) analysed individual milk samples from cows belonging to Holstein Friesian, Ayrshire and Holstein Friesian/Ayrshire crossbred lines for the protein variants. The genotypes observed were αS_1-Cn BB, BC and CC. The locus αS_1-Cn was dominated by allele B (95.5% overall).

β-Casein

Aschaffenburg (1968) could resolve the A variant of β-casein on further electrophoresis in acid gel into A^1, A^2 and A^3 variants. A^3 allele was found rare in Holstein Friesian and Normande breeds. Frequency of A^2 was found to be higher in Ayreshires and Guernseys and also in certain Indian Zebu cattle breeds like Sahiwal, Tharparkar, Deshi, Hariana

and Red Sindhi. 124 of the 126 animals found to be β-casein-A were A^2 homozygous. The frequency of B variant of β-casein was very low in majority of the breeds. C variant of β-casein was not found in Jerseys. This allele was first observed in Guernsey cattle and was found to be the characteristic of Brownswiss and other mountain breeds. In Holstein Friesian the frequency of 'C' variant of β-casein was very low. The 'D' variant of β-casein was found in few Deshi and Boran cows indicating a link between Indian and African Zebu cattle.

Singh and Khanna (1972) by employing Starch urea gel electrophoretic technique reported β-casein variants in two Hariana populations maintained at Izatnagar and Haringhatta. They observed five phenotypes viz. A^1A^1, A^2A^2, BB, A^1A^2, A^1B and A^2B controlled by three alleles β-casein A^1, β-casein A^2 and β-casein B. the phenotype A^2A^2 was the predominant one in both the populations. A^1B was observed only in Izatnagar population. The gene frequencies of β-casein A^1, β-casein A^2 and β-casein B in the Izatnagar animals were 0.051, 0.838 and 0.11 and that for Haringhatta animals were 0.040, 0.804 and 0.156 respectively.

Milk samples from Sahiwal, Rathi and Jersey x Sahiwal crossbred cows were subjected for starch gel electrophoresis by Juneja and Chaudhary (1973) at β-casein locus.

Two phenotypes viz., AA, AB were observed in all the populations the predominant phenotype in all the breeds were AA, though the frequency of heterozygotes AB was higher in Jersey x Sahiwal crossbreds. The frequency of the allele β-casein A was 0.94 in Sahiwal, 0.95 in Rathi and 0.81 in Jersey x Sahiwal. A new rare phenotype casein AD was observed in one of the Rathi cows. The casein D had slower mobility to that of β casein B.

Frequency of B allele of β-casein was reported to be very low in the Zebu breeds (Sahiwal, Tharparkar and Red Sindhi) by Jairam and Nair (1983b). The highly significant differences between the gene frequencies in Zebu cattle (0.04-0.07) and Brownswiss x Sahiwal crossbreds (0.16) indicated that gene frequencies in exotic breed might be quite different from that in Indian cattle.

Ng-Kwai-Hang et al. (1984) by polyacrylamide gel electrophoresis observed that the gene frequency of β-casein variants were β-casein A^1 0.561, β casein A^2 0.421, β-casein A^3 0.011, β-casein B 0.007, in Holstein cows belonging to the Qubec dairy herds. In the β-casein system no C variant was detected and the rare B gene was only in heterozygotes in combination with A^1, A^2 and A^3 in 1.37 per cent of the observations. The most predominant types of β-casein were A^1 and A^2 with frequencies 56.1 and 42.1 per cent respectively.

There were 30.71 per cent homozygous for A^1, 16.63 per cent A^2 and heterozygot combination of A^1, A^2 and A^3 accounted for 51.25 per cent.

The gene frequency of casein variants were estimated by Mc Lean et al. (1984) by the electrophoretic method from 289 Jersey Cows and 249 Friesian cows and were found to be 0.074, 0.564 and 0.362 respectively for β-casein A, A^2 and B respectively in Jersey cows and 0.074, 0.564 and 0.362 for β-casein A^1, A^2 and B respectively in Friesian cows. β-casein A^3 was not detected in both the breeds.

Lin et al. (1986) observed β-casein polymorphism in cows belonging to Holstein Friesian, Ayreshire and Holstein Friesian/Ayreshire crossbred lines. The genotypes observed for β-casein variants were A^1A^1, A^1A^2, A^1A^3, A^1B, A^2A^3 and A^2B. β-casein A^1A^3 and A^1B were significant among Ayreshire and β-casein A^1B was absent among Holstein Friesian cows. At the β-casein locus alleles A^1 and A^2 (44 and 45 per cent respectively) dominated.

κ-Casein

Aschaffenburg (1968) observed two variants of κ-casein viz. A and B in almost all the breeds examined. The A allele predominated in majority of cattle breeds except Jersey, Normande and some African Zebu cattle.

In a study on Sahiwal, Rathi and Jersey x Sahiwal Crossbreds, Juneja and Chandhary (1973) reported two phenotypes viz. AA and AB at the κ-casein locus in the zebu breeds and three phenotypes AA, AB and BB in the crossbreds. The frequency of the allele κ-CN-A was 0.92 in sahiwal, 0.83 in Rathi and 0.54 in Jersey x Sahiwal crosses.

Two phenotypes κ-casein AA and κ-casein AB were reported in Tharparkar, Sahiwal and Red Sindhi Cows (Majumder and Ganguli, 1970). They could not observe BB phenotype in any of the breeds studied. All the zebu breeds were predominantly of AA phenotype. The gene frequency of κ-casein A was 0.63, 0.78 and 0.62 in Tharparkar, Sahiwal and Red Sindhi respectively.

Jairam and Nair (1983b) observed that κ-Cn homozygous B type was absent in both Sahiwal and Red Sindhi cows. The gene frequency of B allele was significantly higher in cross-bred cows.

Lin et al. (1986) found the three genotypes of Casein AA, AB and BB in the breeds of Holstein, Friesian, Ayreshire and Holstein Friesian-Ayreshire cross bred lines. κ-Cn locus was dominated by allele A (65.6%).

The gene frequencies of κ-Cn variants observed in Holstein cows of Qubec dairy herds were reported by Ng-Kwai-Hang et al. (1984). The gene frequencies were K-Cn A 0.744 and κ Cn B 0.256. Holstein cows were approximately 53 per cent homozygous for A, 4 per cent homozygous for B and 43 per cent heterozygous for AB.

β-lactoglobulin

Aschaffenburg and Drewry (1955) observed β-lactoglobulin A and B variants in Shorthorn Friesian, Ayreshire and Guernsey breed of cows. This discovery was an important event in the research on milk protein polymorphism.

Aschaffenburg (1968) reported the distribution of two variants of β-lactoglobulin in breeds of various countries. Zebu breeds tended to be low in the frequency of A variant and the same reached very high in certain Western breeds. B allele predominated in most cases. The C variant of β-lactoglobulin was detected only in the breed of South African Ngumi and in which it was observed in the heterozygous form.

β-lactoglobulin variants A, B and C were observed in all the phenotypic combinations in Hariana Cattle by Singh and Khanna (1972). Herd to herd variation was observed in their

study. The gene frequency of Lg-B in Izatnagar herd was reported to be 0.837 and the same in Haringhatta herd was 0.921.

Juneja and Chaudhary (1973) by starch urea gel electrophoretic method of milk samples from Sahiwal, Rathi and Jersey x Sahiwal crossbred cows reported three phenotypes viz. AA, AB and BB at the β-lactoglobulin locus in all the populations. The homozygous BB was predominent in all the genetic groups. The frequency of β Lg-A was 0.18, 0.21 and 0.32 in Sahiwal, Rathi and Sahiwal x Rathi crossbreds respectively.

Phenotyping of milk samples for β-lactoglobulin allele from cows of indigenous breeds (Hariana, Sahiwal, Kankrej, Ongole, Red Sindhi, Kangayam Gir and Tharparkar), Holstein Friesian and crossbreds of Holstein Friesian with Hariana and Sahiwal was carried out by Singh and Bhat (1980). Holstein cows exhibited β-lactoglobulin A and B. Indigenous and crossbred cattle had three alleles LgA, LgB and LgC. LgB was the most common allele among indigenous breeds (0.44-0.94). Lg A had relatively high frequency among Holsteins (0.57).

Jairam and Nair (1983b) reported significant differences between Tharparker and Sahiwal or Red Sindhi in the β-lactoglobulin gene frequencies. The gene frequency of A

allele in Sahiwal, Tharparkar and Red Sindhi and crossbred cows was 0.23, 0.13, 0.23 and 0.34 respectively. The differences in gene frequency of A allele were significant among Zebu breeds, and they were significantly different ($P<0.01$) from that in crossbred cows.

Ng-Kwai-Hang et al. (1984) observed gene frequency of β-lactoglobulin variants in Holstein cows as β LgA, 0.387 and β-Lg B 0.613. In the Holstein population, 13.44, 50.54 and 36.02 per cent animals were AA, AB and BB respectively.

Lin et al. (1986) phenotyped the milk samples for protein polymorphism of cows belonging to Holstein Friesian, Ayreshire and Holstein Friesian-Ayreshire crossbred lines. The β-lactoglobulin variant observed were AA, AB or BB. β-lactoglobulin locus was dominated by allele B (78.4%).

Linkage studies on milk protein loci

Grosclaude et al. (1964) by analysing data collected in suitable families discovered that linkage relation existed between αS_1 and β-casein loci.

Arave (1967) by phenotyping β-casein for separate A^1, A^2 and A^3 variants could observe that 47 of 51 cows carrying the A^3 allele carried αS_1-casein C allele, and the six cows

homozygous for β-casein A^3 were likewise homozygous for αS_1-casein C.

Aschaffenburg (1968) reported close linkage between κ-casein and the αS_1 and β-casein systems. In Indian zebu cattle he observed that β-A^2 allele closely associated with αS_1-casein C, the predominant αS_1-casein variant in Indian cows.

Hines et al. (1969) confirmed the evidence of linkage between caseins while studying linkages among cattle blood and milk protein polymorphism.

Jairam (1986) could find significant association among milk protein variants in Jersey x Ongole and Friesian x Ongole crossbreds. In Jersey x Ongole crosses β-casein AB was significantly associated with β-lactoglobulin AB. In Friesian crosses αS_1-casein BB was highly associated with β-casein AA, and αS_1-casein BC was significantly associated with β-casein AA. In certain herds of Ongole significant association of αS_1-casein CC type with β-casein type AA and with α-lactalbumin AB was observed at 5 per cent level. There was significant association between κ-casein AA and α-lactalbumin types especially with α-lactalbumin AB type.

Association of milk protein variants with milk yield in cows

Mityuko and Ukolov (1978) reported that milk yields are generally higher in animals homozygous for individual milk proteins. In a study conducted for production of milk in relation to protein polymorphism in Russian Black Pied cows it was found that cows with aggregate genotypes BB/AA/AB/AA or BB/AB/AB for αS_1-Casein, and κ-Casein and β-lactoglobulin had higher milk yield than those with other genotypes. The authors reported after studying the line and aggregate genotype for protein polymorphism on milk production of Black Pied cattle that the highest yielding groups all had αS_1-casein BB, β-casein AA and κ-casein AA.

According to Jairam and Nair (1983a) milk production types did not confer any influence on first lactation yield in combined breed studies. In breed-wise studies significant influence ($P<0.05$) of β-lactoglobulin type was noticed in Tharparkar breed. But the same effect was not observed in Sahiwal and Red Sindhi. β-lactoglobulin types did not confer any influence on the traits among crossbred cows. There was a significant trend that αS_1-casein BB type produced more milk than αS_1-casein BC during the first lactation in Brown Swiss x Sahiwal crosses.

Ng-Kwai-Hang et al. (1984) phenotyped casein of 2045 Holstein cows and whey proteins of 3870 cows distributed in 63 Qubec dairy herds. It was found that milk yield of first lactation was highest in cows carrying the αS_1-casein BB phenotype and lowest in αS_1-casein AB cows. In the β-casein system cows carrying the A^3 gene were superior for milk production. Comparison at the β-casein locus between A^1 and A^2 showed a slight advantage of A^2 over A^1. Variants in κ-casein and lactoglobulin had no significant effects on the milk yield of first lactation.

McLean et al. (1984) studied the effect of milk protein genetic variants on milk yield and composition and found that milk protein genotypes had no significant effect on yields over a complete lactation of milk and fat.

Samarinaenu and Stamatescu (1984) reported that in Romanian Brown cows β-lactoglobulin AA type were superior to type BB and AB in milk yield.

Leonhard-Kluz and Gwozdziewics (1985) found that in Polish Red cows the best yield were obtained for cows with aggregate genotype β-LgB/β-LgB + κ-Cn A/κ-Cn B. The lowest yields of milk and milk constituents were obtained for cows with the κ-Cn A/κ-Cn A type.

Lin et al. (1986) examined the effect of milk protein loci on first lactation production in Holstein bared "H'line, Ayreshire bared A line and crossbred C line between H and A lines. Gene substitution at αS_1-casein locus showed the highest effect on first lactation yields. It was suggested that lactating performance can be improved through milk protein typing by increasing the frequency of B allele at αS_1-casein locus, A^2 allele at β-casein locus and B allele at κ-casein and β-lactoglobulin loci.

Lin et al. (1989) in a study conducted for the relationship of milk protein types to life time performance observed that only the κ-casein locus had significant effects on fixed parity and fixed age total milk and herd life. Cow with BB-κ-casein type outproduced those with AB or AA κ-casein, in three parity total milk by 963 and 1657 kg respectively. Considering the total milk accumulated upto 61 months of age in life, cows with BB κ-casein type and outperformed than counterparts with AB of AA κ-casein types by 1050 and 1923 kg respectively. The authors suggested that the moderate gene frequency of κ-casein B allele in current dairy population can be increased to improve the life time total milk production.

Bech and Kristiansen (1990) reported that in Danish Jersey, Danish Red and Danish Black Pied cows, the distribution of genotypes of different milk protein systems

were different from breed to breed. In Danish Red breed nearly all the cows were homozygous for αS_1-casein B. β-casein genotypes were associated with yield parameters in all breeds. The A_2A_2 genotypes of the β-casein gene produced higher yields of milk fat and protein.

Ng-Kwai-Hang et al. (1990) used polymorphic forms of αS_1-casein, β-casein, κ-casein and β-lactoglobulin as genetic markers for milk yield, percentage of fat, percentage of protein during three lactations in Qubec Holstein cattle. It was observed that αS_1-casein types affected milk yield in the second lactation only (BB>AB>BC). Protein content of milk was influenced by phenotypes of κ-casein and β-lactoglobulin for all three lactations. The replacement of A by B allele at the κ-casein locus would increase protein levels in milk by 0.08, 0.06 and 0.04% respectively.

Milk yield differed significantly between cows with β-lactoglobulin types, AA, BB and AB but was not significantly related to types of αS_1-casein β-casein or κ-casein (Meyer et al., 1990).

Association of milk protein variants with incidence of mastitis

Jairam (1986) studied the influence of milk protein genetic variants on the incidence of mastitis in different breeds. It was reported that in Jersey β-lactoglobulin BB type animals were least affected with mastitis when compared to AA or AB typed cows. In Jersey x Ongole crossbred animals lowest incidence of mastitis was observed in β-lactoglobulin BB and AB typed animals. In Friesian x Ongole cows, α lactalbumin types significantly influenced the incidence of mastitis. α-lactalbumin AB typed animals had the lowest incidence of mastitis.

Vecehiotti et al. (1989) observed the β-lactoglobulin polymorphism in Friesian cows. No relationship between somatic cell count and the three β-lactoglobulin type was apparent in this study.

Meyer et al. (1990) studied the relationship between milk yield, udder health, and milk protein and blood protein polymorphism in German Black Pied cows. Udder health score was not significantly related to any of the milk protein variants.

Materials and Methods

MATERIALS AND METHODS

The present study was carried out on 135 animals belonging to three crosses of local cattle with exotic breeds viz., 50 Jersey cross-breds, 45 Brown-swiss crossbreds and 40 Holstein Friesian crossbreds, maintained at University Livestock Farm, Mannuthy.

Collection of milk samples

The milk samples were collected in four stages of lactation i.e. 1st, 7th, 22nd and 37th week of lactation from each animal in a 25 ml sterilized glass tube. Samples were collected directly from the teats and milk samples of all the four teats were mixed together.

Fractionation of milk

Fractionation of casein was started within an hour of sample collection by using the method of Thompson and Kiddy (1963) with slight modification.

Fractionation of casein

Five ml each of milk samples were centrifuged at 3000 rpm for 15 minute. The skim milk under the fat layer was aspirated with a long hypodermic needle and transferred to

centrifuge tubes. 1 M hydrochloric acid was added to the skim milk samples till the pH was adjusted to 4.6. The samples were again centrifuged at 3000 rpm for 15 minutes and the whey portion was removed. The casein obtained in the tubes was washed four to five times with distilled water, with the pH maintained at 4.6-4.8.

Separation of genetic variants of caseins

The variants of casein were separated by horizonal polyacrylamide gel electrophoresis (Medrano and Sharrow, 1989) with slight modifications. 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 (1961) and Eigel et al. (1984).

Buffers and solutions

Electrode buffer

Tris	12 g
Glycine	57.6 g
Distilled water	2.0 l
pH	8.3

Acrylamide stock solution (A)

Acrylamide	32 g
N'N'Methylene Bisacrylamide	0.8 g

The two reagents were dissolved in 100 ml distilled water and filtered.

Gel buffer stock solution (B)

Tris	9 g
Citric acid	0.9 g
Crystalline Urea	24.24 g
2 mercaptoethanol	0.06 ml
Distilled water	100 ml

Ammonium per sulphate (C)

Fifty milligrams of ammonium per sulphate was dissolved in 50 ml distilled water. Freshly prepared solution was used.

Working gel solution

Composition for the 8% acrylamide gel	
Acrylamide (A)	6.25 ml
Gel Buffer (B)	8.00 ml
Distilled water	2.75 ml
TEMED	0.03 ml
Ammonium per sulphate (C)	8.00 ml

Staining solution

Amido black 10 B	-	1 g
Distilled water	-	930 ml
Acetic acid	-	70 ml

Destaining solution

Methanol	-	2500 ml
Glacial acetic acid	-	500 ml
Distilled water	-	2500 ml

Preserving solution

Acetic acid	-	70 ml
Distilled water	-	930 ml

Procedure

Preparation of gel

A continuous buffer system of 8 per cent acrylamide gel at pH 8.3 was used.

Two plates of equal size, one made up of acrylic sheet and the other one a glass plate was used for the preparation of gel. The acrylic sheet was having 1.5 mm high frame on the three sides which formed the thickness of the gel. On the free side without the frame the acrylic sheet was having

projection to form well on the gel. The glass plate was kept in apposition with the frame of the acrylic sheet with the application of vacuum grease on the frame. Paper clips were applied on all the sides.

Freshly prepared working gel solution was filled into the gap between the plates. Care was taken to avoid the formation of air bubble in the gel.

Preparation of casein sample for electrophoresis

The casein was dissolved in 6 M urea solution for the application of the samples for electrophoresis.

Electrophoresis

Acrylic sheet was carefully removed and the gel was gently placed in the electrophoretic chamber containing the electrode buffer. Whatman filter paper No.1 was used as the wick for completion of circuit connecting the gel and electrode buffer. Enough number of filter papers of equal sizes and same level at the edges were used for uniform voltage gradient. The wicks were wetted well and placed gently on either side of the gel.

Twenty microlitres of casein sample dissolved in 6 M urea solution was charged into the wells. Bromphenol blue was used as marker on one or two wells as indicator. An initial

current of 200 V at 10 mA for 10 minutes followed by 200 V at 25 mA for 5 hours was applied.

Staining, destaining and preservation

The gel was removed from the glass plate and put in the staining solution for 2 hrs. Excess stain was removed by keeping the gel in destaining solution or 12 hrs. Then it was preserved in the preserving solution. The genetic variants were identified by their relative mobility on the gel.

Whey proteins

Whey protein variants were separated by the method of Ng-Kwai-Hang and Kroeker (1984). The method was almost similar to that of casein separation. Few modifications was applied for whey proteins. Crystalline urea @ 0.4 g/ml was added to the supernatant left after casein precipitation Twenty microlitres of the samples were applied to the wells. Twelve percentage acrylamide gel without urea was used for the whey proteins.

Analysis of data

Gene frequencies

The gene frequencies at different loci were calculated by the direct counting method.

Test for goodness of fit

Chi square test for goodness of fit was applied to find out whether the populations were in equilibrium or not with respect to the particular polymorphic system (Snedecor and Cochran, 1967).

The gene frequencies of different loci in different population were compared using X^2-test (Snedecor and Cochran, 1967).

Test for heterozygosity

The heterozygosity in different population was measured as per the method described by Nei and Roy Choudhary (1974).

The heterozygosity of K^{th} locus (hk) could be defined as

$$hk = 1 - jk$$

where

$jk = X^2_1$ is the homozygous at K^{th} locus

and $X_1 = \frac{ni}{n}$, denotes the gene frequency of e^{th} allele at k^{th} locus

$$\hat{H} = \sum_{k=1}^{r} \frac{hk}{r},$$

where r is the number of loci examined.

Linkage studies of milk protein variants

The concept of linkage or association of genes controlling the synthesis of milk proteins implies on the relative distance of different loci situated on the same chromosome (Farrel and Thompson, 1971). The genetic markers of milk proteins identified by the polyacrylamide gel electrophoresis was used to prepare two way contingency table, each showing the combination of different alleles in the two different protein loci. In each genetic group, test for independence between different alleles in each milk protein types from those in other milk protein groups were carried out using the chi square test (Snedecor and Cochran, 1967).

Effect of milk protein variants on milk production

First lactation milk yield for 305 days of the animals under study were recorded. The effect of milk protein variants on individual milk production of these group was analysed by Student's 't' test (Snedecor and Cochran, 1967).

Effect of milk protein on incidence of mastitis

Influence of milk protein variants on the incidence of mastitis in the three genetic groups under study was compared. The percentage distribution of animals affected along with Chi-square test of significance was calculated for each of the protein type (Snedecor and Cochran, 1967).

Results

RESULTS

One hundred and thirty five crossbred cows were typed for the variants of αS_1-casein, β-casein, κ-casein and the whey protein β-lactoglobulin.

αS_1-Casein

αS_1-casein had faster mobility in the gel than β-casein and κ-casein. Three αS_1-casein phenotypes viz., BB, BC and CC in the descending order of mobility towards the anode were observed (Fig.1).

Phenotype and gene frequencies

The genetic group wise frequency of various αS_1-casein phenotypes are given in Table 1. The frequencies of BB phenotype were 0.36, 0.34 and 0.28 in the Jersey crossbreds, Brown Swiss crossbreds and Holstein Friesian crossbreds respectively. In the pooled population the frequency was 0.36. The frequency of BC was 0.54 in Jersey crossbreds, 0.42 in Brown Swiss crossbreds and 0.46 in Holstein Friesian crossbreds and 0.53 in the pooled population. The frequency of CC ranged from 0.06 in Holstein Friesian crossbreds to 0.14 in Brown Swiss crossbreds.

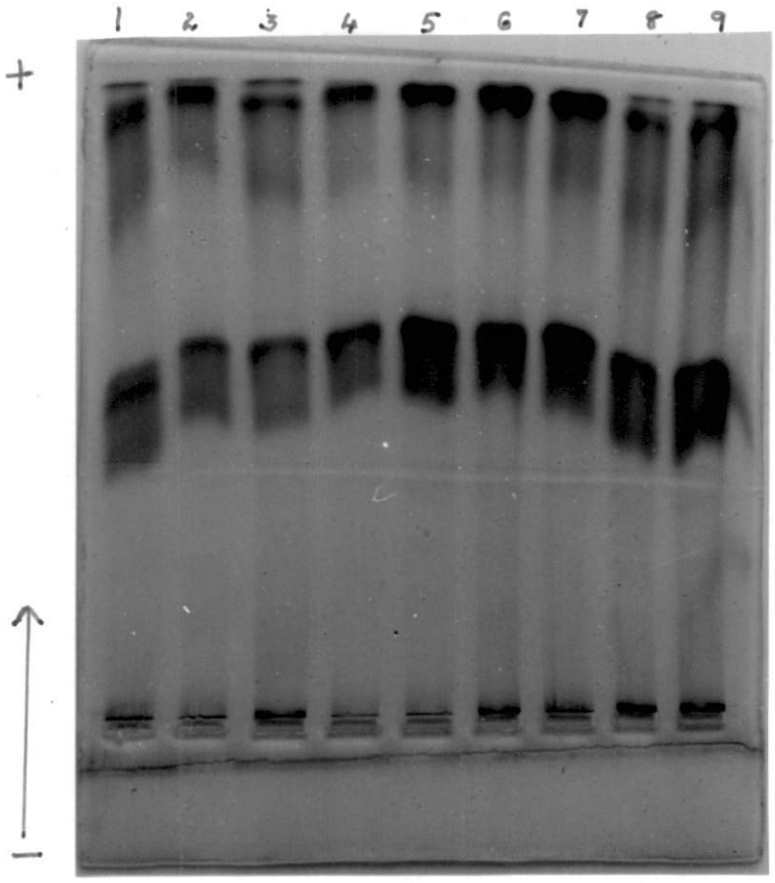


Fig.1 Phenotypes of αS_1 and β-casein

	1	2	3	4	5	6	7	8	9
αS_1-casein	BC	BC	CC	BB	BC	BC	BC	BB	BB
β-casein	AB	AA	AA	AA	AA	AA	AA	AA	AA

Table 1. Phenotype and gene frequencies of αS_1-casein types in different crossbred genetic groups

Population	Sample size	αS_1-Phenotype frequencies			αS_1-Gene frequency	
		BB	BC	CC	B	C
Cross-bred Jersey	50	0.36 (18)	0.54 (27)	0.10 (5)	0.63	0.37
Cross-bred Brown Swiss	45	0.34 (17)	0.42 (21)	0.14 (7)	0.61	0.39
Cross-bred Holstein Friesian	40	0.28 (14)	0.46 (23)	0.06 (3)	0.63	0.37
Pooled cross-breds	135	0.36 (49)	0.53 (71)	0.11 (15)	0.63	0.37

Number of animals is given in parenthesis

Table 2. Observed and expected frequencies of αS_1-casein phenotypes in different crossbred genetic groups

Population		αS_1-Phenotype			X^2 values
		BB	BC	CC	
Cross-bred Jersey	Obs	18	27	5	1.32 NS
	Exp	19.8	23.31	6.8	
Cross-bred Brown Swiss	Obs	17	21	7	0.01 NS
	Exp	16.75	21.45	6.85	
Cross-bred Holstein Friesian	Obs	14	23	3	2.10 NS
	Exp	15.8	18.65	5.48	
Pooled cross-breds	Obs	49	71	15	2.08 NS
	Exp	53.58	62.94	18.48	

NS - Not significant

In all the crossbred genetic groups the B allele was found to be predominant and the frequencies were almost the same (0.63, 0.61, 0.63 and 0.63 in crossbred Jersey, Crossbred Brown Swiss, Crossbred Holstein Friesian and Pooled crossbreds respectively). The frequency of C allele was 0.37, 0.39, 0.37 and 0.37 in crossbred Jersey, crossbred Brown Swiss, crossbred Holstein Friesian and pooled crossbred respectively (Table 1).

Test for genetic equilibrium

All the crossbred populations were found to be in genetic equilibrium with respect to αS_1-casein locus (Table 2). The observed number of animals with different αS_1 casein variants did not differ significantly from the expected number in all the genetic groups.

β-casein

β-casein had an electrophoretic mobility between the κ-casein and αS_1-casein types. Three variants of β-casein were observed in all the crossbred groups studied (AA, AB and BB). AA genotype was fastest in mobility towards the anode (Fig.1).

Phenotype and gene frequencies

Phenotype and gene frequencies of β-casein types in different genetic groups of cattle are given in Table 3. The

Table 3. Phenotype and gene frequencies of β-casein polymorphism in different genetic groups

Population	Sample size	β-casein Phenotype frequencies			Gene frequency	
		AA	AB	BB	A	B
Cross-bred Jersey	50	0.56 (28)	0.38 (19)	0.06 (3)	0.75	0.25
Cross-bred Brown Swiss	45	0.67 (30)	0.29 (13)	0.04 (2)	0.81	0.19
Cross-bred Holstein Friesian	40	0.50 (20)	0.42 (17)	0.08 (3)	0.71	0.29
Pooled cross-breds	135	0.58 (78)	0.36 (49)	0.06 (8)	0.76	0.24

Number of animals is given in parenthesis

Table 4. Observed and expected frequencies of β-casein phenotype in different crossbred genetic groups

Population		β-casein phenotype			X^2 values
		AA	AB	BB	
Cross-bred Jersey	Obs	28	19	3	0.01 NS
	Exp	28.13	18.75	3.13	
Cross-bred Brown Swiss	Obs	30	13	2	0.15 NS
	Exp	29.5	I3.85	1.62	
Cross-bred Holstein Friesian	Obs	20	17	3	0.05 NS
	Exp	20.16	16.47	3.36	
Pooled cross-breds	Obs	78	49	8	0 NS
	Exp	77.98	49.25	7.78	

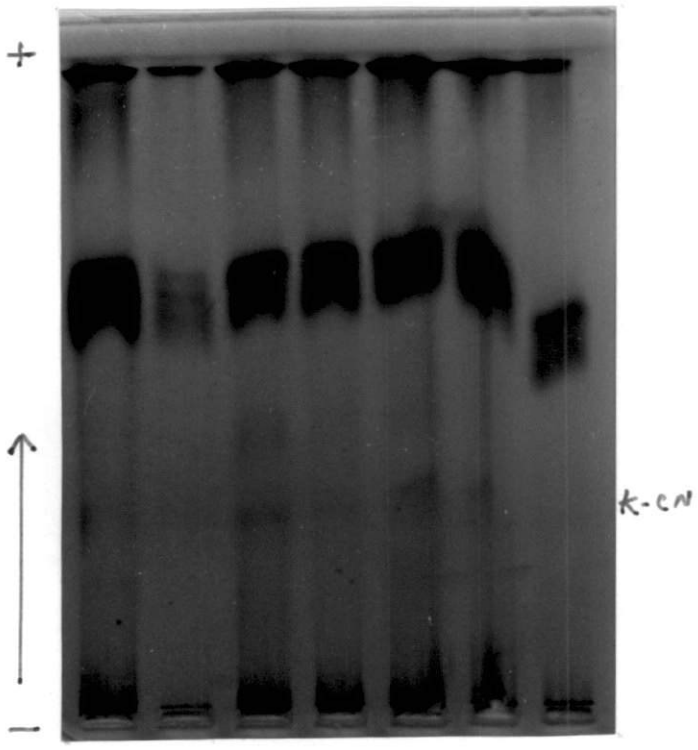
NS - Not significant

frequency of AA phenotype was 0.56, 0.67, 0.50 and 0.58 in crossbred Jersey, crossbred Brown Swiss, crossbred Holstein Friesian and pooled population respectively. The phenotypic frequency of AB was 0.38 in crossbred Jersey, 0.29 in crossbred Brown Swiss, 0.42 in crossbred Holstein Friesian and 0.36 in pooled population. The frequency of BB phenotype was 0.06, 0.04, 0.08 and 0.06 in crossbred Jersey, Crossbred Brown Swiss, Crossbred Holstein Friesian and pooled crossbreds respectively.

Gene frequency of β-casein A allele was found to be very high in crossbred Brown Swiss group (0.81) and almost the same in all other populations (0.75, 0.71, 0.76 in crossbred Jersey, crossbred Holstein Friesian and pooled crossbreds respectively). The B allele was found to be lowest in crossbred Brown Swiss group (0.19) and a higher value of 0.29 was observed in crossbred Holstein Friesian (Table 3).

Test for genetic equilibrium

The results of test for genetic equilibrium at the β-casein locus are furnished in Table 4. It was found that all the populations were in genetic equilibrium at β-casein locus. The observed and expected values were not differed much both for homozygotes and heterozygotes in all the



Fig。2 Phenotypes of κ-casein

κ-casein。 AA

Table 5. Phenotype and gene frequencies of κ-casein types in different cross-bred genetic groups

Population	Sample size	κ-casein Phenotype frequencies			Gene frequency	
		AA	AB	BB	A	B
Cross-bred Jersey	50	0.52 (26)	0.34 (17)	0.14 (7)	0.69	0.31
Cross-bred Brown Swiss	45	0.31 (14)	0.51 (23)	0.18 (8)	0.56	0.44
Cross-bred Holstein Friesian	40	0.38 (15)	0.50 (20)	0.12 (5)	0.63	0.37
Pooled cross-breds	135	0.41 (55)	0.44 (60)	0.15 (20)	0.63	0.37

Number of animals is given in parenthesis

Table 6. Observed and expected frequencies of κ-casein phenotypes in different crossbred genetic groups

Population		κ-casein phenotypes			X^2 values
		AA	AB	BB	
Cross-bred Jersey	Obs	26	17	7	2.11 NS
	Exp	23.81	21.39	4.81	
Cross-bred Brown Swiss	Obs	14	23	8	0.09 NS
	Exp	14.11	22.18	8.70	
Cross-bred Holstein Friesian	Obs	15	20	5	0.188 NS
	Exp	15.88	18.65	5.48	
Pooled cross-breds	Obs	55	60	20	0.299 NS
	Exp	53.58	62.94	18.48	

NS - Non significant

population and the differences were statistically non-significant.

κ-casein

κ-casein, the slowest moving casein type had three variants viz., AA, AB and BB. AA variant had greater mobility than the BB type (Fig.2).

Diagramatic representation of the αS_1-casein, β-casein and κ-casein phenotypes are given in Figure 3.

Phenotype and gene frequencies

Table 5 shows the phenotype frequencies and gene frequencies of the alleles at κ-casein locus. The frequency of κ-casein AA phenotype was 0.52 in crossbred Jersey, 0.31 in crossbred Brown Swiss, 0.38 in Holstein Friesian and 0.41 in pooled population. The frequency of AB phenotype was 0.34, 0.51, 0.50 and 0.44 in crossbred Jersey, crossbred Brown Swiss, crossbred Holstein Friesian and pooled population respectively. The phenotypic frequency of BB in crossbred Jersey, crossbred Brown Swiss, crossbred Holstein Friesian and pooled population was 0.14, 0.18, 0.12 and 0.15 respectively.

The gene frequency of A allele was 0.69, 0.56, 0.63 and 0.61 in crossbred Jersey, crossbred Brown Swiss, crossbred Holstein Friesian and pooled crossbreds respectively. The B

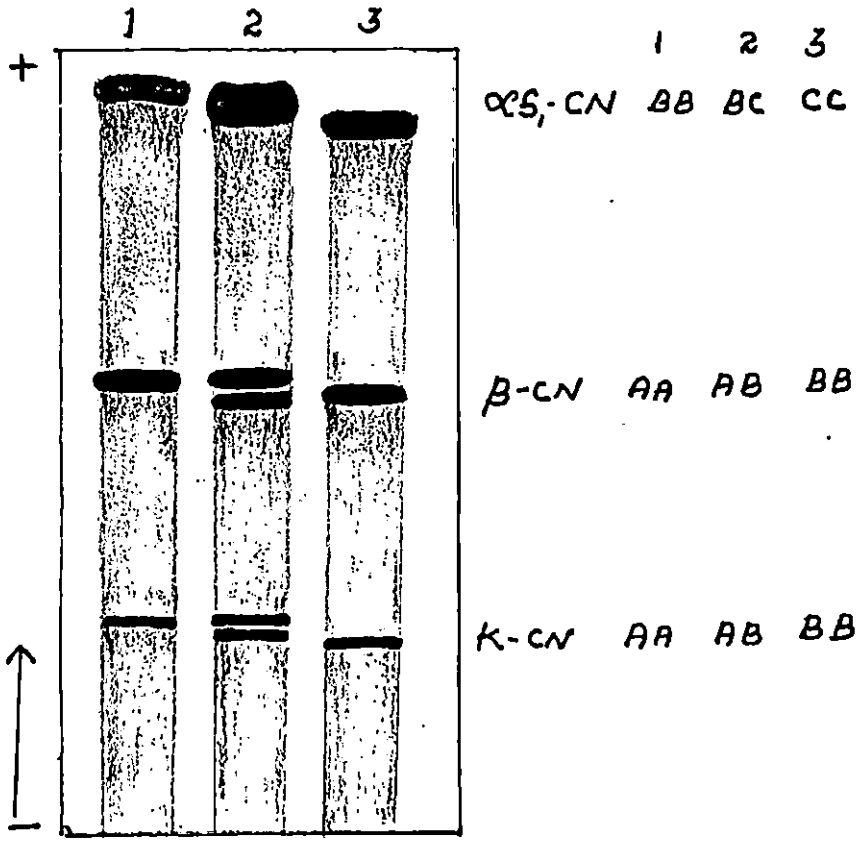


Fig.3 Diagrammatic representation of αS_1, β and κ-casein phenotypes

Table 7. Phenotype and gene frequencies of β-lactoglobulin types in different cross-bred genetic groups

Population	Sample size	β-lactoglobulin Phenotype frequencies			Gene frequency	
		AA	AB	BB	A	B
Cross-bred Jersey	50	0.24 (12)	0.38 (19)	0.38 (19)	0.43	0.57
Cross-bred Brown Swiss	45	0.18 (8)	0.44 (20)	0.38 (17)	0.40	0.60
Cross-bred Holstein Friesian	40	0.25 (10)	0.43 (17)	0.33 (13)	0.46	0.54
Pooled cross-breds	135	0.22 (30)	0.42 (56)	0.36 (49)	0.43	0.57

Number of animals is given in parenthesis

Table 8. Observed and expected frequencies of the β-lactoglobulin phenotypes in different cross-bred genetic groups

Population		β-lactoglobulin Phenotypes frequencies			X^2 values
		AA	AB	BB	
Cross-bred Jersey	Obs	12	19	19	2.53 NS
	Exp	9.25	24.51	16.24	
Cross-bred Brown Swiss	Obs	8	20	17	0.24 NS
	Exp	7.2	21.6	16.2	
Cross-bred Holstein Friesian	Obs	10	17	13	0.84 NS
	Exp	8.46	19.87	11.66	
Pooled cross-breds	Obs	30	56	49	3.18 NS
	Exp	24.96	66.18	43.86	

NS - Non-significant

allele frequency was 0.31, 0.44, 0.37 and 0.37 in crossbred Jersey, crossbred Brown Swiss, crossbred Holstein Friesian and pooled population respectively (Table 5).

Test for genetic equilibrium

The results of Chi-square test for goodness of fit to check the genetic equilibrium of the population under study at the β-casein locus are provided in Table 6. The observed values did not differ significantly from the expected values in any of the genetic groups.

β-lactoglobulin

β-lactoglobulin was subjected to electrophoresis in separate gel containing 12 per cent polyacrylamide. β-lactoglobulin had three variants viz., AA, AB and BB in all the genetic groups of which AA had the maximum mobility (Fig.4). Digramatic representation of the β-lactoglobulin phenotypes are given in Figure 5.

Phenotype and gene frequencies

The frequencies of the different β-lactoglobulin phenotypes are presented in Table 7. The frequency of phenotype AA was 0.24 in crossbred Jersey, 0.18 in crossbred Brown Swiss, 0.25 in crossbred Holstein Friesian and 0.22 in

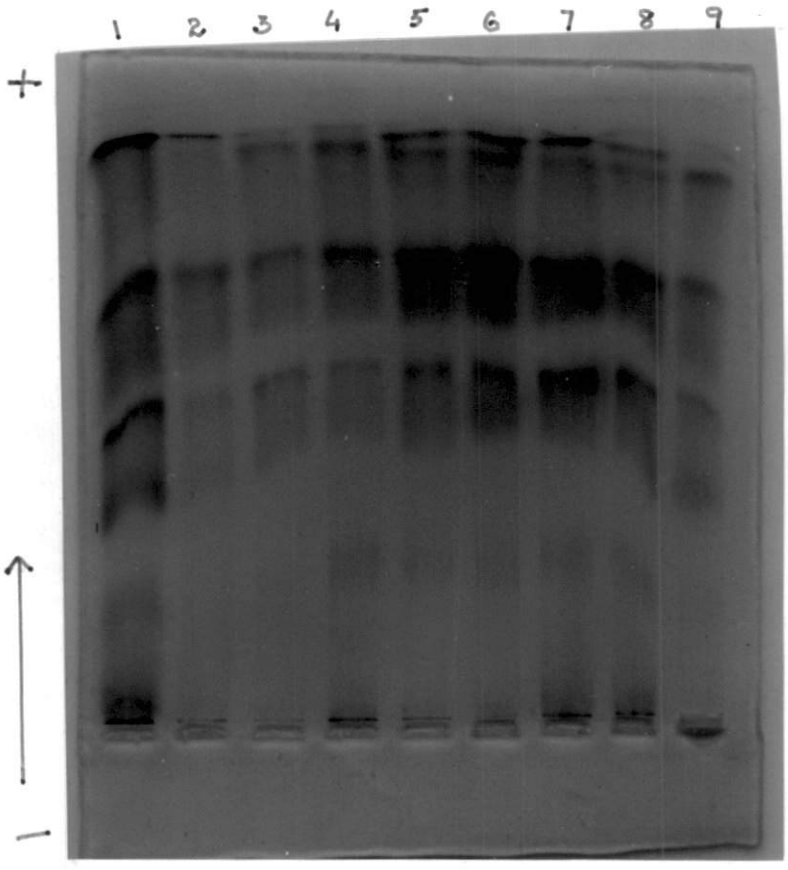


Fig. 4 Phenotypes of β-lactoglobulin

1	2	3	4	5	6	7	8	9
AA	AA	BB	BB	AB	AB	AB	AB	BB

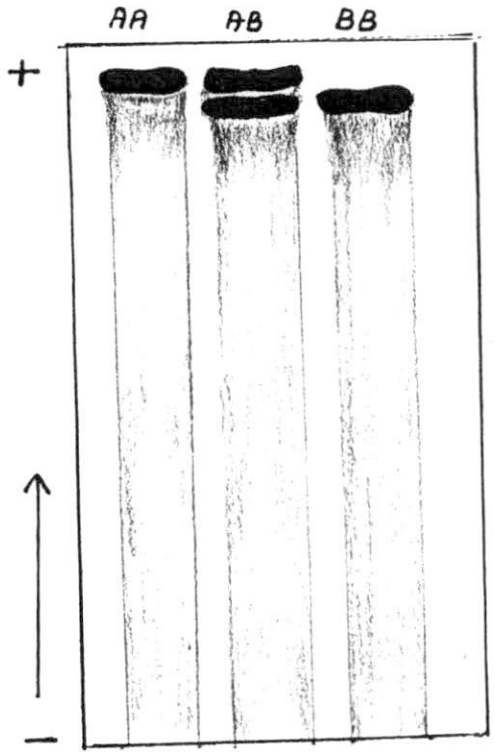


Fig.5 Diagrammatic representation of β-lactoglobulin phenotypes

pooled population. The phenotypic frequency of β-lactoglobulin AB was 0.38, 0.44, 0.43 and 0.42 in crossbred Jersey, crossbred Brown Swiss, crossbred Holstein Friesian and pooled population respectively. Frequency of phenotype BB in crossbred Jersey, crossbred Brown Swiss, crossbred Holstein Friesian and pooled population was 0.38, 0.38, 0.33 and 0.36 respectively.

The gene frequency of β-lactoglobulin A allele was 0.43 in crossbred Jersey, 0.40 in crossbred Brown Swiss, 0.46 in crossbred Holstein Friesian and 0.43 in pooled population respectively (Table 7). The frequency of B allele 0.57, 0.60, 0.54 and 0.57 in crossbred Jersey, crossbred Brown Swiss and crossbred Holstein Friesian and pooled population respectively.

Test for genetic equilibrium at β-lactoglobulin locus

In Table 8 the observed and expected phenotype frequencies and gene frequencies of β-lactoglobulin in different crossbred populations are given. The Chi-square values obtained were non significant in all the genetic groups.

Heterozygosity

The genetic variability of a population is usually

Table 9. Heterozygosity at milk protein loci in crossbred cattle

Population	α-S_1-casein	β-casein	κ-casein	β-lactoglobulin	Average heterozygosity
Cross-bred Jersey	0.4662	0.3750	0.4278	0.4902	0.4400
Cross-bred Brown Swiss	0.4758	0.3078	0.4928	0.4800	0.4346
Cross-bred Holstein Friesian	0.4662	0.4118	0.4662	0.4968	0.4603
Pooled cross-breds	0.4662	0.3648	0.4662	0.4902	0.4469

measured by the average heterozygosity per locus. The heterozygosity in each population were estimated and results are presented in Table 9. The average heterozygosity values were 0.4400 in crossbred Jersey, 0.4346 in crossbred Brown Swiss, 0.4603 in crossbred Holstein Friesian and 0.4469 in pooled population. Among the four loci, maximum heterozygosity was detected at the β-lactoglobulin locus in crossbred Jersey (0.4902) and crossbred Holstein Friesian (0.4968). Whereas in crossbred Brown Swiss κ-casein locus had the maximum heterozygosity (0.4928).

Linkage between the genes controlling synthesis of milk protein

Crossbred Jersey

Combination of different milk protein alleles in Jersey crossbreds was analysed and tabulated in Table 10. As indicated in the table certain milk protein types were significantly associated with each other. αS_1-casein types showed significant association with β-casein types ($P<0.05$). Similarly, κ-casein types were significantly associated with β-lactoglobulin types ($P<0.05$). All the other combinations of milk protein variants exhibited no significant association.

Table 10. Linkage between the genes controlling synthesis of milk proteins in cross-bred Jersey cows

Milk protein types			β-lactoglobulin AA	AB	BB	X^2	κ-Casein AA	AB	BB	X^2	β-Casein AA	AB	BB	X^2
αS_1-Casein	BB	O	4	9	5		9	6	3		9	7	2	
		E	4.32	6.84	6.84	1.2	9.36	6.12	2.52	0.11	10.08	6.84	1.08	1.08
	BC	O	6	9	12		16	8	3		14	12	1	
		E	6.48	10.26	10.26	0.486	14.04	9.18	3.78	0.587	15.12	10.26	1.62	0.62
	CC	O	2	1	2		1	3	1		5	0	0	
		E	1.2	1.9	1.9	0.96	2.6	1.7	0.7	2.11	2.80	1.9	0.3	3.93*
Total	X^2					2.646				2.807				5.63*
β-Casein	AA	O	7	11	10		13	11	4					
		E	6.72	10.64	10.64	0.51	14.56	9.52	3.92	0.399				
	AB	O	4	8	7		11	5	3					
		E	4.56	7.22	7.22	0.237	9.88	6.46	2.66	0.50				
	BB	O	1	0	2		2	1	0					
		E	0.72	1.14	1.14	1.898	1.56	1.02	0.42	0.544				
Total	X^2					2.186				1.443				
κ-Casein	AA	O	3	12	11									
		E	6.24	9.88	9.88	2.26								
	AB	O	6	5	6									
		E	4.08	6.46	6.46	1.266								
	BB	O	3	2	2									
		E	1.68	2.66	2.66	0.908								
Total	X^2					4.434*								

* $P<0.05$

Table 11. Linkage between the genes controlling synthesis of milk proteins in cross-bred Brown Swiss cows

Milk protein types			β-lactoglobulin AA	AB	BB	X^2	κ-Casein AA	AB	BB	X^2	β-Casein AA	AB	BB	X^2
αs_1-Casein	BB	O	2	6	9		4	10	3		14	3	0	
		E	3.02	7.55	6.42	0.34	5.28	8.68	3.02	0.51	11.33	4.91	0.76	2.13
	BC	O	5	10	6		7	11	3		10	9	2	
		E	3.73	9.33	7.93	0.32	6.53	10.73	3.73	0.18	14	6.07	0.93	3.78*
	CC	O	1	4	2		3	2	2		6	1	0	
		E	0.26	3.11	2.64	1.03	2.18	3.58	1.24	1.47	4.66	2.02	0.31	1.13
Total	X^2					1.69				2.16				7.04**
β-Casein	AA	O	4	12	14		9	16	5					
		E	5.33	13.33	11.33	1.09	9.33	15.33	5.33	0.05				
	AB	O	3	3	3		3	7	3					
		E	2.31	5.77	6.42	2.88	4.04	6.64	2.31	0.50				
	BB	O	1	1	0		2	0	0					
		E	0.35	0.88	0.75	1.217	0.62	1.02	0.36	4.45*				
Total	X^2					4.598*				5.01*				
κ-Casein	AA	O	4	6	4									
		E	2.48	6.22	5.29	1.253								
	AB	O	3	10	10									
		E	4.08	10.22	8.66	0.4913								
	BB	O	1	4	3									
		E	1.42	3.56	3.02	0.1785								
Total	X^2					1.923								

* P<0.05 ** P<0.01

Table 12. Linkage between the genes controlling synthesis of milk proteins in cross-bred Holstein Friesian cows

Milk protein types			β-lactoglobulin AA	AB	BB	X^2	κ-Casein AA	AB	BB	X^2	β-Casein AA	AB	BB	X^2
αS_1-Casein	BB	O	4	8	2		7	5	2		9	4	1	
		E	3.5	5.95	4.55	2.21	5.25	7.0	1.75	1.19	7	5.95	1.05	1.21
	BC	O	5	7	11		8	13	2		10	11	2	
		E	5.75	9.78	7.48	1.66	8.67	11.5	2.88	0.51	11.5	9.78	1.73	1.60
	CC	O	1	2	0		0	2	1		1	2	0	
		E	0.75	1.28	0.98	1.46	1.13	3	0.38	2.47	0.16	0.01	0.23	2.0
Total	X^2					6.22*				4.17*				4.81*
β-Casein	AA	O	2	11	7		7	10	3					
		E	5	8.5	6.5	2.54	7.5	10	2.5	0.153				
	AB	O	6	5	6		7	8	2					
		E	4.25	7.23	5.53	1.45	6.38	8.5	2.13	0.097				
	BB	O	2	1	0		1	2	0					
		E	0.75	1.28	0.98	2.71	1.13	1.5	0.375	0.557				
Total	X^2					6.70**				0.787				
κ-Casein	AA	O	4	9	2									
		E	3.75	6.38	4.88	2.79								
	AB	O	5	4	11									
		E	5	8.5	6.5	5.43								
	BB	O	1	4	0									
		E	1.25	2.13	1.625	3.31								
Total	X^2					11.53**								

* P<0.05 ** P<0.01

Crossbred Brown Swiss

Linkage relationship between genes controlling the synthesis of αS_1-casein, β-casein, κ-casein and β-lactoglobulin are tabulated in Table 11. αS_1-casein types were significantly associated with β-casein types as revealed by Chi-square test of independence ($P<0.01$). Similarly β-casein and κ-casein types were found to be significantly associated ($P<0.05$). β-casein types were found associated with β-lactoglobulin types significantly ($P<0.05$).

Crossbred Holstein Friesian

Association of different milk protein genes in Holstein crossbred cows are presented in Table 12. αS_1-casein types were found to be significantly associated with β-lactoglobulin, κ-casein and β-casein ($P<0.05$). Linkage between β-casein protein types and β-lactoglobulin types were also found to be significant ($P<0.01$). κ-casein was also found to be significantly linked with β-lactoglobulin.

Influence of milk protein variants on milk production

The first lactation average standardised milk yield of the three genetic groups viz., crossbred Jersey, Crossbred Brown Swiss and crossbred Holstein Friesian in relation to different αS_1-casein variants are given in Table 13. Effect

Table 13. Association of αS_1-protein types with first lactation yield/305 days

Population	Genotype	Total No.	Mean value (kg)
Cross-bred Jersey	BB	18	1844.819 ± 115.044
	BC	27	1895.268 ± 72.502
	CC	5	2128.620 ± 130.334
Cross-bred Brown Swiss	BB	17	1877.838 ± 73.934
	BC	21	1830.768 ± 105.852
	CC	7	1510.357 ± 114.887
Cross-bred Holstein Friesian	BB	14	1655.925 ± 69.252
	BC	23	1801.133 ± 81.916
	CC	3	1836.816 ± 141.382

Table 14. Influence of β-casein protein types on lactation yield in Kg in different cross-bred population

Population	Genotype	Total No.	Mean value (kg)
Cross-bred Jersey	AA	28	1960.290 ± 89.119
	BB	3	1702.633 ± 255.356
	AB	19	1887.166 ± 67.994
Cross-bred Brown Swiss	AA	30	1825.653 ± 66.675
	BB	2	1642.425 ± 186.760
	AB	13	1733.100 ± 155.424
Cross-bred Holstein Friesian	AA	17	1683.945 ± 69.739
	BB	3	1803.517 ± 192.84
	AB	20	1822.094 ± 92.825

Table 15. Influence of κ-casein protein types on lactation yield in Kg in different cross-bred population

Population	Genotype	Total No.	Mean value (kg)
Cross-bred Jersey	AA	26	1811.659 ± 84.808
	BB	7	2165.140 ± 118.578
	AB	17	1907.876 ± 88.522
Cross-bred Brown Swiss	AA	14	1715.962 ± 85.141
	BB	8	1804.331 ± 179.650
	AB	23	1830.922 ± 97.678
Cross-bred Holstein Friesian	AA	15	1707.871 ± 137.084
	BB	5	1813.95 ± 121.428
	AB	20	1747.188 ± 84.611

Table 16. Influence of β-lactoglobulin types on lactation yield in Kg in different cross-bred population

Population	Genotype	Total No.	Mean value (kg)
Cross-bred Jersey	AA	12	1749.971 ± 81.599
	BB	19	1963.421 ± 101.223
	AB	19	1797.924 ± 103.924
Cross-bred Brown Swiss	AA	8	1869.656 ± 169.078
	BB	17	1753.933 ± 93.353
	AB	20	1622.433 ± 107.824
Cross-bred Holstein Friesian	AA	10	1768.650 ± 97.820
	BB	13	1835.377 ± 136.330
	AB	17	1731.012 ± 84.298

Table 17. Influence of αS_1-protein types on incidence of mastitis in different cross-bred genetic groups

Population	Geno-type	Total	Affe-cted	Percen-tage	X^2
Cross-bred Jersey	BB	18	3	16.66	
	CC	5	1	20	
	BC	27	4	14.81	0.094 NS
Cross-bred Brown Swiss	BB	17	2	11.76	
	CC	7	1	14.28	
	BC	21	4	19.04	0.389 NS
Cross-bred Holstein Friesian	BB	14	3	21.43	
	CC	3	1	33.33	
	BC	23	4	17.39	0.449 NS

NS - Non-significant

Table 18. Influence of β-casein types on incidence of mastitis in different cross-bred genetic groups

Population	Geno-type	Total	Affe-cted	Percen-tage	X^2
Cross-bred Jersey	AA	28	3	10.71	
	BB	3	0		
	AB	19	5	26.30	2.657 NS
Cross-bred Brown Swiss	AA	30	3	10	
	BB	2	1	50	
	AB	13	3	23.0	2.676 NS
Cross-bred Holstein Friesian	AA	20	2		
	BB	3	0		
	AB	17	4		1.891 NS

NS - Non-significant

Table 19. Influence of κ-casein types on incidence of mastitis in different cross-bred genetic groups

Population	Genotype	Total	Affected	Percentage	X^2
Cross-bred Jersey	AA	26	4	15.38	
	BB	7	2	28.57	
	AB	17	3	17.65	0.651 NS
Cross-bred Brown Swiss	AA	14	3	21.42	
	BB	23	3	13.04	
	AB	8	3	37.50	2.245 NS
Cross-bred Holstein Friesian	AA	15	2	13.33	
	BB	20	4	20.00	
	AB	5	1	20.00	0.863 NS

NS - Non-significant

Table 20. Influence of β-lactoglobulin types on incidence of mastitis in different cross-bred genetic groups

Population	Geno-type	Total	Affe-cted	Percen-tage	X^2
Cross-bred Jersey	AA	12	1	8.33	
	BB	19	5	26.31	
	AB	19	1	5.26	2.918 NS
Cross-bred Brown Swiss	AA	8	3	37.65	
	BB	17	3	17.65	
	AB	20	3	15.00	1.068 NS
Cross-bred Holstein Friesian	AA	10	3	30.00	
	BB	13	3	23.08	
	AB	17	4	23.52	0.178 NS

NS - Non-significant

of each variant on milk production was tested by Student's 't' test. All the combinations yielded a non-significant response.

The standardised first lactation milk yield of all the animals under study in relation to different β-casein variants are given in Table 14. Analysis with 't' test revealed non significant effect of casein types on milk yield.

In Table 15 the variants of κ-casein and the average first lactation milk production of cows are furnished. In the 't' test all combinations were found to have no significant effect on milk production.

β-lactoglobulin variants and the average first lactation milk production of cows were given in Table 16. Results of 't' test analysis showed no significant effect.

Influence of milk protein types on incidence of mastitis

The variants of αS_1-casein, β-casein, κ-casein and β-lactoglobulin did not affect the incidence of mastitis (Tables 17, 18, 19 and 20) in any of the populations studied.

DISCUSSION

Caseins

αs_1-Casein

Phenotype and gene frequencies

Present investigation on three different crossbred genetic groups revealed three αs_1-casein phenotypes viz. BB, BC and CC (Table 1). The pooled population revealed a phenotypic frequency of 0.363, 0.526 and 0.111 for BB, BC and CC respectively. With a gene frequency of 0.63 and 0.37 for B and C alleles. The phenotypic frequency for BB was highest in crossbred Jersey (0.36) followed by crossbred Brown Swiss (0.34) and lowest in Holstein Friesian cows (0.28). The frequency of CC phenotype was lowest in Holstein crosses (0.06) and highest in Brown Swiss crosses (0.14). Holstein Friesian crossbreds had a higher frequency of BC type compared to crossbred Brown Swiss but lower than crossbred Jersey. The frequency of B and C alleles were exactly similar in crossbred Jersey and crossbred Holstein Friesian group in the present study. Brown Swiss crossbreds had a higher frequency of C allele.

In the fifth revision of the nomenclature for milk proteins of cow's milk five variants of αs_1-casein has been described viz. A, B, C, D and E in the increasing order of

molecular weight (Eigel et al., 1984). Ng-Kwai-Hang et al. (1990) could observe three alleles, A, B and C at the αS_1-casein locus in a study of 8000 Holstein Friesian cattle. Present study revealed two alleles and three phenotypes. Allele 'A' described by Ng-Kwai-Hang et al. (1990) in Holstein cattle has been reported to be unique for a single blood line originating in the state of Michigan (Aschaffenburg, 1968). Similarly the frequency of A allele was reported to be very rare in Brown Swiss cattle with frequencies ranging from 0 (Kiddy et al., 1964) to 0.06 and 0.03 (Aschaffenburg, 1968). Kiddy et al. (1964) could find only B alleles in crossbreds of Brown Swiss and Holsteins. In Indian breeds the frequency of B allele was very rare (Juneja and Chaudhary, 1973; Jairam and Nair, 1983). Grosclaude et al. (1974) and Thompson et al. (1971) confirmed the predominance of B variant in Bos taurus cattle and C variant in Bos indicus cattle. In the light of above reports it is pertinent to point out that the present study could demonstrate two alleles B and C which fully concurs with the above findings. This view is supported by the findings of Juneja and Chaudhary (1973) who could also find only B and C alleles in Sahiwal x Jersey crosses. The other variants reported by Eigel et al. (1984) apparently appears to be confined to certain breeds or blood lines of cattle.

Frequency of B allele was reported to be 0.59 in Jersey x Sahiwal crosses (Juneja and Chaudhary, 1971) and 0.51 in Brown Swiss x Sahiwal crosses (Jairam and Nair, 1983). Ashaffenburg (1968) reported that Zebu cattle had a frequency of 0.9 for C allele. Juneja and Chaudhary (1973) also reported a frequency of 0.91 of C allele in Sahiwal and Rathi breeds. In Jersey x Sahiwal crosses the frequency of B and C alleles were 0.5. This frequency is an intermediary frequency of the two alleles B and C as found in Bos taurus and Bos indicus respectively. In the present study the frequency of B allele was in the range of 0.61 to 0.63 and the frequency of C allele was between 0.37 and 0.39. As might be expected from the findings of Juneja and Chaudhary (1973) the frequency should have been around 0.5 in the present study also, had the gene frequency of C allele be 0.9 in the local nondescript breed used for synthesis of crossbred Jersey, Holstein or Brown Swiss. The local nondescript cattle used in the production of crossbreds might have had a lesser frequency of C allele. It is also to be seen whether B allele confers any selective advantage over the C allele in the humid tropical conditions of the state among the segregating F_1 crossbreds. The sampling variation as a result of limited number of animals in the present study might also have contributed to the fluctuation of gene frequency towards B allele.

Test for genetic equilibrium

The observed and expected phenotype frequencies among different genetic groups are presented in Table 2. The observed and expected phenotype frequencies at the αS_1-casein locus did not differ significantly indicating that the different population in the present study is in genetic equilibrium at the αS_1-casein locus. This trend is suggestive of a neutral role of αS_1-casein locus in the panmictic population.

β-casein

Phenotype and gene frequencies

Phenotype and gene frequencies of β-casein variants in different genetic groups of cattle are presented in Table 3. There were two variants of β-casein viz. A and B with three phenotypic combinations AA, AB and BB. In pooled population, the phenotypic frequency of AA, AB and BB were 0.5778, 0.3630, 0.0593 respectively. The frequency of AA phenotype was highest in crossbred Brown Swiss (0.67) and lowest in crossbred Holstein Friesian (0.50). Highest frequency of AB phenotype was in Holstein Friesian crossbreds (0.42).

The frequency of A allele was highest in Brown Swiss crossbreds (0.81) and lowest in Holstein Friesian (0.71). Though seven casein variants viz. A1, A2, A3, B, C, D and E

were reported in cattle (Eigel et al., 1984) only A and B could be observed in the present study. The frequency of A allele was reported to be 0.95 in Holstein cattle and in Jersey crossbreds it was found to be between 0.58 and 0.72 (McLean et al., 1984). Jersey x Sahiwal crosses had a frequency of 0.814 for A allele. The frequency of B allele was reported to be very low in Sahiwal, Tharparkar and Red Sindhi (Jairam and Nair, 1983). A and B alleles are the most common alleles reported at this locus in the studies mentioned above. Other alleles reported by Eigel et al. (1984) apparently appear to be rare alleles and a study involving large number of cattle from different places would be required to identify those alleles. The frequency of A allele for Jersey crossbreds reported by McLean et al. (1984) was in agreement with the observations of present study. The frequency of B allele for Jersey crossbreds also concurs with the findings of McLean et al. (1984). In the present study B allele was found to be having a higher frequency compared to the earlier findings in crossbred cattle. The high frequency of B allele in the crossbreds might be due to the higher occurrence of B allele in the local cattle of Kerala. It is to be seen whether B allele may attribute to any selective advantage under the humid tropics.

Test for genetic equilibrium

Chi-square test could not reveal any significant difference between the observed and expected casein pehenotypes (Table 4). The compatibility between expected and observed frequency of the alleles at β-casein locus indicate the genetic stability and genetic equilibrium at this locus. This might be attributable to a neutral role of β-casein allele in the fertility and viability of cattle.

κ-casein

Phenotype and gene frequencies

Phenotype and gene frequencies at κ-casein locus among different genetic groups are presented in Table 5. Three phenotypes AA, BB and AB controlled by two alleles A and B were observed in the present study. A allele was more frequent than B allele in all crossbred groups. The lowest frequency of AA phenotype was observed in crossbred Brown Swiss (0.31) and highest frequency in crossbred Jersey (0.52). The frequency of AB was highest in crossbred Brown Swiss with a frequency of 0.51. Highest frequency of A allele was in crossbred Jersey (0.69) and that of B was in crossbred Brown Swiss (0.44). The observations of Eigel et al. (1984) fully concurs with the present study. Aschaffenburg (1968) reported predominance of A allele in temperate breeds except Jersey

Aschaffenburg (1968) and Jairam and Nair (1983) found predominance of A allele in the few Indian breeds. The present study also fully endorses this view with a higher frequency of A allele. Jairam and Nair (1983) reported that the frequency of B allele is higher in crossbreds compared to Zebu cattle. This observation is supported by the comparatively high frequency of B allele in the present study. Ng-Kwai-Hang (1984) could also find only two alleles A and B at κ-casein locus with respective frequencies of 0.744 and 0.256, though with a smaller frequency of B allele compared to the present study.

Test for genetic equilibrium

Table 6 provides the Chi-square test on the expected and observed phenotype frequencies of κ-casein variants. There was no significant difference between the observed and expected values emphasising genetic equilibrium of the alleles at the locus in all the genetic groups studied. This possibly indicates the neutral role of the alleles at κ-casein locus.

β-lactoglobulin

Phenotype and gene frequency

Three phenotypic classes of β-lactoglobulin viz. AA, BB and AB controlled by two alleles viz. A and B were observed in the present study (Table 2). AA phenotypes were highest in

crossbred Holstein Friesian (0.25) and lowest in crossbred Brown Swiss (0.18). BB phenotype is highest in crossbred Jersey (0.38) and lowest in crossbred Holstein Friesian (0.325). The distribution of heterozygous phenotype AB is highest in Brown Swiss (0.444) followed by crossbred Holstein Friesian (0.425) and lowest in crossbred Jersey (0.38). In all crossbreds, the freqency of B allele was higher compared to that of A allele. The frequency of A allele was highest in crossbred Holstein Friesian (0.46) and lowest in crossbred Brown Swiss (0.40) while crossbred Brown Swiss had the highest frequency of B allele (0.60). Aschaffenburg and Drewry (1955) observed two alleles A and B at β-lactoglobulin locus. Singh and Khanna (1972) observed three alleles A, B and C at the β-lactoglobulin locus in Hariana cattle. Singh and Bhat (1980) found two alleles A and B of β-lactoglobulin locus. They also reported that β-lactoglobulin B is the most common allele among indigenous breeds. β-lactoglobulin A had the highest frequency in crossbred Holstein Friesian. Ng-Kwai-Haung (1984) could also find β-lactoglobulins A and B as the most common alleles with respective frequencies of 0.387 and 0.613. The present observation on the gene and phenotype frequency closely agrees with the above mentioned reports.

Test for genetic equilibrium

A good agreement was obtained between the observed and

expected values of different β-lactoglobulin types in all the genetic groups studied (Table 8). This can be attributed to the genetic stability at this locus. This also indicates the neutral role of β-lactoglobulin alleles in selection.

Heterozygosity

Polymorphism in a population reflects genetic variability. The variation in the population help in the selection and breeding, for it none exists, there would be little scope for selection for further improvement. Rendel (1967) suggested blood groups and protein variants might prove to be very useful tools for estimating variability among the populations.

The genetic variability in the crossbred populations was calculated by estimating the heterozygosity.

Heterozygosity at each loci and average heterozygosity in each population were calculated (Table 9). In all the population except crossbred Brown Swiss, maximum heterozygosity was at the β-lactoglobulin locus. In the crossbred Brown Swiss, maximum heterozygosity was seen at the κ-casein locus.

The overall heterozygosity in different crossbred population indicated that the crossbred Holstein Friesian had

comparatively highest degree of heterozygosity (0.4603) followed by crossbred Jersey (0.4400) and crossbred Brown Swiss (0.4346). It also indicated that average heterozygosity in different crosbreds varied according to the exotic breeds used, though indigenous cattle remained common.

Linkage studies at milk protein loci

The linkage analysis between milk protein loci in crossbred Jersey cattle are given in Table 10. A significant association could be established between αS_1-casein locus and β-casein locus ($P<0.05$). αS_1-casein CC phenotype showed a significant association with β-casein types. κ-casein variants were significantly associated with β-lactoglobulin phenotypes.

Linkage analysis results of milk protein types in crossbred Brown Swiss are furnished in Table 11. It was observed that a significant association existed between αS_1-casein and β-casein locus. Among the αS_1-casein types BC phenotype showed close association with β-casein phenotypes. It could be observed that β-casein and β-lactoglobulin were significantly linked. Linkage between κ-casein protein types were also found significant. Among the casein variants BB phenotype was more linked.

Among crossbred Holstein Friesian group (Table 12) all the milk protein types except β-casein and κ-casein were found to be significantly associated.

The concept of linkage or association of genes controlling the synthesis of milk protein implies the relative distance of different loci situated on the same chromosome and has been clarified by Farrel and Thompson (1971). By analysing segregation data collected in suitable families, Grosclaude et al. (1964) discussed the linkage between αS_1-casein and β-casein loci. Hines et al. (1969) confirmed the evidence of linkage between caseins while studying linkages among cattle blood and milk protein polymorphism. The present study was made as an understanding for possible association of alleles of different protein types and the absence of certain recombination using an indirect method of analysis by preparing two way contingency table (Snedecor and Cochran, 1967) each showing the combination of different alleles in two different protein loci in each breed.

The associations between alleles of αS_1-casein loci and β-casein locus observed in the present study are fully supported by the findings of Grosclaude et al. (1964), Aschaffenburg (1968) and Jairam (1986). This was evident in all the genetic groups studied.

Association between κ-casein and β-lactoglobulin was significant in crossbred Jersey and crossbred Holstein Friesian groups. In crossbred Holstein Friesian group all the milk protein variants except β-casein and κ-casein types were found to be significantly associated.

The population size in the present study was a major and limiting factor for arriving at a valid conclusion with regard to linkage relationship. Before substantiating close associations between milk protein variants it would be worthwhile to undertake extensive study involving large number of animals in all the genetic groups.

Association of milk protein variants with milk yield

In the present study no association could be established between first lactation milk yield/305 days and milk protein variants in all the populations (Tables 13, 14, 15 and 16). The literature on the association between milk protein variants and lactation yield are plenty as against the present observation. Significant association between milk protein variants and lactation yield was reported earlier (Mituko and Ukolov, 1978; Jairam and Nair, 1983; Ng-Kwai-Hang, 1984). Extensive studies involving large number of animals would be required before arriving at meaningful conclusions.

Association of milk protein variants with incidence of mastitis

It can be seen from the Tables 17, 18, 19 and 20 the incidence of mastitis could not be correlated with any of the milk protein variants in the present study. This finding is in agreement with those of Vechiotti *et al*. (1989). However, Jairam (1986) could observe an association between incidence of mastitis and different milk protein variants. In that study Jersey breed of cows with β-lactoglobulin BB type were more resistant to mastitis. In Jersey x Ongole crosses AB type of lactoglobulin also contributed to an increased resistance to mastitis. In the case of Friesian crosses the incidence of mastitis was associated with α-lactalbumin locus. The contribution of different loci to the incidence and pathogenicity of mastitis and its biological significance is yet to be uncovered. It will be worthwhile to note that several recent studies including Vechiotti *et al*. (1989) and Mayer *et al*. (1990) could establish no association between incidence of mastitis and milk protein variants fully concurring with the present study.

Summary

SUMMARY

1. Biochemical polymorphism at four milk protein loci viz. αS_1-casein, β-casein, κ-casein and β-lactoglobulin were studied in 135 crossbred cattle maintained at University Livestock Farm, Mannuthy.

2. Horizontal polyacrylamide gel electrophoretic technique was employed for phenotyping milk proteins based on the zone numbering system.

3. αS_1-casein locus consisted of three phenotypes with two alleles B and C. The BC and BB phenotypes were higher in crossbred Jersey. The B allele had the highest frequency in all the three genetic groups studied viz., crossbred Jersey, crossbred Brown Swiss and crossbred Holstein Friesian with respective frequencies of 0.63, 0.61 and 0.63.

4. Three phenotypes viz. AA, AB and BB determined by two alleles A and B were observed at the β-casein locus. The highest frequency of A allele was in crossbred Brown Swiss (0.81) while the crossbred Jersey and crossbred Holstein Friesian had frequencies of 0.75 and 0.71 respectively. Frequency of AA phenotype was highest in crossbred Brown Swiss (0.67) and AB was highest in crossbred Jersey (0.29).

5. Two alleles A and B and three phenotypes viz. AA, AB and BB were observed at the κ-casein locus in all the genetic groups. Allele A was predominant in all the populations. The frequency of AA phenotype was highest in crossbred Jersey (0.52) and AB genotype in crossbred Brown Swiss (0.51).

6. Two alleles viz. A and B were detected at the β-lactoglobulin locus. The frequency of A allele was highest in crossbred Holstein Friesian (0.46) and that of B allele was highest in crossbred Brown Swiss (0.60). Frequency of AA phenotype was highest in Holstein Friesian crosses (0.25) and that of BB phenotype was highest in crossbred Jersey (0.38) and the frequency of AB highest in crossbred Brown Swiss (0.44).

7. All the population studied were in genetic equilibrium with respect to the αS_1-casein, β-casein and κ-casein and β-lactoglobulin loci.

8. The average heterozygosity was calculated in each genetic group. Maximum heterozygosity was observed in crossbred Holstein Friesian (0.4603). Among the four milk protein loci, the heterozygosity was highest at β-lactoglobulin locus in crossbred Jersey and crossbred Holstein Friesian. In crossbred Brown Swiss maximum heterozygosity was observed at κ-casein locus.

9. Among all genetic groups studied αS_1-casein was found to be highly associated with β-casein locus. In the case of crossbred Jersey αS_1-casein CC phenotype was found to be linked with β-casein locus while in the case of crossbred Brown Swiss αS_1-BC phenotype was associated β-casein types.

10. The milk protein variants were found to be not associated with lactation milk yield or incidence of mastitis in any of the crossbred groups.

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ABSTRACT

The present investigation was undertaken to identify the biochemical polymorphism at different milk protein loci and to establish their inheritance pattern in crossbred cattle. It was also envisaged to analyse the association of different milk protein variants with lactation milk yield and incidence of mastitis. One hundred and thirty five animals belonging to three different crosses of local nondescript cattle viz. crossbred Jersey (50), crossbred Brown Swiss (45) and crossbred Holstein Friesian (40) were typed for milk protein variants by standardising horizontal polyacrylamide gel electrophoresis. The milk protein loci studied were αS_1-casein, β-casein, κ-casein and β-lactoglobulin. Two alleles namely B and C with three phenotypes BB, BC and CC were identified at αS_1-casein locus. B allele had the frequency ranging from 0.61 to 0.63.

β-casein locus exhibited three phenotypes contributed by two alleles A and B. A allele had the highest frequency of 0.81 in crossbred Brown Swiss and it ranged from 0.71 to 0.81 among different crossbreds. Highest frequency of AA phenotype was in crossbred Brown Swiss (0.67) and that of AB phenotype was highest in crossbred Jersey (0.29).

Two alleles namely A and B contributed three phenotypes viz., AA, AB and BB at κ-casein locus among

different crossbreds studied. Crossbred Jersey showed the highest frequency of A allele while crossbred Brown Swiss (0.69) had the highest frequency of B allele (0.44). K-casein AA phenotype had the highest frequency in crossbred Jersey (0.52) and AB phenotype had the highest frequency in crossbred Brown Swiss (0.51).

β-lactoglobulin locus showed two alleles A and B contributing three phenotypes viz., AA, AB and BB. The frequency of A allele was highest in crossbred Holstein Friesian (0.46) and that of B allele in crossbred Brown Swiss (0.60). AA phenotype had the highest frequency in crossbred Holstein Friesian (0.25) while crossbred Jersey had the highest frequency of BB phenotype (0.38) and AB phenotype was highest in crossbred Brown Swiss (0.44).

The observed and expected phenotypes among different genetic groups at all the four milk protein loci viz. αS_1-casein, β-casein, κ-casein and β-lactoglobulin were tested by Chi-square test. All the populations studied were in genetic equilibrium with respect to these four loci. This trend is suggestive of neutral role of the three milk protein loci in the population.

The genetic variability in the crossbred population was calculated by estimating the heterozygosity. In all the population except crossbred Brown-Swiss maximum heterozygosity

was at β-lactoglobulin locus. In the crossbred Brown-Swiss maximum heterozygosity was seen at the κ-casein locus. The overall heterozygosity in different crossbred population indicated that the crossbred Holstein Friesian had comparatively highest degree of heterozygosity (0.4603) followed by Crossbred Jersey (0.4346).

In crossbred Jersey αS_1-casein types showed significant association with β-casein types. κ-casein phenotypes were significantly associated with β-lactoglobulin types. In crossbred Brown Swiss also αS_1-types were found to be linked with β-casein types. β-casein BB phenotype and κ-casein variants were found to be linked. β-lactoglobulin phenotypes and β-casein types were also found associated in crossbred Brown Swiss. In the case of crossbred Holstein Friesian all the milk protein variants except β-casein and κ-casein were found to be associated.

Milk protein variants were not found to be associated with first lactation milk yield or the incidence of mastitis in all the crossbred population studied.

The present study could establish the existence of biochemical polymorphism at αS_1-Casein, β-Casein, κ-Casein and β-lactoglobulin loci in crossbred Jersey, crossbred Brown Swiss and crossbred Holstein Friesian cattle studied.