GENETIC POLYMORPHISM OF MILK PROTEINS IN GOATS

By

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THESIS

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DECLARATION

I hereby declare that the thesis entitled **"GENETIC POLYMORPHISM OF MILK PROTEINS IN GOATS"** 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.

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CERTIFICATE

Certified that this thesis, entitled **"GENETIC POLYMORPHISM OF MILK PROTEINS IN GOATS"** is a record of research work done independently by **Sri. T.V. Raja**, 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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Dedicated to
my beloved sister

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INTRODUCTION

Indian economy is mainly based on agriculture and nearly 80 per cent of the population is engaged directly or indirectly in it. In this sustenance present agro-climatic situation, arising out of the increasing human population is pressing hard on the direct use of land for food production in India and several other countries. So agriculture alone is unable to provide necessary employment and income to the people. Therefore, efforts to improve milk production in the country will provide supplementary source of income to the people and also combat the nutritional deficiencies prevalent among the poorer sections of the society.

Being the second largest contributor to the gross agricultural produce, milk and milk products contribute 3.5 per cent of the national economy. The bulk of milk production in India is in the hands of rural population. Fifty eight per cent of the rural population is constituted by the marginal farmers and landless labourers who cannot afford to maintain crossbred cows and buffaloes because of their high initial investment and maintenance cost. The extremely low purchasing power of the average farmers in the country makes it imperative that these people tend livestock which needs very little capital investment and at the same time can provide a definite and steady income. In this regard the role of goat

farming in upliftment of economy of millions of rural poor is well recognised.

The goat population in India is about 112 million, which is highest in the world. With the inherent characteristics of faster multiplicity together with higher adaptability to even marginal environments and disease tolerance, goats have made themselves acceptable to the rural downtrodden.Goats produce about 2.2 million tonnes of milk which contributes about 4 per cent of the total milk produced in India (Pal and Agnihotri, 1995). Kerala possesses 2.0 million goats with an annual production of 74,000 tonnes of milk, forming about 8.0 per cent of the total milk produced in the state.

The value of goat milk in human nutrition, has so far received very little factual and academic attention in India. Goat milk finds a market due to its superiority in nutritional qualities. It is found suitable even for individuals who are allergic to the protein of cow's milk. In recent years the progressive formulation of various developmental activities in the country has laid great importance on the milk production potentials of goats. The present trend in this regard is to produce crossbreds with exotic breeds like Saanen and Alpine depending on the location and requirements. In Kerala Agricultural University these two breeds were used with

Malabari breed for evolving crossbred goats for increased milk production.

In the field of dairying, studies on milk proteins play a very vital role in understanding the nature of milk and its physicochemical characteristics. The discovery of electrophoretic technique and its introduction of separating the proteins has led to the identification of numerous genetically controlled biochemical variants known as biochemical polymorphism.

Polymorphism in general provides genetic markers. The differences in the genetic polymorphism of milk proteins makes them to be used as a genetic marker to provide materials for studies on the mechanism of gene action and greater understanding of the complex genetic influence in controlling variation in economic traits. Milk protein polymorphisms have been studied in depth since Aschaffenburg's and Drewry's discovery of electrophoretically distinct forms of cattle Beta-lactoglobulin in 1955. Later significant advancement has been made in separating the milk protein variants of different species. Recent studies on the genetic polymorphism of milk proteins as a means for discovering genetically determined differences between various breeds of goats in other countries have opened newer vistas in the research of biochemical genetics of goat milk proteins.

The milk protein can be classified into two classes, the caseins and whey proteins. The caseins consists of Kappa casein and the so called calcium sensitive caseins Alpha S_1, Alpha S_2 and Beta caseins. The major whey proteins are Alpha lactalbumin and Beta lactoglobulin. The goat milk proteins have many significant differences in amino acid composition from the milk of other mammalian species especially in relative proportions of various milk proteins and in their genetic polymorphism. The major protein in cow milk is Alpha S_1 casein but goat milk may differ genetically by having either none (Null type) or much (High type).

The genetic polymorphism of goat milk proteins has great importance for its processing properties. The manufacturing process of the milk product like cheese depends on changes in the state of milk proteins. Therefore much research should be necessarily directed towards the understanding of the nature of milk proteins and their variants.

In view of the above situation the present study was undertaken with the following objectives.

Identification of milk protein variants at Alpha S_1 and Alpha S_2 caseins, Beta casein and Kappa casein loci in Malabari and Alpine x Malabari goats and to study the pattern of inheritance and their association with milk production.

Review of Literature

REVIEW OF LITERATURE

The proteins in goat milk, have been less extensively studied than those in cow milk. However various workers have isolated and characterised the major components of goat milk proteins. Isolation and characterization of the individual proteins of goat milk have followed much the same pattern as for cow's milk. Elucidative approaches made during the past 70 years using the new analytical tools and advanced milk-protein chemistry have helped in disclosing the milk proteins as a heterogenous blend of macromolecular proteins called caseins and soluble proteins called whey proteins.

2.1 General classification of milk proteins

Ganguli (1968) in his review declared the traditional classification of milk proteins into major or main proteins present in higher concentrations and minor proteins present in small quantities. The milk proteins were further classified into colloidal caseins and soluble whey proteins. According to Webb *et al.* (1987) casein may be defined as the protein precipitated by acidifying skim milk to a pH value near 4.6. The proteins remaining after caseins have been removed from the skim milk are known as whey proteins or skim milk proteins. By electrophoresis and by several other techniques the casein was fractionated into Kappa casein and the calcium

sensitive caseins Alpha S_1, Alpha S_2 and Beta casein. Similarly whey proteins were fractionated as alpha lactalbumins comprising major proteins like alpha lactalbumin and beta lactoglobulin and minor proteins such as lactoglobulins consisting of Euglobulin and Pseudoglobulin, serum albumin and proteose peptone fractions.

2.2 Identification of goat milk proteins

2.2.1 Caseins

Isolation of the whole casein fraction of goat's milk protein by the usual acid precipitation was first reported by Tangl (1908).

The free-boundary electrophoretic method when applied in 1939 by Mellander to bovine casein itself, showed that the protein was made up of at least three components, which were designated as Alpha, Beta and Kappa casein in the order of decreasing mobility at pH 7. This observation opened the way to use electrophoresis as a criterion of purity and the actual separation of casein into different fractions soon followed.

Distinct casein fractions in goat's milk were readily recognised by free boundary electrophoresis (Dovey and Campbell, 1952) and paper electrophoresis (Schulte and Muller,

1955; Hofman, 1958a) and these have been designated Alpha and Beta caseins by analogy with cow's milk caseins.

Schulte and Muller (1955) analysed goat, ewe and cow milk proteins by paper electrophoresis. They observed that casein peak was smaller in goats than in cow and ewes milk. The beta casein moved faster in goats and slower in ewes milk than in cow's milk.

Hofman (1958b) was able to separate Alpha and Beta fractions from caprine casein by the method developed by Warner (1944) for bovine casein.

Zittle and Custer (1966) employing the sulphuric acid urea method developed for bovine Kappa casein, isolated a Kappa casein from caprine casein and concluded that in gel electrophoresis it was probably obscured by the beta casein zones.

Parkash and Jenness (1968) in their review described that goat casein was found to lack the distinct gamma casein fraction of cow's casein. With the aid of polyacrylamide gel electrophoresis in 4.5 M urea, they identified the caprine Alpha S casein. They revealed that the caprine Alpha S casein was more heterogeneous in electrophoresis than bovine casein.

2.2.2 Whey proteins

Beta lactoglobulin

Analyses of the proteins of goat's whey by either free boundary or paper electrophoresis demonstrated the presence of a predominant component constituting about 60 per cent of the total whey proteins. This has been identified as being homologous to bovine Beta lactoglobulin and consequently was called caprine Beta lactoglobulin (Parkash and Jenness, 1968).

The first attempt to isolate goat's Beta lactoglobulin in electrophoretically pure form was made by Bain and Deutsch (1948), using successive low temperature ethanol fractionations of goat's milk. The precipitation showed the presence of more than one component at certain pH in free boundary electrophoresis.

2.3 Genetic polymorphism of milk proteins

2.3.1 Caseins

For a long time identification of casein components was one of the main problems, if not the main one, of the milk protein research. It was actually more than 70 years ago that the Danish chemist Linderstrom-Lang (1925) published the first

evidence of casein heterogeneity. From that time, many researchers, using contemporary means and techniques, strove to penetrate casein secrets .

The advent of starch gel electrophoresis by Smithies (1955) and polyacrylamide gel electrophoresis by Peterson (1963) brought about a revolution in the concept of the heterogeneity of casein proteins because of their superb resolving power.

2.3.1.1 Alpha S_1 casein

The Alpha S_1 casein locus in goats is remarkable for its high level of polymorphism and for the fact that clear differences exists in the level of protein synthesized between alleles or group of alleles.

Richardson and Creamer (1975) suggested from an analytical study of caprine casein that there may be no protein corresponding to cattle Alpha S_1 casein.

Ajit Singh and Ganguli (1977) published the electrophoretic studies of goat milk proteins which definitely showed the existence of Alpha S casein and Beta casein fractions by analogy with cow milk casein.

Jenness (1980) in his review reported that goat milk had five principal proteins Alpha lactalbumin, Beta lactoglobulin, Kappa casein, Beta casein and Alpha S_2 casein. However he reported the lacking of caprine Alpha S_1 casein.

Boulanger *et al.* (1984) studied the Alpha S_1 casein polymorphism in Alpine and Saanen crossbreds by using starch gel electrophoresis and identified 3 variants, A, Beta and C controlled by alleles Alpha S_1Cn^A, Alpha S_1Cn^B and Alpha S_1Cn^C respectively.

Grosclaude and Mahe (1986) investigated the polymorphism of caprine Alpha S_1 casein and found that it was controlled by minimum of 5 alleles A, B, C, B^- and O.

Mikkelsen *et al.* (1987) using sodium dodecyl sulphate polyacrylamide gel electrophoresis separated the goat's milk casein into different components and confirmed the existence of Alpha S_1 casein in goat's milk by sequencing the Alpha S_1 casein component.

Grosclaude *et al.* (1987) and Mahe and Groscalude (1989) further investigated the genetic polymorphism of caprine Alpha S_1 casein and established the existence of at least 7 alleles (A, B, C, D, E, F and O) distinguishable on the basis of their electrophoretic mobility together with their relative amount in milk. While alleles Alpha $S_1Cn^{A,B\&C}$ each contributed

approximately 3.6 g Cn/lit of milk (strong alleles)allele E contributed only 1.6 g/lit of milk (intermediate or medium allele), alleles F and D 0.6 g/lit of milk (weak alleles) and allele O appeared to be a null allele.

The biochemistry as well as the molecular basis of caprine Alpha S_1 casein variants A, B, C, D, E and F was actively studied in Jouy-en-Jos-as by Brignon *et al.* (1989, 1990).

Tutta *et al.* (1991) by using an alternative two dimensional polyacrylamide gel electrophoretic method resolved the caprine Alpha S_1 casein into 4 distinct bands and also found that the two dimensional gel pattern allowed a clear distinction both between Alpha S_1 and Alpha S_2 groups and within the components of Alpha S_1 group.

Langley Danysz (1993) confirmed the presence of 7 Alpha S_1 casein variants in caprine (A, B, C, E, F, G and O) and demonstrated the association of O variant of Alpha S_1 casein with their absence in goat milk.

Martin (1993) by studying the genomic data of caprine Alpha S_1 casein demonstrated that the Alpha S_1 casein polymorphism was controlled by at least 14 alleles at Alpha S_1 casein locus distributed into 6 different classes of protein variants divided into 4 levels of expressions.

2.3.1.2 Alpha S_2 casein

Research on caprine Alpha S_2 casein polymorphism started when Bogdanov *et al.* (1972) reported the existence of two Alpha S_2 caseins in electrophoretic examination of casein from goats kept in Belorussia.

Richardson and Creamer (1975) reported for the first time that a component of goat casein was compositionally similar to the minor bovine casein formally called Alpha S_2, Alpha S_3, Alpha S_4, Alpha S_6 casein which was later designated as Alpha S_2 casein.

Boulanger (1976) after a detailed study of heterogeneity of caprine Alpha S_2 casein, stated that the Alpha S_2 casein genetic polymorphism was much more important than Alpha S_1 casein.

The elucidation of the primary structure of Alpha S_2 casein as revealed by Jenness (1980) was of prime importance for better understanding of the casein. According to him the difference between Alpha S_1 casein and Alpha S_2 casein is a disulfide linkage in the former and complete lack of disulfide or thiol in the latter.

The most remarkable observation with heterogeneity of Alpha S_2 casein was made by Boulanger *et al.* (1984) using

starch gel electrophoresis at alkaline pH. The two genetic variants viz. A and B differed slightly in their mobility and occurred singly (A, B) or in pair (AB).

Russo *et al.* (1986) confirmed the presence of Alpha S_2 casein polymorphism in goats. By starch gel electrophoresis and urea at pH 8.6 and 1.7 they analysed the milk samples from 103 Camosciata delle Alpi and 66 Saanen goats. Two Alpha S_2 casein phenotypes AA and AB were observed.

In the year 1987, Mikkelsen *et al.* by using sodium dodecyl sulphate polyacrylamide gel electrophoretic system found that the molecular mass of caprine Alpha S_2 casein was higher than Alpha S_1 components. This finding supported to differentiate and identify the Alpha S_2 caseins from Alpha S_1 caseins.

Chianese *et al.* (1990) while analysing the goat milk casein using polyacrylamide gel electrophoresis at alkaline pH identified a new band migrating ahead of the complex towards the anode. Since the band then obtained presented an isoelectric point intermediate between the Kappa casein and Alpha S_2 casein it was tentatively identified as an Alpha S_2 component. Later Bouniol *et al.* (1994) identified this Alpha S_2 component as the C variant of Caprine Alpha S_2 casein. This was actually obtained by subdividing the Alpha S_2 A allele into two new alleles by using isoelectricfocussing

Tutta *et al.* (1991) by using an alternative 2 dimensional polyacrylamide gel electrophoresis resolved 3 distinct bands of caprine Alpha S_2 casein viz., A, B and C.

Bouniol *et al.* (1993) established the primary structure of caprine Alpha S_2 casein A and Alpha S_2 casein B variants. The main difference was a single amino acid substitution Glu 64/Lys affecting a phosphorylation site.

2.3.1.3 Beta casein

Beta caseins are quantitatively the major protein components of goat milk. The higher concentration of Beta casein than Alpha S casein in whole goat milk was observed by Majumdar and Ganguli (1970).

Genetic variants of Beta casein was first reported by Macha (1970). The four genetic variants viz. A, B, C and D with different electrophoretic mobilities were detected in 194 Czechoslovakian White Polled goats.

Richardson and Creamer (1974) could resolve only two Beta casein variants representing the most prominent electrophoretic bands in goat casein. These had identical amino acid composition and differed only in having five and six phosphate groups respectively. The authors called these components as $Beta_1$ and $Beta_2$ caseins.

Electrophoretic studies by Stasio *et al.* (1983) in 990 goat milk samples revealing monomorphic condition of Beta casein was supported by the absence of polymorphism in Beta casein loci of 103 Camosciata delle Alpi (CA) and 66 Saanen goat (Russo *et al.*, 1986).

The third genetic variant Beta casein O or null Beta casein allele observed in the Criollo breed of Gaudeloupe (Groscalude and **Mahe** , 1986) was confirmed in the Italian Garganica breed by Dall'olio *et al.* (1989).

Sheonarain *et al.* (1992a) typed 179 dairy goats belonging to Barbari and Jamunapari breeds. They had reported two Beta casein variants A and B on the basis of mobility of electrophoretic bands influenced by two codominant genes Beta Cn^{A} and Beta Cn^{B}.

Langly Danysz (1993) could resolve the Beta casein into 3 variants viz. A, B and O. He also observed that the O variant of Beta casein was associated with their absence in goat milk.

Mahe and Grosclaude (1993) made an extensive study on the Beta casein variants in Creole goat of Guadeloupe and reported the existence of 3 variants of Beta casein namely A, B and O. The null allele (0) was found in Alpha S_1Cn, Beta casein haplotype: alpha Alpha S_1Cn B, Beta Cn 0 and

Alpha Alpha S_1-Cn A, Beta Cn O. This suggested the existence of 2 mutations producing a null allele at Beta casein locus.

2.3.1.4 Kappa casein

Methods were developed by Zittle and Custer (1966) to identify Kappa casein among the components of whole goat casein by polyacrylamide gel electrophoresis. Kappa casein was prepared by extraction of whole casein with urea-sulfuric acid and supplemented with ethanol precipitation. Eventhough they were successful in isolating Kappa casein from caprine milk, they could not detect Kappa casein zones in the gel electropherograms. These authors suggested that Kappa casein band was probably obscured by Beta casein zones of goat casein in the electropherogram, since these proteins exhibited similar rate of mobility.

Addeo *et al.* (1978) prepared the whole goat Kappa casein by chromatography and separated it using starch gel electrophoresis. They could identify 5 fractions of whole goat Kappa casein.

Dayhoff (1979) reported the detailed amino acid analysis of caprine Kappa casein. It differed from its bovine counterpart in having chains of 171 instead of 169 amino acid residues.

Russo *et al.* (1986) reported Kappa casein variants in Camosciata delli Alpi goats. They observed the appearance of 2 Kappa casein bands by urea starch gel electrophoresis at alkaline pH.

Di Luccia *et al.* (1990) made an extensive study on the Kappa casein variants of Italian local goats and observed the variation with respect to their electrophoretic mobility and named them as Kappa casein^{A} and Kappa casein^{B} in the order of decreasing mobility.

Chianese *et al.* (1990) reported the occurrence of genetic polymorphism of Kappa casein in the milk of goats reared in Campania.

Law and Tziboula (1993) developed a convenient method for fractionating Caprine casein samples using cation and anion exchange fast protein liquid chromatography. Separation of Kappa casein variants were accomplished with polyacrylamide gel electrophoresis. Two variants viz. A and B were identified which were expressed in a Mendelian way (0.80AA:0.17AB:0.03BB).

Langley Danysz (1993) reporteed the existence of two variants of Kappa casein namely A and B in the milk of caprine species.

2.3.2 Beta lactoglobulin

Sen and Chaudhuri (1962) examined the whey proteins of 75 goats by paper electrophoresis and Bell and Mckenzie (1964) those of 30 Saanens by starch gel electrophoresis. In all cases only a single Beta lactoglobulin band was found.

The preparations from pooled milk used by Phillips and Jenness (1965) and Kalan and Basch (1966) revealed only a single Beta lactoglobulin.

The first report of caprine Beta lactoglobulin polymorphism was reported by Stupnitskii (1967) using agar gel electrophoresis. Kalan *et al.* (1967) also confirmed the heterogeneity of caprine Beta lactoglobulin using DEAE cellulose column chromatography.

Macha (1970) typed 47 Czechoslovakian White Polled dairy goats for Beta lactoglobulin by starch gel electrophoresis. He had reported two Beta lactoglobulin phenotypes designated as AB and BB determined by two alleles namely Beta lactoglobulin A and B of which A had a faster mobility than B towards the anode.

By polyacrylamide gel electrophoresis from the milk of 34 Barbari and 24 Jamunapari goats, Sheonarain *et al.* (1992b) detected that the Beta lactoglobulin was governed by 4 alleles

namely A, B, C and D. They observed six different genotypes viz. AA, AB, AC, AD, BB and CD in Jamunapari goats. Three additional genotypes viz. BC, BD and CC were observed in Barbari goats.

Imafidon *et al.* (1995) detected the existence of two genetic variants of caprine Beta lactoglobulin namely A and B either singly (A or B) or in pairs (AB) in the milk of Sanluis goats.

2.4 Breed distribution and gene frequencies of milk protein variants

2.4.1 Alpha S_1 casein

Stasio *et al.* (1983) phenotyped Alpha S_1 casein for 990 Sardinian goats distributed on 22 farms. They reported the gene frequencies of Alpha S_1Cn^A, Alpha S_1Cn^B and Alpha S_1Cn^C as 0.64, 0.16 and 0.20 respectively.

Boulanger *et al.* (1984) reported 3 genetic variants of Alpha S_1 casein in Saanen and Alpine goats controlled by alleles Alpha S_1-Cn^A, Alpha S_1-Cn^B (with 2 forms differing in synthesis rate) and Alpha S_1^C. The first 2 alleles occurred in all flocks at frequency of 0.08-0.10 and 0.89-0.90 respectively. The allele Alpha S_1-Cn^C occurred only in Saanen with a frequency of 0.03.

Russo *et al.* (1986) reported 4 Alpha S_1 casein phenotypes AA, AB, BB and CC in the Camosciata delle Alpi (CA) and Saanen (SA) breeds of goats by starch gel electrophoresis and urea at pH 8.6. Genotypic frequencies (%) for CA and SA breeds respectively were 8.6 and 12.3 AA, 40 and 26.5 AB, 50 and 59.2 BB and 1.4 and 2.0 CC.

According to Grosclaude and Mahe (1986) there were 5 variants (A, B, C, B^- and O) of the Alpha S_1 series in the order of decreasing electrophoretic mobility. They revealed that the 'O' allele predominated in the French goat population followed by B^-.

Grosclaude *et al.* (1987) reported that in the French Alpine and Saanen dairy breeds, the mutant alleles producing lower amounts of Alpha S_1 casein (Alpha S_1Cn^E and Alpha S_1Cn^F) were largely predominant.

The seventh genetic variant, Alpha S_1Cn^D was observed by Mahe and Grosclaude (1989) in Alpine and Saanen goats. Its frequency in a herd of 198 Alpine goats was 0.025.

In a study on Maltese and Syrian derivative goats, Stasio *et al.* (1990) reported that the gene frequency of Alpha S_1 $casein^A$ was higher than the Alpha S_1 $casein^B$.

The gene frequencies of caprine Alpha S_1 casein variants were estimated in Spanish breeds by Jordana *et al.* (1991) using sodium dodecyl sulphate polyacrylamide gel electrophoresis. They reported the gene frequencies for Murciana Granadina, Malaguena, Payoya and Canaria breeds as follows.

Breed	Number	Allele frequencies					
		A	B	C	E	F	D+O
Murciana Granadina	109	0.08	0.23	-	0.59	0.08	0.02
Malaguena	373	0.09	0.09	-	0.65	0.04	0.13
Payoya	111	0.05	0.19	-	0.76	-	-
Canaria	74	0.28	0.32	-	0.20	-	0.20

The result showed that the E allele (intermediate content of Alpha S_1Cn in milk) was predominant in peninsular dairy breeds, while A and B alleles (high content of Alpha S_1 casein) were more frequent in Canaria breed. The low frequency of F allele (reduced level of Alpha S_1 casein) in Spanish breeds was contrast with the predominance of this allele in Alpine and Saanen breeds.

Stasio *et al.* (1993) observed Alpha S_1 casein polymorphism in Somali Arab goats and revealed that the

frequency of CASA IA, IB, IC, IE, IF and IO alleles were 0.55, 0.22, 0.02, 0.06, 0.13 and 0.02 respectively. They concluded that the Alpha S_1Cn locus in Somali Arab goats was similar to that in the other breeds of Mediterranean origin.

Grosclaude *et al.* (1994) in their review discussed the prevalence of the high, medium and low alleles in the European dairy breeds particularly in the French and Italian Alpine and Saanen breeds and in the Corsican breeds. They reported that in Alpine and Saanen breeds the high alleles were almost absent in goats which have low index or protein percentage, but were in the majority of Alpine breed (0.72) and were frequent in Saanen breed (0.42).

Ricordeau *et al.* (1995) reported that the B allele of Alpha S_1Cn was very rare in Alpine and Saanen breeds and C allele of Alpha S_1Cn was absent in Saanen. The frequency of Alpha S_1Cn^A in Alpine and Saanen breeds was 0.74 and 0.69 respectively.

2.4.2 Alpha S_2 casein

Boulanger *et al.* (1984) described a polymorphism of Alpha S_2 Cn with the two alleles Alpha S_2Cn^A and Alpha S_2Cn^B, the first one being predominant in the Alpine (0.85) and Saanen (0.87) breeds.

Russo *et al.* (1986) observed two Alpha S_2 casein phenotypes in Camosciata delle Alpi (CA) and Saanen (SA) goats, controlled by two codominant alleles viz. Alpha S_2Cn A and Alpha S_2Cn B. The genotypic frequencies (%) for CA and SA breeds respectively were 46.6 and 50.9 AA and 53.4 and 49.01 AB.

Bouniol *et al.* (1994) also studied the Alpha S_1Cn variants in Alpine and Saanen breeds. They reported 3 Alpha S_2Cn variants namely A, B and C with frequencies of 0.85, 0.04 and 0.11 respectively.

2.4.3 Beta casein

Macha (1970) studied Beta casein polymorphism in 194 Czechoslovakian White Polled goats from different herds. He observed Beta casein types AA, AB, AC, AD, BB, BC, BD and DD with the frequency of 0.34, 0.18, 0.22, 0.03, 0.17, 0.05, 0.005 and 0.005 respectively.

By polyacrylamide gel electrophoresis from the milk of 81 Barbari and 98 Jamunapari goats, Sheonarain *et al.* (1992a) observed that the gene frequencies of Beta Cn^A and Beta Cn^B were 0.53 and 0.47 in Barbari and 0.47 and 0.53 in Jamunapari respectively.

Mahe and Grosclaude (1993) studied the Beta casein polymorphism in the Creole goats of Guadeloupe and found 2 new alleles of Beta casein viz. Beta Cn^{B} with a frequency of 0.03 and Beta Cn^{0}, a null allele with a frequency of 0.20, in addition to the common alleles.

2.4.4 Kappa casein

Law and Tziboula (1993) examined the Kappa casein variants by polyacrylamide gel electrophoresis in British Saanen goats. They found 3 phenotypes viz. AA, AB and BB which were explained by the presence of 2 Kappa casein allles viz. Kappa Cn^{A} and Kappa Cn^{B}. The genotypic frequency of AA, AB and BB were 0.80, 0.17, 0.03 respectively.

2.4.5 Beta lactoglobulin

Macha (1970) identified individual variants in the Beta lactoglobulin system by starch gel electrophoresis in Czechoslovakian White Polled goats. They found 2 phenotypes AB and BB with the frequency of 0.62 and 0.38 respectively.

Sheonarain (1992b) reported that the caprine Beta lactoglobulin variants were governed by 4 allleles Beta lactoglobulin^{A}, Beta lactoglobulin^{B}, Beta lactoglobulin^{C} and Beta lactoglobulin^{D}. Six different genotypes AA, AB, AC, AD, BB and CD were identified in both the breeds with genotype

frequencies as 8.82, 29.41, 14.70, 5.88, 5.88 and 8.82 per cent in Barbari and 4.17, 33.33, 29.17, 25.00, 4.17 and 4.17 per cent in Jamumapari goats respectively. Three additional genotypes observed in Barbari goats were BC, BD and CC with the gene frequencies of 5.88, 17.65 and 2.95 respectively. Fast moving Beta lactoglobulin^{A} allele was higher (0.48) in Jamunapari than in Barbari (0.34) whereas the remaining 3 alleles Beta lactoglobulin^{B}, Beta lactoglobulin^{C} and Beta lactoglobulin^{D} were lesser in gene frequencies in Jamunapari than in Barbari.

Barillet *et al.* (1993) reported genetic variants at the Beta lactoglobulin locus in French Lacaune breed. The gene frequencies of Beta lactoglobulin^{A} and Beta lactoglobulin^{B}, were 0.63 and 0.37 respectively.

2.5 Genetic relationship between the genes controlling synthesis of milk proteins

One of the most attractive aspects of milk protein research is the existence of a very close linkage between the milk protein loci, a situation that was first discovered and analysed by Grosclaude *et al.* (1964) in cattle. Information on linkage studies of milk proteins in goats is very very limited.

The existence of close linkage relationship between Alpha S_1 and Alpha S_2 casein proteins of Alpine and Saanen breeds was first demonstrated by Grosclaude *et al.* (1987).

2.6 Association of milk protein variants with milk yield in goats

Many earlier studies have indicated close associations between genetic polymorphism of milk proteins with milk production, milk composition and technological properties.

Macha (1981) studied the genetic polymorphism of Beta casein in the milk of Czechoslovakian White Polled goats and Fawn Polled goats and correlated the variants with the performance. It was observed that goats with Beta Cn BD type produced highest milk yield (1603 kg) and the lowest was for those with the DD type (938 kg).

Remeuf (1989) phenotyped Alpha S_1 casein of 13 Alpine and Saanen breeds of goats. It was found that goats with the A, B or C allele of Alpha S_1 casein had significantly lower milk yield (576 Vs 776 kg).

On the Other hand study of Manfredi *et al.* (1993) to compare the milk yield of goats with different genotypes of Alpha S_1 casein locus showed that goats bearing strong alleles (A, B & C) had produced higher milk yield.

Langley Danysz (1993) also demonstrated the effect of Alpha S_1 casein variants on milk yield and composition. He confirmed that A variant of Alpha S_1 casein was associated with high milk yield.

Barillet *et al.* (1993) analysed the genetic polymorphism of Beta lactoglobulin in the French Lacaune breed and found that there was no relationship between Beta lactoglobulin phenotype and milk yield.

Grosclaude *et al.* (1994) in his review also declared that the polymorphism of casein variants did not affect the milk yield of goats.

Barbieri *et al.* (1995) examined the effect of Alpha S_1 casein locus on dairy performance of Alpine goats. They observed that the Alpha S_1 Cn AA types produced significantly lesser milk yield than AE, AF, EE and EF goats.

Material and Methods

MATERIALS AND METHODS

The main objective of the study was to identify the milk protein variants at Alpha S_1, Alpha S_2, Beta casein and Kappa casein loci in Malabari and its exotic cross viz. Alpine x Malabari. The facilities available at Centre for Advanced Studies in Animal Genetics and Breeding were utilized for the laboratory work. The goats maintained at University Sheep and Goat Farm formed the material for the study.

3.1 Experimental animals

The present study was carried out on 100 dairy goats belonging to Malabari and Alpine x Malabari crossbreds stationed at the University Sheep and Goat Farm, Kerala Agricultural University, Mannuthy (Fig.1A and 1B). Out of these 100 goats 50 belonged to Malabari and 50 Alpine x Malabari.

3.2 Collection of milk samples for the electrophoretic studies of milk proteins

Individual milk samples of 15 ml each were collected in 20 ml clean sterile test tubes, directly from the healthy teats of goats after cleaning the udder and the milk samples of the two teats were mixed together.

Fig. 1.A. Malabari

Fig. 1.B. Alpine x Malabari

3.3 Preparation of milk samples for electrophoretic studies

Fractionation of casein

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

Immediately after bringing the samples to the laboratory the supernatent portion of milk sample was poured out to remove the fat layer on the top. The remaining portion was taken in clean centrifuge tubes (10 ml polythene tubes) and they were centrifuged at 3000 rpm for 15 minutes. The tubes were then kept in test tube stand and stored in refrigerator to solidify the fat on the top of the skim milk, during the hot day time. The skim milk under the fat layer was aspirated with a long hypodermic needle and transferred to centrifuge tubes. Care was taken to avoid the mixing of fat with the skim milk. Sufficient quantity of 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 or five times with distilled water, maintaining the pH at 4.6-4.8.

3.4 Separation of genetic variants of caseins

The variants of casein were separated by horizontal polyacrylamide gel electrophoresis (Medrano and Sharrow, 1989). The genetic variants were identified by their electrophoretic mobility as per the nomenclature of major and minor milk proteins of goat's milk as described by Boulangar *et al.* (1984) and Addeo *et al.* (1988).

3.5 Electrophoresis for milk proteins

Polyacrylamide gel is a three dimensional molecular network. It is made from acrylamide which is a monomer and which undergoes both polymerisation and cross linking through N, N'-methylene-bis-acrylamide commonly called as bis. The gel is formed by mixing acrylamide and bis in buffer solution and adding a catalyst accelerator ammonium persulphate N,N,N',N'-Tetramethyl ethylene-diamine (TEMED).

3.5.1 Buffers and solutions

3.5.1.1 Electrode buffer

Tris	-	12 gm
Glycine	-	57.6 g
Distilled water	-	2.0 lit
pH	-	8.3

3.5.1.2 Acrylamide stock solution (A)

Acrylamide	-	32 g
N',N' Methylene Bis acrylamide	-	0.8 g

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

3.5.1.3 Gel buffer stock solution (B)

Tris	-	9 gm
Citric acid	-	0.9 g
Crystalline urea	-	24.24 g
2 Mercapto ethanol	-	0.06 ml
Distilled water	-	100 ml

3.5.1.4 Ammonium per sulphate (C)

Hundred milligrams of ammonium per sulphate was dissolved in 100 ml distilled water. Freshly prepared solution was used.

3.5.1.5 Working gel solution

Composition for 8 per cent 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

3.5.1.6 Staining solution

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

3.5.1.7 Destaining solution

Methanol	-	2,500 ml
Glacial acetic acid	-	500 ml
Distilled water	-	2,500 ml

3.5.1.8 Preserving solution

Acetic acid	-	70 ml
Distilled water	-	930 ml

3.5.2 Procedure

3.5.2.1 Preparation of gel

A discontinuous buffer system of 8 per cent acrylamide gel (3.5.1.5) 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 the gel. The acrylic sheet was having 1.5 mm high frame on

the three sides which formed the thickness of the gel. One of the closed sides of the acrylic sheet, opposite to the free side (without the frame) was having projections to form the wells 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.

3.5.2.2 Preparation of casein for electrophoresis

The casein was dissolved in 6 M urea solution (36.04 gm of urea dissolved in 100 ml distilled water) for the application of the samples for electrophoresis.

3.5.2.3 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. Bromophenol 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. During the run the temperature was kept constant at 5°C.

3.5.2.4 Staining, destaining and preservation

On completion of the run, 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 for 12 hrs. Then it was preserved in the preserving solution. The genetic variants were identified by their relative mobility on the gel.

3.5.3 Whey proteins

Whey protein variants were separated by the method of Sheonarain *et al.* (1992b) with slight modification. Seven molar urea solution was added to the supernatant left after the casein precipitation to dissolve the whey proteins. Twenty microlitres of the sample was charged into the wells.

Twelve percent acrylamide gel without urea was used for the whey proteins.

3.6 Milk recording

Milk recording was done both in the morning and evening at weekly intervals starting from the first week of lactation to a period of 120 days. These recordings were used to estimate the lactational yield (120 days).

3.7 Analysis of data

3.7.1 Gene frequencies

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

3.7.2 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).

3.7.3 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

$JK = X_i^2$ is the homozygous at K^{th} locus

and

$X_i = \frac{n_i}{n}$, denotes the gene frequency of i^{th} allele at K^{th} locus

$$\hat{H} = \sum_{K=1}^{r} \frac{hK}{r},$$

where r is the number of loci examined.

3.7.4 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).

3.7.5 Effect of milk protein variants on milk production

The estimated lactational yield (120 days) was used to analyse the association of milk protein variants with milk production. Students 't' test (Snedecor and Cochran, 1967) was used as a statistical method for analysis.

Results

RESULTS

One hundred dairy goats comprising of 50 Malabari and 50 Alpine x Malabari Crossbreds were typed for the variants of Alpha S_1 Casein, Alpha S_2 Casein, Beta Casein, Kappa Casein and the whey protein Beta lactoglobulin by employing horizontal polyacrylamide gel electrophoresis.

4.1 Alpha S_1 Casein

The results of the electrophoretic study revealed the presence of Alpha S_1 Casein in the milk samples of dairy goats. Alpha S_1 Casein had faster mobility in the gel than Alpha S_2 and Kappa Casein. The electrophoretic mobility of Alpha S_1 Casein revealed that in goat it resolves into distinct zones on polyacrylamide gel electrophoresis. On the basis of increasing rate of electrophoretic mobility towards the anode the bands were designated as F and S respectively. The different phenotypes of Alpha S_1 Casein were classified by taking into account the presence or absence of any one or more bands. Accordingly three Alpha S_1 phenotypes viz. FF, FS and SS were observed (Figures 2A & 2B).

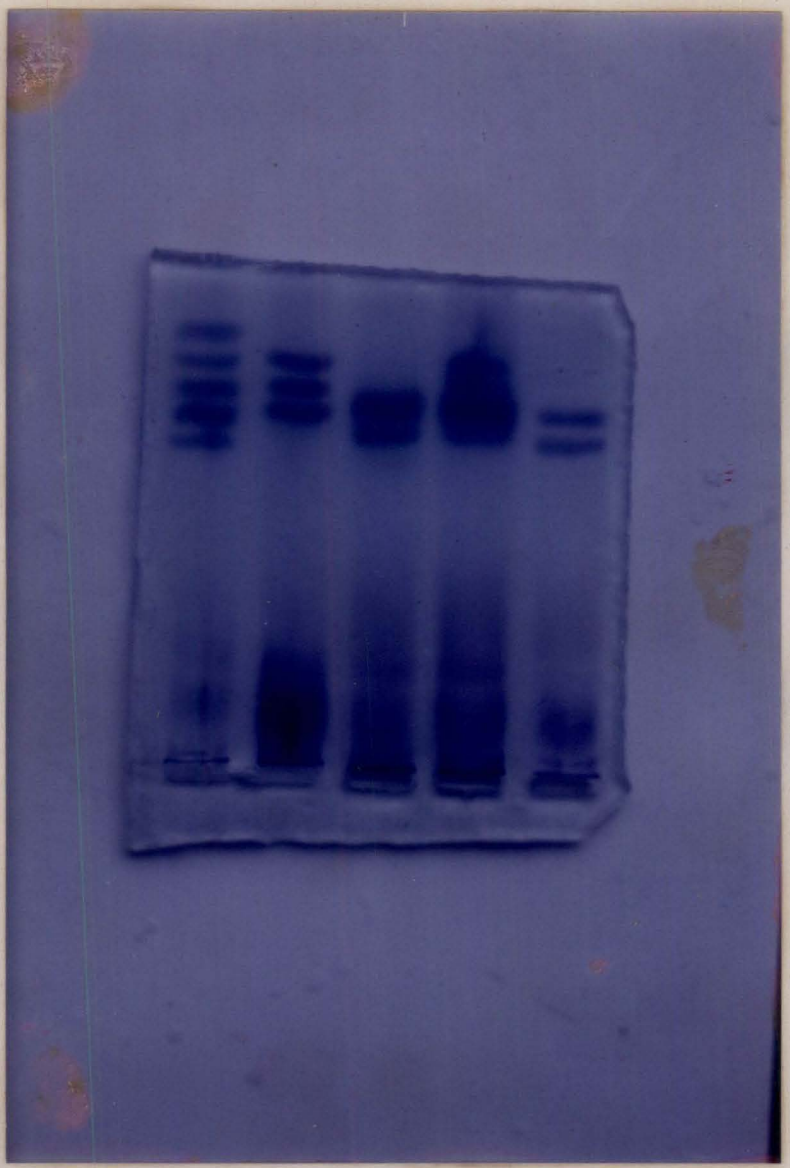


Fig. 2.A. Phenotypes of Alpha S_1 Casein

FS FF SS FS SS

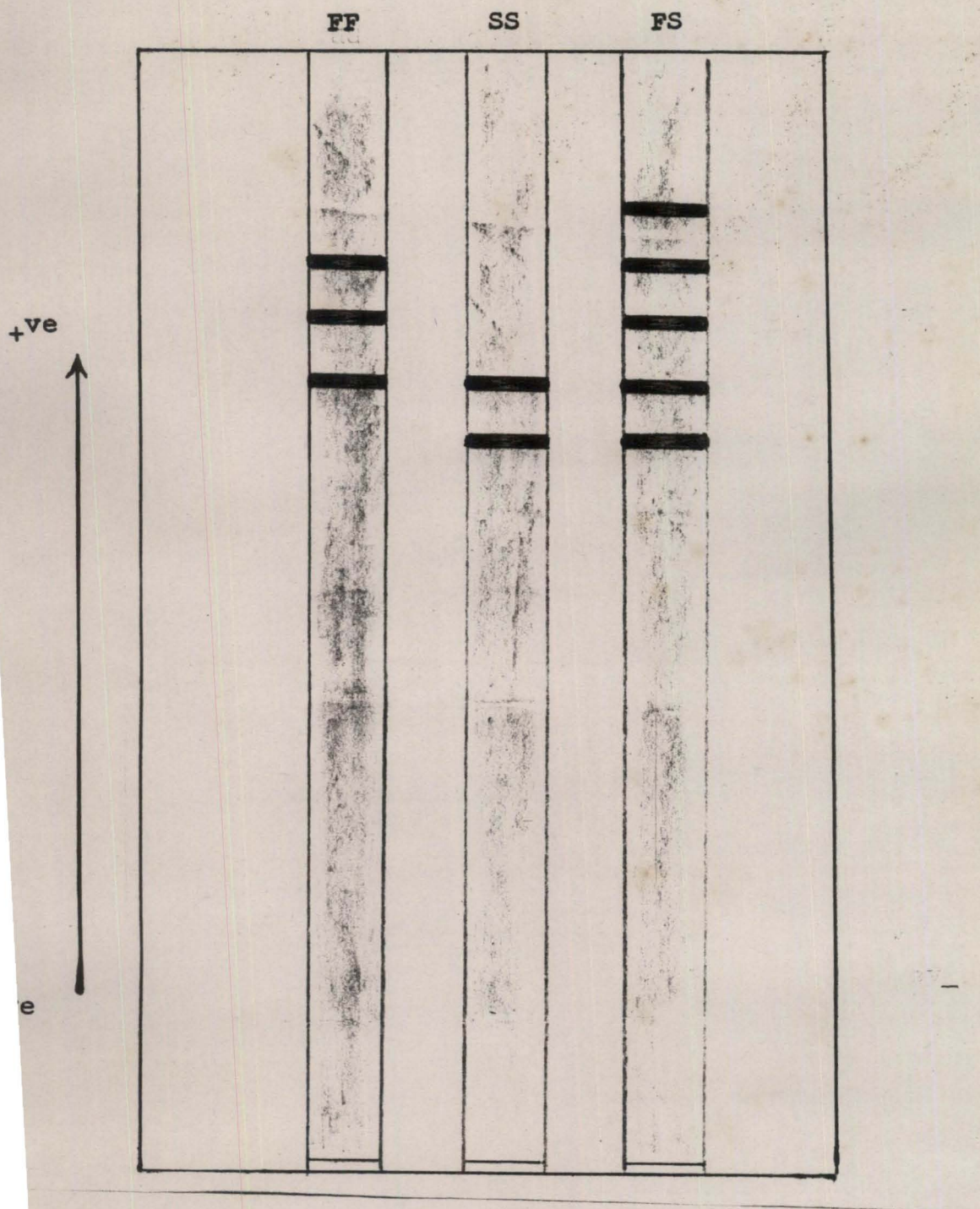


Fig. 2.B. Diagrammatic representation of Alpha S_1 Casein phenotypes

4.1.1 Phenotype/genotype and gene frequencies

The phenotypic frequencies of various Alpha S_1 variants in different populations are presented in Table 1. The frequency of FF phenotype was 0.20 and 0.12 in the Malabari and Alpine x Malabari Crossbreds respectively. The frequency of FS was 0.56 in Malabari and 0.36 in Alpine x Malabari Crossbreds. The frequency of SS phenotype was 0.24 and 0.52 in the Malabari and Alpine x Malabari respectively. The percentage occurrence of various genotypes and gene frequencies are given in Figures 2C and 2D respectively.

In Table 1, are also presented the gene frequencies which were calculated by direct counting method. The frequency of Alpha S_1 casein F was found to be 0.48 and 0.30 in Malabari and Alpine x Malabari goats respectively. The frequency of Alpha S_1Cn S gene was found to be 0.52 and 0.70 in Malabari and Alpine x Malabari crossbreds respectively. In both the genetic groups the S allele was found to be predominant.

4.1.2 Test for genetic equilibrium

The results of the test for genetic equilibrium at the Alpha S_1 casein locus are furnished in Table 2. Both Malabari and Alpine x Malabari populations were found to be in genetic equilibrium with respect to Alpha S_1 casein locus. The observed number of animals with different Alpha S_1 casein

Table 1. Phenotype frequencies and gene frequencies of Alpha S_1 casein types in Malabari and Alpine x Malabari goats

Population	Sample size	Alpha S_1 phenotype frequencies			Alpha S_1 gene frequencies	
		FF	FS	SS	F	S
Malabari	50	0.20 (10)	0.56 (28)	0.24 (12)	0.48	0.52
Alpine x Malabari	50	0.12 (6)	0.36 (18)	0.52 (26)	0.30	0.70

Number in parenthesis indicates number of animals

Table 2. Observed and expected number of goats with different Alpha S_1 casein types

Population		Alpha S_1 phenotype			X^2 value (df=1)
		FF	FS	SS	
Malabari	Obs.	10.0	28.0	12.0	0.742 NS
	Exp.	11.52	24.96	13.52	
Alpine x Malabari	Obs.	6.0	18.0	26.0	1.02 NS
	Exp.	4.5	21.0	24.5	

NS - Non significant
Obs. - Observed
Exp. - Expected

Fig.2.C DIAGRAMMATIC REPRESENTATION OF GENOTYPE FREQUENCIES IN MALABARI AND ALPINE x MALABARI (αS_1 CASEIN)

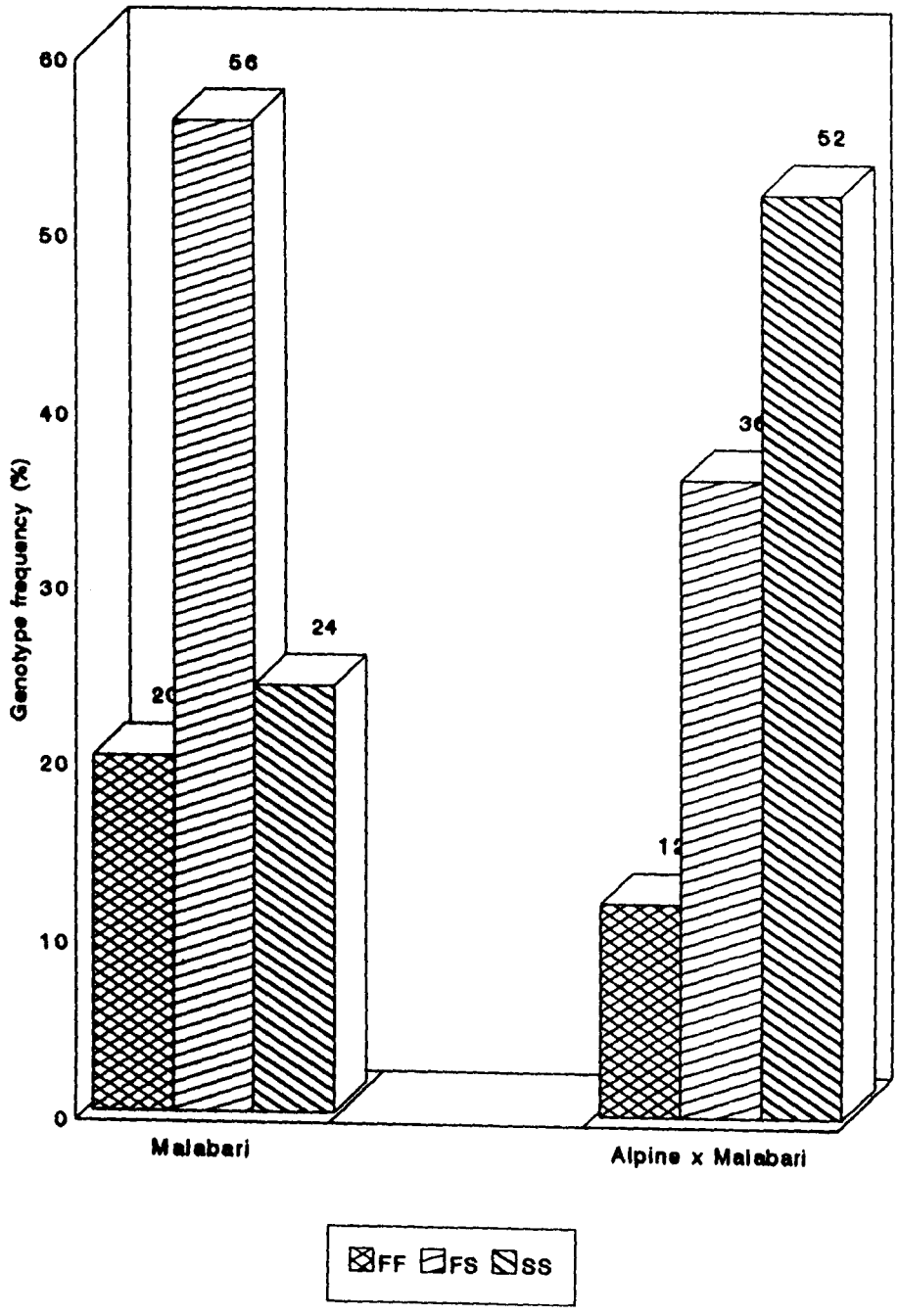
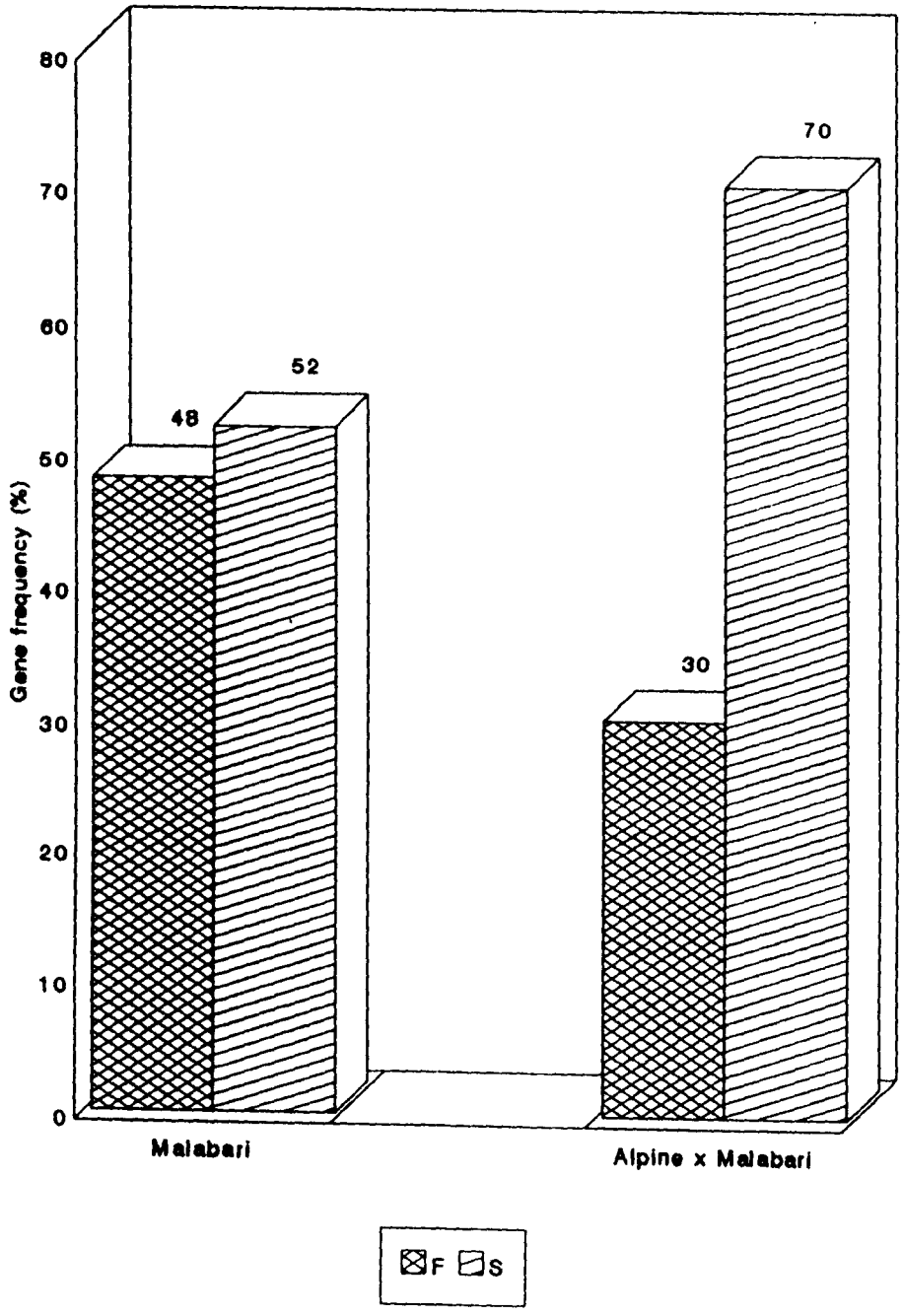


Fig.2.D DIAGRAMMATIC REPRESENTATION OF GENE FREQUENCIES IN MALABARI AND ALPINE x MALABARI (κS_1 CASEIN)



variants did not differ significantly from the expected number in the genetic groups.

4.2 Alpha S_2 Casein

Alpha S_2 casein had an electrical mobility between Kappa casein and Alpha S_1 casein types. Three Alpha S_2 casein phenotypes viz. Alpha S_2CnAA, Alpha S_2CnAB and Alpha S_2CnBB were observed in both the genetic groups studied (Figures 3A and 3B). The Alpha S_2CnAA was faster in mobility while the Alpha S_2CnBB type had slower mobility towards anode. The Alpha S_2CnAB had one component of Alpha S_2 and one of Alpha S_2Cn^B

4.2.1 Phenotypes and gene frequencies

Phenotypes and gene frequencies of Alpha S_2Cn types in different populations are presented in Table 3. The frequency of Alpha S_2CnAA phenotype was higher in Alpine x Malabari Crossbred (0.52) and lower in Malabari (0.40). Comparatively, higher frequency of Alpha S_2CnAB was observed in Malabari (0.44). The frequency of Alpha S_2CnBB phenotype was found to be 0.16 and 0.10 in Malabari and Alpine x Malabari Crossbreds respectively. The diagrammatic representation of the genotype and gene frequencies of Alpha S_2Cn types in different populations are shown in Figure 3C and Figure 3D respectively.

The gene frequency of Alpha S_2Cn^A was found to be more in Alpine x Malabari crossbreds (0.71) and in Malabari it was

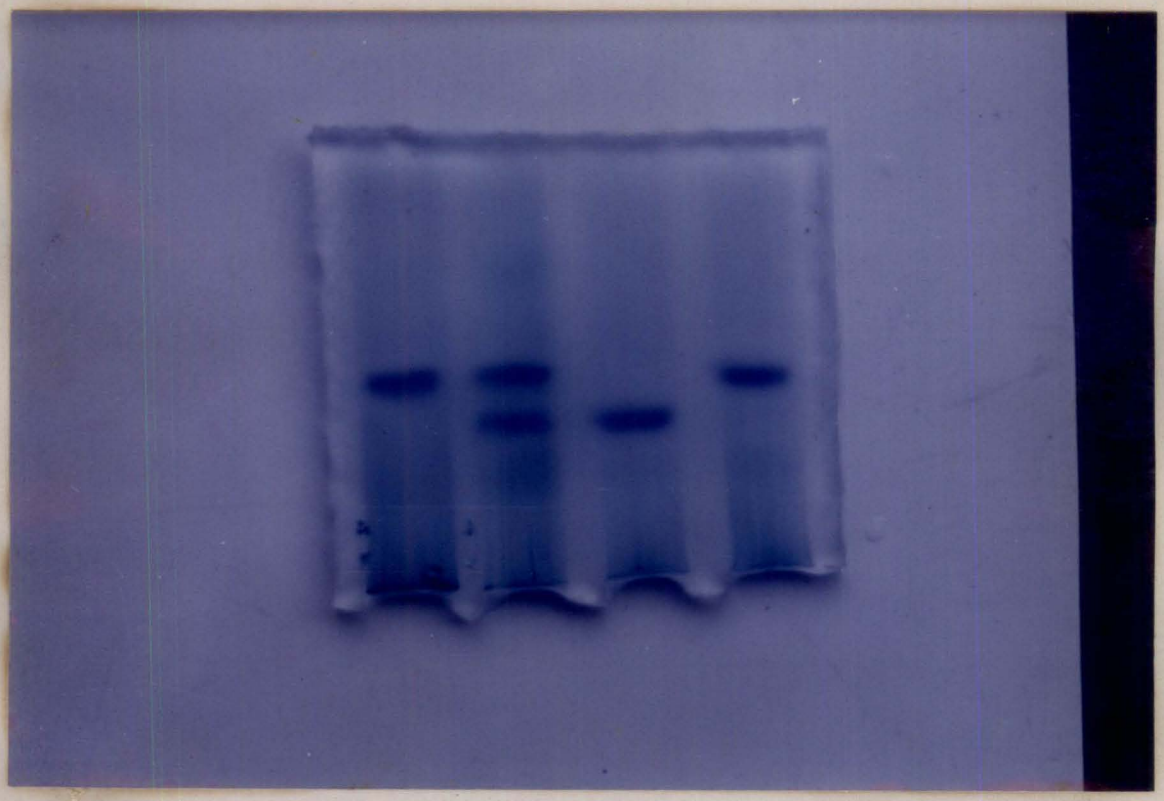


Fig. 3.A. Phenotypes of Alpha S_2 Casein

AA AB BB AA

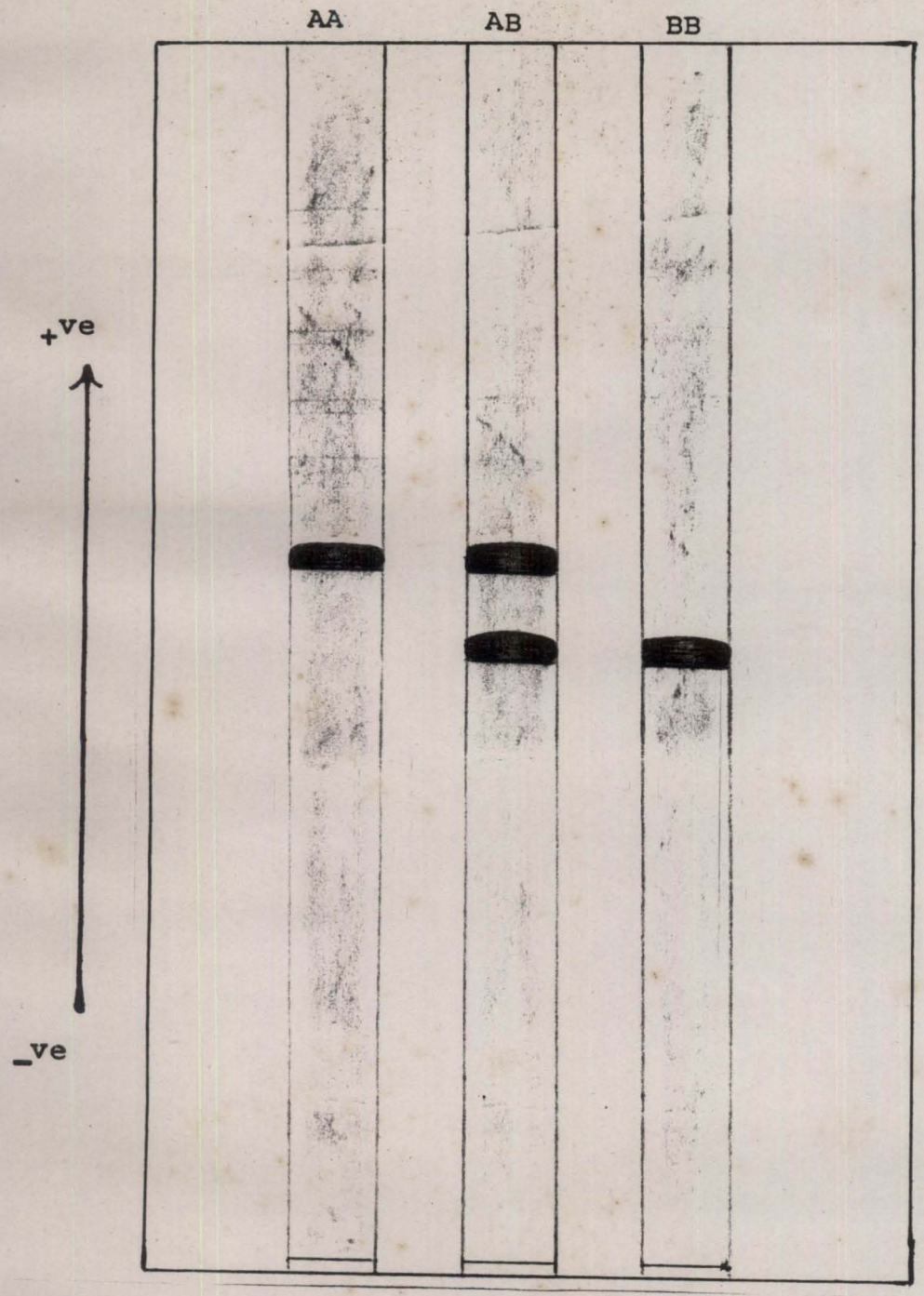


Fig. 3.B. Diagrammatic representation of Alpha S_2 Casein phenotypes

Table 3. Phenotype frequencies and gene frequencies of Alpha S_2 casein types in Malabari and Alpine x Malabari goats

Population	Sample size	Alpha S_2 phenotype frequencies			Alpha S_2 gene frequencies	
		AA	AB	BB	A	B
Malabari	50	0.40 (20)	0.44 (22)	0.16 (8)	0.62	0.38
Alpine x Malabari	50	0.52 (26)	0.38 (19)	0.10 (5)	0.71	0.29

Number in parenthesis indicates number of animals

Table 4. Observed and expected number of goats with different Alpha S_2 casein types

Population		Alpha S_2 phenotype			X^2 value (df=1)
		AA	AB	BB	
Malabari	Obs.	20.00	22.00	8.00	0.22 NS
	Exp.	19.22	23.56	7.22	
Alpine x Malabari	Obs.	26.00	19.00	5.00	0.30 NS
	Exp.	25.21	20.59	4.20	

NS - Non significant
Obs. - Observed
Exp. - Expected

Fig.3.C DIAGRAMMATIC REPRESENTATION OF GENOTYPE FREQUENCIES IN MALABARI AND ALPINE x MALABARI (αS_2 CASEIN)

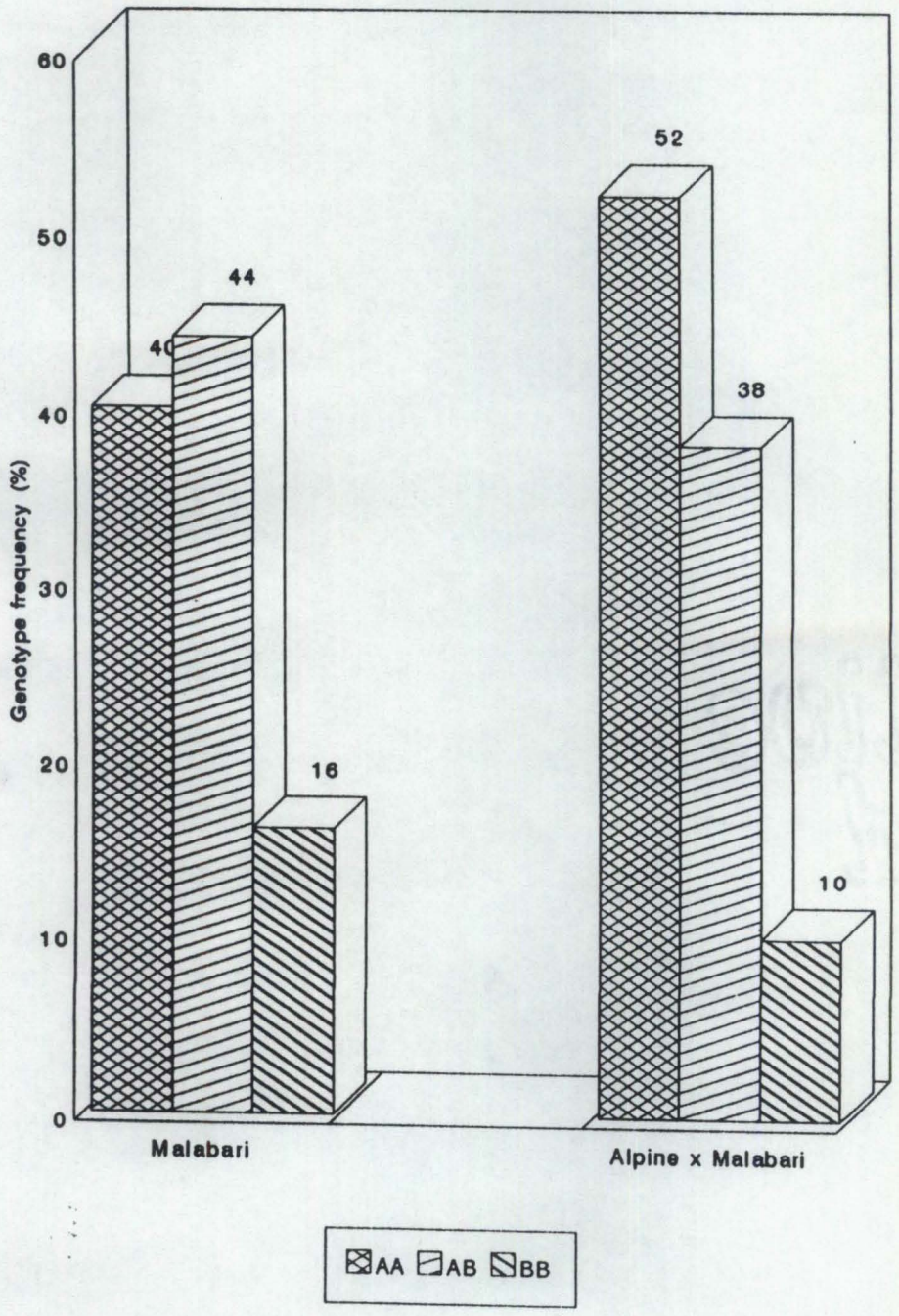
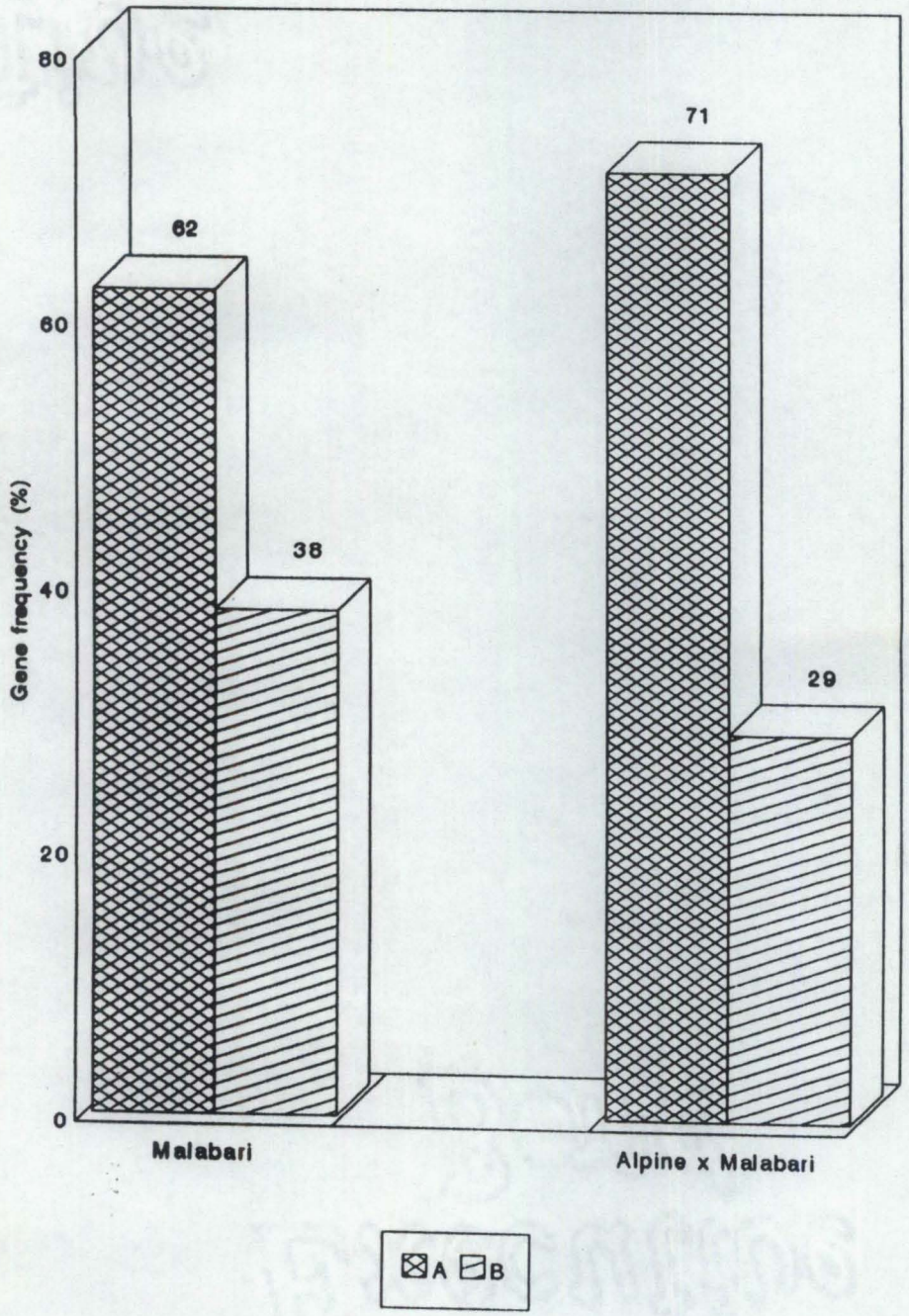


Fig.3.D DIAGRAMMATIC REPRESENTATION OF GENE FREQUENCIES IN MALABARI AND ALPINE x MALABARI (αS_2CASEIN)



0.62. The gene frequency of Alpha S_2Cn^B in Malabari and Alpine x Malabari Crossbreds was 0.38 and 0.29 respectively (Table 3).

4.2.2 Test for genetic equilibrium

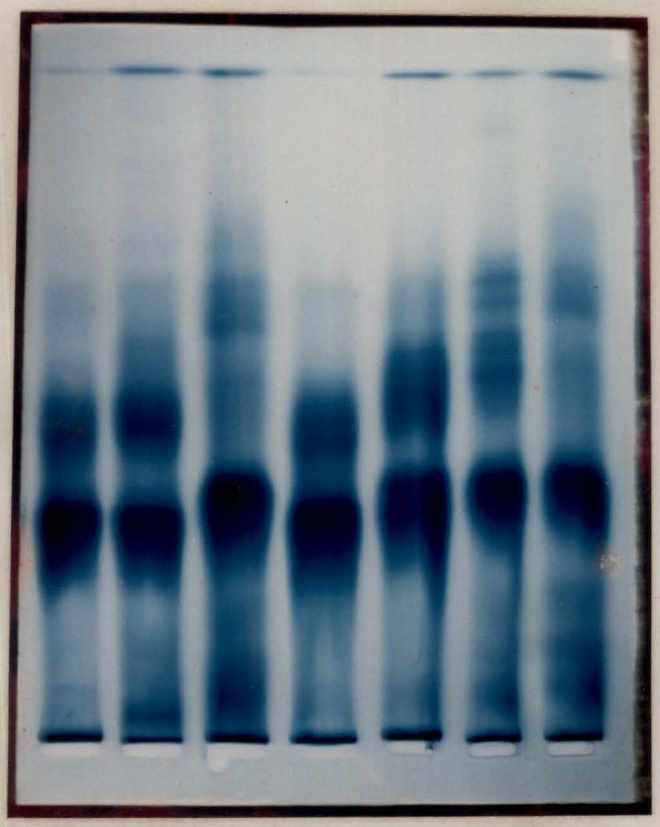
Table 4 shows the observed number of animals with different Alpha S_2 casein types and their expected numbers assuming Hardy-Weinberg equilibrium. A good agreement was obtained between the observed and expected Alpha S_2Cn phenotypes in both Malabari and Alpine x Malabari populations.

4.3 Beta Casein

Beta casein, the slowest moving caprine casein type had three genetic variants viz. AA, AB and BB of which AA variant had faster mobility than BB type (Figures 4A & 4B).

4.3.1 Phenotype and gene frequencies

Table 5 shows the phenotypic frequencies and gene frequencies of the alleles at Beta casein locus. The frequency of Beta casein AA phenotype was 0.18 in Malabari, 0.34 in Alpine x Malabari crossbreds. The frequency of AB phenotype was almost the same in both populations (0.54 in Malabari and 0.52 in Alpine x Malabari). The frequency of Beta Casein BB was found to be higher in Malabari (0.28) and the lower frequency was observed in Alpine x Malabari milk samples (0.14). The percentage wise distribution of genotype and gene frequencies



Beta casein

Fig. 4.A. Phenotypes of Beta Casein.
BB BB AB BB AB AA AA

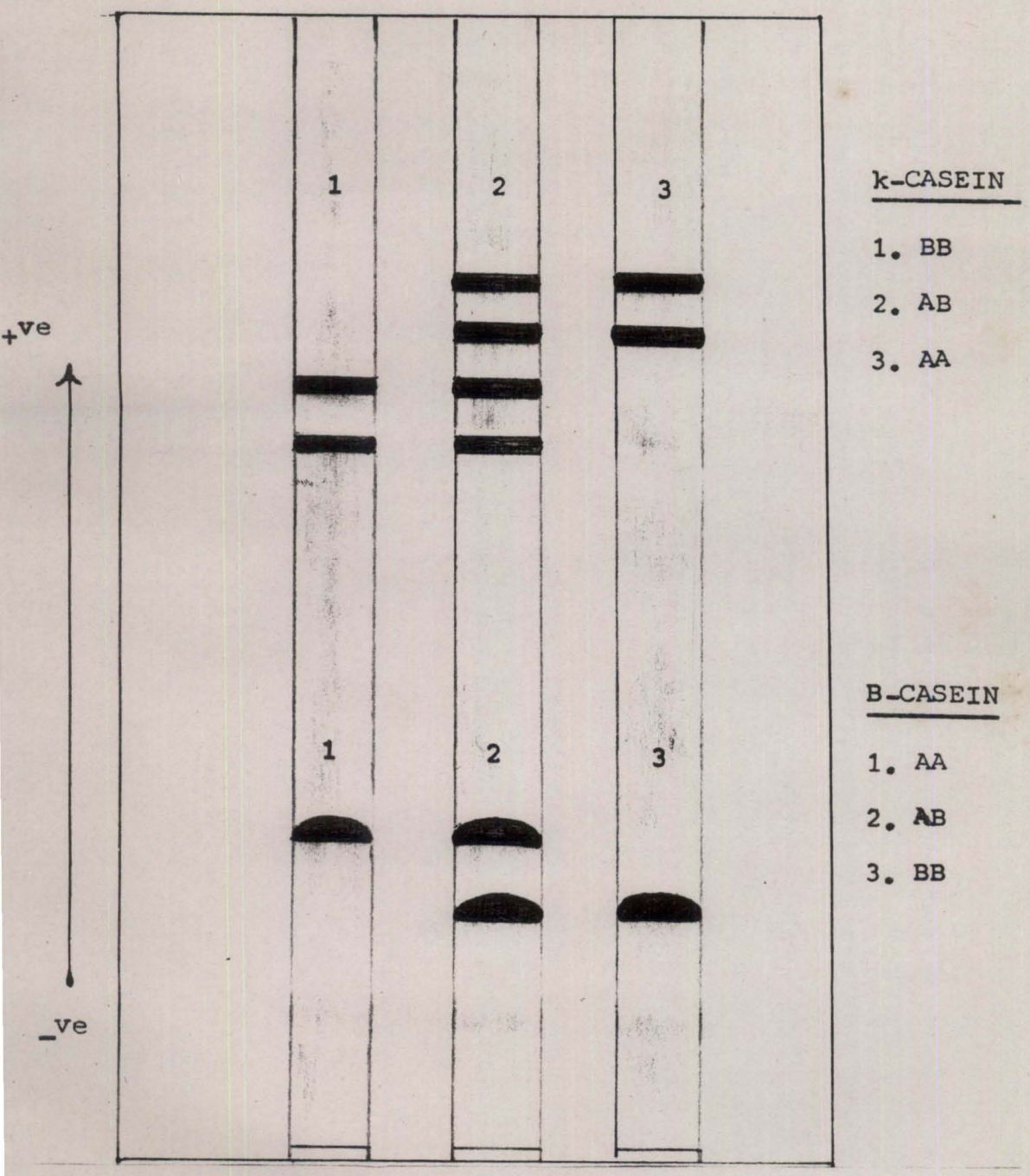


Fig. 4.B. Diagrammatic representation of Beta casein phenotypes.

Table 5. Phenotype frequencies and gene frequencies of Beta casein types in Malabari and Alpine x Malabari goats

Population	Sample size	Beta Casein phenotype frequencies			Beta Casein gene frequencies	
		AA	AB	BB	A	B
Malabari	50	0.18 (9)	0.54 (27)	0.28 (14)	0.45	0.55
Alpine x Malabari	50	0.34 (17)	0.52 (26)	0.14 (7)	0.60	0.40

Number in parenthesis indicates number of animals

Table 6. Observed and expected number of goats with different Beta casein types

Population		Beta Casein phenotype			X^2 value (df=1)
		AA	AB	BB	
Malabari	Obs.	9.00	27.00	14.00	0.41 NS
	Exp.	10.13	24.75	15.13	
Alpine x Malabari	Obs.	17.00	26.00	7.00	0.097 NS
	Exp.	18.00	24.00	8.00	

NS - Non significant
Obs. - Observed
Exp. - Expected

Fig.4.C DIAGRAMMATIC REPRESENTATION OF GENOTYPE FREQUENCIES IN MALABARI AND ALPINE x MALABARI (β CASEIN)

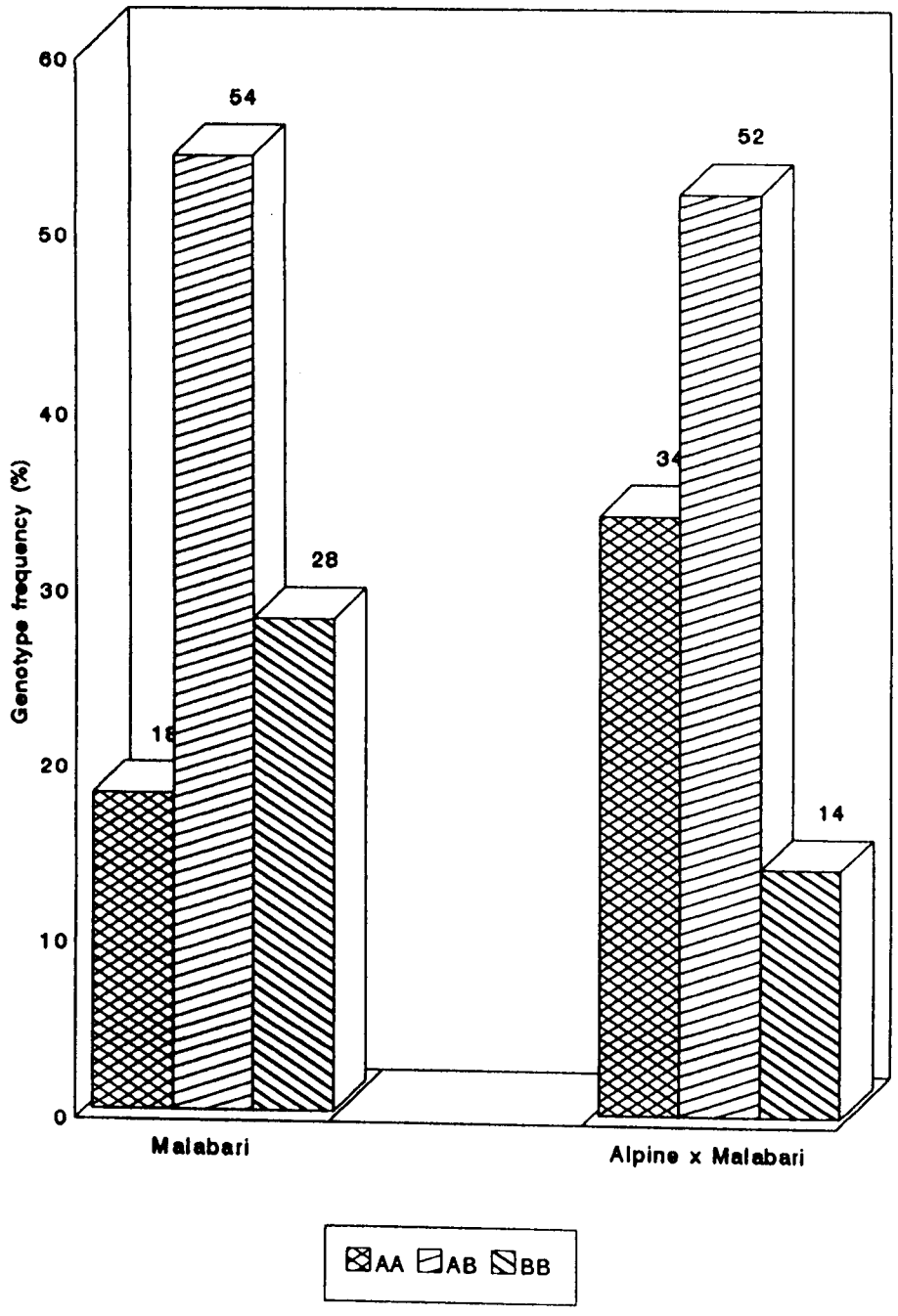
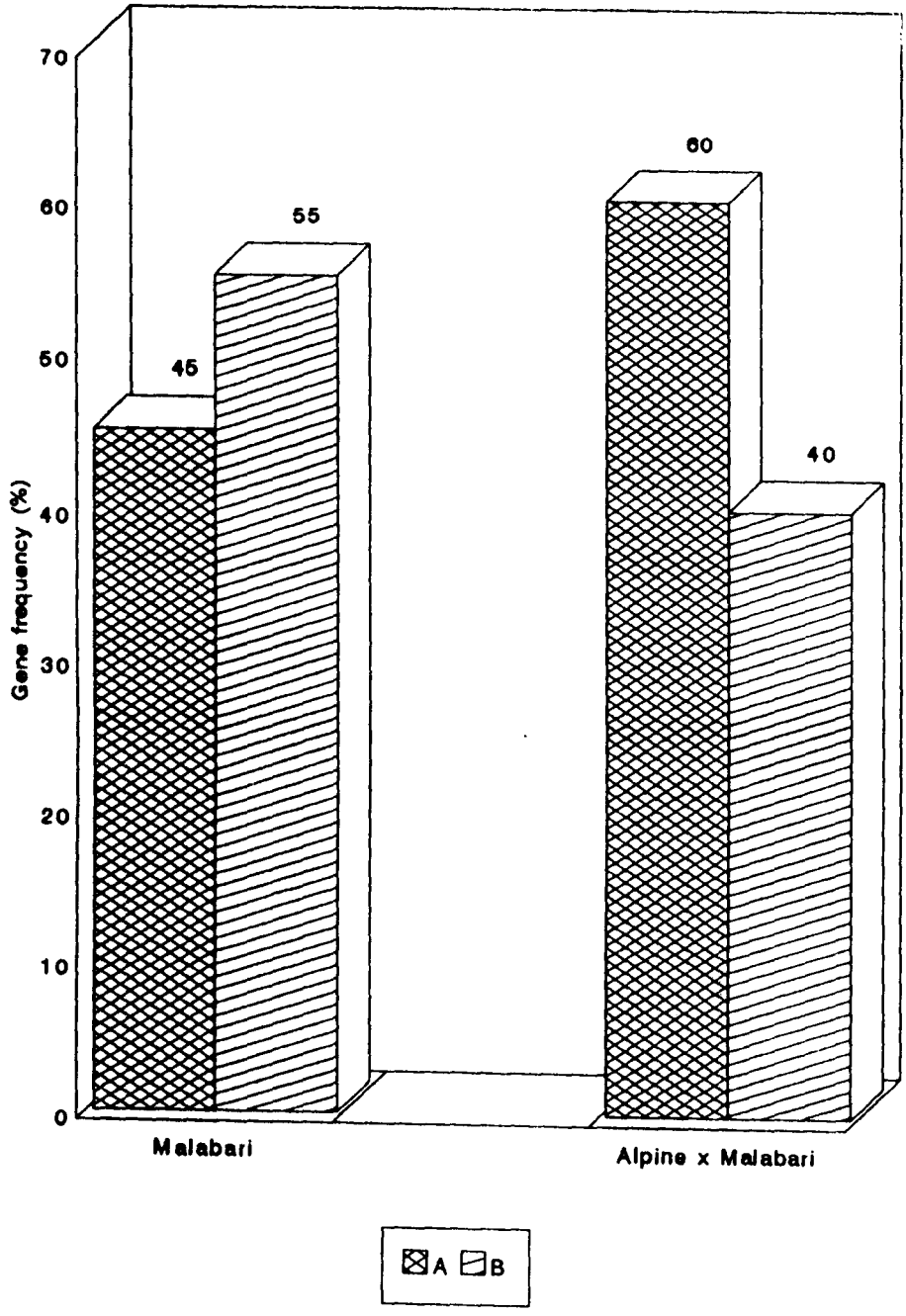


Fig.4.D DIAGRAMMATIC REPRESENTATION OF GENE FREQUENCIES IN MALABARI AND ALPINE x MALABARI (β CASEIN)



of Beta Casein variants are shown in Figures 4C and 4D respectively.

The gene frequency of Beta Casein A was found to be 0.45 and 0.60 in Malabari and Alpine x Malabari Crossbreds respectively. The gene frequency of B allele was 0.55 and 0.40 in Malabari and Alpine x Malabari Crossbreds respectively (Table 5).

4.3.2 Test for genetic equilibrium

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

4.4 Kappa Casein

Kappa casein had an electrical mobility between the Alpha S_2 casein and Beta casein types. Three Kappa casein phenotypes viz. AA, AB and BB were observed in the present study (Figure 5A & 5B). The Kappa casein component having the fastest anodic mobility was designated as KCn^A while the slowest component was called as KCn^B.

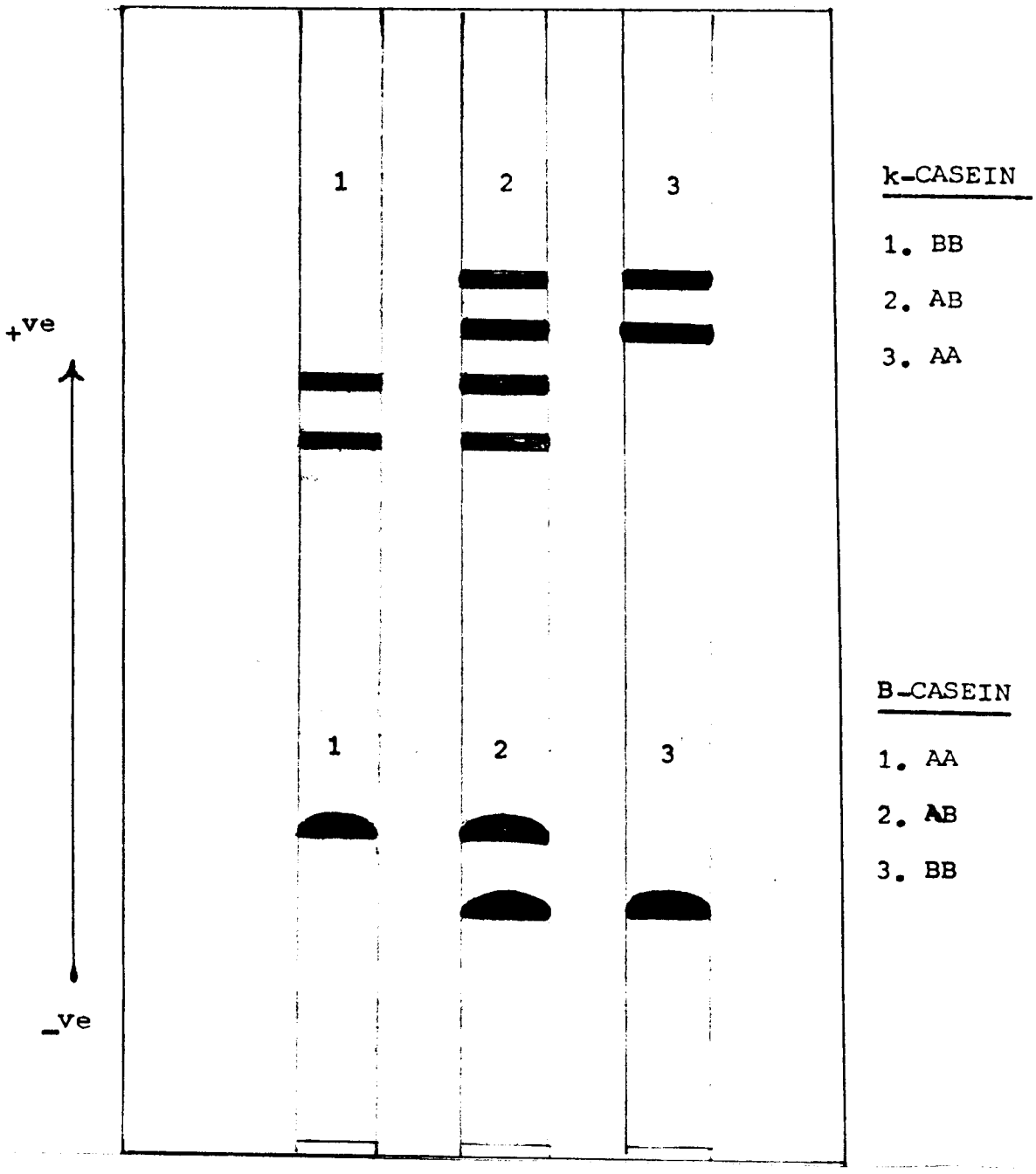


Fig. 5.B. Diagrammatic representation of Kappa Casein Phenotypes.

4.4.1 Phenotype and gene frequencies

Table 7 presents the phenotypic frequencies and gene frequencies of Kappa Cn types observed in the two different populations. The frequency of Kappa CnAA was higher in Alpine x Malabari crossbreds (0.56) compared to Malabari (0.44). The frequency of AB phenotype was 0.48 and 0.40 is the Malabari and Alpine x Malabari crossbreds respectively. The frequency of BB was less in both the genetic groups. It was 0.08 and 0.04 in Malabari and Alpine x Malabari crossbreds respectively. The percentage occurrence of various genotypes and gene frequencies of Kappa casein are furnished in Figures 5C and 5D respectively.

In both the genetic groups the A allele was predominant and the frequency was 0.68 and 0.76 in Malabari and Alpine x Malabari crossbreds respectively. The gene frequency of Kappa casein B was 0.32 in Malabari and 0.24 in Alpine x Malabari crossbreds.

4.4.2 Test for genetic equilibrium

In Table 8 the observed and expected phenotypic frequencies of Kappa casein in two different genetic groups are given. It was found that both the populations were in genetic equilibrium with respect to Kappa casein locus. The observed and expected values did not differ much both for homozygotes

Table 7. Phenotype frequencies and gene frequencies of Kappa casein types in Malabari and Alpine x Malabari goats

Population	Sample size	Kappa Casein phenotype frequencies			Kappa Casein gene frequencies	
		AA	AB	BB	A	B
Malabari	50	0.44 (22)	0.48 (24)	0.08 (4)	0.68	0.32
Alpine x Malabari	50	0.56 (28)	0.40 (20)	0.04 (2)	0.76	0.24

Number in parenthesis indicates number of animals

Table 8. Observed and expected number of goats with different Kappa casein types

Population		Kappa casein phenotype			X^2 value (df=1)
		AA	AB	BB	
Malabari	Obs.	22.00	24.00	4.00	0.53 NS
	Exp.	23.12	21.76	5.12	
Alpine x Malabari	Obs.	28.00	20.00	2.00	0.47 NS
	Exp.	28.88	18.24	2.88	

NS - Non significant
Obs. - Observed
Exp. - Expected

Fig.5.c DIAGRAMMATIC REPRESENTATION OF GENOTYPE FREQUENCIES IN MALABARI AND ALPINE x MALABARI (κ CASEIN)

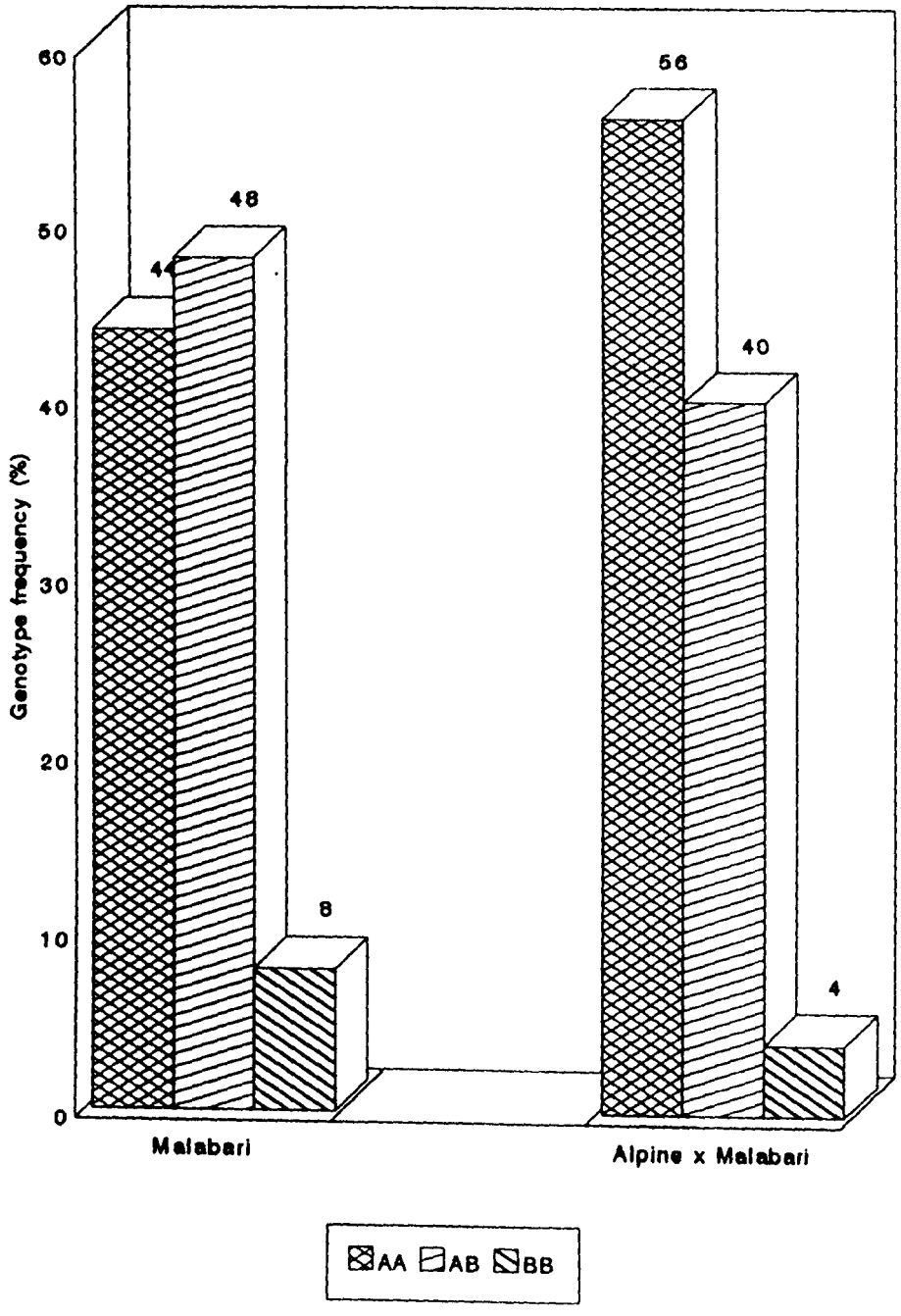
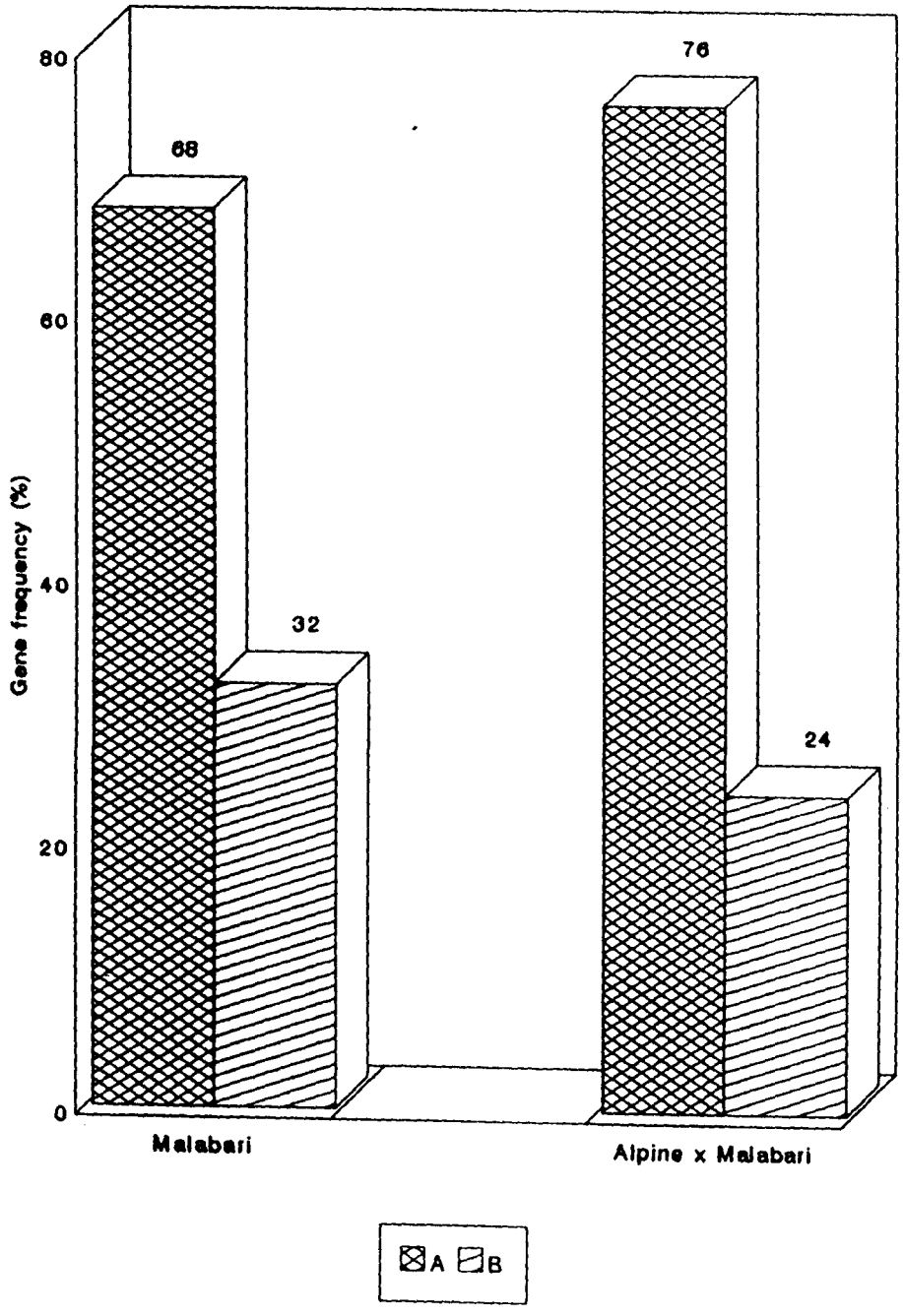


Fig.5.D DIAGRAMMATIC REPRESENTATION OF GENE FREQUENCIES IN MALABARI AND ALPINE x MALABARI (κ CASEIN)



and heterozygotes in both the populations and the differences were statistically non-significant.

4.5 Beta lactoglobulin

The electrophoretic study of Beta-lactoglobulin revealed three phenotypes in the milk of dairy goats. They were identified as Beta lactoglobulin AA, AB and BB phenotypes controlled by two alleles of Beta lactoglobulin A and B (Figure 6A). Diagrammatic representation of the Beta lactoglobulin phenotypes are given in Figure 6B.

4.5.1 Phenotype and gene frequencies

The frequency of different Beta lactoglobulin phenotypes have been presented in Table 9. Higher frequency of Beta Lg AA type was obtained in Alpine x Malabari (0.50). In Malabari the frequency of Beta Lg AA was 0.44. The frequency of Phenotype BB was 0.14 and 0.12 in Malabari and Alpine x Malabari crossbreds respectively. Diagrammatic presentation of various genotypes and gene frequencies of Beta lactoglobulin types are shown in Figures 6C and 6D.

The gene frequency of Beta lactoglobulin^{A} was almost same in both populations (0.65 in Malabari and 0.69 in Alpine x Malabari Crossbreds). The frequency of Beta lactoglobulin^{B} allele was 0.35 in Malabari and 0.31 in Alpine x Malabari.

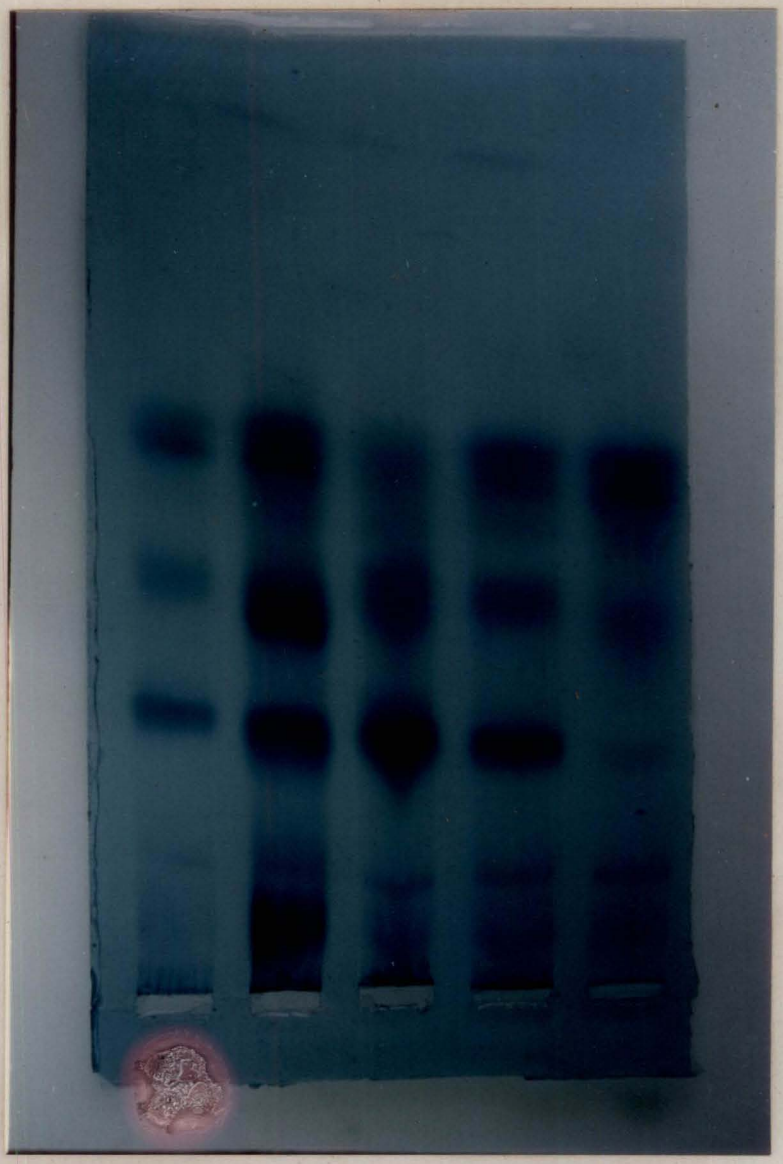


Fig. 6.A. Phenotypes of Beta Lactoglobulin.

AA BB AB AA BB

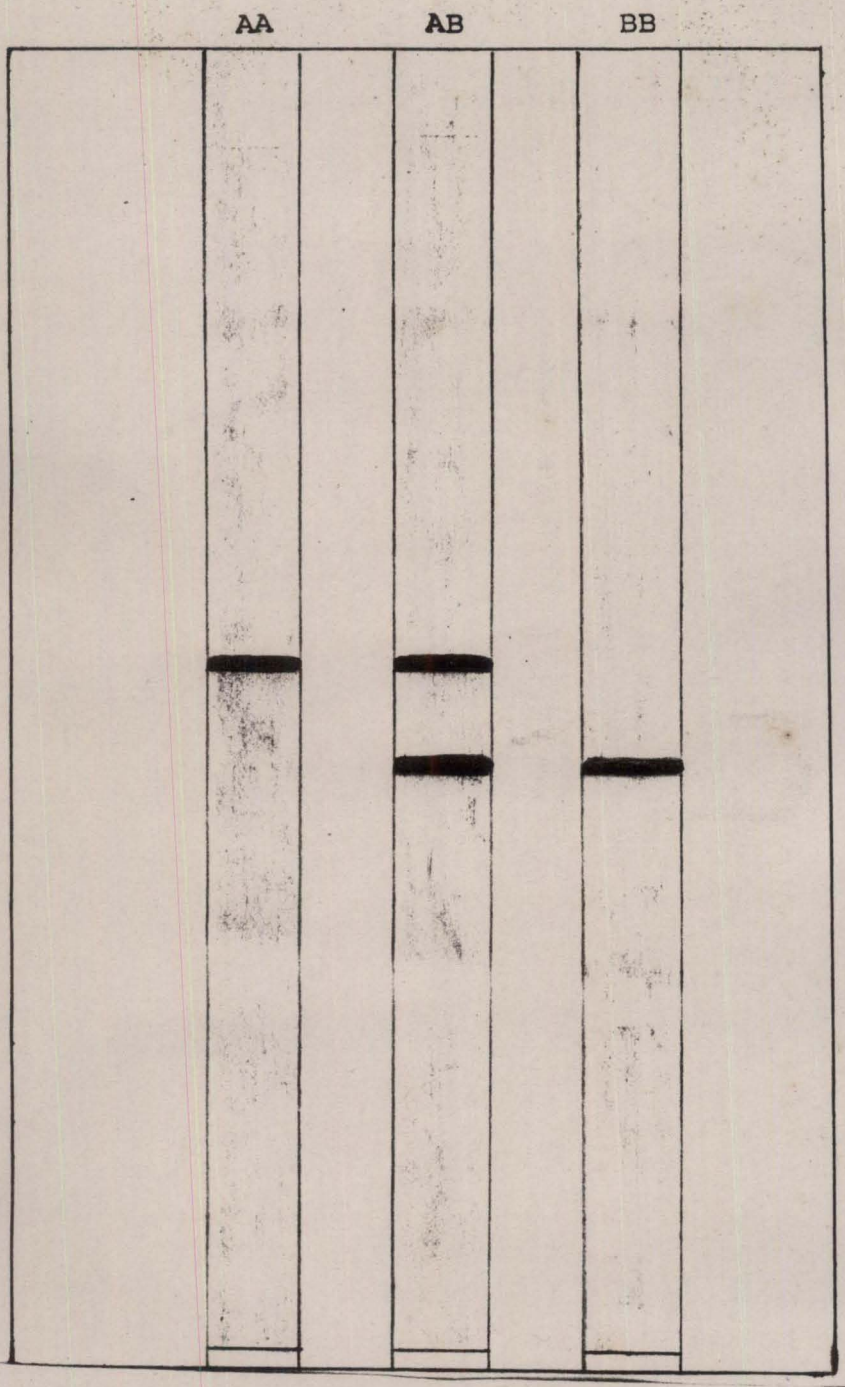


Fig. 6.B. Diagrammatic representation of Beta Lactoglobulin Phenotypes.

Table 9. Phenotype frequencies and gene frequencies of B-lactoglobulin types in Malabari and Alpine x Malabari goats

Population	Sample size	Beta-lactoglobulin phenotype frequencies			Beta-lactoglobulin gene frequencies	
		AA	AB	BB	A	B
Malabari	50	0.44 (22)	0.42 (21)	0.14 (7)	0.65	0.35
Alpine x Malabari	50	0.50 (25)	0.38 (19)	0.12 (6)	0.69	0.31

Number in parenthesis indicates number of animals

Table 10. Observed and expected number of goats with different Beta lactoglobulin types

Population		Beta lactoglobulin phenotype			X^2 value (df=1)
		AA	AB	BB	
Malabari	Obs.	22.00	21.00	7.00	0.29 NS
	Exp.	21.13	22.75	6.13	
Alpine x Malabari	Obs.	25.00	19.00	6.00	0.62 NS
	Exp.	23.81	21.39	4.81	

NS - Non significant
Obs. - Observed
Exp. - Expected

Fig.6.c DIAGRAMMATIC REPRESENTATION OF GENOTYPE FREQUENCIES IN MALABARI AND ALPINE x MALABARI (β LACTOGLOBULIN)

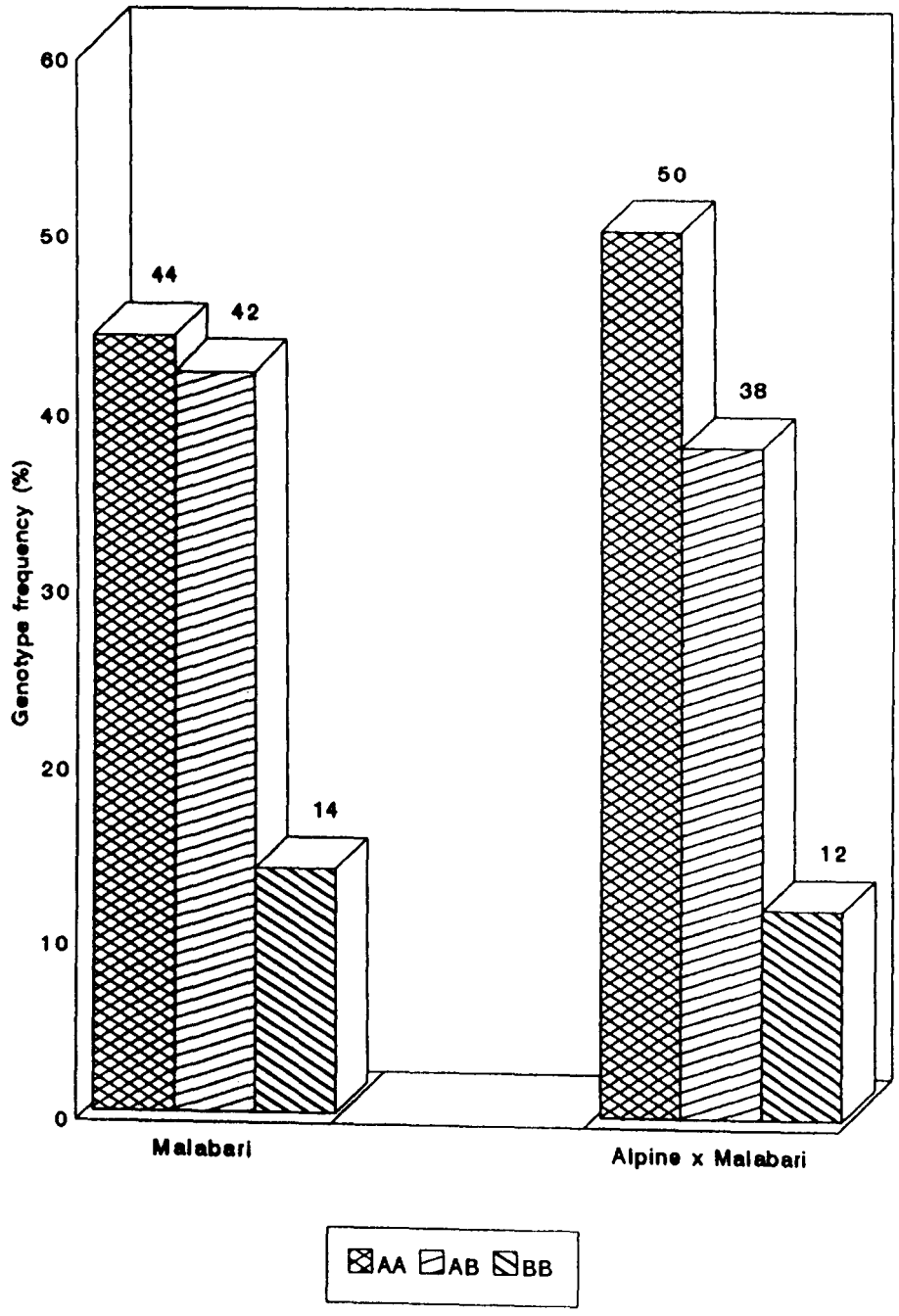
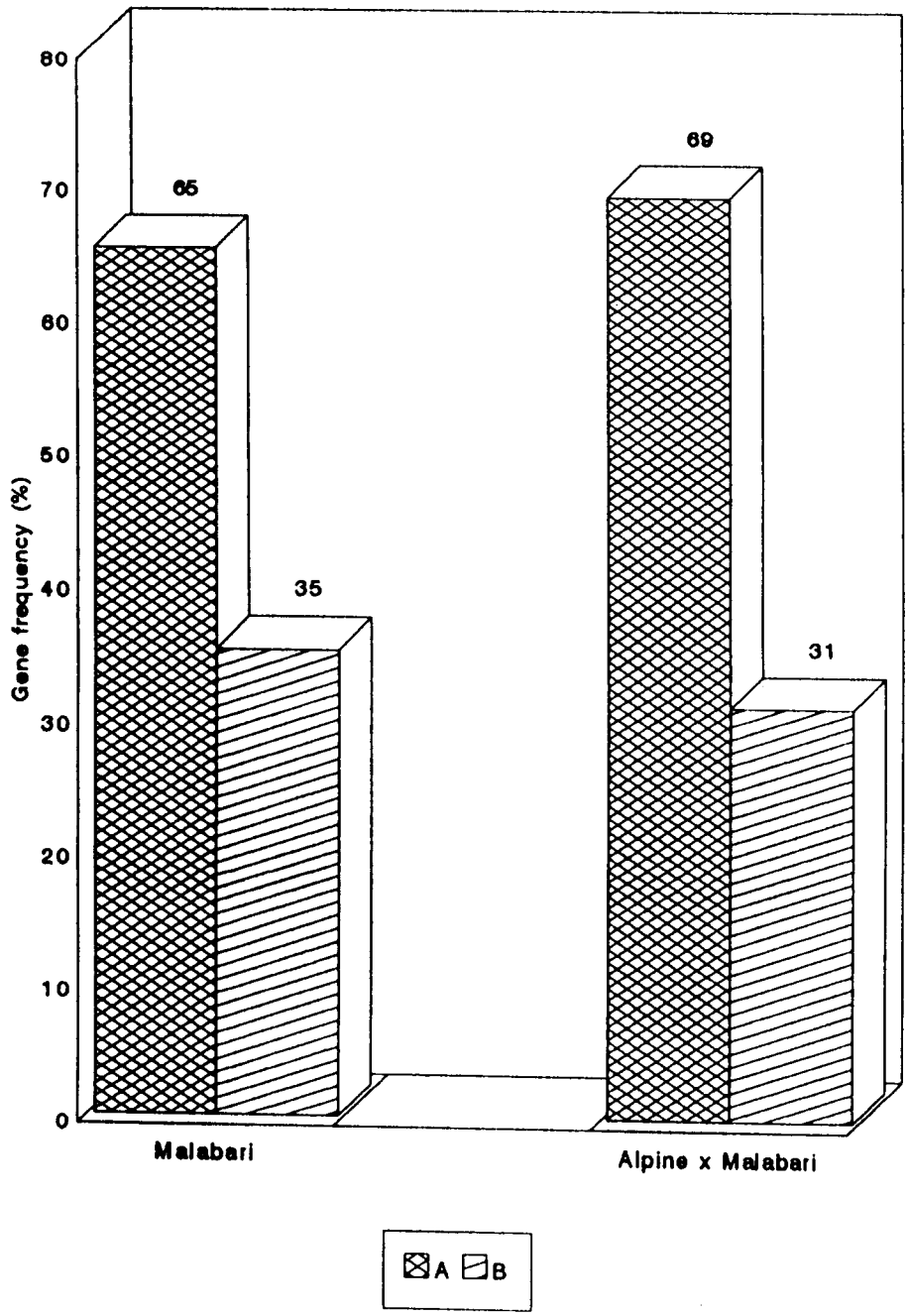


Fig.6.D DIAGRAMMATIC REPRESENTATION OF GENE FREQUENCIES IN MALABARI AND ALPINE x MALABARI (β LACTOGLOBULIN)



4.5.2 Test for genetic equilibrium

Table 10 shows the observed number of animals with different Beta lactoglobulin phenotypes and their expected numbers assuming Hardy Weinberg equilibrium. The result showed that in both populations the observed values were not significantly different from what was expected.

4.6 Heterozygosity

The genetic variability of a population is usually measured by the average heterozygosity per locus. The heterozygosity in each population was estimated by employing the method of Nei and Roychoudhary (1974). The results are presented in Table 11. The average heterozygosity values were 0.4711 in Malabari and 0.4209 in Alpine x Malabari crossbreds. Among the five loci studied, the maximum heterozygosity was detected at Alpha S_1 casein locus in Malabari goats (0.4992). In Alpine x Malabari crossbreds maximum heterozygosity was detected at Beta Cn locus (0.4800).

4.7 Genetic association between the genes controlling the synthesis of milk proteins

4.7.1 Malabari

The various combinations between protein variants of one locus and the variants of other protein loci have been shown in Table 12. As indicated in the table the chi-square test

Table 11. Heterozygosity at milk protein loci in Malabari and Alpine x Malabari goats

Population	Alpha S_1 Casein	Alpha S_2 Casein	Beta Casein	Kappa Casein	Beta lacto-globulin	Average hetero-zygosity
	1	2	3	4	5	6
Malabari	0.4992	0.4712	0.4950	0.4352	0.4550	0.4711
Alpine x Malabari	0.4200	0.4118	0.4800	0.3648	0.4278	0.4209

Table 12. Genetic association between the genes controlling synthesis of milk proteins in Malabari goats

Protein types			Alpha S_2 Casein				Beta Casein				Kappa Casein				Beta lactoglobulin			
			AA	AB	BB	X^2(4 df)	AA	AB	BB	X^2(4 df)	AA	AB	BB	X^2(4 df)	AA	AB	BB	X^2(4 df)
Alpha S_1 Casein	FF	O	1	6	3		1	7	2		4	5	1		5	4	1	
		E	4.00	4.40	1.60	4.06	1.80	5.40	2.80	1.06	4.40	4.80	0.80	0.09	4.40	4.20	1.40	0.206
	FS	O	13	11	4		4	14	10		11	15	2		10	13	5	
		E	11.2	12.32	4.48	0.48	5.04	15.12	7.84	0.89	12.32	13.44	2.24	0.35	12.32	11.76	3.92	0.865
	SS	O	6	5	1		4	6	2		7	4	1		7	4	1	
		E	4.8	5.28	1.92	0.756	2.16	6.48	3.36	2.15	5.28	5.76	0.96	1.10	5.28	5.04	1.68	1.05
						5.296 NS				4.1 NS				1.54 NS				2.121NS
Beta lactoglobulin	AA	O	6	12	4		3	14	5		13	8	1					
		E	8.8	9.68	3.52	1.511	3.96	11.88	6.16	0.829	9.68	10.56	1.76	2.087				
	AB	O	10	8	3		5	9	7		7	11	3					
		E	8.4	9.24	3.36	0.510	3.78	11.34	5.88	1.089	9.24	10.08	1.68	1.664				
	BB	O	4	2	1		1	4	2		2	5	0					
		E	2.8	3.08	1.12	0.906	1.26	3.78	1.96	0.067	3.08	3.36	0.56	1.739				
						2.927 NS				1.985 NS				5.49 NS				

Table 12. (Contd.)

Protein types			Alpha S_2 Casein				Beta Casein			
			AA	AB	BB	X^2(4 df)	AA	AB	BB	X^2(4 df)
Kappa Casein	AA	O	8	11	3		3	15	4	
		E	8.8	9.68	3.52	0.330	3.96	11.88	6.16	1.809
	AB	O	13	7	4		5	10	9	
		E	9.6	10.56	3.84	2.411	4.32	12.96	6.72	1.557
	BB	O	0	3	1		1	2	1	
		E	1.6	1.76	0.64	2.676	0.72	2.16	1.12	0.134
						5.417 NS				3.500 NS
Beta Casein	AA	O	3	4	2					
		E	3.6	3.96	1.44	0.257				
	AB	O	13	11	3					
		E	10.8	11.88	4.32	0.917				
	BB	O	4	7	3					
		E	5.6	6.16	2.24	0.830				
						2.004 NS				

NS - Non significant O - Observed E - Expected

Table 13. Genetic association between the genes controlling synthesis of milk proteins in Alpine x Malabari goats

Protein types			Alpha S_2 Casein				Beta Casein				Kappa Casein				Beta lactoglobulin			
			AA	AB	BB	X^2(4 df)	AA	AB	BB	X^2(4 df)	AA	AB	BB	X^2(4 df)	AA	AB	BB	X^2(4 df)
Alpha S_1 Casein	FF	O	2	2	2		3	2	1		4	2	0		3	3	0	
		E	3.12	2.28	0.6	3.703	2.04	3.12	0.84	0.884	3.36	2.4	0.24	0.429	3.0	2.28	0.72	0.947
	FS	O	11	6	1		5	12	1		11	6	1		11	5	2	
		E	9.36	6.84	1.8	0.746	6.12	9.36	2.52	1.866	10.08	7.2	0.72	0.393	9	6.84	2.16	0.951
	SS	O	13	11	2		9	12	5		13	12	1		11	11	4	
		E	13.52	9.88	2.6	0.285	8.84	13.52	3.64	0.682	14.56	10.4	1.04	0.415	13	9.88	3.12	0.683
						4.734 NS				3.432 NS				1.237 NS				2.581NS
Beta lactoglobulin	AA	O	12	10	3		10	14	1		12	12	1					
		E	13.0	9.5	2.5	0.203	8.5	13.0	3.5	2.127	14.0	10.0	1.0	0.686				
	AB	O	11	6	2		7	9	3		13	5	1					
		E	9.88	7.22	1.9	0.008	6.46	9.88	2.66	0.167	10.64	7.6	0.76	1.489				
	BB	O	3	3	0		0	3	3		3	3	0					
		E	3.12	2.28	0.6	0.832	2.04	3.12	0.84	7.599*	3.36	2.4	0.24	0.429				
						1.373 NS				9.893*				2.604 NS				

Table 13. (Contd.)

Protein types			Alpha S_2 Casein				Beta Casein			
			AA	AB	BB	X^2(4 df)	AA	AB	BB	X^2(4 df)
Kappa Casein	AA	O	17	10	1		8	16	4	
		E	14.56	10.64	2.8	1.605	9.52	14.56	3.92	0.387
	AB	O	9	8	3		9	8	3	
		E	10.4	7.6	2.0	0.710	6.8	10.2	2.8	1.201
	BB	O	0	1	1		0	2	0	
		E	1.04	0.76	0.2	4.136	0.68	1.04	0.28	1.846
						6.451 NS				3.434 NS
Beta Casein	AA	O	8	8	1					
		E	8.84	6.46	1.7	0.735				
	AB	O	13	10	3					
		E	13.52	9.88	2.6	0.083				
	BB	O	5	1	1					
		E	3.64	2.66	0.7	1.673				
						2.491 NS				

O - Observed E - Expected NS - Non significant * - P<0.05

revealed no significant association between genes controlling the synthesis of various caprine milk proteins.

4.7.2 Alpine x Malabari

Table 13 shows the association of different milk protein genes in Alpine x Malabari goats. The observed frequencies of Beta casein types having the combination of Beta lactoglobulin BB were not agreeing with the expected frequencies ($P<0.05$). Overall chi-square value was statistically significant at 5 per cent level. Combination of different alleles in other proteins presented variations were not significant.

4.8 Influence of milk protein variants on milk production

Student's t test was performed for finding out any association between the various milk protein genotypes with lactational milk producdtion (120 days) (Tables 14 to 18). Results of the analysis revealed no significant association between the protein types and the milk production.

4.9 Milk production

The average predicted lactational yield of Malabari and Alpine x Malabari crossbred goats was calculated. In Malabari the average yield was 69 $\pm$ 2.25 kgs and in Alpine x Malabari crossbreds it was 89.65 $\pm$ 2.78 kgs. The highest yield was recorded in Alpine x Malabari goats (128.15 kgs) and the lowest yield was recorded in Malabari goats (42.95 kgs).

Table 14. Influence of Alpha S_1 casein types on lactation yield (120 days) in kg in different genetic groups

Population	Genotype	Total number	Mean value (kg)	t value
Malabari	FF	10	77.35 ± 5.13	A 2.0031 NS
	FS	28	65.89 ± 2.89	B 1.369 NS
	SS	12	68.00 ± 4.60	C 0.3951 NS
Alpine x Malabari	FF	6	84.48 ± 7.02	A 0.7146 NS
	FS	18	91.26 ± 4.93	B 0.5961 NS
	SS	26	89.73 ± 3.88	C 0.2492 NS

NS - Non significant A --> t value between FF & FS

B --> t value between FF & SS

C --> t value between FS & SS

Table 15. Influence of Alpha S_2 casein types on lactation yield (120 days) in kg in different genetic groups

Population	Genotype	Total number	Mean value (kg)	t value
Malabari	AA	20	68.29 ± 3.01	A 0.2636 NS
	AB	22	67.05 ± 3.57	B 1.8170 NS
	BB	8	79.20 ± 7.12	C 1.7650 NS
Alpine x Malabari	AA	26	87.34 ± 4.03	A 1.0785 NS
	AB	19	93.73 ± 4.69	B 0.0988 NS
	BB	5	86.18 ± 3.81	C 0.7966 NS

NS - Non significant A --> t value between AA & AB

B --> t value between AA & BB

C --> t value between AB & BB

Table 16. Influence of Beta casein types on lactation yield (120 days) in kg in different genetic groups

Population	Genotype	Total number	Mean value (kg)	t value
Malabari	AA	9	67.27 ± 5.52	A 0.0546 NS
	AB	27	68.33 ± 2.82	B 0.3926 NS
	BB	14	70.30 ± 5.04	C 0.5096 NS
Alpine x Malabari	AA	17	94.56 ± 4.99	A 1.0882 NS
	AB	26	87.97 ± 3.66	B 1.1386 NS
	BB	7	83.98 ± 8.00	C 0.4879 NS

NS - Non significant A --> t value between AA & AB

B --> t value between AA & BB

C --> t value between AB & BB

Table 17. Influence of Kappa casein types on lactation yield (120 days) in kg in different genetic groups

Population	Genotype	Total number	Mean value (kg)	t value
Malabari	AA	22	64.34 ± 2.92	A 1.7062 NS
	AB	24	71.76 ± 3.19	B 1.1350 NS
	BB	4	74.15 ± 13.33	C 0.2578 NS
Alpine x Malabari	AA	28	90.95 ± 3.64	A 0.4286 NS
	AB	20	88.42 ± 4.75	B 0.5158 NS
	BB	2	83.85 ± 8.10	C 0.4522 NS

NS - Non significant A --> t value between AA & AB

B --> t value between AA & BB

C --> t value between AB & BB

Discussion

Table 18. Influence of Beta lactoglobulin types on lactation yield (120 days) in kg in different genetic groups

Population	Genotype	Total number	Mean value (kg)	t value
Malabari	AA	22	69.48 ± 3.46	A 0.5072 NS
	AB	21	66.97 ± 3.52	B 0.2624 NS
	BB	7	71.32 ± 6.16	C 0.6170 NS
Alpine x Malabari	AA	25	92.15 ± 3.92	A 0.2100 NS
	AB	19	90.91 ± 4.35	B 1.9093 NS
	BB	6	75.26 ± 7.66	C 1.7655 NS

NS - Non significant A --> t value between AA & AB

B --> t value between AA & BB

C --> t value between AB & BB

DISCUSSION

The present study in the Malabari breed of goat and its exotic cross viz., Alpine x Malabari was aimed to investigate the electrophoretic polymorphs of milk proteins using horizontal polyacrylamide gel electrophoresis (PAGE). The electrophoretic phenotyping revealed biochemical polymorphisms in Alpha S_1 casein, Alpha S_2 casein, Beta casein, Kappa casein and Beta-lactoglobulin.

5.1 Alpha S_1 casein

5.1.1 Phenotype and gene frequencies

The various phenotypes and gene frequencies of Alpha S_1 casein were analysed and presented in Table 1. The results revealed the presence of three Alpha S_1 casein phenotypes FF, FS and SS controlled by Alpha S_1 casein^F and Alpha S_1 casein^S alleles. This finding is in agreement with the observations made by Boulanger *et al.* (1984) and Addeo *et al.* (1988).

In the present study phenotypic frequency of FF phenotype was higher in Malabari breed (0.20) compared to Alpine x Malabari crossbred (0.12). The frequency of SS phenotype was lower in Malabari (0.24) and higher in Alpine x Malabari crossbreds (0.52). Malabari had a higher frequency of FS

phenotype (0.56) compared to Alpine x Malabari crossbreds (0.36).

The gene frequency of Alpha S_1 $casein_F$ was found to be higher in Malabari (0.48) than that in Alpine x Malabari crossbreds (0.30). The frequency of Alpha S_1 $casein^S$ was high in both the genetic groups with the value of 0.52 in Malabari and 0.70 in Alpine x Malabari crossbreds.

A high frequency of Alpha S_1 S allele in Alpine x Malabari crossbreds is probably due to higher frequency of S allele in Alpines (Grosclaude *et al.*, 1987 and Jordana *et al.*, 1991).

5.1.2 Test for genetic equilibrium

By assuming the Hardy-Weinberg equilibrium, the expected values of Alpha S_1 casein types were calculated and compared with the observed values. The results of the test for genetic equilibrium at Alpha S_1 casein locus furnished in Table 2 revealed that there were no significant differences existed between the observed frequencies and the expected frequencies for the various Alpha S_1 casein phenotypes viz. FF, FS and SS in the Malabari and Alpine x Malabari crossbred populations. From this we can infer that both the populations are in genetic equilibrium. As no selection of animals was carried out based on its Alpha S_1 casein phenotype, the present results could be expected.

5.2 Alpha S_2 Casein

5.2.1 Phenotype and gene frequencies

Fractionisation of caprine casein revealed two variants of Alpha S_2 casein viz. A and B with three phenotypic combinations AA, AB and BB. The frequency of AA phenotype was higher in Alpine x Malabari crossbreds (0.52) compared to Malabari breed (0.40). The results also revealed the lower frequency of BB phenotype in both the populations studied.

In contrast to the present findings absence of Alpha S_2 casein BB types in Camosciata delle Alpi and Saanen breed was reported by Russo *et al.* (1986). They could observe only two phenotypes viz. AA and AB with the frequencies of 46.6 and 50.9 AA and 53.4 and 49.01 AB in Camosciata delle Alpi and Saanen breeds respectively.

However the existence of Alpha S_2 casein BB was reported in Saanen breeds by Tutta *et al.* (1991) and present findings supports the same.

The gene frequency of Alpha S_2 casein A allele was higher in Alpine x Malabari crossbreds (0.71) compared to Malabari (0.62). These were lower than the frequency observed in Alpine and Saanen breeds which was reported to be 0.85 and 0.87 respectively by Boulanger *et al.* (1984). In the present study the B allele was found to be having higher frequency in Alpine

x Malabari crossbreds (0.29) compared to the earlier findings in Alpine breed of goats (0.04) by Bouniol *et al.* (1994). This higher frequency of Alpha S_2 casein B may be due to the higher occurrence of B allele in the Malabari breed. It is to be seen whether B allele may attribute to any selective advantage under the humid tropics.

5.2.2 Test for genetic equilibrium

Failure to observe significant difference between the observed and expected Alpha S_2 casein phenotypes in both the genetic groups was the indicative of the population presently studied in Hardy-Weinberg equilibrium with respect to the gene frequencies and genotype frequencies. This could be expected as the criteria for selection of goats did not include Alpha S_2 casein types of the animals.

5.3 Beta casein

5.3.1 Phenotypes and gene frequencies

Studies on Beta casein polymorphism in Malabari and Alpine x Malabari goats revealed the existence of three phenotypes viz. AA, AB and BB controlled by two alleles Beta casein A and Beta casein B. The frequency of A allele in Alpine x Malabari was observed to be greater (0.60) than that in Malabari (0.45). The frequency of Beta casein B allele was found to be higher in Malabari breed (0.55).

Sheonarain *et al.* (1992a) observed that the frequency of Beta casein^{A} was higher in Barbari (0.53) and the frequency of Beta casein B was higher in Jamunapari (0.53).

Frequency of AA phenotype was lower in Malabari breed (0.18) and higher in Alpine x Malabari crossbreds (0.34). The frequency of AB phenotype was almost similar in both Malabari (0.54) and Alpine x Malabari crossbreds (0.52).

Though 8 phenotypes were reported in Czechoslovakian white polled goats (Macha, 1970) only three phenotypes could be observed in the present study.

Perusal of the available literature which were few on the genetic polymorphism of caprine Beta casein revealed that the frequency of A allele was 0.53 (Sheonarain *et al.*, 1992a) which closely agrees with the present observations in Malabari and Alpine x Malabari crossbreds.

5.3.2 Test for genetic equilibrium

It was observed that the Beta casein types were not significantly different from their number expected assuming the population in Hardy-Weinberg equilibrium. Hence it can be concluded that, these genetic groups were in Hardy-Weinberg equilibrium with respect to Beta casein locus. This possibly

indicates the neutral role of the alleles at the Beta casein locus.

5.4 Kappa casein

5.4.1 Phenotypes and gene frequencies

Three phenotypic classes of Kappa casein viz. AA, AB and BB controlled by two alleles A and B were observed in the present study (Table 7). AA phenotype was higher in Alpine x Malabari crossbreds (0.56) compared to Malabari (0.44). The genotype frequency of BB was quite low in both Malabari (0.08) and Alpine x Malabari crossbreds (0.04). The distribution of heterozygotes AB was higher in Malabari (0.48) and lower in Alpine x Malabari crossbreds (0.40).

The frequency of A allele was higher in both the populations when compared to the B allele. In Alpine x malabari crossbreds it was 0.76 and in Malabari it was 0.68. The frequency of B allele was higher in Malabari (0.32) as compared to Alpine x Malabari goats (0.24).

Law and Tziboula (1993) reported three Kappa casein phenotypes namely AA, AB and BB in British Saanen goats with the frequency of 0.80, 0.17 and 0.03 respectively, and the present results reveal similar genetic configuration in Malabari goats.

5.4.2 Test for genetic equilibrium

Absence of significant difference between the observed and expected number of phenotypes in any of the genetic group studied indicate that the populations were in genetic equilibrium and were mating at random.

5.5 Beta lactoglobulin

5.5.1 Phenotypes and gene frequencies

Phenotype frequencies and gene frequencies of Beta lactoglobulin variants in different genetic groups are presented in Table 9. There were 2 variants of Beta lactoglobulin viz. A and B with three phenotypic combination AA, AB and BB.

The frequency of AA phenotype in Alpine x Malabari crossbreds was observed to be greater (0.50) than that in Malabari (0.44).

The frequency of AB phenotype in Alpine x Malabari crossbred was observed to be lesser (0.38) than that in Malabari (0.42). The frequency of BB phenotype was more or less same in both the genetic groups (0.14 in Malabari and 0.12 in Alpine x Malabari).

In the present study frequency of Beta lactoglobulin^{B} allele was 0.35 in Malabari and 0.31 in Alpine x Malabari crossbreds. The frequency of Beta lactoglobulin^{B} allele was 0.65 in Malabari and 0.69 in Alpine x Malabari crossbreds.

The frequency of A allele in Malabari and Alpine x Malabari crossbreds observed in the present study agrees with Barillet *et al.* (1993) who observed the higher frequency of Beta lactoglobulin^{A} (0.63) compared to Beta lactoglobulin^{B} (0.37) in French Lacauna breed.

5.5.2 Test for genetic equilibrium

Chi-square test reveal that both the populations were in genetic equilibrium. This trend is suggestive of a neutral role of Beta lactoglobulin types in panmictic population.

5.6 Heterozygosity

Polymorphism in a population reflects genetic variability. The variation in the population provides scope for selection. Rendel (1967) suggested that blood groups and protein variants might prove to be very useful tools for estimating variability between populations.

In the present study heterozygosity at each locus and average heterozygosity in two different populations were calculated for 5 milk protein systems (Table 11).

The heterozygosity observed at the Alpha S_1 casein locus was 0.4992 (Malabari) and 0.4200 (Alpine x Malabari crossbreds).

The average heterozygosity in different populations of goats indicated that Malabari breed had comparatively higher degree of heterozygosity (0.4711) followed by Alpine x Malabari crossbreds (0.4209). From these results inference could be drawn that heterozygosity decreased as the level of exotic blood increased. This could be due to the fact that the crossbreds were produced originally from few number of Alpine bucks.

5.7 Genetic association between the genes controlling the synthesis of milk proteins

The present study attempted to understand the possible association of alleles between different phenotypes and the absence of certain recombinants observed in the protein types using chi square test in the two breeds of goats.

The linkage analysis between milk protein loci in Alpine x Malabari crossbreds is given in Table 13. It was observed

that a significant association existed between Beta lactoglobulin locus with the Beta casein locus. Among the Beta loctoglobulin types BB phenotype showed close association with Beta casein phenotypes ($P<0.05$). In Malabari goats, the combination of Beta casein and Beta lactoglobulin phenotype did not show any significant difference between observed and expected frequencies (Table 12).

The present findings showed no association between Alpha S_1 and Alpha S_2 casein genes. Thus conclusion could be drawn that so called linkage claimed by Grosclaude *et al.* (1987) based on the contingency tables and the chisquare values were not seen here. Certain combinations showing the associations between Beta casein and Beta lactoglobulin genes as studied by chisquare test in Alpine x Malabari crossbreds needs further confirmation using large number of animals to arrive at fool proof conclusions.

5.8 Influence of milk protein variants on milk production

In the present study no association could be established between the lactational yield (120 days) and milk protein variants in both Malabari and Alpine x Malabari crossbred populations (Tables 14 to 18). The literature on the association between milk protein variants and lactational yield are plenty as against the present observation. Significant

association between milk protein variants and lactational yield was reported earlier (Macha, 1981; Remeuf, 1989; Langley Danysz, 1993 and Barbieri *et al.*, 1995).

However, the present work has confirmed the findings of Barillet *et al.* (1993) and Grosclaude *et al.* (1994) who reported the total absence of association between milk protein variants and milk yield in goats.

Before making a final conclusion of absence of associations between milk protein variants and milk yield in the population under study it would be worthwhile to undertake extensive study involving large number of goats.

Summary

SUMMARY

Individual milk samples were typed for the milk proteins Alpha S_1 casein, Alpha S_2 casein, Beta casein, Kappa casein and Beta lactoglobulin. The samples comprised of 50 from Malabari goats and 50 from Alpine x Malabari crossbreds belonging to sheep and goat farm, Kerala Agricultural University, Mannuthy.

Phenotyping of milk proteins was carried out using horizontal polyacrylamide gel electrophoresis (PAGE).

Three phenotypes viz., FF, FS and SS determined by two alleles 'F' and 'S' were observed at the Alpha S_1 casein locus. The FF and FS phenotypes were higher in Malabari breed and phenotype SS was higher in Alpine x Malabari crossbreds. The S allele had higher frequency in both the genetic groups viz., Malabari and Alpine x Malabari with respective frequencies of 0.52 and 0.70.

Three types of Alpha S_2 casein were identified as AA, AB and BB. Higher frequency of A allele was observed in Alpine x Malabari crossbreds (0.71) while Malabari breed had 0.62. Frequency of AA phenotype was higher in Alpine x Malabari crossbred (0.52) and AB phenotype was higher in Malabari (0.44).

Two alleles A and B and three phenotypes namely AA, AB and AB were observed at the Beta casein locus in both the genetic groups studied. Allele B was predominant in Malabari breed (0.55) and in Alpine x Malabari crossbreds allele 'A' was predominant (0.60). The frequency of AA phenotype was higher in Alpine x Malabari crossbreds (0.34) and AB phenotype was higher in Malabari breed (0.54).

Kappa casein locus consisted of three genotypes (AA, AB and BB) with two alleles A and B. Frequency of AA phenotype was higher in Alpine x Malabari crossbred (0.56) and AB was higher in Malabari (0.48). The frequency of BB phenotype was comparatively low in both the populations studied (0.08 in Malabari and 0.04 in Alpine x Malabari). Frequency of 'A' allele was high both in Alpine x Malabri (0.76) and Malabari (0.68).

Two alleles viz., A and B were dectected at the Beta lactoglobulin locus. Allele 'A' was predominant in all the populations studied. Among the three phenotypes observed the frequency of AA phenotype was higher in Alpine x Malabari (0.50) and AB phenotype was highr in Malabari (0.42).

Both the populations studied were in genetic equilibrium with respect to the Alpha S_1 casein, Beta casein, Kappa casein and Beta lactoglobulin loci.

The average heterozygosity was calculated in each genetic group. Maximum average heterozygosity was observed in Malabari (0.4711). Among the five protein loci, the heterozygosity was highest at Alpha S_1 casein locus in Malabari breed. In Alpine x Malabari crossbreds maximum heterozygosity was observed at Beta casein loci (0.4800).

Analysis was done to study the association of genes controlling the synthesis of milk proteins by studying their combinations in two way contingency tables. The combination of Beta lactoglobulin BB type with Beta casein in Alpine x Malabari was appeared to be associated than others.

No significant association could be established between the milk protein variants and the milk yield (120 days).

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* Original not seen

GENETIC POLYMORPHISM OF MILK PROTEINS IN GOATS

By

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ABSTRACT OF A THESIS

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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 Malabari and its exotic cross viz., Alpine x Malabari. It was also envisaged to analyse the association of different milk protein variants with lactation milk yield. Hundred goats belonging to Malabari (50) and Alpine x Malabari (50) were typed for milk protein variants employing horizontal Polyacrylamide Gel Electrophoresis (PAGE). The milk protein loci studied were Alpha S_1 casein, Alpha S_2 casein, Beta casein, Kappa casein and Beta lactoglobulin.

Two Alpha S_1 casein variants, the faster Alpha S_1 casein F and the slower Alpha S_1 casein S with three phenotypes Alpha S_1 casein AA, Alpha S_1 casein AB, and Alpha S_1 casein BB were observed. S allele had the frequency of 0.52 in Malabari and 0.70 in Alpine x Malabari crossbreds. The gene frequency of Alpha S_1 casein F and Alpha S_1 casein S were 0.48 and 0.52 in Malabari and 0.30 and 0.70 in Alpine x Malabari crossbreds, respectively. The frequency of S allele was higher in both the populations.

Alpha S_2 casein locus exhibited three phenotypes viz., AA, AB and BB controlled by two alleles A and B. A allele had the

higher frequency of 0.71 in Alpine x Malabari crossbreds compared to 0.62 in Malabari. Higher frequency of AA phenotype was found in Alpine x Malabari (0.52) and that of AB phenotype in Malabari (0.44).

Two alleles namely A and B contributed three phenotypes viz., AA, AB and BB at the beta casein locus in both the populations studied. Malabari showed higher frequency of B allele (0.55) while Alpine x Malabari had higher frequency of A allele (0.60). Beta casein AB phenotype had higher frequency in Malabari breed (0.54).

Kappa casein locus showed two alleles A and B contributing three phenotypes viz., AA, AB and BB. The frequency of A allele was higher in both the population. The phenotype AA was higher in Alpine x Malabari crossbreds (0.56) while Malabari had higher frequency of AB phenotype (0.48)

Two alleles namely A and B with three phenotypes AA, AB and BB were identified at Beta lactoglobulin locus. The phenotype AA was dominant in Alpine x Malabari crossbreds (0.50) and the frequency of BB phenotype was almost similar in both Malabari and Alpine x Malabari crossbreds (0.14 in Malabari and 0.12 in Alpine x Malabari crossbreds). The gene frequency of Beta lg A was found to be 0.65 in Malabari and 0.67 in Alpine x Malabari crossbreds.

Both the populations studied were in genetic equilibrium with respect to these five milk protein loci. No significant diversity was found to exist between genetic groups.

The genetic variability in the populations was calculated by estimating the heterozygosity. The overall heterozygosity in different populations indicated that the Malabari breed had comparatively higher degree of heterozygosity (0.4711) followed by Alpine x Malabari crossbreds (0.4209). In Malabari maximum heterozygosity was observed at Alpha S_1 casein loci (0.4992). In Alpine x Malabari maximum heterozygosity was observed at Beta casein locus (0.4800).

The study of association between the genes controlling synthesis of milk proteins showed significant association between the Beta casein with the Beta lactoglobulin BB types in Alpine x Malabari crossbreds. All the other combinations of genes did not show any significant association.

Milk protein variants were not found to be associated with the lactational yield (120 days) of the goats.

The present study could establish the existence of biochemical polymorphism at Alpha S_1 casein, Alpha S_2 casein, Beta casein, Kappa casein and Beta lactoglobulin loci in Malabari and Alpine x Malabari crossbreds studied.