PREVALENCE OF YEAST AND YEAST LIKE FUNGI IN BOVINE MASTITIS AND THEIR *IN VITRO* DRUG SENSITIVITY

By

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THESIS

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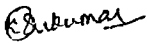
1996

DECLARATION

I hereby declare that this thesis entitled **Prevalence of yeast and yeast like fungi in bovine mastitis and their *in vitro* drug sensitivity** is a bonafide record of research work done by me during the course of research and that this 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



Place Mannuthy
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CERTIFICATE

Certified that this thesis entitled **Prevalence of yeast and yeast like fungi in bovine mastitis and their *in vitro* drug sensitivity** is a record of research work done by Sri K SUKUMAR under my guidance and supervision and that it has not previously formed the basis for the award of any degree fellowship or associateship to him

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K SUKUMAR

Dedicated to
my Parents and Brother

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INTRODUCTION

Alkaline phosphatase is one among the group of nonspecific enzymes capable of hydrolysing a large number of primary phosphate esters in the alkaline pH In vivo these enzymes are associated with calcification and bone formation Through the use of electrophoretic techniques the different forms of alkaline phosphatase termed isoenzymes have been separated and studied These biochemical characters can be utilised as gene markers for early selection of farm animals as the association between them and the production traits is well established This association may be due to pleiotropy linkage or due to interaction between alleles

The introduction of crossbreeding technology has resulted in considerable increase in the proportion of crossbred cattle in Kerala According to the latest Livestock Census (1987) the percentage of crossbreds in the cattle stock is about 51 This technological change has boosted the milk production from about two lakh tonnes in the sixties to 21 lakh tonnes by the year nineties (Thankachan 1995) This has been due to the transformation of local cattle into crossbreds and improvement in management practices

Improvement of economic traits depends mainly on proper selection and scientific breeding The conventional methods of selection based on performance records are time consuming and maintenance of inferior stock for longer duration involves much economic loss This could be avoided if the selection could be done at an early age The forecasting of productivity characters of animals in their early age based on indirect methods has been

a persistent puzzle in improvement of domestic animals Since the discovery of blood groups in domestic animals it has been suggested that blood groups and biochemical traits could be associated with performance and could be valuable for animal improvement In domestic animals if a reasonably full proof and firm association between blood group factors/ polymorphism with production traits is established it would pave the way for rapid improvement through sequential selection by making possible early selection and culling Extensive work has been conducted in recent years on the genetic variations of the proteins enzymes and red cell antigens In any study of population dynamics there is a need for markers by which changes in the genetic variation between different populations may be measured The markers should show simple inheritance and be fairly neutral with regard to the production viability and reproduction The loci controlling the polymorphic proteins and enzymes represent excellent marker loci for application in the parentage control and for the genetic investigations It has been found that the frequencies of the alleles controlling many protein systems in cattle vary from breed to breed As a result the gene frequencies of such alleles at different loci in cattle have been employed in studies of relationship among cattle breeds and breed structure The present study was therefore undertaken with the following objectives

1 To find out the usefulness of alkaline phosphatase as a genetic marker

2 To study the heterogenety of serum alkaline phosphatase variation in crossbred cattle

3 To study association if any between the serum alkaline phophatase polymorphism and traits of economic importance such as milk production and composition of milk

Review of Literature

REVIEW OF LITERATURE

Alkaline phosphatases (AP) [Orthophosphoric monoester phosphohydrolase E C 3 1 3 1] are esterases that catalyse the splitting of phosphate radicals from phosphate monoester molecules optimally at about pH 8 10 They exist in multimolecular forms and as such are designated as isoenzymes As in the case with several other enzymes isoenzymes of alkaline phosphatase can be detected in body fluids These include specific isoenzymes originating from bone liver placenta and intestine They are very stable enzymes and can be frozen with very little or no loss of activity

2 1 Serum Alkaline Phosphatase (SAP) polymorphism in cattle

Differences in electrophoretic mobility of alkaline phosphatase have been found in human serum (Boyer 1961)

Gahne (1963) reported the electrophoretic variations of serum alkaline phosphatases in cattle He observed that data obtained from 263 matings were in accordance with the hypothesis that variation in cattle phosphatase was determined by a genetic system symbolized by F that one allele F^A determine the presence of the enzyme zone A while another allele (or alleles) F^O give rise to zymograms without zone A He had found that the gene frequencies of F^A and F^O in Swedish Red and White bulls were 0 133 and 0 867 respectively Rendel and Stormont (1964) reported alkaline phosphatases polymorphism in sheep and their association with the R O blood groups They had examined blood serum samples from 177

sheep (105 group R 67 group O 5 group 1) by starch gel electrophoresis With a few exceptions it was possible to demonstrate two clearly demarcated zones of phosphatase activity A and B in decreasing rate of migration It was shown that phosphatase activity in zone B was highly correlated with the presence of the soluble blood group substance O and since the O property of serum is inherited as a recessive the author inferred that the B phosphatase band would appear as a recessive trait

Hope (1966) confirmed the association between serum alkaline phosphatase variants and the R O 1 blood group system in the Australian Merino He had found that sera from all animals revealed an A zone thus animals were classified into two groups corresponding to the presence (+) or absence ( ) of the B zone Family data agreed with expectations based on the postulated genotypes for the R O 1 blood group phenotypes grups O being due to a homologous recessive condition at an autosomal locus

Romanov (1972) reported three alkaline phosphatase phenotypes F FS and S controlled by two autosomal codominant genes (P^F and P^P) three breeds of Russian cattle

Amano et al (1973) studied the alkaline phosphatase isoenzymes by starch gel and polyacrylamide gel electro phoresis in cattle They have classified the phenotypes as A or O according to the presence or absence of the fastest moving band [A band] By experimental mating they have found that A and O phenotypes were determined by a pair of autosomal alleles F^A and F^O Their study revealed that the observed frequency of F^A in Holstein Friesian was 0 03

Dragnev (1974) worked on the genetic polymorphism of serum isozymes of alkaline phosphatese in some Bulgarian cattle breeds and tabulated the distribution of alkaline phosphatase phenotypes and gene frequencies

Nandakumaran (1976) typed 286 crossbred animals belonging to Holstein Friesian x Hariana Brown Swiss x Hariana and Jersey x Hariana for alkaline phosphatase They had reported two alkaline phosphatase phenotypes designated as SS and FS determined by two alleles P^F and P^S of which P^F had a faster mobility than P^S The frequency of phenotype SS ranged from 1 00 (Brown Swiss x Hariana crossbreds) to 0 8696 (Jersey x Hariana crossbreds) The frequency of FS ranged from 0 1304 in Jersey x Hariana to 0 in Brown Swiss X Hariana The gene frequencies of P^F in the Holstein Friesian X Hariana Brown Swiss X Hariana Jersey X Hariana and crossbreds of F_2 generation were 0 0072 0 0 0653 and 0 0145 respectively The frequency of P^S in these populations were 0 9928 1 00 0 9347 and 0 9855 respectively They had reported that the frequency of P^S in Holstein Friesian X Hariana was not significantly different from Brown Swiss X Hariana and Jersey X Hariana crossbreds and crossbreds of F_2 generation There was a significant difference in the frequency of PS between Brown Swiss X Hariana and Jersey X Hariana crossbreds P^S was not significantly different Brown Swiss X Hariana and crossbreds of F_2 generation and between Jersey X Hariana and crossbreds of F_2 generation

Walawski et al (1977) reported that the fast migrating variant of the serum alkaline phosphatase was controlled by a dominant gene (A) and slower

migrating variant by its recessive allele (a) He had also found that the enzyme activity was three times greater in cattle with one or two dominant genes compared with the recessive homozygotes

Katholm (1978) had worked in the plasma alkaline phosphatase isoenzymes in cattle and revealed that the fast moving A band is inherited in a polygenic system They had noticed that the A band was more frequently found in males than in females Tandon and Khanna (1978) worked on serum alkaline phosphatase polymorphism using starch gel electrophoresis technique and observed two phenotypes in Hariana cattle and its corssbreds One designated [AkpFS] showed two bands an AkpS band and a faster [AkpF] band They could not observe any AkpFF phenotype The frequency of the Akp^S gene ranged from 0 963 in the exotics to 0 997 in Hariana There appeared to be interaction between alkaline phosphatase and amylase polymorphisms They had noticed more alkaline phosphatase heterozygotes than expected with amylase type A_1A_1 Vijay Shankar et al (1985) had investigated serum alkaline phosphatase polymorphism in about eleven hundred animals belonging to zebu (Sahiwal 76 Tharparkar 42) exotic (Friesian 42 Jersey 27) and their crossbred strains (Karan Swiss 508 and Karan Fries 440) and had observed two phenotypes (SAP A and SAP O) which were controlled by a dominant system where the F^A allele determined the presence of enzyme activity (SAP A) and F^O its absence (SAP O) They had found out that the frequency of the F^A allele to be high among all the breed or genetic groups studied

Mondal et al (1986) reported the heritability of serum alkaline

phosphatase activity as 0 20 ± 0 02 Jana et al (1989) estimated the heritability of blood serum alkaline phosphatase activity as 0 39 ± 0 08 Kirmani et al (1989) could observe two alkaline phosphatase phenotypes by polyacrylamide gel electro phoresis of blood serum of Sahiwal and its cross with exotic breeds The phenotypes were found to be controlled by two alleles (FA and FO)

Ormian and Ormian (1992) studied the biochemical polymorphism of some enzymes in the blood serum of cows and had reported that there were two alleles for alkaline phosphatase They had observed significant deviations of genetic equilibrium for all loci for many enzymes except alkaline phosphatase

Mizuno et al (1992) had reported that bovine porcine and fowl alkaline phosphatases were able to liberate inorganic phosphate from casein phosphopeptides (CPP) and B casein They had found that inhibitors of alkaline phosphatases include Carrageenan (S) mucin pectin and dextran sulphate Among them they had noticed that Carrageenan (S) showed the highest inhibitory action against the decomposition of casein phosphopeptides by alkaline phosphatase and thus may have further value as a food additive

2 2 Alkaline phosphatase level

Bodansky (1933) used glycerol phosphate as substrate and expressed the serum alkaline phosphatase activity in Bodansky unit while King and Armstrong (1934) used phenyl phosphate as substrate and expressed the activity in King and Armstrong unit

Bessey et al (1946) evolved a method of determin ation of serum alkaline phosphate by using p nitro phenyl phosphate as substrate and he expressed the serum alkaline phosphatase activity in millimol of nitrophenol

Kaneko J J and Coxelius C E (1970) reported that the enzyme activity of the serum remains fairly constant over a long period of time with a great range of SAP activities (0 3 114 3 KA units/100 ml) in cattle

Kaneko J J and Coxelius C E (1970) reported an average of 11 8 K A units of SAP per 100 ml of serum with a range of 4 7 62 4 KA units/100 ml

The SAP level in cattle was found to be varying between zero to 488 I U the average being 194 $\pm$ 126 (Kaneko JJ and Coxelius CE 1970)

Antonov and Malchevski (1983) analysed the activity of alkaline phosphatase isoenzymes in the blood serum of cattle sheep and swine and found that in all three species total alkaline phosphatase activity was much greater in young animals than in adults They could not find any difference between adult males and females or between lactating and dry females

Mazumder and Mazumder (1985) noticed that age and season had highly significant effect ($P < 0\ 01$) and breed composition had significant effect ($P < 0\ 05$) on the serum alkaline phosphatase activity in crossbred cattle

Shaker et al (1988) had examined the effect of in vitro addition of

some metal ions on serum alkaline phosphatase in calves They had found that on additon of 10 3 mol/litre of $MgCl_2$ or of 10 5 mol/litre of zinc sulfate significantly increased the enzyme activity at nearly all stages They had found a non significant increases in enzyme activity for barium Mercuric chloride in concentrations above 10 5 mol/litre resulted in non significant inhibitiion of enzyme activity They had recorded significant inhibitory effects for cobalt at all ages particularly at a concentration of 10 3 mol/litre They had also reported that the inhibition of alkaline phosphatase activity by arsenic was strong and pronounced in the first month at 10 3 mol/litre and only about a quarter of the original acitivity persisted They had noticed that with advancing age the enzyme was more resistant to inhibition by arsenic

2 3 Alkaline phosphatase and production traits

The enzyme polymorphism may be applied to the livestock improvement since some of the polymorphic alleles may be linked with the economic traits due to linkage pleiotrophy or due to interaction between alleles (Rendel 1967)

Palanskii and Romanov (1973) reported non significant association between alkaline phosphatase types and milking rate in Ukranian White headed cows

Nandakumaran (1976) compared the alkaline phosphatase types and the various economic traits like age at first calving first and second lactation yield first and second lacation length first and second dry

period intercalving period etc in Jersey X Hariana They could not observe any significant association between alkaline phosphatase types and these economic traits

Agnes (1977) studied the level of serum alkaline phosphatase during pregnancy and found that inspite of a marked increase in total alkaline phosphatase activity at parturition Heat Stable Alkaline Phosphatase (HSAP) levels did not vary significantly He had concluded that in the cow HSAP level had no value in indicating a functional placenta as it did in women

Sengonca (1977) examined the relationship of alkaline phosphatase with fattening performance and carcass characters and had found that alkaline phosphatase activity increased significantly with age and was affected by genotype but not significantly They had observed significant correlations of alkaline phosphatase activity with average daily gain carcass weight dressing percentage and loin eye area Agergaard and Katholm (1978) analysed the plasma alkaline phosphatase activity and isoenzyme composition in calves and had observed that calves with a high plasma alkaline phosphatase activity between 41 and 90 days of age had significantly higher gains than those with a low activity They had concluded that plasma alkaline phosphatase activity was partly genetically controlled since the variation in activity was significantly smaller within groups of half sibs than between groups They had recommended that the determination of alkaline phosphatase activity would provide a useful criterion for the evaluation of potential growth in calves

Katzmann et al (1978) examined the serum alkaline phosphatase activity of young bulls and had reported that serum alkaline phosphatase activity was a poor predictor of carcass characters

Katholm (1978) revealed that the A band a fast migrating band was associated with rapid growth rate

Arole and Kate (1980) revealed that alkaline phos phatase activity was not correlated with mammary weight or body weight They had found that during lactation activity increased to a maximum on day seven and then decreased to basal values by day 30 They had concluded that alkaline phosphatase activity in the rat mammary gland was hormone dependent

Walawski et al (1981) noticed that a few cows carried the dominant gene shown by the presence of faster migrating variant of alkaline phosphatase in blood They had found that the activity was significantly lower in the dominant group than in the recessive homozygous cows (236 9 Vs 308 2 I U $P < 0\ 05$) They had shown that the SAP activity in milk increased significantly during lactation but did not differ significantly between quarters at one sampling

Camas et al (1986) observed a negative correlation (0 595) between serum alkaline phosphatase SAP activity and weight of bones and head ($P<0\ 01$) and a negative correlation (0 405) between SAP activity and hide weight ($P <0\ 05$) Kirmani et al (1989) statistically analysed the influence of serum alkaline phosphatse phenotypes on growth reproduction and production traits and was found to be non significant but the least

squares means of growth reproduction and production traits suggested that SAP O type of animals exhibited faster growth early puberty/ maturity lower breeding efficiency and higher production performance

Jana et al (1989) reported that the genetic correlations of serum alkaline phosphatase activity with lactation and peak milk yield were positive

Schlimme and Thiemann (1992) suggested that it was not possible to indicate a lactophysiological normal activity of alkaline phosphatase in bulk milk but only a range within which enzyme activity can be expected

Materials and Methods

MATERIALS AND METHODS

3 1 Experimental Animals

The animals under study consisted of 110 cows belonging to two crosses of local non descript cattle with exotic breeds viz 56 Holstein Friesian crossbreds and 54 Brown swiss crossbreds maintained at the University Livestock farms at Thumburmuzhi and Mannuthy

3 2 Collection and preparation of serum samples

Ten ml of blood samples were collected in sterile test tubes by venepuncture of jugular vein The samples were labelled packed in ice and transported to the Animal Breeding and Genetics Laboratory College of Veterinary and Animal Sciences Mannuthy The samples were centrifuged at 2000 rpm for 15 minutes and the serum was removed and placed in a small sterile screw capped vial of 3 ml capacity and preserved at 40 C until further analysis

3 3 Electrophoresis for serum alkaline phosphatase

Polyacrylamide gel disc electrophoretic technique of Ornstein (1964) and Davis (1964) with slight modification as described by Tombs and Akroyd (1967) was used to determine the alkaline phosphatase types

Polyacrylamide gel is a three dimentional molecular net work It is made from acrylamide which is a monomer and which undergoes both

polymerisation and cross linking through N N methylene bis acrylamide commonly called as bis The gel is formed by mixing acrylamide and bis in buffer solution and adding a catalyst accelerator ammonium persulphate N N N N tetramethyl ethylene diamine (TEMED)

3 3 1 Preparation of the gel

i Monomer

To 28 g of acrylamide 0 735 g N N methylene bis acrylamide was added and made upto 100 ml with deionized water

ii Small pore buffer (pH 8 9)

To 48 ml of IN HCl 36 6 gms of Tris and 0 23 ml of Tetramethylene tetramethyle ethyline diamine (TEMED) were added and made up to 100 ml with deionized water

iii Catalyst

Freshly prepared 0 14 per cent ammonium per sulphate in deionized water served as a catalyst

3 3 2 Electrode buffer

Lithium hydroxide boric acid buffer system was used for the electrophoresis (Steiner and Joslyn 1979)

Composition of the buffer

Lithium hydroxide 1 5 g/litre (0 036 M) Boric acid 12 0 g/litre (0 194 M) pH 8 25

3 3 4 Preparation of the running gel

A seven per cent running gel was cast in the following proportions

Monomer	2 volumes	Small pore buffer	1 volume
Ammonium per sulphate	4 volume		
Deionised Water	1 volume		

Two plates of equal size one made up of acrylic sheet and the other one a glass plate was used for the preparation of the gel The acrylic sheet had 1 5 mm high frame on three sides and formed the thickness of the gel On the free side without the frame the acrylic sheet was having projections to form wells on the gel The glass plate was kept in apposition with the frame of the acrylic sheet with the application of vacuum grease on the frame Paper clips were applied on all the sides

Freshly prepared working gel solution was filled into the gap between the plates Care was taken to avoid the formation of air bubble in the gel Completion of polymerisation which takes about 20 minutes was indicated by the appearance of a refractile line of demarcation between gel and water Before applying the sample the water layer was removed

3 3 5 Electrophoresis

Acrylic sheet was carefully removed and the gel was gently placed in the electrophoretic chamber containing the electrode buffer Whatman filter paper No 1 was used as the wick for completion of circuit connecting the gel and electrode buffer Enough number of filter papers

of equal sizes and same level at the edges were used for uniform voltage gradient The wicks were wetted well and placed gently on either side of the gel

The serum already prepared and stored in the deep freezer was taken out and thawed Equal quantity of serum and 40 per cent sucrose solution which was used to increase the density formed the sample 20 microlitres of this sample was charged into the wells Bromophenol blue was used as marker on one or two wells as indicator An initial current of 15 mA for one hour followed by 25 mA for 5 hours was applied During the run the temperature was kept constant at 5 C

On completion of the run the gel was removed from the glass plate and put it in a dish and stained for serum alkaline phosphatase as per the method of PaneerSelvam (1983)

The staining solution consisted of

0 05 M Tris HCl buffer (pH 8 5)	100 ml
Polyvinyl pyrrolidone	500 mg
Sodium 1 naphthyl phosphate	100 mg
Fast blue salt BB	100 mg
Magnesium chloride	60 mg
Mangenese chloride and	60 mg
Sodium chloride	2 g

The gels were incubated at 37 C in the staining solution for two hours and then the gels were washed in distilled water and fixed in seven per cent acetic acid and the staining pattern of each sample was noted

3 4 Quantitative Estimation of Alkaline phosphatase

Serum alkaline phosphatase content was estimated by employing the Kind and King s method (1954) as supplied by M/s Mediprob laboratories kits Hyderabad

PRINCIPLE

Serum ALP hydrolyzes phenyl phosphate into phenol and disodium hydrogen phosphate at pH 10 0 The phenol so formed reacts with 4 Aminoantipyrine in alkaline medium in presence of oxidzing agent Potassium ferricyanide to form a red colored complex whose abosrbance is proportional to the enzyme activity

$$\text{Disodium Phenyl phosphate} + H_2O \xrightarrow[\text{pH 10 0}]{\text{ALP}} \text{Phenol} + \text{Disodium Hydrogen Phosphate}$$

$$\text{Phenol} + \text{4 Aminoantipyrine} \xrightarrow[\text{Potassium ferricyanide}]{\text{Alkaline medium}} \text{Red Coloured complex}$$

CALCULATIONS

$$\text{Serum ALP in KA Units/100 ml} = \frac{\text{A of (T) A of (C)}}{\text{A of (S) A of (B)}} \times 10$$

Where A Absorbance

T Test

C Control

S Standard

B Blank

3 5 Estimation of the proportion of gene frequency in different crossbreds

If there is genetic equilibrium the proportion should be less than 1 96 The proportion can be found out by using the formula

$$Z = \frac{p_1 \quad p_2}{pq\,(1/n_1 + 1/n_2)}$$

Where Z – proportion

n_1 – Sample size of first population

n_2 – Sample size of second population

p_1 – Gene frequency of first population

p_2 Gene frequency of second population

$$p = \frac{x_1 + x_2}{n_1 + n_2}$$

x_1 – Sum of observed value of FF + 1/2 FS of first population

x_2 – Sum of observed value of FF+1/2 FS of second population

$q = 1 \quad p$

3 6 Production data

1 Association of serum alkaline phosphatase phenotypes/ levels with milk production and milk composition

The association of serum alkaline phosphatase phenotypes with milk production and milk composition were studied For statistical analysis the t test and Chi square tests described by Snedecor and Cochran (1967) were used

i Milk production

The milk yield for 305 days of the animals under study were recorded

ii Milk composition

a Milk fat by Gerber s method

Gerber s Sulphuric acid 10 ml

Well mixed sample of milk 10 75 ml

Amyl alcohol 1 ml

After taking all the three components into the butyrometer placed on a stand mixed the contents by shaking the butyrometer with stand at 45 angle until all the curd has been dissolved The butyrometers are placed in the centrifuge and are then rotated for 4 5 minutes at 1100 rpm Adjusted the fat column within the scale on butyrometer and recorded the reading

b Milk total solids (Gravimetric method)

Weighed accurately the clean dry empty dish Pipetted 5 ml of milk and transferred into the weighed dish and weighed quickly Placed the dish on a boiling water bath at least for 30 minutes Removed the dish from water bath wiped the bottom and kept the dish in the hot air oven and heated at 100°C for about 3 hours After 3 hours transferred it to a dessicator and allowed in to cool for 30 minutes Weighed the dish

Calculation

$$\text{Total solids per cent} = 100 \times \frac{\text{Weight of solids}}{\text{Weight of milk}}$$

c Milk Solids Not Fat (SNF)

By deducting the milk fat percentage from milk total solids percentage the SNF percentage was calculated

Results

RESULTS

One hundred and ten crossbred cows of Holstein Freisian and Brown Swiss were typed for the variants of Serum Alkaline Phosphatase (SAP) by employing horizontal polyacrylamide gel electrophoresis (PAGE)

4 1 Alkaline phosphatase

4 1 1 Phenotype/genotype and gene frequencies

The electrophoretic mobility of SAP revealed that in cattle it resolves into two distinct zones on polyacrylamide gel electrophoresis The bands were designated as F and S on the basis of increasing rate of electrophoretic mobility towards the anode The activity was observed as dark brown bands on staining with fast blue (Fig 1 and 2) The different phenotypes/genotypes of SAP were classified by taking into account the presence or absence of any one or more bands Accordingly three variant genotypes can be obtained but of the three possible combinations FF was absent in any of the crossbreds presently studied The genetic groupwise frequency of various SAP genotypes are given in Table 1

The frequency of SS genotype was 0 54 (31 out of 57) and 0 66 (35 out of 53) in the Holstein Friesian crossbreds and Brown Swiss crossbreds respectively In the pooled population the frequency was 0 60 (66 out of 110) The frequency of FS genotype was 0 46 and 0 34 in the Holstein Friesian crossbreds and Brown Swiss crossbreds respectively In the pooled population the frequency was 0 40 The percentage occurrence of

various genotypes and gene frequencies are given in Fig 3

The gene frequencies were calculated from the genotype frequencies by direct counting method The gene frequency of P^F was found to be 0 23 and 0 17 in Holstein Friesian crossbreds and Brown Swiss crossbreds respectively The gene frequency of P^S was found to be 0 77 and 0 83 in Holstein Friesian crossbreds and Brown Swiss crossbreds respectively In the pooled population the gene frequencies of P^F and P^S were 0 20 and 0 80 respectively

4 1 2 Test for genetic equilibrium

The results of the test for genetic equilibrium at the serum alkaline phosphatase locus are furnished in Table 2 The Holstein Friesian crossbreds and Brown Swiss crossbreds were found to be in genetic equilibrium at SAP locus In the pooled population the observed and expected values were significantly different both for homozygotes and heterozygotes ($P < 0\ 05$)

4 2 Serum Alkaline Phosphatase (SAP) levels

The SAP levels in different crossbred groups were studied The results revealed that SAP level was not significantly different between Holstein Friesian crossbreds and Brown Swiss crossbreds The mean values of SAP for different crossbred groups are presented in Table 3

The SAP level in Holstein Friesian ranged from 1 00 to 21 11 KA units per 100 ml where as in the case of Brown Swiss it was between 2 63 and

26 11 KA units per 100 ml The mean SAP values for the two crossbreds of Holstein Friesian and Brown Swiss were 8 48 kA and 9 63 KA units per 100 ml respectively The mean SAP value in the pooled population was 9 04 KA units per 100 ml of the serum

4 2 1 Association between Serum Alkaline Phosphatase (SAP) levels and SAP genotypes

The amount of alkaline phosphatase activity in different SAP genotypes of Holstein Friesian crossbreds and Brown Swiss crossbreds are presented in Table 4 The mean SAP levels for the SAP genotype FS were 9 32 KA units per 100 ml and 10 73 KA units per 100 ml for the crossbreds of Holstein Friesian and Brown Swiss respectively The mean SAP levels for the SAP genotype SS were 7 79 KA units per 100 ml and 9 07 KA units per 100 ml for the crossbreds of Holstein Friesian and Brown Swiss respectively There was no significant difference for the serum alkaline phosphatase levels between FS and SS in either Holstein Friesian or Brown Swiss crossbreds

4 2 2 Association between Serum Alkaline Phosphatase (SAP) level and milk production traits

4 2 2 1 Influence of Serum Alkaline Phosphatase (SAP) level on Milk production

The mean milk production for Holstein Friesian crossbreds and Brown Swiss crossbreds were 1702 36 kg and 1961 41 kg respectively There existed a non significant positive correlation (r 0 327) between serum alkaline phosphatase level and the milk production level (305 days) in

Brown Swiss Crossbreds where as a non significant negative correlation (r= 0 251) existed between the serum alkaline phosphatase level and the milk production level in Holstein Friesian crossbreds (Table 5)

4 2 2 2 Influence of Serum Alkaline Phosphatase level on fat percentage of milk

The average milk fat percentage for Brown Swiss and Holstein Friesian crossbreds were 5 24 and 5 22 respectively (Table 6) There existed a non significant negative correl ation between serum alkaline phosphatase level and the milk fat percentage in Brown Swiss Crossbreds (r = 0 18) and Holstein Friesian crossbreds (r 0 02)

4 2 2 3 Influence of Serum Alkaline Phosphatase (SAP) level on Solids Not Fat (SNF) percentage of milk

The average milk SNF percentage in Holstein Friesian crossbreds and Brown Swiss crossbreds were 7 68 (Table 7) There existed a non significant positive correlation between serum alkaline phosphatase level and the milk solids not fat percentage in Brown Swiss Crossbreds (r = 0 03) and in Holstein Friesian crossbreds (r = 0 29)

4 2 2 4 Influence of Serum Alkaline Phosphatase (SAP) level on Total Solids (TS) percentage of milk

The average milk total solids percentage for Holstein Friesian

crossbreds and Brown Swiss crossbreds were 12 90 and 13 02 respectively There existed a non significant positive correlation (r 0 28) between the serum alkaline phosphatase level and the milk total solids percentage in Holstein Friesian crossbreds where as a non significant negative correlation (r − 0 07) between serum alkaline phosphatase level and the milk total solids percentage in Brown Swiss crossbreds (Table 8)

4 2 3 Correlation between Serum Alkaline Phosphatase (SAP) phenotypes/genotypes and milk production traits

4 2 3 1 Correlation between SAP genotypes and Milk Production

Chi square test was performed for finding out any association between the various SAP genotypes with three different levels of milk production such as less than 1500 kg 1500 2000 kg and more than 2000 kg etc (Table 9) It has been observed that no significant difference existed between the patterns of occurrence of the milk production (305 days) and the patterns of number of animals belonging to that particular phenotype/genotype

4 2 3 2 Correlation between SAP genotypes and fat Percentage of milk

Chi square test was performed for finding out any association between the various SAP genotypes with three different levels of milk fat percentage such as less than 5 00 5 10 to 6 00 and 6 10 to 7 00 (Table

10) It has been observed that no significant difference existed between the patterns of occurrence of the milk fat percentage and the patterns of number of animals belonging to that particular phenotype/genotype

4 2 3 3 Correlation between SAP genotypes and Solids Not Fat (SNF) Percentage of milk

Association between the various SAP genotypes with three different levels of milk SNF percentage such as less than 7 50 7 50 to 8 50 and more than 8 50 percentage was found out by performing Chi square test (Table 11) It has been observed that no significant difference existed between the patterns of occurrence of the SNF percentage of milk and the patterns of number of animals belonging to that particular phenotype/genotype

4 2 3 4 Correlation between SAP genotypes and Total Solids (TS) Percentage of Milk

Association between the various SAP genotypes with three different levels of total solids percentage of milk such as less than 13 00 13 10 to 14 00 and more than 14 00 percentage was found out by performing Chi square test (Table 12) It has been observed that no significant difference existed between the patterns of occurrence of the SNF percentage of milk and the patterns of number of animals belonging to that particular phenotype/ genotype

Fig. 1. Genotype of Serum Alkaline Phosphatase

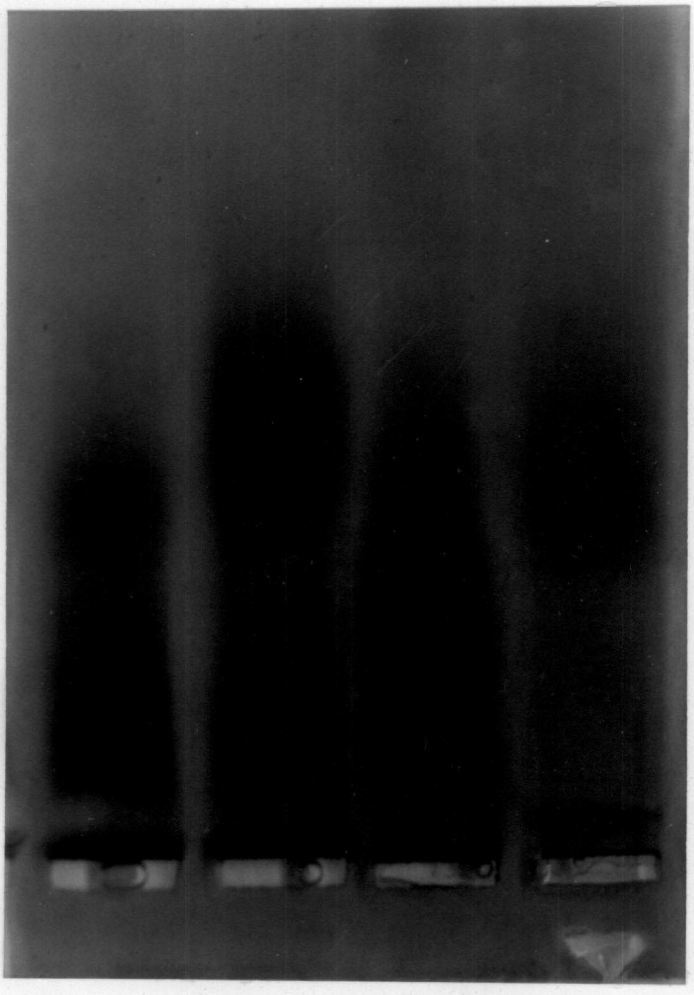

Fig. 2. Diagrammatic representation of Serum Alkaline Phosphatase genotypes

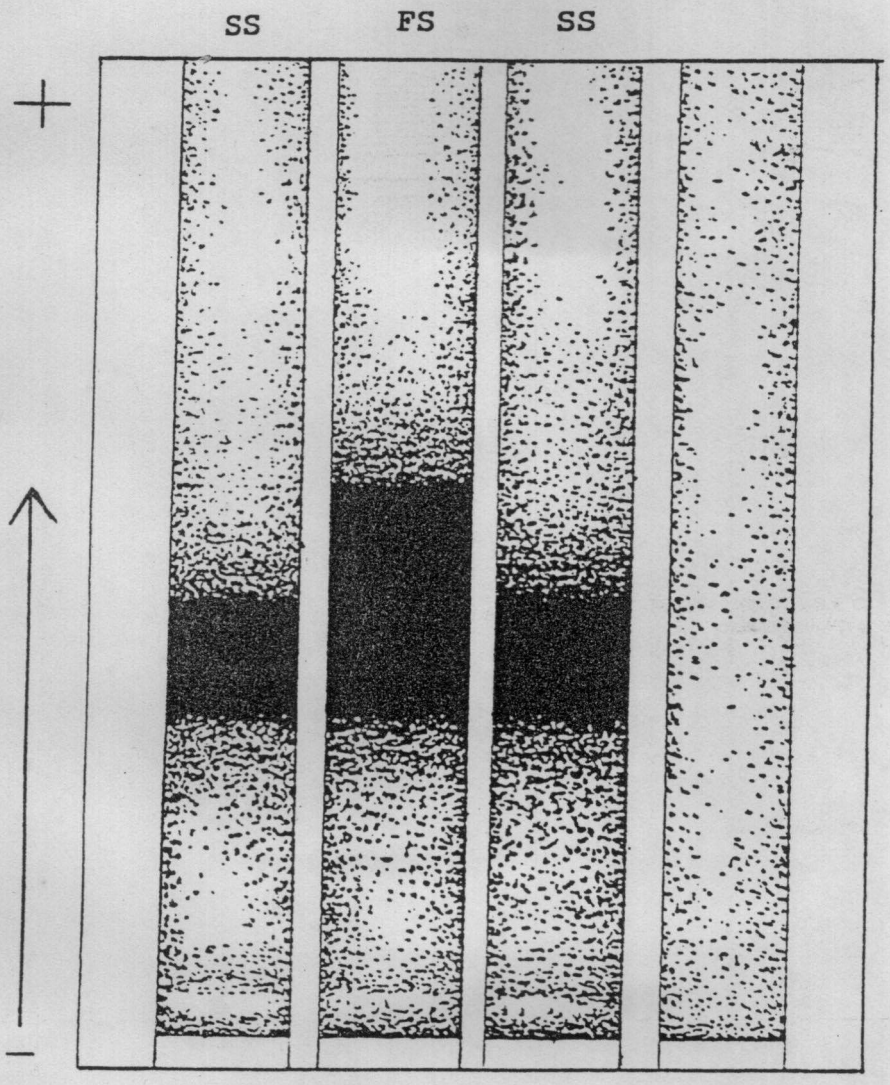

Fig. 3. Diagrammatic representation of Genotype frequencies and gene frequencies in different crossbred genetic groups.

Fig. 3 (A)

GENOTYPE FREQUENCY OF ALKALINE PHOSPHATASE
(BROWN SWISS CROSSBREDS)

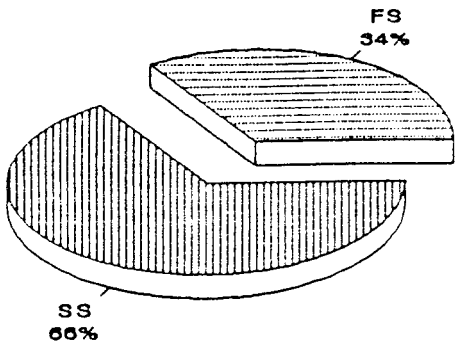

Fig. 3 (B)

GENOTYPE FREQUENCY OF ALKALINE PHOSPHATASE
(HOLSTEIN FRIESIAN CROSSBREDS)

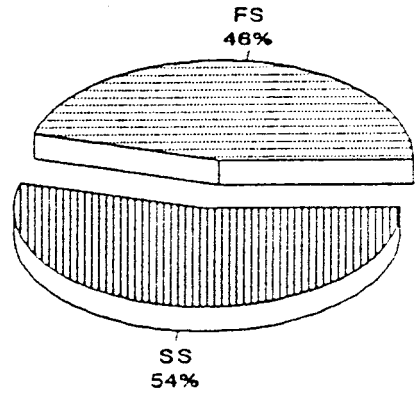

Fig. 3 (C)

GENE FREQUENCY OF ALKALINE PHOSPHATASE
(BROWN SWISS CROSSBREDS)

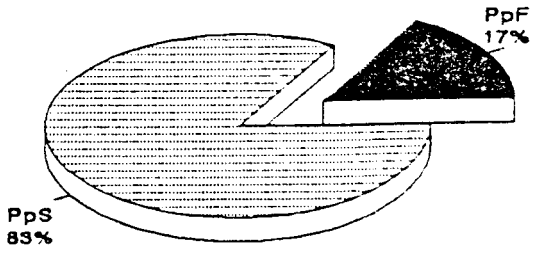

Fig. 3 (D)

GENE FREQUENCY OF ALKALINE PHOSPHATASE
(HOLSTEIN FRIESIAN CROSSBREDS)

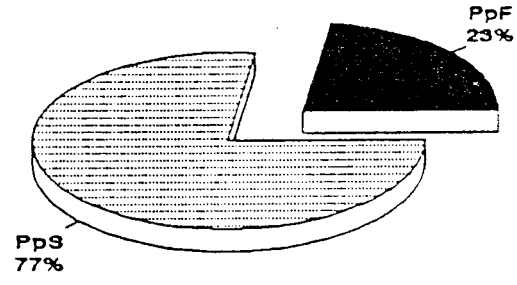

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Table 1 Genotype frequencies and gene frequencies of Sour Alkaline Phosphatase in different crossbred genetic groups

Population	Sample size	SAP-Genotype frequencies			SAP Gene frequencies		Normal deviate	
		FF	FS	SS	P^F	P^S	P^F	P^S
Holstein Friesian crossbreds	57	-	46 (26)	54 (31)	0.43	0.77	7.79	0.79
Brown Swiss crossbreds	53		34 (18)	0.66 (35)	0.17	0.83		
Pooled crossbreds	11		40 (4)	0.60 (66)	0.40	0.60		

Number of animals is given in parenthesis

Table 2 Observed and expected frequencies of Sour Alkaline phosphatase genotypes in different crossbred genetic group

Population		SAP-Genotype frequencies			χ^2 values (4df)
		FF	FS	SS	
Holstein Friesian crossbreds	Obs		26	31	4.89
	Exp	3.01	20.19	33.8	
Brown Swiss crossbreds	Obs		18	35	2.22
	Exp	1.54	14.96	36.50	
Pooled crossbreds	Obs	0	44	66	6.87*
	Exp	4.40	32.8	73.4	

(P < 0.05) * Significant

Table 3 Mean Serum Alkaline phosphatase levels in Holstein Friesian crossbreds and Brown Swiss crossbreds

Population	No of animals	Serum Alkaline phosphatase levels (IA units per 100 ml)			t value (4df)
		Mean ± SE	Maximum	Minimum	
Holstein Friesian crossbreds	57	8.40 ± 0.17	21.11	1.00	1.87
Brown Swiss crossbreds	53	9.62 ± 0.18	26.11	2.63	
Pooled crossbreds	110	9.03 ± 0.16	26.11	1.00	

Table 4 Serum Alkaline Phosphatase levels of different SAF genotypes in Holstein Friesian crossbreds

Breed	SAF genotypes	No of animals	SAF level (IA units per 100 ml) Mean ± SE	t value	df
Holstein Friesian crossbreds	FS	26	9.38 ± 0.16	1.47	55
	SS	31	7.79 ± 0.12		
Brown Swiss crossbreds	FS	18	10.73 ± 0.38	1.08	51
	SS	35	9.72 ± 0.14		

Table 5 Influence of Serum Alkaline Phosphatase level on milk production in different crossbred genetic groups

Breed	No of animals	SAP level units per 100 ml (Mean value)	Milk production (kg) (305 day)	Correlation r	t value	df
Holstein Friesian crossbreds	22	9 80	1702 36	−0 25	1 16	42
Brown Swiss crossbreds	22	9 57	1961 41	-83	1 55	42
Pooled crossbreds	44	9 79	1831 89	0 12	0 78	84

Table 6 Influence of Serum Alkaline Phosphatase level on milk fat percentage in different crossbred genetic groups

Breed	No of animals	SAP level units per 100 ml (Mean value)	Milk fat / Mean value	Correlation	t value	df
Holstein Friesian crossbreds	73	8 00	5 22	−0 02	0 10	44
Brown Swiss crossbreds	17	9 17	5 24	-. 18	0 72	32
Pooled crossbreds	40	8 50	5 23	−0 10	0 60	78

Table 7 Influence of Serum Alkaline Phosphatase level on milk Solids Not Fat percentage in different crossbred genetic groups

Breed	No of animals	SAP level Units per 100 ml (Mean value)	Milk SNF / Mean value	Correlation	t value	df
Holstein Friesian crossbreds	23	8.30	7.68	0.29	1.40	44
Brown Swiss crossbreds	17	9.17	7.68	0.03	.12	32
Pooled crossbreds	40	8.50	7.68	0.15	0.95	78

Table 8 Influence of Serum Alkaline Phosphatase level on total solids percentage of milk in different crossbred genetic groups

Breed	No of animals	SAP level Units per 100 ml (Mean value)	Milk TS Mean value	Correlation	t value	df
Holstein Friesian crossbreds		8.00	12.90	0.28	1.33	44
Brown Swiss crossbreds	17	9.17	13.12	-0.07	0.28	32
Pooled crossbreds	40	8.50	12.96	0.09	.52	78

Table 9 Co elation between Se um Alkaline Phosphatase genotypes and milk p oduction

Milk p oduction kg	No of animals SS	Pe cent	FS	Pe cent	X^2 value (2df)
Less than 1500	6	27 27	3	14 29	
1500-2000	10	45 45	7	33 33	2 98
Mo e than 2000	6	27 27	11	52 38	
Total	22	100 00	21	100 00	

Table 10 Co elation between Se um Alkaline Ph sphatase ge notypes a d milk fat pe centage

Milk fat pe centage	No of animals SS	Per cent	FS	Pe cent	X^2 val e (2df)
Less than 5	12	50 00	8	47 06	
5 1 6	7	29 17	7	41 18	0 92
6 1 7	5	20 83	2	11 76	
Total	24	100 00	17	100 00	

Table 11 Co elation between Se um Alkaline Phosphatase genotypes and Solids not fat pe centage of milk

Milk solids not fat pe centage	No of animals				X^2 value (2df)
	SS	Pe cent	FS	Pe cent	
Less than 7 5	10	41 67	7	58 33	
7 5 8 5	11	45 83	4	33 33	90
Mo e than 8 5	3	12 50	1	8 33	
Total	24	100 00	12	100 00	

Table 12 Co elation between Se um Alkaline Phosphatase genotypes and total solids pe centage of milk

Milk Total solids pe centage	No of animals				X^2 values (2df)
	SS	Pe cent	FS	Per cent	
Less than 13	15	62 5	7	41 18	
13 1-14 0	6	25 00	6	35 29	1 91
Mo e than 14	3	12 50	4	23 53	
Total	24	100 00	17	100 00	

DISCUSSION

Under the conditions of ever increasing intensification in animal husbandry and its transfer on to the industrial production basis the importance of selection and pedigree breeding grows immensely The breeding value of animals depended on their productivity duration of their use and on the quality of their offspring The introduction of genetic markers helps us to follow their inheritance and to study the distribution of genetic material localized in marked chromosomes when evaluating the breeds lines and whole families

In serum the enzyme alkaline phosphatase exists as a mixture of isozymes which are not yet clearly defined The alkaline phosphatase level depends on various factors like genetic physiological and pathological conditions The limitations of this study is the difficulty in eliminating completely the non genetic factors from the study

5 1 Phenotype / Genotype and Gene frequencies

The various phenotypes / genotypes of serum alkaline phosphatase were analysed and tabulated None of the 110 crossbred animals belonging to Brown Swiss cross and Holstein Friesian cross typed for SAP isozyme did reveal the presence of FF genotype Tandon and Khanna (1978) also reported similar observations in Hariana cattle and its crossbreds where they could not observe any AKpFF phenotype Contrary to this observation Romanov (1972) reported three alkaline phosphatase F FS and SS controlled by two autosomal codominant genes (P^F and P^P) in three breeds of Russian cattle

The frequency of SS genotype in Brown Swiss crossbreds were observed to be greater (0 66) than that in Holstein Friesian crossbreds (0 54) (Table 1 Fig 1) The frequency of FS genotype in Brown Swiss crossbreds were observed to be less (0 34) than that in Holstein Friesian crossbreds (0 46) (Table 1 Fig 1) In the pooled crossbreds the genotype frequency of SS and FS were found to be 0 60 and 0 40 respectively

Similar observations were reported by Nandakumaran (1976) where they found out that the phenotype SS was in high frequency in all populations They observed the frequency of phenotype SS ranged from 1 00 (Brows Swiss X Hariana crossbreds) to 0 87 (Jersey X Hariana Crossbreds) They had observed that the frequency of FS ranged from 0 13 (Jersey X Hariana) to zero (Brown Swiss X Hariana)

The average fitness of a population with polymorphism resulting from superior fitness of heterozygotes is less than that of a population in which a single allele performs the same functions as the two different alleles in the heterozygotes [Cain and Sheppard (1954)] On this view polymorphism is a situation that once established is perpetuated by selection between individuals within the population but is a disadvantage to the population as a whole in comparison with another population lacking the polymorphism

Gene frequencies were calculated by direct counting method The gene frequency P^S was found to be higher (0 83) in Brown Swiss crossbreds than that in Holstein Friesian crossbreds (0 77) The gene frequency P^F

was higher (0 23) in Holstein Friesian crossbreds than that in Brown Swiss crossbreds (0 17) The frequencies of P^S and P^F in the pooled population were 0 8 and 0 2 respectively

Gahne (1963) found out that the gene frequencies of F^A and F^O in Swedish Red and White bulls were 0 133 and 0 867 respectively The gene frequency denoted as F^A and F^O are similar to P^F and P^S respectively in this experiment

All the animals examined possessed S band controlled by the P^S allele The reasons for a very high frequency of this allele in most cattle breeds are not yet clear However it focuses to a supposition about its selective advantage

Nandakumaran (1976) reported that the frequency of P^F in the Holstein Friesian X Hariana Brown Swiss X Hariana and Jersey X Hariana were 0 0072 0 0 0653 respectively The frequency of of P^S in these populations were 0 9928 1 and 0 9347 respectively In the present study also a higher frequency level of P^S was obtained in Brown Swiss than that in Holstein Friesian but it was not cent per cent as in the observation made by Nandakumaran (1976) where they got the gene frequency of P^S in Brown Swiss X Hariana as 1 000 This may be due to the change of genetic make up in the local cattle used for crossbreding with the Brown Swiss crossbreds

The inherent tendency of all animal populations or genes is to increase

in numbers But this increase in number is however not infinite since the carrying capacity of the environment always imposes a restriction up on it Thus after reaching the carrying capacity level the population density tends to fluctuate above and below this level and such fluctuations in population between upper and lower limits tend to give some stability to the population Most genes are provided with a unique and intrinsic regulatory gechanism such as self inflicted mortality for controlling the size of the population Limitation of genes on a population is brought about by the action and interaction of two basic regulatory processes namely density independent and density dependent factors Density independent factors are the extrinsic factors which tend to regulate the density of a population under different conditions appearing to act on the population and inflict loss of genes irrespective of the population density Variations in space or cover and favourable weather occur independently of population densities Such ecological or environmental factors influence negatively or positively all the individuals of a population irrespective of density

The density dependent factors are intrinsic or biotic factors and they depend on coaction between individuals within the same population or between individuals within the same population or between populations of different species Density dependent factors may stabilize populations at an asymptote the level of which is determined by the carrying capacity of the environment Some of the important density dependent factors are competition reproductivity predation emigration and disease The combination of factors or any specific factor involved in the density dependent action may vary from species to species and breed to breed

5 1 1 Test for genetic equilibrium

The proportion of loci in the population at which more than one allele exists is called the proportion of polymorphic loci Usually loci with frequencies of less than 0 01 are excluded from the calculation of polymorphism because in a large population virtually every locus has more than one allele present

By assuming the Hardy Weinberg equilibrium the expected values of alkaline phosphatase types were calculated and were compared with the observed values A good agreement was obtained in the two crossbred population of Holstein Friesian and Brown Swiss crossbreds The results of the test for genetic equilibrium at the serum alkaline phosphatase locus furnished in Table 2 revealed that there were no significant differences existed between the observed frequency and the expected frequency for the various SAP phenotypes viz FF FS and SS in the Holstein Friesian crossbreds and Brown Swiss crossbreds From this we can infer that the Holstein Friesian crossbreds and Brown Swiss crossbreds are in genetic equilibrium at SAP locus

We can expect a change in genetic equilibrium if we take either serum alkaline phosphatase as selection criteria or choose a parameter which is having genetic correlation with the serum alkaline phosphatase So far the biochemical polymorphic loci are not at all included in the selection criteria especially that of serum alkaline phosphatase isozyme polymorphism

The proportion of gene frequency between the two crossbreds viz Holstein Friesian crossbreds and Brown Swiss crossbreds calculated by the normal deviate Z (Table 1) revealed that the gene frequency P^F in both the crossbreds were in proportion because there were no significant differences existed for the two gene frequencies of P^F in the two crossbreds Similarly the gene frequency P^S in both the crossbreds were in proportion because there were no significant difference existed for the two gene frequencies of P^S in the two crossbreds Polymorphism is referred to as the existence in a population of individuals with readily discernible differences caused by genes at intermediate frequencies (Falconer 1983) The genes causing polymorphism have usually no obvious advantage of one allele over another all the genotypes being essentially normal or "wildtype" individuals The properties of the genes concerned with polymorphism seem there fore to accord well with the hypothesis that selection is operating on them in favour of the heterozygotes As a general cause of polymorphism however it cannot be taken as fully proved because the superior fitness of hetero zygotes has been demonstrated in relatively few cases

There are many possible reasons for the existence of polymorphism For example the genes may be in a transi tional stage of a change from one extreme to the other as a result of slow environmental change or the intermediate frequencies may be the point of equilibrium between mutation in opposite direction with virtually no selective advantage of one allele over the other Another possible cause of polymorphism lies in the heterogeneity of the environment in which a population lives If the

differences of the environment influence the selection coefficients and another allele in other conditions then polymorphism may result provided that mating is not entirely at random over the range of environments (Levene 1953) The present study of serum alkaline phosphatase polymorphism revealed that in each breed the different phenotypes are in equilibrium or the differences are not statistically significant On pooling the data of Holstein Friesian crossbreds and Brown Swiss crossbreds the observed and expected values were significantly different ($P<0.05$) both for homozygotes and heterozygotes (Table 2) This may be due to the reason that the serum alkaline phosphatase enzyme existed in equilibrium suited for each breed and that equilibrium got disturbed on pooling the results of the SAP genotype frequencies in these two crossbreds

5 2 Serum Alkaline Phosphatase (SAP) levels

Alkaline phosphatase hydrolyzes organic phosphates such as those of glucose or glycerol to yield inorganic phosphate and the organic moiety It is most active at alkaline pH near 9 50 but is also quite active at the pH of blood 7 40 (Kaneko JJ and Corelius CE 1970) It is determined by estimating the phenol liberated from phenyl phosphate (King and Armstrong 1934) To convert the millimolar alkaline phosphatase unit of Bessey Lowrey to King Armstrong units the former is multiplied by a factor of 2 50

A marked increase in serum alkaline phosphatase activity has been reported associated with the approach of parturition (Kaneko J J and Corelius CE 1970) SAP activity has been found to be low during hypomagnesemic tetany (Kaneko JJ and Corelius CE 1970) Kaneko JJ and Corelius CE (1970) reported that increased phosphatase

activity accompanied decrease serum inorganic phosphate levels in 3 to 6 months old calves fed different levels of dietary phosphorus The phosphetase level responded rapidly to a change in the blood level of phosphorus but after an initial change the phosphatase level returned to its "normal level The mean SAP values for the Holstein Friesian crossbreds and Brown Swiss crossbreds revealed that the SAP activity was less in Holstein Friesian crossbreds (8 48 KA units per 100 ml) than that of Brown Swiss crossbreds (9 62 KA units per 100 ml) The SAP values in Holstein Friesian crossbred ranged between 1 00 and 21 11 KA units per 100 ml where as in Brown Swiss crossbreds it was between 2 63 and 26 11 KA units per 100 ml

Although the enzyme activity of the serum of each cow remained fairly constant over a long period of time a great range of activity (0 30 114 30 KA units per 100 ml) was observed in cattle (Allcroft and Folley 1941) The wide range of SAP activities in normal cattle and sheep prohibits its use as an indicator of liver insufficiency or obstructive icterus in these species (Kaneko J J and Cozelus C E 1970) In both cattle and sheep the serum alkaline phosphatase activity progressively decreased with age until maturity was reached Slight elevated levels are observed in pregnancy

5 2 1 Association between SAP levels and SAP phenotypes

The association between two characters that can be directly observed is the correlation of phenotype/genotype values or the

phenotypic/genotypic correlation This is determined from measurements of the two characters in a number of individuals of the populations Consideration of correlated responses suggests that it may sometimes be possible to achieve more rapid progress under selection for a correlated response than from selection for the desired character itself The circumstances most likely to render indirect selection superior to direct selection are chiefly concerned with technical difficulties in applying selection directly to the desired character Two such technical difficulties may be are

a If the desired character is difficult to measure with precision the errors of measurement may so reduce the heritability that indirect selection becomes advantageous

b If the desired character is measurable in one sex only but the secondary character is measurable in both then a higher intensity of selection will be possible by indirect selection

Comparing the serum alkaline phosphatase level for different SAP genotypes (Table 4) revealed that for the FS genotype the SAP activity was higher in Brown Swiss (10 72 KA units per 100 ml) than in Holstein Friesian (9 32 KA units per 100 ml) For the SS genotype also the SAP activity was higher in Brown Swiss (9 07 KA units per 100 ml) than in Holstein Friesian (7 79 KA units per 100 ml) It has been noted that in both the crossbreds viz Holstein Friesian and Brown Swiss the SAP level was found to be higher for the FS genotype Though there is a difference for the SAP levels between the SAP genotypes FS and SS in Holstein

Friesian and Brown Swiss crossbreds that difference is not statistically significant

5 2 2 Association between Serum Alkaline Phosphatase level and milk production traits

5 2 2 1 Influence of Serum Alkaline Phosphatase level on milk production

In the present study no association could be established between milk yield (305 days) and serum alkaline phosphatase (SAP) level The correlation between SAP level and milk production level in Brown Swiss crossbreds were found to be positive but not significant whereas the correlation between SAP level and milk production level in Holstein Friesian crossbreds are found to be negative and not significant (Table 5) Schlimme and Thiemann (1992) suggested that it was not possible to indicate a lactophy siological "normal activity of alkaline phosphatase in bulk milk but only a range within which enzyme activity can be expected

5 2 2 2 Influence of Serum Alkaline Phosphatase level on fat percentage of milk

In both the crossbreds of Holstein Friesian and Brown Swiss the average milk fat percentage was more or less the same In Brown Swiss crossbreds it was 5 24 and in Holstein Friesian it was 5 22 (Table 6) In the both crossbreds a non significant negative correlation existed

between milk fat percentage and the SAP level This may be pointing us to select animals with SAP levels of a few degree less than the normal values

5 2 2 3 Influence of Serum Alkaline Phosphatase level on Solids Not Fat (SNF) percentage of milk

The average milk SNF in both Brown Swiss crossbreds and Holstein Friesian crossbreds was 7 68 (Table 7) In both the crossbreds a non significant positive correlation existed between milk SNF percentage and the SAP level In Holstein Friesian the correlation is (r− 0 29) greater than that in Brown Swiss crossbreds (r= 0 03) From the correlation studies it has been observed that a slightly higher value of SAP activity favours a higher SNF percentage in milk

5 2 2 4 Influence of Serum Alkaline Phosphatase level on Total Solids of milk

The average milk total solids percentage for Brown Swiss and Holstein Friesian were 13 02 and 12 90 respectively (Table 8) A non significant negative correlation (r= 0 07) was existed between SAP level and the milk total solids in Brown Swiss crossbreds where as a non significant positive correlation (r = 0 28) existed between the SAP levels and the milk total solids in Holstein Friesian crossbreds It is risky to arrive at a definite conclusion about the correlations between these two parameters by taking this much number of animals (110) Extensive studies involving large number of animals would be required before arriving at meaningful conclusions

5 2 3 Correlation between Serum Alkaline Phosphatase Phenotypes and milk production traits

5 2 3 1 Influence of serum alkaline phosphatase phenotype/ genotype on milk production

Since there was no significant difference existed between the patterns of occurrence of milk production and the pattern of number of animals belonging to a particular genotype it can be suspected an association between the patterns of milk production and the patterns of number of animals falling to that particular genotype

Out of the total 22 animals belonging to the SS genotype 46 percentage of the animals produced milk ranging 1500 to 2000 kg Only 27 percentage produced more than 2000 kg of milk The rest 27 percentage belonging to the SS genotype produced only less than 1500 kg

Out of the total 21 animals belonging to FS genotype more than 52 percentage of the animals produced more than 2000 kg of milk for the period of 305 days whereas 33 percentage reduced milk ranging 1500 to 2000 kg Only less than 15 percentage of the animals had produced less than 1500 kg of milk From this it can be inferred that the animals belonging to the FS genotype are better milk producers provided all the other factors are satisfactory Palanskii and the Romanov (1973) reported a non significant association between alkaline phosphatase types and milking rate in Ukranian White headed cows

Nandakumaran (1976) compared the various alkaline phosphatase genotypes and the economic traits like first and second lactation yield in Jersey x Hariana crosses They could not observe any significant association between alkaline phosphatase types and these economic traits

5 2 3 2 Influence of Serum Alkaline Phosphatase phenotype/ genotype on fat percentage of milk

There was no significant difference existed between the pattern of occurrence of the milk fat percentage and the pattern of number of animals belonging to a particular genotype it can be suspected an association between the patterns of milk fat percentage and the patterns of number of animals falling to that particular genotype Out of the total 24 animals belonging to the SS genotype 50 percentage of the animals produced milk with less than 5 percentage of fat and 29 percentage of the animals produced milk with fat percentage ranging 5 10 to 6 Only less than 21 percentage of the animals belonging to the SS genotype produced milk with fat percentage ranging 6 10 to 7 Out of the total 17 animals belonging to the FS more than 47 percentage of the animals produced milk with less than 5 percentage of fat where as 41 percentage produced milk with fat percentage ranging 5 10 to 6 and the rest 12 percentage of the animals had produced milk with fat percentage ranging 6 10 to 7 (Table 10)

From this a trend can be noticed that at lower levels of milk fat percentage the performance of both the SS and FS genotypes were

more or less similar where as at fat percentage ranging 5 10 to 6 the performance of FS genotype was better compared to the SS genotype At fat percentage ranging 6 10 to 7 the performance of SS genotype was comparatively better than the FS genotype 5 2 3 3 Influence of Serum Alkaline Phosphatase phenotype / genotype on solids not fat percentage of milk

There was no significant difference existing between the patterns of occurrence of the milk solids not fat percentage (SNF) and the pattern of number of animals belonging to a particular genotype It can be assumed an association between the patterns of milk SNF percentage and the pattern of number of animals belonging to that particular genotype

Out of the total 24 animals belonging to the SS genotype 42 percentage of the animals produced milk with less than 7 50 percentage of SNF where as 46 percentage of the animals produced milk with SNF ranging 7 50 to 8 50 and the rest 12 50 percentage of the animals belonging to the SS genotype produced milk with more than 8 50 percentage of SNF

Out of the total 12 animals belonging to the FS genotype more than 58 percentage of the animals produced milk with less than 7 50 percentage of SNF where as 33 percentage of the animals produced milk with SNF percentage ranging 7 50 to 8 50 and the rest 8 percentage of the animals belonging to the FS genotype only had produced milk with more than 8 50 percentage of SNF (Table 11)

From this a trend can be noticed that the performance of SS genotype is better for producing milk with SNF percentage ranging 7 50 to 8 50 and for producing milk with more than 8 50 percentage of SNF The performance of FS genotype was found to be higher in producing milk with less than 7 50 percentage of SNF

5 2 3 4 Influence of Serum Alkaline Phosphatase phenotype / genotype on total solids (TS) percentage of milk

There was no significant difference existing between the patterns of occurrence of the TS percentage of milk and the pattern of number of animals belonging to a particular genotype A trend can be seen showing an association between the patterns of milk TS percentage and the pattern of number of animals belonging to that particular genotype

Out of the total 24 animals belonging to the SS genotype 62 50 percentage of the animals produced milk with less than 13 percentage of TS where as 25 percentage of the animals produced milk with TS ranging 13 10 to 14 and the rest 12 50 percentage of the animals belonging to the SS genotype produced milk with more than 14 percentage of TS

Out of the total 17 animals belonging to the FS genotype more than 41 percentage of the animals produced milk with less than 13 percentage of TS where as 35 percentage of the animals produced milk with TS percentage ranging 13 10 to 14 and the rest 24 percentage of the animals belonging to the FS genotype only had produced milk with more than 14

percentage of TS (Table 12)

From this a trend can be noticed that the performance of FS genotype is better for producing milk with TS percentage ranging 13 10 to 14 and for producing milk with more than 14 percentage of TS The performance of SS genotype was found to be higher in producing milk with less than 13 percentage of TS

Summary

SUMMARY

1 Biochemical polymorphism at the serum alkaline phosphatase locus was studied in 110 animals belonging to two crosses of local non descript cattle with exotic breeds viz 56 Holstein Friesian crossbreds and 54 Brown Swiss crossbreds maintained inthe University Livestock Farms at Mannuthy and Thumburmuzhi

2 Horizontal Polyacrylamide Gel Electrophorsis (PAGE) technique was employed for phenotyping/genotyping SAP based on the Zone numbering system

3 Two phenotypes/genotypes FS and SS determined by two alleles P^F and P^S were observed at the SAP locus The higher frequency of FS genotype was in Holstein Friesian crossbreds (0 46) than in the Brown Swiss Crossbred (0 34)

4 The genotype FF was absent in both the crossbreds The frequency of SS genotype was higher in Brown Swiss crossbreds (0 66) than that in Holstein Friesian (0 54)

5 The reasons for a very high frequency of P^S allele focuses to a supposition about its selective advantageous The gene frequency P^S in Brown Swiss crossbreds was higher (0 83) than that in Holstein Friesian crossbreds The gene frequency of P^F was higher in Holstein Friesian crossbreds (0 23) than in Brown Swiss crossbreds (0 17)

6 The results of the test for genetic equilibrium at the serum alkaline

phosphatase loci revealed that both the Holstein Friesian crossbreds and Brown Swiss crossbreds are in genetic equilibrium at the SAP loci

7 The proportion of gene frequency between the two crossbreds viz Holstein Friesian crossbreds and Brown Swiss crossbreds calculated by the normal deviate Z revealed that the gene frequency P^F in both the crossbreds were in proportion Similarly the gene frequency P^S in both the crossbreds were in proportion

8 The alkaline phosphatase level was estimated in the serum by employing the Kind and King s method 1954 The mean SAP values for the Holstein Friesian crossbred and Brown Swiss crossbred revealed that the SAP activity is more in Brown Swiss (9 63 KA units per 100 ml) than that in Holstein Friesian crossbreds (8 48 KA units per 100 ml)

9 Comparing the SAP levels for different SAP genotypes revealed that for both the genotypes i e FS and SS the SAP activity was higher in Brown Swiss crossbreds than in Holstein Friesian crossbreds

10 No association could be established between milk yield (305) days and serum alkaline phosphatase level The correlation between SAP level and Milk production level in Brown swiss crossbreds were found to be positive but not significant whereas the correlation between SAP level and milk production level in

Holstein Friesian crossbreds were found to be negative and not significant

11 Analysing the association between serum alkaline phosphatase level and milk production traits revealed that a non significant negative correlation existed between milk fat percentage and SAP level A non significant positive correlation existed between milk SNF percentage and SAP level A non significant negative correlation was existed between SAP level and the milk total solids in Brown Swiss crossbreds where as a non significant positive correlation existed between the SAP level and the milk total solids in Holstein Friesian crossbreds

12 Analysing the correlation between Serum Alkaline Phosphatase phenotypes and the milk production traits revealed that the animals belonging to the FS genotype are better milk producers compared to other genotypes provided that all other factors are satisfactory A trend can be noticed that at lower levels of milk fat percentage the performance of both the SS and the FS genotypes were more or less similar whereas for fat percentage ranging 5 10 to 6 the performance of FS genotype was comparatively better and at fat percentages ranging 6 10 to 7 the performance of SS genotype was comparatively better A trend can be noticed that the performance of SS genotype is better for producing milk with more than 8 50 percentage of SNF It has been noticed that there is a tendency for the FS genotypes for producing milk with higher percentage of total solids

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SERUM ALKALINE PHOSPHATASE POLYMORPHISM IN CROSSBRED CATTLE OF KERALA

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ABSTRACT OF A THESIS

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ABSTRACT

The present investigation was undertaken to identify the the biochemical polymorphism at serum alkaline phospha tase (SAP) loci and to study the heterogenity of SAP variation in crossbred cattle It was also envisaged to analyse the association of SAP variation and traits of economic importance such as milk production and composition of milk One hundred and ten animals belonging to two different crosses of local nondescript cattle viz Crossbred Holstein Friesian (57) and Crossbred Brown Swiss (53) were typed for SAP variance by standardising Horizontal Polyacrylamide Gel Electrophoresis (PAGE) Two genotypes FS and SS were determined The highest frequency of FS genotype was in Holstein Friesian crossbred than in Brown Swiss crossbred The genotype FF was absent in both the crossbreds The highest frequency of SS genotype was in Brown Swiss crossbred than in Holstein Friesian crossbreds

Two alleles namely P^F and P^S with two phenotypes FS and SS were identified as SAP locus P^T allele had the frequency of 0 20 and P^S allele had the frequency of 0 80 in the pooled crossbreds Both the Holstein Friesian crossbreds and Brown Swiss crossbred are in genetic equilibrium at the SAP loci No association could be established between milk yield (305 days) and serum alkaline phosphatase level A non significant negative correlation existed between milk fat percentage and SAP level whereas a significant positive correlation existed between milk SNF percentage and SAP level The correlation between SAP level and milk total solids were found to be negative and non significant in Brown Swiss

crossbreds whereas a non significant positive correlation existed between the SAP level and milk total solids in Holstein Friesian crossbreds

Animals belonging to the FS genotype are better milk producers compared to the SS genotype For higher fat percentages the performance of SS genotype was compara tively better The performance of SS genotype is better for producing milk with more than 8 5 percentage of SNF FS genotype performed better for producing milk having higher percentage of total solids