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GENE ACTION INVOLVED WITH REPRODUCTION AND GROWTH  
OF BRAIN AND MUSCLE IN WEIGHT-SELECTED LINES OF CHICKENS

by

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(ABSTRACT)

Genetic variation influencing reproduction and growth of White Rock chickens was studied. The populations used were lines high (HH) and low (LL) selected for 56-day body weight, reciprocal F<sub>1</sub> crosses (HL and LH), F<sub>2</sub> crosses of the F<sub>1</sub>, and dwarf populations (HD and LD) originating from lines HH and LL.

Inheritance of egg production and egg composition was examined (Experiment 1). Although reciprocal effects were generally unimportant, nonadditive genetic variation was evident for reproductive traits and egg component measures of shell and yolk. Comparisons in Experiment 2 involved dwarf and normal chickens from lines HH and LL. Yolk weight increased while the ratio of albumen to yolk decreased with age. Line and genotype differences were evident for egg shell and yolk traits while line by genotype interactions showed that genetic background influenced expression of dw.

Embryo growth of dwarfs was similar to that of nondwarfs at all ages (Experiment 3). Reduced egg size of dwarf pullets did not reduce embryonic weights. Embryo length and weight and yolk sac weight were greater for line HH than LL. In Experiment 4, allometric growth and

58/12/01 792

cellular content of brains were measured in normal and dwarf chickens from lines HH and LL and reciprocal F<sub>1</sub> crosses from hatch to maturity. Allometric slopes were similar for lines HH and LL with significant heterosis. Within an age, DNA, RNA and protein content (mg/g) were similar for all populations. Brain weight differences were due to equal filling of a greater number cells for line HH than LL. Final brain size was influenced more by embryonic hyperplasia than postembryonic hypertrophy.

The final experiment measured growth and cellular content of pectoralis and gastrocnemius muscles in populations HH, LL and F<sub>1</sub> from hatch to day 273. HH chicks had a smaller DNA unit size and a greater unit number than LL chicks at hatch. During rapid muscle growth, cell size was larger for HH than LL chickens. Pectoralis muscle grew at a faster rate than gastrocnemius muscle. Also cellular filling as measured by the DNA unit size was higher for pectoralis than gastrocnemius muscle through day 4. By day 10 the pattern reversed with values greater for gastrocnemius than pectoralis muscle.

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## TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS . . . . .	iv
TABLE OF CONTENTS. . . . .	v
LIST OF TABLES . . . . .	viii
LIST OF FIGURES . . . . .	x
INTRODUCTION . . . . .	1
REVIEW OF LITERATURE . . . . .	3
 CHAPTER I	
EGG PRODUCTION AND EGG COMPOSITION OF PARENTAL LINES, F <sub>1</sub> and F <sub>2</sub> CROSSES OF WHITE ROCK CHICKENS SELECTED FOR 56-DAY BODY WEIGHT. . . . .	13
Introduction . . . . .	14
Materials and Methods. . . . .	16
Stocks and Husbandry . . . . .	16
Analyses . . . . .	17
Results. . . . .	19
Egg Production and Body Weight . . . . .	19
Egg Composition. . . . .	20
Discussion . . . . .	22
Summary. . . . .	26
 CHAPTER II	
COMPOSITION OF EGGS FROM DWARF AND NONDWARF CHICKENS IN LINES SELECTED FOR 56-DAY BODY WEIGHT . . . . .	33
Introduction . . . . .	34
Materials and Methods. . . . .	35
Analyses . . . . .	36
Results and Discussion . . . . .	37
Age Effects. . . . .	37
Lines and Genotypes. . . . .	38
Summary. . . . .	41

### CHAPTER III

EMBRYO GROWTH OF NORMAL AND DWARF CHICKENS FROM LINES SELECTED FOR HIGH AND LOW 56-DAY BODY WEIGHT . . . . .	46
Introduction . . . . .	47
Materials and Methods. . . . .	49
Analysis . . . . .	50
Results and Discussion . . . . .	52
Embryonic Weights. . . . .	52
Embryonic Length . . . . .	53
Extraembryonic Membranes . . . . .	54
Summary. . . . .	56

### CHAPTER IV

ALLOMETRIC GROWTH AND DNA, RNA AND TOTAL PROTEIN OF BRAINS FROM WHITE ROCK CHICKENS SELECTED FOR 56-DAY BODY WEIGHT . . . . .	60
Introduction . . . . .	61
Materials and Methods. . . . .	64
Analytical Procedures. . . . .	65
Analyses . . . . .	65
Results and Discussion . . . . .	67
Weight . . . . .	67
Cellular content of brain. . . . .	68
Summary. . . . .	71

### CHAPTER V

DNA, RNA AND TOTAL PROTEIN CONTENT OF LEG AND BREAST MUSCLES OF WHITE ROCK CHICKENS SELECTED FOR 56-DAY BODY WEIGHT. . . . .	75
Introduction . . . . .	76
Materials and Methods. . . . .	79
Analytical Procedures. . . . .	80
Analyses . . . . .	80
Results. . . . .	81
Effects of Selection . . . . .	81
DNA Unit Number. . . . .	82

RNA Content. . . . .	82
Protein Content. . . . .	82
Protein/DNA Ratio. . . . .	83
Protein/RNA and DNA/RNA Ratios . . . . .	83
Pectoralis and Gastrocnemius Development . . . . .	83
Discussion . . . . .	85
Summary. . . . .	89
GENERAL SYNTHESIS. . . . .	97
LITERATURE CITED . . . . .	100
APPENDIX . . . . .	111
LIST OF TERMS. . . . .	118
VITA . . . . .	119
ABSTRACT	

## LIST OF TABLES

### CHAPTER I

Table		Page
1	Contrasts among populations for genetic effects. . .	27
2	Means $\pm$ SEM of age (d) and body weight (g) at first egg, % normal eggs, and ovulations and normal eggs per trapday (%). . . . .	28
3	Means $\pm$ SEM of total egg weight and dry shell, dry albumen, dry yolk, and total moisture expressed as a % of total egg weight . . . . .	29
4	Means $\pm$ SEM of dry shell, albumen and yolk expressed as a percentage of their respective wet component. . . . .	30
5	Means $\pm$ SEM of wet and dry yolk to albumen ratios and various egg quality traits. . . . .	31

### CHAPTER II

1	Mean weights (g) of eggs, moisture and dry shell, albumen and yolk at 300 and 360 days of age. . . . .	42
2	Mean % dry matter of shell, albumen and yolk and and albumen/yolk ratios of eggs produced at 300 and 360 days of age. . . . .	43
3	Means $\pm$ SEM of weights of eggs and moisture, dry shell, albumen and yolk expressed on an absolute basis (g) and % of total egg. . . . .	44
4	Means $\pm$ SEM of egg component dry matter, albumen/yolk and shell characteristics . . . . .	45

### CHAPTER IV

1	Means $\pm$ SEM of DNA, RNA, protein (mg/g tissue) and protein/DNA ration of brain tissue . . . . .	72
---	--	----

### CHAPTER V

1	Means $\pm$ SEM of absolute and relative weights of pectoralis and gastrocnemius muscles . . . . .	90
2	Means $\pm$ SEM of DNA and RNA (mg/g tissue) for pectoralis and gastrocnemius muscles . . . . .	91



3	Means $\pm$ SEM of protein (mg/g) and protein/DNA for pectoralis and gastrocnemius muscles . . . . .	92
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## LIST OF FIGURES

### CHAPTER I

Figure	Page
1 Percentage of pullets in production (upper), ovulations/trapday (middle) and normal egg production (lower) for parental lines, F <sub>1</sub> and F <sub>2</sub> crosses . . . . .	32

### CHAPTER III

1 Wet (upper left) and dry (upper right) embryo weights expressed on an absolute (g) basis, relative to egg set weight (lower left) and as a percent dry matter (lower right) for H and L line chickens (* denotes P < .05). . . . .	57
2 Lengths of shank (upper) and embryo (middle) expressed on an absolute basis (mm) and as a ratio of shank/embryo length (lower) for H and L line chickens (* denotes P < .05) . . . . .	58
3 Yolk weight (upper) and the ratio of yolk/egg weight (lower) at start of incubation for H and L lines (* denotes P < .05) . . . . .	59

### CHAPTER IV

1 Brain weights expressed on an absolute basis (upper) and relative to total body weight (lower) for HH, LL and F <sub>1</sub> populations by age. (P - P denotes parental differences P < .05; H - H denotes heterosis P < .05). . . . .	73
2 DNA (upper left), RNA (upper right), protein (lower left) and protein/DNA (lower right) ratios for brain of HH, LL and F <sub>1</sub> populations. P - P denotes parental differences P < .05; H - H denotes heterosis P < .05). . . . .	74

### CHAPTER V

1 Growth of HH, LL and F <sub>1</sub> populations of chickens by age. (P-P denotes parental differences P < .05; H denotes heterosis P < .05). . . . .	93
--	----

- 2 Pectoralis (left) and gastrocnemius (right) muscles expressed as absolute (upper) and relative (lower) to total body weight of HH, LL and F<sub>1</sub> populations by age. (P - P denotes parental differences P < .05; H - H denotes heterosis P < .05). . . . . 94
- 3 DNA (upper left), RNA (upper right), protein (mg/g) (lower left) and protein/DNA ratios (lower right) for pectoralis muscle of HH, LL and F<sub>1</sub> populations by age. (P - P denotes parental differences P < .05; H - H denotes heterosis P < .05). . . . . 95
- 4 DNA (upper left), RNA (upper right), protein (mg/g) (lower left) and protein/DNA ratios (lower right) for gastrocnemius muscle of HH, LL and F<sub>1</sub> populations by age. (P - P denotes parental differences P < .05; H - H denotes heterosis P < .05). . . . . 96

## INTRODUCTION

Embryonic and postnatal growth involves complex physiological and morphological changes directed by the genetic code of the individual. These changes follow a definite sequence beginning with neurological development followed by the formation of bone, muscle, and fat, with synchronization of these changes essential for "normal development". Proper development of neurological material is important for normal growth. Information on brain growth in chickens is limited as are data concerning effects of selection for body weight on brain size. Data on these topics should include measures of cell number and size as well as DNA, RNA and protein content of brain tissue.

Muscle development sequentially follows bone development in normal embryonic and postembryonic growth and is a combination of cellular hyperplasia and hypertrophy. Although contributions of muscle mass through hyperplasia of myoblasts occur embryonically, additional nuclei are incorporated into muscle fibers from satellite cells. Cellular hypertrophy occurs postembryonically and appears as cytoplasmic filling of muscle fibers. Rate of protein accumulation during the period of rapid growth is influenced by the relationship between protein accretion and degradation. Changes in this relationship have an impact on muscle weights and, thus, the quantity and quality of poultry products.

During the past decade, broilers have reached a whole-bird market weight of 1800 g about 1 day earlier with each generation of selection, but have increased fat content. Now that further processing of poultry is economically important, broilers are also marketed at older ages and

heavier body weights. Thus, understanding the timing of muscle mass development is important to continued progress in poultry weight gains. Reported in this dissertation are experiments designed to:

- (1) measure genetic variation in egg production and egg components in chickens,
- (2) study genetic variation of embryonic and early postembryonic growth in chickens, and
- (3) describe muscle and brain growth in terms of cellular hyperplasia and hypertrophy through measures of RNA, DNA and protein content.

A list of abbreviations used in this dissertation is presented on page 118.

## REVIEW OF LITERATURE

Egg Production and Composition. Genetic selection for increased body weight or egg production has resulted in gains advantageous to the poultry industry and the consumer of poultry products. There is, however, a negative genetic correlation between body weight and egg production. Populations selected for egg production are lighter in body weight and those selected for heavy body weight have lower fecundity. This dichotomy has been documented in Japanese quail (Strong et al., 1978; Nestor and Bacon, 1982), chickens (Kinney, 1969; Reddy and Siegel, 1977a; Dunnington and Siegel, 1985) and turkeys (McCartney et al., 1968; Nestor, 1971; Arthur and Abplanalp, 1975; Nestor, 1977).

Chickens selected for increased body weight have more follicles in rapid development than those from egg-laying strains despite a decrease in production of settable eggs. Erratic ovulation and defective egg syndrome (EODES) also increases in weight-selected populations of chickens (Jaap and Muir, 1968). Selection for increased body weight alters synchrony between the ovary and oviduct, resulting in the production and release of more yolks than the oviduct is capable of forming into settable eggs (Jaap and Muir, 1968).

Egg size increases as a correlated response to selection for heavier body weights. Adjustments in egg size result in changes in egg component parts such as albumen (Rhodda et al., 1977; Akbar et al., 1983) and yolk (Cahaner et al., 1986). Age influences are also involved in changes in egg component distribution (Fletcher et al., 1983; Marguerat and Hagger, 1986).

The sex linked dwarfing allele (dw) is effective in stabilizing lipid overproduction and asynchronous of lay for meat-type chickens (Jaap, 1971; Abplanalp, 1984; Brody et al., 1984; Siegel and Dunnington, 1985; Zelenka and Siegel, 1987). Introduction of dw into a population results in reductions in egg weight and size of egg components on an absolute basis (Cherry et al., 1977). Relative to total egg weight, however, shell weight but not yolk or albumen are changed. This conservation of egg composition may be the result of many years of natural selection providing the most desirable yolk to albumen ratio for embryonic development.

Embryonic Growth. Growth of the avian embryo follows a defined process (Hamburger and Hamilton, 1951) which is based on priorities of development. For example, neurological development precedes skeletal development which precedes muscle development. Growth curve analyses have been employed to describe changes occurring from artificial selection in weight-selected lines of Japanese quail (Marks, 1978; Anthony et al., 1986) and chickens (Tzeng and Becker, 1981; Zelenka et al., 1986a). Significant differences in shape of the growth curve, as described by adjustments in hatch weight, points of inflection and asymptotic weights, were found in weight-selected lines relative to other populations.

Embryonic growth curves revealed differences in growth between 14 and 19 days in lines of White Plymouth Rocks selected for high and low juvenile body weight (Coleman et al., 1964). White Plymouth Rock and White Leghorn chickens have a divergence similar to that for the

weight-selected lines between 11 and 19 days of incubation (Bray and Iton, 1962).

Relative embryonic weights are larger in Pekin than in Mallard ducks throughout the embryonic period (Prince et al., 1968). It must be emphasized that, prior to embryonic size limitations of the egg, embryos of heavy-weight lines are larger than their lighter-weight counterparts. Immediately posthatch, growth of the heavier strains exceeds that of smaller-bodied birds (Bray and Iton, 1962), indicating that selection for body weight at a postembryonic age influences embryonic growth. Therefore, differences in growth curves after hatch (Marks, 1978; Anthony et al., 1986; Zelenka et al., 1986a) may be a compounding of differences occurring at the embryonic level.

While introduction of dw into populations of chickens does not influence body weight at hatch, growth by 6 weeks of age was consistently reduced (Hutt, 1959). Therefore, dw does not stop growth at any specific age but rather reduces it over time. The effect of dw within lines of chickens selected for high and low body weight, while similar through 12 weeks of age, is proportionately greater in the low- than in the high-weight line (Reddy and Siegel, 1977b). Moreover, reductions in body weight are greater than those for shank length. Divergence in body weight between normal and dwarf populations (Zelenka et al., 1986a) suggests that differences in body weight may originate during the embryonic period.

Brain:Body Weight Relationships. Interest in the evolution of brain:body weight relationships dates to the late 19th century (Radinsky, 1978). Early findings indicating relatively small brain size in some



extinct mammals sparked interest in relative brain size. The basis for brain size evolution was only theorized until Jerison (1961) provided evidence for evolutionary trends. Subsequently, Jerison (1963) proposed that diversity in brain size evolved just as diversity in average body size did and that, although the trend was toward large brain:body weight ratios, small-brained species existed at all times.

Brain size may be predicted from the allometric formula:

$$\text{brain size} = a(\text{body size})^b$$

where  $a$  and  $b$  are empirically-fitted constants. The allometric coefficient  $b$  is the slope of a line in log-log scale and  $a$  is the intercept (Riska and Atchley, 1985). Allometric slopes within large taxonomic groups generally range from 0.50 to 0.68 (Brody, 1945; Jerison, 1979). Harvey and Bennett (1983) discussed views that brain weight was generally scaled to the 0.66 power of body weight, implying a relation between brain weight and body surface area. Bauchot (1978), Martin (1981), and Hofman (1982) indicated that for large-bodied mammalian species, the relationship does not hold, and proposed a more accurate estimate would be to scale brain weight to the 0.75 power of body weight so as to associate growth and energy utilization. Basal metabolic rates, measured as energy need per unit body weight during a given time period, increase with the 0.75 power of body weight (Harvey and Bennett, 1983). This relationship was discussed by Armstrong (1983) who stated that "the proportion of the available energy directed toward the brain accounts for

much of the observed deviations in relative brain size." Thus, species adaptation appears to depend on the allocation of energy supplied to the brain. Martin (1981) found the brain to body exponent was approximately 0.75 for mammals and approximately 0.56 for birds and most reptiles with the differences due to additional parameters associated with weight and metabolic rate of the egg. On an evolutionary time scale, the limit to adult brain size may be due to the amount of energy the mother can supply to the developing embryo. In the case of egg-laying species, this limit may be the metabolizable energy of the egg (Martin, 1981).

Whereas slopes of distantly-related species approach 0.77, slopes of adults from closely-related species range from 0.20 to 0.40 (Riska and Atchley, 1985). Brain size has changed as a result of body size evolution with brain to body allometric slopes of 0.20 to 0.40 observed in rats and mice selected for body size (Lande, 1979; Atchley, 1984). Riska and Atchley (1985) postulated that the source of correlation between brain and body weight occurs embryonically when both systems are growing rapidly. Changes in early embryonic growth (hyperplastic period) are likely to have a greater influence on brain size than late postembryonic (hypertrophic) changes in body weight. In summary, allometric slope differences between species are due mainly to hyperplastic and hypertrophic changes in body weight. Conversely, the differences in higher taxa (i.e., genera, family, and order) are primarily due to embryonic hyperplastic activity.

Current research relating genetic associations of brain to body weight deals mainly with selection studies involving mice. Brain growth

has been measured as a correlated response to selection for body weight (Atchley, 1984; Leamy, 1985; Lyons and Wahlsten, 1988) and as the selected trait (Fuller and Geils, 1972; Hahn et al., 1979). In the case of divergent selection for body weight or for relative brain weight, significant changes occur in brain weight, both as correlated and direct responses, with no evidence of sexual dimorphism (Fuller and Geils, 1972; Karkowski, 1986). Selection for 42-day brain weight fixed many of the genes involved with brain growth as indicated by the lack of response to reverse selection (Hewitt et al, 1986). Atchley (1984) reported heritabilities of mature brain size of  $0.64 \pm 0.23$  in males and  $0.36 \pm 0.18$  in females with genetic correlations between mature brain size (189 days) and body weight gain prior to 14 days of age of  $0.65 \pm 0.14$  and  $0.36 \pm 0.03$  for males and females, respectively.

Since allometric slope differences between species may be attributed to changes in hyperplastic and hypertrophic cellular activity, responses to selection for brain:body weight ratios could be a function of more rapid brain growth over a prolonged period and/or a modification of the age at which brain growth usually decreases. In comparison to the unselected control population, divergent selection for larger relative brain weight in mice increased rate of brain growth over a prolonged period while selection for decreased relative brain weight reduced the period of rapid brain growth (Fuller and Geils, 1972).

Studies involving ground-nesting birds, although limited, generally indicate a very rapid increase in embryonic brain weight with a corresponding postembryonic decrease in growth relative to body weight.

Embryonic brain weight of Japanese quail increased from  $87 \pm 3$  mg at 9 days of incubation to  $308 \pm 8$  mg at hatch, to  $745 \pm 20$  mg at 42 days of age, with no sexual dimorphism (McFarland and Wilson, 1965). Relative brain sizes of Japanese quail selected for heavier body weights were about half those of the unselected controls, indicating a brain to body weight correlation of close to zero (Ricklefs and Marks, 1984).

Information on the cellular content of nucleic acids in brain tissue, while available for mice, is limited for poultry. Differences in adult brain size for mice divergently selected for brain size may be predetermined by neonatal DNA levels (Dudek and Berman, 1979). Therefore, alterations in rates of neuroblast formation must be different prenatally with differences in adult brain size a combination of changes in cell number and size.

Muscle Growth. Histological studies show increased muscle fiber number in mice selected for heavy body weight (Fowler et al., 1980). Also total muscle RNA and DNA are increased while RNA and DNA concentrations were similar. These trends have also been observed in Japanese quail (Fowler et al., 1980; Campion et al., 1982a) and chickens (Smith, 1963; Lepore et al., 1965; Aberle et al., 1979).

Measures of DNA, RNA and protein/g tissue provide information on cellular dimensions and activity. Concentrations of nucleic acids within muscle, a tissue predominantly consisting of multinucleated cells, are useful in quantifying cell size and number. For example, the total DNA content/g muscle (DNA unit number) can be related to total number of nuclei contained within a sample. DNA content per nucleus of somatic cell

is consistent within a species (Moss et al, 1964; Cheek et al, 1971; Cheek et al., 1985). The volume of cytoplasm per nucleus may be estimated through the ratio of protein:DNA as a nucleus is believed to control a defined volume of cytoplasm (Epstein, 1967; Cheek et al., 1971; Cheek et al., 1985). Since RNA serves as a template for protein synthesis, RNA content may be thought of as the capacity for protein synthesis. Likewise, the ratio of protein:RNA refers to the efficiency of protein synthesis (Millward, 1978). Inaccuracies with these measures may arise through variation among populations in number of non-muscle cells such as adipocytes, fibroblasts and vascular cells. Seventy percent of the total muscle cell mass consists of a homogeneous population of muscle nuclei associated with contractile cells (Laurent et al., 1978).

Although effects of selection for body weight on muscle accumulation in Japanese quail (Fowler et al., 1980; Bacon and Nestor, 1983; Lilja et al., 1985), chickens (Siegel and Dunnington, 1987; Katanbaf et al, 1988) and turkeys (Nestor et al., 1987b) is well documented, little information describing chronological and nuclear origins is available. Embryonic muscle development is in the form of cellular hyperplasia of mesodermal tissue (Goldspink, 1977). Maximum cellular mitotic divisions occur between days 7 and 10 of incubation (Marchok and Herrmann, 1967). As the embryo develops, myoblasts align in rows forming the myotube structure. Innervation of the myotube coincides with cessation of hyperplastic activity of myoblast cells theoretically fixing nuclei numbers within the myotube. Thus, numbers of adult muscle fibers are determined before hatch (Goss, 1966) and classified as determinant i.e.,

cells which can be neither replaced nor multiplied. It has been shown, however, that additional accumulation of nuclei can be derived through satellite cells known to fuse with the muscle fiber (Campion et al., 1982b) and that mitotic activity decreases postnatally (Knizotova et al., 1982).

A positive relationship between DNA content and muscle mass exists for very young chickens (Smith, 1963; Moss, 1968). In later development, however, relationships between muscle mass and nuclei content vary. Although no differences in total DNA of total muscle of fast and slow growing chickens were observed by Moss et al, (1964), Lepore et al. (1965) and Mizuno and Hikan (1971), recent research described the opposite situation (Kang et al., 1985; Tinch and McKay, 1987). In these recent reports, muscle DNA content between heavy and light breeds is similar if comparisons are made among muscles of similar weights.

Postembryonic muscle development occurs primarily through hypertrophy and elongation of preexisting muscle fibers. While most of the weight increases of an organism occur through postembryonic hypertrophy, the potential number and size of muscle myofibrils determine growth potential of total muscle. Muscle differences between broiler and layer populations appears to be due to adjustments in nuclei number rather than cell size (Tinch and McKay 1987).

Muscle deposition is a modification in rate of protein accumulation over time (Jones et al, 1986). Hypertrophy of muscle myofibrils and, thus, total muscle weight are dictated by ratio of accretion to degradation. Young birds undergoing periods of rapid growth have higher

protein accretion and lower degradation than adults (Scanes, 1987). In adult Japanese quail, (Maeda et al., 1985) and chickens (Maeda et al., 1986) reduction in rate of protein accretion is considerably greater than rate of degradation.

## CHAPTER I

### EGG PRODUCTION AND EGG COMPOSITION OF PARENTAL LINES, F<sub>1</sub> and F<sub>2</sub> CROSSES OF WHITE ROCK CHICKENS SELECTED FOR 56-DAY BODY WEIGHT



## INTRODUCTION

Age at which chickens reach sexual maturity may be modified through direct genetic selection (Komiyama et al., 1984), as a correlated trait (Abplanalp et al., 1977; Siegel and Dunnington, 1985), and through nongenetic means such as feeding regimen (Gowe et al., 1960), lighting regimens (Morris and Fox, 1958; 1960), and diet (Zelenka et al., 1985). Regardless of method, thresholds necessary to commence egg production are dependent on age, body weight and carcass composition (Dunnington et al., 1984).

Jaap and Muir (1968) reported that the frequency of erratic ovulations and defective eggs (EODES) can be influenced by genetic alterations in body weight. This syndrome has since been observed in weight-selected populations of Japanese quail (Nestor et al., 1987a), chickens (Jaap and Clancy, 1968; Siegel and Dunnington, 1985), and turkeys (Nestor et al., 1970). Also positively correlated with body weight are egg weight and weights of egg components (i.e., shell, albumen, yolk). Petersen et al. (1986) reported an influence of age and body weight at sexual maturity on egg and component weights. Although chickens from a line selected for low 56-day body weight laid smaller eggs than those from a line selected for high 56-day body weight (Reddy and Siegel, 1977a), changes in component weights were not proportionate.

Correlated responses in egg weight due to genetic selection for egg production resulted primarily through adjustments in total egg albumen (Rhodda et al., 1977; Akbar et al., 1983). Fletcher et al. (1983) and Marguerat and Hagger (1986) reported relative increases in total albumen

in larger eggs, and that eggs produced by older hens were larger and proportionately greater in yolk and yolk dry matter than those from the same hens at younger ages. Also, hens selected for increased abdominal fat laid smaller eggs with relatively larger yolks and greater lipid content (Cahaner et al., 1986). Abplanalp et al. (1984) found positive heterosis for proportion of yolk and negative heterosis for proportion of albumen in eggs produced by crosses of inbred lines of White Leghorns. Therefore, egg weight may not be an appropriate predictor of yolk and albumen size because the ratio of albumen:yolk (A/Y) is subject to change based on genetic influences and age of hen. The purpose of this study was to investigate effects of divergent selection for 56-day body weight on egg production and egg component traits utilizing parental lines and their  $F_1$  and  $F_2$  reciprocal crosses.

## MATERIALS AND METHODS

Stocks and Husbandry. Chickens used in this experiment were progeny from the 28th generation matings of lines developed through divergent selection for high (HH) and low (LL) body weight at 56 days of age (Siegel, 1978; Dunnington and Siegel, 1985). Reciprocal F<sub>1</sub> females (HL, LH) were obtained by crossing populations HH and LL (the sire line is designated first and the dam line second). F<sub>2</sub> populations (HL X HL, HL X LH, LH X HL, LH X LH) were obtained from matings of respective F<sub>1</sub> individuals.

Eggs from age-contemporary parents were incubated in the same machine to produce progeny for all populations. At hatch (March 4, 1986), chicks were wingbanded, vaccinated against Marek's disease and placed in floor pens with forced-air brooding and wood shavings for litter. Continuous lighting was maintained through 14 days of age. From 14 to 56 days of age, a 12 h photoperiod was supplied, after which chicks were moved to other floor pens and exposed to natural day light and length. At 127 days of age, pullets were moved to individual cages in a windowless house and provided with a 14 h photoperiod. Feed and water were supplied ad libitum.

Egg production was recorded daily from the onset of lay to 280 days of age. Oviposits were classified (Middelkoop and Siegel, 1976) as either normal or defective (e.g., double yolk, shell-less, membrane, soft shelled, compressed, or extra-calcified). Weight and age of each female was recorded on the day of her first oviposit. At 300 days of age, 30 producing hens from each population were selected at random and used to

summarize production curves and supply eggs for component analysis. All normal eggs produced for 5 consecutive days were individually identified, collected and weighed (.01 g) daily. Components were measured for eggs produced on days 1, 3 and 5 and specific gravity was determined for those collected on days 2 and 4.

For component analyses eggs were hard-boiled (Lee, 1985) and then cooled for 5 minutes in running tap water. Samples of shell without membrane from the top, middle and bottom of each egg were measured (.001 mm) and their average considered as shell thickness for that egg. Shell with membrane, albumen, and yolk were then separated, placed into tared aluminum pans and weighed (.01 g). Samples were dried to constant weight (A.O.A.C., 1975), reweighed, and then discarded. Total egg moisture was calculated by subtraction of dry weights from total egg weight. Specific gravity was determined on fresh eggs using a modified procedure (Novikoff and Gutteridge, 1949) involving flotation of each egg in 9 solutions of increasing salinity (increments of .005) with 1 having a specific gravity of 1.065.

Analyses. Statistical analyses of egg data were based on mean values for eggs laid by each female during the 5-day period and individual values for body weight and production traits. Population was the main effect and individuals within population the error term. Body and dry egg component weights were transformed to common logarithms and relative component weights and % dry matter of components were transformed to arc sine square roots prior to analyses. Orthogonal contrasts were made among populations to determine parental line (HH vs LL) and reciprocal (HL vs

LH) differences. Heterosis was estimated as percentage deviation of the mean  $F_1$  from the midparent mean of the parental lines. Recombination loss was estimated as percent deviation of  $F_2$  from  $F_1$  mean. Maternal and paternal effects were determined through appropriate contrasts within the  $F_2$  populations. Differences among means were also assessed using Duncan's multiple range test.

## RESULTS

Table 1 provides a summary of analyses conducted for these data. Means and standard errors are provided in subsequent tables.

Egg production and body weight. Mean age at first egg of females reaching sexual maturity by 280 days of age was 53 days later for line LL than line HH (Tables 1, 2). Parental line difference, heterosis and recombination loss were significant. Body weight at first egg was more than two times greater for HH than LL pullets. Weights of F<sub>1</sub> and F<sub>2</sub> individuals were intermediate to parental lines with no heterosis or recombination effects.

LL pullets had a lower intensity of lay than HH pullets (Tables 1, 2) as measured by number of ovulations/trap days (TD). There was also significant heterosis for ovulations/TD. Percent normal egg production was lower for HH than for LL, F<sub>1</sub> and F<sub>2</sub> pullets with both heterosis and recombination loss being significant. Although normal egg production, expressed as % of total TD, was similar for the parental lines, heterosis and recombination loss were significant. Reciprocal, paternal and maternal effects were generally unimportant for any of the above traits.

Production patterns for % pullets in production, % ovulations/TD and % normal egg production are summarized in Figure 1. Lack of reciprocal effects allowed pooling of crosses in the F<sub>1</sub> and in the F<sub>2</sub> generations. Both F<sub>1</sub> and F<sub>2</sub> individuals commenced lay prior to the selected lines. The more gradual onset of lay of F<sub>1</sub> pullets, in contrast to the sudden onset in the line HH, masked differences in age of sexual maturity between these populations. Onset of lay in the F<sub>2</sub> populations

consistently lagged behind that of the  $F_1$ . Not only was onset of lay in line LL delayed but peak % ovulations/TD was lower than for populations HH,  $F_1$  or  $F_2$ . Although % ovulations/TD were similar for HH,  $F_1$  and  $F_2$  pullets, % normal egg production was less for HH pullets which incorporated fewer yolks into normal eggs because of EODES. Percent normal egg production was consistently lower for  $F_2$  than for  $F_1$  pullets.

Egg composition. Eggs from line HH were heavier than those from line LL with significant heterosis and recombination loss (Tables 1, 3). Reciprocal, paternal and maternal effects were not evident. Relative dry shell weights were greater for line LL than HH, with no heterosis, recombination loss, reciprocal or maternal effects. Although % dry albumen was similar for lines HH and LL, paternal, maternal and reciprocal effects were present in the  $F_1$  generation with population LH being lower than HL. This pattern appeared to carry over to the  $F_2$  generation where % dry albumen was generally lower from progeny of LH dams. Percent dry yolk, while similar for the parental lines, exhibited considerable heterosis which was still apparent in the  $F_2$  because of negligible recombination loss. Maternal and reciprocal effects were not present, but paternal effects were significant. Moisture (%) was higher for line HH than LL and heterosis was significant. There was no evidence of recombination loss or reciprocal, paternal or maternal effects.

Shell dry matter, expressed as a % of wet shell weight, was lower in line HH than in other populations (Tables 1, 4). Recovery of % dry matter lost by selection was evident from the significant heterotic response. Percent dry albumen was similar for all populations. Eggs from

HH hens contained more dry yolk matter than those from LL hens. Although heterosis and recombination loss were both significant, there is no obvious reason for  $F_2$  values to be generally lower than the parental lines and  $F_1$  crosses.

Difference in albumen to yolk ratios for line HH than LL on a wet weight basis was reduced when expressed on a dry weight basis (Tables 1, 5). Reciprocal effects and heterosis were significant on both a wet and dry weight basis, with recombination loss greater on a dry than wet weight basis. Paternal and maternal effects were significant with greatest effects appearing in matings incorporating HL sires and HL dams.

Specific gravity of eggs was lower for line HH than line LL (Tables 1, 5). Although there was no heterosis, recombination loss, or reciprocal or maternal effects for specific gravity, paternal effects were significant. Shell thickness, a trait closely associated with specific gravity, provided slightly different information for shell quality. Eggs from parental lines did not vary in shell thickness, yet heterosis and maternal effects were significant.



## DISCUSSION

Divergent selection for body weight at 56 days of age resulted in correlated responses in reproductive traits. Differences between selected lines for age at first egg, % ovulations/TD and % normal egg production were consistent with those observed previously (Reddy and Siegel, 1977a; Dunnington and Siegel, 1985). The higher proportion of abnormal eggs for HH hens agreed with previous observations for these lines (Reddy and Siegel, 1977a; Zelenka and Siegel, 1987) and with those of Jaap and Muir (1968), who reported a high incidence of EODES for meat-type chickens.  $F_1$  crosses exhibited heterosis for age at first egg, % ovulations/TD, % normal eggs and % hen-day egg production with recombination loss being evident for all of these traits except % ovulation/TD. The heterotic effects for % normal egg production was due to restoration of synchrony of lay lost in line HH due to EODES. Similarly heterosis for ovulations/TD was due to increased ovulations by  $F_1$  crosses, especially in comparison to selected line LL.

Production patterns for parental lines revealed differing degrees of delay in onset of lay when compared to crosses. As a group, line HH pullets abruptly reached sexual maturity but LL,  $F_1$  and  $F_2$  pullets had extended periods of onset. The frequency of EODES in HH pullets and underproduction of follicles by LL pullets were evident. Thus, selection altered the parental lines through different means, resulting in similar normal egg production. Recombination loss for age at onset of lay and % normal egg production were evident throughout the production period as  $F_2$  values were consistently lower than the  $F_1$

Body weight at first egg was different for parental lines yet heterosis and recombination effects were not significant. This pattern suggests additive inheritance, a conclusion consistent with that of Zelenka et al. (1986a) who described growth patterns of the F<sub>1</sub> populations as being intermediate to parental lines HH and LL from hatch to sexual maturity. Egg weight is positively correlated with body size (Hale, 1961; Hale and Clayton, 1965; Clayton and Robertson, 1966). Therefore, some of the parental line differences for egg weight may be attributed to pullet size. Nonadditive genetic variance, however, also accounted for heterosis and recombination loss in egg weight.

Although little adjustment in moisture content was realized when total moisture was expressed as a % of total egg weight, moisture of eggs from HH females was 1% higher than those of eggs from the other populations. The greater moisture content of eggs from line HH pullets was due to moisture increases in shell. Moisture content of eggs may be conserved if a reduction in moisture of one component is counterbalanced by an increase in moisture of other components.

Variation in shell thickness did not account for differences in % shell dry matter between parental lines. Rather surface area and/or shell membrane may have been key contributors to the difference. Heterosis in % dry matter appeared to be due to greater shell thickness of F<sub>1</sub> eggs.

Population differences in specific gravity were consistent with previous reports for these lines (Dunnington and Siegel, 1985) and data for shell thickness. The only anomaly was the difference in specific gravity of the parental lines which may have resulted from a violation

of an assumption of the specific gravity test. That is, comparisons may be made if densities of the egg contents are uniform. In this situation the parental line difference in relative total moisture ( $HH > LL$ ) may have been sufficient to decrease density of HH eggs.

Divergent selection for 56-day body weight increased the amount of egg yolk dry matter in line HH relative to line LL. This correlated response was consistent with evidence for lipid overproduction in populations selected for increased body weight and the resultant influence on normal egg production (Jaap and Clancy, 1968; Nestor et al., 1970; Siegel and Dunnington, 1985; Nestor et al., 1987a). Similar results were reported for populations known to overproduce abdominal fat (Cahaner et al. 1986). Positive heterosis for proportion of yolk and negative heterosis for proportion of albumen, on a wet weight basis, was reported for White Leghorns (Abplanalp et al., 1984). My data, on a dry weight basis, were consistent with regard to sign, but significant for yolk only. Differences in yolk dry matter between selected lines did not modify the wet albumen:yolk ratio, suggesting that production of the two are independent. Thus, as was found by Lepore et al. (1963), A/Y ratios expressed on dry weight data were similar for the parental lines. The large negative heterosis found for A/Y ratios suggests that the balance of components found in parental lines may not be ideal.

Increased fitness of  $F_1$  offspring may be a function of increased dry yolk relative to dry albumen. The A/Y ratio may be an important characteristic influencing embryonic survival; days to produce an ova are relatively constant (Zekenka et al., 1986b) as is time for an ova to

traverse the oviduct (Lewin, 1988). Because heritabilities of A/Y are quite variable (Kinney, 1969), it may be that deviation from an optimum A/Y ratio decreases hatchability. This deviation may be one of the factors involved in reduction of hatchability observed in these weight-selected lines (Dunnington and Siegel, 1985), in stocks selected for increased abdominal fat (Cahaner et al., 1986) and in aging populations (Sunde and Bird, 1959; Crittenden and Bohren, 1962) where yolk size increases with age (Fletcher et al., 1983). Further investigations are needed to determine implications of variable egg composition on hatchability and survival of chickens.

## SUMMARY

Egg production and weights of eggs and their components were measured in body-weight selected lines and reciprocal  $F_1$  and  $F_2$  crosses. Nonadditive genetic variation was significant for age at onset of lay, % hen-day ovulations, % hen-day egg production, % normal eggs produced, egg weight, shell thickness, % dry yolk, % total moisture, shell and yolk dry matters and ratios of albumen to yolk (A/Y) both on a wet and dry weight basis. There was recombination loss for all of these traits except % hen day ovulations, % dry yolk, % total moisture, shell dry matter and thickness and wet A/Y ratios. Reciprocal effects were unimportant except for % dry albumen and A/Y ratios. Greater yolk dry matter in the high than low body weight line was consistent with observations of lipid overproduction in fast growing populations of chickens.

Table 1. Contrasts among populations for genetic effects

	Parental line		Reciprocal		Heterosis		Recombination		Paternal		Maternal	
	HH	LL	HL	LH	MP	F1	F1	F2	HL--	LH--	--HL	--LH
Egg Production to 280 Days of Age												
Age First Egg	**		--		**			**	--		--	
BW First Egg	**		--		--			--	**		--	
Ovulation/TD (%)	**		--		**			--	--		--	
% Normal Eggs	**		--		**			**	--		--	
Normal/TD (%)	--		--		**			**	--		*	
Egg Weight	**		--		**			**	--		--	
Egg Components - Percent of Egg Weight												
% Dry Shell	**		--		--			--	*		--	
% Dry Albumen	--		**		--			--	*		**	
% Dry Yolk	--		--		**			--	*		--	
% Total Moisture	**		--		**			--	--		--	
Percent Dry Matter of Component												
Shell	**		--		*			--	*		--	
Albumen	--		--		--			--	--		**	
Yolk	**		--		**			**	**		--	
Ratio of Albumen to Yolk												
Wet	**		**		**			--	**		*	
Dry	--		*		**			*	**		**	
Shell Quality Characteristics												
Specific Gravity	**		--		--			--	*		--	
Thickness	--		--		**			--	*		*	

\*  $p < .05$ , \*\*  $p < .01$ .

-- = no significant difference.

Table 2. Means  $\pm$  SEM of age (d) and body weight (g) at first egg, % normal eggs, and ovulations and normal eggs per trapday (%)

Population	Age at First Egg	Body Weight at First Egg	Ovulations/TD (%)	% Normal Eggs	Normal Eggs/TD (%)
<b>Parental Lines</b>					
HH	173 $\pm$ 1bc	2898 $\pm$ 24a	79.1 $\pm$ 1.7a	82.9 $\pm$ 1.5d	64.2 $\pm$ 1.7cd
LL	226 $\pm$ 2a	1229 $\pm$ 16d	59.6 $\pm$ 1.6d	97.6 $\pm$ .8a	58.4 $\pm$ 1.7d
<b>F<sub>1</sub> Populations</b>					
HL	170 $\pm$ 3bc	1889 $\pm$ 22c	75.2 $\pm$ .2ab	98.1 $\pm$ .6ab	73.3 $\pm$ .2ab
LH	167 $\pm$ 2c	1948 $\pm$ 27bc	77.7 $\pm$ 1.4a	98.9 $\pm$ .4a	76.8 $\pm$ 1.4a
<b>F<sub>2</sub> Populations</b>					
HLxHL	176 $\pm$ 3b	1897 $\pm$ 47c	72.1 $\pm$ 1.7bc	96.7 $\pm$ .7abc	69.0 $\pm$ 1.6bc
HLxLH	176 $\pm$ 3b	1919 $\pm$ 36bc	70.4 $\pm$ 3.0c	94.4 $\pm$ 1.6bc	66.2 $\pm$ 3.2c
LHxHL	175 $\pm$ 3b	1996 $\pm$ 29b	78.6 $\pm$ 1.5a	94.7 $\pm$ 1.1c	73.8 $\pm$ 1.6ab
LHxLH	176 $\pm$ 4b	2007 $\pm$ 28b	72.4 $\pm$ 3.3bc	91.3 $\pm$ 3.9c	66.5 $\pm$ 3.3c
% Heterosis	-15.6**	-7.1	10.2**	9.2**	22.4**
% Recombination	4.5**	1.9	-4.0	-4.3**	-8.2**

\*\* p < .01.

a,b,c,d Means within a column with the same superscript are not different (p > .05).

Table 3. Means  $\pm$  SEM of total egg weight and dry shell, dry albumen, dry yolk, and total moisture expressed as a % of total egg weight

Population	Egg Wt (g)	Shell (%)	Albumen (%)	Yolk (%)	Moisture (%)
<b>Parental Lines</b>					
HH	56.17 $\pm$ .69a	8.56 $\pm$ .15d	7.66 $\pm$ .15ab	15.23 $\pm$ .19c	68.55 $\pm$ .34a
LL	40.42 $\pm$ .65d	9.60 $\pm$ .13ab	7.41 $\pm$ .09bcd	15.50 $\pm$ .17bc	67.49 $\pm$ .18b
<b>F<sub>1</sub> Populations</b>					
HL	48.90 $\pm$ .58bc	9.31 $\pm$ .10abc	7.51 $\pm$ .10abc	15.98 $\pm$ .17ab	67.20 $\pm$ .18b
LH	50.41 $\pm$ .74b	9.23 $\pm$ .12bc	7.07 $\pm$ .11d	16.24 $\pm$ .19a	67.46 $\pm$ .18b
<b>F<sub>2</sub> Populations</b>					
HLxHL	47.09 $\pm$ .68c	9.31 $\pm$ .14abc	7.83 $\pm$ .12a	15.45 $\pm$ .17bc	67.40 $\pm$ .19b
HLxLH	47.86 $\pm$ .56c	9.67 $\pm$ .12a	7.33 $\pm$ .11bcd	15.79 $\pm$ .15ab	67.20 $\pm$ .16b
LHxHL	48.13 $\pm$ .50c	9.11 $\pm$ .12c	7.51 $\pm$ .12abc	16.01 $\pm$ .18ab	67.37 $\pm$ .18b
LHxLH	48.01 $\pm$ .03c	9.18 $\pm$ .13bc	7.31 $\pm$ .10cd	16.10 $\pm$ .20a	67.58 $\pm$ .23b
% Heterosis	2.8**	2.1	-3.2	4.9**	-1.0**
% Recombination	-3.8**	0.5	2.9	-1.8	0.1

\*  $p < .05$ , \*\*  $p < .01$ .

a,b,c,d Means within a column with the same superscripts are not different ( $p > .05$ ).



Table 4. Means  $\pm$  SEM of dry shell, albumen and yolk expressed as a percentage of their respective wet component

Population	Component Dry Matter (%)		
	Shell	Albumen	Yolk
Parental Lines			
HH	85.33 $\pm$ 1.04c	13.30 $\pm$ .23ab	53.67 $\pm$ .23a
LL	92.80 $\pm$ .38a	13.26 $\pm$ .14ab	52.49 $\pm$ .12b
F <sub>1</sub> Populations			
HL	91.49 $\pm$ .98ab	13.44 $\pm$ .13ab	52.40 $\pm$ .20b
LH	90.42 $\pm$ .66b	13.22 $\pm$ .09ab	52.36 $\pm$ .22b
F <sub>2</sub> Populations			
HLxHL	92.47 $\pm$ .50ab	13.80 $\pm$ .17a	52.15 $\pm$ .17bc
HLxLH	92.39 $\pm$ .63ab	13.25 $\pm$ .18ab	51.77 $\pm$ .17cd
LHxHL	91.32 $\pm$ .61ab	13.53 $\pm$ .15ab	51.47 $\pm$ .09d
LHxLH	90.43 $\pm$ .65b	13.13 $\pm$ .16b	51.34 $\pm$ .21d
% Heterosis	2.1*	0.4	-1.4**
% Recombination	0.8	0.8	-1.3**

\* p < .05, \*\* p < .01.

a,b,c,d Means within a column with the same superscripts are not different (p > .05).

Table 5. Means  $\pm$  SEM of wet and dry yolk to albumen ratios and various egg quality traits

Population	Wet Albumen Wet Yolk	Dry Albumen Dry Yolk	Specific Gravity <sup>1</sup>	Shell Thickness (mm)
Parental Lines				
HH	2.03 $\pm$ .03a	.51 $\pm$ .01a	2.37 $\pm$ .14b	.303 $\pm$ .005c
LL	1.90 $\pm$ .03bc	.48 $\pm$ .01ab	4.20 $\pm$ .26a	.301 $\pm$ .005c
F <sub>1</sub> Population				
HL	1.84 $\pm$ .03bcd	.47 $\pm$ .01bc	3.56 $\pm$ .16ab	.314 $\pm$ .004abc
LH	1.73 $\pm$ .03f	.44 $\pm$ .01d	3.25 $\pm$ .16ab	.319 $\pm$ .004ab
F <sub>2</sub> Population				
HLxHL	1.92 $\pm$ .03b	.51 $\pm$ .01a	3.67 $\pm$ .23ab	.312 $\pm$ .004bc
HLxLH	1.82 $\pm$ .03cde	.47 $\pm$ .01bcd	3.85 $\pm$ .25ab	.327 $\pm$ .004a
LHxHL	1.79 $\pm$ .03def	.47 $\pm$ .01bc	3.25 $\pm$ .23ab	.308 $\pm$ .004bc
LHxLH	1.74 $\pm$ .03ef	.44 $\pm$ .01cd	3.23 $\pm$ .27ab	.312 $\pm$ .005bc
% Heterosis	-9.2**	-8.1**	3.7	4.8**
% Recombination	1.8	3.8*	2.8	-0.6

\*  $p < .05$ , \*\*  $p < .01$ .

a,b,c,d,e,f Means within a column with the same superscript are not different ( $p > .05$ ).

<sup>1</sup>Indicates number of solution (1-9) in which the egg first floated.

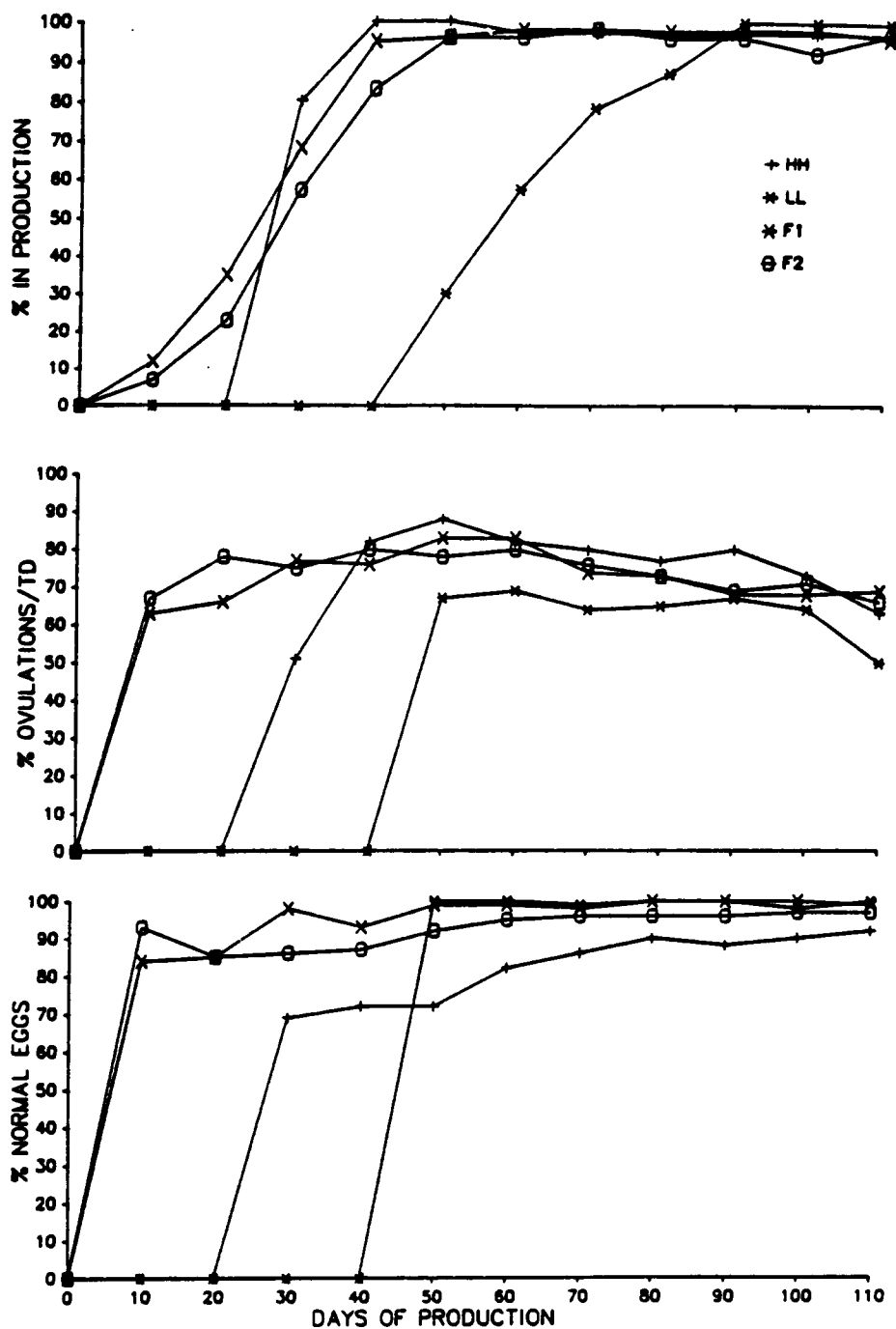


Figure 1. Percentage of pullets in production (upper), ovulations/TD (middle) and normal egg production (lower) for parental lines, F<sub>1</sub> and F<sub>2</sub> crosses.

## CHAPTER II

### COMPOSITION OF EGGS FROM DWARF AND NONDWARF CHICKENS IN LINES SELECTED FOR 56-DAY BODY WEIGHT

## INTRODUCTION

The sex-linked dwarfing allele (dw) can provide several production advantages for female breeders including reduced feed consumption (French and Nordskog, 1973) and more settable eggs (Jaap and Mohammadian, 1969; Reddy and Siegel, 1977b). In the case of the latter, dw seems to have a stabilizing effect on synchrony of ovulation in chickens known to overproduce follicles (Jaap, 1971; Abplanalp, 1984; Brody et al., 1984; Siegel and Dunnington, 1985; Zelenka and Siegel, 1987). Evidence, however, indicates that phenotypic effects of dw on various traits may be influenced by genetic background (Reddy and Siegel, 1977b; Merat, 1982; Lilburn et al., 1986; Zelenka and Siegel, 1987; Martin et al., 1988), resulting in a dwarf genotype by genetic background interaction. Comparisons of egg components between dwarf and nondwarf chickens are generally lacking. Merat (1972) found no difference between normal and dwarf hens in yolk:total egg and yolk:albumen ratios. In contrast, although dw reduced weights of shell, albumen and yolk, only shell was decreased in dw when expressed as a % of total egg weight (Cherry et al., 1977). The purpose of this study was to compare composition of eggs from dwarf and nondwarf chickens in lines divergently selected for body weight at 56 days of age.

## MATERIALS AND METHODS

Chickens used in this experiment were normal (N) and dwarf (D) pullets from White Plymouth Rocks selected for high (H) and low (L) body weight at 56 days of age (Siegel, 1962; Dunnington and Siegel, 1985). The four populations were high normal (HN), high dwarf (HD), low normal (LN), and low dwarf (LD). The dwarfing allele was incorporated into lines H and L in generation 13 by mating  $Dw^{+}/dw$  broiler males to a random sample of females from each selected line (Reddy and Siegel, 1977b). HD and LD populations were then developed by repeated backcrossing of  $Dw^{+}/dw$  males to normal HN and LN females from their respective lines. After 10 generations of backcrossing, HD and LD populations were reproduced each generation by randomly mating dwarf males and females within each population as contemporaries of the selected lines. Comparisons presented in this paper were from the 28th generation of selection.

Eggs from normal and dwarf parents were incubated in the same machine. At hatch (March 4, 1986) chicks were wingbanded, vaccinated against Marek's disease, and placed in windowless floor pens with forced air brooding. Continuous lighting was provided to 14 days of age after which lighting was decreased to a 12 h photoperiod. When 56 days of age chicks were moved to pens with windows and exposed to natural light and day length. At day 127 posthatch, HN and LN pullets were transferred to individual wire cages while HD and LD populations remained in floor pens. In both husbandry settings pullets were exposed to a 14 h photoperiod. Feed and water were supplied ad libitum.

At 300 and 360 days of age, 30 pullets from each population that were in egg production were randomly chosen to supply eggs for determining weights (wet and dry) of shell, yolk and albumen. Normal eggs laid during the sampling period of 5 consecutive days were used. Eggs produced on days 1, 3 and 5 were used for measuring components and specific gravity was determined for those collected on days 2 and 4. Weight of each egg was obtained to the nearest .01 g on the day it was laid. Measures of shell characteristics, albumen and yolk were described in Chapter I.

Analyses. Data were analyzed by analysis of variance using the model:

$$Y_{ijk} = \mu + A_i + L_j + G_k + (AL)_{ij} + (AG)_{ik} + (LG)_{jk} + (ALG)_{ijk} + e_{ijk1}$$

where  $i = 1, 2$  ages,  $j = 1, 2$  lines  $k = 1, 2$  genotypes and  $l = 1, 2, \dots, n$  eggs. Prior to analyses absolute weights were transformed to common logarithms and relative component weights and % dry matters to arc sine square roots. When line by genotype interactions were significant, data were analyzed within lines.

## RESULTS AND DISCUSSION

Significant age by line and age by genotype interactions were rare. In contrast, line by genotype interactions were frequent, implying that although line and dwarfing genotype effects were similar across ages, expression of dw was modified by background genome. Because of this pattern, results are presented by ages and then for line by genotype subclasses with ages pooled.

Age effects. Egg, dry shell, dry albumen and total moisture weights were similar at 300 and 360 days of age, but dry yolk weights increased with age (Table 1). When expressed as a % of total egg weight, moisture and dry albumen weights were also similar at both ages. The increase in dry yolk weight persisted on a relative basis, while decreases in % shell weight became evident. Shell thickness was also reduced, .300 to .291 mm from 300 to 360 days of age - a pattern consistent with findings of Tyler and Geake (1960). Percent dry matter of the shell, however, increased with age (Table 2), a result that contrasted with observations of Marion et al. (1966). Since shell membrane is the variable portion of shell moisture, reductions in shell membrane over time (Tyler and Geake, 1960) may have been responsible for the increased % of shell dry matter.

Percent dry matters of yolk and albumen were similar at 300 and 360 days of age (Table 2). Reductions of albumen to yolk (A/Y) ratios, however, occurred with age both on a wet and a dry weight basis. This observation reflects the stability of albumen content with age and the increases in yolk reported in the previous paragraph. Apparently,



adjustments in efficiency of yolk deposition with age resulted in fewer abnormal eggs (Siegel and Dunnington, 1985; Zelenka and Siegel, 1987) and in eggs containing a greater proportion of yolk.

Lines and genotypes. Line by genotype interaction for egg weight was not significant. Line H pullets laid larger eggs than line L pullets ( $56.0 \pm .4$  vs  $41.1 \pm .3$  g) and nondwarf pullets laid larger eggs than dwarfs ( $50.4 \pm .7$  g vs  $47.2 \pm .8$  g). Moisture content of eggs was less for HD than HN pullets and similar for LN and LD pullets (Table 3), resulting in a line by genotype interaction. When expressed as % moisture, the line by genotype interaction was due to a lack of difference between eggs from HN and HD pullets and 2% less moisture in eggs from LD than from LN pullets.

A significant line by genotype interaction for absolute dry shell weight resulted from lower values for LD than LN pullets and no difference between HD and HN pullets (Table 3). When expressed as relative dry shell weight the interaction was of the crossover type with lower values for dwarfs than normals in line L and higher values for dwarfs than normals in the line H. Shell thickness and specific gravity of eggs were greater for HD than HN pullets and lower for LD than LN pullets (Table 4). These line by genotype interactions were not observed for % shell dry matter which was greater for line L ( $91.0 \pm .6$ ) than line H ( $87.1 \pm .48$ ) and for nondwarf ( $90.6 \pm .4$ ) than dwarf ( $86.0 \pm .2$ ) pullets.

There was a line by genotype interaction for absolute and relative dry albumen weights (Table 3). Although eggs from dwarf pullets contained less dry albumen than those from nondwarfs, the difference was greater

in line H than line L. Relative dry albumen weights were greater for eggs from HN than HD pullets with no differences between eggs from LN and LD pullets.

Absolute dry yolk weights were larger for eggs from H than L pullets ( $9.6 \pm .1$  vs  $7.5 \pm .1$  g) and nondwarfs then dwarfs ( $8.9 \pm .1$  vs  $8.2 \pm .1$  g). The greater relative dry yolk content found in eggs produced by line L ( $18.1 \pm .1$  g) than line H ( $17.2 \pm .1$  g) chickens was consistent with findings of Lepore et al. (1963). Relative dry yolk weights of N pullets tended to be larger than that of D pullets ( $p < .06$ ). Percent dry matter of albumen and yolk was greater for eggs from HN than HD pullets, but similar for eggs from LN and LD pullets (Table 4). Ratios of albumen to yolk (wet and dry) were higher for eggs from HN than HD pullets but similar for LN and LD chickens (Table 4). These interactions showing differences in effects of dw in line H but not line L are consistent with the hypothesis that dw has a stabilizing effect on populations known to overproduce ova (Jaap, 1971; Abplanalp, 1984; Brody et al., 1984; Siegel and Dunnington, 1985; Zelenka and Siegel, 1987).

Line by dwarf genotype interactions observed for egg traits were common and provide evidence of the incongruence of dw in different genotypic backgrounds. Line by dwarf genotype interactions have been reported for other traits in these populations (Reddy and Siegel, 1977b; Martin et al., 1988) and in other populations (Merat, 1982; Lilburn et al., 1986). Modifications in egg composition due to dw may have an influence on hatchability and survivability; i.e., reductions in dry albumen and yolk lead to intermediate albumen to yolk ratios consistent

with increased fitness of  $F_1$  crosses from these lines (Chapter I). When there is high incidence of EODES, crossing and introduction of dw may be effective procedures in bringing yolk production into synchrony and thereby reducing the incidence of EODES. In contrast, effects of these procedures may not be evident or may be reversed in populations where selection has emphasized fecundity.

## SUMMARY

Egg weight and egg components were measured at two different ages in nondwarf and dwarf chickens from lines selected for high and low body weight. Egg, dry shell, dry albumen, total moisture, relative moisture and dry albumen weights as well as albumen and yolk % dry matters were similar at 300 and 360 days of age. Absolute and relative dry yolk weights increased with age while relative shell weight and wet and dry albumen to yolk (A/Y) ratios decreased.

Pullets from the high weight line laid larger eggs with more dry yolk yet less relative dry yolk and shell dry matter than those from the low weight line. Nondwarf pullets produced larger eggs with more absolute and relative dry yolk and shell dry matter than dwarf pullets. Line by genotype interactions were present for all other egg traits measured providing evidence of differential effects of the dwarfing allele in different genotypic backgrounds.

Table 1. Mean weights (g) of eggs, moisture and dry shell, albumen and yolk at 300 and 360 days of age<sup>1</sup>

Trait	Absolute (g)		% of egg weight	
	300 d	360 d	300 d	360 d
Egg	48.8	49.9	-----	-----
Shell	5.5	5.3	11.0	** 10.6
Albumen	4.6	4.7	9.5	9.3
Yolk	8.4	** 9.0	17.3	** 18.1
Moisture	30.4	31.0	62.2	61.9

\*\* p < .01.

<sup>1</sup>Lines and genotypes are pooled within each age.

Table 2. Mean % dry matter of shell, albumen and yolk and albumen/yolk ratios of eggs produced at 300 and 360 days of age<sup>1</sup>

Trait	300 d	360 d
Component dry matter (%)		
shell	87.7	** 90.6
albumen	16.2	15.9
yolk	55.9	56.0
(Albumen/yolk) x 100		
wet	189.0	** 182.3
dry	54.8	** 51.8

\*\*p < .01.

<sup>1</sup>Lines and genotypes are pooled within each age.

Table 3. Means  $\pm$  SEM of weights of eggs and moisture, dry shell, albumen and yolk expressed on an absolute basis (g) and % of total egg<sup>1</sup>

Trait	Absolute (g)				% of egg weight			
	Population				Population			
	HN	HD	LN	LD	HN	HD	LN	LD
Egg <sup>2</sup>	57.1±.4	53.8±.6	41.6±.4	40.3±.5	---	---	---	---
Moisture	36.0±.3	** 34.0±.4	25.0±.3	25.1±.3	63.0±.2	63.1±.2	60.0±.1	** 62.1±.3
Shell	5.9±.1	5.9±.1	5.0±.1	** 4.2±.1	10.3±.1	** 10.9±.1	12.0±.1	** 10.3±.2
Albumen	5.4±.1	** 4.8±.1	4.0±.1	** 3.8±.1	9.5±.1	** 8.9±.1	9.7±.1	9.6±.1
Yolk <sup>2</sup>	9.9±.1	9.2±.1	7.6±.1	7.2±.1	17.3±.1	17.1±.1	18.3±.1	17.9±.2

\*\* p < .01. indicates significant difference (p < .01) between adjacent means.

<sup>1</sup>Ages are pooled within each population.

<sup>2</sup>Indicates no line by genotype interaction for absolute weight, main effects' supplied in text.

Table 4. Means  $\pm$  SEM of egg component dry matter, albumen/yolk and shell characteristics<sup>1</sup>

Trait	Population					
	HN	HD	LN	LD		
Shell characteristic						
Thickness (mm $\times 10^3$ )	300 $\pm$ 3	320 $\pm$ 5	300 $\pm$ 2	** 250 $\pm$ 6		
Specific gravity <sup>2</sup>	2.7 $\pm$ 0.2	4.1 $\pm$ 0.3	4.2 $\pm$ 0.3	** 3.0 $\pm$ 0.2		
Component dry matter (%)						
shell <sup>3</sup>	88.3 $\pm$ 0.5	84.6 $\pm$ 0.9	93.4 $\pm$ 0.3	87.4 $\pm$ 1.2		
albumen	16.0 $\pm$ 0.1	** 15.3 $\pm$ 0.1	16.7 $\pm$ 0.1	16.5 $\pm$ 0.1		
yolk	56.5 $\pm$ 0.2	** 54.9 $\pm$ 0.3	56.0 $\pm$ 0.1	55.8 $\pm$ 0.3		
(Albumen/yolk) $\times$ 100						
wet	193.8 $\pm$ 1.6	** 186.1 $\pm$ 2.3	179.0 $\pm$ 2.0	172.8 $\pm$ 2.7		
dry	55.0 $\pm$ 0.5	** 51.9 $\pm$ 0.6	53.5 $\pm$ 0.6	54.0 $\pm$ 9.0		

\*  $p < .05$ ; \*\* $p < .01$ .

<sup>1</sup>Ages are pooled within each population.

<sup>2</sup>Specific gravity was measured at 300 days.

<sup>3</sup>No line by genotype interaction, main effects supplied in text.



### CHAPTER III

#### EMBRYO GROWTH OF NORMAL AND DWARF CHICKENS FROM LINES SELECTED FOR HIGH AND LOW 56-DAY BODY WEIGHT

## INTRODUCTION

Development of an avian embryo follows an orderly process (Hamburger and Hamilton, 1951) during which the basis for neurological, skeletal, and muscle tissue becomes established and functional. Embryonic growth patterns differed between 14 and 19 days in lines of White Plymouth Rock chickens divergently selected for body weight (Coleman et al., 1964). Bray and Iton (1962) reported differential growth between 11 and 19 days of incubation among White Plymouth Rock and White Leghorn populations of chickens. Relative embryonic weights were larger in Pekin than in Mallard ducks throughout the embryonic period (Prince et al., 1968).

Analysis of growth curves has regained popularity as a method of comparing responses which occur during selection for body weight in lines of Japanese quail (Marks, 1978; Anthony et al., 1986) and chickens (Tzeng and Becker, 1981; Zelenka et al., 1986a). In general, selection for body weight modifies growth curve shape through adjustments in weight at hatch (y-intercept), point of inflection and asymptotic weight (Ricklefs, 1967). Differences in DNA unit number at hatch for White Plymouth Rock chickens selected for 56-day body weight are reported in Chapter V and suggest that variation in growth curves described by Marks (1978), Anthony et al. (1986), Zelenka et al. (1986a) may in effect be a cumulation of differences present at the embryonic level.

Introduction of the sex-linked dwarfing allele (dw) into populations of chickens did not influence body weights at hatch but consistently retarded growth by 6 weeks of age (Hutt, 1959). The effect

of dw within lines of chickens selected for high and low body weight was similar until 12 weeks of age, but thereafter was proportionately greater in the low- than in the high-weight line (Reddy and Siegel, 1977b). Moreover, reductions in body weight were greater than those for shank length and relative reductions of egg size were considerably less than for body weights (Hutt, 1959; Jaap and Mohammadian, 1969; Reddy and Siegel, 1977b).

Growth from hatch to sexual maturity of normal and dwarf chickens of weight-selected lines were described by Zelenka et al. (1986a). Although there were significant differences among growth criteria (i.e., weight at sexual maturity and point of inflection), there was little evidence of when divergence in growth commenced. The purpose of this experiment was to examine embryonic and early postembryonic growth of normal and dwarf chickens in lines divergently selected for body weight.

## MATERIALS AND METHODS

Chickens used in this experiment were normal (N) and dwarf (D) pullets from White Plymouth Rocks selected for high (H) and low (L) body weight at 56 days of age (Siegel, 1962; Dunnington and Siegel, 1985). The four populations were high normal (HH), high dwarf (HD), low normal (LL), and low dwarf (LD). The sex-linked dwarfing allele was introduced into lines H and L in generation 13 by mating  $Dw^+$ / $dw$  males to a random sample of normal females from each selected line (Reddy and Siegel, 1977b). Population HD and LD were then developed by repeated backcrossing of  $Dw^+$ / $dw$  males to normal HH and LL females from their respective lines. After 10 generations of backcrossing, HD and LD populations were reproduced each generation by randomly mating dwarf males and females within each population as contemporaries of the selected lines. Comparisons presented in this chapter were made from the 28th generation of selection for the lines.

Eggs used in this experiment were obtained from age contemporary hens maintained in wire cages in a windowless building with a 14:10h light:dark cycle. HH, HD, LL and LD females were artificially inseminated with pooled semen from their respective population. Eggs were collected daily, individually weighed, then stored at 55 C and 65% relative humidity. Three settings, each including eggs from 8-days, were placed in the same incubator at 37.5 C and 55% relative humidity. This procedure allowed for obtaining data for up to 3 ages on the same day (e.g., day 5, 13 and 21 of incubation). Embryo data were obtained daily from day 5 of incubation to 3 days posthatch from ten fertile eggs in each

population. Each incubated egg was weighed, the embryo minus yolk sac was isolated, blotted dry to remove amniotic fluid and then weighed (.001 g). Yolk sacs drawn into the body cavity were removed by blunt dissection. Lengths of the tarsometatarsus (shank) and total embryo were measured (mm). Body length was the distance from the tip of the beak to the end of the tail bud of the extended embryo. Measurements of shank and body length began on day 12 of incubation, while yolk sacs were weighed (.001 g) from day 13 of incubation to 3 days posthatch. Sexes of embryos were obtained from day 18 of incubation by gonadal examination. Hatched chicks were maintained in wire-floor battery cages under continuous lighting with water available at all times. Embryos, chicks and yolk sacs were oven-dried to a constant weight (AOAC, 1975) and reweighed (.001 g) to determine dry weights.

Analysis. Embryonic growth was analyzed by analysis of variance with age, line and genotype as fixed main effects. Embryo and yolk sac weights and shank and embryo lengths were transformed to common logarithms prior to analysis. Allometric slopes were calculated on a log-log basis. Ratios of wet and dry embryo weight relative to each other and to total egg weight, yolk sac to total egg weight and shank length to total embryo length were transformed to arc sine square roots prior to analysis.

To avoid confounding effects of egg size and embryo dimensions, various mating combinations were compared between normal and dwarf populations within a line. Embryos used in this portion of the study were 18 days and older. Contrasts involving the mating of dams of both genotypes ( $Dw^+/z$  and  $dw/z$ ) to homozygous sires ( $Dw^+/Dw^+$  or  $dw/dw$ ) enabled

measuring the effect of the dw allele in female offspring and the influence of a single dose of dw in male offspring; both contrasts incorporated eggs of the same size. Embryos of the same genotype, growing in different sized eggs, were tested in females through contrasts of matings where the sire of a genotype was mated to either dwarf or nondwarf dams. Similar contrasts were made for males, using heterozygous embryos obtained from reciprocal matings of nondwarf and dwarf chickens.

## RESULTS AND DISCUSSION

Of the 420 contrasts used to measure the effect of dw on embryo traits, 31 were significant. There was no systematic pattern for significance and, hence, they were probably due to chance. A maternal effect of egg size was evident in 41 of the 420 contrasts. They too appeared to be due to chance as no pattern of significance was present for any of the embryo traits. Although egg weight and yolk absorption are known to influence chick weight, in our case embryo weights did not include the yolk sac.

There were no consistent dwarf genotype differences for any of the traits measured from 5 days of incubation to 3 days posthatch which supports the thesis that dw does not influence embryonic and early postembryonic development (Hutt, 1959; Zelenka et al. 1986a). Shank and embryo length were similar for dwarf and normal embryos. These results were consistent with Hutt (1959) who described deviations in skeletal development of dwarf chickens as occurring postembryonically. Line by genotype interactions were also unimportant for embryo traits. Thus, emphasis for the remainder of this chapter is on line differences.

Embryonic Weights. Age by line interactions were significant for embryonic weights because divergence between lines increased with age. Comparisons between lines within ages showed that, commencing on day 10 of incubation, line H embryos were heavier than line L embryos (Figure 1). Embryo weight expressed as a percentage of egg weight at onset of incubation was higher for line L than H from day 5 to 19 of incubation. Lack of differences beyond day 19 indicated that an optimum incubation

of egg space and moisture loss may be necessary to assure normal hatch. Hatch weights of line H chicks containing yolk sac material were 72 % of egg weight at initiation of incubation while that for line L was 68 %. These percentages are consistent with those reported in the literature (e.g., Upp, 1928; Jull and Heywang, 1930; Bray and Iton, 1962).

Although dry embryo weights were significantly different at ages 6, 8 and 10, it was not until day 16 that embryos from line H were consistently heavier than those from line L (Figure 1). Percent embryo to total egg weight was similar to that previously described for wet embryos with percentages consistently higher for line L than H from day 7 to 19 of incubation. The pattern of weight change was similar to that for wet embryo weight except plateauing occurred slightly later. Embryo dry matter, expressed as the ratio of dry/wet embryo was generally higher for line L than H throughout the pre- and posthatch period. This may be related to the higher DNA unit size of LL than HH chicks at hatch (Chapter V).

Embryo Length. Age by line interactions were significant for embryo length but not shank length. Because the ratio of shank to embryo length is important for discussion, all length measures were analyzed between lines for each age. Line differences in shank length were evident as early as 16 days of incubation; however, the patterns were not consistently different until day 19, from which time line H embryos had longer shanks than L embryos (Figure 2). Dimorphism in embryo length became evident on day 14 as line H embryos were longer than those from line L.



Shank length data relative to body length was similar for both lines at all ages (Figure 2). The doubling of the length ratio between 11 and 18 days indicated that prior to day 18 shank length grew at a faster rate than skeletal length. Beyond day 18 shank and body skeleton grew at similar rates as indicated by the plateau in shank:embryo length ratio. The pattern for early rapid leg development by the embryo is consistent with preparation for posthatch locomotion of precocial birds (Provine, 1980).

Extraembryonic Membrane. Line by age interactions were significant for yolk sac measures. Comparisons were therefore made between lines within each age. Yolk sac weights in line H were larger than those from line L from day 12 incubation to 3 days posthatch (Figure 3). This result was expected since yolks are considerably heavier in eggs from line H than L (17.2 g vs 13.4 g respectively). Yolk sac weights expressed relative to egg weight at incubation were generally similar until 20 days incubation when values were greater for line H than L. Since the % dry matter of yolk is greater for eggs from line H than line L pullets (Chapter I), differences in yolk sac absorption between lines do not imply that L line embryos are more efficient in yolk utilization. Actually the opposite may be the case (Lepore et al., 1965).

Deprivation of food during the 3-day posthatch period meant that other than water egg yolk stores were the sole source of nutrients. Although yolk stores were being depleted during embryonic development, it was not until the yolk sac was drawn into the body cavity (20 days) that absorption of yolk was at the highest rate. Utilization of available

yolk by the embryo was in the form of maintenance as indicated by a plateau of body weights early posthatch. Although this period of static body weight is not unusual for line L, which has a tendency toward anorexia (Zelenka et al., 1988), it is quite uncharacteristic of line H (Zelenka et al., 1986a; Katanbaf et al., 1988). Therefore, the postembryonic yolk sac functions as a temporary energy source which insures survival of the newly hatched chick until a suitable food source is located or yolk stores are depleted.

## SUMMARY

Embryonic and early postembryonic growth was measured in normal and dwarf chicks from lines of White Rocks that had been divergently selected for body weight at 56 days of age. Dwarf embryos grew at the same rate as nondwarfs from day 5 of incubation to 3 days after hatch. Similarly, reductions in egg weight associated with the dwarfing allele did not significantly affect embryonic size. Embryonic growth was modified by selection for high and low 56-day body weight. Through much of the growth period embryos from the high line were heavier than those from the low line, but relative to egg weight at onset of incubation embryos from the low line were larger than the high line. Toward the end of incubation, body and shank lengths were longer for high than low line embryos. Yolk sac weights were heavier in the high than the low line. Relative to egg weight, however, yolk sac weights were similar for both lines. After hatch, relative yolk sac weights were less in the low than high-weight line, indicating differential rates of absorption of available yolk stores.

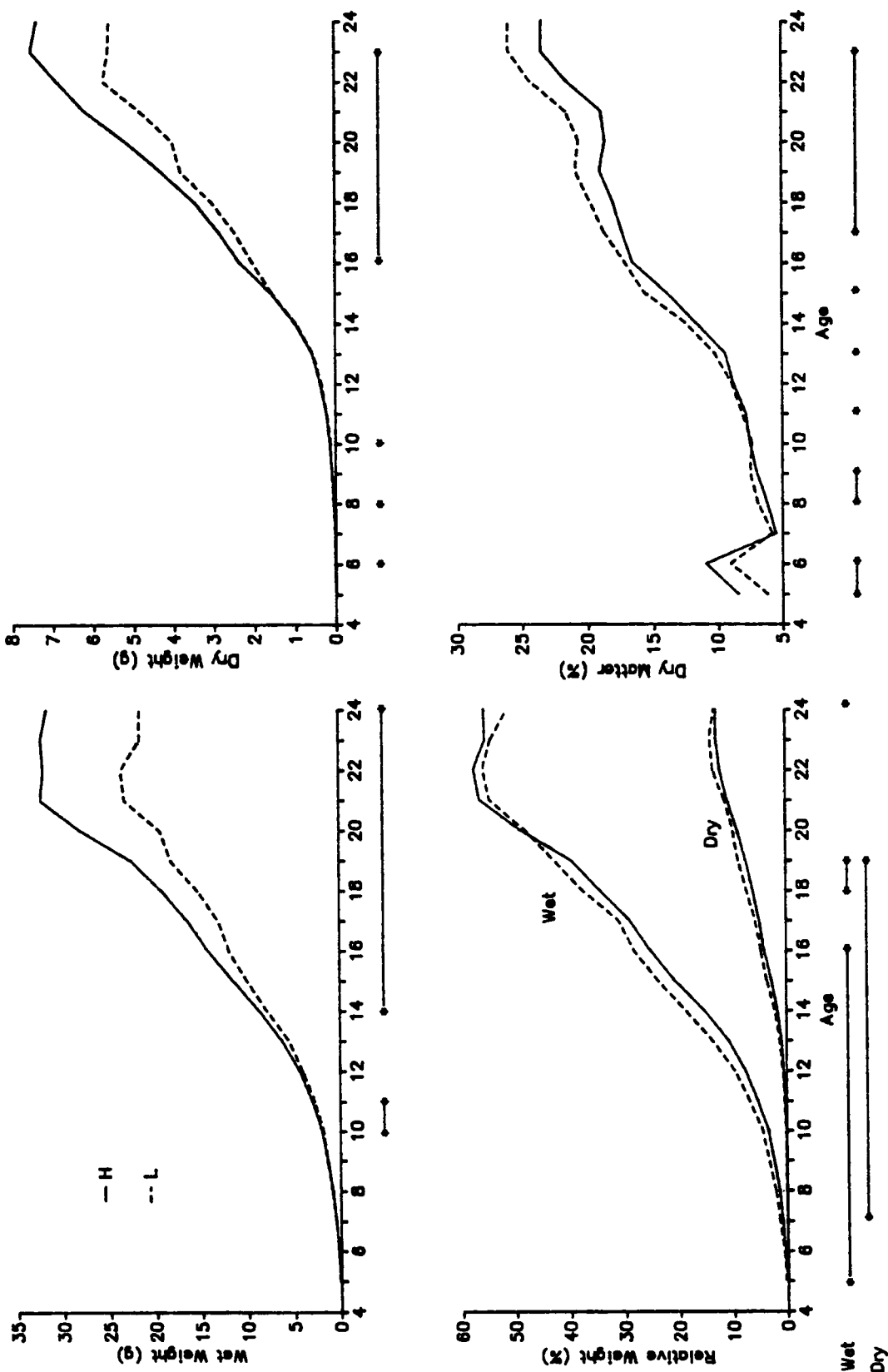


Figure 1. Wet (upper left) and dry (upper right) embryo weights expressed on an absolute (g) basis, relative to egg set weight (lower left) and as a percent dry matter (lower right) for H and L line chickens (\* denotes  $P < .05$ ).

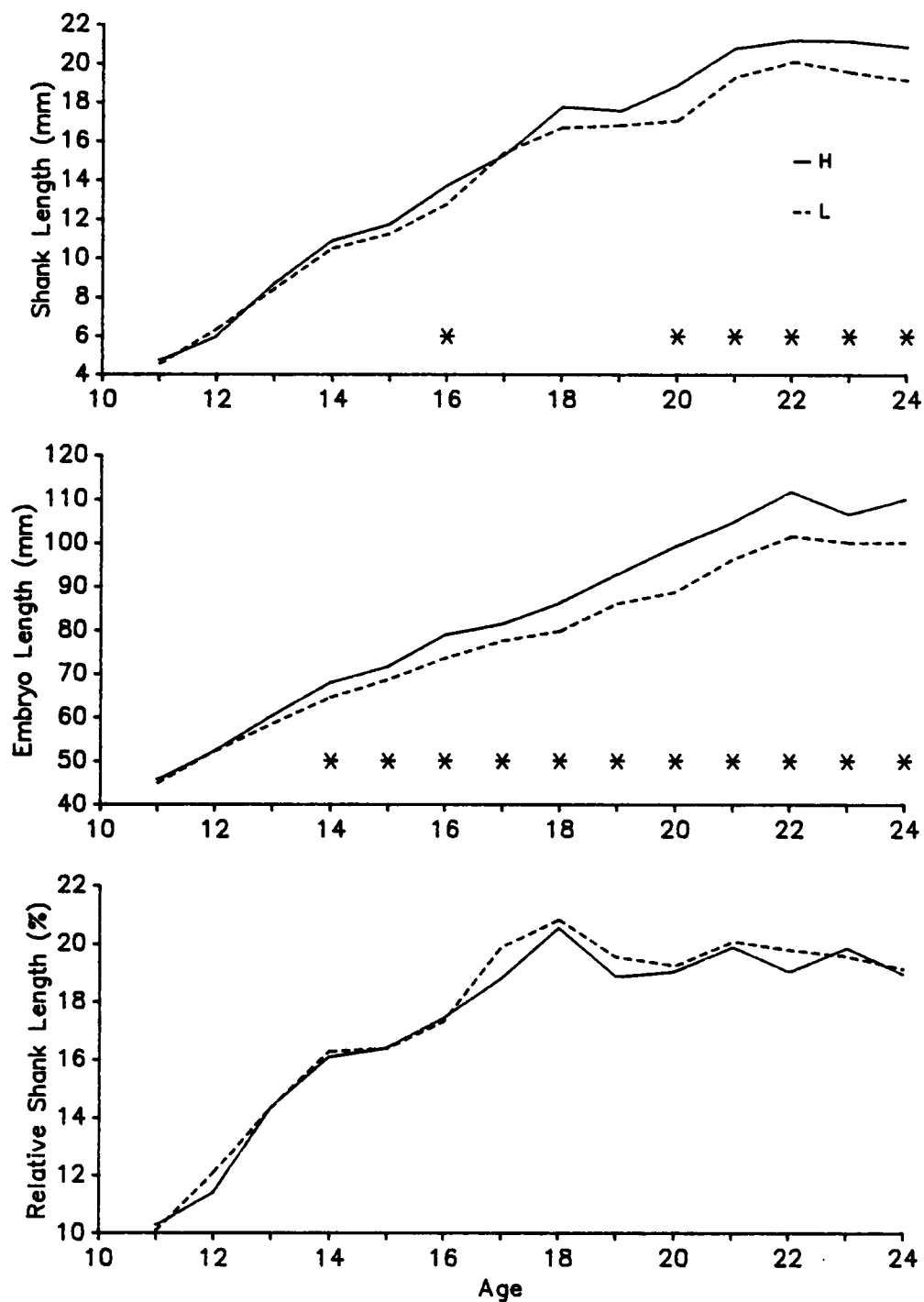


Figure 2. Lengths of shank (upper) and embryo (middle) expressed on an absolute basis (mm) and as a ratio of shank/embryo length (lower) for H and L line chickens (\* denotes  $P \leq .05$ ).

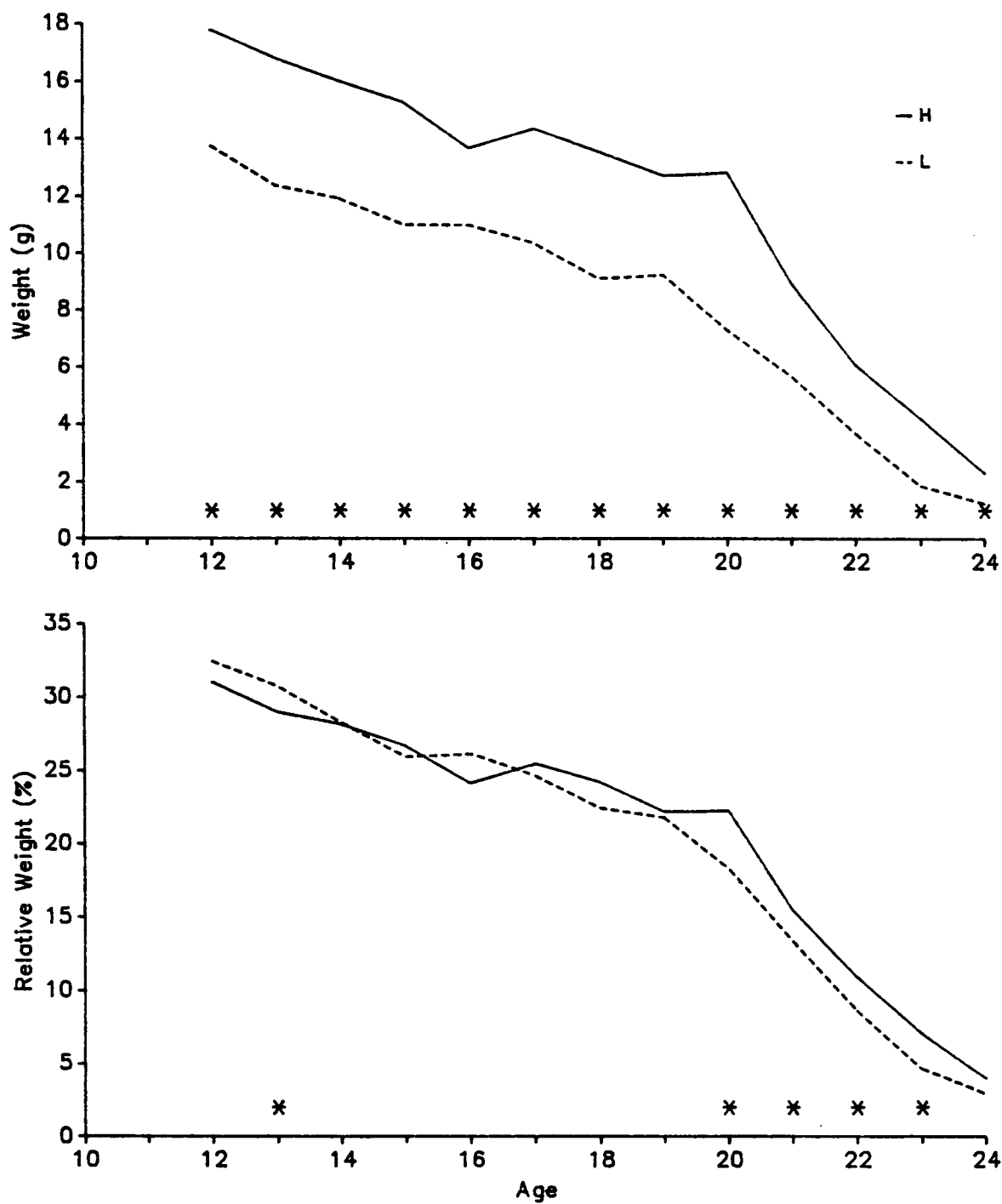


Figure 3. Yolk weight (upper) and the ratio of yolk/egg weight (lower) at start of incubation for H and L lines (\* denotes  $P \leq .05$ ).

## CHAPTER IV

### ALLOMETRIC GROWTH AND DNA, RNA AND TOTAL PROTEIN OF BRAINS FROM WHITE ROCK CHICKENS SELECTED FOR 56-DAY BODY WEIGHT

## INTRODUCTION

Interest in the evolution of brain:body weight relationships dates to the late 19th century (Radinsky, 1978). The basis for brain size evolution was mainly theory until Jerison (1961) provided evidence for evolutionary trends. Subsequently, Jerison (1963) proposed that diversity in brain size evolved as did diversity in body size and although the trend was toward large brain:body weight ratios, species with small brains have existed essentially at all times.

Allometric slopes of brain to body weight (Riska and Atchley, 1985) within large taxonomic groups generally range from 0.50 to 0.68 (Brody, 1945; Jerison, 1979). Although brain weight is generally scaled to the 0.66 power of body weight, implying a relation between brain weight and body surface area (Harvey and Bennett, 1983), the 0.75 power of body weight may provide a more accurate explanation of energetic growth for large-bodied mammals (Bauchot, 1978; Martin, 1981; Hofman, 1982). In general, basal metabolic rates, measured as energy need per unit body weight for a given time period, increase with the 0.75 power of body weight (Harvey and Bennett, 1983). This relationship was discussed by Armstrong (1983) who suggested that the proportion of available energy directed toward the brain accounts for observed deviations in relative brain size.

Since allometric slope differences between species apparently are due to changes in hyperplastic and hypertrophic cellular activity, responses to selection for brain:body weight ratios could result from more rapid brain growth over a prolonged period or a modification of the age



at which brain growth usually decreases. Brain weight has been investigated as a selected trait (Fuller and Geils, 1972; Hahn et al., 1979) and as a trait correlated to body weight (Atchley, 1984). Compared to an unselected control population, selection for larger relative brain weight in mice increased rate of brain growth over a prolonged period, while selection for decreased relative brain weight reduced the period of rapid brain growth (Fuller and Geils, 1972). Riska and Atchley (1985) postulated that the source of correlation between brain and body weight occurred embryonically when both systems were growing rapidly. Changes in early embryonic growth (hyperplastic period) are likely to have a greater influence on brain size than postembryonic (hypertrophic) changes in body weight.

Considerable differences exist among breeds of chickens for structure of the skull (Warren and Smith, 1949). Studies with ground-nesting birds, although limited, indicated a very rapid increase in embryonic brain weight with a corresponding postembryonic decrease in growth relative to body weight. Brain weights of Japanese quail were  $87 \pm 3$  mg at 9 days of incubation,  $308 \pm 8$  mg at hatch and  $745 \pm 20$  mg at 42 days of age with no evidence of sexual dimorphism (McFarland and Wilson, 1965). Although selection increased body weight of Japanese quail by more than 2-fold, absolute brain weights remained the same for the selected and unselected control populations indicating a brain to body weight correlation close to zero (Ricklefs and Marks, 1984).

Additional information on brain growth is needed to better understand genetic mechanisms involved in brain to body weight

relationships. Although gross measurements of brain and body size, as well as cellular characteristics of the brain, are available in mice, there are few for birds. The purpose of this study was to investigate postembryonic brain development through DNA, RNA and protein measures and their relation to brain:body weight ratios.

## MATERIALS AND METHODS

Chickens used in this experiment were progeny from lines selected for 29 generations for high (HH) and low (LL) 56-day body weight (Siegel, 1978; Dunnington and Siegel, 1985). Reciprocal F<sub>1</sub> offspring were obtained through artificial insemination of HH hens with LL semen and LL hens with HH semen. Semen was pooled from at least 10 males within each mating type. The sire line is designated first and the dam line second in identifying matings.

Chicks were obtained from three settings of eggs from age-contemporary dams. Within a setting, all eggs were incubated in the same machine. At hatch chicks were wingbanded, vaccinated for Marek's disease and placed in littered floor pens with forced-air brooding. Lighting was continuous through 14 days of age. From day 14 to 56, the light:dark cycle was 12:12 h, after which chicks were moved to larger floor pens and exposed to natural day length. When 126 days of age, pullets were placed in individual cages with a 14:10 h light:dark cycle. Feed and water were supplied ad libitum.

Chickens were sampled at 1, 2, 4, 7, 10, 14, 18, 24, 32, 56, 128, and 273 days posthatch. The latter age was chosen because pullets from all populations would be sexually mature. Each sampling period incorporated 6 females from each population. Chicks were weighed, killed by cervical dislocation and sex was verified through gonadal examination. The brain from each female was removed and placed on aluminum foil, where it was broken up to form a homogeneous mass, flash frozen in liquid nitrogen and stored at -30 C until analysis.

Analytical Procedures. DNA, RNA and protein content were determined on a subsample of approximately 500 mg of brain tissue. Samples were homogenized in 4 ml sucrose buffer using a Brinkman polytron and liberation of RNA, DNA and protein followed the procedure outlined in Appendix I. Assays were overlapped over ages to minimize confounding of between-assay variation and age. Brain DNA concentration was measured by the Diphenylamine assay (Appendix II) with calf thymus DNA as the working standard. Brain RNA content was analyzed by the Orcinol procedure (Appendix III) with baker's yeast RNA as the working standard. Total brain protein was determined through gravimetric means (Shibko et al., 1967) after RNA, DNA and lipid extractions were complete and each pellet dried to a constant weight (A.O.A.C., 1975).

#### Analyses

Data were analyzed by analysis of variance with age and population as fixed main effects. Brain and body weights were transformed to common logarithms and brain weights relative to body weight and protein/DNA ratios were transformed to arc sine square roots prior to analysis. Orthogonal contrasts were used to differentiate between parental lines (HH vs LL) and reciprocal effects (HL vs LH). Heterosis was estimated as percentage deviation of the combined F<sub>1</sub> mean from the mean for the parental lines. Allometric slopes of brain:body weight were calculated using the formula:

$$\text{brain size} = a(\text{body size})^b$$

where  $a$  and  $b$  were empirically-fitted constants. The allometric coefficient  $b$  is the slope of a line in log-log scale and  $a$  is the intercept (Riska and Atchley, 1985). Slopes were compared by t-test.

## RESULTS AND DISCUSSION

Interactions between age and population were significant for all traits measured. Because of these interactions, data were analyzed within each age.  $F_1$  populations were pooled because of lack of reciprocal effects.

Weight. Body growth was consistent with that of previous descriptions for these populations (Zelenka et al., 1986a; Katanbaf et al., 1988). Briefly, differences between parental lines were present at hatch and increased so that at selection age (56 days) HH chickens were approximately 5 times heavier than those from line LL. That difference was reduced to approximately 2.5-fold for adults. Body weights of  $F_1$  generation crosses were intermediate to parental lines with little evidence of nonadditive genetic variation.

Brain weights increased with age, and brains of HH chicks were heavier than those of LL chicks from 4 to 273 days of age (Figure 1). Expressed relative to total body weight brain weights were greater for LL than HH chicks at all ages. Heterosis for absolute brain weight was present early but not at later ages as changes in body weight were greater than those for brain weight. For relative brain weight, heterosis was negative and significant from 18 to 273 days. This propensity for relative brain weights of  $F_1$  chickens to approach those of parental line HH increased with age.

Allometric slopes (72 df) were similar for lines HH ( $0.328 \pm 0.007$ ) and LL ( $0.336 \pm 0.012$ ). Slopes for the reciprocal  $F_1$  crosses were very similar (HL,  $0.319 \pm 0.007$ ; LH,  $0.317 \pm 0.008$ ) and significantly lower

then those for their parental lines. Deviations in allometric slopes between F<sub>1</sub> crosses and parental lines reflected negative heterosis observed for relative brain weight. These slopes, although higher than the .223 reported for Japanese quail by Ricklefs and Marks (1984), support the same conclusion. Although these selection experiments were in excess of 25 generations and greatly modified body weight, their impact on the brain:body weight relationship was minor compared to that resulting from long term evolution.

Martin (1981) found the brain:body weight exponent was approximately 0.75 for mammals and approximately 0.56 for birds and most reptiles. Differences were due to additional parameters associated with egg weight and metabolic rate. On an evolutionary time scale, the limit to adult brain size may be due to the amount of energy the mother can supply to the developing embryo. In oviparous species, this limit may be the metabolizable energy of the egg (Martin, 1981). Egg weights are greater in line HH (56.2) than LL (40.4) (Chapter I). Lack of reciprocal effects for absolute and relative brain weights indicate that limitations which may exist in metabolizable energy of the egg does not influence brain development. Since neurologic development begins early, embryonic brain size is probably established before growth constraints due to egg size become a factor.

Cellular content of brain. The greater brain DNA (mg/g) for HH than LL chicks at hatch was not present by day 2 or subsequent ages (Figure 2). Brain DNA content which was initially high at hatch exhibited two sharp decreases, one between hatch and 2 days and another between days

7 and 10. One may speculate that the reduced concentrations may be associated with periods of high brain cell death similar to that described for cerebral hemispheres of embryonic chicks (Zamenhof and Marthens, 1978). Heterosis was significant at only 1 of 12 ages (day 18).

Levels of brain RNA (mg/g) were similar for the parental lines except on day 24, and heterosis was significant only on day 32 (Figure 2). High RNA content at and shortly after hatch (Table 1) indicated substantial metabolic activity. There was then a gradual decline in RNA content with no differences between 56, 128 and 273 days of age. a plateau. Brain protein content (mg/g) was generally constant across populations except at on days 4 ( $HH < LL$ ) and 24 ( $HH > LL$ ) with heterosis significant from day 18 through 24. Brain protein content was significantly lower at younger than older ages.

Cell size, as measured by protein:DNA (Table 1), implied that most of the cellular filling had been completed by 10 days and that brain cell size was generally stable thereafter. At hatch and 4 days of age cell size was smaller for HH than LL chicks (Figure 2). These results, plus those previously discussed for DNA content, suggest that HH chicks initially had more and smaller cells than LL chicks. Protein:DNA ratios were similar for parental lines from day 7 to 273 implying similar cellular hypertrophy for both lines. Heterosis was not significant except on day 18. A lack of differences among populations for DNA, RNA, protein and protein:DNA content indicated equal filling and number of cells/g brain weight. Modification of total brain cell content by hatch would, therefore, account for subsequent differences between parental lines in



brain weight. This finding is consistent with that of Riska and Atchley (1985) who described embryonic hyperplastic growth as the key to final brain size.

It appears that much of the framework of cellular content in brain tissue is determined before hatch. This thesis was evident as cell size and number increase at a much lower rate in brain than in muscle and skeletal tissue (Chapter V). Therefore, after hatch hyperplasia rather than hypertrophy is primary in determining brain size, and contributions of hypertrophy to final brain weight is primarily in the form of equal filling of cells regardless of population. Subsequent investigations into allometric changes in brain:body weight should be conducted during the embryonic growth period when brain development is maximum.

## SUMMARY

Brain growth after hatch was measured in lines of White Rock chickens selected for high and low 56-day body weight and their reciprocal  $F_1$  crosses. Absolute brain weights were heavier for high than low line chickens, but brain weights relative to body weights were greater for the line low than high. Allometric slopes for brain:body weight ratios did not differ between selected lines; however, negative heterosis for brain weight caused significantly higher allometric slopes in the  $F_1$  crosses than in either parental line. Populations had similar levels of brain DNA, RNA and protein (mg/g) at all ages, except day 1 for DNA, day 24 for RNA and days 4 and 24 for protein. High-line chicks had more, yet smaller, cells/g brain at hatch than those from the low line. Cellular filling, as measured by protein:DNA ratios was generally completed by day 10. Differences in brain weight at later ages was due to an equal average filling of a greater number of cells in the high then low line. The results imply that embryonic hyperplasia rather than postembryonic hypertrophy dictates brain size in chickens.

Table 1. Means  $\pm$  SEM of DNA, RNA, protein (mg/g tissue) and protein/DNA ratio of brain tissue.

Age (d)	DNA (mg/g)	RNA (mg/g)	Protein (mg/g)	Protein/DNA
1	1.09 $\pm$ 0.07 a	3.60 $\pm$ 0.08 ab	71 $\pm$ 1 de	65 $\pm$ 5 d
2	0.78 $\pm$ 0.05 cb	3.61 $\pm$ 0.11 a	65 $\pm$ 1 f	83 $\pm$ 5 cd
4	0.92 $\pm$ 0.06 b	2.84 $\pm$ 0.06 ef	72 $\pm$ 1 d	78 $\pm$ 4 d
7	0.80 $\pm$ 0.03 bc	3.15 $\pm$ 0.05 cde	69 $\pm$ 1 def	86 $\pm$ 5 cd
10	0.47 $\pm$ 0.02 d	3.20 $\pm$ 0.06 cd	66 $\pm$ 1 ef	140 $\pm$ 8 a
14	0.74 $\pm$ 0.04 c	3.09 $\pm$ 0.18 cdef	77 $\pm$ 2 c	104 $\pm$ 7 bc
18	0.72 $\pm$ 0.06 c	2.87 $\pm$ 0.06 ef	80 $\pm$ 2 bc	111 $\pm$ 29 ab
24	0.71 $\pm$ 0.05 c	3.02 $\pm$ 0.09 def	78 $\pm$ 2 bc	109 $\pm$ 16 ab
32	0.66 $\pm$ 0.04 c	3.34 $\pm$ 0.09 bc	78 $\pm$ 2 bc	119 $\pm$ 8 ab
56	0.67 $\pm$ 0.07 c	3.02 $\pm$ 0.05 def	72 $\pm$ 2 d	107 $\pm$ 9 ab
128	0.69 $\pm$ 0.05 c	2.79 $\pm$ 0.05 f	90 $\pm$ 1 a	130 $\pm$ 6 ab
273	1.13 $\pm$ 0.09 a	2.79 $\pm$ 0.04 f	83 $\pm$ 2 b	73 $\pm$ 6 d

a, b, c, d, e, f Means within a column with the same superscript are not different ( $p > .05$ ).

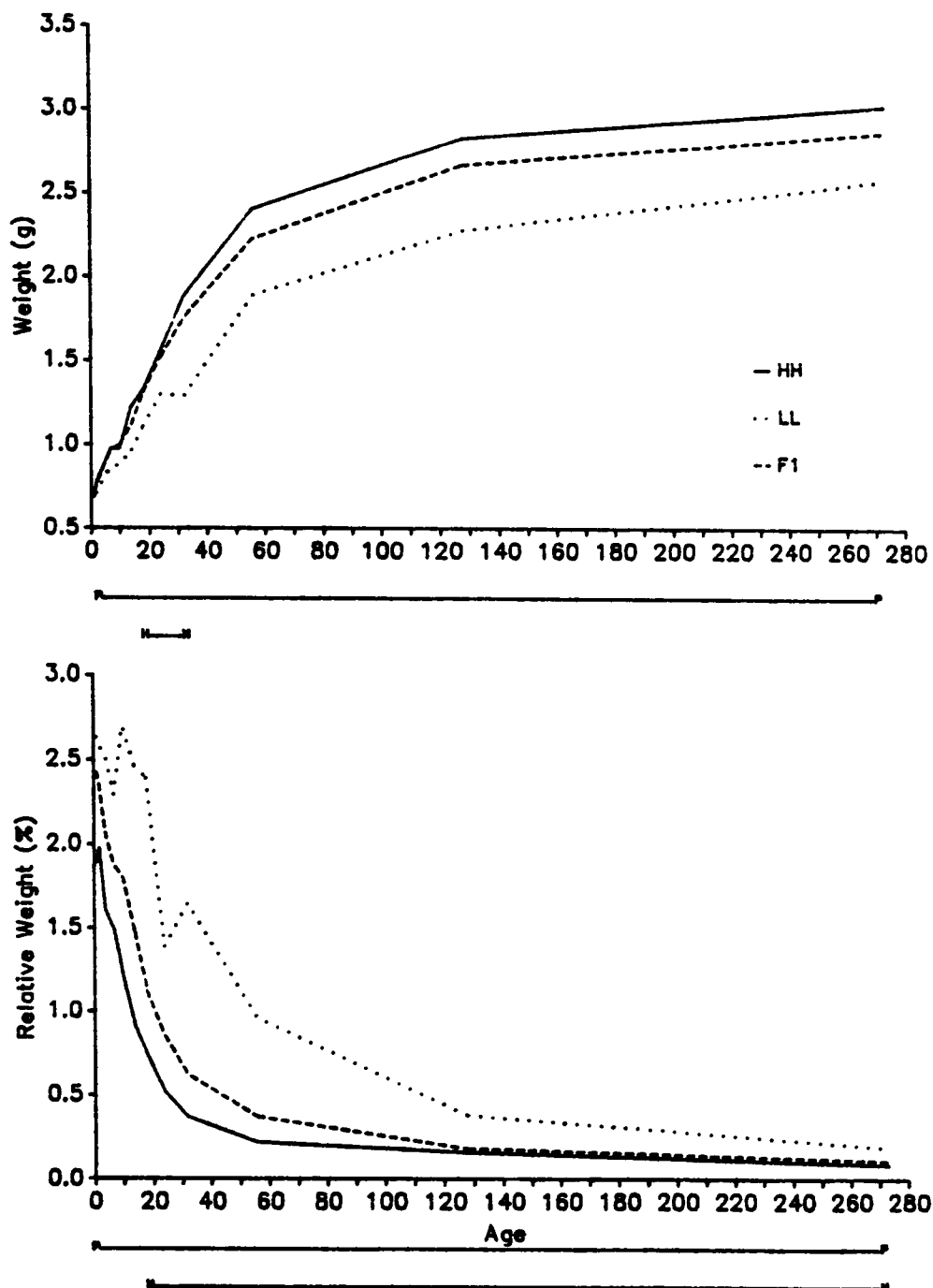


Figure 1. Brain weights expressed on an absolute basis (upper) and relative to total body weight (lower) for HH, LL and F<sub>1</sub> populations by age. (P-P denotes parental differences  $P \leq .05$ ; H-H denotes heterosis  $P \leq .05$ ).

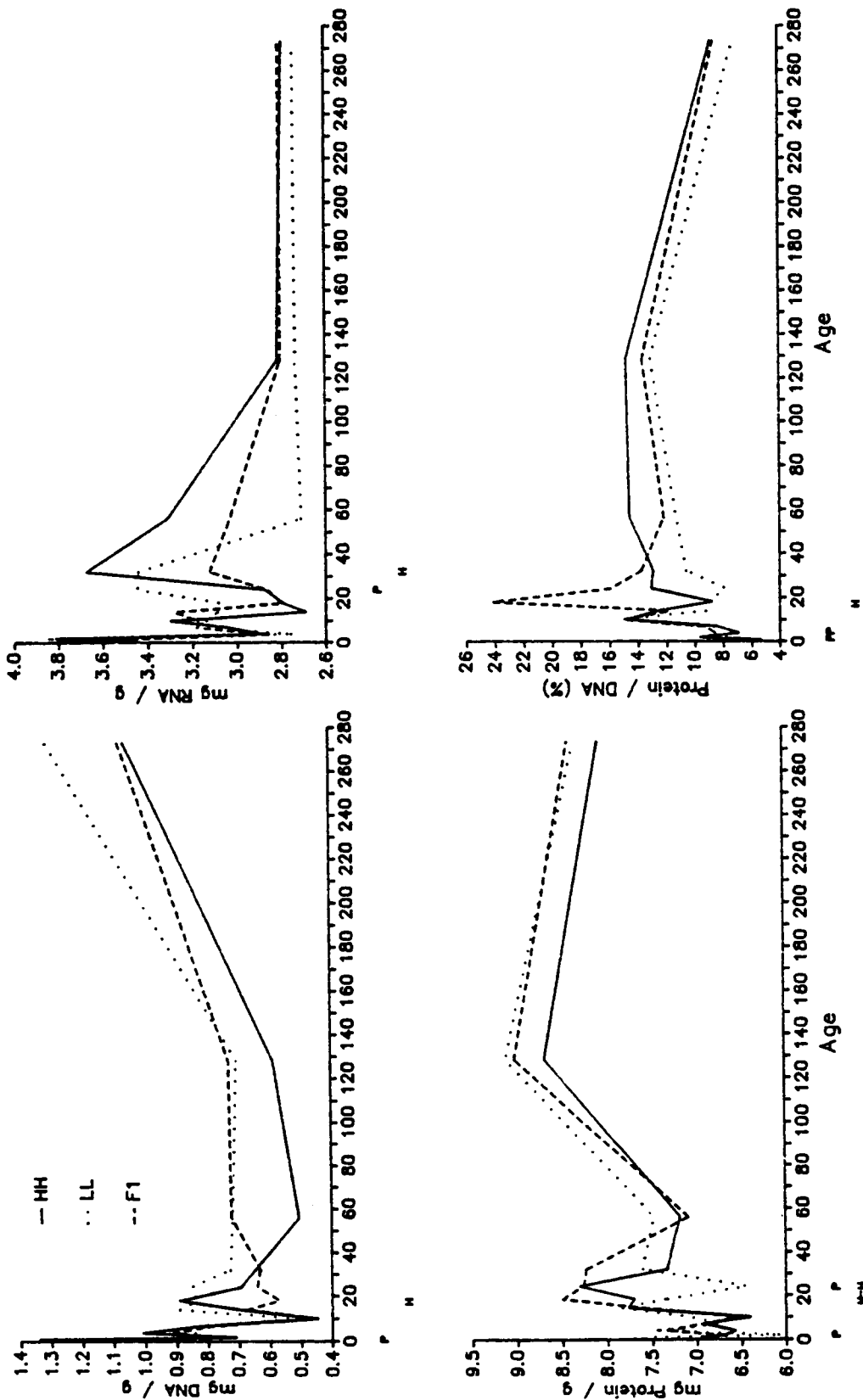


Figure 2. DNA (upper left), RNA (upper right), protein (lower left) and protein/DNA (lower right) ratios for brain of HH, LL and F1 populations by age. (P-P denotes parental differences  $P < .05$ ; H-H denotes heterosis  $P < .05$ ).

## CHAPTER V

### DNA, RNA AND TOTAL PROTEIN CONTENT OF LEG AND BREAST MUSCLES OF WHITE ROCK CHICKENS SELECTED FOR 56-DAY BODY WEIGHT

## INTRODUCTION

Although influences of selection for body weight on muscle accumulation in Japanese quail (Fowler et al., 1980; Bacon and Nestor, 1983; Lilja et al., 1985), chickens (Siegel and Dunnington, 1987; Katanbaf et al., 1988) and turkeys (Nestor et al., 1987b) are well documented, there is little information describing chronological and nuclear origins of muscle. Goldspink (1977) described embryonic muscle development as a period of cellular hyperplasia of mesodermal tissue. As the embryo develops, myoblasts align in rows forming the myotube structure. There is cessation of hyperplastic activity of myoblast cells with innervation of the myotube. This should fix nuclei numbers within the myotube. It has been shown, however, that additional accumulation of nuclei can be derived through satellite cells known to fuse with the muscle fiber (Campion et al., 1982b).

Numbers of adult muscle fibers are determined before hatch (Goss, 1966) and classified as determinant i.e., cells which can be neither replaced nor multiplied. Thus, postembryonic muscle development is primarily through hypertrophy and elongation of preexisting muscle fibers. Although most of the mass increases of an organism occur through postembryonic hypertrophy, the potential number and size of muscle myofibrils determine the growth potential of total muscle. Tinch and McKay (1987) suggested that differences between broiler and layer populations were due to adjustments in nuclei number rather than cell size.

Jones et al. (1986) described muscle deposition as a modification of rates of protein accumulation over time. Hypertrophy of muscle myofibril and, thus, total muscle weight is dictated by the ratio of accretion to degradation. Young birds undergoing periods of rapid growth have higher protein accretion and lower degradation than adults (Scanes, 1987). In adult Japanese quail (Maeda et al., 1985) and chickens (Maeda et al., 1986), the reduction in rate of protein accretion is considerably greater than degradation which ultimately reduces muscle deposition. The point of inflection on the growth curve (Ricklefs, 1967) may coincide with this point of diminishing returns.

Capacity for protein synthesis is controlled by turnover of RNA and therefore, may be defined by RNA concentration. Synthetic efficiency refers to the rate of protein synthesis per unit of RNA (Millward, 1978). Because of the multinucleated state of the myotube, total DNA is not an acceptable measure of myotube number. Rather, total DNA/g tissue (DNA unit number) allows comparisons of nuclei content among populations. The amount of DNA per diploid cell is constant (Moss et al., 1964; Cheek et al., 1971; Cheek, 1985). Similarly, because a nucleus supports a defined volume of cytoplasm (Epstein, 1967; Cheek et al., 1971; Cheek, 1985), protein/DNA ratios can be used to estimate cell size (DNA unit size). DNA unit size varies between muscle types (Millward, 1978), while total muscle DNA is constant with age for poultry (MacDonald and Swick, 1981).

White Rock chickens divergently selected for increased (HH) and decreased (LL) 56-day body weight have extremely different growth patterns (Zelenka et al., 1986a). Although feed intake patterns account



for some of these differences (Siegel et al., 1984), there are other related factors. In the early generations of this selection experiment, differences between lines were reported for muscle cell number and cell size (Lepore et al., 1965). Reasons for these results include modification of embryonic or postembryonic (i.e., satellite cells) hyperplasia causing increased or decreased concentration of nuclei per myotube. Adjustments in duration of hypertrophic growth could also contribute to cellular differences. The purpose of the present investigation was to evaluate, through the use of DNA, RNA, and protein measures, the importance of hyperplasia and hypertrophy on differential muscle growth in weight selected lines of chickens and crosses between them.

## MATERIALS AND METHODS

Chickens used in this experiment were progeny from matings involving lines HH and LL which were developed through divergent selection for body weight at 56 days of age (Siegel, 1978; Dunnington and Siegel, 1985). Parental lines were obtained from S<sub>2</sub> generation matings of lines HH and LL. Reciprocal F<sub>1</sub> offspring were obtained through insemination of HH hens with LL semen and LL hens with HH semen. Semen was pooled from at least 10 males within each mating type. The sire line is designated first and the dam line second in identifying matings.

Data were obtained from three settings of eggs from age-contemporary dams. Within a setting all eggs were incubated in the same machine. At hatch chicks were wingbanded, vaccinated for Marek's disease and then placed in littered floor pens with forced-air brooding. Lighting was continuous through 14 days of age. From day 14 to 56 the light:dark cycle was 12:12 h, after which chicks were moved to larger floor pens and exposed to natural day length. When 126 days of age, pullets were moved to individual cages with a 14:10 h light:dark cycle. Throughout the experiment, feed and water were supplied ad libitum.

Chickens were sampled at 1, 2, 4, 7, 10, 14, 18, 24, 32, 56, 128, and 273 days posthatch. The latter age was chosen because pullets from all populations would be sexually mature. At each age, 6 female offspring from each mating combination were weighed, killed by cervical dislocation and sex verified through gonadal examination. The pectoralis major and minor (pectoralis) and muscles surrounding the tibia (gastrocnemius)

(Chamberlain, 1943) were immediately removed, weighed and flash frozen in liquid nitrogen. Samples were stored at -30 C until analysis.

Analytical Procedures. DNA, RNA and protein content for pectoralis and gastrocnemius muscles were determined on subsamples of approximately 500 mg of tissue. Samples were homogenized in 4 ml of sucrose buffer using a Brinkman polytron and liberation of RNA, DNA and protein followed the procedure in Appendix I. To minimize confounding of age and between assay variation, assays were overlapped among ages. Muscle DNA concentration was measured by the Diphenylamine assay (Appendix II) with calf thymus DNA as the working standard. Muscle RNA content was analyzed by the Orcinol procedure (Appendix III) with bakers yeast RNA as the working standard. Total protein was determined through gravimetric means (Shibko et al., 1967) after RNA, DNA and lipid extraction were complete and each pellet was dried to a constant weight (A.O.A.C., 1975).

### Analyses

Data were analyzed by analysis of variance with age and population as fixed main effects. Body and muscle weights were transformed to common logarithms and relative muscle weights and protein/DNA ratios were transformed to arc sine square roots prior to analyses. Orthogonal contrasts were made among populations to determine differences between parental lines (HH vs LL) and reciprocal effects (HL vs LH). Heterosis was estimated as percentage deviation of the combined  $F_1$  mean from the mean for the parental lines.

## RESULTS

Changes of all traits measured differed among populations over time, resulting in significant age by population interactions. Because of these interactions, data were analyzed within each age and within each population. F<sub>1</sub> populations were combined because reciprocal effects were unimportant for all traits except body weight between days 1 and 7 when the maternal effect on egg size influenced chick weight.

Effects of Selection. Body weights of populations HH, LL and F<sub>1</sub> from hatch to sexual maturity (Figure 1) were consistent with those described by Zelenka et al. (1986a). Growth of pectoralis and gastrocnemius muscles are presented in Figure 2. Percent differences between the parental lines were maximum at 18 days of age with pectoralis and gastrocnemius weights 5.9 and 5.3 times larger, respectively in line HH than LL. By day 273 differences between parental lines were 2.8-fold for pectoralis and 2.4-fold for gastrocnemius muscles. Heterosis for absolute weight of the gastrocnemius muscle was present at 1, 32 and 128 days of age and for pectoralis muscle only at day 128.

Expression of muscle weights relative to total body weight reflected differences in growth patterns between line LL and populations HH and F<sub>1</sub> (Figure 2). Parental line differences for pectoralis muscle were evident at all ages except days 1, 2, 24 and 273. The F<sub>1</sub> chickens mimicked that of parental line HH, with heterosis generally evident from day 10 onward. Growth of gastrocnemius muscles did not follow the same relative pattern as pectoralis muscle through early postembryonic development (Figure 2). Although there were parental line differences

at all ages between day 10 and 128, heterotic effects were present only at 1, 24, 56 and 128 days of age.

DNA Unit Number. DNA/g of pectoralis muscle was high at hatch, rapidly decreased through day 56 and then plateaued (Figure 3). At hatch, values were greater for HH than LL chicks. Differences between parental lines disappeared and reappeared between days 10 and 56 with DNA/g being greater for LL than HH pullets. Heterosis was present at 18, 24, 32 and 128 days of age. Differences between parental lines and heterosis for gastrocnemius muscle were generally similar to those observed for pectoralis muscle (Figure 4).

RNA Content. Highest levels of RNA/g of pectoralis and of gastrocnemius tissue were observed 1 and 2 days posthatch (Figures 3, 4). By day 4, a sharp decline in RNA content had occurred which was followed by a rapid increase and then a steady prolonged decline. Parental line differences for RNA/g pectoralis muscle were present at 1, 4 and 56 days of age with values for LL chicks being higher than those for HH chicks on day 1 and lower on days 4 and 56. Negative heterosis was evident at 10, 18, 24 and 32 days of age. Gastrocnemius RNA/g was greater for LL than HH chicks at 1 and 32 days and lower at 4 and 128 days. Negative heterosis for RNA/g gastrocnemius muscle was present at days 10 and 32.

Protein Content. Parental line differences for protein content of pectoralis muscle were present only at 24 (HH > LL) and 56 (HH < LL) days of age (Figure 3). Heterosis was observed between 18 and 32 days of age with values for crosses being equal to or higher than either parental line. Protein content of the gastrocnemius muscle was greater for LL than

HH chickens on days 4, and 128, and lower on days 10 and 24. Heterosis for gastrocnemius was evident on day 1 and for days 18 through 56.

Protein/DNA Ratio. Although protein/DNA ratios of pectoralis and gastrocnemius muscles were larger for LL than HH chicks at hatch, the pattern quickly reversed (Figures 3, 4). That is, from day 4 to 56 protein/DNA ratios were higher for HH than LL chicks with exceptions being days 18 and 56 for pectoralis and day 18 for gastrocnemius muscle. Heterosis for pectoralis protein/DNA was present from 18 to 128 days of age (except 56 days). In contrast, for gastrocnemius muscle, heterosis was evident only on days 18, 128 and 273.

Protein/RNA and DNA/RNA Ratios. Protein and DNA expressed relative to RNA content did not provide a consistent pattern of significance. Data was therefore not summarized or interpreted for these traits.

Pectoralis and Gastrocnemius Development. Additional analyses compared DNA, RNA and protein of pectoralis and gastrocnemius muscles across ages. For these analysis, data from the populations were pooled with age and muscle type considered as fixed main effects. When differences were found among ages, comparison among means were tested by Duncan's multiple range test.

Pectoralis muscle was consistently heavier than gastrocnemius muscle, both on an absolute and relative to body weight basis at all ages except for absolute weight at hatch (Table 1). Relative pectoralis weights generally increased over time as its relative contribution to total body weight increased at almost every age. Gastrocnemius muscle

did not increase relative to total body weight until after 18 days posthatch.

DNA and RNA content (mg/g) of pectoralis and gastrocnemius muscles decreased with age (Table 2). Change in DNA was greater for the former than the latter while change in RNA was similar for both muscles. DNA unit number was higher in the pectoralis than gastrocnemius muscle from hatch to day 4 (Table 2). For days 7 and 10, DNA unit number was similar for both muscle types after which values were consistently higher for pectoralis than for gastrocnemius muscle. Although similar at hatch, muscle RNA content from day 4 through 10 was greater in pectoralis than gastrocnemius muscle (Table 2); thereafter there were no differences.

From hatch to day 4 protein content was higher in gastrocnemius than pectoralis muscle (Table 3). There was difference on day 7, but on day 10 and thereafter values were greater for pectoralis than gastrocnemius muscle.

DNA unit size, as indicated by protein/DNA ratio (Table 3), indicated that gastrocnemius muscle has a larger unit size than pectoralis to 4 days. A shift then occurred with no difference between muscles on day 7, and then greater values for pectoralis than gastrocnemius muscle from day 10 and subsequent ages. Pectoralis DNA unit size generally increased from hatch to 273 days, while, gastrocnemius unit size remained relatively constant to 14 days and then increased.

## DISCUSSION

Locomotion of precocial birds requires functional use of legs at hatch. Therefore, proper innervation and synchronization of leg muscles must occur prior to hatch (Provine, 1980). Data reported here support this thesis, that at hatch the weight of one gastrocnemius muscle was almost equal to that of the total pectoralis. Gastrocnemius muscle had fewer yet larger DNA units/g tissue than pectoralis muscle, suggesting hypertrophic growth during the embryonic period. Gastrocnemius muscle had a higher protein content than pectoralis muscle at hatch, again supporting the conclusion that leg muscles are more developed than breast muscles at hatch.

Transcription of DNA precedes RNA translation to protein. Thus, elevated levels of DNA/g at hatch for pectoralis muscles allowed transcription of RNA beyond levels found for gastrocnemius muscle. Post-hatch growth of pectoralis muscle was reflected initially as RNA content (mg/g) increased above that for gastrocnemius muscle, indicating the potential for rapid protein synthesis. Zak et al. (1967) found that 85% of RNA isolated from muscle cells was rRNA necessary to translate mRNA. Variable accumulation of RNA among muscle types between days 4 and 10 was reflected in protein content of the respective muscles. Although gastrocnemius muscle had greater protein content than pectoralis from hatch to day 4, protein accumulation, which trailed the elevated RNA content of pectoralis muscle, resulted in protein content becoming greater than that for gastrocnemius. Weights of pectoralis increased more rapidly than gastrocnemius muscle through day 18. Hypertrophic growth



after 18 days still favored the pectoralis as growth of the gastrocnemius was at a slower rate. These results with chickens were consistent with those for Japanese quail (Fowler et al., 1980).

Differential muscle growth between parental lines was apparent at hatch, as DNA unit numbers in pectoralis and gastrocnemius muscles were greater in HH than LL chicks. One may speculate that increased DNA unit number at hatch may provide a vehicle for potential divergence in body mass later in life which could occur by equivalent filling of cells from both lines HH and LL. Minimal change in DNA unit number was observed between 2 and 7 days of age, a period of slow muscle growth both on an absolute and relative basis. Based on information relating the timing of development of supply (e.g., gastrointestinal tract) and consuming (e.g., muscle) organs (Katanbaf et al., 1988), cellular hypertrophy of the latter closely follows the maturation of supply organs. Transition of the digestive system from embryonic absorption of yolk to postembryonic digestion of feed must occur before absorption of nutrients from ingested food can be effectively shuttled from supply to consuming organs.

DNA unit number consistently declined from day 10 to 56 in all populations. Concomitantly, parental line differences for DNA unit size were observed with LL females having smaller values than HH females. Therefore, the difference in rate of growth between HH and LL chickens during this period (Zelenka et al., 1986a) may be attributed mainly to increasing cell size. Differential proliferation and incorporation of nuclei through satellite cells (Campion et al., 1982b), although not

quantified, may have also contributed to muscle mass adjustments between line HH and LL chickens during this stage of rapid development.

Assuming cellular lipid content of muscle remains relatively stable over time and between populations (Cahaner et al., 1987), adjustments in the rates of protein accretion and degradation may have ultimately balanced cell size between line HH and LL. Differences in muscle weight at later ages may be a function of DNA unit number as found at hatch and/or through contributions of nuclei from satellite cells. Although data were not presented, DNA content of the total pectoralis and gastrocnemius muscles increased with age. A similar conclusion was drawn by Tinch and McKay (1987) for muscle unit number in broiler and layer populations of chickens.

Smith (1963) described a strictly additive mode of inheritance for muscle cell size and number. Additive genetic variation was evident in our data as parental line differences in DNA unit number and size at different phases of growth led to differences in muscle weight. Although cell size contributed to differences in body weight during rapid growth, DNA unit number was primarily responsible for differences at hatch and at maturity. In our populations, nonadditive genetic variation was also present during the rapid growth phase with DNA unit size and number for  $F_1$  females similar to that of line HH females. Heterosis for DNA unit size, observed at older ages, was in the form of overdominance. Although genetic variation for absolute muscle weight was primarily additive, nonadditive genetic variation was important at the nuclear level. Future studies emphasizing nuclear proliferation of embryonic muscle tissue

should be attempted in order to investigate the origin of differences in muscle DNA unit numbers at hatch.

## SUMMARY

Pectoralis and gastrocnemius muscles from White Rock chickens divergently selected 29 generations for high and low 56-day body weight were analyzed for DNA, RNA and protein (mg/g) content at 12 ages between hatch and 273 days of age. Dimorphism between lines was maximum at day 18 for both muscle types and then declined with age. High-line chickens generally deposited relatively more muscle tissue than those from the low line. Although nonadditive genetic variation was evident for absolute muscle weights, it was more frequent for muscle weight relative to body weight.

For both muscle types, DNA unit number was larger for the high line than the low immediately after hatch and smaller in the high than low line from day 10 to 56 after which lines were similar. RNA and protein/g muscle were similar for both lines at most ages. Between days 4 and 56, a period of rapid muscle growth, DNA unit size of both muscle types was larger in the high than in the low line. Heterosis was positive for DNA, protein and DNA unit size while negative for RNA content. Pectoralis muscle was heavier and grew at a faster rate than gastrocnemius between day 1 and 273. DNA content was higher in pectoralis than gastrocnemius muscle from hatch to day 4, similar on days 7 and 10 and lower for pectoralis muscle beyond day 10. RNA content was similar at all ages except 4, 7 and 10 days. DNA unit size followed the same pattern as DNA unit number, however, greater nuclei number at hatch for the high line corresponded with low DNA unit size. This pattern suggests a higher rate of cellular filling for pectoralis than gastrocnemius muscle.

Table 1. Means  $\pm$  SEM of absolute and relative weights of pectoralis and gastrocnemius muscles.

Age	Absolute (g)		Relative (g/100g body wt)	
	<u>Pectoralis</u>	<u>Gastrocnemius</u>	<u>Pectoralis</u>	<u>Gastrocnemius</u>
1	0.71 $\pm$ 0.04 i	0.63 $\pm$ 0.03 h	2.27 $\pm$ 0.08 i *	2.04 $\pm$ 0.05 fg
2	0.81 $\pm$ 0.05 i **	0.62 $\pm$ 0.03 h	2.41 $\pm$ 0.09 i **	1.86 $\pm$ 0.05 ih
4	1.42 $\pm$ 0.10 h **	0.90 $\pm$ 0.04 g	3.29 $\pm$ 0.11 h **	2.13 $\pm$ 0.04 ef
7	2.49 $\pm$ 0.18 g **	1.02 $\pm$ 0.06 g	4.66 $\pm$ 0.20 g **	1.92 $\pm$ 0.05 hg
10	2.94 $\pm$ 0.31 g **	0.98 $\pm$ 0.08 g	4.87 $\pm$ 0.23 g **	1.71 $\pm$ 0.03 ij
14	5.63 $\pm$ 0.07 f **	1.44 $\pm$ 0.17 g	6.15 $\pm$ 0.32 f **	1.62 $\pm$ 0.07 j
18	9.15 $\pm$ 1.06 e **	2.58 $\pm$ 0.29 f	7.27 $\pm$ 0.32 e **	2.09 $\pm$ 0.07 efg
24	15.99 $\pm$ 1.45 d **	4.33 $\pm$ 0.41 e	8.37 $\pm$ 0.24 d **	2.26 $\pm$ 0.07 de
32	25.12 $\pm$ 3.06 d **	7.12 $\pm$ 0.80 d	8.13 $\pm$ 0.34 d **	2.37 $\pm$ 0.09 d
56	63.09 $\pm$ 6.91 c **	17.61 $\pm$ 1.87 c	9.75 $\pm$ 0.26 c **	2.76 $\pm$ 0.07 c
128	176.15 $\pm$ 15.57 b **	46.07 $\pm$ 3.76 b	12.70 $\pm$ 0.40 a **	3.40 $\pm$ 0.06 a
273	261.27 $\pm$ 18.81 a **	72.40 $\pm$ 4.81 a	10.84 $\pm$ 0.24 b **	3.05 $\pm$ 0.06 b

\*  $p < .05$ , \*\*  $p < .01$ .

a-j Means within a column with the same superscript are not different ( $p > .05$ ).

Table 2. Means  $\pm$  SEM of DNA (DNA unit number) and RNA for pectoralis and gastrocnemius muscles.

Age	DNA (mg/g)		RNA (mg/g)	
	<u>Pectoralis</u>	<u>Gastrocnemius</u>	<u>Pectoralis</u>	<u>Gastrocnemius</u>
1	2.26 $\pm$ 0.15 a	** 1.46 $\pm$ 0.08 a	4.27 $\pm$ 0.10 a	4.11 $\pm$ 0.07 a
2	1.25 $\pm$ 0.08 b	** 0.93 $\pm$ 0.05 b	4.18 $\pm$ 0.13 a	4.14 $\pm$ 0.13 a
4	1.19 $\pm$ 0.04 b	** 0.98 $\pm$ 0.03 b	3.67 $\pm$ 0.11 c	** 2.99 $\pm$ 0.06 c
7	1.00 $\pm$ 0.06 c	0.99 $\pm$ 0.04 b	4.08 $\pm$ 0.09 ba	** 3.35 $\pm$ 0.05 b
10	0.70 $\pm$ 0.06 d	0.74 $\pm$ 0.03 c	3.80 $\pm$ 0.08 cb	** 3.42 $\pm$ 0.08 b
14	0.65 $\pm$ 0.05 d	* 0.77 $\pm$ 0.04 c	3.57 $\pm$ 0.26 c	3.40 $\pm$ 0.18 b
18	0.55 $\pm$ 0.05 ed	0.66 $\pm$ 0.05 c	3.16 $\pm$ 0.05 d	3.02 $\pm$ 0.07 c
24	0.40 $\pm$ 0.02 fe	** 0.54 $\pm$ 0.03 d	3.08 $\pm$ 0.12 d	3.02 $\pm$ 0.08 c
32	0.31 $\pm$ 0.03 f	** 0.43 $\pm$ 0.03 de	3.56 $\pm$ 0.10 c	3.49 $\pm$ 0.10 b
56	0.26 $\pm$ 0.02 f	** 0.36 $\pm$ 0.03 e	3.19 $\pm$ 0.17 d	3.19 $\pm$ 0.17 cb
128	0.28 $\pm$ 0.02 f	** 0.34 $\pm$ 0.01 e	2.97 $\pm$ 0.05 ed	2.96 $\pm$ 0.05 c
273	0.22 $\pm$ 0.02 f	** 0.31 $\pm$ 0.01 e	2.66 $\pm$ 0.04 e	2.63 $\pm$ 0.04 d

\*  $p < .05$ , \*\*  $p < .01$ .

a-f Means within a column with the same superscript are not different ( $p > .05$ ).

Table 3. Means  $\pm$  SEM of protein and protein/DNA (DNA unit size) for pectoralis and gastrocnemius muscles.

Age	Protein (mg/g)		Protein/ DNA	
	<u>Pectoralis</u>	<u>Gastrocnemius</u>	<u>Pectoralis</u>	<u>Gastrocnemius</u>
1	90 $\pm$ 2 e **	121 $\pm$ 2 d	40 $\pm$ 3 i **	83 $\pm$ 6 f
2	85 $\pm$ 2 e **	117 $\pm$ 1 fed	68 $\pm$ 4 h **	126 $\pm$ 6 ed
4	92 $\pm$ 2 e **	113 $\pm$ 2 ef	77 $\pm$ 3 h **	115 $\pm$ 4 e
7	124 $\pm$ 2 d	122 $\pm$ 2 d	124 $\pm$ 8 g	123 $\pm$ 6 ed
10	124 $\pm$ 3 d **	110 $\pm$ 2 f	177 $\pm$ 13 f **	149 $\pm$ 7 ed
14	137 $\pm$ 5 c **	119 $\pm$ 4 ed	211 $\pm$ 17 f **	155 $\pm$ 8 d
18	169 $\pm$ 6 b **	133 $\pm$ 4 c	307 $\pm$ 60 e *	202 $\pm$ 40 c
24	174 $\pm$ 4 ba **	141 $\pm$ 3 b	435 $\pm$ 33 d **	261 $\pm$ 25 c
32	164 $\pm$ 5 b **	141 $\pm$ 3 b	529 $\pm$ 38 c **	328 $\pm$ 27 b
56	166 $\pm$ 4 b **	141 $\pm$ 4 b	638 $\pm$ 57 b **	392 $\pm$ 31 ba
128	183 $\pm$ 2 a **	158 $\pm$ 2 a	654 $\pm$ 24 a **	465 $\pm$ 21 a
273	173 $\pm$ 2 ba **	146 $\pm$ 2 b	786 $\pm$ 25 a **	470 $\pm$ 13 a

\*  $p < .05$ , \*\*  $p < .01$ .

a-i Means within a column with the same superscript are not different ( $p > .05$ ).

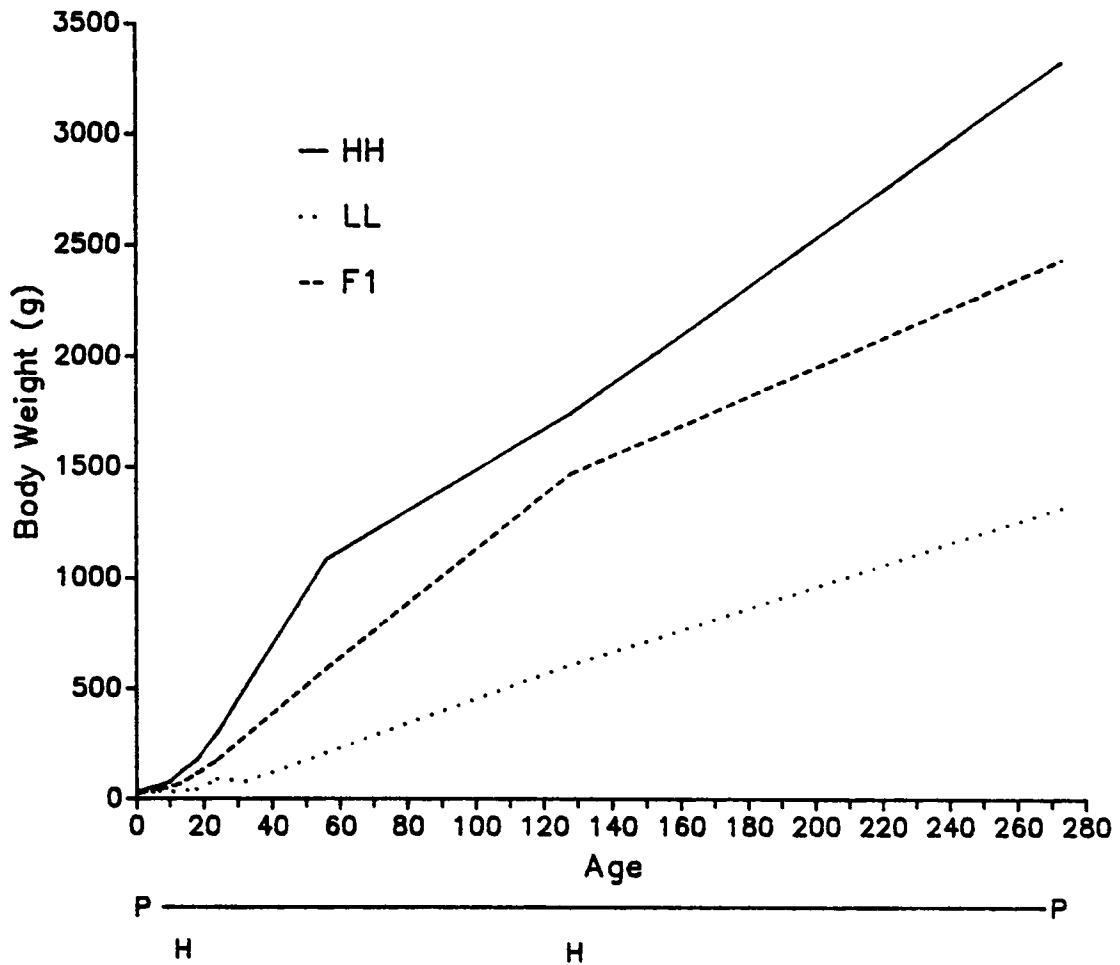


Figure 1. Growth of HH, LL and  $F_1$  populations of chickens by age. (P-P denotes parental differences  $P \leq .05$ ; H denotes heterosis  $P \leq .05$ ).



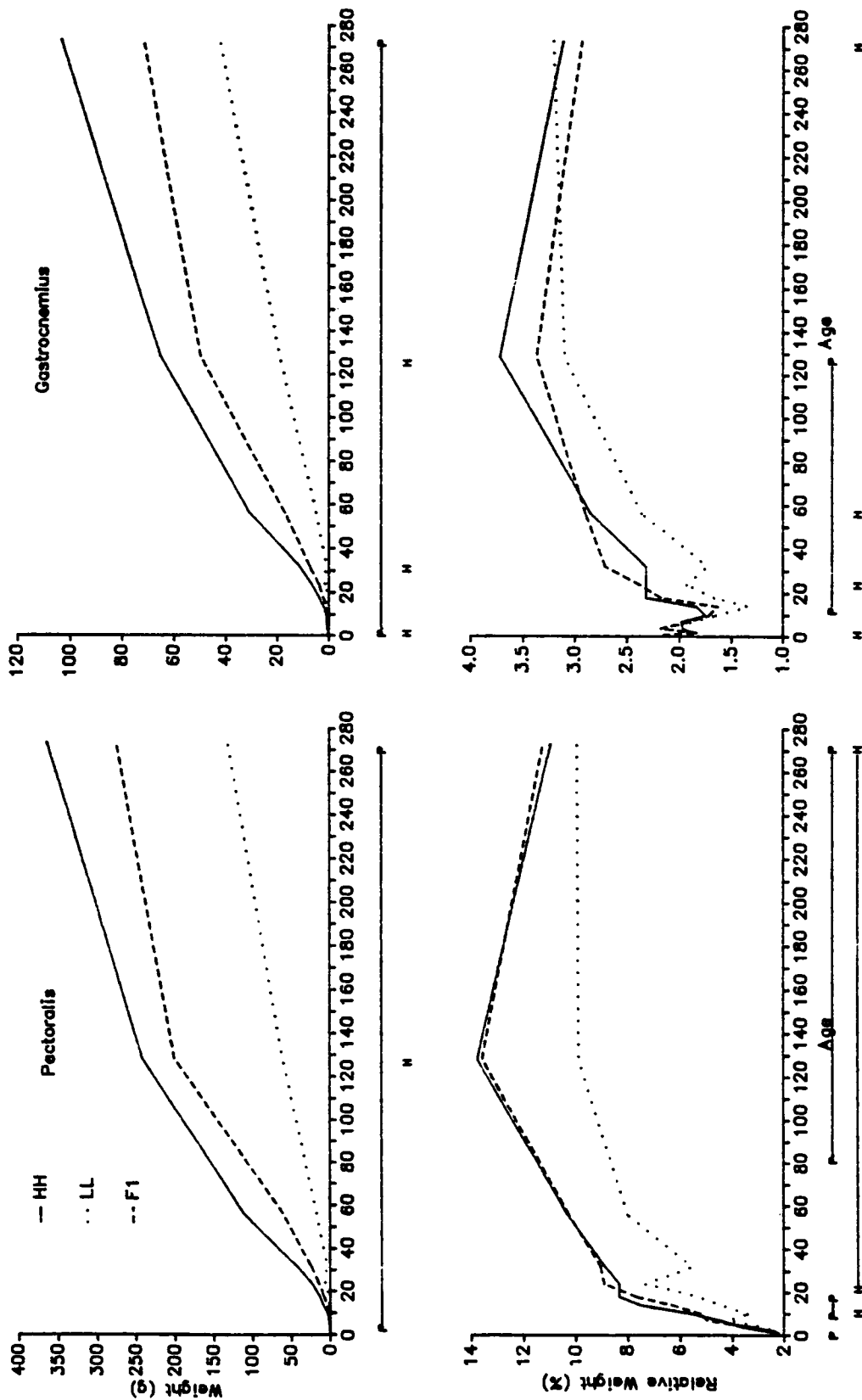


Figure 2. Pectoralis (left) and gastrocnemius (right) muscles expressed as absolute (upper) and relative (lower) to total body weight of HH, LL and F<sub>1</sub> populations by age. (P-P denotes parental differences  $P < .05$ ; H-H denotes heterosis  $P < .05$ ).

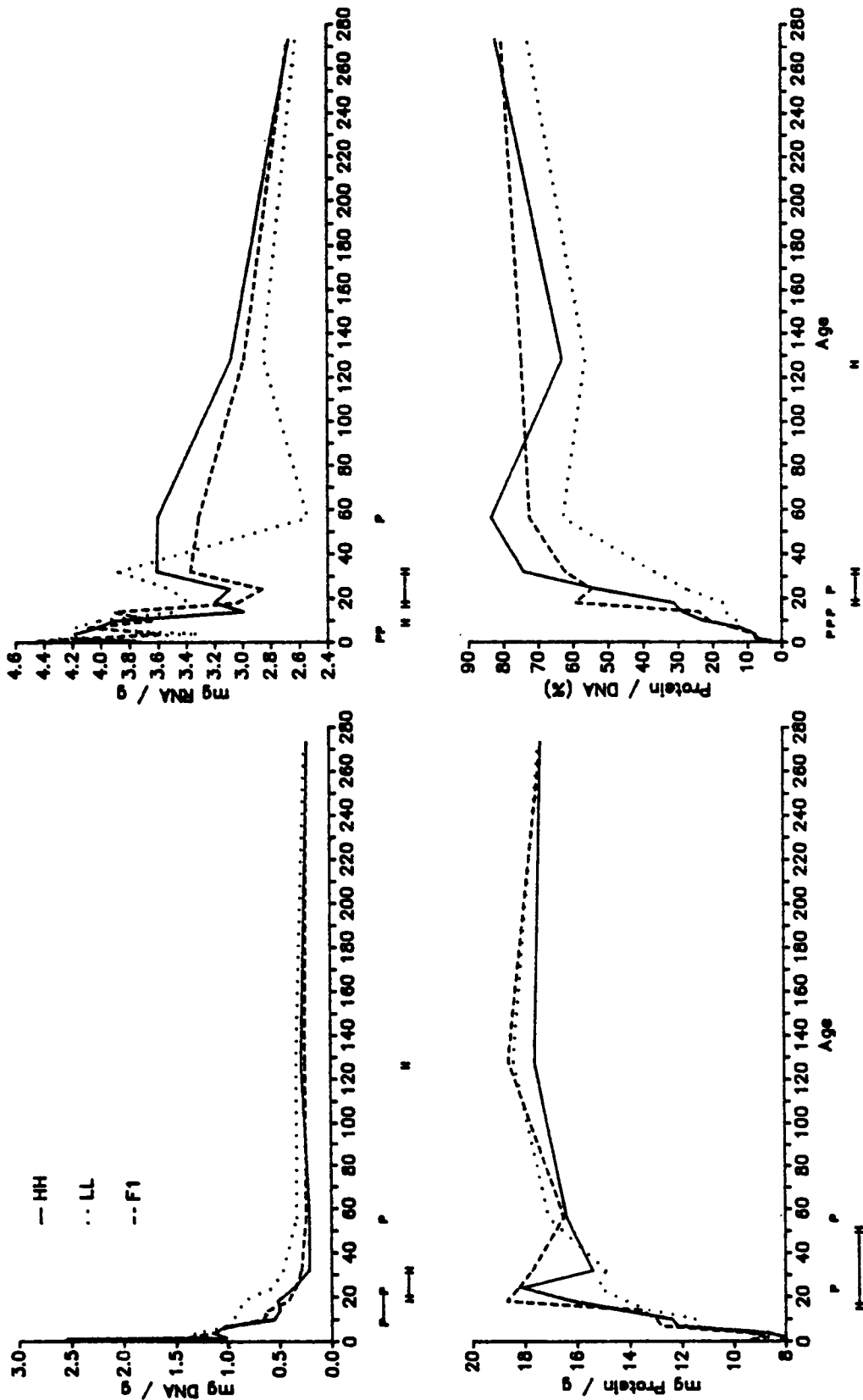


Figure 3. DNA (upper left), RNA (upper right), protein (mg/g) (lower left) and protein/DNA ratios (lower right) for pectoralis muscle of HH, LL and F<sub>1</sub> populations by age. (P-P denotes parental differences  $P < .05$ ; H-H denotes heterosis  $P < .05$ ).

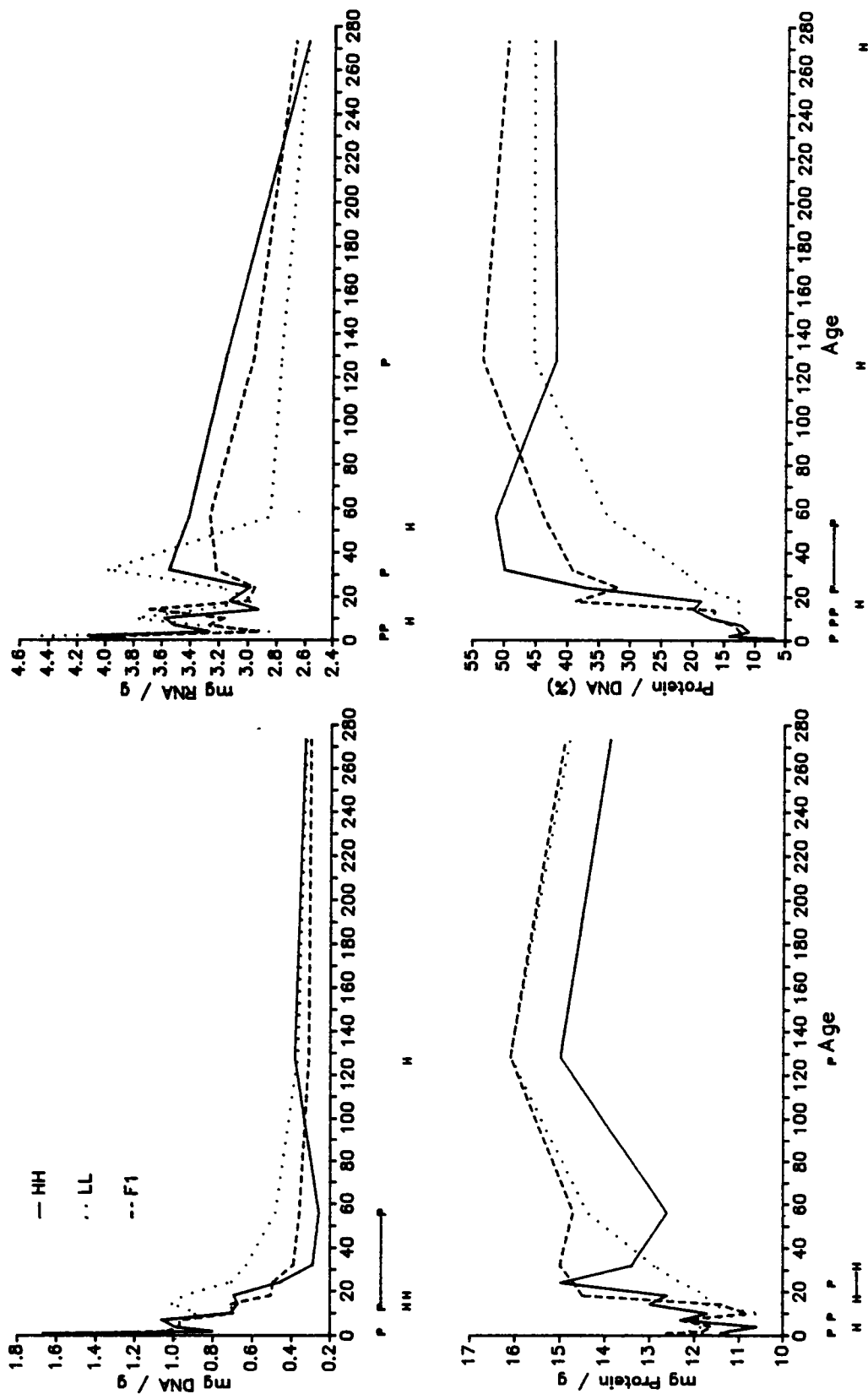


Figure 4. DNA (upper left), RNA (upper right), protein (mg/g) (lower left) and protein/DNA ratios (lower right) for gastrocnemius muscle of HH, LL and F<sub>1</sub> populations by age. (P-P denotes parental differences  $P < .05$ ; H-H denotes heterosis  $P < .05$ ).

## GENERAL SYNTHESIS

Artificial selection for body weight has contributed much to development of the commercial poultry meat industry. Gains in body weight and in efficient utilization of feedstuffs have been associated with correlated responses in obesity and fecundity. Selection for body weight at a specific age modifies growth patterns prior to and subsequent to that age. Although developmental changes appear as differences in body weight, in actuality, changes in tissue masses (i.e., brain, muscle) are due to the interrelating cell dimensions associated with hyperplasia and hypertrophy. Although the balance of hyperplasia and hypertrophy determines final organ size, they do not necessarily follow the same pattern for different tissues.

The experiments reported in this dissertation examined genetic variation associated with growth and reproduction of chickens. Populations used included parental lines of White Plymouth Rock chickens divergently selected for 56-day body weight, their reciprocal  $F_1$  and  $F_2$  crosses and dwarfs derived from the parental lines.

A high incidence of erratic ovulation and defective egg syndrome (EODES) accompanied selection for increased 56-day body weight. The high incidence of EODES in the high line was reduced by introduction of the sex-linked allele for dwarfing and by crossing with the low line. Sexual maturity was delayed in chickens selected for low 56-day weight although the incidence of EODES was low.

Of the egg components studied, shell and yolk were influenced the most by both additive and nonadditive variation. Parental line

differences for yolk dry matter were consistent with lipid overproduction for weight selected populations. Effects of the dw allele on egg composition varied with genetic background. Dwarfs had a lower egg weight and yolk dry matter than nondwarfs indicating the stabilizing effect of dw was similar to that seen in the crosses on yolk deposition.

Reductions in egg weight associated with dw did not reduce embryo weight. Likewise, dwarf embryos grew at the same rate as normal embryos in eggs of comparable weights. Body weight differences associated with dw probably occur postembryonically as described by Hutt (1959) and Zelenka et al. (1986a) and may be a result of differential degrees of muscle cell filling.

Divergence of embryo growth between the high- and low-weight lines occurred before the constraints of egg size became a factor. That is, embryos from the low-weight line filled relatively more of the egg than high-line embryos. During the final three days of incubation, embryos from the high line maintained the same relative egg weight as those from the low line. This pattern suggests growth acceleration by high line embryos prior to day 18. Differences in muscle mass at hatch between the selected lines were primarily due to differences in DNA unit number. This observation suggests that an experiment should be designed to describe embryonic muscle growth in terms of cell size and number. Such an experiment could provide insights as to when differences in muscle growth originate.

Muscle development during the period of rapid growth was through differential rates of filling of muscle cells as noted between muscles

within genetic population. By maturity, however, DNA unit number was constant. Additional research should be conducted to investigate rates of protein accretion and degradation among genetic population and among muscle types. Such studies could add a new dimension to the description of the period of rapid growth.

Allometric slopes of brain to body weight exhibited heterosis. Cellular filling was completed by the 10th day after hatch suggesting brain size was, to a greater extent, determined by embryonic hyperplasia than postembryonic hypertrophy. Since brain development in this context is virtually complete by hatch, a study of embryonic brain growth through gross weights and cell number and size could locate the origin of adult deviations in brain weight.

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## **APPENDIX I**

### **RNA, DNA and Protein Isolation**

METHOD FOR THE SEQUENTIAL QUANTITATIVE SEPARATION OF  
RNA, DNA AND PROTEIN IN BRAIN AND MUSCLE

References: Shibko et al. (1967)

Reagents:

- 1) De-ionized, glass-distilled water
- 2) Buffer- combine 85.5 g sucrose and 0.24 g NaOH in 1 liter of distilled water
- 3) 70% PCA- no modification
- 4) 5% PCA- 72 ml PCA + 928 ml distilled water
- 5) 1.5% PCA- 21.5ml PCA + 978.5 ml distilled water
- 6) 0.3 N NaOH- 12 g NaOH + 1 liter of distilled water
- 7) 0.35% PCA/EtOH- 6 ml PCA (70%) + 994 ml ethanol
- 8) EtOH/Chloroform- 600 ml EtOH + 200 ml chloroform

Procedure:

- 1) Immediately after sacrifice, remove brain, pectoralis and all muscles surrounding the tibia. In larger chickens, a 3 g subsample of the muscle tissue will suffice.
- 2) Freeze samples in liquid nitrogen and store at -30 C.
- 3) Tare tubes to be used throughout the extraction procedure.
- 4) Homogenize approximately 0.5 g of tissue in 4 ml of ice cold buffer solution.
- 5) Add 0.5 ml of ice cold 70% PCA and let stand for 15 min. at 0 C. Centrifuge tubes for 10 min. at 12,000 rpm and pour off supernatant.
- 6) Wash pellet once with 5 ml portion of cold 5% PCA and centrifuge tubes for 10 min. at 12,000 rpm and pour off supernatant.
- 7) Resuspend pellet in 9 ml of 0.3 N NaOH and incubate for 1 h at 37 C.
- 8) Add 1 ml of 70% PCA and cool on ice for 10 minutes.

- Centrifuge tubes for 10 min. at 12,000 rpm  
and save supernatant at 4 C for RNA assay (appendix II).
- 9) Wash pellet once with 5 ml portion of cold 5% PCA.  
Centrifuge tubes for 10 min. at 12,000 rpm and combine  
supernatant with RNA of #8.
  - 10) Resuspend pellet in 4 ml of 1.5% PCA and then incubate  
for 20 min at 90 C.
  - 11) Add 0.25 ml of 70% PCA, and cool on ice.  
Centrifuge tubes for 10 min. at 12,000 rpm  
and save supernatant at 4 C for DNA assay (appendix III).
  - 12) Wash pellet in 5 ml 1.5% PCA and centrifuge tubes for  
10 min. at 12,000 rpm and pour off supernatant.
  - 13) Wash pellet with 10 ml of 0.35% PCA/EtOH solution.  
Centrifuge for 10 min. at 12,000 rpm and pour off  
supernatant.
  - 14) Wash pellet with 5 ml of EtOH/chloroform solution  
Centrifuge for 10 min. at 12,000 rpm and pour off  
supernatant.
  - 15) Wash pellet with 5 ml of EtOH/chloroform solution,  
incubate for 20 min. at 37 C. Centrifuge for 10 min.  
at 12,000 rpm and pour off supernatant.
  - 16) Dry and reweigh tubes and determine protein content as a  
deviation of final tube weight and tare.

## APPENDIX II

### RNA Determination

COLORMETRIC METHOD FOR THE DETERMINATION OF RIBONUCLEIC  
ACID IN BRAIN AND MUSCLE TISSUE

References: Schneider (1957)

Reagents:

- 1) De-ionized, glass-distilled water
- 2) 5% PCA- 72 ml PCA + 928 ml distilled water
- 3) RNA stock solution (500 ug/ml)- dissolve 25 mg of yeast RNA (Sigma) in 50 ml of distilled water. The solution stores for several months at 4 C.
- 4) Prepare standard curve solution concentrations.
- 5) Orcinol Reagent- Dissolve 100 mg of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  and add 100 mg of orcinol (Fisher) into 100 ml of concentrated hydrochloric acid. This reagent should not be prepared until immediately before use.

Procedure:

- 1) Add 1 ml of each of the RNA working standards or 1 ml of RNA of unknown dilutions isolated in Appendix I.
- 2) Add 1 ml of freshly prepared orcinol reagent to each tube.
- 3) Incubate in a boiling water bath for 45 minutes.
- 4) Centrifugation may be necessary at this stage.
- 5) Set spectrophotometer at 670 nm and zero with blank.
- 6) Construct standard curve based on known RNA content against optical densities and extrapolate unknown values.

## **APPENDIX III**

### **DNA Determination**

COLORMETRIC METHOD FOR THE DETERMINATION OF DEOXYRIBONUCLEIC  
ACID IN BRAIN AND MUSCLE TISSUE

References: Burton (1968)

Reagents:

- 1) De-ionized, glass-distilled water
- 2) 1.5% PCA- 21.5ml PCA + 978.5 ml distilled water
- 3) DNA stock solution (400 ug/ml)- weigh 40 mg calf thymus DNA (Sigma) into 100 ml volumetric flask containing 50 ml of 1.5% PCA. Heat for 30 min. at 70 C then cool and dilute to 100 ml with additional 1.5% PCA. The solution may be stored for 3 months at 4 C.
- 4) Prepare standard curve solution concentrations.
- 5) Aqueous Acetaldehyde- Weigh 0.8 g of cooled liquid acetaydehyde and add it to 50 ml of distilled water. Solution stores for several months at 4 C.
- 6) Diphenalamine Reagent- combine the following chemicals in order
  - a) 1.5 g diphenalamine (Fisher)
  - b) 100 ml glacial acetic acid
  - c) 1.5 ml concentrated sulfuric acid
  - d) 1.5ml of 1.6% aqueous acetaldehyde (add right before use)

Procedure:

- 1) Add 1 ml of each of the DNA working standards or 1 ml of DNA of unknown dilutions isolated in Appendix II.
- 2) Add 2 ml of diphenalamine reagent to each tube and cover with parafilm.
- 3) Incubate at room temperature for 20 h.
- 4) Set spectrophotometer at 600 nm and zero with blank.
- 5) Construct standard curve based on known DNA content against optical densities and extrapolate unknown values.



## LIST OF TERMS

- HH - Population selected for increased 56-day body weight.
- LL - Population selected for decreased 56-day body weight.
- dw - Sex linked dwarfing allele.
- HD - Dwarf subpopulation of HH.
- LD - Dwarf subpopulation of LL.
- N - Nondwarf populations HH and LL.
- D - Dwarf populations HD and LD.
- H - High weight populations HH and HD.
- L - Low weight populations LL and LD.
- HL - F<sub>1</sub> population derived by crossing a line HH male and LL female.
- LH - F<sub>1</sub> population derived by crossing a line LL male and HH female.
- TD - Trapday.
- A/Y - Ratio of albumen to yolk.
- EODES - Erratic ovulation and defective egg syndrome.
- DNA unit number - DNA content/g of tissue.
- DNA unit size - The ratio of protein/g to DNA/g of tissue.
- RNA content - RNA/g of tissue.
- Protein content - Protein/g of tissue.

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