GENETIC STUDIES ON CERTAIN ENZYMES OF DAIRY CATTLE

By

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B. V. Sc.

Post-Graduate College of Animal Sciences,

INDIAN VETERINARY RESEARCH INSTITUTE,

IZATNAGAR, (U. P.)

Thesis

Submitted to the Agra University, Agra,

in partial fulfilment of the requirements for the Degree of

MASTER OF VETERINARY SCIENCE (A. H.)

IN

ANIMAL GENETICS & BREEDING

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April 24, 1970.

Certified that the research work contained in the thesis entitled "GENETIC STUDIES ON CERTAIN ENZYMES IN DAIRY CATTLE" by Shri B.M. Paramasiviah, is an original work carried out by him under my supervision and guidance.

(K.J. EAPEN)

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INTRODUCTION

The success of any breeding programme depends primarily on the ability of the individual breeder to evaluate properly the animals that are to be selected as parents of the next generation. In dairy cattle the assessment of productive traits is a difficult task since most of the economic characters are greatly influenced by the environment. It is, therefore, desirable to obtain objective information on genetically dependent efficiency criterion which will serve as a basis for selection even before the animal comes to production. The genetically dependent biochemical variants in animal tissues may provide an answer to this complex problem.

Of the biochemical variants, the study of enzymes, from quantitative genetic aspect, is in vogue. Enzymes are ubiquitous in distribution. They are proteinous, high molecular substances produced by the living organisms. They have a high degree of substrate specificity and are easily effected by changes in pH and temperature. All the chemical reactions that take place in living organisms are brought about in closely controlled sequences. Each step in any sequence is catalysed by the presence of a particular enzyme which brings about the reaction without itself being changes.

The great progress made in recent years in the field

of biochemical genetics has awakened an ever increasing interest in the quantitative genetic aspect of enzymes. It is well established from the work of Beadle and Tatum (1947) that there is a definite relationship between genes and enzymes. Evidence to this effect that enzymes are directly controlled by genes, has been also established by several workers (Petras, 1963; Ashton, 1964; Sick and Nielson, 1964, Hesselholt and Moustguaard, 1965; Heston, 1965; and Pelzer, 1965). There is also sufficient evidence to prove that most of the enzymes are highly heritable (Roderick, 1960; Wilcox et al 1962; Nayudu and Moog, 1960; Angel et al 1967; Yuhas et al 1967 and Karlikov, 1968).

The present investigation is confined to the inheritance of plasma amylase and alkaline phosphatase activities in Red-Danish dairy cattle. This has been taken up to study the genetic nature of these enzymes as a prelude to explore the possibility of using these biochemical traits as a basis for indirect selection in farm animals.

REVIEW OF LITERATURE

Amylase

The enzymatic action of amylase was first discovered by Kirchhoff (1811) in wheat extract. Later on the enzyme was found in human saliva (Leuchs, 1831), in malt (Payen and Persoz, 1833), in human pancreatic juice (Bouchardat and Sandra, 1845), in blood (Magendie, 1846) and in urine (Cohnhein, 1863).

Kuhn (1924) classified amylases into two groups, the α and the β-amylases according to the nature of their reaction on the starch molecule. The α-amylases are found both in plants and animals, whereas the β-amylases only in plants.

Holnberg (1933) showed that it is possible to isolate malt α-amylases from β-amylases by adsorption of the former on starch from cold 50% ethanol in the presence of maltose.

Morris (1942) observed that blood amylases hydrolyse glycogen and starch almost the same rate unlike all other amylases.

Balls *et al* (1948) obtained β-amylase in crystalline form from sweet potatoes. Crystalline form of α-amylases were obtained by Meyer *et al* (1951) from animal tissues.

Bernfeld (1951) made a mention of 4 steps involved during the course of amylase action on amylose namely,(1) a decrease in viscosity,(2) loss of capacity to give blue colour with

iodine, (3) appearance of reducing groups and, (4) formation of maltose and dextrins.

Amylase activities of plasma and serum prepared from human blood were compared by McGeachin et al (1957). They found that when oxalate or citrate was used as an anticoagulent the amylase activity of plasma was 20% lower than that of serum. However, they observed no such differences in other animals.

Masaaki (1957) modified the method of determination of serum amylase, developed by Fuwa.

Sick and Nielsen (1964) electrophoretically demonstrated in mouse three phenotypic variants of salivary amylase which were controlled by 2 co-dominant allelic genes.

Ashton (1964) identified six serum amylase phenotypes in cattle by using starch gel electrophoresis. He concluded that polymorphism is due to three autosomal co-dominant alleles. Hesselholt and Moustgaard (1965) found three phenotypic variants in cattle serum and concluded that the enzyme was controlled by two alleles. Further they discussed the application of serum amylase typing to establish disputed parentage in cattle.

Hesselholt, Larsen and Brauner Nielsen (1966) showed that serum amylase polymorphism in pigs was controlled by 3 co-dominant autosomal alleles. Their investigation on horse sera from Icelandic horses, did not reveal individual serum amylase variants.

Brauner Nielsen (1966) investigated the relationship between serum amylase and I blood group loci in pigs, and found amylase and I system were the products of closely linked autosomal loci. Ashton (1967) correlated types of amylase system with tick infestations in cattle, and found that cattle with amylase type 'C' carry highest and those with type 'B' lowest tick burdens.

Meyer (1967) found 2 alleles to be responsible for amylase polymorphism in German breeds of cattle, using starch gel electrophoresis. However, his investigation did not reveal amylase polymorphism in horses, sheep, goats, dogs and monkeys. Eberman and Bodenscher (1967) showed that serum amylase inheritance in pigs is governed by 3 alleles.

Kirton et al (1968) investigated the effect of added amylase on the fertilising power of bull semen and found that the addition of amylases increased the fertility.

Dinklage (1968) working on serum amylase polymorphism in German improved Landrace pigs showed that serum amylase typing can be used to establish parentage.

Alkaline Phosphatase

Robison (1923) reported this enzyme in ossifying cartilage of rats and rabbits, and discussed the possible significance of this enzyme in bone formation.

Kay (1930) noted a rise in enzyme activity during

healing of fractures in children. Mckeown and Ostergren (1931) observed abnormally high enzymic activity during the healing of rickets in children.

Bodansky and Jaffe (1931), Smith and Maizle (1932) found that certain dietary deficiencies were accompanied by changes in the serum phosphatase of rats.

The action of oxidants and reductants on bovine serum alkaline phosphatase activity (APA) were investigated by Sizer (1942). He found enzyme activity remains unaffected by most reductants and mild oxidants. His results were independent of the source of the enzyme and the substrates used.

Flock and Bollman (1948) found that alkaline phosphatase is supplied to plasma from small intestines by way of lymph in rat.

Young and Underdhal (1948) working on pigs found high APA at birth and by fifth day the activity was reduced to half. However, they found it to level off at the age of about 28 days.

Roche and Thoai (1950) found the enzyme acting optimally at pH values near 9 and hence they named the enzyme 'Alkaline phosphatase'. Ross, Ely and Archer (1951) found the

pH optimum for alkaline phosphatases vary with substrate concentration, the types of the substrate, and the buffer employed.

Tanabe and Wilcox (1960) compared blood serum alkaline phosphatase level in a line of White Leghorn bred for high alkaline phosphatase, with a random bred control line. In both the lines the enzyme level increased rapidly from hatching to a peak at two weeks of age. However, upto 26 weeks the activity was significantly higher in the high line. Upto 14 weeks serum alkaline phosphatase was higher in males but this sex difference was reversed during 18 to 34 weeks. The higher value in adult ♀♀ appears to be related to egg production. In birds that had stopped laying the serum alkaline phosphatase value was low.

Alexander, Kruger and Bogart (1960) investigated the rate of gain and serum phosphatase in Herford and Angus breeds of beef cattle and found a positive correlation between body weight and serum phosphatase level.

Wilcox et al (1962) studied the relationship between serum alkaline phosphatase and egg production. The heritability of serum alkaline phosphatase level in 6 weeks old random-bred White Leghorn as estimated from sire + dam components was 0.36, and a genetic and phenotypic correlation of 0.50 and 0.01 respectively between serum alkaline phosphatase level and egg production. They concluded the serum alkaline phosphatase to be

under genetic control and it can be used as a means of indirect selection for high egg production.

Gahne (1963) established through electrophoretic methods, the genetic control of serum alkaline phosphatase in Swedish Red and White cattle.

Electrophoretic studies of Law and Munro (1965) revealed two mutually exclusive forms, a fast moving and a slow moving variants of alkaline phosphatase in fowl plasma. They studied two inbred lines for two generations and showed that faster moving isozyme was determined by autosomal dominant gene (Ap_2) which was single allelic to recessive gene (Ap_4) responsible for slower isozyme. In heterozygous (Ap_2Ap_4) the (Ap_2) dominant allele prevented the appearance of the product of (Ap_4) allele, so that only fast moving isozyme occurred in the plasma.

Beckman and Nilson (1965) studied the electrophoretic variants of alkaline phosphatase in the sera of different species of birds and their hybrids. The hybrids often showed enzyme patterns representing combination of the parental patterns.

A positive association between serum alkaline phosphatase level and egg production in poultry was noticed by Wilcox and Cloudes (1965) who discussed the possibility of using this as a means of indirect selection for high egg production.

Roussel and Stallcup (1965) studied the influence of age and phosphatase level in blood serum in Holstein bulls and recorded a significant negative correlation with age and enzyme activity.

Performance of a line of fowl selected for high level of alkaline phosphatase at 6 weeks of age was compared by Wilcox (1966) with that of the random bred control from which the line originated. Phosphatase level was significantly higher in the high line at all ages studied and during all 5 selected generations of random breeding. Egg production of high line was significantly greater than in the control. The fertility of egg was significantly low in high line.

Hyre et al (1966) determined the monthly mean alkaline phosphatase level and monthly mean egg production of a White Leghorn population and recorded a fall in both phosphatase level and egg production as laying year progressed. They also noted a highly significant phenotypic correlation between the two traits and a highly significant phenotypic correlation between 8 weeks phosphatase level and that of the annual mean for the same birds during the laying year.

Wilcox (1966) studied zymograms of single serum samples from birds and showed them to contain either a slow or fast moving band or no band. Fast and slow moving bands were controlled by a dominant (AKP) allele and a recessive (akp) allele respectively. He also noted a higher alkaline

phosphatase level at 6 weeks of age in birds with fast bands than with slow bands.

Law (1967) established electrophoretically leucine amino peptidase and alkaline phosphatase variants were controlled by the same gene or by closely linked genes in the plasma of chickens.

Karlikov (1967) mated Large-White pigs with high or low serum APA in all possible combinations and noted a greater litter size for high X high matings than for low X low matings. Litter size was intermediate for heterogeneous matings. Pigling weights at birth and at 1 and 2 months of age were greater for two low A.P. parents than for two high A.P. parents, it was intermediate for heterogeneous parents. He also noted litter size at 2 months was greatest for homogeneous parents, especially for two high alkaline phosphatase parents, and it was lowest for litters of low A.P. ♂♂ X high AP♀♀.

Inherited serum APA of Swedish Red and White cattle was investigated by Gahne (1967). His studies revealed higher APA in adult animals of the 'A' phosphatase type determined by the dominant gene F^A. In animals lacking the 'A' component F locus was responsible for more than half of the variation in phosphatase activity in adult cattle.

Nayudu and Moog (1967) investigated the inheritance of duodenal APA in two inbred strains of Swiss mice. These mice had maintained three to four fold differences in duodenal

phosphatase activity for six generations. The authors noted that enzyme activity was under polygenic control and in one strain the activity was influenced by a maternally inherited factor probably contained in the milk. The heritability based on half sib correlation showed that additive variance makes up about 50-70% of phenotypic variance of duodenal phosphatase activity in low activity strain and 30-45% in high activity strain. The latter strain was more variable both within generations and within litters.

Karlikov (1968) conducted breeding experiments on alkaline phosphatase and acid phosphatase activity in Large-White breeds of pigs and various other breeds of piglings. He observed heritability of alkaline phosphatase calculated from sire component to be 0.221 and 0.239 at 2 and 4 months of age respectively. He also observed that parents with high APA produced progeny with higher APA than did the parents with low APA. High APA X low APA parents produced progeny with intermediate APA.

Eapen and Goswami (1969a) observed sire differences in serum APA in Hariana cattle. They (1969b) further noted that serum APA was heritable to the extent of 4 per cent only, showing a very little additive genetic variability. Goswami et al (1970) found that there were differences in overall serum APA in young and adult Hariana cattle.

MATERIAL AND METHODS

Red Danish cattle belonging to Indo-Danish Project Hessaragatta (Mysore State) constituted the experimental material. The farm is situated at an altitude of 3,000 ft. above the mean sea level and has an average yearly rainfall of 30" and a temperature ranging from 16 to 32°C. 149 offsprings of different ages of both sexes belonging to 12 sire groups were considered for this study.

The animals were maintained at the farm under similar breeding and managerial conditions. Only healthy animals from 3 to 43 months of age were considered for the study.

The feeding schedule for the cattle followed at the farm is given below.

Feeding of cattle

Calves

Age in months	kg. of concentrate	Hay	Grass
3-4 months	3/4	Ad-lib	Ad-lib
5-6 months	1	"	"

Heifers

Age in months	F.U.*	G. Protein
6 - 9	2.0	250
9 - 12	2.5	250
12 - 18	3.0	300
18 - 24	3.5	300
Above 24	4.0	300

(Suitable additions for pregnancy)

*F.U. = Fodder unit = Feeding value as in 1 kg of barley

Cows

Maintenance $\frac{\text{Weight of cow}}{200} + 1.5 = \text{F.U.}$

$\frac{\text{Weight of cow}}{2} = \text{G. Protein}$

Production $\frac{\text{kg milk x fat \%}}{100} = \text{kg butter fat}$

kg Milk x 0.4 + kg butter fat x 15 kg = F.C.M.

Milk Production: For every 2.0 kg milk 1 F.U. and 125 g protein

Pregnancy	F.U.	G. Protein
7 months	0.5	75
8 months	1.0	125
9 months	1.5	225

For growth 1 F.U. extra per day for 1 calf
cows, 1½ F.U. extra per day for 2 calves-cows.

Bulls

8 F.U. and 1200 g protein per day

Composition of the concentrates

	Cows	Calves
Maize crushed	280 kg	150 kg
Milk powder	100 kg	50 kg
Groundnut cake	100 kg	70 kg
Cotton seed cake	70 kg	-
Wheat bran	300 kg	70 kg
Molasses	100 kg	30 kg
Minerals	25 kg	10 kg
Ragi	-	50 kg
Horse gram	-	70 kg
Dicalcium phosphate	-	10 kg

Blood plasma from these Red-Danish animals were used for the genetic study of the enzymes.

For this purpose, the animals were bled from the jugular vein by using sterilized bleeding trocars. About 5 cc of blood from each animal was collected into sterilized heparinated test tubes and transferred to thermos jars containing cracked ice. The samples were centrifuged and plasma separated. The plasma from each blood sample was transferred into a vial, sealed, and stored in refrigerator. These vials were brought to Izatnagar in an Ice-chest. The samples were stored in a deep freeze till the determination was carried out.

Colorimetry (Wootton 1964)

Many substances of biological interest are coloured. Others form coloured derivatives and still others can be made to enter into chemical reactions which yield coloured substances. The measurement of the concentration of coloured substances usually in solution forms the basis of colorimetric analysis.

With a modern photoelectric equipment, a property of the coloured solution is measured which is called its optical density (d). Under suitable conditions, if a coloured solution is illuminated with monochromatic light, its optical density will be proportional to the concentration of the coloured substance multiplied by the depth of the solution in the light

path thus

$$d = K \times C \times L$$

where,

K = constant

C = concentration of solution

L = length of light path

This relation is known as the Lambert Beer Law and is used to compare the concentrations of an unknown test solution with a standard solution measured in the same way. Then

$$d_{test} = K \times C_{test} \times l$$

$$d_{stand} = K \times C_{stand} \times l$$

$$\therefore C_{test} = \frac{d_{test}}{d_{stand}} \times C_{stand}$$

The optical densities of solutions are obtained by ensuring the percentage of the incident light which is transmitted through the solution.

Preparation of solution for measurement

In general it is necessary to prepare three solutions. These are:

(i) The test solution made from the blood filtrate, or other specimen being analysed.

(ii) A standard solution prepared from a known quantity of the substance to be estimated.

(iii) A blank solution containing all the reagents but none of the substance to be estimated.

The blank solution compensates for non-specific colour produced for various reasons, such as traces of impurity in the reagents.

It is important to remember that measurements of transmitted light are required and it is essential to avoid cloudiness, turbidity and bubbles which absorb light. The solution must appear optically quite clear since the photo-electric cell is more sensitive than the eye to small changes in transmission.

Preparation of reagents for amylase determination

(i) Buffered starch substrate (pH 7.0)

35.5 gm of disodium hydrogen phosphate ($Na_2H\ PO_4 12H_2O$) and 4.3 gm of benzoic acid were added to 250 ml of glass distilled water, and brought to boil. 0.2 gm of soluble starch mixed in 5-10 ml of cold glass distilled water and added to the boiling mixture. After boiling for one minute the solution was cooled to room temperature and diluted to 500 ml and stored at 4°C in refrigerator.

(ii) Stock iodine solution (0.1N)

13.5 gm of pure sublimed iodine was dissolved in a solution of 24 gm of potassium iodide in about 100 ml of glass distilled water. The volume was made to one litre with glass

distilled water. The reagent was stored in refrigerator.

(iii) Working iodine solution (0.1N)

50 gm of potassium fluoride was dissolved in a little glass distilled water to which 100 ml of stock iodine solution was added. The total volume was made to one litre with glass distilled water. The solution was stored at 4°C in brown bottle.

Test

Plasma was diluted 1 in 10 with 0.9 per cent saline. 1 ml of buffered starch substrate was pipetted into a test tube and placed in a water bath at 37°. After 3 minutes 0.1 ml of diluted plasma was added into the tube, mixed gently and incubated for exactly 15 minutes. The tube was removed from the bath, 0.4 ml of working iodine solution was added into it, mixed well and 10 ml of glass distilled water was added and mixed again.

Control

1 ml of buffered substrate, 10.1 ml of glass distilled water, and 0.4 ml of iodine were mixed together in the order.

The colours were compared immediately in Klett-Summerson Photo-electric colorimeter with a green filter using glass distilled water as a blank.

The control tube contained 0.4 mg of starch. The amount of starch which had been digested was, therefore,

$\frac{C-T}{C-B} \times 0.4$ mg

The amylase unit (Somogyi unit) is the amount of enzyme digesting 5 mg of starch in these conditions. The amount of enzyme present in 0.01 ml of plasma is

$\frac{C-T}{C-B} \times \frac{0.4}{5}$ units

Thus plasma amylase (Somogyi units per 100 ml) $= \frac{C-T}{C-B} \times \frac{0.4}{5} \times \frac{100}{0.01} = \frac{C-T}{C-B} \times 800$

Preparation of reagents for Alkaline phosphatase determination

Buffer (pH 10.0)

6.3 gm of anhydrous sodium carbonate and 3.36 gm of sodium bicarbonate were dissolved in 1 litre of water, and stored at 4° till used.

Substrate:0.01 M-disodium phen phosphate

2.18 gm of 0.01 M-disodium

dissolved in 1 litre of water. The solution was quickly boiled to kill any organism that might be present. Cooled immediately. 4 cc of chloroform was added to the solution as a preservative and stored at 4°C.

Stock phenol standard (1 mg/ml)

1 g pure crystalline phenol was dissolved in a litre

of 0.1 N-hydrochloric acid and stored at 4° in a brown bottle.

Working phenol standard (1 mg/100 ml)

1 ml of stock phenol standard was diluted to 100 ml with glass distilled water. A few drops of chloroform were added as a preservative and stored in brown bottle at 4°.

0.5-N Sodium Hydroxide

Prepared by dissolving 20 gm of sodium hydroxide per litre in distilled water.

0.5 N-Sodium bicarbonate

Prepared by dissolving 42 gm of sodium bicarbonate per litre in water.

4-Amino antipyrine

6 gm of 4 amino antipyrine was dissolved in a litre of distilled water, and stored in a brown bottle.

Potassium ferricyanide

24 gm of potassium ferricyanide was dissolved in 1 litre of distilled water and stored in brown bottle.

Determination of alkaline phosphatase in plasma

The determination was carried out as per the method described by Wootton (1964).

Method

Phenol released by enzymatic hydrolysis from phen 1 phosphate, under defined conditions of time, temperature and pH was estimated colorimetrically.

Test

1 ml of buffer and 1 ml of phen was taken in a test tube and placed in a water bath at 37° for 3 minutes. 0.1 ml of plasma was added and gently mixed and incubated for exactly 15 minutes. The reaction was stopped by addition of 0.8 ml of 0.5 N Sodium hydroxide control.

Control

1 ml of buffer, 1 ml of substrate and 0.8 ml 0.5 N sodium hydroxide were taken in a test tube and mixed. Then added 0.1 ml of plasma and mixed again.

Standard

1.1 ml of buffer and 1 ml of phen 1 standard (1 mg/100 mg

and 0.8 ml of 0.5 N sodium hydroxide were mixed in a test tube.

Blank

1.1 ml of buffer, 1 ml of distilled water and 0.5 N sodium hydroxide were mixed together in a test tube.

To all the tubes 1.2 ml of 0.5 N sodium bicarbonate was added followed by 1 ml of amino-antipyrine solution and 1 ml potassium ferricyanide solution, all the tubes were agitated well after each addition. Successive addition brings about the required pH for colour development.

The colours were compared immediately with an Ilford 624 green light filter.

The amount of phenol in the standard tube was 10 µg. Thus the phenol produced in 15 minutes in test was

$$\frac{T-C}{S-B} \times 10 \text{ µg}$$

Hence 100 ml of plasma liberate

$$\frac{T-C}{S-B} \times 10 \text{ mg of phenol}$$

Since 1 King-Armstrong unit is the production of 1 mg of phenol in 15 minutes under the conditions of the test

$$\text{Serum alkaline phosphatase (K-A units per 100 ml)} = \frac{T-C}{S-B} \times 10$$

The effects of age and sex were tested by the method

known as 'fitting of the constants' given by Kemptmorne (1952) and Goulden (1952).

The differences due to sex and age groups were corrected by employing constants as used by Hazel and Terril (1945). The female was taken as standard. Subsequently, all the male progenies were brought to the level of female progenies by adding to each male individual the difference between the constants for male and females.

Similarly the age group (10 - 14 months) was held as standard and other age groups were brought to its level.

This was done by adding the difference between standard age group and the other age group to the latter.

Thus the corrected data was grouped sirewise and half sib analysis was carried out to estimate the heritability.

Half Sib Analysis (Falconer 1964)

When a number of sires are each mated to several dams, and only one offspring from each dam is measured to provide the data then the individuals measured form a population of half sibs. An analysis of variance is then made by which the phenotypic variance is divided into observational components attributable to differences between the progeny of different sires (the between sire component σ^2_S) and to differences between individual offspring of the same sire (within sires component σ^2_W).

The Skeleton of half sib analysis is given in Table I.

Table I

Source	D.F.	M.S.	E.M.S.
Between sires	s-1	$M.S_s$	$\sigma^2_W + K\sigma^2_S$
Within sires	p-s	$M.S_w$	σ^2_W

where s = number of sires

p = total number of progenies

K = average No. of progenies per sire

$$= \frac{1}{s-1}\left(P. - \frac{\Sigma P_i^2}{P.}\right)$$

σ^2_S = Sire component of the variance

σ^2_W = the within sire component of the variance

P_i = the number of progenies of i^{th} sire

The Precision of the estimate of heritability is provided by means of its standard error. The formula for the calculation of standard error (Dickerson, 1960) is given below.

$$S.E.(h^2_S) = \frac{4}{(\sigma^2_S + \sigma^2_W)} \sqrt{\frac{2}{K^2}\left[\frac{MS^2_S}{s-1} + \frac{MS^2_W}{P.-s}\right]}$$

Genetic correlation (Falconer, 1964)

Two characters are said to be genetically correlated when they are influenced by same set of genes. The degree of correlation arising from pleiotrophy expresses the extent to which two characters are influenced by the same genes. Some genes may increase both characters, while the others increase one and reduce the other, the former tend to cause a positive correlation, the latter a negative one.

The genetic correlation between any two traits can be calculated by the formula (Falconer, 1964).

$$r_A = COV_{S(XY)} \Big/ \sqrt{\sigma^2_{S(X)}\, \sigma^2_{S(Y)}}$$

where $COV_{S(XY)}$ is the sire component of covariance between X and Y characters.

$\sigma^2_{S(X)}$ and $\sigma^2_{S(Y)}$ are the sire component of variances of X and Y characters respectively.

The standard error of genetic correlation is obtained by the formula

$$SE(r_A) = \frac{(1-r^2_A)}{\sqrt{2}} \Big/ \sqrt{\frac{SE(h^2_X)\,SE(h^2_Y)}{h^2_X\, h^2_Y}}$$

where h^2 is heritability

Components for the calculation of genetic correlation are obtained from Table II.

Table II

Source of variation	D.F.	S.S.	M.S.	Expected mean square	S.P.	M.P.	E.M.P.
Between sires	s-1	$\sum_{i=1}^{s} \frac{Y^2_{i.}}{P_i} - (Y..)^2/p.$	$SS_S/s-1$	$\sigma^2_{W(Y)}+K\sigma^2_{S(Y)}$	$\sum_{i=1}^{s} \frac{Y_{i.}X_{i.}}{p_i} - \frac{Y.X.}{p.}$	$SP_S/s-1$	$COV_{W(XY)}+KCOV_{S(XY)}$
Within sires	$p.-s$	$\left[\sum\sum Y_{ij}^2 - \sum_{i=1}^{s} \frac{Y^2_{i.}}{p_i} \right]$	$SS_W/p.-s$	$\sigma^2_{W(Y)}$	$\sum\sum_{ij} Y_{ij}X_{ij} - \frac{\Sigma Y_{i.}X_{i.}}{p_i}$	$SP_W/p.-s$	$COV_{W(XY)}$
Total	p-1	$\left[\sum\sum Y_{ij}^2 - (Y..)^2/p \right]$			$\sum\sum_{ij} Y_{ij} X_{ij} - \frac{Y..X..}{p.}$		

s = number of sires
P_i = number of progenies of i^{th} sire
$P.$ = Total number of progenies
Y_{ij} = The observation of Y character on j^{th} progeny of i^{th} sire
X_{ij} = The observation of X character on j^{th} progeny of i^{th} sire
σ^2_W = Within sire component of variance
σ^2_S = Sire component of variance
COV_W = within sire component of covariance
COV_S = Sire component of covariance
The dot in place of a suffix denotes summation over that suffix

The estimates of mean, standard error and correlations were obtained by using methods given by Snedecor (1956).

Phenotypic correlation coefficient

The phenotypic correlation coefficient between two characters (X and Y) can be calculated by the formula

$$r = \frac{\text{Covariance XY}}{\left[\text{(Variance X)(Variance Y)}\right]^{\frac{1}{2}}}$$

$$r = \frac{\text{Corrected sum of products XY}}{\left[\text{(Corrected sum of squares X)(Corrected sum of squares Y)}\right]^{\frac{1}{2}}}$$

$$= \frac{\Sigma XY - \frac{(\Sigma X)(\Sigma Y)}{n}}{\left[(\Sigma X^2 - \frac{(\Sigma X)^2}{n})(\Sigma Y^2 - \frac{(\Sigma Y)^2}{n})\right]^{\frac{1}{2}}}$$

where n = Number of pairs of observations

The estimated correlation coefficients were tested for significance by referring Table of Fisher and Yates (1963).

RESULTS

The basic data used for the estimation of heritability, genetic and phenotypic correlation are given in Appendix.

1. Constants

The calculated constants for correcting age groups and sexes are given in Tables III and IV.

Table III

Age Groups		Estimates of constants	
		Amylase	Alkaline phosphatase
1	Upto 9 months	-9.4	5.22
2	10 - 14 "	-0.4	-0.70
3	15 - 19 "	2.3	-3.02
4	20 & above months	7.5	-1.50

Table IV

Sexes	Constants	
	Amylase	Alkaline phosphatase
Male	1.32	-0.518
Female	-1.32	+0.518

2. Heritability

After making corrections for age groups and sex the pooled data were subjected to half sib snalysis. An heritability for amylase activity was found to be 0.08 ± 0.05. However, for alkaline phosphatase activity no heritability could be estimated.

Analysis of variance for uncorrected and corrected data for amylase and alkaline phosphatase activity are shown in Tables V, VI, VII and VIII.

Table V

Analysis of variance of Amylase activity (before correction)

Source of variation	D.F.	S.S.	M.S.
Between sires	11	11036.12	1003.28
Within sires	137	143208.67	1045.32

Table VI

Analysis of variance of Amylase activity (after correction)

Source of variation	D.F.	S.S.	M.S.	Components	Heritability
Between sires	11	12587.49	1144.32	$\sigma^2_S = 18.78$	$h^2_{(S)} = 0.080 \pm 0.00$
Within sires	137	126072.47	920.24	$\sigma^2_W = 920.24$	

Table VII

Analysis of variance of Alkaline phosphatase activity (before correction)

Source of variation	D.F.	S.S.	M.S.
Between sires	11	557.24	50.658
Within sires	137	4328.90	31.598

Table VIII

Analysis of variance of Alkaline phosphatase activity (after correction)

Source of variation	D.F.	S.S.	M.S.	Components	Heritability
Between sires	11	251.21	22.837	σ^2_s = Not estimable	Not estimable
Within sires	137	3209.61	23.427		

3. Genetic correlation

No genetic correlation of amylase activity with milk yield and butter fat could be estimated.

Similarly alkaline phosphatase activity could not be genetically correlated with milk yield and butter fat.

Components for the calculation of genetic correlation are shown in Tables IX, X, XI and XII.

Table IX

Analysis of covariance - Milk yield (B) and Amylase(E)

Source of variation	D.F.	Milk yield		Amylase activity		Milk yield and Amylase		Genetic correlation
		M.S.	Components	M.S.	Components	M.P.	Components	
Between sires	5	2345152.48	$\sigma^2_{S(B)} = 312624.49$	845.11	Not estimable	15906.54	$COV_{S(BE)} = 1851.75$	Not estimable
Within sires	30	503794.22	$\sigma^2_{W(B)} = 503794.22$	923.24	"	4999.68	$COV_{W(BE)} = 4999.68$	"

Table X

Analysis of covariance - Butter fat (C) and Amylase (E)

Source of variation	D.F.	Butter fat		Amylase activity		Butter fat & amylase		Genetic correlation
		M.S.	Components	M.S.	Components	M.P.	Components	
Between sires	5	3969.36	$\sigma^2_{S(C)} = 513.59$	845.11	Not estimable	606.99	$COV_{S(CE)} = 69.57$	Not estimable
Within sires	30	944.29	$\sigma^2_{W(C)} = 944.29$	923.24	"	197.24	$COV_{W(CE)} = 197.24$	"

Table XI

Analysis of covariance - Milk yield (B) and Alkaline phosphatase (D)

Source of variation	D.F.	Milk yield		Alkaline phosphatase		Milk yield & Alkaline phosphatase		Genetic correlation
		M.S.	Components	M.S.	Components	M.P.	Components	
Between sires	5	2345152.48	$\sigma^2_{S(B)} = 312624.49$	8.15	Not estimable	-1108.80	$COV_{S(BD)} = -104.37$	Not estimable
Within sires	30	503794.22	$\sigma^2_{W(B)} = 503794.22$	9.83	"	- 494.06	$COV_{W(BD)} = -494.06$	"

Table XII

Analysis of covariance - Butter fat (C) and Alkaline phosphatase (D)

Source of variation	D.F.	Butter fat		Alkaline phosphatase		Milk yield and Alkaline phosphatase		Genetic correlation
		M.S.	Components	M.S.	Components	M.P.	Components	
Between sires	5	3969.36	$\sigma^2_{S(C)} = 513.59$	8.15	Not estimable	-49.26	$COV_{S(CD)} = -5.13$	Not estimable
Within sires	30	944.29	$\sigma^2_{W(C)} = 944.29$	9.83	"	-19.06	$COV_{W(CD)} = -19.06$	"

Correlation coefficients of alkaline phosphatase and amylase with different economic characters, are presented in Table XIII.

Table XIII

Correlation coefficients of alkaline phosphatase and amylase with different economical characters

Correlations	No. of observations	Alkaline phosphatase		Amylase	
		Milk yield	Butter fat	Milk yield	Butter fat
Genetic correlation	36	Not estimable	Not estimable	Not estimable	Not estimable
Phenotypic correlation	36	-0.2146	-0.2034	0.2448	0.2283

Mean and standard error of amylase and alkaline phosphatase activities are shown in Tables XIV and XV.

Mean and Standard error of Amylase activity in the progenies of different sires

Sire	Amylase Activities					
	No.	Male	No.	Female	No.	Overall sexes
Absalon	12	168.00 ± 9.555	19	150.32 ± 7.927	31	157.16 ± 6.203
Hugo	10	140.6 ± 8.839	11	151.63 ± 8.159	21	146.38 ± 5.976
Dan	9	145.44 ± 8.517	11	144.09 ± 10.027	20	144.70 ± 6.539
Saxo			9	152.33 ± 9.680	9	152.33 ± 9.680
Soren	6	145.83 ± 8.432	10	145.80 ± 10.699	16	145.81 ± 7.195
Jens	6	145.83 ± 7.245	11	148.73 ± 10.479	17	147.71 ± 7.080
Kloves	3	140.33 ± 13.994	4	149.50 ± 29.940	7	145.57 ± 16.928
Thyhoej			5	167.00 ± 14.942	5	167.00 ± 14.942
Michael	3	114.66 ± 19.358	5	142.60 ± 18.099	8	132.13 ± 11.498
Peter	2	125.50 ± 5.518	4	151.50 ± 20.555	6	142.83 ± 14.177
Thy Gann			4	150.25 ± 10.491	4	150.25 ± 10.491
Benny	5	177.40 ± 21.331	-		5	177.40 ± 21.331
Overall	56	149.71 ± 4.2134	93	149.75 ± 3.4093	149	149.74 ± 2.6437

Table XV

Mean and Standard error of Alkaline phosphatase activity in the progenies of different sires

Sire	Alkaline Phosphatase					
	No.	Male	No.	Female	No.	Overall sexes
Absalon	12	8.58 ± 1.651	19	12.94 ± 1.378	31	11.26 ± 1.113
Hugo	10	12.50 ± 2.997	11	10.27 ± 2.337	21	11.33 ± 1.891
Dan	9	11.22 ± 1.978	11	8.18 ± 0.614	20	9.55 ± 0.985
Saxo			9	8.11 ± 2.553	9	8.11 ± 2.553
Soren	6	9.33 ± 0.882	10	12.80 ± 1.373	16	11.50 ± 0.998
Jens	6	7.67 ± 1.202	11	12.91 ± 1.897	17	11.05 ± 1.417
Kloves	3	12.33 ± 1.202	4	19.75 ± 2.090	7	16.57 ± 1.925
Thyhoej			5	7.20 ± 1.576	5	7.20 ± 1.576
Michael	3	18.00 ± 4.046	5	10.60 ± 1.545	8	13.38 ± 2.102
Peter	2	11.00 ± 3.007	4	8.50 ± 1.500	6	9.33 ± 1.318
Thy Gann			4	6.50 ± 1.040	4	6.50 ± 1.040
Benny	5	10.20 ± 1.067	-		5	10.20 ± 1.067
Overall	56	10.63 ± 0.8052	93	10.97 ± 0.5813	149	10.84 ± 0.4709

Correlations coefficient of alkaline phosphatase and amylase activities with age are shown in Table XVI.

Table XVI

Sex	No. of offsprings	Alkaline phosphatase with age	Amylase activities with age
Male	53	-0.10813	0.04708
Female	96	-0.56580**	0.29386**
Overall	149	-0.39547**	0.21569**

** indicates significant at 1% level of significance.

DISCUSSION

Of late, genetic study of enzymatic activity is drawing increasing attention. The enzymes catalyse all the biochemical reaction in the body. Proof to the effect that enzymes are controlled by gene are given by various workers (Beadle and Tatum, 1947; Petras, 1963 and Ashton, 1964). There is also sufficient evidence to prove that many of the enzymes are highly heritable (Roderick, 1961, Wilcox et al, 1962; Nayudu and Moog, 1967, Yuhas et al, 1967 and Karlikov, 1968). The enzyme activity has been used as a criterion for indirect selection for economic traits in poultry (Wilcox et al, 1962) and in swine (Karlikov, 1968). Therefore, there is enough justification in attempting this inheritance study of amylase and alkaline phosphatase activity in blood plasma of dairy cattle.

Amylase - The reasons that prompted this enzyme study are listed below.

1. Amylase is an enzyme that hydrolysis starch and glycogen into disaccharides, thus play a key role in carbohydrate metabolism.

2. It is present in all the body fluids.

3. This enzyme is not influenced by sex, age, hunger, thirst, physical activities and diet (Abderhalden, 1961).

4. Amylase polymorphism has been reported in several farm animals (Ashton, 1964; Sick and Nielsen, 1964; and

Hesselholt and Moustgaard, 1965).

5. There are inadequate information on the quantitative genetic aspect of this enzyme.

In present study the heritability of plasma amylase activity was found to be 0.08 with a S.E. of 0.005. This estimate is though low is a precise one. However, on mice the heritability estimate have been reported to be 0.237 (Purohit et al, 1969). The present heritability estimate of amylase although quite precise, can not be confirmed due to absence of similar work in dairy cattle.

Genetic correlation of amylase with milk yield and butter fat were not estimable because of large sampling error resulting from small number of samples. However, it is possible to suggest from the present investigation that genetic correlation can be estimated from a large sample.

A positive phenotypic correlation were found between amylase and milk yield, and amylase and butter fat which were insignificant at 5 per cent of significance.

An attempt was made to correlate age with enzyme activity. It was positive and insignificant at 5 per cent level of significance in males and positive and significant at 1 per cent level of significance in females. This sex difference observed in correlation between age and enzymic activity defies explanation.

Alkaline phosphatase

The genetic study of this enzyme was considered for the reasons mentioned below.

1. The enzyme plays an important role in calcium and phosphorus metabolism and in bone formation.

2. Considerable information is available on the genetics of this enzyme in animals (Alexander et al 1960, in cattle; Wilcox et al 1962, in poultry; Gahne 1963, in cattle Nayudu and Moog 1967 in mice and Karlikov 1967 in swine).

In the present study no heritability could be obtained which can be attributed to sampling error or perhaps due to small sample size. However, in other breeds of cattle like Hariana extensive studies have been made in the quantitative genetic aspect of this enzyme. Eapen and Goswami observed a heritability of 0.04 and repeatability of 0.22 in Hariana cattle. It is, therefore, likely that the estimate of heritability could have been obtained in case investigation was conducted on large sample size. It may be mentioned that heritability estimates on other species of animals have been reported by several workers (Wilcox et al 1962, in poultry; Yuhas et al 1967 in mice, Nayudu and Moog 1967 in mice and Karlikov 1968 in swine).

In the present investigation no genetic correlation could be estimated between alkaline phosphatase and milk yield

and alkaline phosphatase and butter fat. The reason could be due to sampling variation or due to less number of observations, or due to both. However, it is suggested that large sample size may overcome this difficulty.

A negative and insignificant phenotypic correlation was found between the enzyme with milk yield and butter fat. Correlation of this enzyme with age was found to be negative and insignificant at 5 per cent level of significance in males, and negative and significant at 1 per cent level of significance in females. The former results are contrary to the findings of Roussel and Stallcup (1965), who observed a negative and highly significant correlation in Holstein bulls. Goswami and Eapen (1970) had also observed a considerable effect of age on the activity of this enzyme in Hariana cattle.

On the basis of the present investigation it is not possible to draw any valid inferences unless further work is undertaken.

SUMMARY

149 dairy cattle of both sexes of different ages belonging to 12 sire groups of Red-Danish dairy breed of the Indo-Danish Project at Hessargatta formed the experimental material. Blood plasma from these animals was used for the estimation of amylase and alkaline phosphatase activity. The enzyme determination were carried out as per the method described by Wootton (1964). By using constants (Hazel and Terril, 1945) the differences due to age group and sexes were removed and the pooled data were used for the estimation of heritabilities by half sib analysis (Falconer, 1965). The heritability of plasma amylase was found to be 0.080 and that of alkaline phosphatase activity unestimable. Genetic correlation for these enzymes with milk yield and butter fat were unestimable and phenotypic correlation were found to be insignificant at 5 per cent level of significance. The correlations of amylase with age was positive and insignificant at 5 per cent level of significance in males, but positive and significant at 1 per cent level of significance in females. In case of alkaline phosphatase the correlation with age was negative and insignificant at 5 per cent level of significance in males, and negative and significant at 1 per cent level of significance in females.

On the basis of present investigation it is not possible to draw any useful conclusion unless further work is pursued on these lines. However, it is suggested that further work may be undertaken with large sample to draw any valid conclusions.

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APPENDIX

Sire	Progeny No.	Sex	Age in months	Alkaline phosphatase activity KAU/ 100 ml	Amylase Somogyi units/ 100 ml	Corrected for 305 days	
						Milk yield in kgs	Butter fat in kgs
1. Absalon	52	♂	7	14	192		
	95	♂	8	18	137		
	146	♂	9	4	180		
	158	♂	13	3	140		
	148	♂	14	5	149		
	122	♂	15	9	238		
	125	♂	14	4	208		
	126	♂	15	8	136		
	128	♂	14	20	170		
	151	♂	15	5	177		
	167	♂	18	5	160		
	168	♂	19	8	129		
	66	♀	9	28	103		
	73	♀	9	15	109		
	74	♀	8	20	97		
	81	♀	4	15	116		
	108	♀	9	21	156		
	112	♀	13	10	171		
	98	♀	13	14	146		
	99	♀	14	13	143		
	100	♀	14	10	131		
	107	♀	17	16	124		
	113	♀	18	8	167		
	117	♀	24	8	189		
	12	♀	42	8	168	3596	150.3

APPENDIX (Contd.)

Sire	Progeny No.	Sex	Age in months	Alkaline phosphatase activity KAU/ 100 ml	Amylase Somogyi units/ 100 ml	Corrected for 305 days	
						Milk yield in kgs	Butter fat in kgs
	21	♀	43	5	229	4122	174.1
	23	♀	41	5	203	4760	195.2
	34	♀	38	7	133	3927	166.4
	38	♀	29	15	163	3015	120.6
	44	♀	28	10	140	1343	55.1
	48	♀	41	18	168	4440	186.0
2. Hugo	77	♂	8	7	100		
	92	♂	3	39	140		
	143	♂	9	11	114		
	141	♂	11	10	135		
	142	♂	9	7	120		
	152	♂	12	8	177		
	153	♂	12	10	174		
	171	♂	24	10	180		
	172	♂	23	12	140		
	177	♂	24	11	126		
	57	♀	9	15	171		
	82	♀	4	32	139		
	106	♀	9	8	127		
	116	♀	12	5	189		
	97	♀	23	9	190		
	5	♀	27	8	140	3296	136.4
	6	♀	25	9	156	3435	145.5
	19	♀	33	5	123	3611	157.4
	28	♀	23	7	178	2061	86.6
	36	♀	27	10	143	3617	153.8
	43	♀	41	5	112	4029	171.8
	3	♀	32	5	153	3964	166.9

APPENDIX (Contd.)

Sire	Progeny No.	Sex	Age in months	Alkaline phosphatase activity KAU/ 100 ml	Amylase Somogyi units/ 100 ml	Corrected for 305 days	
						Milk yield in kgs	Butter fat in kgs
3. Dan	121	♂	15	4	185		
	124	♂	15	4	166		
	127	♂	18	9	147		
	156	♂	13	9	117		
	165	♂	19	7	97		
	170	♂	34	15	149		
	174	♂	33	15	150		
	181	♂	21	19	146		
	183	♂	26	19	152		
	102	♀	12	9	154		
	103	♀	13	6	109		
	109	♀	17	8	115		
	110	♀	17	11	130		
	134	♀	14	12	133		
	159	♀	15	8	111		
	136	♀	15	7	123		
	1	♀	38	7	171	2690	113.6
	9	♀	38	9	170	3133	129.9
	10	♀	35	5	219	4471	186.0
	39	♀	43	8	150	2952	122.8
4. Saxo	2	♀	31	7	114	2577	106.2
	7	♀	24	10	140	2713	115.3
	14	♀	27	6	213	2954	122.5
	16	♀	31	12	155	2280	92.5
	17	♀	33	6	129	2824	119.4
	18	♀	31	11	135	3351	165.5
	29	♀	30	8	171	2851	115.1
	37	♀	32	8	167	2088	90.9
	47	♀	27	5	147	3018	120.7

APPENDIX (contd.)

Sire	Progeny No.	Sex	Age in months	Alkaline phosphatase activity KAU/ 100 ml	Amylase Somogyi units/ 100 ml	Corrected for 305 days	
						Milk yield in kgs	Butter fat in kgs
5. Soren	53	♂	6	12	140		
	64	♂	7	8	126		
	67	♂	6	12	141		
	155	♂	9	9	149		
	157	♂	11	7	134		
	118	♂	14	8	185		
	49	♀	7	15	140		
	56	♀	8	19	206		
	86	♀	6	18	119		
	72	♀	9	17	109		
	160	♀	9	11	123		
	115	♀	9	10	185		
	104	♀	14	7	147		
	120	♀	17	7	147		
	132	♀	14	11	176		
	76	♀	8	13	106		
6. Jens	69	♂	8	13	132		
	119	♂	15	5	178		
	145	♂	10	6	137		
	147	♂	13	6	149		
	154	♂	14	7	149		
	178	♂	20	9	130		
	54	♀	8	23	201		
	62	♀	7	20	147		
	83	♀	4	9	136		
	105	♀	10	15	113		
	130	♀	17	8	181		
	133	♀	14	13	137		
	137	♀	18	6	169		
	138	♀	16	7	151		
	139	♀	16	7	189		
	161	♀	11	22	129		
				12	83		

APPENDIX (contd.)

Sire	Progeny No.	Sex	Age in months	Alkaline phosphatase activity KAU/ 100 ml	Amylase Somogyi units/ 100 ml	Corrected for 305 days	
						Milk yield in kgs	Butter fat in kgs
7. Kloves	58	♂	7	14	168		
	84	♂	4	10	130		
	144	♂	9	13	123		
	55	♀	8	26	238		
	88	♀	7	18	124		
	89	♀	8	18	130		
	163	♀	9	17	106		
8. Thy Hoej	22	♀	37	5	210	4771	206.8
	25	♀	37	7	140	4901	200.3
	26	♀	38	4	165	4191	178.1
	27	♀	38	7	190	4229	176.5
	45	♀	38	13	130	4611	195.0
9. Michael	93	♂	3	23	134		
	1	♂	6	21	134		
	75	♂	10	10	76		
	79	♀	6	14	135		
	131	♀	14	8	109		
	129	♀	14	12	208		
	164	♀	11	13	150		
	166	♀	14	6	111		
10. Peter	175	♂	25	8	120		
	180	♂	26	14	131		
	111	♀	7	12	171		
	135	♀	16	6	121		
	140	♀	23	6	114		
	13	♀	27	10	200		

APPENDIX (contd.)

	Sire	Progeny No.	Sex	Age in months	Alkaline phosphatase activity KAU/ 100 ml	Amylase Somogyi units/ 100 ml	Corrected for 305 days	
							Milk yield in kgs.	Butter fat in kgs
11.	Thy Gann	8	♀	35	4	154	4767	193.4
		11	♀	38	6	174	4111	173.0
		31	♀	35	9	123	3350	139.9
		32	♀	38	7	150	3634	152.1
12.	Benny	68	♂	9	9	168		
		70	♂	8	14	138		
		94	♂	8	8	129		
		149	♂	10	9	212		
		150	♂	13	11	240