STUDY OF GENETIC DIVERSITY IN MALABARI GOATS (*Capra hircus*) UTILIZING BIOCHEMICAL AND IMMUNOLOGICAL MARKERS

By

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DECLARATION

I hereby declare that the thesis entitled "Study of Genetic Diversity in Malabari Goats (*Capra hircus*) Utilizing Biochemical and Immunological Markers" 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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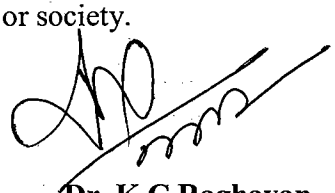
CERTIFICATE

Certified that the thesis entitled "Study of genetic diversity in Malabari goats (*Capra hircus*) utilizing biochemical and immunological markers" is a record of research work done independently by Dr.K.A.Bindu under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship, other similar title to her of any other University or society.



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1. INTRODUCTION

The domestic goat *Capra hircus* is universally reckoned as an important livestock species that alternates as a good source of milk, meat, fibre and skin. Goats have had their unparalleled roles in different facets of human civilization as depicted in history. The special emphasis laid on goats owes it to its contribution to the agrarian economy and the positive role it has played in offering livelihood security to the small, landless and marginal farmers.

The relevance of goat rearing assumes greater significance in a country like India due to its unique potential in bolstering the efforts to poverty alleviation as well as economic upliftment of the rural poor. The promising prospects of goat rearing as regards to employment generation, financial autonomy and raising of living standards of the rural poor can directly translate into poverty alleviation. The easiness in management of this species attaches special significance, since it works out to the benefit of the weaker sections of the society including women who can take it up as a source of self-employment, income generation, food security and household nutrition.

India is a repository of 20 well-recognized breeds of goats that have evolved in respect to the needs of a variety of geographical and climatic situations. Each of these different breeds has specific importance of its own, relevant to the different agro climatic zones to which they belong and hence warrants specificity from the research point of view. The geographically evolved goat breeds thus display genetic divergence in their production, reproduction, adaptability, heat tolerance and potential to disease resistance. Lack of information on the characteristics and extent of existing diversity in indigenous breeds has lead to their underutilization, replacement and dilution through cross

breeding. This necessitates conservation of genetic variation as well as estimation of the genetic uniqueness of the individual breeds at genomic level.

Goat production in Kerala is centered mainly on its native breed "Malabari", a dual-purpose goat of North Kerala. This breed is supposed to have originated centuries back by mixing of native feral goats with Arab, Surti and Mesopotamian goats along with the native goats of Western coast (Kaura, 1952). The breed owes its name to the area where they belong, extensively distributed in the Malabar area of Kerala constituted by districts such as Kasaragode, Kannur, Kozhikode and Malappuram. The animals have predominant breed characteristics of white and a combination of white with black and brown. They are mostly long eared and horned with convex forehead and have rounded udders with funnel shaped pointed teats. Variation exists within this breed as far as most traits of economic importance are concerned. There exist significant difference between populations of this breed with regard to traits of economic importance and hence the data obtained from any particular population cannot be extrapolated to the breed as a whole. Hence this genetic diversity has to be studied in detail in different populations in all possible ways. In addition to helping further selection and improvement, this throws some light on the evolution of a breed as such.

One way to study this genetic diversity is by the determination of genetic variability through polymorphic studies. Polymorphism in a population assures a pool of genetic variability, for if none exists, there would be no progress made through selection and breeding. This accentuates the need to study polymorphism between breeds as well as within breeds. Polymorphism studies can be undertaken at various levels, viz. expressed protein studies to the genic level studies. Protein studies date back to quite some time now while the latest trends stand at molecular level studies.

In many mammalian species, genetic polymorphism observed at the level of blood proteins has been well studied. The protein variants have their use in the

study of origin and evolution of breeds of livestock. These markers have proved to be useful for parentage determination and population analysis (Groselande *et al.,* 1990). Biochemical polymorphisms are used to study the variability between populations and to estimate genetic racial divergence.

Microsatellite marker study is a new approach in studying variations at DNA level. Microsatellite markers are present in the genome and the number of repeats within many of them is highly variable in a population of a particular species. Microsatellite polymorphisms are now used for a wider range of applications in genetics including construction of genetic linkage maps, linkage mapping of quantitative traits and disease resistance genes, diagnosis of genetic disorders, paternity testing and forensic studies and also for the selective breeding in livestock. The microsatellite markers are of much use in studying differences between breeds as well as within breeds at molecular level.

Selection for immune responsiveness and disease resistance has often been ignored by animal geneticists because of the difficulty in measuring these traits. Immune response, the strategic defense system in the fight against microbial invasions, is of primary importance to the survival and optimum performance of an animal. Serum gamma globulin levels have often been related with health, better performance and survival of all species of livestock. An understanding of the influence of disease resistance, immune responses and production traits is very important in selection of future breeding plans.

The difference within a breed whether at biochemical, immunological or gene level will be reflected in biometrical traits of the animals. Any study to discriminate different populations within a breed will be complete only when the populations are compared based on these economic traits.

With these views a study was planned in Malabari goats with the following objectives.

1. Study the genetic similarities and differences among three populations of Malabari goats utilizing biochemical polymorphisms and immune responsiveness and to estimate the relationship of biochemical variants with traits of economic importance (body weight and milk yield) and immune responsiveness with disease incidence.

2. Study the polymorphic pattern of three populations of Malabari goats utilsing Microsatellite markers.

3. Estimate the gene and genotype frequencies of variants in different sub populations.

4. Study the phylogenetic relationship existing among these groups by estimating genetic distances.

Review of Literature

2. REVIEW OF LITERATURE

The sustainable use of indigenous breeds, the genetic variation existing in these breeds and the potential for genetic improvement has so far been exploited only to a limited extent. Malabari is a potential dual-purpose goat breed of Kerala and it exhibits a lot of phenotypic and biometrical variations. This study is focused on examining these variations by utilizing biochemical, molecular, biometrical, and immunological means. The recent literature available on this subject is briefly reviewed under the following divisions:

2.1 Biochemical Polymorphism

2.2. Polymorphism of Microsatellite Markers

2.3. Biometrical and Reproduction Traits

2.4. Immune Response

2.1 BIOCHEMICAL POLYMORPHISM

The genetic variation caused by immuno-haematological traits and biochemical polymorphisms is classified under the heading "Genetic polymorphism". It has significance in dealing with genetic, ecological and physiological studies and are useful in breeding economic livestock if the polymorphic traits bear relationship with the production or reproduction traits (Khanna, 1979). They constitute a very useful tool for studies on evolution, relationship and structure of breeds (Elmaci and Uzatici, 2002)

2.1.1 Haemoglobin Polymorphism

The molecular weight of haemoglobin in most species varies from 66000 to 69000. Each haemoglobin molecule consists of four polypeptide chains normally occurring in two pairs of identical chains. The haemoglobin heterogeneity in goat has been reported to be due to two different alpha chains.

Braide and Enyenihi (1969) conducted studies on the haemoglobin types in three Nigerian goat breeds namely, West African Dwarf, Mambilla and Kano Brown and reported the presence of three haemoglobin types in goats for the first time ever as against the two haemoglobin types reported till then. The types identified included one with fast migration towards the anode, another with relatively slower migration and a third haemoglobin with intermediate electrophoretic mobility.

Enyenihi (1974), studying on the phenotype and gene frequencies of polymorphic haemoglobins of Nigerian goat breeds, reported that starch gel electrophoresis of the Sahel goat's haemoglobin, using a continuous buffer system, yielded at least four electrophoretically distinct types, three of which were identical with those of Red Sokoto (Maradi) and Kana Brown breeds, the fourth type being extremely slow in its migration towards the anode.

Heamoglobin polymorphism in Barbari and Jamunapari goat breeds was studied by Joshi *et al.* (1975), who reported the existence of three polymorphic haemoglobin types, namely, homozygous Hb^{A} and Hb^{B} type and heterozygous Hb^{A} Hb^{B} of which Hb^{A} type was predominant in both breeds (89.5 per cent in Barbari and 90.0 per cent in Jamunapari). The distribution of Hb^{B} was 2.64 and 1.43 and of Hb_{AB} was 7.89 and 8.57 per cent, respectively in Barbari and Jamunapari breeds.

Singh *et al.* (1977) conducted studies in non-descript native goats, Barbari, Beetal and Barbari- Beetal crosses using horizontal starch gel electrophoresis and found out two variants of haemoglobin, the faster Hb^{A} and the slower Hb^{B} and two haemoglobin phenotypes. The Hb^{A} variant was seen with very low frequency of occurrence in non-descripts, while it was completely absent in Barbari, Beetel and their crosses.

Haemoglobin types in Muzaffaranagri breed of sheep and its crosses with Suffolk and Dorset were studied by Bhat (1978) using starch gel electrophoresis, who found that Hb^{B} types were more common than those of Hb^{A}. They reported no significant departure from Hardy- Wienberg frequencies in the population.

Mostaghni (1979) studied haemoglobin polymorphism in goats of Iran electrophoretically and found out three haemoglobin types, viz. haemoglobin A, B and C. According to him Hb^B type was predominant (61.5 per cent) and had the highest gene frequency (0.577). Gene frequencies of Hb^A and Hb^C were 0.194 and 0.229, respectively.

A total of 230 goats of Jamunapari, Black Bengal and Barbari breeds were typed for haemoglobin by Baruah and Bhat (1980) by horizontal starch gel electrophoresis who found out two phenotypes of haemoglobin, Hb_{AA} and Hb_{AB} in Barbari goats. In Jamunapari and Black Bengal animals, only Hb_{AA} type was observed.

Tucker and Clarke (1980) reported two types of haemoglobin, Hb^A and Hb^B in sheep.

Buvanendran *et al.* (1981), studying haemoglobin polymorphism and resistance to helminthes in Red Sokoto goats, detected three haemoglobin variants and five phenotypes. The observed proportions of haemoglobin phenotypes in animals over one year of age differed from the expected numbers calculated on the basis of genetic equilibrium, while in kids there was good agreement between observed and expected values.

According to Bhat *et al.* (1983), Barbari breed of goats were of Hb_{AA} and Hb_{AB} types whereas Jamunapari animals were of only Hb_{AA} type.

In Hungarian native goats, the frequency of Hb^A (0. 954) was higher than Hb^B (0.096) as reported by Fesus *et al.* (1983), who also noticed no difference between the expected and observed numbers of the individual haemoglobin types.

Morera *et al.* (1983) observed high frequency of Hb^B (0.673) as against Hb^A (0.327) in Spanish Merino sheep.

Barbancho *et al.* (1984) studied the electrophoretic variance of genetic markers in the blood of four Spanish goat breeds, viz. Granadina, Murciana, Malaguena and Serrana Andaluza. They found haemoglobin Hb^A and Hb^B variants in Spanish goat breeds. The frequencies of Hb^B allele in both the Spanish Granadina (0.154) and the Malaguena (0.156) goat breeds were higher than the other breeds studied.

Biochemical polymorphism of Jamunapari was studied by Bhat (1985) by starch gel electrophoresis and reported the presence of two phenotypes of haemoglobin (Hb_{AA} and Hb_{AB}), with a gene frequency of 0.99 (Hb^A) and 0.01 (Hb^B), respectively.

Shamsuddin *et al.* (1986) studied haemoglobin polymorphism in Malabari and its Saanen and Alpine half breds and reported two haemoglobin phenotypes Hb_{AA} and Hb_{AB}, controlled by two alleles Hb^A and Hb^B. Hb_{AA} had only one band and was faster in mobility towards anode. Hb_{AB} had one component of Hb^A and other of Hb^B. The frequency of Hb_{AA} was highest in Saanen half breds (0.985) and lowest in Alpine halfbreds (0.934). Higher frequency of Hb_{AB} was observed in Alpine halfbreds and Hb_{BB} was not observed in any of the three genetic groups. The gene frequencies of Hb^A and Hb^B were 0.975 and 0.025, respectively in Malabari goats.

Reddy *et al.* (1988), studying on distribution of gene frequencies of Hb^A and Hb^B alleles in Nellore and Nellore x Dorset halfbred sheep, failed to record Hb^A and found that majority of the animals belonged to Hb^B type. The gene frequency of Hb^B allele was 0.991 in Nellore breed and 0.904 in Nellore x Dorset cross breds.

While studying on blood groups and biochemical polymorphism in the Namaqua sheep breed, Clarke *et al.* (1989) reported the presence of common haemoglobin variants (Hb^A and Hb^B), with Hb^B predominating.

Kilgour *et al.* (1990), conducting studies on Hb types in sheep, classified most of the samples into the common types, Hb_{AA}, Hb_{BB} or Hb_{AB}.

Naitana *et al.* (1990) conducted studies on haemoglobin phenotypes of the wild European Mouflon sheep living in the island of Sardinia and characterised the sheep phenotypes by the anodally migrating haemoglobin, Hb^A and the cathodally migrating haemoglobin Hb^B or by the combination of the two.

Haemoglobin polymorphism in domesticated goats in the USA, namely, Alpine, Angora, Nubian, Saanen and Spanish was studied by polyacrylamide gel electrophoresis (Wang *et al.,* 1990). Significant difference in the allelic frequencies of haemoglobin, among these breeds was reported.

Kumar *et al.* (1991) observed Hb^A and Hb^B in UAS strain, Bannur breed and non-descript sheep, where the frequency of Hb^B was higher.

Nguyen *et al.* (1992) reported predominance of Hb^A allele (frequency 0.697) over Hb^B in Rambouillet, as against in Spanish Merino wherein Hb^B (frequency 0.662) predominated over Hb^A.

A rather common haemoglobin variant in the Sardinian and Altamurana sheep breeds which appeared to be produced under the control of an allele at the Hb^B locus due to a neutral amino acid substitution was detected by Manca *et al.* (1993). The variant was referred to as the Hb I.

Pepin and Nguyen (1994) examined five goat breeds, viz. French Alpine, French Saanen, Guadeloupean Cresole, Guinean and West African Sahel breeds for electrophoretic variants of haemoglobin and found that Hb^D occurred in each of the five breeds but its allelic frequency was only noticeable in Saanen (0.15) and West African Sahel (0.27). According to them the blood proteins appeared not to be highly polymorphic in the goats tested and the inter population variability at the protein loci was not as striking as might be expected from the origin of the populations examined.

Predominance of Hb^B type allele (0.94) over that of Hb^A which was of low occurrence (0.06) in Konya Merino sheep was reported by Boztepe *et al.* (2000).

Canatan and Boztepe (2000) observed two haemoglobin phenotypes Hb_{AA} and Hb_{AB} in Turkish hair goat by starch gel electrophoresis. The frequencies of Hb^A and Hb^B were 0.815 and 0.185, respectively. The observed phenotypic numbers of the two haemoglobin types were not significantly different from the expected numbers on the basis of Hardy-Weinberg equilibrium.

Elmaci and Uzatici (2002) reported haemoglobin Hb^A and Hb^B in Turkish sheep breeds with the Hb^B allele predominant over the Hb^A allele. All populations studied were reported to be in Hardy-Weinberg equilibrium.

A and B types of haemoglobin in Bakeravali and Pashmina goats were observed by Menrad *et al.* (2002). The allelic frequencies of Hb^B were higher in all the populations compared to Hb^A types.

Elmaci (2003) studied haemoglobin variants in the hair goats of Turkey and observed two different haemoglobin phenotypes by starch gel electrophoresis. The frequencies of haemoglobin Hb^A and Hb^B were 0.83 and 0.17, respectively. Population was found in Hardy-Weinberg equilibrium..

Two different haemoglobin types, Hb_{AB} and Hb_{BB} were identified in Akkaramen and Awassi breeds of sheep by Yildiz *et al.* (2003). No Hb_{AA} phenotype was observed in both breeds. The frequencies of Hb^B allele were 0.92 and 0.91 in Akkaramen and Awassi breeds, respectively. The observed genotypic numbers of the three haemoglobin types did not differ significantly from the expected numbers based on Hardy-Wienberg equilibrium.

2.1.2 Polymorphism of Blood Proteins

2.1.2.1 Transferrin

The biochemical polymorphism of blood serum proteins in farm animals has been investigated by many workers utilizing the methods of electrophoresis. Genetic control of transferrin was first reported by Ashton and Mc Dougall (1958) who suggested it as β globulin polymorphism resulting from two co-dominant alleles, β^A and β^B. Transferrin is a specific iron binding protein, whose major function is transportation of iron to bone marrow and tissue storage organs. Transferrin also participates directly in the regulation and control of iron absorption and protects from iron intoxication.

Complete absence of Tf^B allele was reported by Efrenov and Braend (1965) in Norwegian goats, Watanabe and Suzuki (1966) in Saanen Swiss goats and Tjankov (1972) in Toggenburg breed.

Singh *et al.* (1977) studied the transferrin polymorphism in Barbari, Beetal, Barbari - Beetal crosses and non-descript locals, using starch gel electrophoresis and revealed the presence of two variants and three phenotypes. The gene frequency of transferrin Tf^B was higher in all the populations and was highest in Beetal goats.

Trivedi and Bhat (1978) studied transferrin polymorphism in Muzaffaranagri breed of sheep (*Ovis aries)* and its crosses with Suffolk and Dorset and observed five variants,viz. Tf^A, Tf^B, Tf^C, Tf^D and Tf^E.

Baruah and Bhat (1980) identified three transferrin phenotypes, Tf_{AA} Tf_{AB} and Tf_{BB} in Jamunapari, Black Bengal and Barbari breeds of goats and observed the gene frequencies of Tf^A and Tf^B as 0.27 and 0.73, 0.37 and 0.63, and 0.44 and 0.56, respectively.

Studies on transferin polymorphism in Alpine, Saanen, Nubian, Alpine X Beetal and Saanen X Beetal cross-breds, by Trehan *et al.* (1981), indicated that it was controlled by at least three co-dominant alleles. Starch gel electrophoresis revealed Tf_{AA}, Tf_{BB}, Tf_{AB}, Tf_{AC} and Tf_{BC} variants. Tf_{AA} and Tf_{BB} were represented by two bands each on starch gels. Faster band of Tf_{BB} corresponded with slower band of Tf_{AA}. Phenotypes of Tf_{AB} and Tf_{BC} were represented by three bands each and Tf_{AC} was represented by four bands. No significant differences were observed between expected and observed phenotypic frequencies.

Bhat *et al.* (1983) reported the occurrence of transferrin phenotypes, Tf_{AA} and Tf_{BB} in Jamunapari and Barbari breeds of goats.

Two allelic variants for transferrin, Tf^{A} (0.588) and Tf^{B} (0.412) in Hungarian native goat breeds were detected by Fesus *et al.* (1983).

Morera *et al.* (1983) detected five transferrin variants, Tf^{A}, Tf^{B}, Tf^{C}, Tf^{D} and Tf^{E} in Spanish Merino sheep.

Barbancho *et al.* (1984) studied transferrin polymorphism in four breeds of Spanish goats, viz. Granadina, Malaguena, Serrana Andaluza and Murciana and noticed three transferrin alleles, Tf^{A}, Tf^{B} and the rare Tf^{C}. In Granadina breed, a departure from the Hardy- Weinberg equilibrium was noticed in the population. In all the breeds studied, Tf^{A} had a much higher frequency than Tf^{B}.

A new electrophoretic variant of transferrin, Tf^{L} was discovered in sheep by horizontal polyacrylamide gel electrophoresis by Archibald and Webster (1986).

Bhat (1986a) described transferrin Tf^{A}, Tf^{B} and Tf^{C} in Jamunapari breeds and detected that the gene frequency of Tf^{B} was greater than that of Tf^{A} or Tf^{C}.

Erhardt (1986), by vertical discontinuous polyacrylamide electrophoresis, identified Tf^A, Tf^G, Tf^B, Tf^C, Tf^D, Tf^M, Tf^E, Tf^Q and Tf^P in sheep.

Pashmina goats, viz. Chegu and Changthangi were studied for transferrin polymorphism by Bhat (1987) and reported the gene frequencies of Tf^A, Tf^B and Tf^C as 0.79 and 0.19, 0.02 and 0.66, and 0.30 and 0.04, respectively.

Three alleles of transferrin, Tf^A, Tf^B and Tf^C were detected in Jhakrana, Kutchi, Marwari and Sirohi breeds of north Western India and a relatively high frequency of Tf^C allele in Jhakrana breed was recorded by Kumar and Yadav (1988).

Shamsuddin *et al.* (1988) conducted electrophoretic studies on transferrin polymorphism in Malabari goats and its exotic cross breds and identified transferrin phenotypes, Tf_{AA}, Tf_{AB}, Tf_{BB} and Tf_{AC}, controlled by alleles Tf^A, Tf^B and Tf^C in the order of descending mobilities towards the anode and they failed to observe Tf_{BC} and Tf_{CC} out of the six possible phenotypes. The study further revealed that Tf_{AB} had the highest number in all the genetic groups and that the phenotype AC was observed only in Saanen and Alpine halfbred goats. Further more, the frequency of Tf^B allele was higher (0.55) than that of Tf^A in Malabari gaots, whereas in Saanen halfbred and Alpine half bred goats Tf^A had the highest frequency followed by Tf^B and Tf^C. The allele Tf^C was found only in exotic crossbreds and was not reported in any of the Indian breeds.

Clarke *et al.* (1989), studying on blood groups and biochemical polymorphism in the Namaqua sheep breed, identified transferrin variants Tf^A, Tf^B, Tf^C and Tf^D.

Casati *et al.* (1990) observed seven variants for transferrin in Italian sheep breeds namely, Sarda, Comisana, Bergamasca, Gentile di Puglia and Massese.

Genetic characteristics of Bangladeshi sheep as based on biochemical variation were studied by Tsunoda *et al.* (1990) and found that of the six alleles of Tf locus, Tf^A,

Tf^B, Tf^C, Tf^E and Tf^F were present in Bangladeshi sheep with the Tf^D missing and Tf^E being the most common of all populations.

Wang *et al.* (1990) conducted studies on transferrin polymorphism in domesticated goats in USA and observed A and B alleles in the population. Allele A had a much higher occurrence than allele B. Significant difference in the allelic frequency distribution was observed only between Spanish and Alpine breeds.

Five transferrin alleles (Tf^A Tf^B Tf^C Tf^D and Tf^E) occurred in Spanish Merino and three (Tf^A $Tf^D$and Tf^E) in French Rambouillet, indicating a lower degree of genetic polymorphism in French Rambouillet compared to Spanish Merino as reported by Nguyen *et al.* (1992).

Menrad *et al.* (1994) reported the presence of three phenotypes, Tf_{AA}, Tf_{AB} and Tf_{BB} in Boer and German improved Fawn goats. The frequencies of Tf^A and Tf^B were 0.58, 0.95 and 0.42, 0.05, respectively.

Three variants of transferrin named A, B and C were observed by Pepin and Nguyen (1994) in five goat breeds, namely, French Alpine, French Saanen, Guadeloupean Creole, Guinean and West African Sahel. Tf^A and Tf^B were found in Alpine, Saanen, Guinean and West African goat breeds. Tf^C was extremely rare and was found only in one animal of West African goat breeds. Allelic frequencies of Tf^A were 0.99, 0.93, 0.83, 0.99 and 0.95 and of Tf^B were 0.01, 0.07, 0.17, 0.01 and 0.05, respectively in Alpine, Saanen, Guadeloupean, Guinean and West African goat breeds.

Boztepe *et al.* (2000) observed six transferrin alleles (Tf^A, Tf^B, Tf^M, Tf^D, Tf^E and Tf^S) and reported that Tf locus was not in Hardy-Wienberg equilibrium.

Canatan and Boztepe (2000) identified three alleles for transferrin in Turkish hair goats. The frequencies of Tf alleles were 0.64, 0.347 and 0.014, respectively for Tf^A,Tf^B and Tf^C.

Menrad *et al.* (2002) studied transferrin variants in Pashmina and Bakervali goats by horizontal polyacrylamide gel electrophoresis and found that allelic frequency of transferrin deviated significantly from Hardy - Wienberg equilibrium. Tf^{A} Tf^{B} and Tf^{C} were observed in the population.

2.1.2.2 Albumin

Albumin is one of the most important serum proteins in blood. This protein is of great importance because of its relative abundance, homogeneity, osmotic and transport functions.

Tucker (1968) observed three types of albumin FS, SW and WW in Clunforest, Dorsethorn, Welsh mountain, Soay, Merrino cross and Finnish Landrace sheep. Albumin with fastest mobility was described as F, the slowest as W and that with intermediate mobility as S. The findings also suggested that majority of the samples of the Finnish Landrace had identical albumin patterns (Type SS) though three additional types (Types FS, SW and WW) were also found, of which the slowest albumin (W) was a new type. It was further found that except for one FS type identified in the Welsh Mountain breed, all the sheep in other breeds were of the SS type. In Finnish Landrace, the occurrence of the three albumin zones was controlled by three co-dominant alleles Al^{F}, Al^{S} and A^{W} of which the frequencies of Al^{F} and Al^{W} were low (0.017 and 0.11, respectively) accounting for the relative absence of FF or FW types.

Monomorphic bands for albumin in Australian Merino and Poll Dorset Sheep were observed by Manwell and Baker (1977).

Singh *et al.* (1977) observed no polymorphism for albumin in Barbari, Beetal, their crosses and non-descript native goats and noticed only one band for albumin in starch gel electrophoresis.

According to Tucker and Clarke (1980), each albumin variant in sheep was composed of two electrophoretic bands, a weak anodal and a strong cathodal band. They also opined that sheep Al^S had a similar mobility to goat Al^A .

Morera *et al.* (1983) observed two albumin phenotypes SS and SF in Spanish Merino sheep by starch gel electrophoresis, with a high frequency of Al^S allele (0.989).

Two albumin types, Al^F and Al^S in four breeds of Spanish goats, viz. Granadena, Murciana, Malaguena and Serrana Andaluza were described by Barbancho *et al.* (1984). A high frequency of the allele Al^F was reported by them in the Serrana Andaluza breed.

Shamsuddin *et al.* (1986) observed no polymorphism with regard to Albumin in the serum of Malabari goats and its Saanen and Alpine halfbreds by starch gel electrophoresis. Two bands were observed in all the animals tested and the slower band was densely stained as compared to the faster band which was lighter.

Bhat (1987) reported that the Pashmina goats, Chegu and Changthangi showed two bands for albumin in starch gel electrophoresis indicating absence of polymorphism in the loci tested.

Clarke *et al.* (1989), studying on blood groups and biochemical polymorphism in the Namaqua sheep breed, reported that all samples tested were of the commonly occurring albumin Al^S phenotype.

Two alleles were reported for albumin (Al^F and $Al^{S)}$ in Spanish Merino sheep by Tunon *et al.* (1989).

Casati *et al.* (1990) studied genetic polymorphism in five Italian sheep breeds namely, Sarda, Comisana, Bergamasca, Gentile di Puglia and Massese using seven blood protein loci and observed only one band (S) for albumin in all the breeds except in

Gentile di Puglia, where in the gene frequencies of S and F bands were 0.996 and 0.004, respectively.

Genetic characteristics of Bangladeshi sheep as regards biochemical variation were studied by Tsunoda *et al.* (1990), who identified the presence of Al^A allele in addition to Al^C, which was absent in all European breeds studied.

Vankan and Bell (1992), studying genetic polymorphism in Cashmere goats using 12 per cent polyacrylamyde gel, observed two albumin phenotypes and found the frequencies of Al^A and Al^B to be 0.02 and 0.98, respectively.

According to Erhardt and Simianer (1993) albumin polymorphism occurred only in some breeds of sheep and the frequency of the variant alleles was very low using discontinuous polyacrylamide gel electrophoresis.

Pepin and Nguyen (1994) observed monomorphic albumin in five goat breeds, viz. French Alpine, French Saanen, Guadeloupean Creole, Guinean and West African Sahel goats.

According to Ertugrui and Akyuz (2000), the allele frequencies of Al^F and Al^S in Angora goats were 0.289 and 0.711, respectively. They concluded that the Angora goats raised in villages in Turkey had maintained their genetic variability in spite of decrease in their number.

Menrad *et al.* (2002) found out new variants for albumin in Bakervali goats, Al^D in Udhampur and Al^V in Jammu, with frequencies of 0.333 and 0.238, respectively and reported that both phenotypes occurred in high frequencies in a few flocks only.

2.1.2.3 Cerruloplasmin (Cp)

Cerruloplasmin, the copper binding α 2 globulin contains about 95 per cent of serum copper and contains eight atoms of copper per molecule. Cerruloplasmin (Cp) was first isolated from porcine plasma. Cp is not a single enzyme but a series of isoenzymes.

No polymorphism for Cerruloplasmin was observed in Barbari, Beetal, their crosses and non-descript natives (Singh *et al.,* 1977), in Australian Merino sheep (Manwell and Baker,1977), in sheep of Netherland (Buis and Tucker, 1983), in Hungarian native breeds of goats (Fesus *et al.,*1983), in Jamunapari and Sirohi goat breeds (Bhat, 1986a), in Changthangi and Chegu breeds (Bhat, 1987) and in Spanish goat breeds (Tunon *et al.,* 1989).

Khanna (1979) reported that no polymorphism was observed in Indian buffaloes when serum samples were subjected to starch gel electrophoresis for Cerruloplasmin typing.

Six different Cp phenotypes in cattle were observed by Przytulski and Klemke (1981) who reported that the frequencies of Cp^{A}, Cp^{B} and Cp^{C} were 0.347, 0.018 and 0.635, respectively.

Two Cerruloplasmin phenotypes Cp_{AA} and Cp_{BB} were identified in hair goats of Turkey with allelic frequencies of Cp^{A} and Cp^{B} as 0.97 and 0.03, respectively by Elmaci (2003). The phenotype, $Cp_{AB,}$ was absent in the population. The study further revealed significant differences between the expected and observed number of Cerruloplasmin phenotypes.

2.1.2.4 Amylase

Mammalian amylases are mainly alpha-amylases which hydrolyse starch and glycogen by splitting central glucosidic linkages. Serum amylase was described as the site of starch degrading activity, the system being controlled by co-dominant alleles.

Three amylase phenotypes were observed in Angora goats by Fechter and Pretorius (1970) who designated them as A for the fast moving type, S for the slow moving type and AS for the combination of two types.

Singh *et al.* (1977) observed single band for amylase in Beetal, Barbari, their crosses and nondescript natives. Amylase was found to be monomorphic in the goat population studied.

Khanna (1978) conducted studies on amylase polymorphisms in Indian buffaloes by starch gel electrophoresis and reported the occurrence of three forms designated as Amy^C, Amy^A and Amy^B in the order of decreasing mobility towards anode. The gene frequency for Amy^A ranged from 0.85 to 1.00, and for Amy^C and Amy^B was from nil to 0.3 and nil to 0.12, respectively. He further opined that the differences in the gene frequencies amongst different populations were not very much marked.

No polymorphism for amylase was detected by Morera *et al.* (1983) in Spanish Merino sheep.

Bhat (1986a) studied the electrophoretic variants of Jamunapari and Sirohi goats by starch gel electrophoresis and observed two variants for amylase. The frequency of Amy 1 was more than that of Amy 2.

Malabari goats and its Saanen halfbreds and Alpine halfbreds were studied for electrophoretic variants of amylase using 11 per cent hydrolysed potato starch by Shamsuddin *et al.* (1986). Polymorphism in amylase system was not observed in any of

the three genetic groups and each amylase phenotype was represented by a single band in starch gel.

Tunon *et al.* (1989) reported no polymorphism in majority of the Spanish goat breeds. Very low occurrence of Amy^B allele was reported in Berciana (0.02), Negra Serrana (0.06), Malaguena (0.01) and Canaria (0.01).

No polymorphism for amylase was detected by Menrad *et al.* (1994) in German improved Fawn and Boer goats.

Menrad *et al.* (2002) reported no polymorphism for amylase in Changthangi goats and described the presence of a new phenotype with alleles Amy^A and Amy^C, detected in two animals of Bakervali and Pashmina goat breeds.

Three amylase phenotypes AA, AB and BB were reported in the native hair goat population of Turkey by Elmaci (2003). The population was not in Hardy-Wienberg equilibrium for amylase locus. The frequencies of Amy^A and Amy^B were 0.983 and 0.017, respectively.

2.1.2.5 Carbonic Anhydrase

In mammals, the different isozymes vary in their tissue and subcellular distributions and in their susceptibility to inhibitors. Carbonic anhydrase enzymes have different roles in mammals, being implicated in ammonia transport, bone resorption, gastric acidity, muscle contraction, gluconeogenesis, renal acidification, and normal brain development. Carbonic anhydrase functions as an effective attentional gate that controls signal transfer through the neural network, in addition to being involved in signal processing and memory storage.

Tucker and Clarke (1980) reported that all the sheep studied, possessed one single band designated as CA^S for carbonic anhydrase. A rare faster migrating variant, CA^F has also been described by them.

No polymorphism for carbonic anhydrase was observed in sheep breeds of Netherlands by Buis and Tucker (1983).

Morera *et al.* (1983) reported two variants for carbonic anhydrase, (CA^S and CA^F) with a high frequency of CA^S (0.952).

Casati *et al.* (1990) observed only one band for carbonic anhydrase in Sarda breeds, suggesting absence of polymorphism.

Nguyen *et al.* (1992) reported CA^S and CA^F alleles in Rambouillet and Spanish Merino sheep. The frequencies of CA^S were 0.907 and 0.982, and of CA^F, 0.093 and 0.019 in Rambouillet and Spanish Merino, respectively.

Pepin and Nguyen (1994) revealed that carbonic anhydrase was monomorphic in five breeds of goats, viz. French Alpine, French Saanen, Guadeloupean Cresole, Guinean and West African Sahel.

2.1.3 Serum Potassium Polymorphism

Potassium plays major role in physiology. It is responsible for the maintenance of osmotic pressure, electron transport and for many other body processes. It also influences development of membrane potential and carbohydrate metabolism.

Dev *et al.* (1979) studied the blood potassium in goats and observed that the gene frequencies for high potassium (HK) and low potassium (LK) types were 0.82 and 0.18, respectively.

The blood potassium in 208 goats of Iran was assayed using flame photometry by Mostaghni (1979) and it was confirmed that 87. 5 per cent of the goats were of HK type and 12.5 per cent were of LK type. The mean whole blood concentrations of potassium in the high and low blood groups were 21.2 ± 2.3 and 8.9 ± 1.4 meq/l, respectively.

Bhat *et al.* (1983) typed Jamunapari and Barbari herds of Indian goats for potassium concentration (meq/l) by flame photometry. The mean whole blood concentrations of potassium were 18.5 ± 0.45 and 20.47 ± 0.38 meq/l in Barbari and Jamunapari, respectively. According to them, the mean potassium concentration obtained showed a normal distribution and no bimodality with respect to potassium types and these breeds belonged to high potassium type.

Two distinct potassium types, low (LK) and high (HK), were identified in the indigenous goat breeds of Rajasthan (Khan and Taneja, 1983). The concentration of potassium of the LK type varied from nine to nineteen milliequivalents per litre and in HK from 21 to 34 meq/l.

Bhat (1986a) studied potassium alleles of Jamunapari and Sirohi goats and two alleles, high and low were reported. Jamunapari had only high potassium type which got fixed during the course of evolution of the breed.

Bhat (1986b) conducted studies in Muzaffarnagari breed of sheep and its cross breds with Suffolk and Dorset breeds and identified two separate groups of animals on the basis of whole blood potassium. The mean concentration of potassium in the high potassium animals in Muzaffarnagari and its cross-breds varied from 30.95 ± 3.57 to 32.45 ± 3.41 meq/l. In the animals with low potassium type, the mean potassium varied from 11.2 ± 0.56 to 13.82 ± 0.6 meq/l.

High potassium and low potassium phenotypes in Chokla ewes with similar body weights were reported by More and Rawat (1986).

The mean potassium concentration in the whole blood was 29.05 ± 0.39 meq/l in Pashmina goats and the breed had only high potassium type animals (Bhat and Singh 1987).

Tunon *et al.* (1987) reported two types of red cell potassium, the dividing line being set at 45 meq/l of potassium and that K^L was dominant over K^H. The mean concentration of potassium in LK animals was 24.8 ± 0.30 meq/l where as the mean HK concentration was 71.4 ± 0.32 meq/l.

Clarke *et al.* (1989), studying on blood groups and biochemical polymorphism in the Namaqua sheep breed, reported an unusual distribution in the red cell potassium concentrations in that, instead of the bimodal distribution found in sheep, the Namaquua sheep showed a single distribution but with a mean potassium value of 57 mmol /l cells. They were neither typically LK nor HK type.

Tsunoda *et al.* (1990), studying the genetic characteristics of Bangladeshi sheep, based on biochemical variation, found that the frequency of K^h allele ranged from 0.353 to 0.935 with a mean of 0. 607, slightly lower than that in Indian sheep.

Bayon *et al.* (1994) who investigated potassium levels in Spanish Churra Sheep, found that all the animals studied were of the low potassium type and that the mean potassium concentration was 9.89 ± 0.25 mmol.

Roy *et al.* (1997) reported the mean potassium concentration in goats of Bihar as 4.2 ± 0.12.g / dl.

2.1.4 Blood Glutathione Polymorphism

Glutathione is a tripeptide of glycine, glutamic acid and cysteine. In the blood, almost all of the reduced glutathione (GSH) is found within the erythrocytes.

Agar *et al.* (1974) conducted studies on glutathione polymorphism in Angora, Anglo Nubian, British Alpine, Saanen and Toggenberg breeds of goats and reported the existence of two GSH types similar to those present in sheep. They classified goats with GSH values under 60 mg/100ml red blood cells as GSH low type and those with higher values than this as GSH high type and found that GSH high type were predominant in all the breeds studied by them.

According to More *et al.* (1980) the mean values for high GSH in Toggenberg and Anglo Nubian goats were 107.9 ± 17.4 and 88.4 ± 16.9 mg/100ml red cells, respectively and no low GSH types were found in these breeds. The GSH concentration in the red cells of high type was 90.9 ± 17.1, 78.3 ± 12.8 and 110.7 ± 13.3 mg/100ml red cells, respectively in Saanen, Angora and Alpine breeds. Corresponding values for low GSH were 52.4 ± 6.5, 54.7 ± 5.8 and 55.2 ± 6.4 mg/100ml red cells, respectively. The goats examined were mainly of GSH high type, however the Jamunapari breed was found to have relatively more number of low GSH animals (37.5per cent).

Gall (1981) observed that the concentrations of GSH in GSH low types of Saanen, Alpine and Angora were 52 , 55 and 55 mg/100 ml red blood cells while in the high type were 91 , 78 and 111 mg/100 ml red blood cells, respectively.

Reddy and Krishnan (1986) reported that GSH high and low types were controlled by a single pair of autosomal alleles, with the gene for GSH high type being dominant over low type. They found that GSH low type predominated in all the genetic groups studied and for the total population as well. The gene frequency of GSH^{h} allele was 0.81 for the total population of sheep studied and GSH concentration in high or low type did not differ significantly between genetic groups.

Clarke *et al.* (1989), studying on blood groups and biochemical polymorphism in the Namaqua sheep breed reported bimodal distribution in red cell GSH concentrations, 44 per cent of sheep having red cells with less than 50 mg/ 100 ml red cells.

No polymorphism could be observed for GSH loci in Spanish goats by Tunon *et al.* (1989).

Bayon *et al.* (1994) estimated erythrocyte reduced glutathione (GSH) levels in blood by DTNB method in Spanish Churra sheep. In the total population, 53 per cent animals exhibited low GSH type with a mean value of 25.11 ± 1.17mg/100ml red cells and 47 per cent showed the high GSH type with a mean value of 84.90 ± 1.31mg/100ml red cells. The values of GSH concentrations ranged from 3.77 to 52.95 mg/100ml red cells in the low GSH type and from 55.27 to 114.46mg/100ml red cells in the high GSH type.

2.1.5 Genetic Distance

Genetic distance calculated using allele frequencies is a powerful method for detecting the effects of natural selection among different genetic groups. Measures of genetic distance provide the best available objective description of the genetic differentiation between and within breeds. Genetic distancing helps to identify genetically unique breeds so that they may be prioritized for breed conservation purposes. It also provides insight into the history of animal domestication.

Katsumata *et al.* (1981) conducted studies on the blood protein gene constitution in seven populations of the Japanese Saanen goats and found that the Nei's genetic distance between these populations were in the range of 0.0004 to 0.0065 (average:0.0031) suggesting a fairly uniform gene constitution.

Ordas and Primitivo (1986) studied eleven populations of three Spanish dairy breeds using biochemical polymorphisms and estimated the genetic distance utilizing the method proposed by Nei.

Tunon *et al.* (1989) found out genetic distance between Spanish goat breeds by studying blood genetic systems and found out distance values using Nei's genetic distance, ranging from 0.003 (Granadina-Malaguena) to 0.097 (Murciana-Retinta).

Casati *et al.* (1990) studied genetic distance among five most important Italian sheep breeds namely, Sarda, Comisana, Bergamasca, Gentile di Puglia and Massese, using seven blood protein loci and observed close genetic relationship between Bergamasca and Gentile di Puglia which were native to northern and southern Italy, respectively. This finding stood proof of the fact that Bergamasca rams were used on several Gentile di Puglia populations to increase the body size of the latter.

Tsunoda *et al.* (1990) studied genetic characteristics of Bangladeshi sheep based on biochemical variations and found that the genetic distance coefficient in the three populations ranged from 0.0413 to 0.0721, with a mean of 0.0521 for 20 loci and it appeared that the Bangladeshi sheep had a high genetic difference from the European sheep breeds examined.

Pepin and Nguyen (1994) calculated the genetic differences between breeds of goats based on biochemical polymorphism in five goat breeds namely French Saanen, French Alpine, Guadeloupean Creole, Guinean and West African Sahel. Genetic differences were studied by calculating the genetic distances. The smallest distance was observed between the two European breeds of Alpine and Saanen, both of which originated from Switzerland. Saanen and Guinean goats were separated from each other by the largest distance. The Guadelopean Creole appeared to be closer to African than to European breeds.

2.2. POLYMORPHISM OF MICROSATELLITE MARKERS

Microsatellites are tandem repeats of DNA sequences of only a few base pairs (1-6 bp) in length, usually repeated 15 to 30 times. The term microsatellite was introduced to characterize the simple sequence stretches amplified by polymerase chain reaction (PCR). These are also known as simple sequence length polymorphism (SSLP), sequence tagged sites (STS) and short tandem repeats (STRs). The short length makes microsatellites amenable to amplifications by polymerase chain reaction and subsequent

separation by polyacrylamyde gels with the resolution of alleles differing by as little as single base pairs (Beckmann and Weber, 1992).

Kemp *et al.* (1993) reported that a set of six bovine microsatellites, based on $(CA)_n$ repeats, were highly polymorphic and thus represented valuable markers for genome mapping. Four of the six were polymorphic in sheep and two were polymorphic in goats.

Estoup *et al.* (1994) observed that microsatellites permit a highly precise dissection of the genetic structure of populations. They were easy to isolate and the PCR based typing of alleles could be readily automated.

Gill *et al.* (1994) reported that microsatellites have clear advantages over other DNA markers by having multiple alleles and high heterozygosity, which made them highly informative for genetic analysis. In addition, the loci were small enough to be analyzed using polymerase chain reaction.

Ciampolini *et al.* (1995) opined that, for animal breeding and for preservation of natural resources, the approach of using a congruous number of microsatellites might be a very efficient technique for studying the genetic similarities between and within breeds. Another advantage of the use of microsatellites was that the intra group genetic similarity could be calculated without dividing the group and so also the genetic similarities could be obtained by direct counting of the microsatellite alleles.

Kemp *et al.* (1995) used a panel of 81 polymorphic bovine microsatellite markers and reported polymorphic information content (PIC) values ranging from 0.37 to 0.89 in cattle.

Simple sequences repeats (SSR) or microsatellites, detected by polymerase chain reaction amplification, primed by unique flanking sequences had played an increasingly important role in parentage control, population studies, linkage analysis and genome mapping (Arranz *et al.*, 1996)

Saitbekova *et al.* (1999) studied genetic diversity in eight Swiss goat breeds, using 20 bovine microsatellites on 20 to 40 unrelated animals per breed and concluded that microsatellites were powerful tools to differentiate between goat breeds. From the comparison of microsatellite analysis with breeding histories, they opined that microsatellites gave the correct answer, regardless of whether the breeds were closely related or not. In the study they observed that ETH 10, ILSTS 005, ILSTS 030 and INRA 063 were monomorphic.

Yang *et al.* (1999) estimated PIC to vary from 0.37 to 0.89 in Chinese goat breeds using microsatellite markers.

Genetic relationships among six populations of Merino sheep were investigated using microsatellites by Diez-Tascon *et al.* (2000). The PCR was performed using a primer end-labelled with (γ^{33}P) ATP and T_4 polynucleotidekinase. Twenty microsatellites were amplified, their products electrophoresed on sequencing gels and the bands of DNA visualized by autoradiography. The dendograms constructed on the basis of microsatellite allelic frequencies showed that populations that shared selection criteria, tended to cluster together.

Li *et al.* (2000) proposed microsatellites as the best markers for evaluating the genetic diversities of domestic animals because of their abundant even distribution in the genome, high polymorphism and ease of genotyping. They studied the genetic relationships and diversities between four Chinese indigenous pig breeds and one Australian commercial pig breed and concluded microsatellites as the best tools as compared to cytogenetic, biochemical genetics, mitochondrial DNA and randomly amplified polymorphic DNA.

Kim *et al.* (2002) calculated the PIC values which ranged from 0.35 to 0.62 in three Korean goat breeds.

2.2.1 Genetic Distance based on microsatellite markers

Arranz *et al.* (1998) genotyped five indigenous Spanish breeds of sheep for 19 microsatellites and revealed that the greatest genetic variation was in Merino sheep and the lowest in Awassis with respect to allelic frequencies. The dendogram obtained based on genetic distances showed a large differentiation between Awassi breed and the Spanish breeds as was expected from their genetic origin.

Saitbekova *et al.* (1999) studied genetic diversity in eight Swiss goat breeds using PCR amplification of 20 bovine microsatellites on 20 to 40 unrelated animals per breed. The average heterozygosity within populations was higher in domestic goat (0.51 to 0.58) than in Ibex (0.17) and Bezovar goat (0.19). Analysis showed that all this Swiss goats were closely related where as Creole breed, Ibex and Bezovar goats were clearly distinct from all the eight Swiss breeds. Allelic frequencies were used to generate the genetic distance matrix for each pair of populations using the computer programme, PHYLIP.

Yang *et al.* (1999) analyzed five Chinese indigenous goat breeds (Tibetan, Neimonggol, Liaoning, Taihang and Matou. A neighbour-joining tree was constructed using Nei's standard genetic distance (1978). In the tree, Neimonggol and Liaoning were grouped together, then with Taihang, while Tibetean and Matou individually had their own branch. The result was in accordance with the known history and geographical origin of the breeds.

Diez-Tascon *et al.* (2000) found out genetic relationships among six populations of Merino sheep using microsatellite markers. Genetic variation was highest amongst the Spanish and Portugese populations. The dendrograms constructed on the basis of microsatellite allelic frequencies showed that populations that shared selection criteria, tend to cluster together and that the smallest genetic distance (0.086), was estimated between Spanish and Portugese Black Merino. There was little differentiation between the Iberian populations with least distances ranging from 0.135 to 0.086.

Menrad *et al.* (2002) calculated genetic distance between Pashmina and Bakerwali goats using the standard genetic distance by Nei as ranging from 0.002 to 0.080. Lower distance values were found between the sub-populations within the respective breeds.

Tao *et al.* (2005) investigated serum protein variants in Chinese pigs and reported that the relationship between Durocs and Hunan indigenous pigs was found to be more distant than those among the three Hunan indigenous pig populations by calculating the average heterozygosity and genetic distance using Nei and Choudhary (1974) formulae.

2.3 BIOMETRICAL AND REPRODUCTION TRAITS

2.3.1 Litter Size

Shanmughasundaram (1957) reported a high prolificacy rate in Malabari goats with 58.5 per cent incidence of twins and 42 per cent of single births out of 103 kidding.

Moulick *et al.* (1966) reported that the average litter size at birth varied from 1.5 ± 0.5 to 2.53 ± 0.12 in Black Bengal goats.

According to Biswas *et al.* (1995) twinning rate in Pashmina was not common and was about to 3.25 per cent.

Stephen and Rai (1995) studied the prolificacy of Malabari goats and its crosses with Alpine and Sanen breeds and recorded the average litter size at birth as 1.4 in all genetic groups.

The study of Tomar *et al.* (1995) on 215 parturition records of 97 goats of Jamunapari and Barbari breeds, concluded that the average litter size was 1.4 with 38.15 per cent twins and 2.3 per cent triplets.

Raghavan *et al.* (1999) reported that the average litter size at birth in Malabari was 1.98 ± 0.081.

According to Nandakumar *et al.* (2003) the average litter size at birth in Malabari goats based on 232 kidding was 1.5 ± 0.01.

2.3.2 Body weight

Haas (1978) reported that the birth weight of Boer crosses and East African goats as 2.6 and 2.3 kg, respectively.

Mukundan (1980) documented the birth weight of Malabari kids and Saanen half-breds as 1.71 ± 0.06. and 1.88 ± 0.1 kg, respectively.

Raghavan (1980) observed that the average birth weights of Malabari and Malabari Alpine halfbreds were 1.71 ± 0.03 and 2.04 ± 0.02 kg, respectively.

Nagpal and Chawla (1984) observed that the birth weights in Beetal and Alpine goats were 3.15 ± 0.06 and 3.22 ± 0.13 kg, respectively in intensive system of management.

Mishra and Ghei (1989) observed that the birth weight of local Sikkim goat was 1.6 ± 0.05 kg

Mishra (1991) studied the performance traits in a flock of Sikkim local goats for a period of 3 years. He observed that the birth weights in Sikkim local goats (1.6 kg.) were comparable with that of Assam local and Malabari goats.

According to Malik *et al.* (1993), the body weights at first and third month of age in Black Bengal goats were 2.64 ± 0.21 and 5.4±0.14 kg, respectively. The respective

values in Mangolian and Mangolian X native goats were 13.46 ±1.039 and 9.96±1.03 kg, respectively (Yao *et al.*, 1994).

Ebozoye and Nigerie (1995) reported that mean body weights at 30 and 90 days in West African Dwarf Goats (WAD) and WAD X Red Sokoto goats were 2.87 and 4.87 in WAD kids and 3.80 and 6.05kg, respectively in half bred kids.

Stephen and Rai (1995) analysed the data on the body weight in Malabari and its crosses with Alpine and Saanen goats, born during 1974-1988 at the AICRP on Goats, Mannuthy. The body weights at six months of age in pure bred Malabari, Alpine X Malabari (AM) and Saanen X Malabari (SM) were 9.3 ± 0.16, 10.9 ± 0.11, and 11.3 ± 0.14 kg, respectively.

Gokhale *et al.* (1996) documented that the mean body weights at first and third month of age in non-descript and graded Sirohi goats were 5.29 ± 0.6, 14.95 ± 2.07 and 10.44 ±1.19 kg, respectively. The average body weights of crossbred kids of Black Bengal and Ganjam at first and third month were 4.13 ± 0.34 and 7.81 ± 0.83kg, respectively.

Mohapatra and Nayak (1996) reported that the body weights at six month of age in Black Bengal and Ganjam kids were 9.48 ± 0.97, 12.59 ± 1.15 kg, respectively.

Goonewardene *et al.* (1997) reported that the body weight of Barbari kids at three months of age was 8.10 ± 0.31 kg.

Deb (1998) observed that the body weight at six months in Pashmina goats was 12.2±0.21kg.

In Marwari the body weight of kids at six month of age was 15.69±1.12 kg (Patel *et al.*, 1999)

Sivakumar and Thiagarajan (1999) recorded the body weights of Tellichery Goats from birth to 18 months of age, maintained at the Livestock Research Station in Kattupakkam between 1991 and 1994 and observed that the birth weights of goats were 2.06 ± 0.03 and 1.7 ± 0.04 kg for males and females, respectively.

Tarparia *et al.* (1999) analysed the body weight of goats reared in Aravati region of Rajasthan. They documented the body weight at 180 days was 15.22±0.12 kg.

Thiruvenkadan *et al.* (2000) recorded the body weight of Kanni Adu goats of Tamil Nadu at six months as 12.7± 0.38kg. The respective figure in Jamunapari was 14.86±0.19 (Roy *et. al.*, 2001).

2.4 IMMUNE RESPONSE

There are two major types of immune response, those involving the antibody, which are humoral immune responses and those independent of antibody, called cell-mediated immune response. The pioneering work of Glick and Jaap (1956) led to an understanding of immune system.

2.4.1 Humoral Immune Response

Domestic animals vary in genetic susceptibility to disease, which points the way to effective control of some disease through the development of some genetically resistant stock (Hutt, 1958).

The complexity of the immune response and the many genes involved make it difficult to use immune response as a selection tool. A good response to one antigen does not necessarily predict a good response to another antigen (Benacerraf and Mc Devitt, 1972).

Balcarova *et al.* (1973), Vander Zipp (1978) and Prevzner *et al.* (1973, 1975 and 1981) detected genetic differences in immune response in poultry.

Antigen presentation, site of entry, response of antigen binding cells and regulation of response affect the immune response in birds (Tizard, 1979).

The studies of Almid *et al.* (1980), on the quantitative antibody response to diphtheria toxoid in goats demonstrated that the antibody response could significantly be modified by selective breeding over two to three successive generations.

Mallard *et al.* (1982) used immune response traits as selection markers for improving disease resistance. This strategy was based on the assumption of genetic variation in the traits used and genetic correlation between the markers and disease resistance.

Rothschild *et al.* (1984) reported that the immune responses in different breeds vary by using different antigens.

In most cases, susceptibility to infections is of polygenic origin. However, there are examples of single genetic loci that provide protection against certain infectious agents (Muller and Brem, 1991).

The feasibility of selective breeding depends in part upon the heritability of the trait or traits used to measure disease resistance as well as the amount of variation among animals (Staer *et al.* 2001).

In pigs, Buchanan (1980) observed breed differences in the immune response to sheep RBC (SRBC). Seigel and Gross (1980) reported that additive genetic variation was noticed for high and low antibody titres at five days after SRBC inoculation. They reported that high immune response to sheep red blood cells is negatively correlated with body weight in chicken. Vander Zipp and Leenstra (1983) found that mean total antibody titre to SRBC was highest on seventh day after primary immunization.

. The rapid accumulation of experimental studies demonstrating the analogies between genetic control of specific and general antibody synthesis preludes the

formulation in near future of a unified theory of genetic regulation of immune response (Biozzi *et al.* 1985). They concluded that the mammalian immune responses to complex immunogens such as sheep red blood cells are controlled by polygenic inheritance as evidenced by studies in mice using SRBC as test antigen. Ubosi *et al.* (1985) reported that the peak antibody titre was reached in six days after primary immunization with SRBC in chicken. Mounton *et al.* (1988) reported that the mean antibody titre to SRBC was 6.6 ± 1.8 $\log_2$ in mice resistant to *Salmonella typhinurium* while it was 8.1 ± 2.8 $\log_2$ in susceptible ones. Pinard *et al.* (1992) reported that a selection experiment generated high and low response lines to SRBC in chicken. The mean post primary antibody titre was 4.73 $\log_2$ in the base population. After nine generations of selection, antibody titre was 10.62 $\log_2$ in high response group and 1.94 $\log_2$ in low responder lines.

2.4.2 Cell-mediated Immune Response (CMI)

Cellular immunity is a function of many types of leucocytes including T cells (Nabholz and Mc Donald, 1983), macrophages (Adams and Hamilton, 1984), LAK cells (Andriole *et al.,* 1985) and NK cells (Herberman *et al.,* 1986). A large number of genetic factors influence the cell mediated immune response. Knudtson and Lamount (1989) found that IL –2 production and mitogen response to concavalin (Con A) were not always associated, suggesting that more than one gene or more than one mechanism were likely to influence the mitogenic response of T lymphocytes.

Stiffel *et al.* (1977) selected mice on the basis of lymphocyte stimulation using phytohaemaglutinin (PHA) as mitogen. After six generations 3.8 times difference could be observed between high and low responder groups to PHA. By using PHA, CMI could be obsertved as early as three to fourteen days of age (Corrier and Deloach, 1990). The cutaneous responses to PHA in dogs as indicated by increased skin thickness was 0.777 ± 0.362mm.

2.4.3 Total Protein and Globulin

More and Rawat (1986) reported the total protein concentration in Chokla sheep to be between 6.74 ± 0.12 to 7.38 ± 0.11g/dl.

Sharma *et al.* (1990) observed the total protein content in female Pashmina goat sera as 7.6 ± 0.2 and in males as 8.6 ± 0.2 g/dl.

Total protein and total immunoglobulin in the blood plasma of goats of Punjab was estimated by Mandal (1997) who reported the mean values as 6.12 ± 0.30 g / dl and 3.32 ± 0.08 g/ dl, respectively.

Roy *et al.* (1997) studied the total protein in the goats of Bihar and reported the protein concentration in the blood as 7.97 ± 0.51 g/dl.

The total protein and gamma globulin content in post partum Nubian Goats was 7.86g/dl and 2.40g/dl, respectively (Chen *et al.,* 1998).

Kaushih and Bugalia (1999) reported that the total protein content in goats during early pregnancy was 11.34 ± 0.85 g/dl.

Eswari *et al.* (2000) reported the serum protein, albumin and globulin levels in sheep as 6.08 ± 31, and 2.82 ± 0.16 and 3.26 ± 0.17 g/dl, respectively.

As reported by Kaushish *et al.* (2000), the serum protein, albumin and globulin levels in Jakrana, Marvari and Sirohi averages to 6.12, 2.57 and 3.27gram percent, respectively.

Magesh and Vasu (2000) estimated the serum protein, albumin and globulin levels in goats of Namakkal region of Tamilnadu as 6.70 ± 0.17, 2.70 ± 0.12 and 4.00 ± 0.22 g/dl, respectively.

According to Devi *et al.* (2003), the total protein concentration in goats was 6.017 ± 0.178 g/dl.

Patil *et al.* (2004) reported the mean serum total protein in Osmanabadi goats as 7.01 ± 0.15 g /dl.

Materials and Methods

3. MATERIALS AND METHODS

Blood samples collected from 300 goats, registered under the All India Co-ordinated Research Project on Malabari Goat Improvement, belonging to Tanur, Thalassery and Badagara centers of Kerala state formed the materials for the study.

3.1 HAEMOGLOBIN POLYMORPHISM

Three hundred goats, one hundred each belonging to three different centers, viz. Tanur, Thalassery and Badagara were typed for haemoglobin using Vertical Polyacrylamide Gel Electrophoresis (PAGE).

3.1.1 Polyacrylamide Gel Electrophoresis (PAGE)

Five milliliters of whole blood was collected aseptically from each animal in centrifuge tubes containing heparin (5000 IU/ml) as anticoagulant.The samples were analyzed within 24hrs after collection.

The whole blood was centrifuged; cell pellets were collected and washed with normal saline. Gel slabs were made using eight percent acrylamide gel mix. Tris borate EDTA buffer was used in the top and bottom reservoirs. The cell pellet was diluted with distilled water (1:10). Fifteen microlitres of the diluted cell pellet was loaded in the wells and electrophoresed at 100 V for three hours. The gel was stained with Coomassie Brilliant Blue for 30 minutes and was destained overnight.The allelic frequencies were estimated by the method of Nguyen *et al.* (1992).

3.2 POLYMORPHISM OF BLOOD PROTEINS

3.2.1 PAGE for protiens

SDS PAGE was used for visualizing albumin and transferrin bands. Eight percent of the resolving gel and five percent of the stacking gel were used for the preparation of the Sodium Dodecyl Sulphate (SDS) polyacrylamide gel. Tris-glycine electrophoresis buffer was used in the top and bottom reservoirs. Fifteen microlitres each of the serum samples were loaded in the wells and electrophoresed at 85 Volts for four hours. The gel was stained with Coomassie Brilliant Blue for 30 minutes and was distained overnight in destaining solution.

Native PAGE was employed for typing cerruloplasmin, amylase and carbonic anhydrase. Tris borate EDTA buffer was used in the top and bottom reservoirs. Fifteen microlitres of the serum was loaded in the wells and electrophoresed at 85 V for three hours.

The allelic frequencies were estimated by the method of Nguyen *et al* (1992). The genetic differences between different populations were calculated as described by Balakrishnan and Sanghvi (1968).

3.3 SERUM POTASSIUM POLYMORPHISM

3.3.1 Estimation of Serum Potassium

Serum was separated from five milliliter of blood, collected in centrifuge tubes from the jugular vein of goats. Potassium concentration in the serum was estimated by flame photometry as per the method of Oser (1965). The serum samples were diluted 1:10 with deionised water. A stock standard solution containing 10 meq of potassium was prepared by dissolving 0.746 g of potassium chloride and making up the volume to one litre with de-ionized water. Working standards equivalent to 5, 10, 20 and 40 meq of potassium / litre at a dilution of 1:100 were prepared by diluting 0.5, 1, 2, and 4 ml of stock solution to 100 ml, respectively. The flame photometer reading was initially

adjusted to zero by spraying de-ionized water. The potassium standard solution was then atomized and the readings of the flame photometer were recorded separately for each batch of serum samples.

On the basis of the classification made by Nandakumaran (1989) in Malabari goats, the goats having potassium concentration above 22meq/litre were classified as high potassium and those falling below 22meq / litre categorised as low potassium type since no bimodality could be observed in the frequency distribution curve. The frequency distribution of potassium phenotypes is presented in figure (1).

Economic traits considered for the present study were (a) growth trait viz. body weight at the age of six months and one year and (b) production trait, viz.first lactation yield.

3.4 BLOOD GLUTATHIONE POLYMORPHISM

3.4.1 Estimation of Glutathione Concentration in Blood

For estimating Glutathione and packed cell volume, five millilitre of blood was collected in centrifuge tubes containing one millilitre of Acid Citrate Dextrose (ACD) anticoagulant. The samples were analysed within 24 hrs after collection.

Glutathione level in the whole blood was estimated by the method of Beutler *et al.* (1963), using spectrophotometer at a wavelength of 412 nm. The concentration of GSH/100ml of red blood cells was calculated from the whole blood hematocrit values. Reagents used in the method were as follows:

1.Precipitating solution

Dissolved 1.67g of glacial metaphosphoric acid, 0.2g disodium/dipotassium ethylene diamine tetra acetic acid (EDTA) and 30g of sodium chloride in 100 ml distilled water.

2. Phosphate solution

A solution of 0.3molar Na_2HPO4 was prepared in distilled water (53.4gof $Na_2HPO_4.2H_2O$/litre of distilled water).

3. DTNB Reagent

Dissolved 40 mg of 5. 5' – dithiobis (2-nitrobenzoic acid) in 100 ml of one percent sodium citrate solution.

4. Reagent blank

Reagent blank was prepared with 8 ml of the phosphate solution, 2 ml of the diluted precipitating solution (3 parts to 2 parts of distilled water) and one ml of DTNB reagent. The reagent blank was used to set the optical density of the spectrophotometer to zero before reading the optical densities of the samples.

0.2 ml of whole blood was added to 1.8 ml of distilled water. Three millilitre of the precipitating solution was mixed with the haemolysate. The mixture was allowed to stand for about five minutes to precipitate all the proteins and then filtered. Two millilitre of the filtrate was added to 8 ml of the phosphate solution in the cuvette. To this, one millilitre of the DTNB solution was added. The optical density was then measured in the spectrophotometer after setting the optical density to zero with the reagent blank.

Glutathione (GSH) level was calculated on the basis of molar extinction coefficient of 13,600 and molecular weight of 307. The glutathione content of blood (mg per 100 ml red blood cells) was calculated by the following formula when 0.2 ml of blood sample was used.

GSH mg/ 100 ml RBC: OD/13,600 x 5/0.2 x 11/2 x 307 x 100 x 100/PCV = OD x 310.4 x100/PCV (Beutler *et al.*, 1963).

OD= optical density of the spectrophotometer

PCV = Packed cell volume. (Packed cell volume was determined by the microhaematocrit tube method.)

The classification of goats into two distinct glutathione types as suggested by Agar *et al.* (1974) was followed. Goats with GSH values below 60 mg / 100 ml RBC were classified as low glutathione type (GSH low type) and those with values of 60 mg/ ml RBC and above as high glutathione type (GSH high type). The classification was necessitated because there was no bimodality observed as evident from the frequency curve (fig.2).

The gene frequencies at different loci and phenotypic frequencies were calculated by direct counting method. The genetic differences between different populations were calculated as per the method described by Balakrishnan and Sanghvi (1968).

3.5 POLYMORPHISM OF MICROSATELLITE MARKERS

3.5.1 Isolation of DNA from Whole Blood

Blood samples (5ml each) were collected from the jugular vein aseptically using sterile disposable syringes and transferred immediately into sterile disposable centrifuge tubes containing EDTA (1mg/ml of blood) as anticoagulant. The samples were brought in ice to the laboratory and stored at 4^0C till further processing.

DNA was extracted from whole blood using the standard phenol chloroform extraction procedure (Sambrook *et al.*, 1989) with modifications.

3.5.2 PCR Analysis

3.5.2.1 Template DNA

Working solutions of DNA samples were prepared from the DNA stock solution by diluting with sterile triple distilled water to get a final concentration of 50 ng/µl. One microlitre of this working solution was used for every 10 µl PCR assay.

3.5.2.2 Selection of Primers

A set of ten microsatellite markers was selected from available literature and the primers were custom synthesized. Three markers, viz. INRA063, ILSTS030 and HUJ 1177 which exhibited comparatively higher degree of polymorphism among those typed, were chosen for the study. The sequences of the forward and reverse primers for each locus are as follows.

INRA063

F 5'- ATTTGCACAAGCTAAATCTAACC -3'

R 5'- AAACCACAGAAATGCTTGGAAG -3'

ILSTS030

F 5'- CTGCAGTTCTGCATATGTGG -3'

R 5'-CTTAGACAACAGGGGTTTGG -3'

HUJII77

F 5'- TCCATCAAGTATTTGAGTGCAA -3'

R 5'- ATAGCCCTACCCACTGTTTCTG -3'

3.5.2.3 Incorporation of Radioactivity: End-Labelling of Primers

For visualizing the PCR products by autoradiography, one of the primers was radiolabeled. The forward primer for each marker was radiolabeled at the 5' end with γ^{32} P-ATP. The reaction was carried out with the DNA End-labeling Kit.

The procedure for end labeling was as follows:

The following components were added to a 0.2ml-microcentrifuge tube in the order.

10X Polynucleotide kinase (PNK) buffer	-	1 µl
Forward Primer (20p*M*/µl)	-	1 µl
T_4 Polynucleotide kinase (5U/µl)	-	0.5µl
γ^{32} P-ATP (10mCi/ml)	-	1 µl
Nuclease free water	-	6.5µl

The mixture was incubated at 37°C for 30 minutes. The final volume was made up to 40µl. One microlitre of this end-labeled primer was used for every 10µl PCR assay with sterile distilled water.

3.5.2.4 PCR conditions

The PCR conditions for each microsatellite loci were standardized separately. Each reaction was carried out in 10µl volume. PCR reaction was set up with 1µl of 10 X PCR buffer (15mM $MgCl_2$, 100m*M* Tris-pH 8.3, 500m*M* KCl), 200µ*M* of each dNTPs, 1µl of end-labeled and diluted forward primer, 5p*M* of reverse primer and 0.3U of Taq DNA polymerase. Concentration of $MgCl_2$ used was 1.5mM for all the primers selected. The reaction mixture was mixed well and subjected to amplification in a thermal cycler (Techne Flexigene). The thermal cycling involved 35 cycles each consisting of denaturation at 94°C for one minute, annealing at 60°C for INRA063 and ILSTS 030, 58°C for HUJ 1177 for one minute and extension at 72°C for one minute. This was followed by a final extension for five minutes at 72°C. The samples were then cooled down to 4°C and stored at -20°C till further analysis.

3.5.3 Sequencing M13 Bacteriophage DNA

Determination of the exact size of alleles necessitated comparison with a sequencing ladder from M13. Single stranded M13 phage DNA was sequenced using the

DNA Sequencing Kit Version 2.0 (Amersham Biosciences Corporation, USA) according to the manufacturer's instructions.

3.5.4 Denaturing Polyacrylamide Gel Electrophoresis

The radioactively labeled PCR products were subjected to electrophoresis on six per cent denaturing polyacrylamide gels for better resolution. Denaturing Polyacrylamide Gel Electrophoresis (PAGE) was performed on the Vertical Sequencer (Consort, Belgium) as described by Biggin *et al.* (1983). The gels were set between two glass plates (41 x 33cm) separated by 0.35 mm thick spacers.

The gel was prepared by mixing 60ml of 0.5X TBE gel mix (6 percent Acrylamide, 6*M* urea, 0.5X TBE) and 125µl each of 10 percent Ammonium persulphate solution (APS) and N, N, N', N', Tetra Methyl Ethylene Diamine (TEMED) in a beaker. The gel was allowed to set for an hour before electrophoresis. The upper and lower electrode tanks were filled with IX Tris Borate EDTA (pH 8.3) buffer (TBE 0.045*M* Tris borate, 0.001*M* EDTA) to the required level.

The PCR products were mixed with 3.5µl formamide loading buffer denatured at 95°C for 5 min and cooled immediately in ice. About 4µl each of this mixture was loaded into each well. Sequenced products of M13 DNA, which were also denatured at 95°C for five minutes, were loaded simultaneously in the middle or side wells.

The gels were electrophoresed at 40W for three hours maintaining a temperature of around 40°C. The gels were dried in a gel drier at 80°C for one and a half hours.

3.5.5 Autoradiography

The Sarran wrap was removed after drying and the gel was set for autoradiography with X-ray film (Kodak, 35.6 x 43.2cm) in a cassette (Kiran Hypercassette) fitted with an intensifying screen. The X-ray film was developed after 24 to 48 hours depending on the intensity of radioactive signal.

3.5.6 Development of X-Ray Film

The X-Ray film was developed in the dark room. Developing was done by transferring the film serially into I X developer solution (Kodak) for three to five minutes, one per cent acetic acid for a minute followed by washing in distilled water and finally into fixer solution (Kodak) for six to ten minutes. The developed film was washed thoroughly in running water and dried.

3.5.7 Microsatellite Typing

The genotypes of animals were determined for each microsatellite loci by comparing the sizes of alleles with M 13 sequencing ladder. The G, A, T and C sequences were read from the bottom to the top in order. The allele sizes were assigned corresponding to the G, A, T, C bands. The frequency at each locus was determined by direct counting.

3.5.8 Statistical Analysis

3.5.8.1 Heterozygosity

Heterozygosity is a measure of usefulness of the marker. Heterozygosity was calculated by the method of Ott (1992).

$$He = 1 - \sum_{i=1}^{k} p_i^2$$

where p_i is the frequency of i^{th} allele at a locus. Markers with higher heterozygosity values are more useful.

3.5.8.2 Polymorphic Information Content (PIC)

The polymorphic information content expresses informativeness or usefulness of a marker for linkage studies. The PIC values of the markers were calculated as;

$$PIC = 1-[\sum_{i=1}^{k} p_i^2] - \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} 2p_i^2 p_j^2$$

Where p_i and p_j are the frequencies of i^{th} and j^{th} alleles, respectively (Botstein *et al.*, 1980).

3.5.8.3 Genetic Distance

The genetic distance between the three populations were computed using Nei's method (1978). The distance measures were derived using the software POPGENE .

3.5.8.4 Dendrogram

A dendrogam representing the relationships of the animals belonging to the three goat populations was constructed using the POPGENE software.

3.6 BIOMETRICAL TRAITS

The litter size of goats was recorded as singles, twins, triplets and quadruplets. The body weight of kids was recorded at birth, and at completion of one month, three months, six months and also at one year of age. These data were analysed as described by Snedocor and Cochran (1985).

3.7 IMMUNE RESPONSE

3.7.1 Humoral Immune Response

3.7.1.1 Test antigen preparation

Sheep red blood cells (SRBC) were chosen as the test antigen, since they were complex and apparently harmless antigens. One hundred milliliters of blood was collected from a single sheep in Alsever's solution and was washed thrice in sterile

phosphate buffered saline (PBS0.01M, pH 7.2) by repetitive centrifugation (1500 rpm for 10 minutes). The red blood cells were suspended in fresh PBS to get a final concentration of 20 percent (V/V) and stored at 4 ^{0}C in sterile glass containers until immunization / antibody titration.

3.7.1.2 Administration of antigen

For immunisation, test antigen was injected intravenously at the rate of 0.5 ml per kilogram body weight through juguar vein.

3.7.1.3 Collection of the blood for serum

From blood samples collected at day zero, seven, fifteen and twenty-one days post immunisation, sera were separated and stored in tightly capped storage vials at -5^{0}C until used.

3.7.1.4 Anti SRBC titre assay

Serum antibody titre to SRBC was titrated by microhaemolytic test as described by Hines (1985). The test was carried out in 96 welled microtitre plates .To each well 0.05 ml of dilute serum was added followed by 0.025 ml of two percent suspension of SRBC in PBS and 0.025ml of fresh guinea pig serum was added as the source of complement. The microtitre wells were covered and the plates were shaken well. Readings of the test were taken after two hours of incubation at 30^{0}C. The extent of haemolysis was read as follows:

0: All cells intact and settled at bottom. Supernatant was clear

1: Nearly twenty percent of cells lysed. Supernatant was reddish coloured.

2: Nearly fifty percent of cells lysed. The intact cells formed a small button or ring at the bottom. Supernatant was red.

3: Nearly ninety per cent of cells lysed. Supernatant was bright red which on shaking became cloudy.

4: All cells were lysed. Whole liquid was bright red and retained the brightness even

after shaking.

The antibody was recorded as the highest dilution of serum giving reaction to the extent of two.

Antibody titres to SRBC were transformed to $\log_e$ of antibody titre plus one so that antibody responses measured were normally distributed (Burton *et al.*, 1989).

3.7.2 Cell Mediated Immune Response

The cell-mediated immune responses were assessed by cutaneous responses to the intradermal injection of Phytohaemaglutinin-M (PHA-M).

3.7.2.1 PHA Skin Test

In *vivo* T-lymphocyte response to PHA-M was assessed as described by Wilkie *et al.* (1991) with suitable modifications. The skin on the neck region was clipped and cleaned with alcohol. The thickness of the skin was measured using a skin fold caliper. Phytohaemaglutinin (Sigma Chemicals) was dissolved in sterile saline and diluted to contain 50µg in 0.1ml. To ensure uniformity of the reagent, the prepared solution was kept frozen at -30 ^{0}C until half an hour before use. A 0.1ml quantity was injected intradermally. The skin thickness was recorded at 24, 48 and 72 hours after injection. The increase in skin thickness was expressed in millimetre and tabulated.

3.8 ESTIMATION OF TOTAL PROTEIN, ALBUMIN AND GLOBULIN

Total protein and albumin were estimated by biuret method (Weichelbaum, 1946) and bromocresol green method (Doumas, 1971) respectively using standard kits from Agappe. Serum globulin level was calculated by the method described by Benjamin (1985). Statistical analysis was done as described by Snedocor and Cochran (1985).

Results

4. RESULTS

4.1 HAEMOGLOBIN POLYMORPHISM

A total of 300 blood samples, 100 each from goats belonging to three different populations at Tanur, Thalassery and Badagara were used for the study of haemoglobin polymorphism using vertical non-denaturing polyacrylamide electrophoresis.

On electrophoresis, the haemoglobin bands showed distinct movement towards anodic end of the electrophoretogram and two electrophoretically distinct haemoglobin variants were identified. The fast moving one was designated as Hb^A while the slow moving one was Hb^B. Individual animals possessed either one or both the haemoglobins and were accordingly designated as Hb_{AA}, Hb_{BB} and Hb_{AB}. All the three haemoglobin phenotypes (Hb_{AA}, Hb_{AB} and Hb_{BB}) were observed in the present study (Fig.3). The haemoglobin pattern of animals belonging to three different centers is given in table 1.

Among 300 animals typed, 293 animals were of Hb_{AA}, six were of Hb_{AB} and only one of Hb_{BB} phenotype with a gene frequency of 0.987 and 0.012 with regard to Hb^A and Hb^B, respectively. The chi square test revealed that the population as a whole was not in Hardy-Weinberg equilibrium (Table 1).

All the animals belonging to Tanur and Badagara were of Hb_{AA} type. But the goat population of Thalassery possessed Hb_{AA}, Hb_{AB} and Hb_{BB} phenotypes. Out of 100 animals typed, six animals were of Hb_{AB} and only one was of Hb_{BB} phenotype. The gene frequency of Hb^A and Hb^B were 1 and 0 for Tanur and

Badagara populations, respectively. The respective figures were 0.962 and 0.038 for Thalassery population.

4.2 POLYMORPHISM OF BLOOD PROTEINS

4.2.1 Transferrin

Blood samples of 300 goats belonging to three different goat populations, 100 each at Tanur, Thalassey and Badagara were electrophoresed using discontinuous vertical polyacrylamide electrophoresis.

On electrophoresis, transferrin variants showed distinct movement towards anodic end of the electrophoretogram revealing two electrophoretically distinct transferrin types. The fast moving one was designated as Tf^A and slow moving band was designated as Tf^B in accordance with the nomenclature of Trehan *et al.* (1981). Different transferrin phenotypes observed in the goat populations under the present study are depicted in figure 4. Phenotypic frequencies and gene frequencies of transferrin types in goats of Tanur, Thalassery and Badagara are presented in table 2.

Individual animals were found to possess either one or both the transferrin types. Transferrin AB phenotype could be observed in Tanur and Badagara populations, unlike in Thalassery population, where all the animals typed were of Tf_{AA} type. No Tf_{BB} phenotype could be detected in the present study. Phenotypes Tf_{AA} were represented by two bands each on the polyacrylamide gel, while phenotypes AB were represented by three bands. Out of the 100 animals studied in the Tanur area, 99 animals were of Tf_{AA} type and only one was of Tf_{AB}. In Thalassery all the 100 animals belonged to Tf_{AA} type. In Badagara, of the 100 animals studied, 95 were of Tf_{AA} while five belonged to Tf_{AB} type.

The gene frequency of Tf^A was high in Tanur and Badagara populations (0.995 and 0.974, respectively) while that of Tf^B was 0.005 and 0.026, respectively. In pooled population, gene frequency of Tf^A and Tf^B were 0.990 and 0.010, respectively. In the total population studied, a predominance of the Tf^A variant could be detected. The chi square values revealed that the population was in Hardy-Wienberg equilibrium (Table.2).

4.2.2 Albumin

Two bands each were observed for albumin in all the animals studied. A fast moving band designated as Al^F and a slow moving band Al^S, revealing absence of polymorphism at albumin locus (Fig.5).

4.2.3 Cerruloplasmin, Amylase and Carbonic Anhydrase

A total of 300 animals were typed for cerruloplasmin, amylase and carbonic anhydrase on polyacrylamide gel. Single band could be observed for cerruloplasmin, amylase and carbonic anhydrase in all the animals studied, indicating absence of polymorphism at the three loci studied (Fig.6).

4.3 SERUM POTASSIUM POLYMORPHISM

The concentration of potassium in milliequalents per litre (meq/l) in serum of goats belonging to three different populations at Tanur, Thalassery and Badagara is presented in table 3. The mean serum potassium concentration in the pooled population was 4.18± 0.09 meq/l. The concentrations of serum potassium in Tanur, Thalassery and Badagara were 3.45±0.11, 4.52±0.20 and 4.60±0.14 meq/l, respectively (Fig.7).

Among the three goat populations tested, the highest mean concentration for potassium was detected in Badagara population and the lowest in Tanur with the Thalassery population averaging more towards that of Badagara population. Least square analysis of variance of potassium concentration in different populations showed that there is significant difference between goat populations.

4.4 BLOOD GLUTATHIONE (GSH) POLYMORPHISM

The concentration of erythrocyte glutathione (mg/100ml red blood cell) was estimated in 300 goats belonging to three different localities, viz. Tanur, Thalassery and Badagara to find out the polymorphism at this locus. The mean GSH concentrations in mg/100ml RBC are presented in the table 4.

The mean high GSH concentrations in different populations are depicted in figure 8. The mean GSH concentrations were 94.45 mg/100ml RBC for high and 33.16 mg/100ml RBC for low GSH groups in the pooled population. The mean values of high GSH were 103.19, 76.27 and 103.90 mg/100ml RBC for Tanur, Thalassery and Badagara, respectively. For low GSH the respective values were 34.40, 29.63 and 35.47 mg/100ml RBC. The number of goats in each GSH groups and the corresponding gene frequencies are given in table 5.

The gene frequencies of GSH^H were 0.45, 0.09 and 0.30 in Tanur, Thalassey and Badagara goat populations, respectively whereas the respective gene frequencies of GSH^h were 0.55, 0.91 and 0.70. In the pooled population, the gene frequency of GSH^H and GSH^h were 0.27 and 0.73, respectively. In all the

sub-populations as well as in the pooled population the frequency of GSH^h was found to be higher.

In the pooled goat population, 46.33 per cent of the animals belonged to high GSH where as 53.67 per cent of the animals were categorised to low GSH type. The percentage of GSH high type in Tanur, Thalassery and Badagara populations were 70, 17 and 51 where as low GSH type were 30, 83 and 49, respectively. In Thalassery population, unlike the other two sub-populations, the percentage of GSH low phenotype was found to be higher.

Least square analysis of variance of GSH values revealed the existence of significant difference between the different sub- populations.

4.5 GENETIC DISTANCE BETWEEN POPULATIONS

Out of the eight loci tested, in three goat populations of Tanur, Thalassey and Badagara, polymorphism could be observed only at haemoglobin, transferrin and glutathione loci. Cerruloplasmin, albumin, amylase, serum potassium and carbonic anhydrase were found to be monomorphic. Genetic distances calculated using the method described by Balakrishnan and Sanghvi (1968) was found to be 0.1249, between Tanur and Badagara, while that between Tanur and Thalassery was 0.6690 and between Badagara and Thalassery was 0.3351. Genetic distance values between different sub populations based on allelic frequencies are presented in table 6.

Genetic distances were also calculated using the allelic frequencies of polymorphic loci using computerized GENDIST program of PHYLIP version 3.63. Genetic distance between Tanur and Badagara was found to be 0.0092, while that between Tanur and Thalassery was 0.0498 and between Badagara and

Thalassery was 0.0166. Genetic distance values are presented in table 7.

4.6 POLYMORPHISM OF MICROSATELLITE MARKERS

4.6.1 Yield and quality of DNA

The average yield of DNA obtained from five milliliter of whole blood sample was 350.05 ± 10.05 μm. The concentration of DNA averaged about 700.11 ± 20.10 μm /ml. The ratio of optical density at 260nm and 280 nm was constantly around 1.70±0.006, indicating good deproteinisation. Values are presented in table 8. Out of the ten-microsatellite markers tested, seven markers (TGLA53, ILSTS 005, INRA 005, ETH 10, ILSTS 011, BM 720 and INHA) exhibited very low degree of polymorphism in goats. Three primers (INRA 063, ILSTS 030 and HUJ 1177) were found to be polymorphic.

4.6.2 INRA 063 locus

A total of 16 genotypes were observed for INRA 063 in Tanur and 11 each in Thalassery and Badagara populations, respectively (Table.9). A total of 28 genotypes were observed in the pooled population.

A total of ten, five and eight alleles could be observed for INRA 063 locus in goats of Tanur, Thalassery and Badagara, respectively. The allele size and frequencies at INRA 063 locus are presented in table 10. The allele size ranged from 155 to173 bp in Tanur, 163 to 171bp in Thalassery and 157 to173 bp in Badagara populations. The maximum number of alleles (10) was observed in

Tanur population and the minimum (5) in Thalassery. Badagara recorded eight alleles. Six alleles (163/155, 163/159, 165/157, 169/163, 173/163 and 173/165) were specific to Tanur population while three alleles each were specific to the other two populations (163/163, 165/159 and 171/171 in Thalassery and 167/165,169/157and 169/167 in Badagara). The allele size of 163 bp occurred in highest frequency (0.28125) in Tanur, 167 bp topped at a frequency of 0.234375 in Badagara and 171 bp in Thalassery at 0.527778. Two alleles found exclusively in Tanur were 155 and 159 bp. Likewise alleles 157, 161 and 173 were absent in Thalassery population (Fig.8).

The genotypes and their frequencies at this locus are summarised in table 11. In Tanur, 169/163 genotype was observed with maximum frequency of 0.187500 while in Thalassery, 171/163 genotype recorded the highest frequency of 0.277778 and in Badagara, 169/167 genotype recorded highest with frequency of 0.156250. The maximum values for heterozygosity (0.847) and PIC (0.843) were observed in Tanur while the lowest were recorded in Thalassery (0.656 and 0.652, respectively). In Badagara, values for heterozygosity and PIC were 0.826 and 0.815, respectively. The details of heterozygosity and PIC are summarized in table 12.

4.6.3 HUJ 1177 Locus

The highest polymorphism was observed at this locus (Fig.9). The number of alleles observed for HUJ 1177 loci in Tanur, Thalassery and Badagara populations were 15, 14 and 13, respectively. The allele size ranged between 196 bp to 228 bp in Badagara, 188 bp to 228 bp in Tanur and 194 bp to 230 bp in Thalassery. Two alleles each were found specific for Tanur (188 and 204 bp) and Thalassery (200 and 230 bp) populations. Allele 226 bp was found exclusive for

Badagara population. The allele size and frequency at the same locus are summarized in table 13.

Twenty-seven genotypes were recorded in goat populations of Tanur, while 24 each were recorded in Thalassery and Badagara populations (Table.9). The genotypes and their frequencies are presented in table 14. Both values for heterozygosity and PIC in Tanur and Badagara were recorded as 0.894 and 0.880, while at Thalassery the heterozygosity and PIC values stood at 0.909 and 0.907, respectively. The heterozygosity and PIC values at HUJ 1177 loci are presented in table 15.

4.6.4 ILSTS 030 Locus

The maximum number of alleles at this locus was observed in Tanur (11) whereas the minimum was recorded in Thalassery (7) while Badagara recorded nine. The allele size ranged between 156bp to180 bp in Tanur, 164 bp to184 bp in Thalassery and 162 bp to180 bp in Badagara. The allele size and frequency are summarized in table 16. The polymorphism at ILSTS 030 locus is depicted in figure 10.

A maximum number of 18 genotypes were observed for ILSTS030 locus in goats of Badagara, while the lowest number of nine was recorded in Thalassery. In Tanur 16 genotypes were recorded (Table.9). The genotypes and their frequencies of ILSTS 030 are summarised in table 17. The highest values for heterozygosity (0.850) and PIC (0.848) were detected in Tanur population, while the lowest (0.793 and 0.775) was recorded in Thalassery. The heterozygosity and PIC values were 0.828 and 0.823, respectively in Badagara. The heterozygosity and PIC values of ILSTS 030 are presented in table 18.

4.6.5 Genetic Distance based on Microsatellite Markers

4.6.5.1 Genetic Distance (POPGENE Version 1.31 according to Nei's Formula, 1978)

The genetic distance between Thalassery and Badagara was found to be 0.5729, between Thalassery and Tanur was 0.7795 and between Badagara and Tanur recorded as 0.8401. The maximum genetic distance was recorded between Badagara and Tanur, while the minimum was recorded between Thalassery and Badagara. Genetic distance matrix is presented in table 19.

4.6.6 Dendrograms

The dendrogram of relationship between the three different goat populations based on POPGENE program (Nei's, 1978) showed one cluster, grouping Thalassery and Badagara populations. The dendrogram is presented in figure 11.

4.7 BIOMETRICAL AND REPRODUCTION TRAITS

4.7.1 Litter Size

Prolificacy percentages of goats in Tanur,Thalasery and Badagara are given in table 20 and figure 12. The percentage of single kids born in Tanur during 2004-05 was 22.71 while in Thalassery and Badagara were 34.15 and 34.82, respectively. The twin births in Tanur, Thalassery and Badagara were found to be 58.95, 54.46 and 50.37 per cent, respectively. The percentage occurance of triplets was higher in Tanur (15.72) when compared to Thalassery (10.05) and Badagara (14.44). The percentage of quadruplets born in Tanur (2.62) was found to be the highest when compared to Thalassery (1.34) and Badagara (0.37).

4.7.2 Body Weight

The body weight data are presented in table 21 and figure 13. The mean birth weights recorded in kilogram in Tanur, Thalassery and Badagara were 1.73 ± 0.04, 1.88 ± 0.03 and 1.86 ± 0.05 kg, respectively. The respective body weights recorded at first month of age were 4.42 ± 0.04, 5.37 ± 0.17 and 4.5 ± 0.15 kg. At three months, the values for body weight were 8.83 ± 0.30, 10.02 ± 0.24 and 9.52 ± 0.26 kg, respectively. Weight recorded at sixth month of age in the respective populations were 17.83 ±1.54, 21 ± 0.47 and 21.36 ± 0.55 kg. The body weights at first year of age were 18.37 ± 2.07, 27.00 ± 1.75 and 25.58 ± 0.76 kg, respectively. The percentage of multiple births in goat populations of Tanur, Thalassery and Badagara were found to be 0.76, 0.66 and 0.68 respectively (Table.22). On an average the goats of Thalassery and Badagara were found to be heavier when compared to Tanur but the litter size was high in Tanur animals when compared to the other two populations

4.8 IMMUNE RESPONSE

4.8.1 Humoral Immune Response

The antibody titre ($1+\log_e$) to sheep RBC is documented in table 23 (Fig.14). None of the goat populations under the present study showed naturally occurring antibodies to sheep RBC as evidenced by the pre-immunisation titre values. On seventh day after primary immunisation, the antibody titre in goat population of Tanur, Thalassery and Badagara rose to mean values of 3.24 ± 0.05, 3.23 ± 0.06 and 3.33 ± 0.06, respectively. The fifteenth day post immunisation antibody response in Tanur, Thalassery and Badagara had mean values of 2.92 ± 0.05, 2.81 ± 0.05 and 2.87 ± 0.05, respectively. The twenty-first day antibody

response in Tanur, Thalassery and Badagara populations had mean titres of 2.55 ± 0.06, 2.52 ± 0.06 and 2.54 ± 0.06, respectively.

The least square analysis of variance of immune response on seventh, fifteenth and twenty first day revealed no significant difference between different sub-populations.

4.7.1.1 Effect of Diseases

The effect of antibody response to SRBC on 7th, 15th and 21st day after primary immunisation was not found to be significant for the occurrence of diseases like diarrhea and pneumonia (Table.24).

4.7.2 Cell - mediated Immune Response

4.7.2.1 Cutaneous Response To Intradermal Injection to Phytohaemagluttinin – M

The cutaneous response to intradermal injection to Phytohaemagluttinin –M (PHA-M) is given in table 25 and figure 15. The pre injection skin thickness in goat populations of Tanur, Thalassery and Badagara had mean values of 1.34 ± 0.01, 1.33± 0.02 and 1.34 ± 0.02mm. The mean increase in skin thickness at 24 hours of intradermal injection of (PHA-M) in goat populations of Tanur, Thalassery and Badagara were1.84 ± 0.04mm, 1.86 ± 0.03mm and1.95 ± 0.04mm, respectively. The 48 hour mean increases in skin thickness in the respective populations were 1.69 ± 0.02, 1.72 ± 0.02 and 1.76 ± 0.02 mm. The 72 hour skin thickness were 1.61 ± 0.02, 1.62 ± 0.02 and 1.65 ± 0.02 mm in Tanur, Thalassery and Badagara populations. The least square analysis of variance revealed that the pre and post immunization skin thickness at 0, 24, 48 and 72 hours were non

significant between different sub populations.

4.8 TOTAL PROTEIN, ALBUMIN AND GLOBULIN

4.8.1 Total Protein

The mean concentrations of total protein in goat populations of Tanur, Thalassery and Badagara were found to be 4.75±0.32, 5.54±0.37 and 6.01±0.22g/dl, respectively (Fig.16 and Table.26).The highest concentration was recorded in Badagara population while the lowest was in Tanur. The least square analysis of variance of total protein concentration revealed significant difference between the sub-populations.

4.8.2 Albumin

The concentration of albumin in goat populations of Tanur, Thalassery and Badagara averaged to be 2.41 ± 0.06, 2.41 ± 0.10 and 2.73 ± 0.10 g/dl, respectively (Fig.16 and Table.26). The highest mean value was recorded in Badagara, while more or less same values were obtained from Tanur and Thalassery. The least square analysis of variance of albumin concentration revealed significant difference between the sub- populations.

4.8.3 Globulin

The mean concentration of globulin in goat populations of Tanur, Thalassery and Badagara were 2.34 ± 0.31, 3.13 ± 0.31 and 3.28 ± 0.22 g/dl, respectively (Fig.16and Table.26). Among the three goat populations tested, the highest mean concentration for globulin was detected in Badagara population and the lowest in Tanur with the Thalassery population averaging more towards that of

Badagara population. The least square analysis of variance of globulin concentration revealed significant difference between populations.

Table1. Phenotype frequencies and Gene frequencies of Haemoglobin variants in Malabari goats

Population	No. of animals	Pheneotype frequencies			Gene frequencies		Chi square
		Hb_{AA}	Hb_{AB}	Hb_{BB}	Hb^A	Hb^B	
Tanur	100	100	0	0	1	0	0.00
Thalassery	100	93	6	1	0.962	0.038	5.52*
Badagara	100	100	0	0	1	0	0.00
Pooled	300	97.67(293)	2.00(6)	0.33(1)	0.987	0.012	24.73**

* $p \geq 0.05$

**$p \geq 0.01$ Observed number in the paranthesis

Table 2. Phenotype frequencies and gene frequencies of Transferrin types in Malabari goats

Population	No.of animals	Phenotype frequencies		Gene frequencies		Chi Square
		Tf_{AA}	Tf_{AB}	Tf^A	Tf^B	
Tanur	100	99	1	0.995	0.005	0.003*
Thalassery	100	100	0	1	0	0.000
Badagara	100	95	5	0.974	0.026	0.068*
Pooled	300	98 (294)	2 (6)	0.990	0.010	0.030*

*$P \leq 0.05$ Observed number in the paranthesis

Table 3. Mean concentration of serum potassium (meq/l.) in Malabari goats

Population	Number of animals	Mean
Tanur	100	3.45 ± 0.11
Thalassery	100	4.52 ± 0.20
Badagara	100	4.60 ± 0.14
Pooled	300	4.18 ± 0.09

Table 4. Mean GSH concentrations (mg/100ml RBC) in Malabari goat populations

Population	Mean	
	High GSH	Low GSH
Tanur	103.19	34.40
Thalassery	76.27	29.63
Badagara	103.90	35.47
pooled	94.45	33.16

Table 5. Distribution of GSH phenotypes and gene frequencies of GSH^H and GSH^h in Malabari goats

Population	Percentage of animals			Gene frequency	
	Total	Low GSH	High GSH	GSH^H	GSH^h
Tanur	100	30	70	0.45	0.55
Thalassery	100	83	17	0.09	0.91
Badagara	100	49	51	0.30	0.70
Pooled	300	53.67(162)	46.33(138)	0.27	0.73

Observed number in the paranthesis

Table 6. Genetic distance between populations (Balakrishnan and Sanghvi, 1968) using allelic frequencies of protein polymorphic loci

Population	Thalassery	Tanur	Badagara
Thalassery	*****		
Tanur	0.6690	*****	
Badagara	0.3351	0.1249	*****

Table 7.Genetic distances between populations (PHYLIP)

Population	Thalassery	Tanur	Badagara
Thalassery	*****		
Tanur	0.0498	*****	
Badagara	0.0166	0.0092	*****

Table 8. Yield and purity of DNA isolated from the blood of Malabari goats belonging to Tanur, Thalassery and Badagara

Parameters	Mean
Concentration μm /ml	700.11 ± 20.10
Yield / 5ml	350.05 ± 10.05μm
OD ratio (260/280nm)	1.70 ± 0.006

Table 9. Number of genotypes observed in Tanur, Thalassery and Badagara goat populations for different markers

Marker loci	Tanur	Thalassery	Badagara
INRA063	16	11	11
ILSTS 030	16	9	18
HUJ 1177	27	24	24

Table 10. Allele size and frequency at INRA 063 Locus in Malabari goats

Sl. No.	Allele size	Tanur	Badagara	Thalassery
1	155	0.015625	0.0000000	0.000000
2	157	0.078125	0.015625	0.000000
3	159	0.031250	0.0000000	0.000000
4	161	0.093750	0.031250	0.000000
5	163	0.281250	0.046875	0.166667
6	165	0.109375	0.187500	0.166667
7	167	0.078125	0.234375	0.083333
8	169	0.171875	0.203125	0.055556
9	171	0.078125	0.140625	0.527778
10	173	0.062500	0.140625	0.000000

Table 11. Genotypes and frequencies at INRA063 locus in Malabari goats

Sl. No.	Genotype	Frequency		
		Tanur	Thalassery	Badagara
1	163/155	0.031250	0.000000	0.000000
2	163/157	0.093750	0.111111	0.000000
3	163/159	0.031250	0.000000	0.000000
4	163/163	0.000000	0.027778	0.000000
5	165/157	0.062500	0.000000	0.00000
6	165/159	0.000000	0.027778	0.000000
7	165/161	0.031250	0.027778	0.000000
8	165/165	0.031250	0.000000	0.09375
9	167/159	0.031250	0.027778	0.000000
10	167/163	0.062500	0.000000	0.093750
11	167/165	0.000000	0.000000	0.093750
12	167/167	0.031250	0.000000	0.031250
13	169/157	0.000000	0.000000	0.031250
14	169/161	0.093750	0.027778	0.000000
15	169/163	0.187500	0.000000	0.000000
16	169/165	0.000000	0.055556	0.031250
17	169/167	0.000000	0.000000	0.156250
18	169/169	0.031250	0.000000	0.062500
19	171/161	0.062500	0.000000	0.062500
20	171/163	0.093750	0.277778	0.000000
21	171/165	0.000000	0.111111	0.062500

Table 11. (Continued) Genotypes and frequencies at INRA063 locus in Malabari goats

22	171/167	0.000000	0.083333	0.062500
23	171/171	0.000000	0.027778	0.000000
24	173/163	0.062500	0.000000	0.000000
25	173/165	0.031250	0.000000	0.000000
26	173/169	0.000000	0.000000	0.062500
27	173/171	0.000000	0.000000	0.012500
28	173/173	0.000000	0.000000	0.032500

Table 12. Heterozygosity and PIC value at INRA 063 locus in Malabari goat populations of Tanur, Thalassery and Badagara

Populations	No.of alleles	Heterozygosity	PIC
Tanur	10	0.847	0.843
Thalassery	5	0.656	0.652
Badagara	8	0.826	0.815

Table 13. Allele size and frequency at the HUJ 1177 locus in Malabari goat populations of Tanur, Thalassery and Badagara

Sl. No	Allele size (bp)	Tanur	Thalassery	Badagara
1	188	0.027027	0.000000	0.000000
2	194	0.108108	0.102941	0.000000
3	196	0.081081	0.102941	0.013889
4	198	0.067568	0.073529	0.125000
5	200	0.000000	0.029412	0.000000
6	202	0.040541	0.058824	0.166667
7	204	0.013514	0.000000	0.000000
8	208	0.148649	0.014706	0.000000
9	210	0.040541	0.000000	0.027778
10	212	0.175676	0.088235	0.013889
11	214	0.013514	0.058824	0.027778
12	216	0.121622	0.000000	0.138889
13	218	0.013514	0.029412	0.041667
14	220	0.094595	0.044118	0.055556
15	222	0.000000	0.132353	0.069444
16	224	0.040541	0.117647	0.069444
17	226	0.000000	0.000000	0.055556
18	228	0.013514	0.117647	0.194444
19	230	0.000000	0.029412	0.000000

Table 14. Genotypes and frequencies at HUJ1177 locus in the goat populations of Tanur,Thalassery and Badagara

Sl.No.	Genotype	Frequency		
		Tanur	Thalasserry	Badagara
1	188/188	0.270270	0.000000	0.000000
2	194/194	0.054054	0.029412	0.000000
3	194/198	0.027027	0.000000	0.000000
4	196/194	0.000000	0.029412	0.000000
5	196/196	0.027027	0.058824	0.000000
6	198/194	0.027027	0.029412	0.000000
7	198/198	0.027027	0.029412	0.027778
8	200/200	0.000000	0.029412	0.000000
9	202/194	0.000000	0.058824	0.000000
10	202/202	0.027027	0.000000	0.055556
11	204/194	0.027027	0.000000	0.000000
12	208/194	0.027027	0.000000	0.000000
13	208/196	0.027027	0.000000	0.000000
14	208/198	0.000000	0.029412	0.000000
15	208/212	0.054054	0.000000	0.000000
16	208/228	0.027027	0.000000	0.000000
17	210/210	0.027027	0.000000	0.000000
18	212/196	0.054054	0.058824	0.000000
19	212/198	0.027027	0.000000	0.000000
20	212/202	0.000000	0.029412	0.000000

Table 14. (Continued) Genotypes and frequencies at HUJ1177 locus in the goat populations of Tanur,Thalassery and Badagara

21	212/208	0.027027	0.000000	0.000000
22	212/212	0.027027	0.000000	0.000000
23	214/198	0.000000	0.029412	0.027778
24	214/202	0.000000	0.029412	0.027778
25	214/208	0.027027	0.000000	0.000000
26	216/196	0.027027	0.000000	0.000000
27	216/198	0.000000	0.000000	0.083333
28	216/202	0.027027	0.000000	0.027778
29	216/208	0.081081	0.000000	0.000000
30	216/212	0.000000	0.000000	0.027778
31	216/216	0.027027	0.000000	0.000000
32	218/216	0.000000	0.000000	0.027778
33	218/218	0.000000	0.029412	0.000000
34	218/224	0.027027	0.000000	0.000000
35	220/194	0.000000	0.029412	0.000000
36	220/208	0.027027	0.000000	0.000000
37	220/210	0.027027	0.000000	0.000000
38	220/212	0.135135	0.029412	0.000000
39	220/214	0.000000	0.029412	0.000000
40	220/218	0.000000	0.000000	0.027778
41	220/220	0.000000	0.000000	0.055556
42	222/296	0.000000	0.000000	0.027778
43	222/202	0.000000	0.000000	0.055556

Table 14. (Continued) Genotypes and frequencies at HUJ 1177 locus in the goat populations of Tanur,Thalassery and Badagara

44	222/210	0.000000	0.000000	0.027778
45	222/212	0.000000	0.029412	0.000000
46	222/222	0.000000	0.088235	0.000000
47	224/202	0.000000	0.000000	0.083333
48	224/212	0.000000	0.029412	0.000000
49	224/214	0.000000	0.029412	0.000000
50	224/216	0.054054	0.000000	0.000000
51	224/224	0.000000	0.058824	0.000000
52	226/198	0.000000	0.000000	0.027778
53	226/202	0.000000	0.000000	0.027778
54	226/210	0.000000	0.000000	0.027778
55	226/216	0.000000	0.000000	0.027778
56	228/198	0.000000	0.000000	0.055556
57	228/214	0.000000	0.000000	0.027778
58	228/216	0.000000	0.000000	0.083333
59	228/222	0.000000	0.058824	0.027778
60	228/224	0.000000	0.000000	0.027778
61	228/228	0.000000	0.088235	0.083333
62	230/244	0.000000	0.058824	0.000000

Table 15. No. of alleles, Heterozygosity and PIC value at HUJ 1177 locus in goat populations of Tanur, Thalassery and Badagara

Population	No.of alleles	Heterozygosity	PIC
Tanur	15	0.894	0.894
Thalasserry	14	0.909	0.907
Badagara	13	0.880	0.880

Table 16. Allele size and frequency at ILSTS 030 locus in goat populations Tanur,Thalassery and Badagara

Allele size	Tanur	Thalassery	Badagara
156	0.045455	0.000000	0.000000
158	0.106061	0.000000	0.000000
160	0.257576	0.000000	0.000000
162	0.030303	0.000000	0.044118
164	0.136364	0.064516	0.014706
166	0.136364	0.000000	0.191176
168	0.030303	0.274194	0.220588
170	0.045455	0.290323	0.073529
172	0.166667	0.16129	0.102941
174	0.030303	0.000000	0.073529
178	0.000000	0.048387	0.029412
180	0.015152	0.112903	0.250000
184	0.000000	0.048387	0.000000

Table 17. Genotypes and frequencies at ILSTS 030 locus of Malabari Goat Populations of Tanur, Thalassery and Badagara

Sl. No.	Genotype	Frequency		
		Tanur	Thalassery	Badagara
1	158/156	0.30303	0.000000	0.000000
2	160/158	0.060606	0.000000	0.000000
3	160/160	0.121212	0.000000	0.000000
4	162/162	0.000000	0.000000	0.029412
5	164/164	0.060606	0.000000	0.000000
6	166/158	0.121212	0.000000	0.000000
7	166/160	0.060606	0.000000	0.000000
8	166/164	0.090909	0.000000	0.000000
9	166/166	0.000000	0.000000	0.117647
10	168/162	0.000000	0.000000	0.029412
11	168/164	0.060606	0.129032	0.000000
12	168/168	0.000000	0.000000	0.147059
13	170/160	0.060606	0.000000	0.000000
14	170/168	0.000000	0.161290	0.029412
15	170/170	0.000000	0.032258	0.029412
16	172/156	0.060606	0.000000	0.000000
17	172/160	0.090909	0.000000	0.000000
18	172/162	0.060606	0.000000	0.000000
19	172/166	0.000000	0.000000	0.029412
20	172/168	0.000000	0.193548	0.000000
21	172/172	0.030303	0.000000	0.088235

Table 17. (Continued) Genotypes and frequencies at ILSTS 030 locus of Malabari Goat Populations of Tanur, Thalassery and Badagara

22	174/166	0.000000	0.000000	0.029412
23	174/168	0.000000	0.000000	0.029412
24	174/170	0.030303	0.000000	0.000000
25	174/172	0.030303	0.000000	0.000000
26	174/174	0.000000	0.000000	0.029412
27	178/170	0.000000	0.16129	0.000000
28	178/178	0.000000	0.000000	0.029412
29	180/164	0.000000	0.000000	0.029412
30	180/166	0.000000	0.000000	0.058824
31	180/168	0.000000	0.032258	0.088235
32	180/170	0.000000	0.129032	0.058824
33	180/172	0.030303	0.000000	0.000000
34	180/174	0.000000	0.000000	0.029412
35	180/178	0.000000	0.064516	0.000000
36	180/180	0.000000	0.000000	0.116747
37	184/170	0.000000	0.096774	0.000000

Table 18. Heterozygosity and PIC value at ILSTS 030 locus in Malabari goat populations of Tanur, Thalassery and Badagara

Populations	No.of alleles	Heterozygosity	PIC
Tanur	11	0.850	0.848
Thalassery	7	0.793	0.775
Badagara	9	0.828	0.823

Table 19. Genetic distance based on microsatellite markers (Nei ,1978)

Population	Thalassery	Tanur	Badagara
Thalassery	*****		
Tanur	0.7795	*****	
Badagara	0.5729	0.8401	*****

Table 20. Litter Size (in percentage) in Malabari goat populations of Tanur, Thalassery and Badagara

Sl.No.	Litter size	Populations		
		Tanur	Thalassery	Badagara
1.	Single	22.71	34.15	34.82
2.	Twins	58.95	54.46	50.37
3.	Triplets	15.72	10.05	14.44
4.	Quadruplets	2.62	1.34	0.37

Table 21. Body weight (kg) of Malabari goats

Populations	Birth Weight	One Month weight	Three Month weight	Six months weight	One year weight
Tanur	1.73±0.04	4.42±0.04	8.83±0.30	17.83±1.54	18.37±2.07
Thalassery	1.88±0.03	5.37±0.17	10.02±0.24	21.00±0.47	27.00±1.75
Badagara	1.86±0.05	4.50±0.15	9.52±0.26	21.36±0.55	25.58±0.76

Table 22. The percentages of multiple births in Malabari goat populations

Parameters	Tanur	Thalassery	Badagara
Multiple Births	0.76	0.66	0.68

Table 23. Mean antibody response to sheep RBC in Malabari goat populations of Tanur,Thalassery and Badagara

Population	Pre-immunisation titre	1+ $\log_e$ of titre		
	0 day	7th day	15th day	21st day
Tanur	0	3.24±0.05	2.92±0.05	2.55±0.06
Thalassery	0	3.23±0.06	2.81±0.05	2.52±0.06
Badagara	0	3.33±0.06	2.87±0.05	2.54±0.06

Table 24. Least square analysis of variance of antibody response to SRBC on disease incidence.

SOURCE	DF	MEAN SQUARES		
		7^{th}	15^{th}	21^{st}
CENTRE	2	0.647150**	0.077509 **	0.005721**
INCIDENCE	2	0.008907**	0.013971 **	0.028577**
REMAINDER	57	0.062785	0.060401	0.075183

**P≥0.05

Table 25. Mean increase in skin thickness in millimetre at 24, 48 and 72 hours in Malabari goat populations

Populations	Pre injection Thickness	24hours	48hours	72hours
Tanur	1.34±0.01	1.84±0.04	1.69±0.02	1.61±0.02
Thalassery	1.34±0.02	1.86±0.03	1.72±0.02	1.62±0.02
Badagara	1.33±0.02	1.95±0.04	1.76±0.02	1.65±0.02

Table 26. Mean concentrations of Total Protein, Albumin and Globulin in g/dl in Malabari goat populations

Population	Total Protein	Albumin	Globulin
Tanur	4.75±0.32	2.41±0.06	2.34 ±0.31
Thalassery	5.54±0.37	2.41±0.10	3.13 ±0.31
Badagara	6.01±0.22	2.73±0.10	3.28±0.22

Fig.1 Frequency distribution curve for potassium in the pooled population of Malabari goats

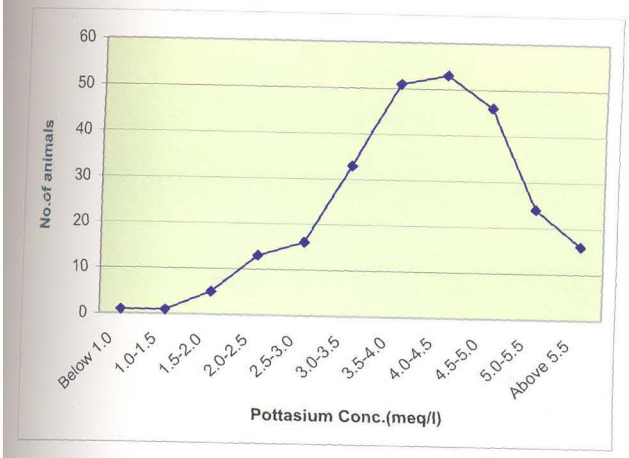


Fig..2 Frequency distribution curve of GSH concentration in the pooled population of Malabari goats

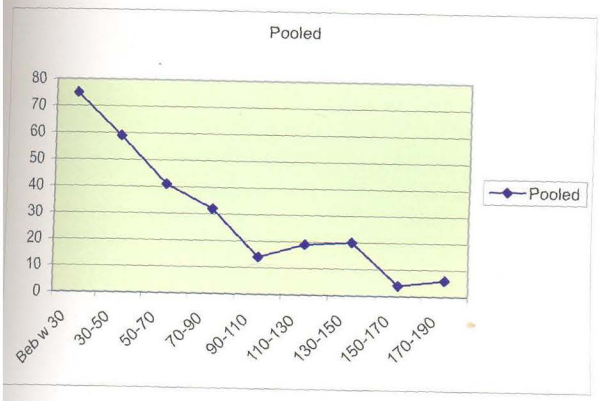


Fig..3 Haemoglobin pattern in Malabari goats

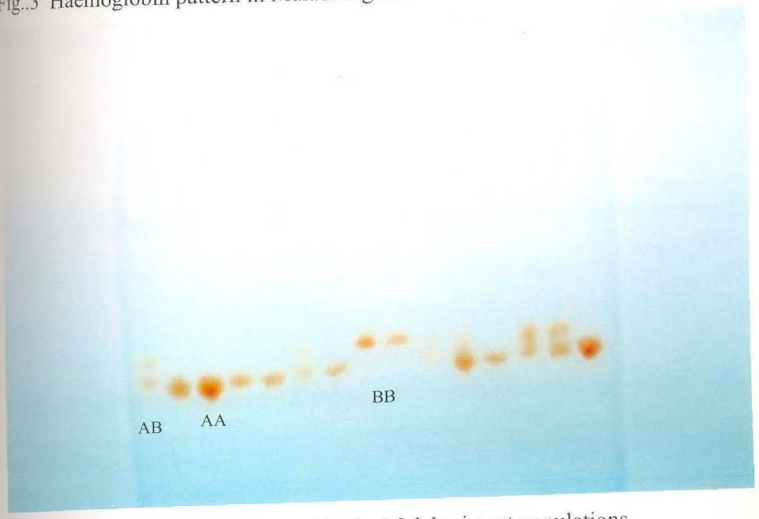


Fig.4 Transferrin variants observed in the Malabari goat populations

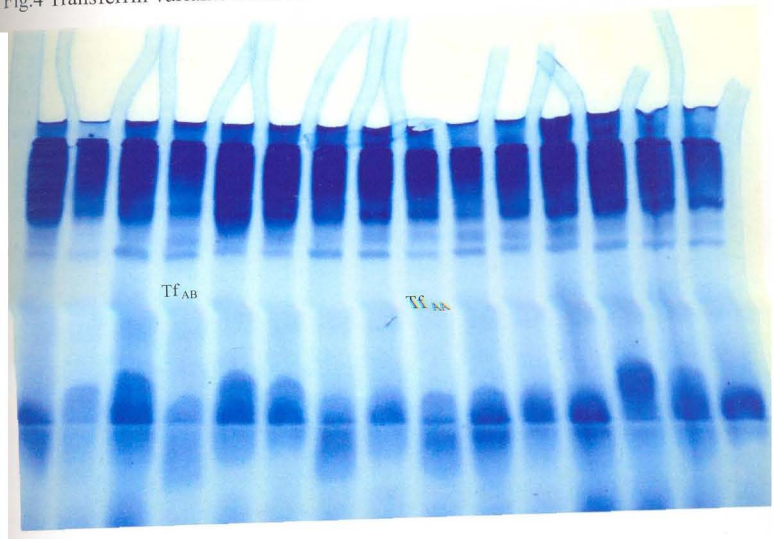


Fig.5 Albumin bands observed in Malabari goat populations

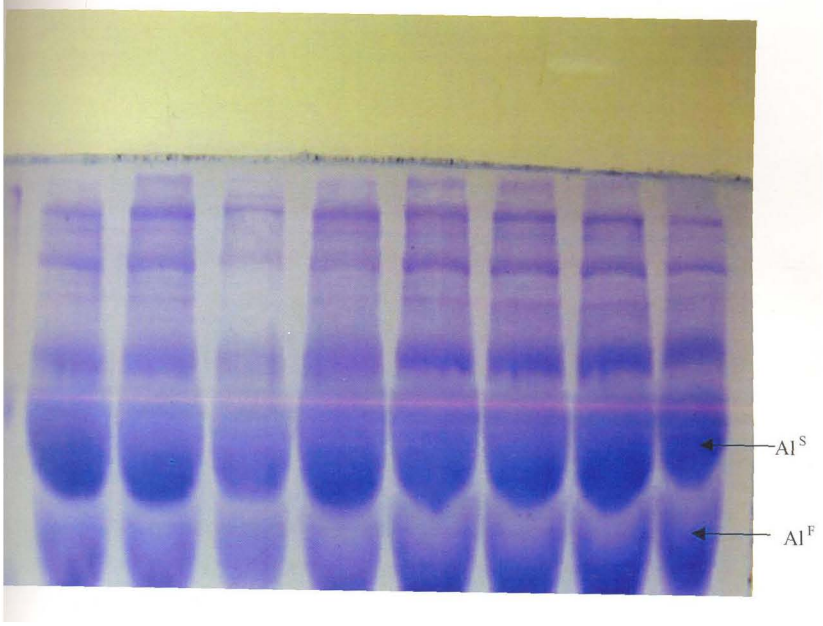


Fig.6 Cerruloplasmin, amylase and carbonic anhydrase bands in Malabari goat populations

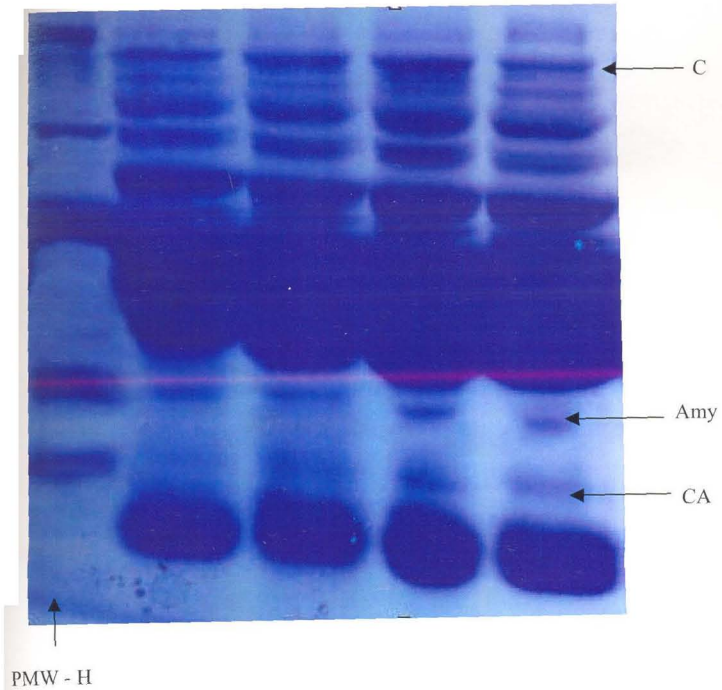


Fig.7 Mean potassium concentrations in Malabari goat populations

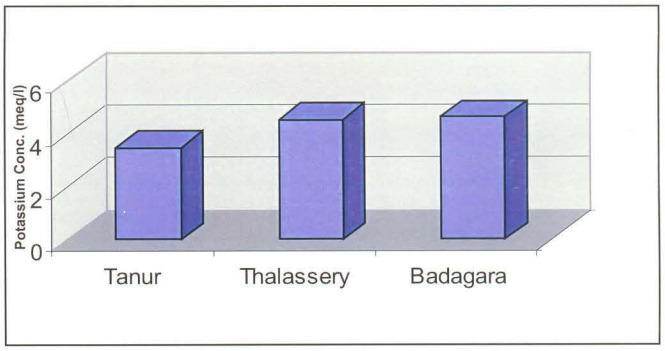
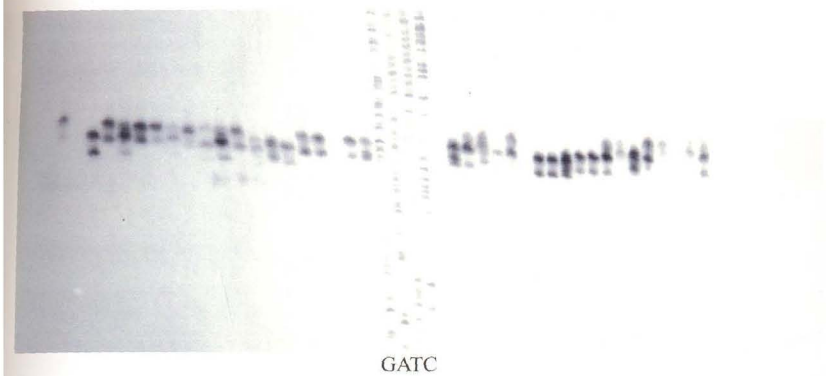
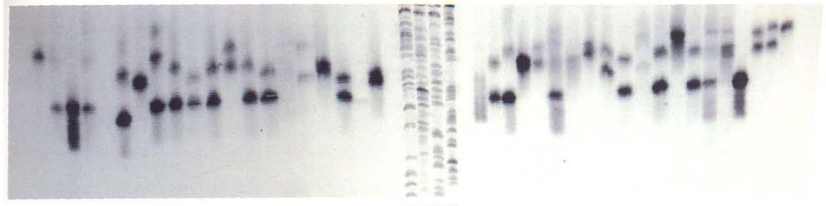


Fig.8 Autoradiograph showing polymorphism at INRA 063 locus
GATC represents M13 sequence used as marker



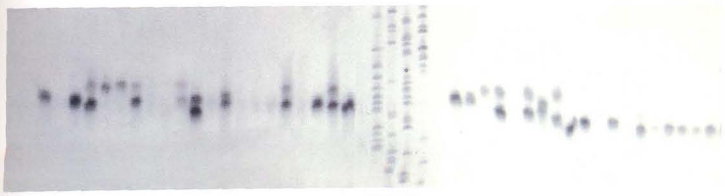
GATC

Fig.9 Autoradiograph showing polymorphism at HUJ 1177 locus
GATC represents M13 sequence used as marker



GATC

Fig.10 Autoradiograph showing polymorphism at ILSTS 030 locus
GATC represents M13 sequence used as marker



GATC

Fig.11 Dendrogram based on Nei's (1978) genetic distance

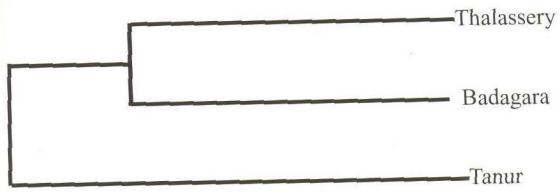


Fig.12 Litter Size in Malabari goats

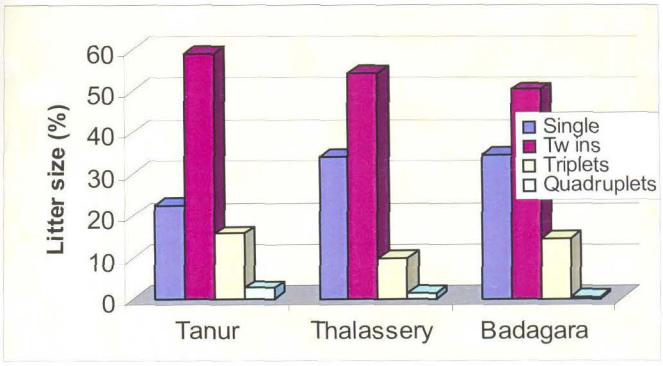


Fig.13 Body weight in Malabari goats

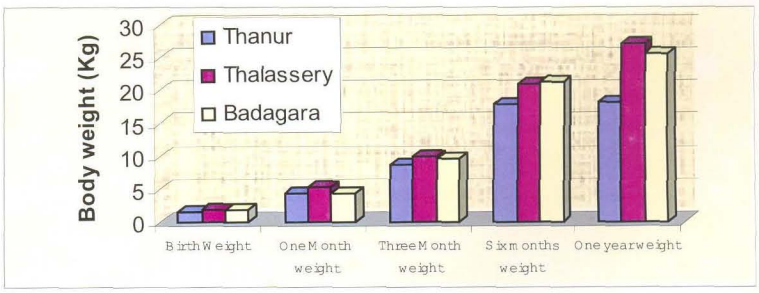


Fig.14 The mean antibody titre ($1+\log_e$) to sheep RBC on 0day, 7th day, 15th day and 21st day

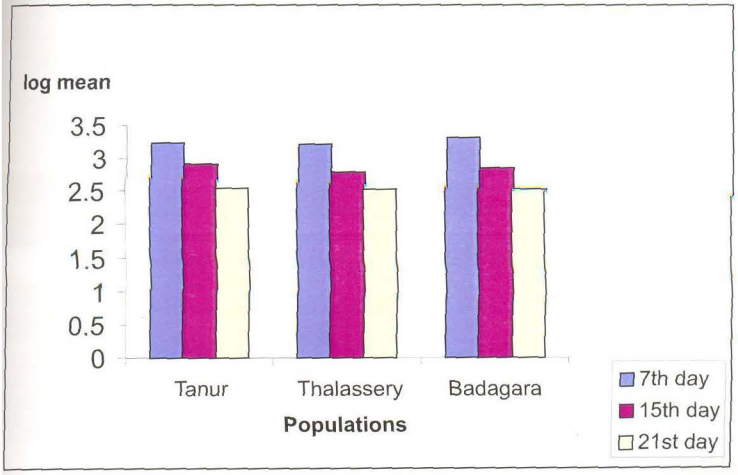


Fig.15 The mean skin thickness to intradermal injection to Phytahaemagluttinin – M (PHA-M) on 0hr,24hr,48hr and 72hr

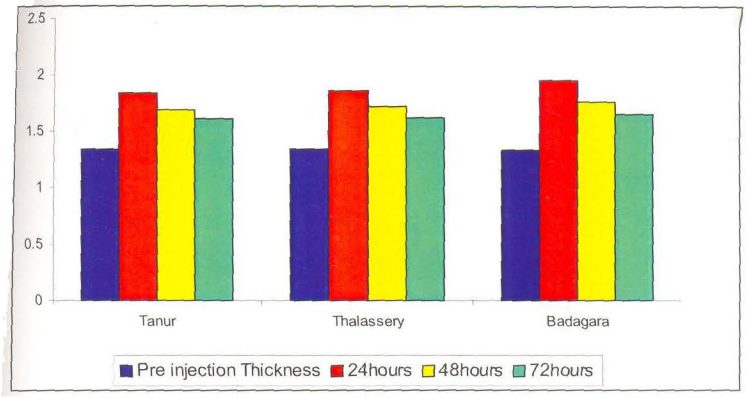
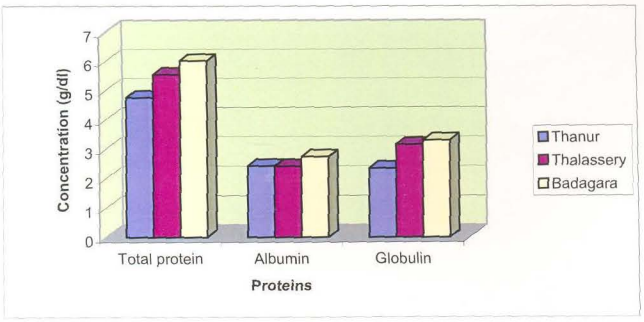


Fig.16 Total Protein, Albumin and Globulin concentrations in Malabari goat populations



Discussion

5. DISCUSSION

5.1 HAEMOGLOBIN POLYMORPHISM

Three hundred Malabari goats belonging to three different centers, viz Tanur, Thalassery and Badagara were typed for haemoglobin, using Vertical Polyacrilamide Gel Electrophoresis (PAGE).

Genotypic frequencies of 97.67, 2.00 and 0.33 per cent was observed for Hb_{AA}, Hb_{AB} and Hb_{BB} in Malabari goat population under study. All goats belonging to Tanur and Badagara were found to be of Hb_{AA} type with an allelic frequency of Hb^A as one. In Thalassery, the frequencies of Hb^A and Hb^B were found to be 0.962 and 0.038, respectively. The frequencies of Hb ^{A} and Hb^B variants in the pooled population were 0.987 and 0.012, respectively indicating a predominance of Hb ^{A} in the population.

Similar findings in other Indian goat breeds have been reported by Joshi *et al.* (1975) in Barbari and Jamunapari goat breeds, Baruah and Bhat (1980) in Jamunapari and Black Bengal goats and Bhat (1985) in Jamunapari goats.

In exotic breeds, a clear predominance of Hb^A variant over Hb^B has been established by Fesus *et al.* (1983) in Hungarian native goats, Barbancho *et al.* (1984) in Spanish goat breeds and Canatan and Boztepe (2000) and Elmaci (2003) in Turkish goats.

In the present study, no Hb $_{BB}$ phenotype could be observed in Tanur and Badagara goat populations. Shamsuddin *et al.* (1986) made similar findings in Malabari, Saanen halfbreds and Alpine halfbreds. The study conducted by Canatan and Boztepe (2000) in goats of Turkey, which revealed no BB phenotype, is also in agreement with that of the present study. The absence was attributed to the inability of the animals of BB phenotype to survive in rural regions of Toros Mountains. The authors also reported that the absence of BB phenotype in Hb loci had a selective advantage.

Most of the world's goat breeds are fixed for the Hb^A allele according to many workers (Efrenov and Braend, 1965 and Bhat *et al.*, 1983).

The present work revealed that the pooled population under study was not in Hardy-Weinberg equilibrium. Significant deviations from Hardy-Weinberg equilibrium refer to a deficiency of heterozygous genotypes in the populations. Similar findings have also been reported by Menrad *et al.* (2002) in Pashmna goats and Buvanendran *et al.* (1981) in Red Sokoto goats above one year of age.

Association between haemoglobin types and traits of economic importance were not studied, as the number of animals with Hb^B allele was too small.

Perusal of the literature available on the haemoglobin polymorphism in Indian as well as exotic goat breeds showed that Hb $_{AA}$ is the predominant type in almost all the goat breeds.

The absence or negligible presence of Hb^B allele in goats, indigenous as well as exotic, may be indicative of either adaptive preference of Hb ^{A} allele to Hb ^{B} allele or species characteristic.

A predominance of Hb ^{A} variant was observed in the population under study, as already established in some of the Indian goat breeds, viz. Jamunapari, Barbari and Black Bengal goats.

5.2 POLYMORPHISM OF BLOOD PROTEINS

5.2.1 Transferrin

In the present study, SDS PAGE was performed to study the transferrin polymorphism which revealed two variants, Tf ^{A} and Tf ^{B}. In the goat populations of Tanur, Thalassery, Badagara and pooled population, the gene frequencies of Tf ^{A} observed were 0.995, 1.000, 0.974 and 0.990, respectively indicating the predominance of Tf ^{A} allele in the population. In Thalassery all the animals typed were of Tf $_{AA}$ type.

The above finding is in agreement with the observations of Fesus *et al.* (1983) who reported that majority of the goat breeds in the world have gene frequency of Tf^A more than that of Tf^B. Many other workers who also gave the gene frequencies of Tf^A have reported on similar lines while working on various breeds of goats in India, viz. Baruah and Bhat (1980) in Black Bengal goats and Bhat (1987) in Chegu and Changthangi goats.

Similar results in exotic breeds were given by Fesus *et al.* (1983) in Hungarian native goats, Menrad *et al.* (1994) in Boer and improved Fawn goats and Canatan and Boztepe (2000) and Elmaci (2003) in hair goats of Turkey.

The present findings with regard to transferrin locus are in agreement with those made on most of the goat populations in the world.

In contradiction to the present findings with regard to the gene frequency, predominance of Tf^{B} allele has been reported by Singh *et al.* (1977) in Barbari, Beetal, their crosses and nondescript locals and Baruah and Bhat (1980) in Jamunapari and Barbari goats.

In Thalassery population, complete absence of Tf^{B} was evident, with all the animals belonging to Tf_{AA} type. This is in agreement with the findings made by Efrenov and Braend (1965) in Norwegian goats, Watanabe and Suzuki (1966) in Saanen Swiss goats and Tjankov (1972) in Toggenberg breed, where Tf^{B} allele was found to be completely absent.

As against the finding of only two alleles with regard to transferrin locus as evinced by the present study, many authors have reported the presence of more than two variants for transferrin alleles in goats, viz. Bhat (1987) in Pashmina goats and Kumar and Yadav (1988) in Jhakrana, Kutchi, Marwari and Sirohi goats.

More than two alleles for transferrin locus were observed in exotic goat breeds by Trehan *et al.* (1981) in Alpine, Saanen and Nubean and Pepin and Nguyen (1994) in West African goats.

In the present study, the transferrin locus was in Hardy-Weinberg equilibrium which is in agreement with Trehan *et al.* (1981), in Alpine, Saanen, Nubian, Alpine x Beetal and Saanen x Beetal cross breds.

Association between transferrin types and traits of economic importance were not studied, as the number of animals with Tf^{B} allele was too small.

The present findings suggest that the goat populations under study are more close to some of the other Indian breeds, viz. Black Bengal, Chegu and Changthangi goats.

5.2.2 Albumin

The Malbari goat population under the present study, was screened for albumin variants using SDS PAGE and two bands each were observed in all the animals studied from Tanur, Thalassery and Badagara, indicating absence of polymorphism at this locus. One fast moving band Al^F and one slow moving band Al^S were present in all animals tested. The present study agrees with the findings of Shamsuddin *et al.* (1986) in Malabari goats and its Saanen and Alpine half breds. Singh *et al.* (1977) also have reported absence of polymorphism in Barbari, Beetal, their crosses and non-descript native goats.

Two albumin variants in goats have already been reported by Barbancho *et al.* (1984) and Tunon *et al.* (1989) in Spanish goat breeds, Vankan and Bell (1992) in Cashmere goats and Ertugrui and Akyuz (2000) in Angora goats, but with higher degree of polymorphism at the locus. As regards the albumin locus, the three populations studied were homogenous, indicating absence of polymorphism, similar to some other Indian breeds like Barbari and Beetal goats, as already reported.

5.2.3 Cerruloplasmin, Amylase and Carbonic Anhydrase

Serum samples of goats were subjected to polyacrylamide gel electrophoresis, to study the polymorphism at cerruloplasmin, amylase and carbonic anhydrase loci.

5.2.3.1 Cerruloplasmin

In the present study no polymorphism could be observed at cerruloplasmin locus in any of the goats tested. Single band was detected in all the populations. In Indian breeds similar findings were reported by Fesus *et al.* (1983) in Beetal, Barbari, their crosses and non descript natives, Bhat (1986a) in Jamunapari and Sirohi breeds and Bhat (1987) in Changthangi and Chegu breeds. Similar findings were observed in exotic breeds by Singh *et al.* (1977) in Hungerian native goats and Tunon *et al.* (1989) in Spanish goat breeds.

In contrast to the above findings polymorphism at cerruloplasmin locus were reported by Elmaci (2003) in hair goats of Turkey. But the frequency of the variant allele was very low (0.027).

The findings of the present work suggests that cerruloplasmin locus is monomorphic, indicating absence of polymorphism as has been reported in other Indian breeds like Beetal, Barbari, Jamunapari, Sirohi, Changthangi and Chegu breeds.

5.2.3.2 Amylase

In goats of Tanur, Thalassery and Badagara a single band could be observed for amylase locus indicating the absence of polymorphism. This finding is in agreement with the reports in Indian goat breeds by Singh *et al.* (1977) in Beetal, Barbari, their crosses and non descript natives, Shamsuddin *et al.* (1986) in Malabari and Bhat (1987) in Changthangi and Chegu goats.

The present work is also in agreement with the studies conducted in exotic breeds by Morera *et al.* (1983) in Spanish Merino sheep, Shamsuddin *et al.* (1986) in Saanen and Alpine halfbreds with Malabari, Tunon *et al.* (1989) in Spanish goats and Menrad *et al.* (1994) in German improved Fawn and Boer goats.

In contrast to the above findings polymorphism for amylase locus was observed in Angora goats (Fechter and Pretorius, 1970), Jamunapari and Sirohi goats (Bhat, 1986a) and in Turkish hair goats (Elmaci, 2003). Two variants for amylase locus were observed by all the above workers and in all the studies the frequency of the variant allele was very low.

Bhat and Baruah (1980) reported the presence of two amylase variants Am-1 and Am-2 in Jamunapari and Barbari goats. The frequency of Am-2 was very low in both the breeds (0.005 and 0.020 in Jamunapari and Barbari, respectively).

The present study indicates that the amylase locus in Malabari goats is monomorphic, as already reported in other Indian breeds viz.,Beetal, Barbari, Changthangi and Chegu goats.

5.2.3.3 Carbonic Anhydrase

In the present study a single band could be observed for all the goat populations under study. Polymorphysm at this locus could not be observed. This finding is in agreement with the

observations of Casati *et al.* (1990) in Sarda breeds and Pepin and Nguyen (1994) in five breeds of goats viz. French Alpine, French Saanen, Guadeloupean Creole, Guinean and west African Sahel.

5.3 SERUM POTASSIUM POLYMORPHISM

Flame photometry was adopted to estimate potassium concentration in the serum samples in the present study.The mean serum potassium concentration in the pooled goat population was found to be 4.18±0.09 meq/l. Tunon *et al.* (1987) have reported the bimodality in the frequency distribution curve as the basis for the classification of animals into different categories as to low and high potassium types. Hence a frequency curve of potassium was plotted for the whole population as well as for the individual populations under study, which failed to reveal bimodality and the animals could not be categorised to low and high potassium types on a strict basis. This finding agrees with Clarke *et al.* (1989) who reported that in Namaqua sheep a single distribution could be observed indicative of neither LK nor HK, and with Nandakumaran (1989) in Malabari and its Saanen cross-breds. Since classification based on bimodality could not be done in the present study, goats having potassium concentration above 22meq/l were classified as high potassium type (HK) and those falling below 22meq/l were designated as low potassium (LK) type as was done by Nandakumaran (1989) in Malabari goats.

In the present study potassium concentration ranged between 1.3 to 15.7 meq/l, all indicative of low potassium types. Nandakumaran (1989) reported high and low potassium types in Malabari and its Saanen and Alpine halfbreds with a predominance of low potassium in all the genetic groups. The author further reported that among Malabari, the percentage of LK type was 77.78.

Absence of polymorphism has also been reported by Bhat *et al.* 1983 in Jamunapari and Barbari herds, who opined that unlike in sheep, all goat breeds may not exhibit polymorphism at potassium locus. So also, Bhat (1986a) reported that in Jamunapari breed, only HK type could be detected which got fixed during the course of evolution of this breed. Further, studies in Pashmina goats by Bhat and Singh (1987) agrees with the above finding, where in only HK type was observed indicating absence of polymorphism at potassium locus, with a mean serum potassium concentration of 29.05meq/l.

Many of the goat breeds of the world showed the predominance of HK type in goats. But a reverse trend is noticed in the present study. According to Kaura (1952), centuries ago, Arab merchants who came to Kerala for trade brought with them Mesopotamian goats which were crossed to local and Kutch strain in large numbers along the Malabar sea cost and thus it can be seen that the Malabari goats owes its origin to Arabian and Mesopotamian goats. During the course of time the low potassium types might have got fixed in the population, which may be due to some linkage to some traits of economic importance or due to some adaptive importance. The absence of HK type in the present study substantiates the historical view expressed by Kaura (1952) on the evolution of Malabari goats from Arab and Mesopotamian Goats.

Association between potassium types and traits of economic importance were not studied, as the animals could not be categorized into different types on the basis of potassium concentrations.

5.4 BLOOD GLUTATHIONE (GSH) POLYMORPHISM

The concentration of erythrocyte glutathione (mg/100ml red blood cells) was estimated in goats by the method of Beutler *et al.* (1963), using spectrophotometer at a wavelength of 412 nm to find out the polymorphism if any at this locus. On the whole, the glutathione concentration ranged from 29.63 to 103.19 mg/100 ml red blood cells. Tucker and Kilgour (1972) have reported classification as to high and low glutathione based on the bimodality evident from the frequency distribution curve plotted to find out the polymorphism at this locus. But the frequency distribution curve plotted in this study does not reveal any clear-cut bimodality. Hence as per the classification of Agar *et al.*(1974) the animals with GSH concentration less than 60 mg /100 ml RBC were classified as low glutathione (GSH^h) and animals having GSH concentration above 60mg/100mlRBC as high glutathione type (GSH^H). GSH^H and GSH^h are controlled by a single pair of autosomal alleles, the gene for GSH^H being dominant to GSH^h type.

The present study pointed to a predominance of the low GSH type on the whole. The low GSH type animals (49 per cent) existed in almost equal proportions as the high GSH type in Badagara, while in Tanur, the low GSH type animals were in lesser proportions (30 per cent). The Thalassery population however had a marked predominance of the low GSH type (83 per cent), establishing a predominance of low GSH type (53.67 per cent) in the pooled population in the final analysis.

More *et al.* (1980) has reported that Jamunapari animals had relatively more number of low GSH animals. So also, Nandakumaran (1989) has stated that in Malabari goats, the number of low GSH type was relatively more when compared to Saanen and Alpine half-bred.

In goats of Badagara, a place of geographical proximity to Thalassery, more or less equal proportions of GSH high (51 per cent) to GSH low types (49 per cent) were detected with the GSH high type gaining only a slight edge. The reason here in may be attributed to the relatively close confinement of these two populations. Moreover a process of natural selection on grounds of economic traits possibly having some linkage to the gene for low glutathione type might be going on in this breeding tract resulting a predominance of low glutathione type in the populations on the whole. Further more, it should be noted in this context that Malbari goats also have Jamunapari blood in which the GSH low types predominates, as established already.

In contrast to the findings at Thalassery and Badagara, a clear predominance of high glutathione type was noticed in the goat population of Tanur (GSH high 70 per cent and GSH low 30per cent), indicating a deviation from that of Thalassery and Badagara populations, though the general trend in gene frequency remains much the same as that of the other two centres. High incidences of GSH high type was reported in Saanen (91 per cent), Angora (74 per cent) and British Alpine (93 per cent) by Agar *et al.* (1974) who also reported that all the animals of Anglonubean and Toggenberg were of GSH high type. More *et al.* (1980) also has reported high percentage of GSH high type in Saanen, Angora and Alpine breeds. Tunon *et al.* (1989) has however reported the absence of polymorphism with regard to GSH loci in Spanish goats.

The frequency distribution of GSH allele in the present study showed a predominance of GSH^{h} allele in all the populations. The gene frequency of GSH^{h} allele was found to be 0.73, 0.55, 0.91 and 0.70, respectively in pooled population, Tanur, Thalassery and Badagara. This finding is in agreement with Nanadakumaran (1989) who reported a high gene frequency for GSH^{H} allele in Saanen and Alpine halfbreds with values of 0.62 and 0.66, respectively. Based on the present study, it can be concluded that natural selection might have happened for GSH low type animals or artificial selection might have been practiced for some traits of economic importance with some possible linkage to GSH^{h} allele resulting in considerable increase in the percentage of animals of GSH low type in the population.

The least square analysis of low GSH type showed that no significant difference was appreciable between populations. In the pooled populations also, no significant difference was observed between GSH types and between populations. This finding agrees with that of Reddy and Krishnan (1986) who reported that GSH concentration in high or low types did not differ significantly between genetic groups. Nanadakumaran (1989) also has reported that though there was difference in gene frequency among genetic groups, it was not significant.

5.5 GENETIC DISTANCE

In the present study genetic distances between different populations were computed using Nei's method (1978), based on polymorphic loci, viz. haemoglobin, transferrin and glutathione. Genetic distance between Tanur and Thalassery was 0.0498, between Tanur and Badagara was 0.0092 where as between Badagara and Thalassery was 0.0166. In this study the maximum genetic distance was found between Tanur and Thalassery population which is in agreement with the geographical distance between the populations.

The data obtained from studies of blood genetic systems should be combined with other data for more accurate interpretations as regards to phylogenetic relationships.

Pepin and Nguyen (1994) calculated genetic distances based on the variation in allelic frequencies between breeds, and concluded that the results were in close agreement with data from history and geographic origin of the breeds examined. Nguyen *et al.*(1992) opined that the observed genetic differences between Rambouillet and Spanish Merino could be attributed to the evolutionary change due to random drift in the small and closed flock of Rambouillet.

Our findings are in agreement with Casati *et al.*(1990) who reported that the Nei's distance values, calculated based on polymorphism at different blood protein loci ranged from 0.0124 to 0.0599.

Similarly genetic distance was calculated by Tsunoda *et al.*(1990) and revealed conspicuous differences among the three regional populations of Bangladesi sheep. They concluded that though slight differences exist between different populations of Bangladeshi breed, it is considered that no great difference among the populations were present as a whole.

European sheep breeds had a genetic distance ranging from 0.0194 to 0.0613, similar to the result of the present study.

Tunon *et al.* (1989) calculated the genetic distances between 14 Spanish goat breeds in similar way using Nei's distance and opined that the distance values ranged from 0.003 to 0.097.

The genetic distances between different populations were also calculated using the method described by Balakrishnan and Sanghvi (1968). Accordingly, the maximum genetic distance of 0.6690 was recorded between Tanur and Thalassery populations, which correlate with the geographical separation between the two places. The minimum distance recorded was between Thanur and Badagara (0.1249) and are found to be the related populations. The distance between Thalassery and Badagara was calculated as 0.3351. Nei (1978) emphasized that the dendrogram only represents genetic relationship between breeds, but may or may not show the true evolutionary history of populations, especially when they are not completely isolated.

Protein polymorphic markers being based on limited number of expressed genes, the genetic distance calculated by this method fails to give a conclusive picture. The only possible conclusion that could be arrived at from the above studies is the existence of a relationship between these populations. It further suggests that the Tanur population is distinctly different from the other two populations. Hence an attempt was made to study the different populations at molecular level, using microsatellite markers.

The two clear advantages that microsatellites have over other DNA markers is that they have multiple alleles as well as high heterozygosity frequencies , which make them highly informative for genetic analysis (Gill *et al.*,1994). The values of genetic distance obtained from microsatellite data might be more precise when compared to that obtained from protein markers (Arranz *et al.*, 1996).

5.6 POLYMORPHISM OF MICROSATELLITE MARKERS

Microsatellites have proved to be useful polymorphic markers for the analysis of the genetic relationships. The usefulness of microsatellite markers for the estimation of genetic distances among closely related populations has been documented in numerous studies (Ciampolini *et al.*, 1995 and Arranz *et al.*, 1996). The microsatellites are powerful tools to differentiate between

breeds and they are giving the correct answer regardless of whether the breeds are closely related or not. (Saitbekova *et al.*,1999).

There is high level of similarity between cattle, sheep and goat chromosomes (Vaiman *et al.*,1996) and bovine microsatellites very well amplify and detect polymorphism in goats. Saitbekova *et al.* (1999) and Kumar *et al.* (2005) reported that caprine DNA amplified very well with bovine microsatellite primers.

The number of alleles at different marker loci and their frequencies are indicators of genetic variability and form the basis of all genetic diversity indices for the estimation of genetic distances and construction of phylogenetic trees.

Ten microsatellite primers (TGLA 53, INRA005, INRA 063, ILSTS 005, ILSTS 030, ILSTS 011, HUJ 1177, ETH 10, INHA and BM 720) were tested in the present study. Out of these, only 3 primers (INRA 063, ILSTS 030 and HUJ 1177) were found to be polymorphic. Two primers (INHA and BM 721) failed to produce PCR amplification. Among the primers, which had successful amplification, five demonstrated no polymorphism or very little polymorphism. Microsatellite, the tandem repeat loci, can be generally considered to be hyper variable in length. This variability is a reflection of a general mechanism, most likely slippage occurring during DNA repair or replication processes. At these loci, a mutation may alter the size of an allele by adding or deleting one or more repeats Slippage mutations are sufficiently frequent to maintain a high degree of polymorphism within populations, but not frequent enough to occur in successive generations. The mutation rate is exceptionally high, implying a higher degree of polymorphism (Tauz, 1989). Three markers detecting higher number of alleles were chosen for further analysis. Moore *et al.*(1991) reported that the degree of polymorphism detected by a particular microsatellite in any species was variable. Saitbekova *et al.* (1999) successfully used bovine primers for genetic diversity studies in goats. Yang *et al.* (2004) also reported the use of sheep microsatellites for similar studies in goat population.

5.6.1 INRA 063 Locus

The maximum number of alleles (ten) for INRA 063 locus was observed in goats of Tanur, while eight and five alleles were recorded in Badagara and Thalassery populations, respectively with a size range of alleles from 155 to 173 bp. These findings were in accordance

with the work of Saitbekova *et al.* (1999), who observed seven alleles at INRA 063 locus with a size range of 154 to 168 bp in goats.

Pepin *et al.*(1995) reported that these bovine primers could produce strong amplification with a single band in homozygous individuals or with two bands in heterozygous individuals, and absence of non-specific bands with goat DNA. They detected six alleles with an average size of 170bp in goats. Chenyambuga *et al.*(2004) reported ten alleles with a size range of 141to 179 bp in goats of Subsaharan Asia.

With regard to INRA locus allele, 163 bp was more predominant in Tanur, while allele 167 and 171 bp predominated in Badagara and Thalassery, respectively. For INRA 063 locus, the most frequent alleles occurred towards the upper extreme of allele size in all the populations.The values for heterozygosity at this locus ranged from 0.656 to 0.847, in the total population.

5.6.2 HUJ 1177 Locus

This bovine microsatellites marker was found to be the most polymorphic of all the markers typed for the present study, detecting 19 alleles in total, with a size range of 188 to 230bp. Twenty seven genotypes were recorded in the goat populations of Tanur while 24 genotypes were recorded in Thalassery and Badagara populations. Allele size of 211bp was reported in cattle by Shalom *et al.*(1994). Vallejo *et al.*(2003) observed the presence of six alleles among 23 Holstein Friesian bulls typed at the locus.

All the three goat populations demonstrated high heterozygosity values which were in accordance with the expectations of microsatellite loci. Heterozygosity values ranged from 0.880 to 0.909 in the goat populations studied. These high values indicate the suitability of these markers for diversity studies.

5.6.3 ILSTS 030 Locus

In the present study the bovine microsatellite primer ILSTS 030 was well amplified with caprine DNA. A total of eighteen genotypes were observed in Badagara populations where as sixteen and nine genotypes were recorded in Tanur and Thalassery populations, respectively. Eleven, seven and nine alleles were observed in Tanur, Thalassery and Badagara at a size range

of 156 to 184 bp. This finding is in accordance with the observations of Saitbekova *et al.*(1999) who reported that this bovine primer was very well amplified in goats with a total number of 19 alleles and with an average size of 175bp. Allele size range reported at this locus in Marwari goat breed was reported to be 164 to 174 bp (Kumar *et al.*, 2005).

The disadvantage with this primer was the presence of shadow bands. According to Murray *et al.*(1993) shadow bands were produced in PCR of DNA sequences containing CA dinucleotide repeat. They found that shadow bands appeared due to two base pair deletions occurring randomly in the CA repeat region during PCR. They attributed the reason for the production of shadow bands to slippage synthesis or recombination events.

For ILSTS 030 locus the most frequent alleles in Tanur, Thalassery and Badagara were 160, 170 and 180 bp, respectively. The common alleles were in the medium size range. These results are in accordance with the data previously reported for microsatellites by other authors (Forbes *et al.*, 1995).

The high mean number of alleles per loci is an indication of high genetic variability within breeds (Sodhi *et al.*, 2003). The mean number of alleles is dependent on sample size because of the presence of unique alleles which occur in low frequencies in the population and also because of the number of observed alleles tends to increase with increase in population size (Kotze *et al.*, 2004). Heterozygosity values ranged from 0.850 to 0.973 in the goat populations studied.

The occurrence of a few highly frequent alleles in the different populations could be suggestive of probable linkage to traits of economic importance. Another reason for the predominance of certain alleles in the populations may be a higher rate of inbreeding within the populations. This is even more likely since the numbers of males in the populations are considerably low.

The number of alleles in the whole population ranged from five to fifteen. The highest mean number of alleles per locus as well as high allelic size range was noticed in Tanur goat population. Hence Tanur goat population can be considered to be more genetically diverse than the other groups based on the number of alleles and size range.

5.6.4 Genetic Distance

The genetic distance was calculated using Popgene programme according to Nei's formula (1978) between different goat populations under study. The genetic distance between Tanur and Thalassery was found to be 0.7795, Thalassery and Badagara 0.5729 and between Tanur and Badagara 0.8401. These results revealed that Thalassery and Badagara populations are more closely related when compared to Tanur population.

From the genetic distance values calculated, it is evident that genetic distance between Tanur and Thalassery and Tanur and Badagara are more than Thalassery to Badagara., which is in accordance with the geographical distribution of the breedable area. This can be substantiated by the fact that because of the geographical proximity, close breeding may be occurring between Thalassery and Badagara populations and they are found to be closely related. Tanur population is different from the other two goat populations.

Kemp *et al.* (1995) recommended that since distance measures cannot account for the consequences of artificial selection on morphological or economic traits or for natural selection for fitness and cannot accurately measure the time since divergence from a common ancestral population, they should only be used as an initial guide to population structure and breed differentiation. Arranz *et al.*(1996) observed that genetic distance calculated from microsatellite marker system is usually higher when compared to the other marker systems because of greater variation at these loci. The present findings also support the observations of Arranz *et al.*(1996). The values of genetic distance calculated using microsatellite markers are higher when compared with the genetic distance values calculated using protein polymorphic markers.

5.6.5 Dendrogram

The dendrogram calculated from the genetic distance data using Popgene programme grouped Thalassery and Badagara goat populations in one cluster, suggesting closer relationship between them. This finding is justified by the geographic proximity between these populations. It was found that the set of microsatellite markers used in the study could be used satisfactorily for genetic diversity studies in goats. However use of more number of polymorphic markers and raising the sample size might aid in a better differentiation between related populations.

In the present study genetic parameters were obtained separately for protein markers as well as for microsatellite markers and the results were compared. Estimates of genetic distance from microsatellites were greater than those obtained using protein markers. Large differences were found between the two types of markers, genetic variability at microsatellite marker loci being much greater than that of protein markers. The number of alleles per locus ranged from five to fifteen in the former case, and from one to three in the latter. The numbers of alleles at the three microsatellite loci (36) were much greater than that found at eight protein markers (13). However, results of different studies involving protein markers have shown considerable variation because of the number of loci sampled and variations in the percentage of the monomorphic systems (Arranz *et al.,* 1996). Genetic distances calculated (Nei's formula 1978) from microsatellite markers are 62.27 fold greater than from protein markers. The latter gave values ranging from 0.0092 to 0.0498 where as distances based on microsatellite data varied from 0.57 to 0.84. The genetic distance values calculated based on microsatellite marker studies was more precise in respect of the geographical distribution than that obtained by protien marker studies.

This goes in agreement with the earlier report by Arranz *et al.,* 1996, to the effect that values of genetic distance obtained from microsatellite data might be more precise when compared to that obtained from protein markers. The findings of this work agrees with those of Kemp *et al.*(1995) as well, who calculated the time of divergence between sheep breeds using eight microsatellites and compared the results with those obtained by Manwell and Baker (1977) using 30 biochemical markers and concluded that microsatellite loci gave more accurate values than protein markers of the time when species or populations are separated from a common ancestor. The present study further agrees with the authors in that microsatellite might be a better indicator of evolutionary relationships than protein polymorphisms within a particular species.

The genetic distance values calculated based on microsatellite marker studies precisely reflected the relationship between the different populations, as regards their physical profile and geographical proximities.

5.7 BIOMETRICAL AND REPRODUCTION TRAITS

5.7.1 Litter Size

The percentage of twin births was higher when compared to singles, triplets and quadruplets in all the populations studied. Similar results were reported by Shanmugasundaram

(1957) in Malabari goats. In contradiction, Biswas *et al.*(1995) reported that twinning rate (3.25%) was very less in Pashmina goats. In the present study it was found that the percentage of multiple births was higher in Tanur compared to the other populations, indicating higher prolificacy .The multiple births and litter size influence the genetic gain and economy of goat production.

5.7.2 Body Weight

The present study indicated that Thalssery and Badagara goats were much heavier than that of Tanur animals. The mean birth weight recorded in Tanur, Thalassery and Badagara was 1.73 ± 0.04, 1.88 ± 0.03 and 1.86 ± 0.05 kg, respectively. At three months, the values for body weight were 8.83 ± 0.30, 10.02 ± 0.24 and 9.52 ± 0.26kg. Sixth month weight recorded in kilograms in the respective populations were 17.83 ± 1.54, 21.00 ± 0.47 and 21.36 ± 0.55.

The data on litter size and body weight indicated that goats of Tanur area are different from that of Thalassery and Badagara.

5.8 IMMUNE RESPONSE

5.8.1 Humoral Immune Response

On seventh day after primary immunisation, the antibody titre in goat population of Tanur, Thalassery and Badagara rose to mean values of 3.24±0.05, 3.23±0.06 and 3.33±0.06, respectively. The fifteenth day post immunisation, antibody response in Tanur, Thalassery and Badagara had mean values of 2.92±0.05, 2.81±0.05 and 2.87±0.05, respectively. The twenty-first day antibody response in Tanur, Thalassery and Badagara populations had mean titres of 2.55±0.06, 2.52±0.06 and 2.54±0.06, respectively.

In the present study, maximum antibody response was detected on seventh day of immunisation. The titre gradually decreased from seventh day to twenty first day of immunisation.

The result in this study are in close conformity with that of Vander Zipp and Leenstra (1983) who found that mean total antibody titre to SRBC was highest on seventh day after primary immunisation. Ubosi *et al.* (1985) reported that following fourth day of primary injection of chicken with SRBC, differences in response could be noticed. Peak value was reached at six days in all the populations following primary immunisation with SRBC. The magnitude of

antibody titre ($1+\log_e$) closely agrees with that obtained in calves against human red blood cells (Burton *et al.*, 1989). The present study did not reveal any significant difference between populations in antibody response to SRBC. The absence of significant difference in antibody response can be considered as a species specific phenomena or a result specific to this study. It can be elucidated that the humid tropical stress might have contributed to an immunosuppressant effect so that final resolutions to the immune response capacity characteristic to this native breed could not be fully expressed.

The effect of antibody response to SRBC on 7^{th}, 15^{th} and 21^{st} day after primary immunization was not found to be significant for the occurrence of diseases like diarrhea and pneumonia. Burton *et al.* (1989) showed that prevalence of diarrhoea was negatively correlated with high primary antibody response against HRBC. Chicken selected for four generations of early high antibody response to *Escherichia coli* showed greater resistance to challenge with *E.Coli* (Piticovski *et. al.*, 1989).

The overall incidence of disease was substantially low possibly due to the emergence of resistant animals under natural selection of humid tropical stress reducing the variability to the occurrence of diseases.

5.8.2 Cell-mediated Immune Response

The mean increase in skin thickness at 24 hours of intradermal injection of (PHA-M) in goat populations of Tanur, Thalassery and Badagara were1.84±0.04, 1.86±0.03 and1.95±0.04mm, respectively. The 48 hour mean increases in skin thickness in the respective populations were 1.69±0.02, 1.72±0.02 and 1.76±0.02 mm. The 72 hour skin thickness values were 1.61±0.02, 1.62±0.02 and 1.65±0.02 mm in Tanur, Thalassery and Badagara populations. The least square analysis of variance revealed that the pre and post immunization skin thickness at 0, 24, 48 and 72 hours were non significant between different sub populations

Numerous factors differentiate genetic difference in acquired or innate cell mediated immune responses. The responses to these differences are likely to relate to a variety of genes including those determining cytokine receptors or the adhesion proteins. The cellular T-lymphocyte dependant immune response has also been reported to be polygenically regulated.

The PHA responses were not significant possibly due to the over riding and multifactorial influences of climate, diseases and management practices.

However, PHA responses being an index of generalized CMI status, duration and intensity of this trait has tremendous potential of being used as a tool in the development of disease resistant strains.

5.9 TOTAL PROTEIN, ALBUMIN AND GLOBULIN

In the present study the mean concentrations of total protein, albumin and globulin in goat populations ranged from 4.75±0.32 to 6.01±0.22, 2.41±0.06 to 2.72±0.10 and 2.34±0.31 to 3.28±0.22 g/dl, respectively. Similar ranges were observed in Nubian goats (Chen *et al.*, 1998), in Jakrana, Marwari and Sirohi goats (Kaushish *et al.*, 2000) and in goats of Namakkal region of Tamilnadu (Magesh and Vasu, 2000). The mean concentration of globulin in goat populations of Tanur, Thalassery and Badagara were 2.34 ± 0.31, 3.13 ± 0.31 and 3.28 ± 0.22 g/dl, respectively. The least square analysis of variance of total protein, albumin and globulin concentration revealed significant difference between the sub-populations. The globulin level was found lowest in Tanur population as compared to the other two populations. This might possibly be a population specific characteristic or even a reflection of an impact of some area specific stress, probably climatic, effect of pesticides or sub clinical infections of hither to unidentified etiology.

Overall in the present study, Malabari goats at the three different centres, viz.Tanur,Thalassery and Badagara were subjected to studies based on biochemical, immunological and microsatellite markers. In addition to that the populations were also compared on the basis of biometrical traits. No significant variations were evident with regard to the immunological profile.

Out of the eight biochemical markers tested, only three were found to be polymorphic and the genetic distance calculated could not reveal any precise information about their relationships. Hence microsatellite marker studies was resorted to, which clearly indicated that Tanur population was different from the other two populations. This finding was reconfirmed by the result of the analysis of the biometrical traits. Though all populations under study had all predominant physical characteristics of the Malabari breed, the Tanur population stood apart as regards the biometrical characteristics, like litter size and body weight and characteristics perceivable at the molecular level. It could well be inferred that this population might have evolved through mixing up of the local nondescript Tanur goats with original Malabari goats. Be it from a breeding point of view or that concerning economy, the high prolificacy exhibited by the

Tanur population is a desirable and welcome characteristic. The need for more research activities directed at exploring the chances of conserving and developing such unique populations within a breed has to be identified as the need of the hour and hence be addressed without delay. From the perspective of the average small farmer of India as well, who is rather concerned at the economic traits than the breed characteristics, it is highly necessary to instigate investigations on the possibilities and potentialities of such prolific populations.

Summary

6. SUMMARY

The possibility of existence of differences between the populations of Malabari goats offers scope for studies in this regard. The present study aimed at studying the genetic similarities and differences among three populations of Malabari goats utilizing immune responsiveness, biochemical and molecular markers. The phylogenetic relationship existing among these groups has also been investigated by estimating genetic distances based on biometrical traits, biochemical and microsatellite polymorphism. With these objectives, three hundred Malabari goats, hundred numbers from each population belonging to Tanur, Thalassery and Badagara were studied.

The proteins haemoglobin, transferrin, albumin, cerruloplasmin, amylase and carbonic anhydrase were studied by polyacrylamide gel electrophoresis. Three phenotypes were observed for haemoglobin, viz. Hb_{AA}, Hb_{AB} and Hb_{BB}. All the animals belonging to Tanur and Badagara were of Hb_{AA} type while the goat populations of Thalassery possessed Hb_{AA}, Hb_{AB} and Hb_{BB} phenotypes with gene frequencies of 0.092 and 0.038 as regards Hb^{A} and HB^{B}, respectively. In the pooled population, the gene frequency of Hb^{A} and Hb^{B} were 0.987 and 0.012, respectively. The Hb^{A} variant was noticeably predominant in all the populations studied.

On SDS PAGE electrophoresis, two transferrin variants were observed, suggestive of two phenotypes Tf_{AA} and Tf_{BB}. No Tf_{AB} could be detected. The gene frequency of Tf^{A} was found to be higher in Tanur, Badagara and pooled populations (0.995, 0.974 and 0.990, respectively). The gene frequencies of Tf^{B} were recorded as 0.005, 0.026 and 0.010, respectively in Tanur, Badagara and pooled population. In the total population, a predominance of Tf^{A} variant was evident.

Two bands each were observed for albumin locus in all the animals studied, indicating absence of polymorphism. Absence of polymorphism for cerruloplasmin,

amylase and carbonic anhydrase in all the populations studied was indicated by the appearance monomorphic bands for each of these proteins.

The mean concentration of potassium in the pooled population was 4.18±0.09 meq/l. The mean potassium concentrations in Tanur, Thalassery and Badagara were 3.45 ± 0.11., 4.52. ± 0.20 and 4.60 ± 0.14 meq/l, respectively. The least squares analysis of variance of potassium concentrations showed that there existed significant difference between different sub populations. All the animals studied were of low potassium type.

The animals were classified as low and high glutathione types. The mean values for low and high glutathione types in Tanur, Thalassery and Badagara and pooled populations were 34.40 and 103.19, 29.63 and 76.27, 35.47 and 103.90; and 33.16 and 94.45mg/100 ml RBC, respectively. The gene frequencies of GSH^{H} and GSH^{h} in Tanur, Thalassery, Badagara and pooled populations were 0.45 and 0.55, 0.09 and 0.91, 0.30 and 0.70; and 0.27 and 0.73, respectively. The least square analysis of glutathione concentrations showed significant variation between populations. In the pooled population, majority of the animals were categorized as low GSH type (53.67 per cent).

Out of the eight biochemical markers tested, polymorphism could be observed only for haemoglobin, transferrin and glutathione loci. Genetic distances calculated using the allelic frequencies of these polymorphic loci, by the method described by Balakrishnan and Sanghvi (1968), were found to be 0.3351 between Thalassery and Badagara, 0.6690 between Thalassery and Tanur and 0.1249 between Badagara and Tanur.

Genetic distance was also calculated using the computerized GENDIST programme of PHYLIP version 3.63. The genetic distance between Tanur and Badagara was found to be 0.0092 while that between Tanur and thalassery was 0.0498 and between Badagara and Thalassery was 0.0166. Genetic distance values calculated using the method described by Balakrishnan and Sanghvi (1968) were found to be higher when compared to the values obtained using the GENDIST programme.

Protein polymorphic markers failed to give a conclusive picture of genetic relationship. Hence an attempt was made to study the different populations at molecular level, using microsatellite markers.

Microsatellite analysis was also performed by PAGE, using radio labeled isotope, to find out the similarities and differences at molecular level between the different sub - populations. The average yield of DNA obtained from 5 ml of whole blood sample was 350.05 ±10.05 μm. The concentration of DNA averaged about 700.11 ± 20.10μm/ml.

Ten microsatellite markers tested were TGLA53, ILSTS 030, ILSTS 011, ILSTS 005, INRA 005, INRA 063, ETH 10, HUJ 1177, BM 720, and INHA, out of which INRA 063, HUJ 1177and ILSTS 030 were found to be polymorphic.

A total of ten, five and eight alleles were observed for INRA 063 locus in Tanur, Thalassery and Badagara populations, respectively. Heterozygosity and PIC values calculated were 0.847and 0.843, 0.656 and 0.652, and 0.826 and 0.815, respectively. Six genotypes were found to be specific for Tanur population. The allele of 163 bp occurred in highest frequency in Tanur. Two alleles found exclusively in Tanur were of 155 and 159 bp. The maximum number of genotypes as well as alleles were observed in Tanur compared to the other populations.

With regard to HUJ 1177 locus, 15, 14 and 13 alleles were detected in Tanur, Thalassery and Badagara populations with heterozygosity and PIC values of 0.894 in Tanur and 0.880 in Badagara populations. In Thalassery population, heterozygosity and PIC values were 0.909 and 0.907, which were higher when compared with the two other populations studied. Two alleles were specifically found in Tanur populaton. The maximum number of genotypes as well as alleles was observed in Tanur compared to the other populations.

The third microsatellite marker ILSTS 030 gave rise to eleven, seven and nine alleles, respectively in Tanur, Thalassery and Badagara populations. Heterozygosity and

PIC values were highest in Tanur (0.850 and 0.848) while lowest values (0.793 and 0.775) were recorded in Thalassery population. In Badagara the values were 0.828 and 0.823, respectively. Maximum number of alleles for this locus was observed in Tanur.

The genetic distances calculated on the basis of the polymorphic microsatellite markers by computerized GENDIST programme were 0.5729 (between Thalassery and Badagara), 0.7795 (between Tanur and Thalassery) and 0.8401(between Badagara and Tanur).

The microsatellite studies suggest a definite relation between Thalasserry and Badagara much in accordance with the geographical proximity between the two places. The finding with regards to biometrical traits also was reiterative of the close relationship between the two populations. Tanur population was found to be widely separated from the other populations as regards the genetic distance.

With regard to litter size, the percentage occurance of kidding with singles, twins, triplets and quadruplets were higher in Tanur when compared to Thalassery and Badagara, during the year 2004-2005. However the body weights of goats at Thalassery and Badagara were higher than that of Tanur population. The mean birth weights recorded in Tanur, Thalassery and Badagara were 1.73 ± 0.04, 1.88 ± 0.03 and 1.86 ± 0.05Kg, respectively. The corresponding body weights at one year of age were18.37 ± 2.07, 27.00 ± 1.75 and 25.58 ± 0.76 kg, respectively. The percentage of multiple births in goat populations of Tanur, Thalassery and Badagara were found to be 0.76, 0.66 and 0.68, respectively. On an average, the goats of Thalassery and Badagara were heavier in comparison to Tanur goats, though the prolificacy remained higher in Tanur animals than that of the other two populations.

The different sub-populations under the present study were also screened for the antibody response to SRBC by microhaemolytic test. Naturally occurring antibody to SRBC was absent in all the populations. The highest concentration of antibody was observed on day seventh after primary immunization, apparent by the mean antibody titre

in goat populations of Tanur, Thalassery and Badagara (3.24 ± 0.05, 3.23 ± 0.06 and 3.33 ± 0.06), respectively. On 15th day the titre stood reduced at 2.92 ± 0.05, 2.81 ± 0.05 and 2.87 ± 0.05, respectively. The concentration was the lowest on day 21st, as detected by the mean antibody titre of 2.55 ± 0.06, 2.52 ± 0.06 and 2.54 ± 0.06 in the respective sub populations. The least square analysis of variance of immune response on seventh, fifteenth and twenty first day revealed no significant differences between the different sub-populations. The effect of antibody response to SRBC on the 7th, 15th and 21st days after primary immunisation was not found to be significant for the occurrence of diseases like diarrhea and pneumonia.

The cutaneous response to intradermal injection to Phytohaemagglutinin – M (PHA-M) was also studied to find out the difference if any in and between the various sub populations under the present study. The pre injection skin thickness in goat populations of Tanur, Thalassery and Badagara had mean values of 1.34 ± 0.01, 1.34 ± 0.02 and 1.33 ± 0.02mm, respectively. The mean increase in skin thickness was maximum at 24 hours of intradermal injection of PHA-M in Tanur, Thalassery and Badagara populations (1.84 ± 0.04, 1.86 ± 0.03 and 1.95 ± 0.04 mm, respectively). The skin thickness reduced to 1.69 ± 0.02, 1.72 ± 0.02 and 1.76 ± 0.02 mm after 48 hours. The values for 72-hour skin thickness stood at 1.61 ± 0.02, 1.62 ± 0.02 and 1.65 ± 0.02 mm in Tanur, Thalassery and Badagara populations. The least square analysis of variance revealed that the pre and post immunisation skin thickness at 0, 24, 48 and 72 hours were non significant between different sub-populations.

Total protein and albumin were estimated by biuret method (Weichelbaum, 1946) and bromocresol green method (Doumas, 1971) respectively using standard kits from Agappe. Serum globulin level was calculated by the method described by Benjamin (1985).

The mean concentrations of globulin in goat populations of Tanur, Thalassery and Badagara were 2.34 ± 0.31, 3.13 ± 0.31 and 3.28 ± 0.22g/dl, respectively. The highest mean concentration for globulin was detected in Badagara population and the lowest in Tanur. The least square analysis of variance of globulin concentration revealed

significant difference between populations. The level of globulin in these populations indicates a higher disease resistance in Malabari goats.

The present study revealed that the Tanur goat population was different from the Thalassery and Badagara goat populations. The high prolificacy exhibited by the Tanur population is a desirable and welcome characteristic which needs to be explored further with a view to conservation and development for the benefit of the average Indian farmer.

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STUDY OF GENETIC DIVERSITY IN MALABARI GOATS (*Capra hircus*) UTILIZING BIOCHEMICAL AND IMMUNOLOGICAL MARKERS

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ABSTRACT

Goat populations of Tanur, Thalassery and Badagara were studied for biochemical polymorphisms, immunological and microsatellite markers to investigate the similarities and differences between these populations.

With regard to biochemical markers tested, polymorphism was observed only for haemoglobin, transferrin and glutathione loci. Two variants were observed for haemoglobin, Hb^{A} and Hb^{B} with a frequency of 0.987 and 0.012, respectively, suggestive of three phenotypes, viz. Hb_{AA}, Hb_{AB} and Hb_{BB}, and indicating the predominance of Hb^{A} in the pooled population. Hb^{B} variant was observed only in the Thalassery population (gene frequency 0.038).

Two variants for transferrin (Tf^{A} and $Tf^{B)}$ were detected with a predominance of Tf^{A} in the population. All the goats from Thalassery population belonged to Tf_{AA} type. In the present study only two phenotypes as regards transferrin locus could be observed, (Tf_{AA} and Tf_{BB}) with the notable absence of Tf_{AB}. No polymorphism was observed for albumin, cerruloplasmin, amylase and carbonic anhydrase loci in all the animals tested.

The animals were classified as low and high glutathione types based on the values obtained for blood glutathione concentration. In the pooled population, majority of the animals belonged to low GSH type (53.68 per cent). The least square analysis of glutathione concentrations showed significant variation between populations.

With regard to potassium loci, all the animals in the present study belonged to low potassium type, with the mean potassium concentration of the pooled population recorded at 4.18 ± 0.09 meq/l. The least square analysis of variance of potassium concentrations showed that there existed significant difference between different sub-populations.

Genetic distance was calculated as described by Balakrishnan and Sanghvi (1968), using the allelic frequencies of protein polymorphic loci. Genetic distance between Tanur and Badagara was found to be 0.1249 , while that between Tanur and

Thalassery was 0.6690 and between Badagara and Thalassery was 0.3351. The only possible conclusion that could be arrived at from the above studies is the existence of a relationship between these populations. Hence an attempt was made to study the different populations at molecular level, using microsatellite markers. Three markers, viz. INRA 063, HUJ 1177and ILSTS 030 were found to be polymorphic. Based on the genetic distances, it was found that Thalssery and Badagara were closely related than Tanur population.. This finding, much in agreement with biometrical traits, reiterates the close relationship between the Thalassry and Badagara populations. On an average, the goats of Thalassery and Badagara were heavier in comparison to Tanur goats, though the prolificacy remained higher in Tanur animals than the other two populations.

The different sub-populations under the present study were also screened for the antibody response to SRBC. The highest concentration of antibody was observed on day seventh after primary immunization. The titre gradually reduced by the 15^{th} day, reaching the lowest values on 21^{st} day of post immunization. The effect of antibody response to SRBC on the 7^{th}, 15^{th} and 21^{st} days post immunization was not found to be significant for the occurrence of diseases like diarrhoea and pneumonia.

The cutaneous response to intradermal injection to Phytahaemagluttinin – M (PHA-M) was also studied to find out the differences, if any in and between the various sub- populations under study. The values for skin thickness were maximum at 24 hours post-intradermal injection of PHA-M and were recorded as 3.24±0.05, 3.23±0.06 and 3.33±0.06 mm in Tanur, Thalassery and Badagara, respectively. The skin thickness reduced considerably after 48 hours and reached 1.61±0.02, 1.62±0.02 and 1.65±0.02 mm, respectively at 72 hours. The least square analysis of variance revealed that the values for pre and post immunization skin thickness at 0, 24, 48 and 72 hours were non significant between different sub-populations.

Total protein, albumin and globulin concentrations also were estimated. The highest mean concentration for globulin was detected in Badagara population

(3.28±0.22g/dl) and the lowest in Tanur (2.340 ± 0.31g/dl). The least square analysis of variance of globulin concentration revealed significant difference between populations.

Though all populations under study had all predominant physical characteristics of the Malabari breed, the Tanur population stood apart as regards the biometrical characteristics, like litter size and body weight and charecteristics perceivable at the molecular level. It could well be inferred that this population might have evolved through mixing up of the local nondescript Tanur goats with original Malabari goats. The study reiterates the need for more research activities directed at exploring the chances of conserving and developing such unique populations within a breed.

Annexure

ANNEXURE

Solutions for SDS-PAGE

1. 30% acrylamide stock solution 29:1

Acrylamide	29 g
N. N –Methylene bisacrylamide	1 g

Make up to 100 ml with distilled water.

2. Resolving gel (8%) – 40ml

Water	18.5 ml
30% acrylamide stock solution	10.7 ml
1.5 M Tris (pH 8.8)	10 ml
10% SDS	0.4 ml
10%APS	0.4 ml
TEMED	0.024 ml

3. Stacking gel 5% -10 ml

Water	6.8 ml
30% acrylamide stock solution	1.7 ml
1.0M tris (pH 6.8)	1.25 ml
10 %SDS	0.1ml
10 % APS	0.1 ml
TEMED	0.01 ml

4. SDS gel loading buffer (2X)

0.05 M Tris (pH 6.8)	2.5 ml
10% SDS	4ml
Glycerol	2 ml
Mercaptoethanol	0.2 ml
Bromophenol blue	0.5 mg

Make up to 10 ml with distilled water

5. 5X TGE

Tris base	15.1 g
Glycine	94 g

Dissolved in 900 ml water

10% SDS	50 ml

Make up the volume to 1000 ml with water

6. Staining solution

Coomasie brilliant blue	1.25 g
Methanol	227 ml
Glacial acetic acid	46 ml
Distilled water	237 ml

7. De staining solution

Methanol	1500 ml
Glacial acetic acid	500 ml
Distilled water	up to 5000 ml

8. Fixative

Methanol	250 ml
Glacial acetic acid	60 ml
Distilled water	up to 1000 ml

Solutions for Native PAGE

1 30% acrylamide stock solution 29:1

Acrylamide	29 g
N. N –Methylene bisacrylamide	1 g

Make up to 100 ml with distilled water.

2 10 XTBE

Tris Base	108 g
Boric acid	55 g
EDTA	9.3g

Dissolve in 700ml water. Adjust the pH to 8.3 and make up to 1000ml.

3 8% gel (70 ml)

30% acrylamide stock solution	18.62 ml
Water	36.89 ml
5X TBE	14 ml
10%APS	0.49 ml
TEMED	25 µl