AN ABSTRACT OF THE THESIS OF

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Title: Genetic Studies of Reproductive and Biochemical Traits in Turkeys (Meleagris gallopavo) Divergently Selected for Semen Ejaculate Volume.

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The genetic control of changes in unselected traits of Wrolstad Medium White turkeys divergently selected for semen ejaculate volumes (SEV) for 16 generations (G) was studied. Genetic parameters were estimated in G 10 to 14 for embryonic mortality (EM), the incidence of pipped eggs, and for total plasma cholesterol (PC) and high density lipoprotein cholesterol (HDLC) in 16-week old birds of G 15. An attempt at determining enzyme polymorphisms in the two lines was performed in G 16. A multivariate discriminant analysis procedure was established in an attempt to classify birds as low or high SEV based on fertility and incubation records in G 10 and 12.

It was determined that a more reliable, yet flexible method of estimation of variance components for heritability of embryonic mortality in turkeys is a likelihood procedure. The mean heritability estimates were -.03 and .10 for embryonic mortality in the early (Days 1-10) and late (Days 21-28), respectively. Estimates of
heritability for the incidence of pipped eggs were .21 and .08 in the low and high lines respectively.

Since there were no line differences (P>.05) for PC and HDLC, data was pooled from low and high SEV lines and h² was computed to be -.03 and .26, respectively. Genetic correlation among PC, HDLC, and 16 week body weight (BW) varied from .05 between PC and HDLC, .13 PC and BW and -.34 HDLC and BW. No polymorphisms were observed for the enzymes examined in the present study.

The discriminant function developed to categorize birds as low or high volume semen producers, had a moderate (.55) to high (.75) hit ratio for classification of birds as low or high volume producers in G 10 and 12.

It was concluded that divergence in unselected traits, embryonic mortality and the incidence of pipped eggs, in the low and high SEV lines had a negligible additive genetic control. Cholesterol, though a major intermediate in the biosynthesis of steroid hormones, in turkeys at 16 weeks of age is not a suitable biochemical marker for SEV. Although genetic control in turkeys appears to be negligible for PC, there is a moderate and significant hereditary influence on HDLC. With a misclassification rate of .30-.40, fertility and incubation records, as demonstrated here can be used to classify birds as low or high volume semen producers.
GENETIC STUDIES OF REPRODUCTIVE AND BIOCHEMICAL TRAITS IN TURKEYS (Meleagris gallopavo) DIVERGENTLY SELECTED FOR SEMEN EJACULATE VOLUME

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Typed by Edward J. Smith
DEDICATION

To the memories of my mother, Ye Yema, and my sister Ngo Princess, both put our happiness above theirs, and in the process lost it all.
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Genetic Studies of Reproductive and Biochemical Traits in Turkeys (Meleagris gallopavo) Divergently Selected for Semen Ejaculate Volume

CHAPTER I

Introduction

Genetic selection for increased body weight in domesticated turkeys (Meleagris gallopavo) has led to a precipitous decline in the natural mating capability of males. Artificial insemination has thus become the predominant method of reproduction for turkeys. This predominance justifies emphasis on increased volumes of semen ejaculate as a critical male selection trait within a turkey breeding program (Nestor and Brown, 1971; Nestor, 1984). Aggressiveness, a traditional male characteristic is now, as a result, of less importance to primary breeders in selecting males.

Studies by Cecil and Bakst (1988) reported that extensive individual variation exists between toms in their capacity to produce semen. This difference in semen volume has been reported to be largely genetic in origin (Nestor, 1976; Hales et al., 1989). Heritabilities (h²) reported for semen ejaculate volume (SEV) have ranged from moderate, .45 (Hales et al., 1989) to high, .64 (Nestor, 1976) and .86 (Carson et al., 1955). The large h² values suggest that
(Carson et al., 1955). The large $h^2$ values suggest that increased semen production in turkeys can be accomplished through genetic selection. An increased semen volume may lead to improved management, reduced feed and housing costs as fewer males would be required. Selection for SEV, a sex limited trait, is made directly in males after the onset of sexual maturity and indirectly in females by using records of their male relatives. If the ability to identify high semen producing males at an age prior to sexual maturity could be accomplished, substantial cost savings to the turkey industry would result. Phenotypic markers, if genetically associated with SEV, could allow for possible selection for semen production before sexual maturity (Maeda et al., 1978; Merat, 1990). Theoretically, this identification of low and high volume semen producers can also be accomplished by a multivariate statistical procedure, discriminant analysis (DA). The technique of DA can be used to develop discriminant function, that could enable categorization of toms as either low or high volume semen producers using reproductive and biochemical traits.

The vas deferens of the male turkey is the primary anatomical structure that differs between low and high semen producers (Van Krey, 1990; Froman and Engel, 1991). This structural difference could have arisen from the effects of steroid hormones such as testosterone (Bahr and Bakst, 1987). Diurnal changes in steroid hormone levels,
however, make them difficult to measure reliably and the time of day when measurements are made is critical (Hafez, 1987). Bacon et al. (1987) reported that in mature males, daily testosterone secretions are pulsatile. Cecil and Bakst (1986), however, observed no significant differences when testosterone levels where compared between low and high semen producing males at 32 weeks of age (WOA). It is probable that at 32 WOA, development in the vas deferens is complete, which may explain why no differences in testosterone levels were observed. Cholesterol, a principal intermediate in the biosynthesis of steroid hormones, and reduced daily pulsatile variation can be investigated as an alternate indicator for SEV. Such work should ideally be performed prior to sexual maturity or before males are photostimulated.

A long standing interest in agriculture for both industry and academic researchers concerned with genetic improvement is the nature of inheritance of quantitative traits (QTs) such as SEV (Thoday, 1961; Edwards et al., 1987; Patterson et al., 1988). Understanding the inheritance of quantitative traits involves first determining the chromosomal location of genes influencing QTs, normally referred to as quantitative trait loci (QTL). It is only when genes at QTL are clearly defined will research emphasis be placed on gene transfer as a viable genetic improvement technique for avian species (Sheldon,
Biochemical and molecular markers possess a substantial potential for facilitating an understanding of this inheritance (Kurnitt et al., 1982; Murray et al., 1984). Advances in molecular biology and genetic engineering methods for the transfer of genes in eukaryotic systems permit identification of pleiotropic and linked genes for QTs worthy of the large cost in time and money (Shuman, 1990).

To establish a potential gene pool for transfer of genetic material between domestic animals including poultry, there is an increasing need for research aimed at identifying markers for economic traits. These markers can be morphological, biochemical, molecular or quantitative but should be inherited as discrete or Mendelian characters (Shuman, 1990). Economically important QTs usually emphasized by selection programs in poultry production for which markers can be essential include body weight (Nestor, 1984); fertility (Lake, 1969); hatchability (Hutt, 1969) and disease resistance (Washburn et al., 1971).

Biochemical markers have been identified and successfully applied to the improvement of QTs in poultry. Griffin and Whitehead (1982) and Whitehead and Griffin (1985) used serum triglyceride and very low density lipoprotein levels (VLDL) to select for leanness in chickens and turkeys. The VLDL concentrations are reported to have a high positive phenotypic and genetic correlations
with abdominal and total body fat. Alleles at certain loci of the major histocompatibility complex (B-locus) have been shown to be associated with disease resistance in chickens (Gavora, 1990; and Lamont and Dietert, 1990). Hemoglobin type has also been implicated with resistance to Marek's disease in chickens (Washburn et al., 1971). In turkeys, however, information on biochemical variants and the possible association with QTs is limited (Savage, 1990).

Molecular markers have been investigated for some traits in chickens. Dunninton et al. (1990) reported that through DNA fingerprinting, line specific DNA banding patterns were observed in White Plymouth Rock chickens selected for low and high 8-week body weight. When adequate restriction fragment length polymorphisms (RFLPs) are identified in avian species, then a more innovative approach to identify quantitative trait loci in avian species will be possible (Soller and Beckman, 1986). Though work on RFLPs in livestock continues, only a limited number has as yet been identified in poultry (Sheldon, 1980; Crittenden, 1981; Shuman, 1990). Beckman and Soller (1983) discussed features that make RFLPs, when identified and association with QTL established, attractive as genetic markers in breeding programs. While it is easier to obtain DNA in research programs, it would be rather difficult for both small and major primary poultry breeders to use DNA analysis as selection aids in breeding. In humans, however,
the application of RFLP is extensive in medical research, gene mapping and detection of genetic defects (Kan and Dozy, 1978; Botstein et al., 1980; Murray et al., 1984). The chromosomal location of the gene for Duchenne Muscular Dystrophy was determined using RFLPs (Hill et al., 1982). The advantages of this type of research in livestock production has to be carefully balanced against the large investments of money and time required to identify even a single RFLP (Kingston et al., 1983; Murray et al., 1984).

Polymorphism at enzyme loci or isozymes offer the potential for these to be used as biochemical markers for QTs. A recent review by Merat (1990) suggested that isozymes reported to be associated with QTs in poultry, can be used as markers. A Report by (Ranjan et al., 1974) suggests that alkaline phosphatase can be used as a marker for semen production in chickens; males homozygous recessive for the slow (electrophoretic mobility) allele were superior. Alleles of cholinesterase differed significantly in lines selected for divergent growth rates in avian species (Grunder and Merritt, 1977). Thus it is possible to select for an economically important quantitative trait using correlated traits that have mono or polygenic inheritance (Ranjan et al., 1974; Rao et al., 1980). The phenotype of the correlated trait can be metric, cytological, biochemical or molecular.

In the turkey research program of the Oregon
Agricultural Experiment Station, a unique divergent selection program for SEV was initiated in 1975. Through 17 generations of selection for semen volume, the low and high SEV lines continue to diverge (Smith et al., 1991). This divergence suggests the presence of large number of segregating genes in both populations influencing SEV. Changes in unselected traits in the low and high SEV lines (Savage et al., 1984) are probably due to factors other than random genetic drift.

Thus the objectives of this thesis are to:

1. Estimate genetic parameters for embryonic mortality in the low and high SEV lines in order to determine the additive genetic control of changes in unselected traits.

2. Compare within the low and high SEV lines, embryonic mortality, including the incidence of pipped eggs in five generations, G 10 to 14 inclusive.

3. Evaluate plasma (PC) and high density lipoprotein cholesterol (HDLC) levels as markers for semen ejaculate volume.

4. Compare allele frequencies of selected enzymes in the low and high SEV lines.

5. Develop a discriminant function that could be used to establish a criterion for classifying birds as low or high volume semen producers based upon fertility, hatch of fertile, embryonic mortality and the
incidence of pipped eggs.
REFERENCES


CHAPTER II

Literature Review

A. Semen Ejaculate Volume

Semen ejaculate volumes (SEV) produced by avian species is regarded as low (Van Krey, 1990). This factor, in combination with the predominance of artificial insemination in the turkey industry, make SEV of primary importance to turkey breeders and researchers. Lacking consensus however, is the role of SEV in fertilization. Some researchers suggest that semen functions only as a medium for transporting spermatozoa. Thus selection for SEV does not necessarily translate into increased fertility (Cherms, 1968).

1. Volume and concentration of semen: Though unsubstantiated, it is believed that 100 million spermatozoa per insemination is sufficient to sustain optimum fertility for a 2-week period in turkeys (Van Krey, 1990). The average SEV for mature male turkeys or toms is .4 ml with a concentration greater than a billion spermatozoa (Carson et al., 1955; Cecil and Bakst, 1988). A ten-fold dilution will therefore be essential for good management to maintain optimum fertilization. The dilution
can be effected either by artificial diluents or entirely by seminal plasma. The production of a high ejaculate semen volume is both an economic and a management concern. Males with higher SEV can inseminate more hens with each ejaculate. The availability of such males may also mean fewer breeder toms.

The indirect economic benefits of SEV has led to several investigations as to the influence of environmental and genetic factors on semen production in turkeys. Though Carson et al. (1955) reported significant differences among sires for SEV and sperm concentration, reports of other studies indicate, however, that spermatozoal cell concentrations in ejaculates of varying volumes are the same for low and high volume semen producers (Cherms, 1968; Nestor and Brown, 1971; Cecil and Bakst, 1984).

2. Genetic influence on SEV: Buss (1971) reported differences in semen volume among six varieties of turkeys. Average semen ejaculate volumes ranged from .07 mL for the Reds to .24 mL for the Greys. Within the Broad Breasted Bronze variety, the families evaluated in the study differed significantly in the average SEV. The mean family ejaculate semen volume varied from .09 to .19 mL at each collection.

   Reported heritability estimates for SEV vary from .30 (Carson et al., 1955) and .45 (Hales et al. 1989), to .70
(Nestor, 1976). The large individual variation in SEV reported by Cecil and Bakst (1984) can therefore be attributed to heredity. Selection primarily for SEV has also been highly successful, a further indication of the large additive genetic control of this trait (Nestor, 1976; Smith et al., 1991).

3. Semen quality: Effect of semen volume on semen quality, expressed as percentage bent sperm per ejaculate, has also been investigated. In a study by Nestor and Brown (1976), low SEV line males had greater variability for semen quality. In males selected for semen quality over 4 generations, Shanskova, (1976) reported significant concurrent increases in semen volume of about 50 percent. Other factors reported to affect semen quality include diet (Nilipour, 1987).

B. Selection

1. Selection limit: Selection designed to bring about improvement in a specific trait(s) by changing the average population genotype has a limit (Falconer, 1989). Theoretically, this limit is reached when genetic variation in the population is at its minimum. The time required to reach this limit is influenced primarily by the number of loci contributing to the phenotypic expression of the trait.
under selection (Lande, 1981). At the selection plateau, the predominant genotype of the population is that of the trait been selected for. The form this genotype assumes at the selection limit is difficult to define. Fixation at the loci influencing the trait in question does not necessarily indicate that the genotypes are homozygous. Frequently, if the number of loci is large, and the relative contribution of each locus to the trait small, near other than complete homozygosity is attained (Roberts, 1966; Falconer, 1971). When populations under long-term selection continue to diverge, as it is for the low and high SEV lines in the OSU Turkey Research program (Smith et al., 1991), a large variation exists from the contribution of many loci and there is a continuous segregation of genes (as for those affecting SEV) (Lande, 1981). In effect the selection limit has not been reached for SEV. This continued response to selection for SEV, also suggests that small effective population size, as suggested by Hales et al. (1989) and Smith et al. (1991), or the resulting drift cannot adequately account for changes observed in the unselected traits (Lande and Arnold, 1983; Jones et al., 1968).

2. Genetic parameters: Improvement of traits through selection is largely dependent upon the amount of genetic variation in that character. Genetic variation for a specified trait in a population is measured by statistical
parameters, heritability \( (h^2) \) and genetic correlation. Heritability has been considered to be among the most important properties of a quantitative trait (Falconer, 1989). Heritability measures the extent to which an individual's genotype influences the phenotype. In effect, it determines the extent to which the phenotype reflects the breeding value of the individual. Methods of estimating \( h^2 \) have been the subject of many reports and reviews (Henderson, 1953; Hartley and Rao, 1967; Rao, 1971; Searle, 1971; Henderson, 1973; Harville, 1977; Thompson, 1979; Kennedy, 1981; Becker, 1984; Swallow and Monahan, 1984; Henderson, 1986; Falconer, 1989). Methods of estimation include regression of offspring values on one parent, or on mid-parents and half and full-sib correlations. Estimates based on half-sib correlation and regression of offspring on sire/father are considered the most reliable (Mather and Jinks, 1977; Hill and Nicholas, 1974). Experimental conditions have been suggested under which estimation of \( h^2 \) can be considered reliable (Hill, 1971). The design of experiments necessary for estimating genetic parameters with high precision, primarily concern the number of individuals or families on which the trait can be measured.

Genetic correlations between quantitative traits are of considerable interest to breeders. Positive correlations enable selection to emphasize fewer traits, while a negative relationship does not allow for simple selection.
A genetic correlation arising from linkage is transient, lasting only the first few generations of selection. The linkage therefore arises only from the presence of both loci on the same chromosome. This association, depending on the distance between the linked loci on the chromosome, is severed by recombination after a few generations. Correlations due to pleiotropy, a more persistent relationship, suggests a major gene affecting quantitative traits (Mather and Jinks, 1977). If two traits are affected by the same differences in environmental conditions, environmental correlations result (Falconer, 1989). Estimation of correlations are by procedures similar to that for heritability (Mather and Jinks, 1977).

C. Reproductive and Hatchability Traits

1. Fertility: The proportion of eggs fertilized at each mating, is a reproductive trait that is difficult to define (Buss, 1989). Researchers and commercial breeders differ in the definition and consideration of the extent of fertility in avian species. It is not uncommon for the reported percentage fertility to be 90 and 75 from research and commercial hatcheries respectively. Reports of the effect of SEV on fertility have primarily been based on records from research hatcheries (Nestor, 1976).

In a rather extensive review, Lake (1969) discussed
the relative importance of factors that influence fertility. These include physiology, management, environment and genetics all or some of which influence male and female breeders. A direct effect of semen volume and quality on fertility is yet to be established. McCartney (1956) and McCartney et al. (1958) reported no significant differences in fertility between lines of turkeys selected for semen quality. Saeki and Brown (1962) and Cherms (1968) in separate studies reported that yellow semen, considered an indication of lower quality, results in a considerable decrease in fertility.

In a recent review, Buss (1989), discussed genetic factors influencing fertility in turkeys. Inbreeding and outcrossing were both reported to have significant effects on fertility. Kondra and Shoffner (1955) reported 78 percent fertility for the Beltsville Small White but only 58 percent from a cross with the Broad Breasted Bronze variety. Work from McCartney and Chamberlain (1961), cited by Buss (1989) suggests that maternal and individual heterosis have significant effects on fertility. Higher percent fertility was reported for F1 dams. It has also been suggested that duration of fertility after a single insemination has a large genetic correlation with fertility. Buss (1989) reported the average of published genetic correlation estimates to be .40. Varietal differences in fertility have also been reported. Sampson
and Wilson (1944) compared two varieties with average fertilities of 65 and 82 percent. Harper and Parker (1950) compared fertility among ten families and noted that fertility varied from 39 to 100 percent based upon biweekly inseminations. Reports of the influence of selection for some traits including body weight, egg number, clutch length and broodiness on fertility are inconsistent (Buss, 1989). Through 5 generations of selection for semen volume, Nestor (1976) reported no directional change in fertility. Estimates of heritability for percentage fertility have ranged from .28 (Koneva, 1976) to .34 (Nestor et al., 1972).

2. Hatchability: The physiological basis of a relationship, if any, between semen volume and hatchability is difficult to interpret. Such a relationship is however possible if genes influencing these quantitative traits have pleiotropic effects. Semen volume is a characteristic of the tom and while hatchability is considered a trait of the hen and or the embryo.

Several factors have been reported to influence hatchability. Length of preincubation storage is reported to have a significant effect on hatchability (Abplanalp and Kosin, 1953). Asmundson (1947) observed that hatchability declined with eggs stored for periods longer than 8 days. Preincubation storage for less than 2 days also depresses
hatchability (Christensen and Bagley, 1989).

The effect of varying incubation conditions on hatchability is the subject of numerous papers, most recently by Christensen and Bagley (1989). Optimum incubation temperature for hatchability of turkey eggs for the first 24 days is 37.5 and 36.9 °C in the last 4 (Insko, 1949; Moreng and Avens, 1985).

Physiological changes in the embryo leading to reduced hatchability as a result of incubation temperature fluctuation have been described by Christensen and Bagley (1989).

Reported varietal and breed differences support claims that heredity influences hatchability (Abplanalp and Kosin, 1953; Buss, 1989). Other studies, however, suggest that changes in hatchability are often due to significantly large maternal and environmental effects (McCarty and Chamberlain, 1961). Earlier studies by Kondra and Shoffner (1955) showed that heterosis had no effect on hatchability. The average reported heritability estimates of .25 (Buss, 1989) suggests a low additive genetic influence on hatchability. Estimates of $h^2$ also differed among breeds. Nestor et al. (1972) reported $h^2$ of .17 for Medium Whites, while Koneva (1976) reported estimates of .10 for the Large white and .40 for the Broad Breasted Bronze.
3. Embryonic mortality: It is difficult to discuss hatchability and embryonic mortality (EM) as separate and independent characteristics. Hatchability is a result of no/reduced embryonic mortality. In general the two can be considered as cause and effect; EM or lack thereof is cause, and hatchability is the effect. As cause, EM can be divided into three periods, distinguished primarily by peaks in mortality during the incubation period (Christensen and Bagley, 1989). Embryonic mortality within days 1-10, 11-20, and 21-28 of incubation are referred to as early (D1), mid (D2) and late (D3), respectively (Shook, et al., 1971). Christensen (1978) reported that 38.5 percent of all embryonic deaths occur in the early (D1) incubation period. Work from the Nicholas Turkey Breeding Farms suggests that EM in mid (D2) incubation period is lowest, accounting for only 20 percent of all embryonic deaths (Anonymous, 1979). In the late (D3) incubation period, the average reported EM was 39.6 percent (Christensen, 1978). In their review of EM, Christensen and Bagley (1989) suggest that embryonic mortality within D1, D2, and D3 incubation periods is a non-random event. Genetic and environmental, including management practices could therefore be used to influence the magnitude of EM in any of the periods. Factors reported to influence EM include age of hen (Christensen, 1978). Younger hens had a higher incidence of embryonic mortality in the D1 period.
while older hens had significantly more deaths in the D3 period. Christensen and Bagley (1989) speculated that this difference could be a result of older hens laying eggs with thinner shells. These embryos would be affected by higher conductance and water vapor loss with altered oxygen/carbondioxide exchange. Smith et al. (1991) however reported no differences in eggshell quality as indicated from specific gravity measurements between lines of turkeys differing in D3 mortality.

Incubation conditions are considered most critical in the D1 period. Temperature deviations in this period are more severe due to alterations in metabolic rate, acid-base balance, extracellular fluids and an increased pressure on the output of the developing circulatory system (Buckland, 1969; Simkiss, 1980). Unlike temperature, humidity another incubation factor, is critical throughout the entire incubation period. Insko and Martin (1935) suggested that to minimize EM the mean humidity should be maintained at 54 percent. The primary physiological effect of changes in humidity is water loss. Simkiss (1980) argues that variation in humidity should not be of much concern as temperature since embryos have an extensive physiological flexibility to buffer against water loss. Ventilation and turning of eggs are the other factors reported to significantly influence EM in any incubation period (Christensen and Bagley, 1989). The embryos survival is
therefore dependent on a complex response to the interaction of these factors within D1, D2 and D3 incubation periods.

In turkeys, a direct effect on EM from maternal nutritional deficiencies has not been established. In chickens, however, Beer (1969) reported that nutrition, is the primary indirect cause of EM. Embryonic deaths in early, mid or late incubation periods can result from lesions due to parasites that stem from nutritional deficiencies.

Though the role of heredity on embryonic mortality is the subject of many research reports (Kondra and Shoffner, 1955; McCartney, 1962; Arora and Kosin, 1966) only Shook et al. (1971) defined this role according to incubation periods. For the Broad Breasted Bronze, $h^2$ estimates for EM were .03, .75 and .06 in D1, D2 and D3 incubation periods, respectively. The estimates suggest a negligible genetic control on EM in D1 and D3 during incubation. The same source (Shook et al., 1971) however reported significant increase of about 9 percent in EM when selection emphasized reduced hatchability. Later studies by McCartney (1962) indicate that improvement from selection for reduced EM in turkeys can be very slow.

D. Markers for Quantitative Trait Loci

Economically important traits in livestock including
poultry are mostly polygenic. Gelderman (1975) has suggested that the chromosomal location of these genes be referred to as quantitative trait locus (QTL). Genes at a QTL generally contribute small but additive effects to the phenotypic expression of the trait. The small effect makes it rather difficult to identify and assess individual loci involved in the expression of polygenic traits (Falconer, 1989).

Several investigators have sought to understand the molecular basis for the inheritance of quantitative traits (QT) in livestock. This understanding, it is believed will facilitate improvement from selection emphasizing QTs (Gelderman, 1976; Kluge and Gelderman, 1982; Shuman, 1990; Soller and Beckman, 1986). Biochemical and molecular markers of monogenic inheritance have been suggested as a means for understanding hereditary control of QTs. The effect of the marker gene on the QT could either be through pleiotropy or from linkage. In the latter, the expression of the marker gene is observed only in a limited number of populations and is transient (Merat, 1990). If pleiotropic and the effect significant, the gene is considered a major gene (Hartman, 1988). Body weight, a polygenic trait in poultry is profoundly influenced by the dwarf (\textit{dw}) gene in chickens, for example (Guillaume, 1976; Merat, 1984). The \textit{dw} allele can therefore be regarded as a major gene.
1. Markers and genetic selection: Markers, biochemical, molecular or morphological traits used as indicators of other traits including QTs, are primarily for indirect selection. Indirect selection through markers is justified for traits that: have low heritability, are sex and age limited and / or influenced, and difficult to measure (Shuman, 1990). When the effect of a marker is large, its transfer through genetic engineering to other animals may be justifiable. Merat (1990) reviewed other circumstances for which the large expense involved in identifying markers may be justified. Improvement through traditional selection practices is very slow for traits that are difficult to measure. In poultry these include feed efficiency, embryonic mortality including pipped eggs and persistency of egg production. For these QTs, markers such as the molecular type, may facilitate progress in selection. Other circumstances for which markers are essential include unfavorable correlations among traits making direct selection difficult. Rate of chick growth, for example, has a negative phenotypic correlation with hen feed efficiency (Merat, 1990). Though markers are becoming very popular with researchers concerned with the genetic improvement of poultry, some workers have cautioned against the heightened interest, based on the limited polymorphism often reported at marker loci and the irregular chromosomal distribution of these genes (Bulfield, 1985). This irregularity makes
tracking of segregating genes for QTs rather difficult. Gelderman (1975 and 1976) suggested methods and experimental conditions required to define markers for QTs. Two approaches have been proposed to establish a relationship between a marker and a QT. In the first method, marker frequency is compared among established lines of QTs. In a randombred population animals are categorized as low and high for a quantitative trait, marker frequency between these is then compared (Stuber, et al., 1980). The second approach involves establishing defined phenotypes for the markers, and subsequently comparing the defined groups for QTs (Falconer, 1989). In animal studies, the latter approach is preferred particularly when the effective population size is small and random genetic drift is a concern.

2. Biochemical markers: Biochemical traits expressed in different tissues and inherited as discrete characters can be used as markers for QTs. For body fat in broilers, biochemical molecular markers have been established. Griffin and Whitehead (1982) reported that circulating levels of plasma very low density lipoprotein (VLDL) can be used to select against fatness. The mean phenotypic correlation between VLDL and body fat was reported to be .68. Reports from this group also suggest that levels of plasma triglyceride can be used as a marker to select for
leanness in turkeys (Whitehead and Griffin, 1985). Dunnington et al. (1990) reported significant differences in deoxyribonucleic acid (DNA) banding pattern between low and high body fat lines in broilers. Though these could be considered markers, knowledge of the inheritance of the line specific banding patterns would be necessary to confirm this deduction. Wilcox and Shaffner (1963) reported that White Leghorn lines divergently selected for serum cholesterol levels did not differ in reproductive performance over that of a randombred population.

Though biochemical markers for other QTs have also been investigated, results are inconsistent and often contradictory. Reports by Stratil (1967), Kushner et al. (1974) and Moiseeva et al. (1977) indicate that the ovalbumin genotype AA confers a higher hatchability than either AB or the BB genotypes in chickens. This superiority, however, could be the result of a heterozygote advantage and therefore not a true marker. Heterozygote advantage is also advanced as the reason heterozygotes at the B (Major Histocompatibility) complex in chickens have lower embryonic mortality. Though biochemical variants in the blood of turkeys have been reported, no attempts have yet been made to relate these to QTs (Savage, 1990; and Merat, 1990).

Isozymes, variants of an enzyme, have also been the subject of many investigations with the primary objective
of establishing an association with QTs. The majority of the information involves alkaline phosphatase. Rao et al. (1980) reported no differences in egg production and hatchability traits among alleles at the alkaline phosphatase (akp) locus. Pingel and Ranjan (1975) (cited by Merat, 1990) and Ranjan et al. (1974) determined that the slow allele, akp', was associated with higher semen ejaculate volume. For liver esterase in chickens, Maeda et al. (1978) reported differences in fertility and age at first egg.

3. Molecular markers: Several recent reviews addressed the applications of genetic engineering and molecular biology to poultry through identification of markers and genes affecting QTs (Bulfield, 1985; Shuman, 1990; Soller and Beckman, 1986). Molecular markers have the advantage of being less influenced by sex, age or tissue type.

Of significant potential as a molecular marker in poultry are restriction fragment length polymorphisms (RFLPs). Shuman (1990) defined this class of markers as originating from changes in DNA sequences as a result of single base pair changes or from the effects of modifying enzymes. Homologous DNA will thus differ in restriction sites which on cleavage will produce fragments of varying lengths or RFLPs. The changes in DNA resulting in RFLPs may originate from point mutation, sequence inversion, and base
insertion or deletion. It is thus possible that RFLPs may be frequent in genomes except for highly inbred lines and populations subjected to severe bottlenecks through several generations (Botstein et al., 1980). Extensive use is made of RFLPs as markers in prenatal diagnosis of genetic diseases in humans (Kan and Dozy, 1978; Kurnitt et al., 1982). Soller and Beckman (1986) suggest that the effect of an RFLP locus on an economically important QT is to facilitate selection and fixation at the QTL. Introduction of such loci to other populations is thus easier and more rapid. By using probes of genes with known effects on QTs, such as growth hormone (body weight) or an allele at the MHC locus (resistance to Marek Disease), there is a greater probability of accurately detecting an RFLP locus associated with a QTL.
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CHAPTER III

A Comparison of Four Methods of Variance Component Estimation for Heritability of Embryonic Mortality in Turkeys¹

EDWARD J. SMITH and THOMAS F. SAVAGE

Department of Animal Sciences
Oregon State University
Corvallis, Oregon 97331-3402

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ABSTRACT

A comparison of four methods for variance component estimation to compute heritability of embryonic mortality in the early (Days 1 to 10) and late (Days 21 to 28) incubation period, was performed using Henderson's Method 3, maximum likelihood (ML), restricted maximum likelihood (REML), and minimum variance quadratic unbiased estimator (MIVQUE). Incubation records from Wrolstad Medium White turkeys divergently selected for semen ejaculate volume were the source of data for this study. Negative sire components of variance were obtained for early mortality by both Henderson's Method 3 and the MIVQUE procedure. Estimates of the sire variance component for the late mortality period were lower (P<.05) for ML than those obtained using Henderson's Method 3, REML, and MIVQUE. It was concluded that likelihood procedures (ML and REML) are superior for estimating variance components and genetic parameters from unbalanced data.

(Key words: heritability, Henderson's Method 3, maximum likelihood, restricted maximum likelihood, minimum variance quadratic unbiased estimator)
INTRODUCTION

Knowledge of genetic parameters form the basis for making decisions in poultry breeding programs. Selection progress is a function of the heritability of the traits involved. The greater the heritability estimate, the less intense the selection pressure necessary to achieve significant genetic gain (Falconer, 1989). Heritability and genetic correlations can be estimated by parent-offspring regression and intraclass correlation of observations on sibs. Estimation of genetic parameters by full- or half-sib correlations is based on partitioning variance into its causal components. Procedures for estimating variance components differ in their degree of simplicity and their reliability. In general the choice of a method for estimation of variance components is influenced most by simplicity rather than reliability (Falconer, 1989). The traditional approach for estimation of variance components involves ANOVA procedures (Fisher, 1921 and Henderson, 1953). For mixed models, ANOVA procedures involving Henderson's Methods 2 and 3 are adequate when data are balanced (Searle, 1971). In Henderson's Method 2, data are first adjusted using least square estimates of fixed effects prior to ANOVA. Henderson's Method 3 involves
solving for variance components by equating mean squares estimated by least squares analysis to their expectations.

Data obtained in most animal breeding programs are generally unbalanced, which makes ANOVA estimators of variance components relatively inefficient. Although they remain unbiased, these procedures can no longer be considered minimum variance estimators (Swallow and Monahan, 1984). Inherent in the unbiased characteristics of ANOVA estimators is the situation that these estimators can result in negative values. Searle (1971) discussed different strategies for avoiding negative variance estimates. Alternative methods, including likelihood and minimum variance quadratic unbalanced estimation (MIVQUE) (Rao, 1971), with less restriction on experimental design (acceptable efficiency for both balanced and unbalanced designs) and a non-negativity constraint, have been suggested (Brown and Burgess, 1984; Swallow and Monahan, 1984; Henderson, 1986).

Likelihood methods to estimate breeding values and variance components in animal breeding programs have become widely used alternatives (Thompson, 1979; Kennedy, 1981). Harville (1977) reviewed maximum (ML) and restricted maximum likelihood (REML) methods of variance component estimation. The ML estimates of variance components
maximize the likelihood functions over the parameter's space (heritabilities range from 0 to 1 and variance components are positive). It has been suggested (Kennedy, 1981) that whereas selection tends to bias ANOVA estimators of variance components, the likelihood procedures are valid for populations under selection. Restricted maximum likelihood, a modification of the ML procedure, partitions the likelihood function into portions that are variant and invariant relative to the fixed effects. The invariant portion of the likelihood function is maximized to prevent a loss in degrees of freedom that result when fixed effects are estimated using either ML or ANOVA procedures (Patterson and Thompson, 1971). For random models, ML and REML procedures both yield similar estimates (Swallow and Monahan, 1984). In a recent report, Beaumont (1991), using simulated data for reproductive traits in poultry, indicated that REML is more efficient than Henderson's Method 1.

The MIVQUE procedure suggested by Rao (1971) has been used rarely in estimating variance components within poultry breeding programs. When iterated, MIVQUE estimations of variance components are biased because the estimates are limited to the parameter space. However, the MIVQUE procedure of Rao (1971) is not iterative, and like
ANOVA estimators, it has the advantage of being unbiased. This MIVQUE method requires postulation of a priori values for variance components and does not have a non-negativity constraint, unlike likelihood procedures.

The objectives of the present study were to compare estimation of variance components and of heritability by Henderson's Method 3, ML, REML, and MIVQUE procedures using embryonic mortality data obtained from a divergent selection program for semen ejaculate volume (SEV) in Wrolstad Medium White turkeys.
MATERIALS AND METHODS

Birds

Artificial incubation records for Wrolstad Medium White turkeys during Generation 15 of divergent selection for SEV, low and high, respectively, were the body of data for this study. A description of the selection and management programs for the birds has been reported (Hales et al., 1989; and Smith et al., 1991). In the present study, embryonic mortality in the early (Days 1 to 10) and late (Days 21 to 28) incubation periods was evaluated using incubation records (six biweekly egg settings) for each of 10 sires each mated to 10 hens per SEV line.

Model

To estimate variance components for embryonic mortality in early and late incubation periods a mixed linear model describing the data was used:

\[ y_{ijk} = \mu + L_i + S_{ij} + e_{ijk} \]  \hspace{1cm} [1]  

where \( y_{ijk} \) is arc sine transformed percentage embryonic mortality at kth hatch; \( \mu \) is the population mean; \( L_i \) is the
fixed effect of the $i^{th}$ semen ejaculate volume line; $s_{ij}$ is the random effect of the $j^{th}$ tom within the $i^{th}$ SEV line; and $e_{ik}$ is the residual error attributed to the $k^{th}$ hatch record. Using matrix algebra, this equation assuming all genetic variation is additive, can be expressed as

$$y = Lb + Zs + Ie$$

where $y$ is the column vector of percent embryonic mortality, $L$ is the design matrix for line effects, $Z$ and $I$ are design matrices for sires and residual error: and $b$, $s$, and $e$ are column vectors with contributions attributed to fixed line effects and random effects due to sire and error, respectively. The normal equation for [2] is then expressed as

$$L'Ly = L'y$$

Henderson's Method 3

The ANOVA procedure used to estimate sire ($\sigma_s^2$) and error ($\sigma_e^2$) variance components was the fitting constants procedure of Henderson commonly referred to as Method 3 (Henderson, 1953). This approach involves substituting sum of squares in the ANOVA procedure with quadratic forms involving least square solutions for sire plus error effects (Searle, 1971). Briefly, the $\sigma^2$ are solved for by
equating reductions in sum of squares,
$Y' L (L' L)^{-1} L' Y$, and the differences between these to their
expectations under the full model as follows (Searle, 1971):

$$\sigma_s^2 = \frac{[R(s/b) - \sigma_c^2 r(L; L) - r(L)]}{\text{tr} [Z' Z - Z' L (L' L)^{-1} L' Z]} \quad [4]$$

$$\sigma_c^2 = \frac{(y'y - b' L' y - s' z' y)}{N_h - r(L; Z)} \quad [5]$$

where $R(s/b)$ is the additional sire sums of squares after
fitting line effects $b$; $r$ is the number of linearly
independent columns of $L$; $\text{tr}$ is the sum of the diagonal
elements of the matrix or trace operator, and $N_h$ is the
total number of incubation records.

**Likelihood methods**

The ML estimation procedure for a mixed model was
derived by Hartley and Rao (1967). In the present study,
Henderson's (Henderson, 1973) mixed model equations are
applied to the estimation of sire and error components of
variance. Assuming normality ML variances are obtained as

$$\sigma_s^2 = \frac{[s's + \sigma_c^2 \text{tr}(Z'Z + I\lambda)^{-1}]}{n},$$

$$\sigma_c^2 = \frac{(y'y - b'L'y - s'z'y)}{N_h} \quad [6]$$
where \( n_s \) is the number of sires and \( \lambda \) is \( \sigma^2_s/\sigma^2_e \). In iteration, convergence occurs when computed \( \lambda \) is equal to the variance ratio of the previous cycle; \( b \) and \( s \) are solutions to the mixed model equations at convergence as in [3].

Restricted maximum likelihood, like ML, is an iterative procedure; REML corrects the bias in ML that arises from estimating fixed effects. The rationale for REML is that all the information relevant in estimating variance components is combined in any set of \( N_h - p \) linearly independent error contrasts, where \( p \) is the number of fixed effects and the error contrast is defined as a linear combination of observations whose expectation is zero (Scheffe, 1959). In the present study, variance components are estimated as REML using an analog of Henderson's mixed model equations (Harville, 1977). Applying this analog to the model for embryonic mortality gives

\[
\sigma_s^2 = \frac{[s's + \sigma^2_e \text{tr}(Z'Z + I\lambda)]}{n_s} \\
\sigma_e^2 = \frac{(y'y - b'L'y - s'Z'y)/[N_h - r(L)]}{[N_h - r(L)]} \quad [7]
\]
**Minimum Variance Quadratic Unbiased Estimator**

In the "varcomp" procedure of SAS Software (SAS Institute, 1988) a priori values are set at 0 and 1, for $\sigma_s^2$ and $\sigma_e^2$, respectively. With the assumption of normality after arc sine transformation of percentage embryonic mortality in D1 and D3, the vector of MIVQUE is

$$\sigma^2 = \begin{bmatrix} \sigma_s^2 \\ \sigma_e^2 \end{bmatrix}$$

where $\sigma_s^2$ and $\sigma_e^2$ are determined from algebraic equations as in Sorensen and Kennedy (1986).

Estimates of the calculated variance components and heritability were compared by the "Waller-Duncan" multiple comparison option in SAS® Software (SAS Institute, 1988). A separate analysis was made for early (Days 1 to 10) and late (Days 21 to 28) embryonic mortality.
RESULTS AND DISCUSSION

Estimates of sire component of variance for early embryonic mortality determined by Henderson's Method 3 and the MIVQUE procedure were negative but estimates based upon the ML and REML with non-negativity constraints were both zero (Table III.1). Negative variance components obtained for embryonic mortality, Days 1-10, in this study, are hard to interpret, and, as some researchers have suggested, have no value in predicting response to selection (Searle, 1971). However, Nelder (1977) reported that negative estimates may be useful in breeding programs, as these are inherent in certain sampling schemes and can also be a reflection of the inadequacy of the model. The negative estimates reported here might be due to the small number of hatches included in the analysis. It is possible that such estimates would result if heritability for the trait under consideration were low, as it is in the present study. Likelihood estimation procedures, ML and REML, provide estimates confined to the parameter's space. This attribute makes ML and REML methods biased. Hayes and Hill (1981) suggested that this bias cannot be adjusted for in actual field data. Maximum likelihood has the added disadvantage of not accounting for the loss of degrees of
freedom for estimating fixed effects (Patterson and Thompson, 1971). As the number and level of fixed effects increases, the bias becomes more significant. The REML method combines the desirable properties of ML with accountability for loss in degrees of freedom when fixed effects are first estimated. The primary advantage of Henderson's Method 3 as with all ANOVA estimators is that this method is unbiased. However, a consequence of using Henderson's Method 3, as shown (Table III.1) for embryonic mortality in D1 is that negative estimates are often obtained for variance components (Searle, 1971).

Estimates of sire and error variance components for embryonic mortality between 21 and 28 days of incubation are presented in Table III.2. Maximum likelihood estimates of $\sigma_s^2$ and $\sigma_e^2$ were smaller than estimates using Henderson's Method 3, REML, and MIVQUE procedures. The lower estimate from the ML procedure may arise due to its inherent bias under mixed models (Sorensen and Kennedy, 1986). Similar estimates of sire and error variance components were obtained from REML, MIVQUE, and Henderson's Method 3 procedures. This may suggest that the data in the present study were only slightly unbalanced. However, estimates from REML and MIVQUE are not necessarily equal to Henderson's Method 3 sire and error variance components for
a mixed model involving extremely unbalanced data.

Heritability estimates for embryonic mortality in the early and late incubation periods (Tables III.1 and III.2), compare favorably to published estimates (McCartney, 1962; and Shook et al., 1971). The large standard errors associated with the heritability estimates are possibly the result of the small sample size used in the present study. This would suggest that irrespective of the method of estimation reliable estimates for quantitative genetic parameters are more likely with larger data sets. An alternative method of estimation reported to be more efficient is offspring-parent regression, when measurements are made on both parents and offspring (Hill and Nicholas, 1974).

The use of computer programs has greatly enhanced the calculation of variance component estimation by procedures like ML and REML. The "varcomp" procedure of the SAS® system (SAS Institute, 1988) has an option for estimating variance components in mixed models by the different procedures discussed in the present study. The results presented here suggest that likelihood methods, though biased, are more flexible and should be the method of choice over the fitting constants procedure of Henderson (Method 3) for unbalanced data in mixed models.
ACKNOWLEDGEMENTS: The authors express their appreciation to P. E. Bernier and D. R. Thomas, Departments of Animal Sciences and Statistics, respectively, for their critical comments and suggestions.
TABLE III.1  Sire ($\sigma_s^2$) and error ($\sigma_e^2$) variance components and heritability estimates ± SE for embryonic mortality in early incubation period (Days 1 to 10)

<table>
<thead>
<tr>
<th>Method of estimation</th>
<th>Variance component</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\sigma_s^2$</td>
<td>$\sigma_e^2$</td>
</tr>
<tr>
<td>Henderson's Method</td>
<td>-4.56$^a$</td>
<td>251.35$^a$</td>
</tr>
<tr>
<td>Maximum likelihood</td>
<td>.00$^b$</td>
<td>251.48$^a$</td>
</tr>
<tr>
<td>Restricted maximum</td>
<td>.19$^b$</td>
<td>251.49$^a$</td>
</tr>
</tbody>
</table>

Likelihood

Minimum variance quadratic

**unbiased estimation**  -4.24$^a$  251.56$^a$  -.07 ± .07$^a$

*a,b* Estimates in a column with no common superscript are significantly different (P< .05).
<table>
<thead>
<tr>
<th>Method of estimation</th>
<th>Variance component</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\sigma^2_s$</td>
<td>$\sigma^2_e$</td>
<td>$h^2$</td>
</tr>
<tr>
<td>Henderson's Method</td>
<td>9.09(^a)</td>
<td>339.31(^a)</td>
<td>.10 $\pm$.11(^a)</td>
</tr>
<tr>
<td>Maximum likelihood</td>
<td>7.56(^b)</td>
<td>339.24(^b)</td>
<td>.09 $\pm$.14(^b)</td>
</tr>
<tr>
<td>Restricted Maximum Likelihood</td>
<td>9.01(^a)</td>
<td>339.25(^a)</td>
<td>.10 $\pm$.14(^a)</td>
</tr>
<tr>
<td>Minimum variance quadratic unbiased estimator</td>
<td>9.79(^a)</td>
<td>339.13(^a)</td>
<td>.11 $\pm$.09(^a)</td>
</tr>
</tbody>
</table>

\(^a,b\) Estimates in a column with no common superscripts are significantly different ($P<.05$).
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CHAPTER IV

Genetic Variation in the Incidence of Pipped Eggs in Turkeys Selected for Low and High Semen Ejaculate Volume¹

EDWARD J. SMITH, THOMAS F. SAVAGE, and JAMES A. HARPER

Department of Poultry Science
Oregon State University
Corvallis, Oregon 97331-3402

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Genetic variation in embryonic mortality expressed as embryos that pip their eggshell but do not hatch, was investigated in turkeys selected for low and high semen ejaculate volume (SEV). Through five generations (Generations 10 to 14, inclusive) mean heritability estimates for pipped eggs were .21 and .08 in the low- and high-SEV lines respectively. Estimates of sire, dam and within hatch components of variance suggest greater environmental and maternal effects than genetic influences on the incidence of pipped eggs in turkeys.

(Key words: heritability, pipped eggs, genetic variation, semen ejaculate volume, turkeys)
The influence of heredity on semen ejaculate volume (SEV) has been reported to vary from moderate to high (Nestor, 1976; Hales et al., 1989). Selection emphasizing a single economic trait such as SEV often results in rapid genetic progress (Becker, 1984; Falconer, 1989). Of interest in this type of selection program is the population change in unselected traits of economic importance. Savage et al. (1984) reported that with divergent selection for SEV in turkeys, significant line differences (P<.05) were also observed in the incidence of embryos that died during the process of pipping of the egg shell for hatching was observed.

Pingel and Schubert (1983) have reported limited phenotypic associations between egg and semen production traits. However there have not been any reports regarding genetic correlations between egg traits and semen production in turkeys. Heritability estimates for egg traits in turkeys are generally reported to be low (Nestor et al., 1972). Work by McCartney (1962) suggests that reducing the incidence of embryonic deaths through selection can be slow. Christensen (1978) obtained significant improvement in selection for reduced embryonic deaths at 3, 4, and 6 days of incubation but none in days 24 to 28 day, whereas Shook et al. (1971) reported that
selection for increased embryonic mortality reduced the hatchability of fertile eggs in Broad Breasted Bronze turkeys. Shook et al. (1971) also reported heritability values ranging from -.16 to .02 for embryonic mortality in the late incubation period; however there is no literature describing genetic factors influencing embryonic deaths in pipped eggs. The objective of the present study was to determine the heritability for embryos that pipped the egg but did not hatch in Wrolstad Medium White turkeys divergently selected for SEV.
MATERIALS AND METHODS

Birds and Management

Hatchability records of medium white turkeys selected for low- and high- SEV for 14 generations at the Oregon Agricultural Experiment Station were the source of data for the present study. A description of the selection program including management of the birds in the OSU turkey program, has been previously described (Hales et al., 1989). Briefly, 10 toms were each mated to 10 dams for a total of 100 hens within each line. Eggs from each hen were incubated in six consecutive bi-weekly settings. Hatchability records were from eggs incubated inclusively from 1985 to 1989 (Generations 10 to 14). Pipped eggs were defined as embryos that break the eggshell but do not hatch. Deaths in pipped eggs occurred between Days 27 and 29 of incubation. Embryo malposition in the pipped eggs was not determined.

Specific gravity (SG) of eggs in both lines during the fifteenth generation was measured by a modification of the procedure of Arscott and Bernier (1961). Within 24 h after lay, the eggs were collected and stored at 15°C for 6 h. The eggs were then immersed in water for about 20 seconds and subsequently a series of salt solutions with increasing densities from 1.056 to 1.100 in .004 intervals.
STATISTICAL ANALYSIS

Prior to data analysis, percentages of pipped eggs were transformed to arc sine values as the distribution was binomial. Nested analysis of variance was performed within each line among full and half-sib hatches (SAS Institute, 1988). Variance components of pipped eggs were estimated within each line using a random effects model (Becker, 1984):

\[ P_{yk} = \mu + s_i + d_j + h_{jk} \]

Where \( P_{yk} \) = percentage pipped eggs; \( \mu \) = effect common to all observations; \( s_i \) = random effect of the \( i^{th} \) sire; \( d_j \) = random effect of the \( j^{th} \) dam mated to the \( i^{th} \) sire; and \( h_{jk} \) = random residual attributed to the \( k^{th} \) unhatched pipped progeny within dam.

Estimates of heritability were based on half-sib analysis of variance using both sire and dam components (Table 1). Standard errors for the heritability estimates were computed based on Dickerson's approximation (Becker, 1984).

With the assumption that the base population (G0) was randomly mating and heterozygous, genetic drift within the two subpopulations after 14 generations was estimated using the procedure of Kimura, (1955):
He = [1-(1/2Ne)]^n

Where: He is the heterozygosity and Ne is the effective population size in each line within each generation. In a pedigreed population Ne is estimated as \(1/Ne = (3/16M) + (1/16F)\), where M is number of males used for mating; F is the number of females; and n is the total number of generations.
RESULTS AND DISCUSSION

The mean percentage pipped eggs for generations 10 to 14 are summarized in Figure IV.1. Significant differences (P< .05) between the two lines (low > high) for the incidence of pipped eggs were observed as was divergence in mean SEV between lines (P<.01) within this same period (Hales et al.,(1989).

Average heritability estimates (sire component) for pipped eggs were .21 in the low line and .08 in the high line. The standard errors of the estimates were large and may be due to the small number of sires and dams. The heritability estimates reported in the present study may be biased by selection emphasizing SEV. The heritability values suggest variability in the incidence of pipped eggs to be primarily environmental. This inference is supported by the lower estimates of heritability from sire and dam components of variance (Table IV.2).

In the current study, a time trend in the proportion of total, within-generation genetic variance was used as a measure of the effect of selection for SEV on the incidence of pipped eggs. However, the change in the dam components of variance through the generations was not consistent in both lines (Table IV.3). Except for Generations 13 and 14 in the low line, the dam component of variance was larger than the sire component within both lines. The within-
hatch components of variance were larger than the dam and sire components for both lines in all five generations.

The design of the mating system used in the SEV study reported here did not allow for statistical estimates of correlation between SEV and percent pipped eggs. However the direction of change in variance components through the generations suggest a negative relationship between SEV and percentage pipped eggs and suggests that hatchability is influenced by a complex of environmental factors (Ogasawara et al., 1963; Nestor, 1977 and 1984). A study by Abplanalp and Kosin (1953), indicates that these environmental effects are largely maternal. The difference in the incidence of pipped eggs between the two lines is perhaps a function of divergent selection for SEV as the hens of both lines were maintained in same breeder house and all eggs were incubated and hatched in the same unit. Other unselected phenotypic traits that have diverged with selection for SEV through the generations include body weight (low > high) and egg size (Savage et al., 1984). For body weight, the high line was heavier and therefore expected according to Strong and Nestor (1980) to have reduced embryonic mortality (including the incidence of pipped eggs). In an attempt to identify other physical differences that might explain the line differences in pipped eggs, SG of low- and high- line eggs in Generation 15 was measured. Mean SG was not different (P>.05) between
the lines, 1.092 and 1.089, low and high lines respectively. This observation suggests that shell quality may not be a cause of the observed differences in the incidence of pipped eggs.

The asymmetry in heritability estimates for pipped eggs might be a result of random genetic drift, linkage disequilibrium, inbreeding or differential environmental effects in both lines. Estimated heterozygosity in Generation 14 was 87% in both lines, which indicates that most of the segregating loci in the two populations are still heterozygous. This may explain why after 14 generations of selection for SEV, a significant divergence in both SEV and the incidence of pipped eggs is still observed. It appears from the estimated heterozygosity that drift is negligible. Also the lack of trend in fitness traits such as percent hatch of fertile eggs (data not shown) for the two populations suggests that these factors did not significantly affect the populations. The asymmetry in the heritability estimates is therefore suggested to be from the effect of selection for SEV on physical characteristics including egg size and body weight. Reports suggest that these traits are related to hatchability of fertile eggs (Christensen, 1978). Such traits were better for the high line.

There are two implications of the present study. First, selection emphasizing reduced incidence of pipped
eggs may not lead to significant improvement in this trait. Second, hatchability may not be increased by direct selection for decreased pipping incidence. The normal practice of selecting males for high semen volume, is reported here to have the added advantage of reducing embryonic deaths in the late incubation period. These deaths have been reported to account for about 40 percent of all embryonic deaths (Christensen, 1978).
ACKNOWLEDGMENTS We thank Drs. P.E. Bernier, and S. Knapp for their comments and suggestions and L.W. Mirosh for assistance in determining specific gravity.
Figure IV.1. Mean incidence of percentage pipped eggs that did not hatch in Medium White turkeys selected for low (white bar) and high (black bar) semen ejaculate volumes between generations 10 and 14.
TABLE IV.1. Model of analysis of variance for incidence of pipped eggs in low and high semen volume lines

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Mean squares</th>
<th>Interpretation of variance components</th>
<th>Degrees of freedom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sires</td>
<td>$\sigma_s^2 + \sigma_d^2 + \sigma_h^2$</td>
<td>$\sigma_s^2 = .25 \sigma_g^2$</td>
<td>$N_s - 1$ 9</td>
</tr>
<tr>
<td>Dams</td>
<td>$\sigma_d^2 + \sigma_h^2$</td>
<td>$\sigma_d^2 = .25 \sigma_g^2 + \sigma_c^2$</td>
<td>$N_d - N_s$ 90</td>
</tr>
<tr>
<td>hatches</td>
<td>$\sigma_h^2$</td>
<td>$\sigma_h^2 = \sigma_e^2 + .5 \sigma_g^2$</td>
<td>$N_h - N_d$ 500</td>
</tr>
</tbody>
</table>

$\sigma_g^2$ is the additive genetic variance; $\sigma_e^2$ is the variance due to the environment; $\sigma_c^2$ is the variance arising from maternal effects; and $N_s$, $N_d$, and $N_h$ are the number of sires, dams, and hatches respectively.
<table>
<thead>
<tr>
<th>Generation</th>
<th>Line</th>
<th>Sire</th>
<th>Dam</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>L</td>
<td>.01 ± .03</td>
<td>.47 ± .43</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>.08 ± .05</td>
<td>.18 ± .30</td>
</tr>
<tr>
<td>11</td>
<td>L</td>
<td>.10 ± .08</td>
<td>.56 ± .48</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>.12 ± .05</td>
<td>.26 ± .11</td>
</tr>
<tr>
<td>12</td>
<td>L</td>
<td>.16 ± .08</td>
<td>.31 ± .26</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>.13 ± .17</td>
<td>.08 ± .04</td>
</tr>
<tr>
<td>13</td>
<td>L</td>
<td>.45 ± .63</td>
<td>.26 ± .28</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>.06 ± .05</td>
<td>.47 ± .39</td>
</tr>
<tr>
<td>14</td>
<td>L</td>
<td>.31 ± .24</td>
<td>.29 ± .32</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>.01 ± .03</td>
<td>.13 ± .04</td>
</tr>
<tr>
<td>Mean</td>
<td>L</td>
<td>.21 ± .21</td>
<td>.38 ± .35</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>.08 ± .07</td>
<td>.22 ± .18</td>
</tr>
</tbody>
</table>
TABLE IV.3. Components of variance within five generations in low (L) and high (H) semen volume line

<table>
<thead>
<tr>
<th>Generation</th>
<th>Line</th>
<th>Sire</th>
<th>Dam</th>
<th>Hatch</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>L</td>
<td>5.18</td>
<td>45.90</td>
<td>331.51</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>8.06</td>
<td>18.38</td>
<td>374.94</td>
</tr>
<tr>
<td>11</td>
<td>L</td>
<td>9.25</td>
<td>52.16</td>
<td>308.59</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>11.90</td>
<td>25.66</td>
<td>361.67</td>
</tr>
<tr>
<td>12</td>
<td>L</td>
<td>15.53</td>
<td>30.14</td>
<td>341.45</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>8.70</td>
<td>5.46</td>
<td>250.66</td>
</tr>
<tr>
<td>13</td>
<td>L</td>
<td>40.95</td>
<td>24.20</td>
<td>301.86</td>
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<tr>
<td></td>
<td>H</td>
<td>4.51</td>
<td>38.59</td>
<td>282.34</td>
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<tr>
<td>14</td>
<td>L</td>
<td>26.46</td>
<td>24.64</td>
<td>285.51</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>.49</td>
<td>9.89</td>
<td>295.51</td>
</tr>
</tbody>
</table>
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CHAPTER V

Genetic Variation in Total Plasma and High-Density Lipoprotein Cholesterol and Body Weight in Medium White Turkeys

EDWARD J. SMITH and THOMAS F. SAVAGE

Department of Animal Sciences,
Oregon State University,
Corvallis, Oregon 97331-3402

'Southern Regional Research Project Number 213. Oregon Agricultural Experiment Station Technical Paper Number 9738.

ABSTRACT

Plasma total cholesterol (PC) and its high-density lipoprotein cholesterol (HDLC) fraction were determined in 700 16-wk-old Medium White turkeys in Generation 15 of selection for low and high semen ejaculate volumes (SEV). The birds examined were from pedigree matings involving 10 sires mated to 100 dams in each line. Mean PC and HDLC were not different between lines and between sexes within lines. Genetic parameters were estimated for PC, HDLC, and BW. Sire component heritabilities estimated based on pooled data from low and high SEV lines for PC and HDLC were -.03 and .26, respectively. Genetic correlations were .05 between PC and HDLC; .13 between PC and BW; and -.34 between HDLC and BW. It was concluded that variation in PC in 16-wk-old Medium White turkeys is largely due to environmental variation and that the genetic component is largely non-additive. However, a moderate additive genetic component was observed for HDLC. Further, determination of total PC and HDLC at 16 wks of age does not exhibit a potential value as an indicator of the males future SEV potential.

Key words: Semen ejaculate volume, heritability, genetic correlation, cholesterol, high-density lipoprotein cholesterol
INTRODUCTION

In turkeys (Meleagris gallopavo) the cause of the difference in semen volume between males regarded as low and high producers is undetermined. It has been reported, however, that the capacity for semen storage in the two groups is different (Cecil and Bakst, 1984). These workers suggested that steroid hormones may, during the male's development, influence semen production and its temporal storage. High semen volume producers are reported to have higher storage capacity for semen in the ductus deferens, which constitutes a large percentage of the total ejaculate (Cecil and Bakst, 1984). It has therefore been reasoned that one of the effects of androgens may be in the development of the ductus deferens. Cecil and Bakst (1988) in a subsequent study reported no differences in testosterone levels of 35- to 40-wk old randombred turkeys, classified as low and high volume semen producers. Diurnal changes in plasma levels of testosterone (de Kretser, 1982) suggests that the measurement of other related but more consistent blood parameters may be necessary to establish the role of steroid hormones in semen production in turkeys. Because cholesterol is a major precursor in the biosynthesis of steroids (Cook, 1958), the possibility that this intermediate could be an indicator of the ability to produce high or low volume of semen should be investigated.
Establishing this relationship requires discerning the relative importance of heredity on both SEV and cholesterol levels and distribution. Genetic control of semen production has been reported to be high (Hales et al., 1989; Nestor, 1976).

Factors that influence plasma cholesterol (PC) in chickens and turkeys have been reported to include diet (Burger et al., 1984), age (Estep et al., 1969; Kelley and Alaupovic, 1976), sex (Krista et al., 1970), and breed (Krista et al., 1978). Undetermined in turkeys, however, is the role of heredity on PC (Alaupovic and Dashti, 1983), although hereditary control of PC in other animal species has been reported, including chickens (Cherms et al., 1960; Sutton et al., 1983), humans (Robertson, 1981), and baboons (Kammerer et al., 1984). Although very little is known about the factors influencing the distribution of cholesterol among lipoprotein fractions, indications are that such knowledge as related particularly to high-density lipoprotein cholesterol (HDLC) in turkeys is more significant because it is a predictor of spontaneous dissecting aneurysm, a concern in the turkey industry (Kelley and Alaupovic, 1976; and Pagnan et al., 1980).

Estimates of genetic parameters for PC and serum cholesterol (SC) in avian species have been reported. Bumgardner (1955), as reported by Cherms et al. (1960), obtained a heritability estimate of .42 for SC in New
Hampshire chickens. In two separate studies, Cherms et al. (1960) and Wilcox et al. (1963) reported heritabilities for SC in White Leghorns of .30 and .25, respectively. Hollands et al. (1980), using 9 to 10-wk-old White Leghorns, reported combined heritability estimates for PC of .28 and .20 in males and females respectively, in five generations of random mating. Burke (1986), in the fourth generation of a divergent selection program for PC in four-week-old Coturnix quail, reported mean heritabilities of -.07 and .38 in the low and high lines, respectively. Though no studies have been reported for turkeys, the relationship between PC, SC and economic traits in chickens have also been investigated. Wilcox et al. (1963) reported a phenotypic correlation of -.19 between SC and BW at 6 WOA. At 8 WOA, a genetic correlation of .37 between SC and BW was obtained. Genetic correlations of -.13 and .02 between PC and BW in chickens at 21 and 71 WOA, respectively, were reported by Hollands et al. (1980).

Smith and Savage (1991) in a preliminary report described a study involving PC and its HDLC fraction in turkeys selected for low and high semen ejaculate volumes. The rationale for that study was that variation in cholesterol, a precursor in the biosynthesis of steroids, may be influenced by changes in SEV through long-term selection. Thus, the present report describes estimates of genetic parameters for PC, HDLC, and BW a performance trait
reported to influence PC in other animal species (Kammerer et al., 1985).
MATERIALS AND METHODS

Population Structure

Wrolstad Medium White turkeys used in the study were from Generation 15 of divergent selection for SEV. Hales et al. (1989) have previously described the genetic structure and management of the lines involved in this study. Mean SEV for the low and high line males in Generation 15 were .24 and .60, mL respectively. A total of 350 birds, males and females, from each line representing 200 pedigree matings and managed according to standard Oregon State University practices were used. Data from measurements on a minimum of three progeny selected at random from each pair mating was used in the analysis.

Divergence for SEV in the two lines continues to be significant, indicating large variation and the presence of segregating genes affecting semen volume in the two populations (Smith et al., 1991).

Total Plasma and High Density Lipoprotein Cholesterol Analyses

Both low- and high-line populations were housed on the same grass-covered range and fed on a 15% crude protein (3148 kcal/kg, ME) grower mash ration. The birds were fasted for 12 to 16 h prior to blood collection but were permitted ad libitum access to water. Blood was collected
from the brachial vein by venipuncture into 3-mL sodium heparin vacuum blood collection tubes\(^1\) and immediately placed in crushed ice.

Plasma samples obtained by centrifugation at 700 x g for 10 minutes were subsequently divided into equal aliquots for PC and HDLC analyses. The PC determinations were performed either immediately or the aliquots were stored at -80 C and analyzed within 5 days of collection. All HDLC determinations were performed on samples stored at -80 C and analyzed within 5 days of collection.

Total PC was determined by the enzymatic procedure of Allain \textit{et al.} (1974). Fifty microliters of plasma was mixed with 2 ml of the Cholesterol Reagent\(^2\) and incubated at 37 C for 25 min. Absorbance of the resulting quinoneimine product was measured at 500 nm and the cholesterol concentration determined.

Quantitation of plasma HDLC was performed by a modification of the polyethylene glycol (PEG, Molec. Wt. 6000) precipitation procedure of Demacker \textit{et al.} (1980). To 200 \(\mu\)L of plasma, PEG was added to a final concentration of 80 mM, then thoroughly mixed using a vortex for 5 min, followed by centrifugation at 10,000 x g for 15 min. Cholesterol concentration in the supernatant, the HDLC

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\(^1\)Terumo Medical Corporation, Elkton, MD 21921.

\(^2\)Diagnostic Chemicals Limited, Charlottetown, PEI, C1E 1BO, Canada.
fraction, was determined by the previously described procedure of Allain et al. (1974). A dilution factor of 3 was used to adjust for the reductions in plasma HDLC due to addition of PEG. The handling of the plasma samples and subsequent determination of PC and HDLC conformed with the criteria of the Center for Disease Control Lipid Standardization program (Lipid Research Clinics Program, 1982).

Statistical Analysis

Sire, dam, and error variance components for PC, HDLC, and BW were estimated according to Henderson's Method 2, (Henderson, 1953). This procedure allowed for the correction of body weight data for differences between sexes within lines. A mixed model was used in the analysis of the data (SAS Institute, 1988):

\[ y_{ijkl} = \mu + L_i + s_{ij} + d_{jk} + e_{ijkl} \]

where \( y_{ijkl} \) is total PC, HDLC, or 16-wk BW on the lth progeny; \( L_i \) is the fixed effect of the \( i \)th SEV line; and \( s_{ij}, d_{jk}, \) and \( e_{ijkl} \) are random effects due to the \( j \)th sire, \( k \)th dam, and \( l \)th residual error, respectively.

Sire, dam, and error variance components were used to estimate heritabilities for PC and HDLC and to calculate phenotypic and genetic correlations among PC, HDLC, and BW
(Falconer, 1989).

Standard errors were estimated according to Dickerson's approximation as reported by Becker (1985).
RESULTS AND DISCUSSION

Mean PC, HDLC, and BW values are presented in Table V.1. Mean total PC concentration varied from 2.45 to 2.78 mM in the low-SEV females and males and from 2.55 to 2.68 mM in high-line females and males, respectively. Differences in mean PC levels between lines and between sexes within lines were not significant (P>0.05). Sex differences in BW was adjusted for using least squares. Sex and line interaction for BW was not significant. These results are consistent with published reports that nonlaying male and female turkeys have similar PC levels (Krista et al. 1978). The mean levels of PC in both lines suggest that the strain of turkey used in this study, Wrolstad Medium White could be hypotensive. Levels of PC in hypertensive turkeys, such as the Broad Breasted Bronze, are twice those reported in this study (Speckman and Ringer, 1962).

Line and between sex within lines differences were also not significant for HDLC (Table V.1). Based upon the results presented, the HDLC concentrations determined constitute between 7 and 7.5% of PC. Line differences in PC and HDLC were not significant (P>0.05), suggesting that selection for SEV may not influence PC and its distribution in 16-wk-old turkeys, even though a major precursor for the biosynthesis of steroid hormones (Cook, 1958). As with the
reports of Cecil and Bakst (1988), attempting to associate testosterone levels with semen production, the results of the current study do not indicate that PC measurement at 16 wk of age is a viable biochemical indicator for SEV. It is plausible, however, that genetic variation in cholesterol levels influences steroid biosynthesis, but that the latter plays no significant role in semen production nor in the storage capacity of the ductus deferens in turkeys. There is, however, adequate published evidence from exogenous testosterone administered to male birds that suggest a role for steroid hormones in semen production (Lake and Furr, 1971; Fujihara and Nishana, 1976; and Fujihara et al. 1983).

With line and sex differences not significant for PC and HDLC, the data from both lines were pooled to estimate variance components and genetic parameters. The heritability estimate of .31 (Table V.2) for BW is lower than the mean of recent reported estimates (Buss, 1990). Sire component heritabilities estimated for PC and HDLC were -.03 and .26, respectively (Table V.2). For PC, the heritability estimate suggests that individual differences in PC levels are influenced by factors other than additive genetic variation. This inference is bolstered by the higher estimates of heritability derived from the dam components of variance (Table V.2). This outcome is consistent with the report of Burger et al. (1984) that
diet and environmental factors significantly influence PC. Wilcox et al. (1963) reported a heritability estimate of .36 for SC, based upon studies with 6-wk-old chickens, indicating an additive genetic control. The difference between the study of Wilcox et al. (1963) and the estimate reported in the current study could be due to species and age differences as well as the fact that the chicken populations were selected for levels of serum cholesterol. Heritability estimates of serum cholesterol can be influenced by age (Kammerer et al., 1984). For baboons selected for high serum cholesterol, heritability estimates varied from .16 to .37 at 6 and 24 mo of age, respectively.

The HDLC heritability value determined in this study, .19 based on dam variance components was lower than the sire estimate of .26 (Table V.2). These heritability estimates suggest that genetic factors influence the expression of HDLC. Similar conclusions were derived from studies involving baboons (Kammerer et al. 1984) and humans (Robertson and Cumming, 1979).

Genetic and phenotypic correlations between PC and HDLC for the combined lines and sexes at 16 wk of age were .05 and .31, respectively (Table V.3). Although the phenotypic correlation was moderate, it suggests that the distribution of cholesterol among lipoprotein fractions may be controlled largely by nongenetic factors. The results
significantly by PC. It would appear from the results presented here for correlations among PC, HDLC, and BW that selection for BW will influence HDLC but not PC. Birds with larger BW had significantly lower HDLC, which could imply a higher risk of spontaneous dissecting aneurysms in turkeys, considering that lower HDLC is reported to be associated with coronary artery disease in humans (Sing and Moll, 1990). The results from this study suggest that HDLC could be a physiological marker for BW improvement in turkeys; however, this would require further study.
ACKNOWLEDGMENTS The authors express their appreciation to P. Rossignol, Department of Entomology, for laboratory facilities to carry out part of the work and to P.E. Bernier and R. Wander of the Departments of Animal Sciences, and Nutrition and Food Management, respectively, for their comments and suggestions.
TABLE V.1. Mean ± SE total plasma cholesterol, high density lipoprotein cholesterol and body weights at 16 weeks of age in low and high semen ejaculate volume lines

<table>
<thead>
<tr>
<th></th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Number of Birds</td>
<td>125</td>
<td>225</td>
</tr>
<tr>
<td>Total plasma cholesterol mM</td>
<td>2.78 ± .67</td>
<td>2.45 ± .14</td>
</tr>
<tr>
<td>High density lipoprotein cholesterol mM</td>
<td>.37 ± .08</td>
<td>.35 ± .11</td>
</tr>
<tr>
<td>Body weight Kg</td>
<td>5.85 ± 1.33\textsuperscript{a}</td>
<td>4.15 ± 1.92\textsuperscript{b}</td>
</tr>
</tbody>
</table>

\textsuperscript{a,b} means within rows with the same superscript are not different (P>.05)
Table V.2. Heritabilities ± SE of 16-wk body weight, total plasma cholesterol, and high density lipoprotein cholesterol in Medium White turkeys based on sire ($h^2_s$) and dam ($h^2_d$) components of variance

<table>
<thead>
<tr>
<th>Trait</th>
<th>$h^2_s$</th>
<th>$h^2_d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total plasma cholesterol</td>
<td>-.03 ± .08</td>
<td>.15 ± .11</td>
</tr>
<tr>
<td>High density lipoprotein cholesterol</td>
<td>.26 ± .14</td>
<td>.19 ± .09</td>
</tr>
<tr>
<td>Body weight</td>
<td>.31 ± .19</td>
<td>.25 ± .17</td>
</tr>
</tbody>
</table>
Table V.3. Estimates of genetic (above diagonal) and phenotypic correlations (below diagonal) among total plasma cholesterol (PC), high density lipoprotein cholesterol HDLC), and 16-wk body weight (BW) in Medium White turkeys

<table>
<thead>
<tr>
<th>Trait</th>
<th>PC</th>
<th>HDLC</th>
<th>BW</th>
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</thead>
<tbody>
<tr>
<td>PC</td>
<td>.05</td>
<td>.13</td>
<td></td>
</tr>
<tr>
<td>HDLC</td>
<td>.31*</td>
<td></td>
<td>-.34*</td>
</tr>
<tr>
<td>BW</td>
<td>.23*</td>
<td>.10</td>
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* P< .05.
REFERENCES


CHAPTER VI

Classification of Turkeys as Low or High Semen Volume Producers Using Discriminant Function Analysis

ABSTRACT

Medium White turkeys in generation (G) 10 of divergent selection for semen ejaculate volume were classified as low or high volume semen producers, using a multivariate discriminant analysis technique. The discriminant function was generated from records of hen fertility, embryonic mortality by incubation periods and the incidence of pipped eggs. A hit ratio, the proportion of birds correctly categorized as low or high volume semen producers, of .60 was obtained. The discriminant function was then used to classify birds in G 12 of selection. A misclassification rate of .28 and .48 was obtained for the low and high semen producing birds, respectively. It was concluded that the relatively low rates of misclassification, indicates a potential method for selecting turkeys for semen ejaculate volume based on fertility and incubation records.
INTRODUCTION

Selection of male turkeys for semen ejaculate volume (SEV) is usually performed after the birds have attained sexual maturity at 32 weeks of age (Cecil and Bakst, 1984). If the identification of males prior to this age could be achieved, it would result to savings, both in time and financial cost. There are two approaches for this identification to be accomplished. First, if a biochemical and/or molecular marker associated with SEV could be identified, then efficient selection could theoretically be performed at any age (Shuman, 1990). The successful application of discriminant analysis (DA) in marketing (Crask and Perrault, 1977; Massy, 1965; Perrault et al., 1977); and medical research (Solberg, 1975; Ellis and Goldberg, 1979; Ionasescu et al., 1980; Percy et al., 1982; Plomteux, 1980) suggests that this statistical technique could also be used to classify birds as low or high volume semen producers. The theoretical basis of DA has been the subject of numerous reviews (Cochran and Hopkins, 1961; and Kendall and Stuart, 1968). In general, DA provides information on the distance or the distinction between two groups, based on quantitative differences in specific traits. In the use of this procedure, the primary objective is to identify variables or traits that best distinguish two populations or groups (Hair, et al., 1987).

In the present study, DA was applied to reproductive
and hatchability traits in the classification of male turkeys as low or high volume semen producers. The validity of the discriminant function developed from observations on birds in G 10 of selection for SEV was tested by categorizing birds produced in G 12.
MATERIALS AND METHODS

Incubation records

Records of reproductive and hatchability traits on birds in generation 10 and 12 of divergent selection for semen ejaculate volumes were the source of data for this study. The origin and management of the populations have been previously described (Hales et al., 1989; and Smith et al., 1991). To maintain the lines in each generation, 10 toms are each mated to ten hens, and incubation data collected from 6-biweekly hatches.

Traits included in the analysis were percent hen fertility, hatch of fertile eggs, embryonic mortality in early (Days 1-10) and late (Days 21-28) incubation periods, and the incidence of pipped eggs.

Statistical procedures

Reproductive and hatchability traits included in the analysis consisted of arc sine transformations of percent fertility, hatch of fertile, embryonic mortality in early (Days 1 to 10, D1) and late (Days 21-28, D3) incubation periods and the incidence of pipped eggs. It is expected that DA will provide the extent to which these traits determine the distance between the low and high SEV lines.
A stepwise method, where only variables for which low and high lines differ significantly (P< .05) were selected to estimate the discriminant function. The stepwise procedure only allows variables that maximize the Mahalanobis squared (D^2) distance through a default F-value (SAS Institute, 1988) to be included in the model (Anderson, 1958; Hair et al., 1987). The model used in the analysis assuming near normality after arc sine transformation is:

\[ L(x) = c_0 + c_1 f + c_2 d + c_3 e + c_4 p \]

Where \( L(x) \) is the linear discriminant function normally referred to as a discriminating score, \( c_0 \) is a constant and \( c_1 \) through \( c_4 \) are the weighting coefficients for percent fertility (f), embryonic mortality in D1 (d), D2 (e), and pipped eggs (p) respectively. A turkey in generation 12 is designated as low if \( L(x) \) (Table VI.1) has a value > 22.08 and as high if < 22.08. The extent to which the low and high lines are separated using the variables included in estimating the discriminant function \([L(x)]\) is determined by \( D^2 \), the Mahalanobis squared distance (Blacklith and Reyment, 1971). The validity of the analysis developed using generation 10 data was tested using records of birds in generation 12.
RESULTS AND DISCUSSIONS

A summary of the analysis based on which the discriminant function was developed in G 10 is presented in Table VI.1. The low and high SEV lines did not differ (P > .05) for percent hatch of fertile eggs and biweekly egg production. These traits were thus not included in developing the discriminant function. The matrix developed from DA for reclassification of G 10 birds is presented in Table VI.2. Using a prior probability of .5, that is, at sexual maturity each bird has an equal chance of being categorized as a low or high volume semen producer, the total error of classification was .36 (or 36 percent). The hit ratio, or how well the discriminant function reclassified low and high line birds, was .64. The misclassification rates were .31 and .41 in the low and high lines, respectively. The criterion of classification is presented in Table VI.1. Variables or traits for which the low and high SEV lines differed significantly (P < .05) include, fertility, EM in D1 and D3, and pipped eggs. These were included in the model used for classification. It would appear that the degree of misclassification is large. However, a better than 50 percent chance of correctly identifying birds as low or high volume semen producers would reduce the need for maintaining a large flock of males to sexual maturity. The discriminant
function developed here is better able to identify birds as low rather than high volume semen producers. This procedure could therefore, theoretically be used to cull low SEV birds in a population before sexual maturity.

Using the discriminant function developed in G 10, birds in G 12 were classified into low and high SEV lines (Table VI.2). In the low line, the misclassification rate was .28 but .48 in the high. However the hit ratio of .65 suggests that DA can be used with a high degree of reliability to distinguish between low and high volume semen producers. Based on actual measurements of volume of semen produced by birds at sexual maturity in G 12, .52 and .48 proportion of birds were classified as low and high lines, respectively. It would thus appear that the prediction of .60 and .40 as the proportions of low and high semen producers in G 12 based on DA of G 10 data is significant and acceptable.

The traits for which the low and high lines differed in G 10 (Table VI.1) were also used to develop a discriminant function based on records of birds in G 12. This would indicate the general applicability of the discriminant function developed based on these traits for categorizing birds as low or high volume semen producers (Table VI.4). Based on the hit ratio of .65, classification from DA using incubation records in G 12, to also suggest that the identification of male turkeys based on
reproductive and hatchability data cannot be considered wholly as a chance event.
Table VI.1. Multiple discriminant analysis of data in generation 10 of divergent selection for semen ejaculate volume

<table>
<thead>
<tr>
<th>Line</th>
<th>Number of Settings</th>
<th>Trait</th>
<th>p value$^1$</th>
<th>Coefficient$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>488</td>
<td>% Fertility</td>
<td>.0001</td>
<td>.72</td>
</tr>
<tr>
<td>High</td>
<td>586</td>
<td>% Mortality (DI)</td>
<td>.003</td>
<td>.64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>% Mortality (DIII)</td>
<td>.03</td>
<td>.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>% Pipped eggs</td>
<td>.003</td>
<td>.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>% Hatch of fertile eggs</td>
<td>.61</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Biweekly egg production</td>
<td>.44</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Constant</td>
<td>-</td>
<td>-31.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Squared distance ($D^2$)</td>
<td>-</td>
<td>.085</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Discriminant score</td>
<td></td>
<td>22.08</td>
</tr>
</tbody>
</table>

$^1$ Probability of non-significant effect on semen production

$^2$ Relative contribution of trait to the discriminant score
Table VI.2. Classification matrix from multiple discriminant analysis of data in generation 10 of divergent selection for semen ejaculate volume

<table>
<thead>
<tr>
<th>From line</th>
<th>To line</th>
<th>Low</th>
<th>High</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Low</td>
<td>313</td>
<td>220</td>
<td>533</td>
</tr>
<tr>
<td></td>
<td>Misclassification rate</td>
<td>.31</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>Low</td>
<td>148</td>
<td>337</td>
<td>485</td>
</tr>
<tr>
<td></td>
<td>Misclassification rate</td>
<td>-</td>
<td>.41</td>
<td></td>
</tr>
<tr>
<td>Error rate</td>
<td></td>
<td>.31</td>
<td>.41</td>
<td>.36</td>
</tr>
</tbody>
</table>
Table VI.3. Classification matrix of generation 12 birds based on generation 10 discriminant function

<table>
<thead>
<tr>
<th>From line</th>
<th>To line</th>
<th>Low</th>
<th>High</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td></td>
<td>382</td>
<td>151</td>
<td>533</td>
</tr>
<tr>
<td>High</td>
<td></td>
<td>227</td>
<td>258</td>
<td>485</td>
</tr>
</tbody>
</table>

- Misclassification rate = .28
- Error rate = .48
Table VI.4. Classification matrix from multiple discriminant analysis of data in generation 12 of divergent selection for semen ejaculate volume

<table>
<thead>
<tr>
<th>From line</th>
<th>To Line</th>
<th>Low</th>
<th>High</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td></td>
<td>273</td>
<td>215</td>
<td>488</td>
</tr>
<tr>
<td></td>
<td>Misclassification rate</td>
<td>-</td>
<td>.44</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td></td>
<td>240</td>
<td>346</td>
<td>586</td>
</tr>
<tr>
<td></td>
<td>Misclassification rate</td>
<td>.41</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Error rate</td>
<td>.44</td>
<td>.41</td>
<td>.43</td>
</tr>
<tr>
<td></td>
<td>Squared distance</td>
<td>-</td>
<td>-</td>
<td>.44</td>
</tr>
</tbody>
</table>
REFERENCES


CHAPTER VII

SUMMARY AND CONCLUSIONS

1: Genetic variation in low and high semen ejaculate volume lines.

From incubation records on embryonic mortality, methods of estimation of variance components for genetic parameters were compared. The likelihood procedures, maximum and restricted maximum likelihood, were shown to give estimates of heritability within the parameter space. Negative estimates were obtained from minimum variance quadratic unbiased estimation and Henderson's Method 3 procedures. It is suggested that likelihood procedures be used for unbalanced genetic data in poultry selection programs. Low heritability estimates were obtained for embryonic mortality (EM) in early (Days 1-10) and late (Days 21-28) using combined data from low and high semen ejaculate volume (SEV) lines. Similarly, low though differing estimates were obtained for the incidence of pipped eggs in the two lines. The low heritability values indicate that any observed changes in embryonic mortality, the incidence of pipped eggs and total plasma and high density lipoprotein cholesterol resulting from selection for SEV has a limited genetic basis. The low values also suggest that improvement by genetic selection for reduced
EM and the incidence of pipped eggs would be slow. Thus it is recommended that traditional selection programs not be the primary emphasis for the improvement of EM and the incidence of pipped eggs in turkeys. Such practices should be accompanied by stronger management and environmental control.

2: Biochemical markers

Cholesterol, a major intermediate in steroid biosynthesis was compared between the lines. No differences were observed in plasma (PC) and high density lipoprotein (HDLC) concentrations between sexes within the low and high lines. Levels of PC and HDLC would therefore not be suitable prognosticators of SEV production in 16 week-old turkeys. The heritability estimates for plasma, -.03 and high density lipoprotein cholesterol concentrations, .26 also showed that the hereditary control of individual variation in PC of the populations at 16 weeks of age is negligible, but significant though low for HDLC. Genetic improvement of PC and HDLC through selection, can therefore be slow. A moderate, -.34, but significant negative genetic correlation was obtained between HDLC and body weight. Therefore, the concentration of HDLC in 16-week old turkeys is a possible biochemical marker for improving body weight in turkeys.

Although no enzyme polymorphisms were observed at the
enzyme loci studied, further studies may be necessary using the methods developed in the present work to determine the frequency of alleles at these loci. Such information could assist in defining the role of genetic drift and the environment on changes in unselected traits in the low and high SEV lines.

3: Discriminant analysis
A discriminant score was developed based upon fertility and incubation records in generation 10 to classify males as low or high volume semen producers. A misclassification rate of .41 and .31 was obtained in the low and high lines, respectively. Birds in generation 12 were classified based on the discriminant function from generation 10. A misclassification rate of .28 and .48 in the low and high lines, respectively, was reported. Based on these results, it is possible to identify birds for their potential SEV capabilities based primarily on fertility and incubation records.
BIBLIOGRAPHY


Lande, R., 1981. The minimum number of genes contributing to quantitative variation between and within populations. Genetics 99:541-553.


APPENDIX

Isozyme Variation in Medium White Turkeys Divergently Selected for Semen Ejaculate Volumes

ABSTRACT

Detection of Isozyme variation in Wrolstad Medium White turkeys selected for divergent semen ejaculate volume was attempted using cellulose acetate electrophoresis. Plasma and red blood cells were obtained from 20 birds at 5 weeks of age in each SEV line, and 50 birds, 10 males and 40 females respectively, in each line at 39 weeks of age. Though distinct protein bands were observed for the ten enzymes examined, no polymorphism or variants of the same enzyme, were reported. It is suggested that with availability of funds this study should be continued using methods described here.
Minimal information is known about allozymes in turkeys (*Meleagris gallopavo*) (Merat, 1990; Savage, 1990). Reports of changes in allele frequencies at polymorphic protein loci, in turkeys, that may result from long-term selection for quantitative traits are even fewer (Merat, 1990; and Savage, 1990). Ranjan et al. (1974) reported that the frequency of alleles at the serum alkaline phosphatase locus in chickens vary with selection for performance efficiency index in layers. In a randombred population of chickens, allelic frequency at the alkaline phosphatase locus (akp) was reported to vary with semen production in chickens (Ranjan et al., 1974; Rao et al., 1980). In mammalian species there is extensive literature on biochemical variants that have aided in the establishment of linkage relationships for most chromosomes (Garnett and Falconer, 1975; Harris and Hopkinson, 1976; Harris, 1981). An initial step in establishing linkage maps in turkeys, would therefore require an understanding of the relationship and inheritance of isozymes and other biochemical variants as has been shown for humans and other mammalian species.

Established turkey populations from a long-term
selection experiment for low and high semen ejaculate volumes (SEV) are available only at the Oregon State University Agricultural Experiment Station (Smith et al., 1991). These populations offer a unique opportunity to study the effects of long-term selection for SEV on frequencies of alleles at enzyme loci.

Thus in the following limited study, variation in 10 enzyme systems in turkeys divergently selected for SEV were examined. It was anticipated that information obtained may aid in distinguishing genetic effects due to linkage disequilibrium and random genetic drift on correlated changes in the low and high SEV lines. If allozyme frequency could be determined and correlated with SEV, the type of gene action involved in the expression of this quantitative trait (QT), could be inferred.
Population

The strain of birds used in the study was Wrolstad Medium White turkeys in generation 17 of divergent selection for SEV. The origin, history and management of these populations have been described (Hales et al., 1989; Smith et al., 1991). The birds evaluated consisted of two ages, 5 and 39 weeks of age with mean body weights of 742 and 10,487 g, respectively.

Electrophoretic assays

Selection of enzymes for use in this study was based on the likelihood of variation or polymorphism for each enzyme system. The loci selected for study (Table VI.1) have been reported to be polymorphic in other avian species (Merat, 1990).

Blood was collected from the brachial vein into heparinized 3 mL vacutainer tubes, immediately placed on ice, then centrifuged at 3000 X g for 5 minutes. The plasma was subsequently decanted and the red blood cells (RBC) were washed twice in .75 percent saline. The plasma and RBC
were stored separately at -80 C until analysis. Cellulose acetate enzyme electrophoresis (Helena Laboratories, Beaumont, Texas) was performed according to documented procedures of Kohn, (1976); and Richardson et al. (1986). Electrophoretic separation times varied from 30 to 60 minutes at 200 V. Cellulose acetate plates of dimension 60 mm X 70 mm were used. Specific protocols including soaking and electrophoretic separation buffers and stain recipes are presented in Appendix I. Lactate dehydrogenase and phosphoglucomutase isoenzymes were detected on the same plate using a common staining procedure.

Electrophoretic conditions and Stain recipes were adapted from Richardson et al., (1986), for enzymes examined in the study. Except otherwise noted, electrophoretic separation conditions were as follows: Gel plates were soaked: 45 min-24 hours; Position of sample application was: cathodic with migration being anodic; Voltage: 180-240; separation: 20-60 min depending on the assay. To each stain recipe 2 mL of 1 % agar is added before pouring on plate.

Adenylate kinase:

6 mg Adenosine Di-Phosphate (ADP); 2 mg α-D-Glucose

2 mL stain buffer: .1M Tris HCl pH 8; prepared as follows:
22.2 g Trizma base, 125 mL 1N HCl, and dH₂O up to 2 L; Adjust pH with concentrated HCl.

100 μl 1 % Nicotinamide Adenine Dinucleotide Phosphate (NADP), 1 M MgCl₂, 1 % Methyl Thiazolyl Blue (MTT), 1 % Phenazine Methosulphate (PMS); 10 μl Hexokinase (2 IU); 4 μl Glucose-6-Phosphate Dehydrogenase (2 IU).

α-Amylase:

2 mL starch solution; 2 mL 1% agar; 1.5 mL Stain buffer: .05M Phosphate pH 6.9 made as follows: .6g KH₂PO₄, .65g anhydrous Na₂HPO₄, .08g NaCl, and dH₂O to 200 mL.

Incubate plate in solution for 20 minutes, then carefully peel off the agar overlay. Place plate in Iodine solution for about 5 minutes, then score after thorough washing. The starch and iodine solutions are prepared as follows:

<table>
<thead>
<tr>
<th>Starch solution</th>
<th>Iodine solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>33 mL 1 M K₂HPO₄</td>
<td>16.6 g KI, 25.4g I₂</td>
</tr>
<tr>
<td>66 mL 1 M KH₂PO₄</td>
<td>2 L Water</td>
</tr>
<tr>
<td>900 mL distilled water</td>
<td>Diluted twice for use</td>
</tr>
<tr>
<td>600 mg NaCl; 10 g Soluble starch.</td>
<td></td>
</tr>
</tbody>
</table>
Acid Phosphatase:
5 mg α-Naphthyl acid phosphate; 5 mg Fast Blue BB salt;
2 mL Stain buffer: .05M Citric acid, pH 4.0.

Alkaline phosphatase*:
10 mg α-Naphthyl acid phosphate
2 mL Stain buffer; .05 M Tris HCl pH 8.6 made as follows:
2 g Tris, 1 mL HCl (1N), 2 g NaCl, dH₂O to 300 mL; adjust pH
with 1N HCl. 100 µl 1M MgCl₂; 100 µl 1M MnCl₂; 10 mg Fast
Blue BB.
* This order of addition of stain components was found to
give a better resolution of protein bands.

Glucose-6-Phosphate Dehydrogenase:
6 mg Glucose-6-Phosphate 2 ml .1M Tris HCl pH 8; 100 µl 1
% NADP, 1M MgCl₂, 1 % MTT¹ and 1 % PMS².

Hexokinase:
2 mg α-D-Glucose; 6 mg Adenosine Tri-Phosphate; .1M Tris
HCl pH 8.6; 100 µl 1% NADP, 1M MgCl₂, 1% MTT, 1% PMS; and 2
IU Glucose-6-Phosphate Dehydrogenase.
Lactate Dehydrogenase and Phosphoglucomutase:
2 mL .1 M Tris HCl pH 8; 300 μl .1 M Sodium Lactate pH 7.4, prepared as follows (Kohn, 1976): 1 mL lactic acid to 11 mL .1 M NaOH, heat solution to 90 C, cool and reheat, after cooling adjust pH to 7.4 using NaOH; 10 mg Glucose-1-Phosphate; 1.5 mL 1% NADP; 300 μl 1M MgCl₂, 1% MTT, and 1% PMS; 5 μl G6PDH (2 IU).

Malate Dehydrogenase:
2 mL .1 M Tris HCl pH8; 200 μl Malate; 100 μl 1% NAD, 1% MTT, 1% PMS.

Phosphoglucoisomerase:
1 mL .1 M Tris HCl pH 8; 1 mL 1% NAD³; 10 mg Fructose-6-phosphate; 300 μl 1% MTT¹, 1% PMS²; 10 μl G6PDH⁴.

---
¹ MTT: Methyl Thiazolyl Blue;
² PMS: Phenazine Methosulphate;
³ NAD: Nicotinamide Adenine Dinucleotide;
⁴ G6PDH: Glucose-6-Phosphate Dehydrogenase
RESULTS AND DISCUSSION

Although distinct protein bands (and presumably alleles for each system) were observed at each enzyme locus, there were no variants or polymorphism for the 10 enzymes studied. Five bands or isozymes were observed for LDH; 3 for MDH, ADK and α-Amy; 2 for AKP, PGM, G6PDH, PGI and PGM; and 1 for Acp and Hex. An evaluation of whether the populations of low and high SEV lines were in Hardy-Weinberg equilibrium was not made. Such a determination could have provided further evidence on the extent of genetic drift in the two lines.

The results presented here are regarded to be limited both in scope and validity. Frequency of alleles at the loci of enzymes was not determined, as no polymorphism was observed for all of the enzymes in the study. The absence of variation in the enzyme systems may suggest homogeneity after long term selection for SEV. The most plausible explanation could be that the inadequacy of the separation method and or experimental conditions in this study did not allow for better resolution of protein bands (Beck et al., 1975; Richardson et al., 1986). May be more enzymes need to be screened for variation, in order to determine whether selection for semen volume influenced allele frequencies at
the loci. Positive results however are not in themselves conclusive or significant. One would expect allozyme frequencies to change with selection for a quantitative trait, if allozyme loci were initially in linkage disequilibrium with the selected trait (Soller and Plotkin-Hazan, 1977). With several selection experiments, however, the effects of drift and linkage disequilibrium on changes in allozyme frequencies can easily be distinguished from that brought about by selection (Stuber et al., 1980; Edwards et al., 1987).

It is hoped that with adequate funding this investigation can be continued. Establishing a relationship between a biochemical or molecular marker with SEV, may facilitate use of genetic engineering to increase semen production from tom turkeys. The abundance of literature on the design and interpretation of experiments to determine markers for QTs in other organisms including plants (Tanksley and Rick, 1980), Drosophila (Jayakar et al., 1977) and animals but not including poultry (Gelderman, 1975), should make the application suitable to avian studies (Savage et al., 1970). Enzyme markers hold the advantage or the potential of being numerous, so that these may be widely distributed throughout the genome and the alleles at these loci may be codominant (Tanksley et
al., 1982). Unlike classical monogenic markers like comb type, creeper and frizzling in avian species, enzyme markers rarely have visible effects. This eliminates the possibility of confounding the effects of the quantitative trait with that of the marker (Jayakar et al., 1977; Tanksley and Rick, 1980).
Table A.1. Gene-enzyme systems studied and allozyme type in Wrolstad Medium White turkeys

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Gene Symbol</th>
<th>Buffer System</th>
<th>Tissues</th>
<th>Number of Bands</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenylate Kinase</td>
<td>adk</td>
<td>1</td>
<td>RBC</td>
<td>3</td>
</tr>
<tr>
<td>α Amylase</td>
<td>amy</td>
<td>2</td>
<td>RBC</td>
<td>3</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>acp</td>
<td>3</td>
<td>RBC</td>
<td>1</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>akp</td>
<td>4</td>
<td>Plasma</td>
<td>2</td>
</tr>
<tr>
<td>Glucose-6-phosphate dehydrogenase</td>
<td>gpd</td>
<td>1</td>
<td>RBC</td>
<td>2</td>
</tr>
<tr>
<td>Hexokinase</td>
<td>hk</td>
<td>3</td>
<td>Plasma</td>
<td>1</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>ldh</td>
<td>1</td>
<td>Plasma</td>
<td>5</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td>mdh</td>
<td>3</td>
<td>Plasma</td>
<td>3</td>
</tr>
<tr>
<td>Phosphoglucoisomerase</td>
<td>pgi</td>
<td>4</td>
<td>Plasma</td>
<td>2</td>
</tr>
<tr>
<td>Phosphoglucomutase</td>
<td>pgm</td>
<td>3</td>
<td>Plasma</td>
<td>2</td>
</tr>
</tbody>
</table>

1Buffer systems for soaking cellulose acetate plates and electrophoresis tank (Table A1.2).
Table A.2. Buffers for electrophoresis and soaking cellulose acetate plates:

<table>
<thead>
<tr>
<th>Code</th>
<th>Buffer</th>
<th>Recipe/100 ml of buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>.02M Phosphate, pH 7.0</td>
<td>.42 g Na₂HPO₄·12H₂O</td>
</tr>
<tr>
<td></td>
<td></td>
<td>.13 g NaH₂PO₄·2H₂O</td>
</tr>
<tr>
<td>2</td>
<td>.025M Tris-Glycine pH 8.5</td>
<td>.30 g Tris</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.41 g Glycine</td>
</tr>
<tr>
<td>3</td>
<td>.05M Tris-Maleate pH 7.8</td>
<td>.60 g Tris</td>
</tr>
<tr>
<td></td>
<td></td>
<td>.23 g Maleic acid</td>
</tr>
<tr>
<td>4</td>
<td>.02M Tris-EDTA-borate-MgCl₂ pH 7.8</td>
<td>1.82 g Tris</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pH 7.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.86 g Na₂EDTA</td>
</tr>
<tr>
<td></td>
<td></td>
<td>.95 g anhydrous MgCl₂</td>
</tr>
</tbody>
</table>

¹ Buffer systems presented in Table A1.1.
REFERENCES


