GENETIC STUDIES ON SERUM ALKALINE PHOSPHATASE AND HAEMOGLOBIN IN TWO STRAINS OF WHITE LEGHORN

By

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THESIS

Submitted in partial fulfilment of the
requirement for the degree

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DECLARATION

I hereby declare that the thesis entitled "GENETIC STUDIES ON SERUM ALKALINE PHOSPHATASE AND HAEMOGLOBIN IN TWO STRAINS OF WHITE LEGHORN" is a bona fide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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CERTIFICATE

Certified that the thesis entitled "GENETIC STUDIES ON SERUM

ALKALINE PHOSPHATASE AND HAEMOGLOBIN IN TWO

STRAINS OF WHITE LEGHORN" is a record of research work done

independently by **Sri. S. Sankaralingam** under my guidance and supervision

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INTRODUCTION

Modern developments in analytical biochemistry have opened up fresh research areas for animal breeders. Techniques like electrophoresis, chromatography and quantitative enzyme assays have made possible the direct detection of genes segregating in population at molecular or gene product level.

Poultry breeders still rely mainly on the conventional selection methods. But it must be emphasized that these selections were mostly carried out in the absence of sufficient basic information about the key biochemical and physiological steps involved in the manifestations of these traits. With increasing information on these basic aspects, we may well identify the genetic variations of profound practical utility.

In the genetic improvement of poultry, the additive genetic variance and non-additive genetic variance have been exploited to a great extent. In future, breeding programme for egg production and egg quality traits needs some new variants for reinforcing the existing selection methods. An approach based on biochemical polymorphism is perhaps one of the ways to achieve this objective.

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 and birds in their early age based on indirect methods has been a persistent puzzle in improvement of domestic animals and birds. Since the discovery of blood groups, it has been suggested that blood groups and biochemical polymorphism could be associated with production performance and could be used for improvement of livestock and poultry. In domestic animals and birds, if a reasonably firm association between blood group factors/biochemical polymorphism and production traits is established, it would pave the way for rapid improvement through sequential selection, by making possible early selection and culling.

It has been found that the frequencies of the alleles controlling many protein systems in poultry vary from breed to breed and strain to strain. As a result, the gene frequencies of such alleles at different loci in poultry have been employed in studies of relationship among poultry breeds and breed structure.

Although a great deal of work on the association of polymorphism with growth and egg production traits has been carried out, comparatively very little

work has been done in this regard with the egg quality traits and with the fitness traits like fertility, embryonic mortality and hatchability.

Several enzymes occur in different forms within individual tissues. Alkaline phosphatase is one among the group of non-specific enzymes capable of hydrolysing a large number of primary phosphate esters in the alkaline pH. It exists in the blood not as simple entity but in multimolecular forms which are immunologically and chemically different and these different forms are designated as iso-enzymes. Alkaline phosphatase enzyme is an important constituent and plays an important role in calcium metabolism, especially during egg shell formation and rapid bone growth.

Haemoglobin is a protein which is vital for every living creature. It helps in transfer of oxygen from lungs to different tissues of the body. It exists inside the red blood cells (RBCs) not as simple entity but in multimolecular forms which are chemically different and these different forms are designated as polymorphs. Gene controlled haemoglobin heterogeneity is rare among avian species. Its occurrence and the inheritance pattern have been studied in detail by many workers like Washburn et al. (1968), Maeda et al. (1975) and Dimri et al.(1979b). However, only scant information is available regarding its relationship with production traits and fitness traits like fertility, embryonic mortality and hatchability.

With this background, present investigation was undertaken with the following objectives:

1.   To ascertain the alkaline phosphatase and haemoglobin polymorphism and their gene frequency in IWP and IWN strains of White Leghorn belonging to two successive generations.

2.   To study the usefulness of alkaline phosphatase and haemoglobin as genetic markers.

REVIEW OF LITERATURE

With the help of starch gel electrophoresis, agar gel electrophoresis, agarose gel electrophoresis, paper electrophoresis and polyacrylamide gel electrophoresis a large number of proteins and enzymes can possibly be identified. Gene determined polymorphism of atleast 17 proteins and 26 enzymes were reported in chicken by Singh (1987) and Grunder (1990).

2.1 Serum Alkaline Phosphatase (SAP) polymorphism

Alkaline phosphatase is a widely distributed enzyme with many glycoprotein and sialoprotein residues which catalyse the hydrolysis of phosphate monoesters under alkaline condition. Using electrophoretic procedures Law and Munro (1965) reported existence of genetically controlled fast and slow type alkaline phosphatase isozymes in chicken. They assigned the symbol Ap^2 for the fast band and Ap^4 for the slow band. The former was found to be completely dominant over the latter.

Wilcox (1966) named these two fast and slow isozymes as Akp (fast) and akp (slow). He found that the bands were located between transferrin and albumen bands and also no bird was found to have both fast and slow bands together. He also detected few sera which were producing no band. This was

supported by the findings of Nair et al. (1974) and Singh et al. (1976). Singh et al.(1976) indicated very low alkaline phosphatase activity, due to that reason perhaps no bands were evident in the electrophorograms.

According to Ashton et al. (1966) the symbols Akp^F and Akp^S were applied to the genes for the fast type and the slow type respectively.

Chaudhary et al. (1971) found each serum sample had either a slow or a fast band when stained for alkaline phosphatase. Engh and Wilcox (1971), Chaudhary (1972), Banerjee et al.(1974), Nair et al. (1974), Ranjan et al. (1974), Shabalina (1974), Garcia and Carbonell (1975), Tamaki et al. (1975), Tamaki and Watanabe (1977), Tanabe et al. (1977), Mathur et al. (1979), Amin et al. (1980), Gasparska et al. (1980), Rao et al. (1980), Patil et al. (1981), Amin et al. (1983), lotsov et al. (1983), Mathur et al. (1983), Singh (1986), Banerjee et al. (1987), lotova et al. (1989), Goswami et al. (1990), Mazumder and Mazumder (1991), Parmar et al. (1991), Shukla et al. (1991) and Ahlawat et al. (1994) also obtained Similar type of result in chicken.

Savage et al. (1970) separated fast moving and slow moving bands in Japanese quail by polyacrylamide disc electrophoresis.

Tamaki and Tanabe (1970) revealed two types of electrophoretic patterns of alkaline phosphatase isozymes in the case of starch gel and agar gel

electrophoresis of the chicken plasma. The first type contained two bands, a fast migrating band and a slow migrating band. The plasma of second type possessed only a slower migrating band. Similar finding was also obtained by Pal et al. (1994). But Tamaki and Tanabe (1970) revealed presence of two types of alkaline phosphatase isozymes by polyacrylamide gel electrophoresis, the plasma of first type had two fast moving bands and the plasma of second type had two slow moving bands. These bands were located between α - globulin and γ - globulin areas.

Savage et al. (1971) observed five different isozymes of alkaline phosphatase in four lines of chicken by using polyacrylamide disc electrophoresis.

Maeda et al. (1972) reported six alkaline phosphatase isozyme phenotypes in the serum of Japanese quail. These were grouped into two regions, Akp-1 which showed only one band in all individuals and Akp-2 region which showed six different phenotypes (AA, AB, BB, BC, AC and CC) controlled by three autosomal co-dominant alleles, Akp-2^A, Akp-2^B, Akp-2^C.

Beck et al. (1975) revealed 13 distinct patterns (zymograms) of turkey alkaline phosphatase. The isozyme possessing the highest mobility was classified as band number one as suggested by Brewer (1970). The remaining

bands were numbered in the order of decreasing mobility with the slowest moving band assigned number 13.

Jain et al. (1976) observed five serum alkaline phosphatase isoenzymes in chicken by polyacrylamide disc electrophoresis and designated as Akp-1 to Akp-5, including Akp-5^F (fast migrating) and Akp-5^S (slow migrating) in the order of decreasing electrophoretic mobility. Akp-5^F and Akp-5^S bands never occurred together in any of the birds.

Gasparska (1977) observed seven phenotypes of alkaline phosphatase in chicken by starch-gel electrophoresis namely Ap^1, Ap^{1}Ap^2, Ap^{1}Ap^4, Ap^2, Ap^{2}Ap^4, Ap^3 and Ap^4. The Ap^1 allele was found to be dominant to Ap^3 and incompletely dominant to the Ap^2. The Ap^1 and Ap^2 alleles appeared to be dominant to Ap^4.

Tamaki et al. (1977) revealed that the zymogram in alkaline phosphatase isozymes of each type was composed of two electrophoretic bands, F(or S) and B-bands.

Kimura et al. (1979) reported a second plasma alkaline phosphatase system characterized by the presence or absence of a single zone migrating anodally to the alkaline phosphatase system of Law and Munro (1965). The system called alkaline phosphatase-2 is controlled by two autosomal alleles

with dominance, Akp-2° is dominant to akp-2^{a}. Similar finding was reported by Okada et al. 1988) and Maeda et al. (1992).

Dimri et al. (1980) observed seven different alkaline phosphatase isozymes in the serum of Japanese quail.

Washburn et al. (1980) observed the F, S, B bands and a new isozyme (F^{1}) migrating at a faster rate than the previously reported F band in a randombred chicken population by polyacrylamide gel electrophoresis.

Mazumder and Mazumder (1982) observed three different alkaline phosphatase phenotypes Viz. alk-F, alk-S+f and alk-S in poultry by starch-gel electrophoresis. The alk-F and alk-S types had only one zone of different mobility and alk-S+f had two zones.

Ali et al. (1993) identified 15 electrophoretic patterns in alkaline phosphatase polymorphism of chicken.

Abraham (1995) revealed that both fast moving (F) and slow moving (S) bands occurred together in the serum of an individual cattle. However, scanning the literature revealed no such finding in poultry.

2.1.1 Phenotypic, genotypic and gene frequencies of Akp alleles

2.1.1.1 Phenotypic and Genotypic frequencies of Akp alleles

Shabalina (1974) identified the frequencies of ALP^F/ALP^F, ALP^F/ALP^S and ALP^S/ALP^S genotypes as 0.216, 0.498 and 0.286 for Cornish, 0.237, 0.497 and 0.266 for White Plymouth Rock and 0.032, 0.274 and 0.693 for Leghorns respectively.

Rao et al. (1980) reported higher phenotypic frequency of Fast band (0.85) in high plasma alkaline phosphatase level birds than control birds (0.60).

Washburn et al. (1980) revealed the phenotypic frequency of F, S, SF, F^1 and B band as 0.465, 0.590, 0.000, 0.005 and 1.000 respectively in a randombred chicken population.

Mazumder and Mazumder (1982) found the relative percentage of alk-F, alk-S and alk-S+f as 36.9, 53.2 and 9.9 percent in White Leghorn, 80.2, 16.1 and 3.7 percent in White Cornish, 26, 46 and 28 percent in New Hampshire and 53.9, 40.2 and 5.9 percent in broiler breed respectively.

Vataliya (1986) indicated a decline in genotypic frequency of FF and FS from 0.14 and 0.47 to 0.13 and 0.46 respectively and also increase of SS from

0.39 to 0.41 in the first generation which was subjected to selection for part-year production.

Abraham (1995) reported that the frequency of `SS' genotype was 0.54 and 0.66 in the Holstein Friesian crossbreds and Brown Swiss crossbreds respectively and `FS' genotype was 0.46 and 0.34 respectively.

2.1.1.2 Gene frequency

Wilcox (1966) found the gene frequency of fast band (0.18) isozyme to be lower than the slow band (0.82) isozyme in a randombred White Leghorn population. Similar finding was observed by Chaudhary et al. (1971), Banerjee et al. (1974), Shabalina (1974), Amin et al. (1980), Patil et al. (1981), Vataliya (1986), Banerjee et al. (1987) and Goswami et al. (1990) in White Leghorn population.

Tamaki and Tanabe (1970) reported the gene frequencies of White Plymouth Rock population as 0.36 and 0.64 for the Akp^F and Akp^S alleles.

Engh and Wilcox (1971) reported Akp Fast band frequency ranging from 0.29 to 0.96 in 14 commercially available egg type strains.

Shabalina (1974) found the frequencies of Akp^F gene in cornish and White Plymouth Rock as 0.464 and 0.486 respectively.

Jain et al. (1976) observed the distribution of Akp-1, 2, 3, 4 and 5 as 60, 30, 92, 50 and 100 percent respectively. Fast band frequency (Akp-5^F) was 0.32 and 0.36 for strain 1 and 2 against 0.68 and 0.64 for slow band (Akp-5^S) respectively.

Singh et al. (1976) revealed that the gene frequency of fast and slow isozymes in the Rhode Island Red flock was found to be 0.17 and 0.83 respectively.

Tanabe et al. (1977) identified that the gene frequency of Akp^F appeared to be higher in breeds with White ear lobes than in those with red ear lobes.

Maeda et al. (1980) found the gene frequency of Akp-1 as 1 and the gene frequency of Akp-2^A, Akp-2^B and Akp-2^C as 0.00, 0.92 and 0.08 respectively in Japanese quail population.

Rao et al. (1980) revealed increase in Akp^F gene frequency as 0.61 in birds selected for high plasma alkaline phosphatase compared to control birds having 0.37.

Washburn et al. (1980) identified the frequencies of S and F as 0.77 and 0.23 respectively. The B band was observed in all individuals of Athens-Canadian randombred population.

Amin et al. (1983) observed increase of SAP^F allele frequency from 0.178 to 0.257 by two years of selection for egg number upto 40 weeks of age (EN40) in White Leghorn.

Singh (1986) found the frequency of Akp^F and Akp^S in Aseel population as 0.045 and 0.955, and in Kadaknath as 0.042 and 0.958 respectively. Almost similar finding was observed by Pal et al. (1994).

Okada et al. (1988) revealed the gene frequency of Akp (Fast) allele as 0.150 and 0.780 in Dhaka and Chittagong division native chicken of Bangladesh and also revealed the gene frequency of $Akp-2^o$ as 0.690 and 0.678 as against 0.310 and 0.322 of $Akp-2^a$ allele in Dhaka and Chittagong division. Almost similar result was observed by Maeda et al. (1992) in native and red jungle fowls in Nepal.

Parmar et al. (1991) observed the gene frequency of fast and slow isozyme types as 0.42 and 0.58 in dwarf broiler birds.

Shukla et al. (1991) identified the gene frequency of pooled White Leghorn strain as 0.74 and 0.26 for SAP^F and SAP^S alleles respectively.

Ahlawat et al. (1994) found the gene frequencies of Akp^S and Akp^F isozymes in female birds as 0.77 and 0.23 and in male birds as 0.81 and 0.19 respectively in Nicobari fowl.

Abraham (1995) found the gene frequency of Akp^F as 0.23 and 0.13 in Holstein Friesian crossbreds and Brown Swiss crossbreds respcetively.

2.1.2 Inheritance of AKP

Wilcox (1966) revealed 100 percent fast band chicks in Akpakp X AkpAkp cross, 3:1 fast and slow band chicks in Akpakp X Akpakp cross, 1:1 in Akpakp X akpakp cross, 100 percent fast band chicks in akpakp X AkpAkp cross, 1:1 in akpakp X Akpakp cross and 100 percent slow band chicks in akpakp X akpakp cross. This observation revealed autosomal complete dominance of Akp gene over akp. Almost similar result was obtained by Tamaki and Tanabe (1970), Banerjee et al. (1974), Mathur (1978) and Banerjee et al. (1987).

Other than the above said crosses Washburn et al. (1980) made cross between F^1 sire (which was moving faster than F band) with dam having no difference and got 100 percent of F^1 progeny.

2.1.3 SAP types and activity

Wilcox (1966) found significantly higher SAP level (P<0.05) in birds showing fast band (77.1 mmoles nitrophenal/litre/hr) than slow band (37.9 mmoles/nitrophenol/litre/hr) at 6wks of age. Similar result was obtained by Chaudhary et al. (1971), Nair et al. (1974), Tamaki et al. (1975), Singh et al.

(1976), Gasparska et al. (1980), Rao et al. (1980), Iotsov et al. (1983), Vataliya (1986), Banerjee et al. (1987) and Goswami et al. (1990).

Tamaki and Tanabe (1970) found significantly higher Akp level in Akp^F birds at four (P<0.01) and eight (P<0.05) weeks of age. The activity of the enzyme was found to decrease and no significant difference was noticed between F and S birds as age advances.

Mazumder and Mazumder (1991) identified significantly higher enzyme activity (P<0.01) in the fast type chicken than slow type even at 44 wks of age in four strains of White Leghorn.

Abraham (1995) observed no significant difference for the serum alkaline phosphatase level between 'FS' and 'SS' in either Holstein Friesian (9.32 Vs 7.79 KA units per 100ml serum) or Brown Swiss (10.73 Vs 9.07 KA units per 100ml serum) crossbreds.

2.1.4 SAP types and production traits

Wilcox (1966) reported significantly higher (P<0.05) body weight in fast band birds (418g) than the slow band birds (381g) at six weeks of age in White Leghorn. Similar report was made by Parmar et al. (1991) in a dwarf broiler breed.

Chaudhary et al. (1971) reported birds exhibiting fast band were having higher body weight upto 16wks, high egg production and earlier sexual maturity. However, from 16wks onwards there was no difference for body weight, weight at sexual maturity and egg weight. Almost similar result was obtained by Chaudhary (1972) and Mathur et al. (1979).

Ranjan et al. (1974) observed hens with phenotype S had significantly higher egg production (270 days production) than those with F.

Garcia (1975a) and Garcia (1975b) revealed no significant effects of the genes at the serum alkaline phosphatase loci on body weight at 6th week, age at 1st egg, laying performance or egg weight in Rhode Island Red hens. This result was supported by Tamaki and Watanabe (1977), Rao et al. (1980) and Amin et al. (1983) except significant increase in egg weight of S band birds.

Jain et al. (1976) observed significant positive correlation between the number of serum alkaline phosphatase isozymes and egg production. As the number of serum alkaline phosphatase isozyme increased egg production also increased in both the strains. He also observed positive relationship between the fast band isozyme Akp-5[F] and egg production (75.27 Vs 51.52/100 days), age at sexual maturity (167.00 days Vs 178.60 days) and first egg weight (34.16 Vs 29.82g) than Akp-5[S] in White Leghorn layers. Almost similar finding was reported by Singh et al. (1976).

Patil et al. (1981) divided the White Leghorn birds into above-average-group and below-average-group with regard to number of eggs laid upto 280 days of age. The birds with Akp^F isozyme laid on an average 18.71% more eggs than the overall average as against 12.03% more eggs by those with Akp^S isozyme type. Likewise in below-average-group the birds with Akp^F laid on an average 14.37% less eggs than overall average as against 22.90% by Akp^S birds.

Banerjee et al. (1987) found that at all stages of growth S line had the body weight around 92 to 98% of that showed by F line, however the difference became statistically significant (P<0.05) only after four months of age. Age at sexual maturity was found to be significantly low (P<0.05) in F birds that has contributed for the increase in egg production.

Shukla et al. (1991) observed no significant difference between Akp isozymes in body weight, egg number and egg weight. However, they revealed significantly lower (P<0.05) average age at first egg in fast birds than the slow.

2.1.5 SAP types and egg quality traits

Engh (1966) observed lower albumen quality and more blood spots in the eggs of Fast band birds.

Rathore et al. (1979) while studying comparative performance of alkaline phosphatase in White Leghorn with regard to egg quality traits reported significant difference between fast and slow isozyme groups at five percent level for egg weight only, the rest of the differences between the groups for other egg quality traits being non-significant.

Patil et al. (1981) reported the birds with Akp^F isozyme type had significantly higher Haugh unit score (70.43 Vs 68.49) as well as yolk index (0.437 Vs 0.432) than those with Akp^S isozyme type. The reverse was true for shell thickness (0.344 Vs 0.345mm). Similarly very little variation in egg quality traits between the two Akp isozymes was observed by Mathur et al. (1983).

2.1.6 SAP types and reproduction traits

Ranjan et al. (1974) observed cocks with phenotype S had significantly greater semen volume than those with F.

Garcia and Carbonell (1975) reported eggs from hens with the genotype Ap^{4}Ap^4 (fast) had a significantly higher hatchability than those from Ap^{2}Ap^2 (slow) hens (72 Vs 58%). Almost similar report was made by Mathur et al. (1983), Banerjee et al. (1987) and Iotova et al. (1989).

Mathur et al. (1979) found no significant difference between fast and slow type isozyme groups for reproduction traits of randombred White Leghorn population. Similar result was already reported by Mina (1977) and also by Parmar et al. (1991).

2.2 Haemoglobin polymorphism

Haemoglobin is a conjugated protein enclosed in erythrocytes, performing the vital role of oxygen transportation to all body tissues. Its easy availability helped in understanding its structure, genetics and population aspects among galliform species. Haemoglobin multiple molecular forms of fowls can be divided into three distinct categories. The earliest embryonic haemoglobins are associated with primitive generations of erythrocytes derived from the blood islands and attained maximum activity at five days of incubation. The definitive embryonic forms develop around fourth day of incubation in the yolk sac, embryonic spleen, kidney and pancreas and finally the adult variant forms (Pal,1992).

Johanson and Dunlap (1955) reported occurrence of two electrophoretic components viz. F (fast) and S (slow). Similar findings have also been reported by Dunlap et al. (1956), who named them as α and β haemoglobins, followed by Saha et al. (1957) named as Hb_1 (slow) and Hb_2 (fast).

Rodan and Ebaugh (1957) found three haemoglobin components in some of their stocks, suggesting that chickens can have more than two haemoglobin components. But first clear resolution of the fowl haemoglobin into three zones viz. one major and one or two minor components, through starch block electrophoresis were obtained by D'Amelio and Salvo (1959).

D'Amelio and Salvo (1961) identified two distinct embryonic haemoglobin (E_1 & E_2) components present at 68 hours of embryonic development. By 88 hours of embryonic development, they found three additional haemoglobin fractions which were identical to those present in haemoglobin of adult chicken. Almost similar finding was made by Manwell et al. (1963) but they were unsuccessful in finding chicken with more than two haemoglobin components. Washburn (1968b) and Denmark and Washburn (1969) also made similar finding to that of D'Amelio and Salvo (1961), in addition they identified 'Trace' band (T) also in chicken embryos.

Lowe and Washburn (1969) observed neither the trace component nor the persistence of the embryonic component in the Coturnix with the abnormal Type II haemoglobin.

Washburn (1968a) found three different types of haemograms in the Athens-Canadian randombred line of chicken. These haemoglobin types have been designated as Type I (homozygous normal), Type II (homozygous

mutant) and Type III (heterozygous). All the types contained a single major band (M), although both haemoglobin Type I and II had a single minor band their electrophoretic properties were different with the mutant band (m_2) migrating at faster rate than the normal minor band (m_1). These differences in the minor haemoglobin components have been shown to be due to allelic co-dominant genes. Haemoglobin Type III had two minor bands, one with the same migration rate as the minor band (m_1) of Type I and the other with the same migration rate as the minor band (m_2) of Type II. Similar finding was reported by Lowe and Washburn (1971) and Washburn and Yen (1976) in chicken and also by Maeda et al. (1977), Maeda et al. (1978), Dimri et al. (1979a), Dimri et al. (1979b), Agarwal et al. (1980), Ghosh et al. (1990) and Ghosh et al. (1992) in Japanese quail. At the same time Washburn (1968a) found only normal haemoglobin type in Barred Rock, Game bird, New Hampshire, White Rock and White Wyandott breeds.

Washburn et al. (1968) reported only haemoglobin Type I and Type III. There was no mutant type of haemoglobin observed by him. Similar report was made by Mazumder and Mazumder (1982) and Singh et al. (1988) in chicken.

Washburn (1976) found only normal haemoglobin type in 20 strains including a number of randombred population of exotic breeds, commercial layers, broilers, Gallus gallus and Athens-Canadian randombred population. But the Ottawa meat control strain from which the Athens-Canadian

randombred population was developed, had all the normal, mutant and heterozygous haemoglobin types.

Singh (1986) observed that haemoglobin was monomorphic in indigenous fowl breeds of India. This was supported by Pal et al. (1994).

Okada et al. (1988) revealed that the Hb-2 loci was monomorphic and the Hb-1^A was observed only in the populations of Chittagong division, not in Dhaka division of Bangladesh. Hb-1^B was present in both divisions at high frequency (0.78 to 1). Almost similar finding was observed by Maeda et al. (1992) in native and red jungle fowls in Nepal. Ardiningsasi et al. (1993) found only Hb-1^B in Japanese quail and no Hb-1^A was found.

Singh and Singh (1988) found no polymorphism of haemoglobin loci in Guinea fowl.

Mazumder and Mazumder (1989) found normal and heterozygote types of haemoglobin in chicken, only normal type in desi fowls and guinea fowls and normal, heterozygote and mutant types of haemoglobin in quails.

2.2.1 Phenotypic/genotypic and gene frequencies of haemoglobin

Washburn (1968a) revealed the gene frequency of haemoglobin in Barred Rock, Game bird, New Hampshire, White Rock and White Wyandott as

1 for the Type I haemoglobin due to the absence of Type II and III. Similar report was made by Singh (1986) and Pal et al. (1994) in indigenous fowls of India and also by Singh and Singh (1988) in Guinea fowl.

Washburn et al. (1968) observed only AA and AB haemoglobin genotypes, the genotypic frequency was 0.916 and 0.084 respectively. Gene frequency of A and B haemoglobin loci was 0.958 and 0.042 respectively. Almost similar finding was made by Singh et al. (1988).

Maeda et al. (1975) studied the changes of phenotypic and gene frequencies in the four successive generations of randombred quail population. In the first generation the phenotypic frequency of AA, AB and BB was 0.88, 0.11 and 0.01 respectively. It was found that the phenotype BB disappeared from the second generation. Gene frequency of Hb-1[B] was reduced from 0.066 in the first generation to 0.012 in the fourth generation.

Washburn et al. (1980) reported no significant difference in gene frequency of haemoglobin mutant alleles even after 13 generations by maintaining it as a non-selected randombred population (0.060 to 0.053) in Athens-Canadian randombred population of chicken and also in quails after 15 generations of random mating.

Dimri et al. (1979b) found all AA, AB and BB haemoglobin phenotypes in the Japanese quail. The genotypic frequency of them was 0.79, 0.19 and 0.02 respectively. The gene frequency of A and B loci was 0.88 and 0.12 respectively.

Okada et al. (1988) reported the Hb-1^B gene frequency in Hb-1 locus as 1 in the chickens of Dhaka division and the same was 0.778 to 0.988 in the Chittagong division of Bangladesh against the gene frequency of Hb-1^A (0 and 0.222 to 0.012). Almost similar report was made by Maeda et al. (1992) in native and red jungle fowls in Nepal and Ardiningsasi et al. (1993) in quails.

Although Mazumder and Mazumder (1989) did not find any haemoglobin polymorphism in broilers, desi fowls and guinea fowls, he observed that the gene frequency of mutant gene was 0.04 and 0.15 in White Leghorn and quails respectively.

Ghosh et al. (1990) observed all three strains of Japanese quail had two co-dominant alleles (A & B), the frequency of A being higher than that of B in all strains. The frequency of the BB genotype was higher than the expected (P<0.05) in the meat line. The other two layer strains were in genetic equilibrium.

2.2.2 Haemoglobin inheritance

Lowe and Washburn (1969) reported that the phenotypic ratios from the back-cross (Type I x Type III) were 7 Type I and 8 Type III and from Type III x Type III cross were 33 Type I, 53 Type III and 25 Type II birds. These ratios were not significantly different (P<0.05) from the expected 1:1 and 1:2:1 ratio for Type I, Type III and Type II respectively.

Maeda et al. (1975) found the result of the possible crosses among the AA, AB and BB phenotypes were in good agreement with the expected number from Mendalian law without exception. Similar finding was also made by Maeda et al. (1977), Maeda et al. (1978) and Dimri et al. (1979b).

2.2.4 Haemoglobin types and production traits

Lowe and Washburn (1971) found no significant differences between the three haemoglobin genotypes and egg weight, age at sexual maturity. Both hen day and hen housed production of homozygous abnormal genotype was significantly less than the heterozygote or normal at one percent level. Similar result was also obtained by Maeda et al. (1977).

Dimri et al. (1979a) observed an equal influence of three haemoglobin genotypes on weekly body weight upto 8wks of age and serum alkaline

phosphatase level. However serum cholesterol level was more in HbAB type than the other two.

Ghosh et al. (1992) reported a significant effect of haemoglobin genotypes on body weight, quails of the B type were heaviest.

Zhang et al. (1992) revealed that body weight and body measurements were not related to heterozygocity at haemoglobin loci.

2.2.6 Haemoglobin types and reproduction traits

Cucchi and Sangiorgi (1969) observed that hatchability was significantly less in the case of embryos homozygous for mutant haemoglobin. This was later supported by Shabalina (1976).

Lowe and Washburn (1971) found a slight but statistically non-significant advantage in percentage hatch on total and fertile egg set for the A_2A_2 genotype. Fertility was somewhat superior for the heterozygous cross (A_1A_2 x A_1A_2), but this advantage was lost because of the greater degree of embryonic mortality in Athens Canadian randombred population.

Maeda et al. (1977) found significant difference (P<0.05) among the haemoglobin genotypes crosses (AA X AA, AA X BB, BB X BB) on fertility as 83.3, 72.9 and 62.9%, on hatchability of fertile egg set as 64.7, 76.1 and

66.2% and on hatchability of total number of eggs set as 53.9, 55.5 and 41.7 in Japanese quail.

Agarwal et al. (1980) observed significantly (P<0.05) higher fertility for eggs obtained from AA X AA type matings. Lower embryonic mortality and higher hatchability were noted among AA type zygotes. The hatchability values were lowest with respect to eggs from BB X BB matings in Japanese quail. Similar finding was made by Dimri et al. (1979a).

Material and Methods

MATERIALS AND METHODS

The research was undertaken to study the polymorphism of serum alkaline phosphatase and haemoglobin types and its relationship with traits of economic importance. The experiments were conducted at the All India Coordinated Research Project (AICRP) on Poultry improvement, Mannuthy centre, as a part of on-going research project on selection for improving the egg production in IWP and IWN strains of White Leghorn. These populations were under intra-population selection studies for the past 15 years at this centre.

3.1 Serum Alkaline Phosphatase

3.1.1 Experimental birds

The birds under study consisted of 168 selected (by Osborne Index) female breeders, 18 male breeders of IWP strain and 129 female breeders and 16 male breeders of IWN strain in S15 generation and also 100 IWP and 100 IWN females from the population of S16 generation maintained at AICRP on Poultry improvement, Mannuthy centre.

3.1.2 Collection and preparation of serum sample

Three ml of blood samples were collected in sterile test tube by venupuncture of wing vein using 26 gauze sterile needles. The samples were labelled and transported to laboratory. The blood samples were kept undisturbed for few hours and allowed for coagulation. The coagulated samples were centrifuged at 3000 rpm for 15 minutes and the serum was removed with pipette and placed in a sterile screw capped vial of 2ml capacity and stored in the deep freezer at $-40^{\circ}C$ until further analysis.

3.1.3 Electrophoresis for Serum Alkaline Phosphatase

Polyacrylamide gel 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 polymorphism.

3.1.3.1 Preparation of the gel

i. Monomer

To 28g of acrylamide, 0.735g N,N-methylene bis acrylamide was added and made upto 100ml with deionized water.

ii. Small pore buffer (pH 8.9)

To 48ml of 1N Hcl, 36.6g of Tris and 0.23ml of Tetramethylene diamine (TEMED) were added and made upto 100ml with deionized water.

iii. Catalyst

Freshly prepared 0.14% Ammonium persulphate in deionized water served as a catalyst.

3.1.3.2 Electrode buffer

Lethium hydroxide – boric acid buffer system was used for the electrophoresis (Steiner and Joslyn, 1979).

Composition of the buffer

Lithium hydroxide   - 1.5g/litre (0.036M)

Boric acid   - 12.0g/litre (0.194M)

The pH of the buffer was adjusted to 8.25 before use.

3.1.3.3 Preparation of the running gel

From the monomer, small pore buffer, catalyst and water, the 7% running gel was cast in the following proportions.

Monomer	- 2 volumes
Small pore buffer	- 1 volume
Ammonium persulphate	- 4 volumes
Deionized water	- 1 volume

Two plates of equal size, one made up to acrylic sheet and the other one a glass plate were used for the preparation of 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 pored through the top of the acrylic sheet into the gap between plates. Care was taken to avoid the formation of air bubble in the gel. Completion of polymorphism, which takes about 20 minutes was indicated by the appearance of a refractile line of demarcation between gel and water. Before removing the gel the water layer was discarded.

3.1.3.4 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 size 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. 20 microlitres of this serum sample were charged into the wells. Bromophenol blue was used as marker on one or two wells. An initial current of 15mA for one hour followed by 25mA for five hours was applied. During the run, the temperature was kept constant at $5^{\circ}C$.

On completion of the run, the gel was removed from the glass plate and put in a tray and stained with Fast blue BB salt as per the method of Ramesh and Rajasekarasetty (1980).

The staining solution consisted of

0.05M Tris-Hcl buffer (pH 8.5)	- 100ml
Polyvinyl pyrrolidone	- 500mg
Sodium 1-naphthyl phosphate	- 100mg
Fast blue salt BB	- 100mg
Magnesium chloride	- 60mg
Manganese chloride and	- 60mg
Sodium chloride	- 2g

In this staining solution the fast blue BB salt acts as a dye coupler and 1-naphthyl phosphate as substrate.

The gels were incubated at 37°C in the staining solution for one hour and then the gels were washed in distilled water and fixed in 7% acetic acid and the staining pattern of each sample was noted.

3.1.4 Quantitative Estimation of Alkaline Phosphatase

Serum samples were collected from 34 IWP hens (19 F birds and 15 S birds) and 30 IWN hens (10 F birds and 20 S birds) of S15 generation at the age of 50 weeks and tested of SAP level.

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

3.1.4.1 Procedure

Pipette into the test tubes labelled Blank(B), Standard(S), control(C) and Test(T) as follows:

	(B)	(S)	(C)	(T)
Working Buffered substrate	1.0ml	1.0ml	1.0ml	1.0ml
Deionized water	3.1ml	3.1ml	3.1ml	3.1ml
Incubate for 3 minute at 37°C				
Serum	---	---	---	0.1ml
Phenol standard(3)	---	0.1ml	---	---
Incubate for 15 minutes at 37°C				
Colour Reagent(2)	2.0ml	2.0ml	2.0ml	2.0ml
Serum	---	---	0.1ml	---

After each addition of reagent, solutions were mixed well and absorbance for Blank(B), Standard(S), Control(C) and Test(T) were measured against deionized water on spectrophotometer at 510nm.

3.1.4.2 Calculations

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

Where A - Absorbance

T - Test

C - Control

S - Standard

B – Blank

3.2 Haemoglobin

3.2.1 Experimental birds

The birds under study consisted of 100 hens each of IWP and IWN strains belonging to S15 generation, maintained at AICRP on Poultry improvement, Mannuthy centre.

3.2.2 Collection and preparation of haemoglobin

Three ml blood samples were collected in sterile test tube having EDTA as anticoagulant, by venupuncture of wing vein using 26 gauge sterile needles. The samples were labelled and transported to the laboratory. The samples were centrifuged at 3,000 rpm for 30 minutes and plasma was removed by aspiration and then red blood cells (RBCs) were washed with normal saline and again centrifuged, the supernatant was discarded. The procedure was repeated for four times to remove the plasma entrapped between the RBCs completely. Nine volumes of double distilled water was

then added to RBCs to make 10 percent solution. This haemolysed mixture containing the cellular debris and haemoglobin was immediately centrifuged for five minutes at 11,500 rpm. The resultant sample was then examined for the demarcation between the haemoglobin solution and the "gel", which consisted of cellular stroma, nuclei and trapped haemoglobin. The haemoglobin solution above the demarcation was pipetted out and used within 24 hours after collection and never frozen.

3.2.3. Electrophoresis for haemoglobin

Polyacrylamide gel electrophoresis technique of Gahne et al. (1977) with suitable modification was tried to determine the haemoglobin polymorphism. But separation of chicken haemoglobin types were not clear with this technique. Finally Agar gel electrophoresis was carried out as per Maeda (1996).

3.2.3.1 Electrode buffer (pH 8.6)

For routine assay of haemoglobin, tris – EDTA – citric acid buffer was used

Tris	-45.375g (0.249M)
EDTA	- 4.500g (0.008M)
Citric acid	-11.650g (0.037M)

Distilled water upto   -1000ml

3.2.3.2 Preparation of the gel

For the gel preparation the bridge buffer was diluted one sixth and the pH was then adjusted to 8.6. 1.5g of agar and 4.0g of polyvinyl pyrrolidone were dissolved in 200ml of the diluted buffer. This solution was heated in a water bath till frothing subsides. Then the solution was pored on a glass plate in an uniform manner, the glass plate was kept at $5^{o}C$ for solidification of the gel.

3.2.3.3 Electrophoresis

After solidification wells were made in one side of the gel. 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 size and same level at the edges were used for uniform voltage gradient. The wicks were wetted well and placed gently on either side of the gel.

Twenty microlitres of already prepared haemoglobin samples were charged into the wells. The red colour of the haemoglobin itself acted as marker. The electrophoresis was performed at $5^{o}C$ for about 3 hours at 12.5 mA/cm^{2}. After the electrophoresis, the gel plates were stained by brilliant blue for five hours.

The staining solution consisted of

Brilliant blue	- 1.25g
Methanol	- 227.00ml
Glacial acetic acid	- 46.00ml
Distilled water	- 227.00ml

Dye was dissolved in the solution of methanol and distilled water. Acetic acid was then added and stored in dark bottle.

After staining for five hours the gel was destained with the destaining solution for 12 hours and the staining pattern of each sample was noted.

Destaining solution consisted of

Ethanol	- 1500.00ml
Acetic acid	- 500.00ml
Distilled water	- 5000.00ml

3.3 Collection of Data

3.3.1 Inheritance pattern of SAP isozymes

Inheritance pattern of serum alkaline phosphatase isozymes was studied by making different matings between fast and slow moving types. In IWP strain three males with fast band and eight males with slow band were mated

in various combinations to 10 females with fast band and eight females with slow band and the distribution of two types in 182 offspring from these matings was studied.

In IWN strain two males with fast band and two males with slow band were mated in different combinations to four females with fast band and five females with slow band and the distribution of two types in 124 offspring from these matings was studied.

3.3.2 Production traits

Data pertaining to the following production traits were collected from the records maintained at the AICRP on Poultry improvement, Mannuthy centre.

a. Age (days) at sexual maturity

b. Body weight (g) at 20 and 40 weeks of age

c. Egg production upto 40 and 60 weeks of age

d. Egg weight (g) at 32 and 40 weeks of age

The egg production data of both IWP and IWN strains of S15 and S16 generations were divided into 21-40 weeks and 41-60 weeks production and then analysed to find the relationship between SAP types and egg production during these periods.

3.3.3 Egg quality traits

Eggs numbering 222 were collected from 39 IWP and 35 IWN hens in three consecutive days at the age of 50 weeks. Egg weight and shape index were recorded and then the eggs were broken and the following internal quality traits were studied:

 a. Albumen index

 b. Yolk index

 c. Shell thickness

 d. Haugh unit score

3.3.4 Reproduction traits

Eggs numbering 2,500 from 89 IWP hens and 2,000 from 64 IWN hens of S15 generation, were collected and stored at 18°C for 10 days. After 10 days of collection, eggs were incubated and candled at 7th and 18th days of incubation. The infertile eggs, eggs with dead embryos and unhatched eggs were collected during candling and also after 21 days of incubation. They were broken and data on infertility, early embryonic mortality and dead in shell percentage were collected. Data on hatchability were gathered from the records of AICRP on Poultry improvement, Mannuthy centre.

3.4 Analysis of Data

3.4.1 Gene and genotypic frequencies of SAP isozymes

The gene and genotypic frequencies of Serum alkaline phosphatase were calculated from phenotypic frequencies using following formulae.

$$p^2 + 2pq + q^2 = 1$$

$$p + q = 1$$

3.4.2 Association of serum alkaline phosphatase phenotypes with SAP level, production traits, egg quality traits and reproduction traits

The association of serum alkaline phosphatase phenotypes with SAP level, production traits, egg quality traits and reproduction traits were studied. For statistical analysis, the 't' test described by Snedecor and Cockran (1967) were used.

RESULTS

One hundred and sixty eight hens and 18 cocks of IWP and 129 hens and 16 cocks of IWN strains belonging to S15 generation (selected based on Osborne index) and 100 hens each from the population of IWP and IWN strains belonging to S16 generation were typed for the Serum Alkaline Phosphatase (SAP) variants by horizontal Polyacrylamide gel electrophoresis (PAGE).

One hundred hens each from the selected population of IWP and IWN strains were typed for Haemoglobin (Hb) polymorphism by Agar gel electrophoresis.

4.1 Serum Alkaline Phosphatase Polymorphism

The electrophoretic mobility of SAP revealed that in IWP and IWN strains, it resolves into two distinct zones. The bands were designated as Fast (F) and Slow (S). The activity was observed as dark brown bands on staining with fast blue BB salt (Fig 1A and 1B). Two distinct phenotypic classes were observed, one as fast moving and another as slow moving type. None of the samples possessed fast and low bands together.
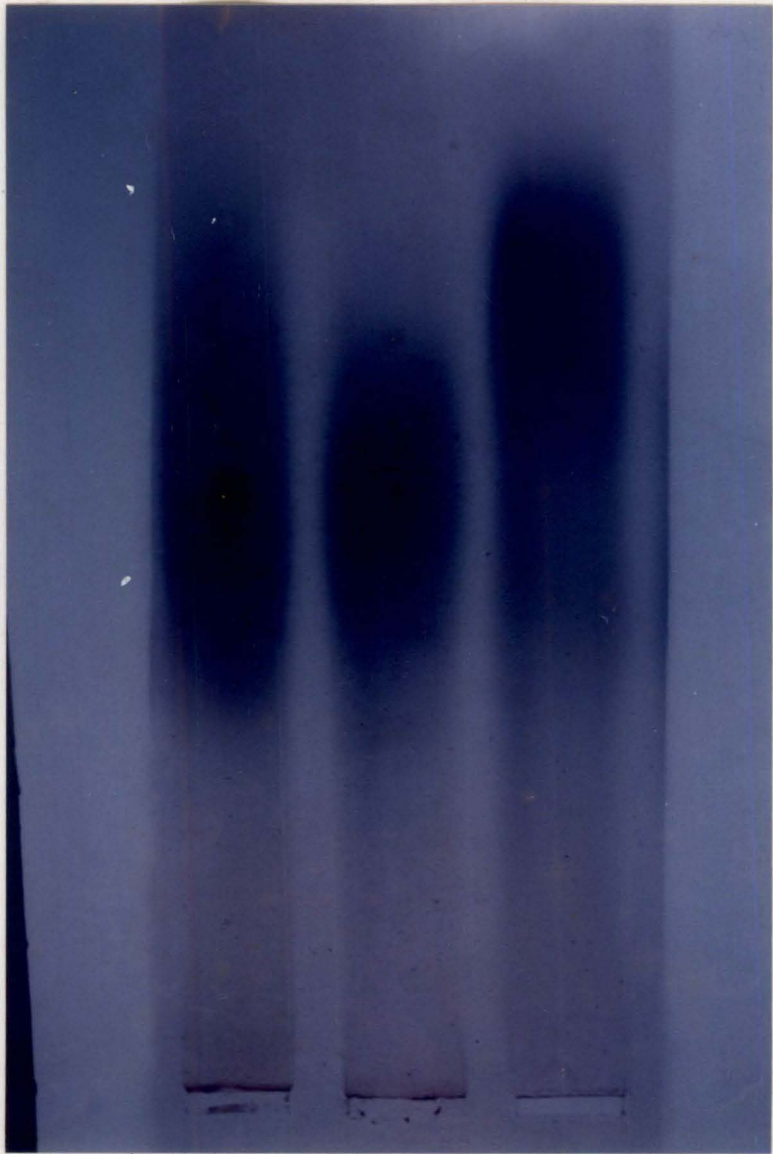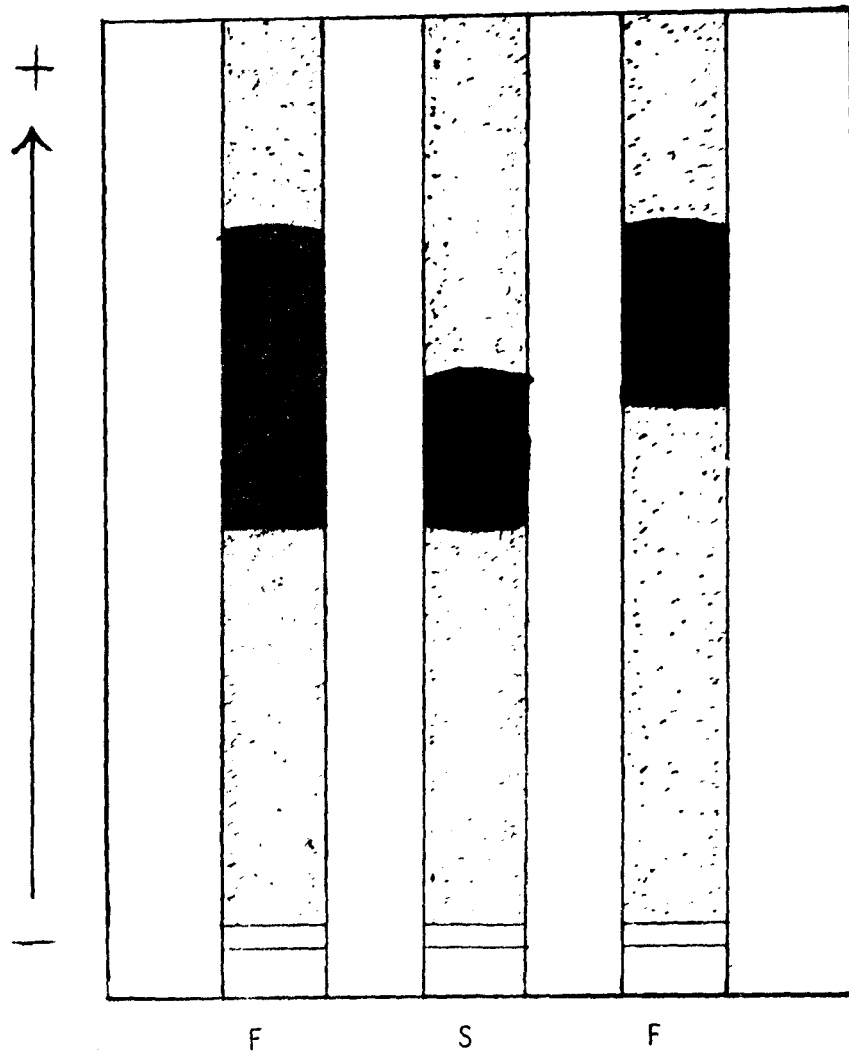
Fig.1(A). Phenotypes of Serum Alkaline Phosphatase

Fig. 1(B. Diagrammatic representation of Serum Alkaline Phosphatase Phenotypes

4.1.1 Phenotypic, genotypic and gene frequencies of SAP isozymes

The frequency of Fast and Slow band phenotypes were 0.66 and 0.34 in IWP strain and 0.15 and 0.85 in IWN strain of S15 generation. In S16 generation, the Fast and Slow band frequencies were 0.58 and 0.42 in IWP strain and 0.24 and 0.76 in IWN strain (Table 1 and Figure 2).

The genotypic frequencies were calculated from the phenotypic frequencies. The frequency of FF, FS and SS genotypes were found to be 0.17, 0.49 and 0.34, respectively in IWP strain and 0.01, 0.14 and 0.85, respectively in IWN strain of S15 generation. In S16 generation the FF, FS and SS frequencies were 0.12, 0.46 and 0.42, respectively in IWP and 0.02, 0.22 and 0.76, respectively in IWN strain (Table 2 and Figure 3).

The gene frequencies were calculated from the phenotypic and genotypic frequencies. The gene frequency of F and S were observed to be 0.42 and 0.58 in IWP and 0.08 and 0.92 in IWN strain of S15 generation. In S16 generation the F and S gene frequencies were 0.35 and 0.65 in IWP strain and 0.13 and 0.87 in IWN strain (Table 2 and Figure 4).

4.1.2 Inheritance pattern of SAP isozymes

Different matings were made between fast and slow moving types to determine the inheritance pattern. In IWP strain three males with fast band

Table 1. Phenotypic frequencies of Serum Alkaline Phosphatase in IWP and IWN strains of White Leghorn

Generation	Strain	No. of birds tested	Phenotypic frequency Fast(FF & FS)	Slow(SS)
S15	IWP	168	0.66(111)	0.34(05⁷)
	IWN	129	0.15(019)	0.85(110)
S16	IWP	100	0.58(058)	0.42(042)
	IWN	100	0.24(024)	0.76(076)

NB: Number of birds is given in the parenthesis

Table 2. Gene and genotypic frequencies of Serum Alkaline Phosphatase in IWP and IWN strains of White Leghorn

Generation	Strain	Gene frequency		Genotypic frequency		
		F	S	FF	FS	SS
S15	IWP	0.42	0.58	0.17	0.49	0.34
	IWN	0.08	0.92	0.01	0.14	0.85
S16	IWP	0.35	0.65	0.12	0.46	0.42
	IWN	0.13	0.87	0.0⁻	0.22	0.76

Fig.2 PHENOTYPIC FREQUENCY OF SERUM ALKALINE PHOSPHATASE IN IWP AND IWN STRAINS

S15 GENERATION

IWP

IWN

S16 GENERATION

IWP

IWN

Fig.3 GENOTYPIC FREQUENCY OF SERUM ALKALINE PHOSPHATASE IN IWP AND IWN STRAINS

S15 GENERATION

FS
0.49

FF
0.17

SS
0.34

IWP

FS
0.14

FF
0.01

SS
0.85

IWN

S16 GENERATION

FS
0.46

FF
0.12

SS
0.42

IWP

FS
0.22

FF
0.02

SS
0.76

IWN

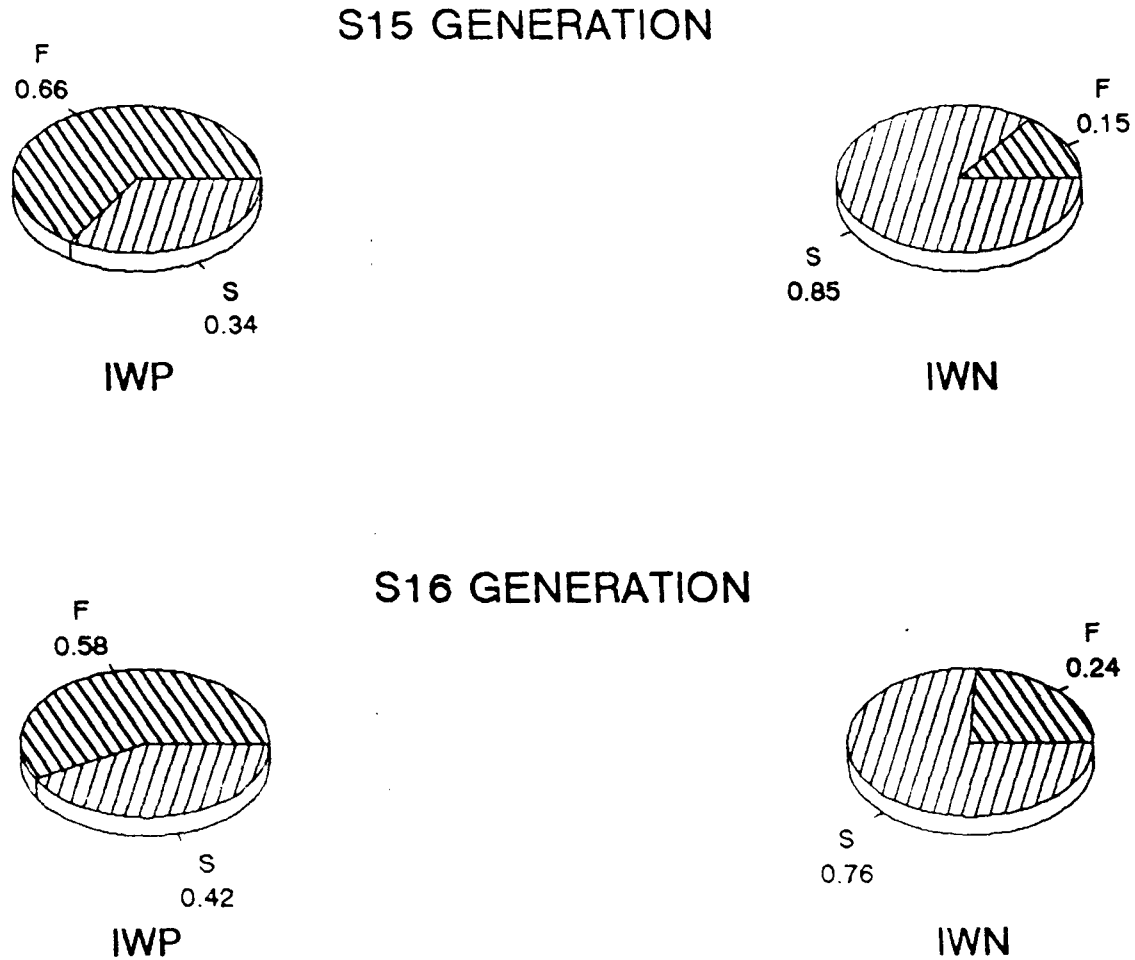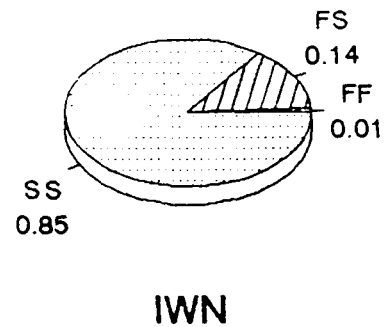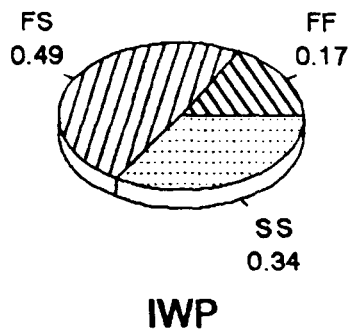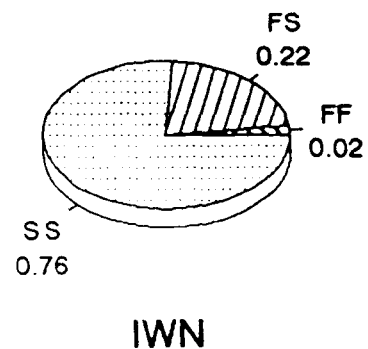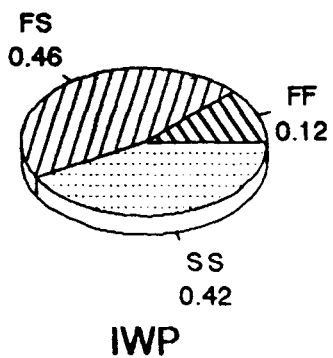# Fig.4　GENE FREQUENCY OF SERUM ALKALINE PHOSPHATASE IN IWP AND IWN　STRAINS

S15 GENERATION

IWP

IWN

S16 GENERATION

IWP
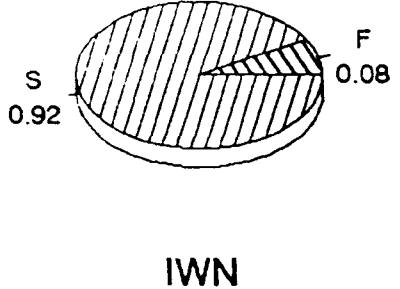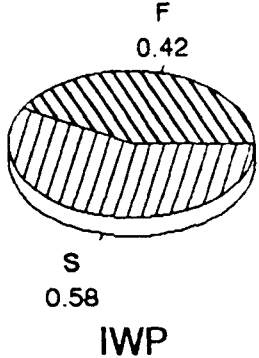
IWN
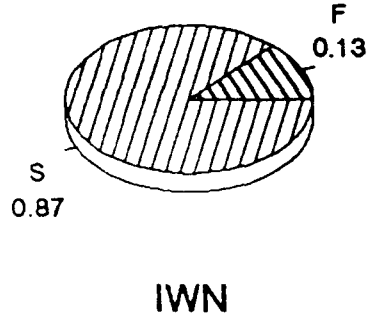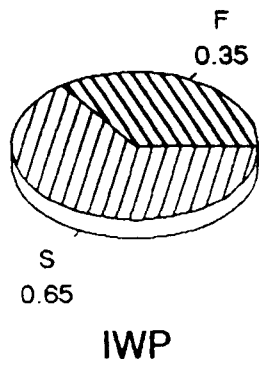
(Akpakp) and eight males with slow band (akpakp) were mated in various combinations to 10 females with fast band (AkpAkp and Akpakp) and eight females with slow band (akpakp) as mentioned in the Table 3. The matings made between akpakp x AkpAkp resulted in 100 percent fast band birds (48 fast progenies), akpakp x Akpakp resulted in 1:1 F and S offspring (28 fast and 24 slow progenies), Akpakp x akpakp also resulted in 1:1 F and S offspring (20 fast and 22 slow progenies), akpakp x akpakp resulted in 100 percent slow band birds (40 slow progenies).

In IWN strain, two males with fast band (AkpAkp and Akpakp) and two males with slow band (akpakp) were mated in various combinations to four females with fast band (Akpakp) and five females with slow band (akpakp) as mentioned in Table 4. The crosses made between AkpAkp x akpakp resulted in 100 percent fast band birds (30 fast progeny), Akpakp x akpakp resulted in 1:1 F and S birds (20 fast and 24 slow progeny), akpakp x Akpakp also resulted in 1:1 F and S birds (27 fast and 23 slow progenies).

4.1.3 SAP isozyme types and SAP level

The amount of alkaline phosphatase activity was 97.5 ± 1.72 and 93.03 ± 1.3 KA units per 100ml serum in F and S birds, respectively of IWP strain. The same was 88.96 ± 10.69 and 68.24 ± 5.99 KA units per 100ml serum in F and S birds, respectively of IWN strain (Table 5 and Figure 5). The alkaline

Table 3. Results of various types of matings among different Serum Alkaline Phosphatase type birds in IWP strain

Phenotype		Genotype(inferred)		No. of Progeny		X^2
Sire	Dam	Sire	Dam	Fast	Slow	
Slow	Fast	akpakp(3)	AkpAkp(5)	48	0	0
Slow	Fast	akpakp(3)	Akpakp(5)	28	24	0.308
Slow	Slow	akpakp(2)	akpakp(4)	0	40	0
Fast	Slow	Akpakp(3)	akpakp(4)	20	22	0.095

NB:Number of sires and dams are given in the parenthesis.

Table 4. Results of various types of matings among different Serum Alkaline Phosphatase type birds in IWN strain

Phenotype		Genotype(inferred)		No. of Progeny		X^2
Sire	Dam	Sire	Dam	Fast	Slow	
Slow	Fast	akpakp(2)	Akpakp(4)	27	23	0.320
Fast	Slow	AkpAkp(1)	akpakp(2)	30	0	0
Fast	Slow	Akpakp(1)	akpakp(3)	20	24	0.364

NB: Number of sires and dams are given in the parenthesis.

Table 5. Mean SAP levels of different SAP type birds in IWP and IWN strains

Strain	SAP level (KA units/100ml serum)			t value
	Fast type Mean ± SE	Slow type Mean ± SE	Over all Mean ± SE	
IWP	97.54 ± 1.72 (19)	93.03 ± 1.30 (15)	95.55 ± 1.17[1] (34)	2.00
IWN	88.96 ± 10.69 (10)	68.24 ± 5.99 (20)	75.14 ± 5.55[2] (30)	1.83

NB: Number of observation is given in the parenthesis
Overall means of showing different numerical superscripts differ significantly (P<0.05)

Fig.5   MEAN SAP LEVEL IN DIFFERENT SAP TYPE BIRDS
OF IWP AND IWN STRAINS

S15 GENERATION
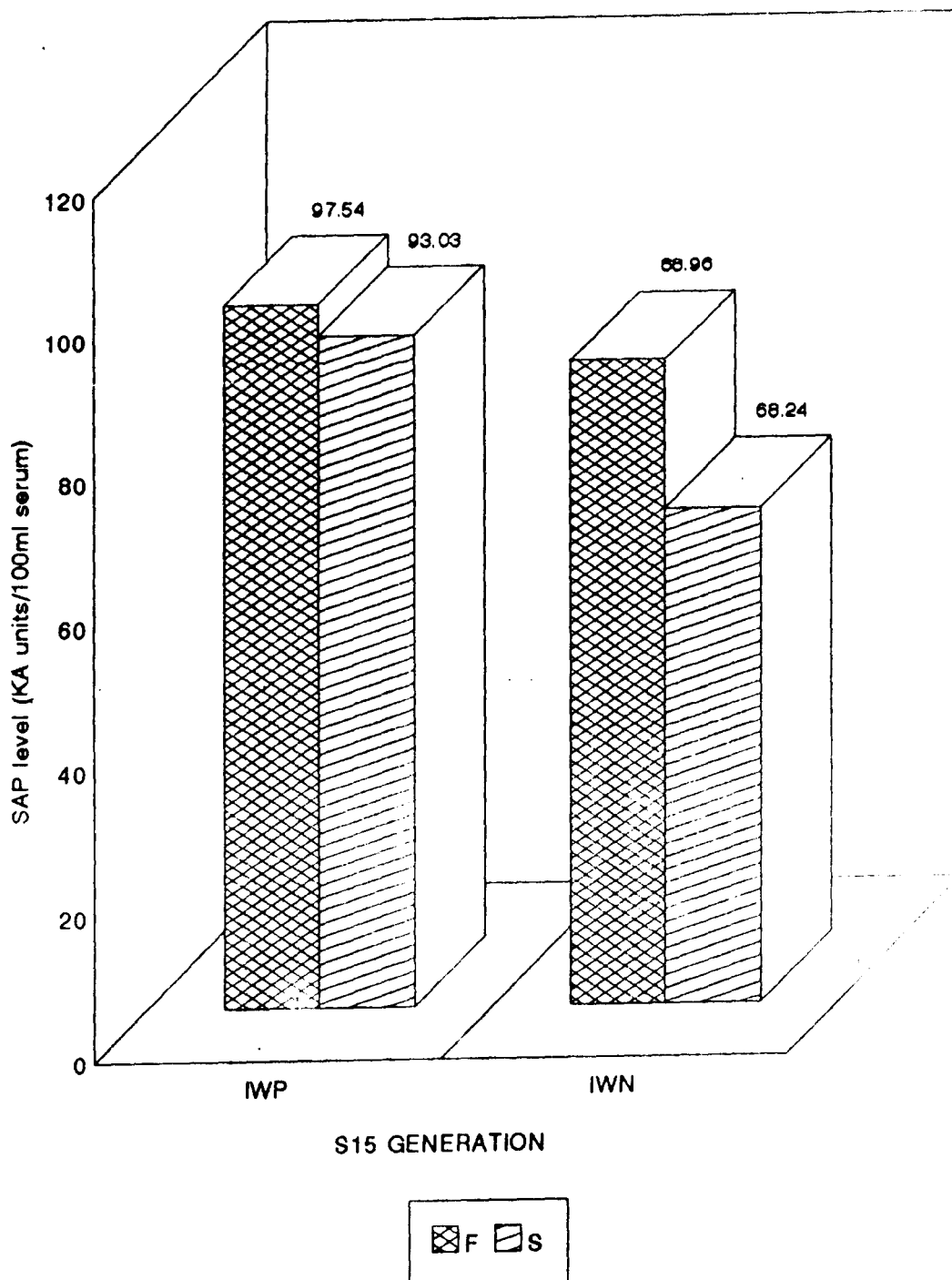
phosphatase activity was numerically higher in F birds than S birds in both IWP and IWN strains, whereas SAP level was significantly (P<0.05) higher in IWP birds (95.55 ± 1.17) than IWN birds (75.14 ± 5.55).

4.1.4 SAP isozyme types and production traits

The age at first egg, body weight at 20th and 40th weeks, egg number upto 40 and 60 weeks and egg weight at 32nd and 40th weeks of the two SAP type birds in IWP and IWN strains are presented in Table 6 and 7, respectively.

4.1.4.1 Age at sexual maturity

The mean age at first egg of F and S birds were 157.7 ± 1.21 and 156.8 ± 1.68 days in IWP strain and 167.8 ± 2.96 and 162.9 ± 1.50 days in IWN strain of S15 generation. The same was 145.7 ± 1.14 and 146.5 ± 1.45 days in IWP strain and 150.8 ± 2.45 and 150.2 ± 1.01 days in IWN strain of S16 generation. There was no significant difference for the mean age at first egg between F and S birds in IWP and IWN strains of both S15 and S16 generation. Mean age at sexual maturity of IWP (157.0 ± 0.97 and 146.1 ± 0.89 days) was significantly (P<0.05) less than IWN (163.7 ± 1.36 and 150.4 ± 0.96 days) in the S15 and S16 generations, respectively.

Table 6. Influence of SAP types on production traits in IWP and IWN strains of S15 generation

Production traits	IWP			IWN		
	Fast moving Mean±SE	Slow moving Mean±SE	Over all Mean±SE	Fast moving Mean±SE	Slow moving Mean±SE	Over all Mean±SE
	(111)	(57)	(168)	(19)	(110)	(129)
Age at 1st egg (days)	157.0±1.21	156.8±1.68	157.0±0.97^2	167.8±2.96	162.9±1.50	163.7±1.36^1
Body weight at 20th week(g)	1371.80±13.33	1375.79±18.43	1373.16±10.77^1	1251.58±31.30	1293.64±13.98	1287.44±2.80^2
Body weight at 40th week(g)	1651.62±17.72	1610.00±27.35	1637.50±14.97	1620.53±48.28	1619.09±18.76	1619.30±17.43
Egg number upto 40 weeks	100.9±1.23	99.1±1.81	100.3±1.02^1	86.6±2.96	91.4±1.48	90.7±1.34^2
Egg number upto 60 weeks	209.2±1.64	206.3±2.13	208.3±1.30^1	196.5±3.33	196.7±2.11	196.7±1.86^2
Egg weight at 32nd week(g)	53.89±0.17	53.82±0.23	53.87±0.14^1	53.05±0.41	53.07±0.22	53.07±0.20^2
Egg weight at 40th week(g)	54.08±0.24	54.35±0.37	53.17±0.20	53.23±0.64	53.11±0.57	53.12±0.50

NB: Number of observations are given in the parenthesis.
Over all mean in row with different numerical superscripts between the strains differ significantly(P<0.05).

Table 7. Influence of SAP types on egg production traits in IWP and IWN strains of S16 generation

Production traits	IWP			IWN		
	Fast moving Mean±SE	Slow moving Mean±SE	Over all Mean±SE	Fast moving Mean±SE	Slow moving Mean±SE	Over all Mean±SE
	(58)	(42)	(100)	(24)	(76)	(100)
Age at sexual maturity(days)	145.7±1.14	146.5±1.45	146.1±0.89[2]	150.8±2.45	150.2±1.01	150.4±0.96[1]
Body weight at 20th week(g)	1387.93±12.60	1387.38 27.58	1387.70±13.61	1322.08±29.40	1371.18±17.46	1359.40±15.10
Body weight at 40th week(g)	1727.59±22.50	1753.33±31.82	1738.40±18.62[1]	1666.67±36.46	1667.76±27.72	1667.50±22.73[2]
Egg number upto 40 weeks	107.2±1.47	102.5±2.45	105.3±1.35	99.5±2.39[b]	105.9±1.17[a]	104.4±1.09
Egg number upto 60 weeks	202.7±2.86	190.8±5.65	197.7±2.94[2]	208.2±4.63	207.5±2.32	207.7±2.07[1]
Egg weight at 32nd week(g)	49.04±0.43	47.80±0.53	48.52±0.34[1]	47.79±0.61	47.56±0.31	47.61±0.28[2]
Egg weight at 40th week(g)	52.26±0.40	51.12±0.63	51.78±0.35	52.96±0.51	52.30±0.37	52.46±0.31

NB: Number of observations are given in the parenthesis.
   Means in rows with different alphabetic superscripts within the strains differ significantly(P<0.05).
   Overall means in rows with different numerical superscripts between the strains differ significantly(P<0.05).

4.1.4.2 Body weight

The mean body weight at 20 weeks for F and S birds were 1371.80 ± 13.33 and 1375.79 ± 18.43g in IWP strain and 1251.58 ± 31.30 and 1293.64 ± 13.98g in IWN strain of S15 generation. The same was 1387.93 ± 12.60 and 1387.38 ± 27.58g in IWP and 1322.08 ± 29.40 and 1371.18 ± 17.46g in IWN strain of S16 generation.

The mean body weight at 40 weeks for F and S birds were 1651.62 ± 17.72 and 1610.00 ± 27.35g in IWP strain and 1620.53 ± 48.28 and 1619.09 ± 18.76g in IWN strain of S15 generation. In S16 generation the results were 1727.59 ± 22.50 and 1753.33 ± 31.82g in IWP strain and 1666.67 ± 36.46 and 1667.76 ± 27.72g in IWN strain. There was no significant difference for body weight at 20 and 40 weeks of age between F and S birds in either IWP or IWN strains of White Leghorn.

A significantly (P<0.05) higher mean body weight at 20 weeks was noticed in IWP (1373.16 ± 10.77g) than IWN (1287.44 ± 2.80g) strain in S15 generation and a significantly (P<0.05) higher body weight at 40 weeks was noticed in IWP (1738.40 ± 18.62g) than IWN (1667.5 ± 22.73g) strain in S16 generation.

4.1.4.3 Egg production

The average egg production upto 40 weeks of age for F and S birds were 100.9 ± 1.23 and 99.1 ± 1.81 in IWP strain and 86.6 ± 2.96 and 91.4 ± 1.48 in IWN strain of S15 generation. In S16 generation the results were 107.2 ± 1.47 and 102.5 ± 2.45 in IWP and 99.5 ± 2.39 and 105.9 ± 1.17 in IWN strain.

The average egg production upto 60 weeks of age for F and S birds were 209.2 ± 1.64 and 206.3 ± 2.13 in IWP strain and 196.5 ± 3.33 and 196.7 ± 2.11 in IWN strain of S15 generation, the same were 202.7 ± 2.86 and 190.8 ± 5.65 in IWP strain and 208.2 ± 4.63 and 207.5 ± 2.32 in IWN strain of S16 generation. It was observed that no significant difference existed between the SAP types for egg production except in IWN strain of S16 generation, where S birds were having significantly (P<0.05) higher egg production upto 40 weeks of age than F birds.

In S15 generation the average egg production upto 40 weeks and 60 weeks were high in IWP birds (100.3 ± 1.02 and 208.3 ± 1.30, respectively) than IWN birds (90.7 ± 1.34 and 196.7 ± 1.86, respectively). In S16 generation the average egg production upto 40 weeks was non-significantly higher in IWP birds (105.3 ± 1.35) than IWN birds (104.4 ± 1.09), whereas the average egg production upto 60 weeks was significantly higher in IWN birds (207.7 ± 2.07) than IWP birds (197.7 ± 2.94).

4.1.4.4 Egg weight

The mean egg weight at 32nd week of age for F and S birds were 53.89 ± 0.17 and 53.82 ± 0.23g in IWP strain and 53.05 ± 0.41 and 53.07 ± 0.22g in IWN strain of S15 generation, the same were 49.04 ± 0.43 and 47.80 ± 0.53g in IWP strain and 47.79 ± 0.61 and 47.56 ± 0.31g in IWN strain of S16 generation.

The mean egg weight at 40th week of age for F and S birds were 54.08 ± 0.24 and 54.35 ± 0.37g in IWP strain and 53.23 ± 0.64 and 53.11 ± 0.57g in IWN strain of S15 generation. In S16 generation the same were 52.26 ± 0.40 and 51.12 ± 0.63g in IWP strain and 52.96 ± 0.51 and 52.30 ± 0.37g in IWN strain. There was no significant difference for egg weight at 32nd and 40th week of age between F and S birds in either IWP or IWN strains of White Leghorn.

There was significantly ($P<0.05$) higher 32nd week egg weight in IWP strain (53.87 ± 0.14 and 48.52 ± 0.34g) than IWN strain (53.07 ± 0.20 and 47.61 ± 0.28g) in S15 and S16 generations, respectively. Whereas egg weight at 40th week of IWP strain (53.17 ± 0.20 and 51.78 ± 0.35g) was not significantly different from IWN strain (53.12 ± 0.05 and 52.46 ± 0.31g).

4.1.4.5 Egg production at different intervals

The average 21-40 weeks egg production of F and S birds were 100.9 ± 1.23 and 99.1 ± 1.81 in IWP strain and 86.6 ± 2.96 and 91.4 ± 1.48 in IWN strain of S15 generation. The same of S16 generation were 107.2 ± 1.47 and 102.5 ± 2.45 in IWP strain and 99.5 ± 2.39 and 105.9 ± 1.17 in IWN strain (Table 8 and Figure 6 and 7).

The average 41-60 weeks egg production of F and S birds were 108.3 ± 1.10 and 107.6 ± 1.53 in IWP strain and 110.0 ± 1.91 and 105.2 ± 1.22 in IWN strain of S15 generation. The same of S16 generation were 97.2 ± 1.70 and 88.3 ± 3.39 in IWP strain and 108.8 ± 3.07 and 101.6 ± 1.61 in IWN strain (Table 8 and Figure 6 and 7).

It was observed that no significant difference existed between SAP types for mean 21-40 weeks egg production except in IWN strain of S16 generation, where the slow type birds were having significantly (P<0.05) higher 21-40 weeks production than fast type birds. However, the mean 41-60 weeks egg production of fast type birds were significantly (P<0.05) higher than slow type birds in IWP an IWN strains of both S15 and S16 generations (except in IWP strain of S15 generation where the difference was non-significant).

Table 8. Influence of SAP types on egg production at different intervals in IWP and IWN strains.

Genera-tion	Interval	IWP			IWN		
		Fast moving Mean±SE	Slow moving Mean±SE	Over all Mean±SE	Fast Moving Mean±SE	Slow moving Mean±SE	Over all Mean±SE
S15	2?-40wks	100.9±1.23 (111)	99.1±1.81 (57)	100.3±1.02[1] (168)	86.6±2.96 (19)	91.4±1.48 (110)	90.7±1.34[2] (129)
	41-60wks	108.3±1.10 (111)	107.6±1.53 (57)	108.1±0.89 (168)	110.0±1.91[a] (19)	105.2±1.22[b] (110)	105.9±1.09 (129)
S16	21-40wks	107.2±1.47 (58)	102.5±2.45 (42)	105.3±1.35 (100)	99.5±2.39[b] (24)	105.9±1.17[a] (76)	104.4±1.09 (100)
	41-60wks	97.2±1.70[a] (58)	88.3±3.39[b] (42)	93.5±1.82[2] (100)	108.8±3.07[a] (24)	101.6±1.61[b] (76)	103.3±1.45[1] (100)

NB: Number of observations are given in the parenthesis.
Means in rows with different alphabetic superscripts within the strains differ significantly($P < 0.05$).
Overall means in rows with different numerical superscripts between the strains differ significantly($P < 0.05$).

Fig.6   SAP TYPES AND EGG PRODUCTION AT DIFFERENT INTERVALS IN IWP & IWN STRAINS
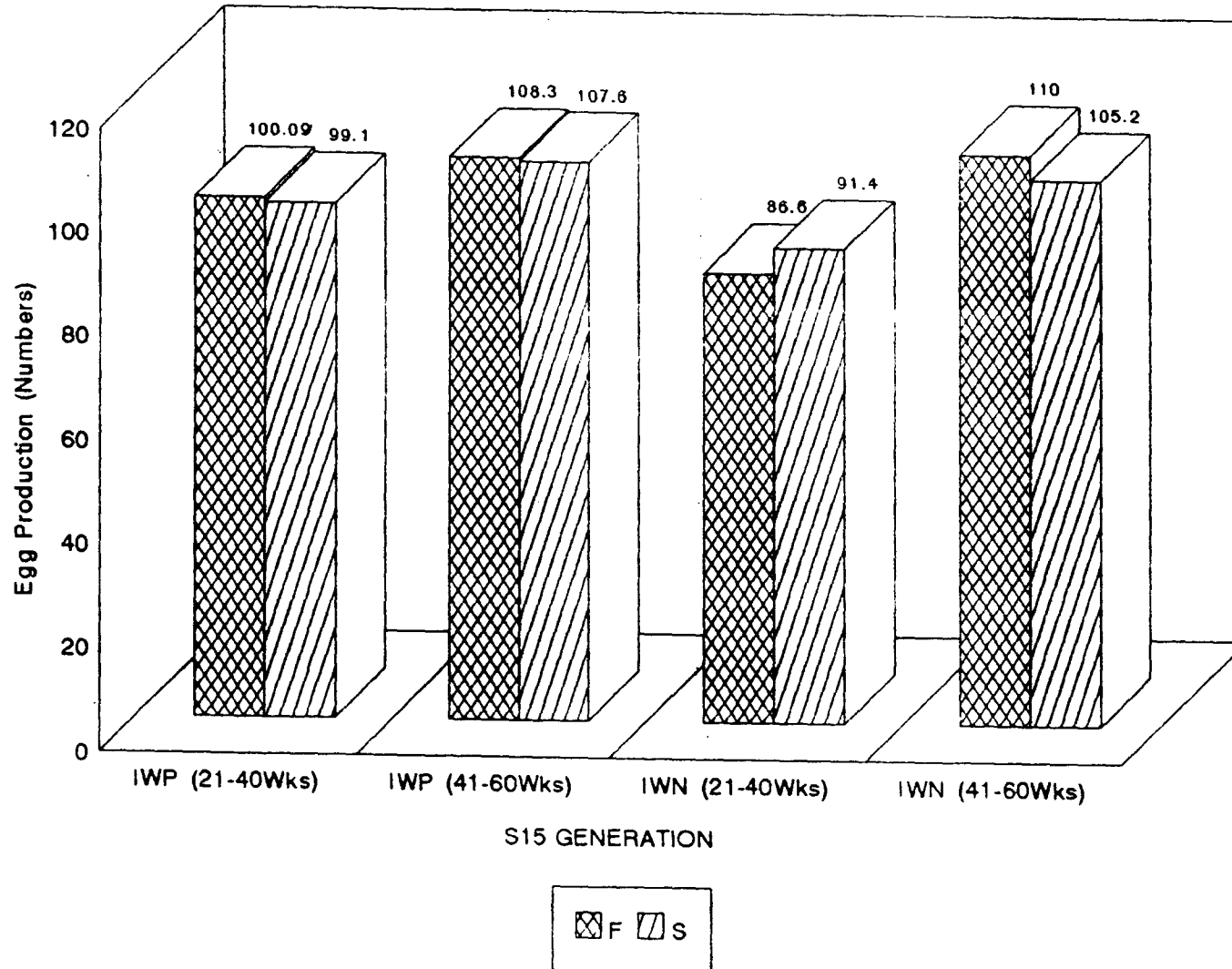
Fig.7 SAP TYPES AND EGG PRODUCTION AT DIFFERENT INTERVALS IN IWP & IWN STAINS
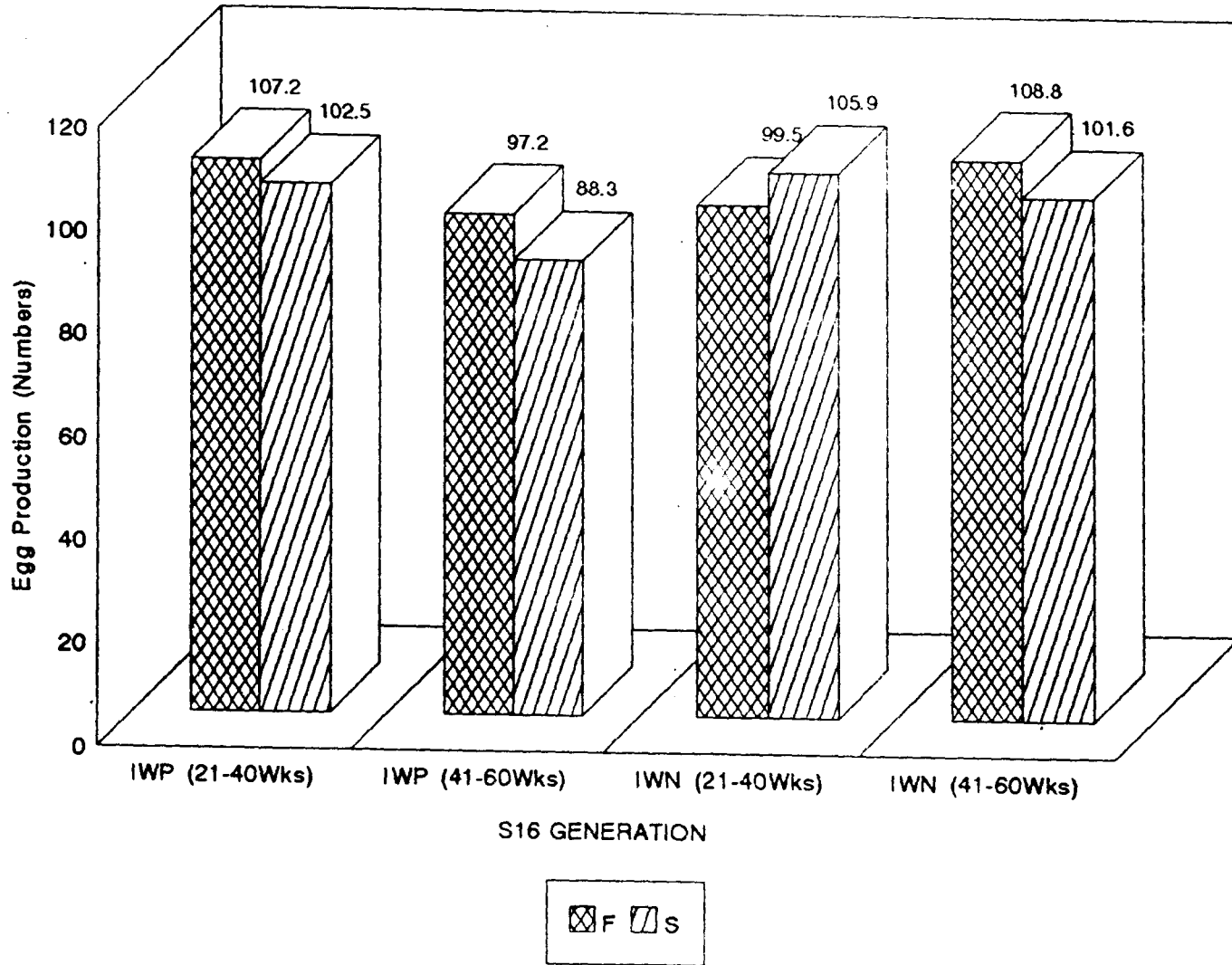
4.1.5 SAP isozyme types and egg quality traits

The relationship between SAP types and egg quality traits were analysed and presented in Table 9. The differences between the eggs of F and S type birds belonging to IWP and IWN strains were not significant for mean shape index (75.16 ± 0.77, 75.53 ± 0.66 and 74.18 ± 1.31 and 73.89 ± 0.79, respectively), albumen index (0.078 ± 0.003, 0.083 ± 0.004 and 0.108 ± 0.005 and 0.106 ± 0.004, respectively), yolk index (0.419 ± 0.003, 0.428 ± 0.006 and 0.419 ± 0.005 and 0.417 ± 0.004, respectively) and haugh unit score (79.94 ± 1.06, 81.64 ± 1.94 and 89.37 ± 1.44 and 88.38 ± 1.28, respectively). However the eggs of F type birds were having significantly (P<0.05) higher shell thickness (0.317 ± 0.004 and 0.319 ± 0.005) than the S birds (0.299 ± 0.005 and 0.303 ± 0.004) in IWP and IWN strains respectively.

Albumen index and haugh unit score were significantly (P<0.05) higher in eggs of IWN strain than IWP strain. Shape index, yolk index and shell thickness were not showing any significant difference between IWP and IWN strains.

4.1.6 SAP isozyme types and reproduction traits

The mean performance of F x F, F x S, S x F and S x S matings were 88.10 ± 1.27, 87.67 ± 1.79, 79.40 ± 3.53 and 85.96 ± 1.12 percent for fertility,

Table 9. Influence of SAP types on egg quality traits in IWP and IWN strains of S15 generation

Egg quality traits	IWP			IWN		
	Fast moving Mean±SE	Slow moving Mean±SE	Over all Mean±SE	Fast moving Mean±SE	Slow moving Mean±SE	Over all Mean±SE
	(21)	(18)	(39)	(14)	(21)	(35)
Shape index	75.16±0.77	75.53±0.66	75.33±0.51	74.18±1.31	73.89±0.79	74.00±0.69
Albumen index	0.078±0.003	0.083±0.004	$0.080±0.003^2$	0.108±0.005	0.106±0.004	$0.107±0.003^1$
Yolk index	0.419±0.003	0.428±0.006	0.423±0.003	0.419±0.005	0.417±0.004	0.418±0.003
Shell thickness(mm)	$0.317±0.004^a$	$0.299±0.005^b$	0.309±0.004	$0.319±0.005^a$	$0.303±0.004^b$	0.310±0.003
Haugh unit score	79.94±1.06	81.64±1.94	$80.72±1.06^2$	89.37±1.44	88.38±1.28	$88.77±0.95^1$

NB: Number of observations are given in the parenthesis.
Means in rows with different alphabetic superscripts within the strains differ significantly($P<0.05$).
Overall means in rows with different numerical superscripts between the strains differ significantly($P<0.05$).

50.72 ± 3.44, 46.46 ± 4.28, 44.83 ± 3.51 and 36.47 ± 5.71 percent for hatchability on total egg set, 57.12 ± 3.51, 53.28 ± 4.64, 56.53 ± 3.42 and 42.24 ± 6.35 percent for hatchability on fertile egg set, 22.39 ± 3.01, 24.51 ± 4.16, 23.88 ± 2.67 and 23.09 ± 5.61 percent for early embryonic mortality and 20.49 ± 2.41, 22.21 ± 3.15, 19.59 ± 2.76 and 34.67 ± 5.05 percent for dead in shell respectively, in IWP strain (Table 10 and Figure 8).

The mean performance of F x F, F x S, S x F and S x S matings were 90.61 ± 1.68, 87.85 ± 1.63, 72.53 ± 7.29 and 79.68 ± 2.27 percent for fertility, 54.39 ± 2.35, 48.68 ± 3.23, 36.15 ± 2.84 and 40.83 ± 3.09 percent for hatchability on total egg set, 59.99 ± 2.16, 55.00 ± 3.07, 51.35 ± 3.29 and 50.07 ± 3.47 percent for hatchability on fertile egg set, 24.02 ± 0.95, 28.05 ± 1.96, 34.54 ± 5.58 and 34.02 ± 3.09 percent for early embryonic mortality and 15.99 ± 1.11, 16.95 ± 3.67, 14.11 ± 6.39 and 15.91 ± 1.63 percent for dead in shell respectively in IWN strain (Table 11 and Figure 9).

In both IWP and IWN strains, fertility of F x F and F x S matings were significantly ($P<0.05$) higher than S x F mating. Hatchability on total egg set was significantly ($P<0.05$) higher in F x F mating than S x F mating in IWN strain. Dead in shell percentage was significantly ($P<0.05$) higher in eggs from S x S mating than F x F, F x S, and S x F matings in IWP strain. All other correlations were not significant.

Table 10. Different SAP type matings and reproduction traits in IWP strain of S15 generation

Reproduction traits(%)	F x F Mean±SE	F x S Mean±SE	S x F Mean±SE	S x S Mean±SE
	(43)	(24)	(12)	(10)
Fertility	88.10±1.27[a]	87.67±1.79[a]	79.40±3.53[b]	85.96±1.12
Hatchability (total egg set)	50.72±3.44	46.46±4.28	44.83±3.51	36.47±5.71
Hatchability (fertile egg set)	57.12±3.51	53.28±4.64	56.53±3.42	42.24±6.35
Early embryonic mortality	22.39±3.01	24.51±4.16	23.88±2.67	23.09±5.61
Dead in shell	20.49±2.41[b]	22.21±3.15[b]	19.59±2.76[b]	34.67±5.05[a]

NB: Number of observation is given in the parenthesis
     Means in row with different alphabetic superscripts differ significantly(P<0.05).

Fig.8 DIFFERENT SAP TYPE MATINGS AND REPRODUCTION TRAITS IN IWP STRAIN

S15 GENERATION

Legend: ⊠ F x F   ⧄ F x S   ▢ S X F   ⧅ S x S
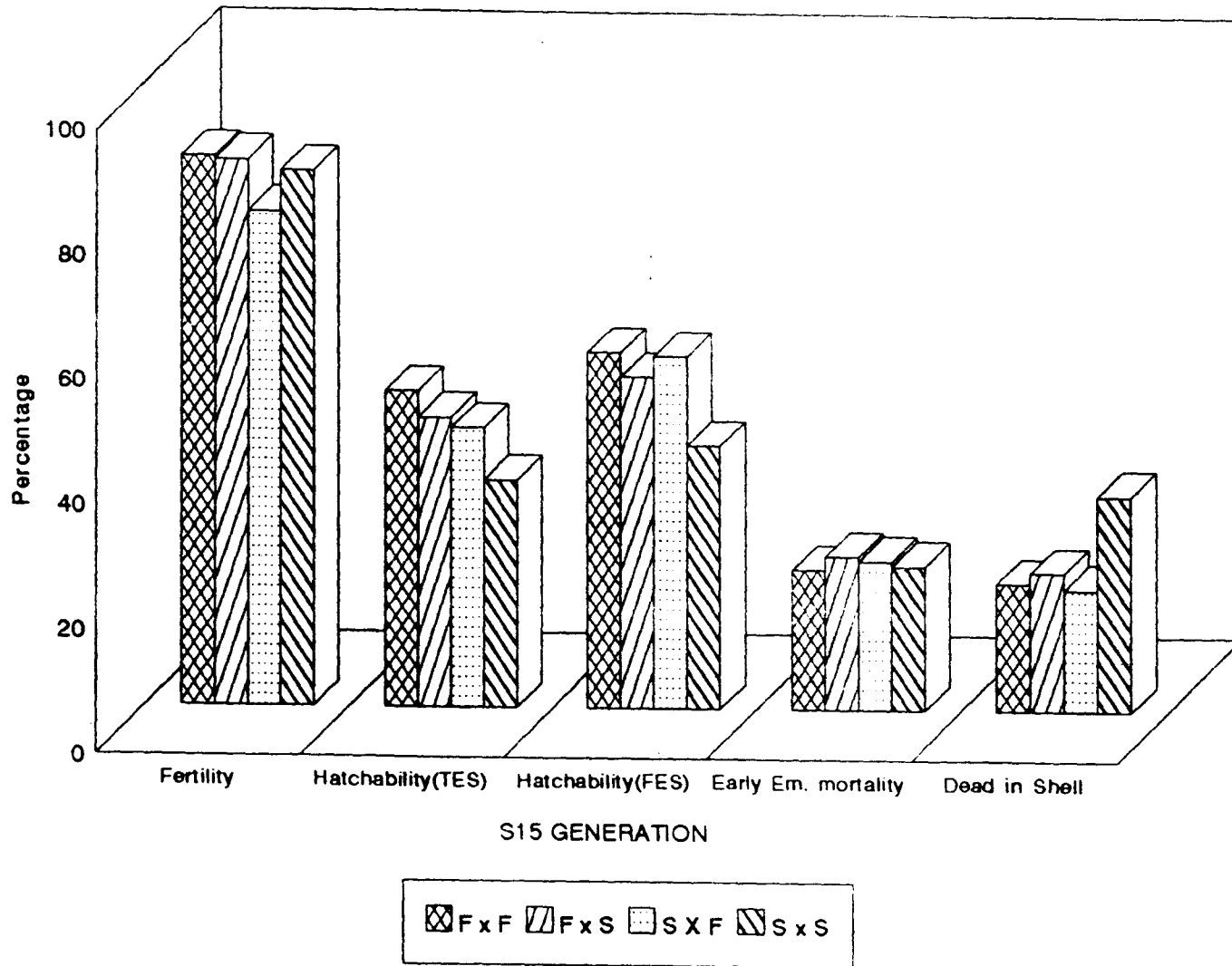
Table 11. Different SAP type matings and reproduction traits in IWN strain of S15 generation

Reproduction traits(%)	F x F Mean±SE	F x S Mean±SE	S x F Mean±SE	S x S Mean±SE
	(6)	(12)	(8)	(38)
Fertility	90.61±1.68[a]	87.85±1.63[a]	72.53±7.29[b]	79.68±2.27
Hatchability (total egg set)	54.39±2.35[a]	48.68±3.23	36.15±2.84[b]	40.83±3.09
Hatchability (fertile egg set)	59.99±2.16	55.00±3.07	51.35±3.29	50.07±3.47
Early embryonic mortality	24.02±0.95	28.05±1.96	34.54±5.58	34.02±3.09
Dead in shell	15.99±1.11	16.95±3.67	14.11±6.39	15.91±1.63

NB: Number of observation is given in the parenthesis
      Means in row with different alphabetic superscripts differ significantly(P<0.05).

Fig.9 DIFFERENT SAP TYPE MATINGS AND REPRODUCTION TRAITS
IN IWN STAIN

S15 GENERATION

⬜FxF ⬜FxS ⬜SXF ⬜SxS

70
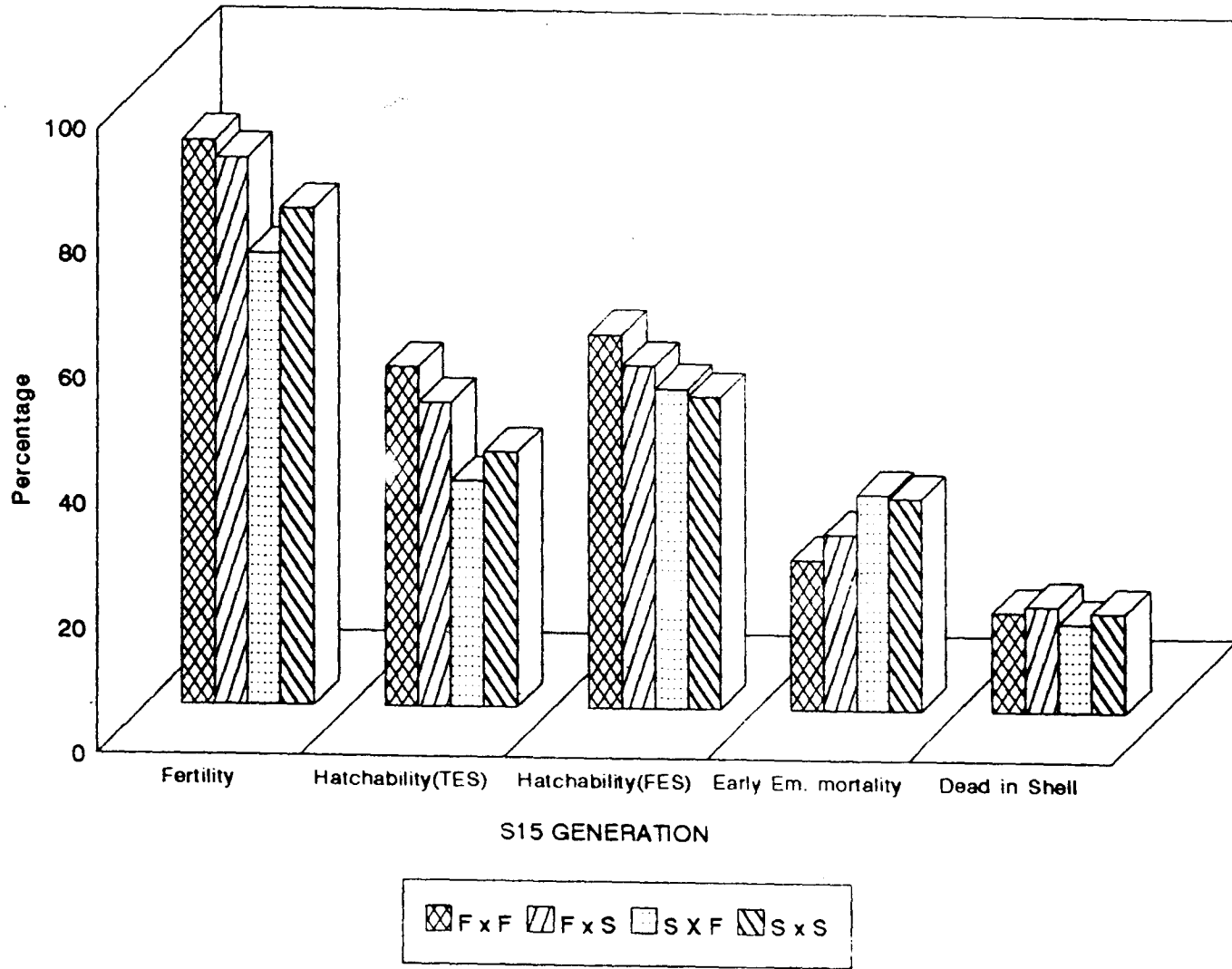
Table 12. Influence of strains on reproductive traits in S15 generation

Reproductive traits(%)	Strain		t value
	IWN Mean±SE	IWP Mean±SE	
	(89)	(64)	
Fertility	81.35 ± 1.76^2	86.57 ± 0.96^1	2.61
Hatchability(total egg set)	42.99 ± 2.06	47.18 ± 2.20	1.34
Hatchability(fertile egg set)	52.09 ± 2.20	54.47 ± 2.35	0.71
Early embryonic mortality	31.94 ± 2.03^1	23.24 ± 1.95^2	3.03
Dead in shell	15.85 ± 1.40^2	22.42 ± 1.64^1	3.05

NB: Number of observation is given in the parenthesis.
       Means in rows with different numerical superscripts differ significantly
       (P<0.05).

The mean fertility, early embryonic mortality and dead in shell percentage were having significant (P<0.05) difference between IWP and IWN strains of White Leghorn (Table 12).

4.2 Haemoglobin Polymorphism

Electrophoresis of haemoglobin revealed one slow moving major (M) and one fast moving minor (m_1) component in all birds (Figure 10A and 10B). Mutant minor band (m_2) which migrates faster than the normal minor band (m_1) was not found in any of the 200 haemoglobin samples of IWP and IWN.

Fig.10(A).   Zymogram of Haemoglobin
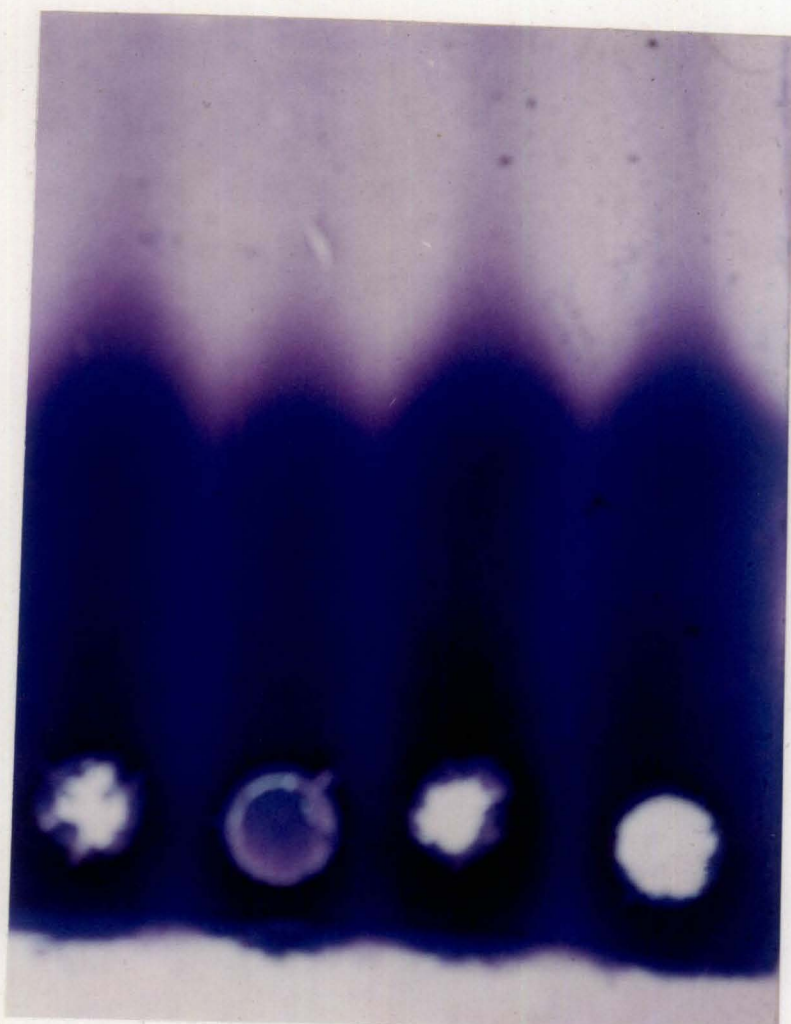
Fig. 10(B)   Diagramtic representation of Hemoglobin Zymogram
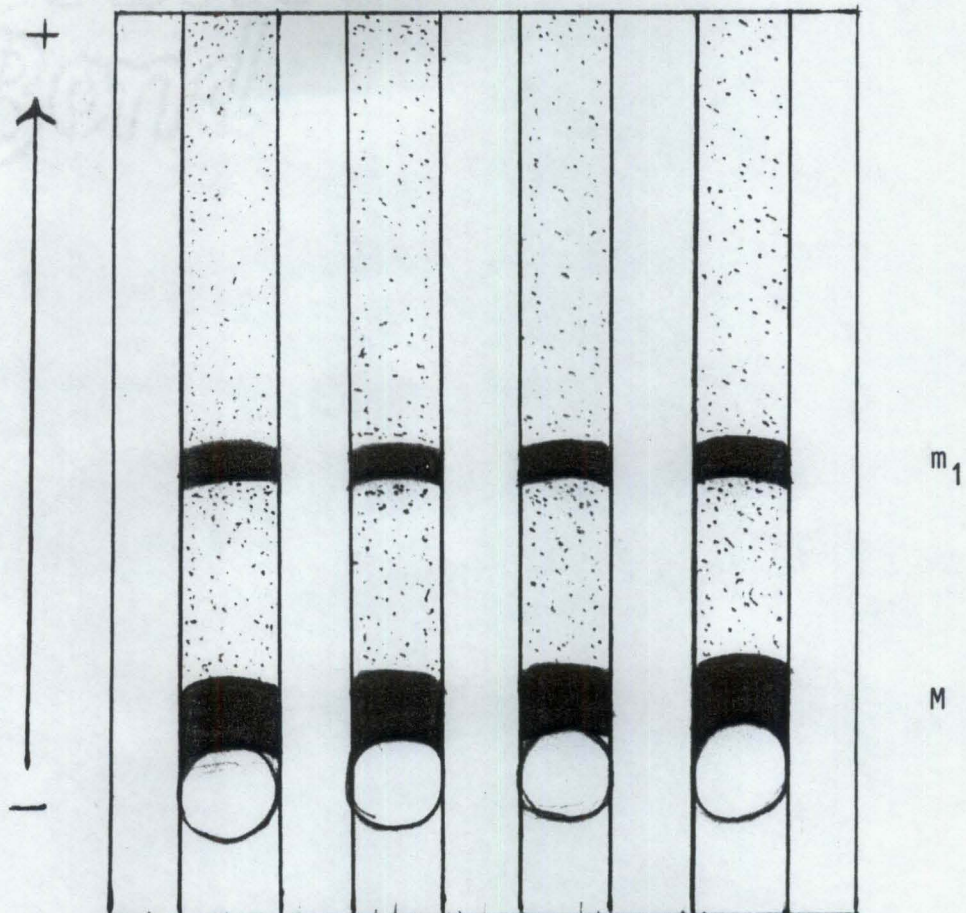
Discussion

DISCUSSION

The results of the present investigation have been discussed under following headings.

5.1. Serum alkaline phosphatase polymorphism

5.1.1 Phenotypic, genotypic and gene frequencies of   SAP isozymes

5.1.2 Inheritance pattern of SAP isozymes

5.1.3 SAP isozyme types and SAP level

5.1.4 SAP isozyme types and production traits

5.1.5 SAP isozyme types and egg quality traits

5.1.6 SAP isozyme types and reproduction traits

5.2 Haemoglobin polymorphism

5.1 Serum Alkaline Phosphatase Polymorphism

In the present study, the SAP resolved with two distinct bands viz. Fast (F) and Slow (S) on the basis of electrophoretic mobility. Polyacrylamide Gel Electrophoresis (PAGE) showed two different phenotypes, the fast and slow types. Parmar et al. (1991), Shukla et al. (1991) and Ahlawat et al. (1994) also reported that each sample had either a fast or a slow band when stained for alkaline phosphatase. On the contrary, Savage et al. (1971) observed five

different isozymes of alkaline phosphatase in four lines of chicken by using Polyacrylamide disc electrophoresis.

5.1.1 Phenotypic, genotypic and gene frequencies of SAP isozymes

The various phenotypes and genotypes of serum alkaline phosphatase were calculated and tabulated (Table 1 and 2). None of the birds were found to have both fast and slow bands. Wilcox (1966) and Chaudhary et al. (1971) also observed that F and S bands never occurred together in the serum of an individual chicken. But both were present when individual sera containing fast and slow bands were combined into a composite sample (Wilcox, 1966). Contrary to this Tamaki and Tanabe (1970) reported both fast migrating and slow migrating bands in the sera of a single bird. Phenotypic frequency of fast and slow band birds were 0.66 and 0.34 in IWP and 0.15 and 0.85 in IWN strain respectively, of S15 generation. The same of S16 generation were 0.58 and 0.42 in IWP and 0.24 and 0.76 in IWN strain, respectively.

The genotypic frequencies of 'FF', 'FS' and 'SS' in S15 generation were 0.17, 0.49 and 0.34 in IWP and 0.01, 0.14 and 0.85 in IWN strain, respectively of S15 generation. The same were 0.12, 0.46 and 0.42 in IWP and 0.02, 0.22 and 0.76 in IWN strain, respectively of S16 generation.

If 'F' is considered as a dominant allele of 'S', the gene frequency of 'S' is determined by (genotypic frequency S)$^{1/2}$. The frequencies of AKP^F and AKP^S would be 0.42 and 0.58 in IWP strain and 0.08 and 0.92 in IWN strain, respectively of S15 generation. The same would be 0.35 and 0.65 in IWP strain and 0.13 and 0.87 in IWN strain, respectively of S16 generation.

The observation of a higher gene frequency of F allele in the IWP strain in comparison to IWN strain was in line with the observations of Engh and Wilcox (1971). They reported a wide range of Fast band frequencies ranging from 0.29 to 0.96 in different commercial strains selected for egg production. Thus the great variation in fast band frequencies among stocks selected for egg production suggests that there is no direct association of alkaline phosphatase phenotype and egg production. The general conviction among geneticists is that traits which vary among populations ultimately have adaptive significance. The adaptive significance of alkaline phosphatase isozymes remains obscure. This might also be a reason for the variation in F gene frequency between IWP and IWN.

On the contrary, Shukla et al. (1991) reported that the gene frequency from data pooled over strains were 0.74 and 0.26 respectively, for SAP^F and SAP^S alleles. Banerjee et al. (1987) throughout the study found low F band frequency as compared to S bands due to the greater mortality percentage in F birds.

The wide variation in F gene frequency between IWP and IWN strains may be due to the low egg production upto 40th week in F birds than S birds in IWN strain and the same was equal in F and S birds of IWP strain. This is discussed in detail under the sub-heading Egg production (5.1.4.3).

5.1.2 Inheritance pattern of SAP isozymes

The inheritance pattern of different matings within IWP an IWN strains was studied and results are given in the Table 3 and 4 respectively. The matings made between akpakp x AkpAkp resulted in 100 percent F offspring, akpakp x Akpakp and Akpakp x akpakp resulted in 1:1 F and S progenies, akpakp x akpakp resulted in 100 percent S progeny. The results were best explained by the presence of an autosomal completely dominant and recessive allele, the symbols being Akp^{F} and Akp^{S}. Numbers observed fit this hypothesis well, as evidenced by Chi-square analysis. Similar observations were also made by Wilcox (1966), Tamaki and Tanabe (1970), Banerjee et al. (1974), Mathur (1978) and Banerjee et al. (1987). Their findings were also best explained by Mendalian inheritance for completely dominant gene.

5.1.3 SAP isozyme types and SAP level

The amount of alkaline phosphatase activity was measured for F and S birds of IWP and IWN strains and presented in the Table 5. Although there

was no significant difference between F and S birds due to high standard error, there was apparent difference between F and S birds. The overall SAP level between IWP and IWN strains was significantly different. This observation falls in line with those of Wilcox (1966) and Goswami et al. (1990). Mazumder and Mazumder (1991), identified significantly ($P<0.05$) higher SAP level in the fast type chicken than slow type even at 44 weeks of age in four strains of White Leghorn. Similar observation was made by Tamaki and Tanabe (1970) at four and eight weeks of age. But they also observed that the activity of the enzymes decreased and no significant difference was noticed between F and S birds as age advanced.

There may be significant differences in enzyme activity even between homozygous and heterozygous fast birds. A detailed study to confirm this may be worth while as it may lead to a novel approach for detecting heterozygosity at the level of a gene product having potential economic importance.

5.1.4 SAP isozyme types and Production traits

The relationship between SAP isozyme types and production traits were analysed and the results are presented in Table 6, 7 and 8 and also discussed below:

5.1.4.1 Age at Sexual Maturity

There was no significant difference between F and S birds for age at sexual maturity. This observation is in conformity with studies of Garcia (1975b), Tamaki and Watanabe (1977) and Rao et al. (1980). On the contrary, Banerjee et al. (1987) and Shukla et al. (1991) identified significant reduction in age at first egg of F type birds than S.

A significant (P<0.05) difference was noticed between IWP (157.0 ± 0.97 and 146.1 ± 0.89 days) and IWN (163.7 ± 1.36 and 150.4 ± 0.96 days) strains in both S15 and S16 generations, respectively with respect to this trait.

5.1.4.2 Body weight

From Table 6 and 7, it could be seen that the body weight at 20th and 40th week are not significantly different between F and S birds in both IWP and IWN strains of S15 and S16 generation. This result was in agreement with those of Shukla et al. (1991), who also observed no significant difference between Akp isozymes and body weight. Chaudhary et al. (1971) reported birds exhibiting fast band have higher body weight upto 16 weeks, from 17 weeks onwards there was no difference for body weight.

A significant (P<0.05) difference was noticed between IWP and IWN with respect to this trait as well.

5.1.4.3 Egg production

It could be seen from Table 6 and 7 that the egg number upto 40 weeks and also upto 60 weeks were not showing any significant difference between F and S birds in both IWP and IWN strains of S15 and S16 generation. Rao et al . (1980) and Goswami et al . (1990) also did not find any significant difference for part-year and full-year production between F and S birds of White Leghorn. However, Banerjee et al. (1987) found significant difference for both 40th week and 60th week production between F and S type birds of White Leghorn. Ranjan et al. (1974) observed hens with phenotype S had significantly higher egg production (270 days production) than those with F.

In S15 generation IWP was having significantly higher egg production than IWN. On the contrary, in S16 generation IWN was having significantly (P<0.05) higher 60th week egg production than IWP.

5.1.4.4 Egg weight

The data in Table 6 and 7 revealed that there was no significant difference for egg weight at 32nd week and 40th week between F and S birds in both IWP and IWN strains of S15 and S16 generations. This observation is in accordance with Chaudhary et al. (1971), Garcia (1975b), Rao et al. (1980), Goswami et al. (1990) and Shukla et al. (1990).

IWP birds had significantly (P<0.05) higher 32nd week egg weight than IWN birds.

5.1.4.5 Egg production at different intervals

In the present study, which is probably the first of its kind, alkaline phosphatase phenotypes were found to be correlated with egg production at different intervals. The egg production data were divided into 21-40wk production and 41-60wk production and then analysed to find the relationship between SAP polymorphism and egg production at different intervals. The results are presented in Table 8, which revealed that in IWP strain 21-40wk and 41-60wk production of F birds were equal to or higher than S birds in both the generations. This may be the reason why higher gene frequency of F was observed in IWP strain. In IWN strain the 21-40wk egg production of F birds were lesser than S type birds. But 41-60wks egg production of F birds were significantly higher than S birds in IWN strain of S15 generation and IWP and IWN strains of S16 generation. In IWP strain of S15 generation the 41-60wk production of F and S birds showed very little difference. This result tends to suggest that the lesser frequency of F gene in IWN strain may be due to the natural selection in operation during fourteen generations. The gene frequency of F can be maintained or improved if the selection is performed for extended period of time. This observation is in conformity with studies of Ranjan et al. (1974), who reported hens with phenotype S had significantly

higher part-year egg production (270 days production) than those with F. Vataliya (1986) indicated a decline in genotypic frequency of FF and FS from 0.14 and 0.47 to 0.13 and 0.46 and also increase of SS from 0.39 to 0.41 in the first generation which was subjected to selection on part-year production (40wk). But they did not evaluate the relationship between SAP types and 41-60 week egg production.

Contrary to this, Amin et al. (1983) observed increase of SAP^F allele frequency from 0.178 to 0.257 by two years of selection even for 40wk egg number.

5.1.5 SAP isozyme types and egg quality traits

The egg quality study was made in both IWP and IWN srains of S15 generation at the age of 50 weeks. The details are presented in the Table 9 and discussed below:

5.1.5.1 Shape index

From Table 9, it could be seen that there was no significant difference between F and S types in IWP and IWN strains of White Leghorn. There was no significant difference between strains also.

5.1.5.2 Albumen index

Albumen index did not show significant difference between F and S birds of IWP and IWN strains. This observation is in agreement with that of Mathur et al. (1983), who reported only little variation in albumen index between Fast and Slow lines which was not statistically significant. Engh (1966) observed lower albumen quality and more blood spots in the eggs of Fast band birds than slow band birds. But the albumen index of IWN strain was significantly (P<0.05) higher than IWP.

5.1.5.3 Yolk index

There was no significant difference in yolk index between F and S type birds of IWP and IWN strains and also between IWP and IWN strains. This observation is in accordance with Mathur et al. (1983), who observed very little variation in yolk index between F and S type birds. However, Patil et al. (1981) reported the birds with Akp^F type had significantly (P<0.05) higher yolk index (0.437 Vs 0.432) than those with Akp^S.

5.1.5.4 Shell thickness

The shell thickness of eggs from fast band birds was significantly (P<0.05) higher than that of slow band birds in both IWP and IWN strains. Mathur et al. (1983) found only very little variation in egg shell thickness

between Fast and Slow lines. On the contrary Patil et al. (1981) reported the birds with Akp^F isozyme had slightly lower (0.344 ± 0.003mm) egg shell thickness than Akp^S birds (0.345 ± 0.003mm).

5.1.5.5 Haugh unit score

There was no significant difference between fast and slow moving birds of IWP and IWN strains for haugh unit score. This result falls in line with the report of Mathur et al. (1983). However, Patil et al. (1981) found significantly higher haugh unit score for the birds with Akp^F isozyme type (70.43 ± 0.81) than Akp^S (68.49 ± 0.93). But the eggs from IWN were showing significantly (P<0.05) higher haugh unit score (88.77 ± 0.95) than IWP (80.72 ± 1.06).

5.1.6 SAP isozyme types and reproduction traits

Crosses were made between different phenotypes (F x F, F x S, S x F, S x S) of IWP and IWN strains of White Leghorn belonging to S15 generation (selected based on Osborne index) and their reproductive study was made in detail, the results were presented in the Table 10, 11 and 12.

5.1.6.1 Fertility percentage

From Table 10 and 11, it is evident that fertility percentage of F x F and F x S matings were significantly (P<0.05) higher than S x F mating in IWP and

IWN strains. S x S mating showed non-significant increase in fertility than S x F mating. Iotova et al. (1989) found significantly (P<0.05) higher fertility in FF hen's egg than SS. On the contrary, Mathur et al. (1979) made test mating of Slow male x Slow female and Fast male x Fast female, they found no significant difference in fertility between these matings. Parmar et al. (1991) also found no significant variation between fast and slow type birds for fertility.

5.1.6.2 Hatchability percentage on Total egg set

From Table 10 and 11, it could be seen that although there was no significant difference among crosses in both IWP and IWN for hatchability percentage on total egg set (except F X F mating which shows significantly higher hatchability than S X F mating in IWN strain), F x F mating showed best hatchability followed by F x S, S x F and S x S. This observation lends support to the study of Parmar et al. (1991), who reported the effect of alkaline phosphatase isozyme types on hatchability as non-significant. But Banerjee et al. (1987) and Iotova et al. (1989), found significantly higher hatchability on total egg set for eggs laid by fast type hens than the slow type.

5.1.6.3 Hatchability percentage on fertile egg set

From Table 10 and 11, it could be observed that although there was no significant difference among the matings, F x F mating exhibited highest

hatchability and S x S mating exhibited the lowest hatchability on fertile egg set in both strains. This observation is in agreement with that of Mathur et al. (1979), who did not find any significant difference between F x F and S x S crosses for hatchability. Contrary to this Mathur et al. (1983) found significant difference between Slow and Fast lines for hatchability.

The overall hatchability of both IWP and IWN strains were low (54.47 ± 2.35 and 52.09 ± 2.20 percent, respectively). This lower hatchability might have been due to the age of the birds (80wks), pre-incubation storage temperature and pre-incubation storage time (1-10 days). This was supported by the findings of Sankaralingam et al. (1996), who reported that the hatchability was negatively correlated with pre-incubation storage time and the reduction in hatchability with increase in storage time was mainly due to early embryonic mortality (at 80wks of age) in IWP and IWN strains of White Leghorn.

5.1.6.4 Early embryonic mortality percentage

Although there was no significant difference among the matings for early embryonic mortality in both IWP and IWN strains, it was high in eggs from S x F and S x S matings in IWN strain of White Leghorn (Table 10 and 11).

5.1.6.5 Dead in shell percentage

From Table 10 and 11, it can be seen that there was no significant difference among the mating groups for dead in shell percentage, except S x S mating which showed significantly (P<0.05) higher dead in shell percentage compared to F x F, F x S and S x F matings.

The higher early embryonic mortality and dead in shell percentage could have contributed to the low hatchability percentage of eggs from S x S mating than other mating groups. Mathur et al. (1983) found significant difference between Slow and Fast line on mortality during incubation.

5.2 Haemoglobin Polymorphism

The results of electrophoresis revealed no polymorphism in haemoglobin in both strains of White Leghorn. The zymogram (Figure 10A and 10B) of haemoglobin showed one slow moving major (M) and one fast moving minor (m₁) components in all birds tested. The fast moving minor component m₂ was not identified in any of the birds of both IWP and IWN strains of White Leghorn. This result is in agreement with the findings of Washburn (1976), who found only normal haemoglobin type in 20 strains including a number of randombred population of exotic breeds, commercial layers, broilers, Gallus gallus and Athens Canadian randombred population. Singh (1986) and Pal et

al. (1994) observed that haemoglobin was monomorphic in indigenous fowl breeds of India.

However, this finding is at variance with the studies of Rodan and Ebaugh (1957), Washburn (1968a), Washburn (1968b), Mazumder and Mazumder (1989), who reported more than one haemoglobin type in chicken.

Due to the monomorphism of haemoglobin in the present study, comparisons between haemoglobin polymorphism and economic traits could not be attempted.

The findings tend to conclude that the birds having gene Akp^F perform better in respect of egg production, egg quality and reproduction traits. The egg production at the later stage of life was found to be high in F type birds and hence it is desirable to follow selection on extended testing periods than relying on 40 weeks egg records.

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SUMMARY

An experiment was conducted to find biochemical polymorphism at the serum alkaline phosphatase and heamoglobin locus, their inheritance pattern and to determine the influence of SAP and haemoglobin polymorphism on production, egg quality and reproduction traits of IWP and IWN strains of White Leghorn.

6.1 Alkaline phosphatase polymorphism

1. Biochemical polymorphism at the serum alkaline phosphatase locus was studied in 168 hens and 18 cocks of IWP and 129 hens and 16 cocks of IWN strains belonging to S15 generation (selected based on Osborne index) and 100 hens of IWP and 100 hens of IWN strains from the populations of S16 generation.

2. Horizontal polyacrylamide Gel Electrophoresis (PAGE) technique was employed to identify SAP phenotypes in poultry.

3. Two phenotypes F and S determined by two alleles Akp^F and Akp^S were observed at the SAP locus. The F phenotypic frequency in IWP strain

(0.66 and 0.58) was higher than that of IWN strain (0.15 and 0.24) in both S15 and S16 generations, respectively.

4. The expected gene frequency of F was higher in IWP birds (0.42 and 0.35) than IWN birds (0.08 and 0.13) in S15 and S16 generations, respectively.

5. The reason for a very low frequency of Akp^F allele in IWN birds may be due to the natural selection in operation during the past fourteen generations. The frequency of Akp^F can be maintained or slowly increased by selection based on extended testing period.

5. Different matings were made between fast and slow moving types and the progenies were tested for their SAP type. The results of the matings revealed that the fast band type is determined by an autosomal completely dominant gene over slow band type and it follows a Mendalian pattern of inheritance.

7. The alkaline phosphatase level was estimated in the serum by employing the Kind and King's method (1954). The mean SAP values for the F and S birds revealed that the SAP activity was more in F type birds (97.54 and 88.96 KA units per 100ml serum) than that of S type birds (93.03 and 68.24 KA units per 100ml serum) in IWP and IWN strains.

8. Critical analysis of the correlation between SAP types and the production traits like age at sexual maturity, body weight at 20th and 40th week, egg production upto 40 and 60 weeks and egg weight at 32nd and 40th week revealed that the birds belonging to the F type were better producers compared to S type birds. But the birds belonging to IWP strain were superior than IWN strain in almost all the above said production traits in both S15 and S16 generations (except egg production upto 60 weeks, which was significantly higher in IWN strain than IWP strain in S16 generation). It can be surmised that high frequency of fast moving allele in IWP is associated with the above production traits.

9. Correlation between serum alkaline phosphatase phenotypes and egg production at different intervals (21-40 week and 41-60 week of age) revealed that the 21-40 week egg production of F birds were equal to or less than the S birds in both the strains of S15 and S16 generations. But the 41-60 week egg production of F birds were significantly higher than S birds. This study revealed that selection based on extended testing period may maintain or improve the gene frequency of F in both IWP and IWN strains of White Leghorn than relying on records upto 40 weeks of age. This is very likely to improve the production performance of both the strains.

10. Analysis of the association between SAP types and egg quality traits like egg weight, shape index, albumen index, yolk index, shell thickness and haugh unit score at 50th week of age revealed no significant difference between them except shell thickness. The eggs from F type birds had significantly higher shell thickness than S type in IWP and IWN strains of White Leghorn.

11. Analysis of the relationship between different SAP type matings and reproduction traits like fertility percentage, hatchability percentage on total egg set, hatchability percentage on fertile egg set, early embryonic mortality percentage and dead in shell percentage revealed that feritility of F x F and F x S matings were high followed by S x S and S x F. The hatchability (on fertile egg set) was high in F x F followed by F x S, S x F and S x S.

12. Comparison of strains for reproductive traits revealed higher fertility, hatchability and lower early embryonic mortality percentage in IWP strain than IWN strain. The lower fertility and higher early embryonic mortality in IWN strain may be due to its increased frequency of S gene, which might have resulted in reduced shell thickness due to the low SAP level, which ultimately could have caused increased moisture loss from egg and increased microbial contamination prior to and during pre-incubation

storage time. This could have caused death of the embryo in pre-detection stage or in the early embryonic stage.

6.2 Haemoglobin Polymorphism

1. Biochemical polymorphism at the haemoglobin locus was studied in 100 hens each of IWP and IWN strains which belonged to S15 generation.

2. Agar gel electrophoresis was carried out to find polymorphism among haemoglobin samples.

3. The results of electrophoresis revealed no polymorphism in haemoglobin locus in both strains of White Leghorn. The zymogram of haemoglobin showed one slow moving major (M) and one fast moving minor (m_1) component in all birds tested.

4. Due to the monomorphism of haemoglobin in the present study, comparison between haemoglobin polymorphism and economic traits could not attempted.

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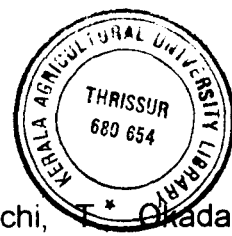
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GENETIC STUDIES ON SERUM ALKALINE PHOSPHATASE AND HAEMOGLOBIN IN TWO STRAINS OF WHITE LEGHORN

By

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ABSTRACT OF A THESIS

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ABSTRACT

The present investigation was undertaken to identify the Serum Alkaline Phosphatase (SAP) and haemoglobin (Hb) polymorphism and also aimed to find their association with production, egg quality and reproduction. Four hundred and ninety seven hens of two different strains of White Leghorn Viz. IWP (168 and 100) and IWN (129 and 100) belonging to S15 and S16 generations, respectively were typed by Horizontal Polyacrylamide Gel Electrophoresis (PAGE). Two phenotypes, Fast and Slow were determined. Higher frequencies of Fast phenotype were observed in IWP strain (0.66 and 0.58) than IWN strain (0.15 and 0.24) in both S15 and S16 generations, respectively. Hundred hens each of IWP and IWN strains were tested for haemoglobin polymorphism with Agar gel electrophoresis, which revealed no polymorphism.

Two alleles namely Akp^F and Akp^S with two phenotypes Fast and Slow were identified as SAP locus. Akp^F allele had the frequency of 0.42 and 0.35 in IWP strain and 0.08 and 0.13 in IWN strain of S15 and S16 generations, respectively. Different matings between Fast and Slow moving types revealed that the Fast band is determined by an autosomal completely dominant gene over Slow band bird. The mean SAP level for the F and S birds revealed that the SAP activity was more in F type birds (97.54 and 88.96 KA units per 100ml

serum) than that of S type birds (93.03 and 68.24 KA units per 100ml serum) in both IWP and IWN strains respectively.

The association between SAP types and egg quality traits revealed no significant difference between them except shell thickness. The eggs from F type birds had significantly higher shell thickness than S type in IWP and IWN strains of White Leghorn. Correlation between different SAP type matings and reproduction traits revealed that the fertility of F x F and F x S matings were highest followed by S x S and S x F. The hatchability (on fertile egg set) was high in F x F followed by F x S and S x F, the least hatchability was observed in S x S cross.

The findings tend to conclude that the birds having gene Akp^F perform better in respect of egg production, egg quality and reproduction traits. The egg production at the later stage of life was found to be high in F type birds and hence it is desirable to follow selection on extended testing periods than relying on 40 weeks egg records.

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