

GENOTYPE BY FEEDING REGIMEN INTERACTIONS

IN GROWTH-SELECTED CHICKENS

by

Neil P. O'Sullivan

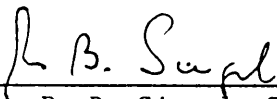
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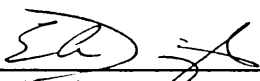
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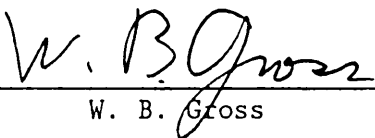
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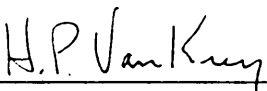
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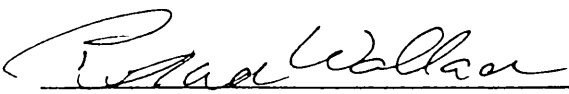
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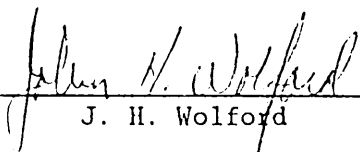
  
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(ABSTRACT)

Growth, reproduction, and immunocompetence were measured in lines of chickens maintained under different feeding regimens. Populations included a commercial broiler breeder parent line segregating at a sex-linked feathering locus ( $K, k^+$ ) and 4 experimental lines of which 2 had undergone 32 generations of divergent selection for 56-day body weight and 2 were their sublimes in which selection has been relaxed for 5 generations.

Mild feed restriction of the broiler line from 7 to 27 days of age reduced carcass fat and heterophil:lymphocyte ratios, and increased immune organ weight, antibody titer to sheep red blood cell (SRBC) antigen and livability than *ad libitum* fed birds. Body weights were similar by 56 days of age, and there was sexual dimorphism for rate of accelerated growth.

Long term obesity, but not short term weight gain, was detrimental to reproductive performance, feed utilization, response to SRBC, and resistance to *Escherichia coli*, lymphoid leucosis and livability of broiler breeder dams. Poorer quality crumbles also reduced reproductive performance. An association between an endogenous viral gene encoding

for avian leucosis virus (*ev21*) and the sex-linked *K* allele of the Z chromosome was confirmed in the broiler genome. Reproductive performance and feed utilization were inferior for *K*/<sup>-</sup> than *k*<sup>+/-</sup>, notwithstanding a pleiotropic effect of *K* associated with heavier egg and embryo weights. Variation in residual feed consumption was influenced by feathering genotypes and management practices. Increases in hatchability for the initial period after onset of lay were due to a reduction in early embryo deaths. Egg and 18-day embryo weights, ratios of embryo:egg and yolk:albumen, and proficiency of lipid transfer also increased but the latter was not associated with higher hatchability.

Selection for 56-day body weight resulted in a divergence between lines at 21 days of age of 404% for body weight, 279% for feed intake and 138% for feed conversion ratio. Genotype by feeding regimen interactions were observed for growth and appetite development. Early posthatch growth of small intestine was highly correlated with subsequent growth of demand organs. Selection had also resulted in correlated changes in cell size of muscles, but not liver or small intestine which increased in size due to cell hyperplasia. Correlated changes in feed intake mediated synthesis and secretion of digestive enzymes.



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## INTRODUCTION

Increased growth potential and overconsumption of feed have resulted in broiler chickens reaching market weight at progressively younger ages. Achieving market weight at younger ages results in less feed being needed for maintenance and corresponding improvements in feed efficiency. Because positive genetic and phenotypic relationships between broiler and adult body weight result in highly undesirable adult body weights, feed restriction programs have become routine as a partial solution to controlling body weight of broiler breeders. These feed restriction programs have a positive influence on both durability and performance of males and females during the reproductive stage of life. Studies with mice show that feed restriction programs can enhance immunological vigor and reduce immunological involution normally associated with aging (Ogura *et al.*, 1989). Although some data are available for chickens (e.g., Han and Smyth, 1972; Katanbaf *et al.*, 1987), additional information is needed on effects of feed restriction on reproduction and immunocompetence in different growth selected populations of chickens.

Further processing of poultry has encouraged increased market weight, and hence, increased age at marketing. This additional outlet for poultry meat means chickens are marketed at several different weights and provides an incentive for rearing of sexes separately. Use of the sex-linked early (*k+*) and late (*K*) feathering alleles facilitates rapid separation of sexes by phenotype based on feather development at hatch. Association on the Z chromosome of allele *K* with an endogenous virus that

increases the incidence of infection with avian leukosis virus has been reported in Leghorn chickens (Bacon *et al.*, 1988). Whether this association exists and its possible consequences in the background genome of broiler breeders requires further study.

A negative genetic correlation between body weight and egg number increases the importance of maximizing hatchability of settable eggs from broiler breeders. With genetic progress in growth of broilers being approximately 3% per year, producers must avail themselves of genetically superior replacement breeders, rather than recycling existing breeding flocks. Yet, age of dam is related to hatchability with lower values observed during the early periods of egg production. The ratio of yolk:albumen changes with age (Anthony *et al.*, 1989a) and lipid transfer from yolk sac to embryo is reduced in the embryos from egg produced by young breeders (Noble, 1987). Relationships among age of female, yolk:albumen ratio and transfer of lipids from the yolk sac to the developing embryos via the liver requires further study to determine if these factors lead to lower hatchability of fertile eggs.

The relationship among feed intake, utilization and residual feed consumption has been studied extensively in egg type layers. The residual component of feed consumption has a moderate to high heritability and low correlations with economically important traits (Luiting and Urff, 1991a,b). There is, however, a dearth of information on this trait in meat-type chickens. Research in this area has potential not only for improvements of feed efficiency independent of body weight changes in the growing broiler, but as benefits to adult broiler breeders. Feeding

programs, feed consistencies, and alleles to facilitate autosexing all play major roles in broiler breeder production and data on their interrelationships are lacking.

Feed efficiency, when measured as a correlated response to selection for growth rate or body weight, is confounded by correlated changes in feed intake. Research on feed efficiency and growth of lean and fat tissue under conditions free of relative differences in feed intake should provide insights into the nature of these traits and how they may have been influenced by selection for growth. Such an understanding should contribute to the development of breeding programs which utilize correlations between body weight and feed efficiency. Understanding partitioning of feed resources prior to and after the age of selection in chickens selected for body weight will become increasingly important as broilers are slaughtered at different weights to meet retailing needs.

Synchronization of growth as mediated by the ontogeny of supply and demand organs and their genetic and environmental interactions are essential to maximize growth potential. Production systems designed to control biologically the synchrony of growth will facilitate further development of heavier broilers at younger market ages. Although the development of supply organs and the synthesis and secretion of digestive enzymes from the pancreas to the small intestinal chyme may be related, presently the relationships are not clear. Because reduced age to market weight has been characteristic of developments in broiler breeding, the

first 3 weeks posthatch when development of these systems is underway are critical.

A major objective of the research reported in this dissertation was to determine the effects of alleles at the sex-linked feathering locus on growth, body composition, reproductive performance, feed utilization, and immunoresponsiveness in young and adult meat-type chickens. A second major objective was to measure correlated responses in populations of chickens selected for juvenile body weight while minimizing feed intake differences between populations.

## REVIEW OF LITERATURE

### *Body Weight Selection*

Selection for juvenile body weight at market age has been reviewed for meat-type chickens (Cahaner and Siegel, 1986; Carte, 1986) experimental populations of chickens (Siegel and Dunnington, 1987; Chambers, 1990), turkeys (Buss, 1990), other galliforms (Marks, 1990), waterfowl (Pingel, 1990), and mice (McCarthy and Roberts, 1989). Long-term intense selection for this trait shows little if any evidence of plateauing. Body weight at market age is the primary trait in selection of meat-type chickens (Siegel and Dunnington, 1988), and decreases of one day each year since 1950 have been reported (Gyles, 1989). This trait is of moderate heritability and is easily measured in large populations (Cahaner and Siegel, 1986). Continuous long-term selection for increased juvenile body weight has resulted in chickens that eat at near gut capacity (Nir *et al.*, 1978). This overconsumption of food is a result of altered control of brain and peripheral appetite (Burkhart *et al.*, 1983; Lacy *et al.*, 1985; Denbow, 1989) which leads to obesity (McCarthy and Siegel, 1983; Katanbaf *et al.*, 1989c) and reproductive complications (Siegel and Dunnington, 1985). Differences in fast and slow growing lines of chickens may be due, in part, to altered growth of intestinal segments (Katanbaf *et al.*, 1988b) and altered ability to synthesize and secrete pancreatic digestive enzymes (Nitsan *et al.*, 1989)



### *Responses in Feed Efficiency and Consumption*

Numerous studies with mice (Fowler, 1962; Rahnefeld *et al.*, 1965; Lang and Legates, 1969; Timon and Eisen, 1970; Sutherland *et al.*, 1970; Jara-Almonte and White, 1973; Bakker, 1974; Brown and Frahm, 1975; Eisen, 1977; Roberts, 1981), chickens (Lepore, 1965; Siegel and Wisman, 1966; Proudman *et al.*, 1970) and quail (Marks, 1980; 1991) have demonstrated positive correlated responses in feed efficiency with selection for body weight at a fixed age, or for weight gain under *ad libitum* feeding. Realized genetic correlations between growth rate and feed conversion (the inverse of feed efficiency) of  $-.51$  and  $-.50$  were reported by Wilson (1969) and by Pym and Nicholls (1979), respectively. There is a large increase in feed consumption correlated to selection for increased body weight or age at which market weight is achieved in meat-type chickens (McCarthy and Siegel, 1983). Studies with force-fed chickens (Nir *et al.*, 1978) show that meat-type stocks selected for market body weight eat to near gut capacity. This capacity to maximize consumption of food relative to body weight results in smaller relative maintenance costs.

Selection for growth under restricted feeding regimens has been conducted in mice (Falconer and Latyszewski, 1952; Falconer, 1960; McPhee *et al.*, 1980; Hetzel and Nicholas, 1986; McPhee and Trappett, 1987; McCarthy and Roberts, 1989). These experiments were based on the expectation that selection for increased growth rate in an environment where feed intake was restricted would minimize differences in food intake. The thesis is that differences in efficiency of food utilization

for gain would form the main source of variation, and thus, selection would act on genes more involved with feed efficiency than feed intake, which occurs when selection is under *ad libitum* feeding (Roberts, 1979; 1981). This approach has not been productive because genotype by environment interactions occur when lines selected on one regimen are placed on other feeding regimens (Hetzel and Nicholas, 1986; McCarthy and Roberts, 1989).

Mice and chickens from lines selected for rapid growth under *ad libitum* feeding show consistent increases in food consumption (McCarthy, 1982; Siegel and Dunnington, 1987). Changes in feed efficiency in such growth selected lines are due, mainly, to more energy being available for growth over maintenance requirements (Hayes and McCarthy, 1976; Yüksel, 1979). Siegel and Wisman (1966) pair-fed chicks from a line selected for high body weight (HW) to the feed intake level of the low weight (LW) selected line. Each HW chick was fed the amount of feed eaten the previous day by its LW partner proportional to their respective body weights. There was an advantage in feed efficiency for HW chicks relative to LW chicks. Because superior feed efficiency was masked by intake, this advantage was not observed under *ad libitum* feeding. A number of generations later, comparisons with these lines demonstrated an advantage in feed efficiency in line HW relative to line LW under *ad libitum* feeding (Owens *et al.*, 1971).

Zelenka *et al.* (1988) reported that some LW females did not become sexually mature due to anorexia. The effect of this inability to reproduce on selection is described by Siegel and Dunnington (1987).

Although these females may not consume sufficient food *ad libitum* to achieve minimum physiological thresholds of body weight, fat and/or lean tissue to reach sexual maturity (Zelenka *et al.*, 1986a; 1987), they commence egg production when force-fed (Zelenka *et al.*, 1988). Rates of lipolysis have been accelerated relative to lipogenesis in line LW compared to line HW (Calabotta *et al.*, 1985). Whether this pattern prevents thresholds for fat tissue from being reached by LW females requires further study. The possibility that fatty acid oxidation and glycolysis are also factors in reproduction was reported for hamsters (Schneider and Wade, 1989). These cycles apparently control entry into estrous by the provision of metabolic fuels. Additional data are needed to determine whether appetite, rate of tissue maintenance or a combination of both are critical factors in the unique growth characteristics of LW chickens.

Burkhart *et al.* (1983) reported altered hypothalamic food satiety mechanisms in chickens from lines HW and LW with an absence of response to lesioning of the ventromedial hypothalamus in the line HW. Such lesioning typically results in hyperphagia (Lepkovsky and Yasuda, 1966). The response of HW chickens to force-feeding (Barbato *et al.*, 1984; Robey *et al.*, 1988) demonstrated that gut capacity was almost fully utilized when they had *ad libitum* access to feed. Their results also showed an effect of age on ability to accommodate an increased food intake with the potential for force-feeding greater in adults than growing chickens. This information, coupled with the anorexia observed in line LW (Zelenka *et al.*, 1988), shows that selection dramatically altered appetite in these

lines to divergent extremes. Such correlated responses may oppose natural selection in terms of fitness in both of these lines (Dunnington *et al.*, 1986; Dunnington, 1990).

### *Responses in Carcass Composition*

Pym (1982) discussed a deficiency in understanding of rates at which lean and fat tissues were maintained in selected populations of meat-type chickens up to and after age of selection. Experiments with lines HW and LW (Calabotta *et al.*, 1983; 1985) suggested that under *ad libitum* feeding the higher rate of lipolysis relative to lipogenesis in the LW than the HW line accounts for the higher rate of fat deposition in the latter. Anthony *et al.* (1989b) found both additive and nonadditive genetic variation for muscle accumulation in HW and LW chicken. Changes in cell unit size (protein/DNA) in both muscles studied (pectoralis and gastrocnemius) were most pronounced during the period of autoaccelerated growth in HW relative to LW chickens. Rate of filling was greater after hatch in pectoralis than gastrocnemius muscles, as the latter muscle is vital for locomotion immediately after hatch. Brain cell unit size and rate of filling, in contrast to muscle tissues, were unresponsive to selection for body weight (Anthony *et al.*, 1991b). Postnatal growth of birds is determined by distribution of growth among organs (Lilja, 1981; Prescott *et al.*, 1985). Characterizations of lines HW and LW and their F<sub>1</sub> cross (Katanbaf *et al.*, 1988b) showed that growth of organs was not proportional to body weight but varied with age and was amenable to alteration by selection.

Studies with mice selected bidirectionally for high and low body weight at different ages indicated that under *ad libitum* feeding, those from high weight lines are leaner at or before the age of selection and then become fatter at later ages than mice from low weight lines (Hayes and McCarthy, 1976; Roberts, 1981). Because selection for body weight resulted in correlated changes in feed consumption in both chickens (Siegel and Dunnington, 1987) and mice (McCarthy, 1982), rates of efficiency of lean and fat tissue deposition under *ad libitum* feeding are confounded with changes that occur in relative feed intake.

#### *Response in Humoral Immunity*

Body weight and antibody titers to sheep erythrocytes (SRBC) are negatively correlated in chickens (Martin *et al.*, 1988; van der Zijpp *et al.*, 1987; Siegel *et al.*, 1982; 1984). Artificial selection for antibody production to SRBC has been studied in chickens (Siegel and Gross, 1980; Dunnington *et al.*, 1984b; van der Zijpp and Nieuwland, 1986; Pinard *et al.*, 1990) and in mice (Biozzi *et al.*, 1970; Feingold *et al.*, 1976). These selection experiments show that responses in antibody titers to SRBC are influenced by polygenic systems, as well as major loci such as the major histocompatibility complex, and that selection resulted in a measure of immunoresponsiveness which was associated with resistance to certain diseases.

Bidirectional selection for body weight in White Rock chickens has led to correlated changes in antibody responses to SRBC antigen with consistently lower titers in line HW than LW (Martin *et al.*, 1988;

Dunnington *et al.*, 1989). These lines are at divergent extremes in appetite (Burkhart *et al.*, 1983; Zelenka *et al.*, 1988) and, therefore, may provide further insights into effects of diet on immunological function that are not readily available from less diverse populations. Diet is a potential source of variation in immunological responsiveness (Gross and Newberne, 1980) and has generated considerable interest in recent years (see review by Cook, 1989). Also, there is considerable genetic variation for size of immunological organs (Yamamoto and Glick, 1982).

The effect of selection for body weight on production of 2-mercaptoethanol resistant (MER) antibodies and 2-mercaptoethanol sensitive (MES) antibodies, measures of immunoglobulin G (IgG) and immunoglobulin M (IgM), respectively, in chickens (Delhanty and Solomon 1966), may further our understanding of the ability of these stocks to meet immune challenges. In White Rock chickens, van der Zijpp *et al.* (1983) reported a very high MES antibody response after a primary immunization with SRBC. Because only IgG is passed through the egg (Darbyshire, 1987), the level of passive immunity given to the chick by its dam could potentially be affected by selection for body weight. Additional data are needed to clarify relationships between body weight selected lines and titer response in terms of IgG and IgM.

### *Broiler Breeders*

When fed *ad libitum* broiler breeders overconsume feed and much of the excess is deposited as fat. The resulting obesity contributes to

reproductive complications and a deterioration of health (McDaniel *et al.*, 1981; Siegel and Dunnington, 1985; Wilson and Harms, 1986; Katanbaf *et al.*, 1989a). The age at which sexual maturity is reached depends on chronological age and minimum body weight requirements being fulfilled (Dunnington *et al.*, 1983; 1984a) and carcass components being in physiologically balanced proportions (Bornstein *et al.*, 1984; Brody *et al.*, 1984; Zelenka *et al.*, 1988). Restriction of feed intake during the rearing period controls body weight and delays onset of egg production of broiler breeders (Pym and Dillon, 1974; Pearson and Herron, 1982; Bornstein *et al.*, 1984; Soller *et al.*, 1984; Robbins *et al.*, 1986; Katanbaf *et al.*, 1989b). This delay in onset of lay increases egg size (Blair *et al.*, 1976; Leeson and Summers, 1983; Katanbaf *et al.*, 1989b), resulting in more settable eggs. Body weight is reduced and livability improved by feed restriction during rearing and lay (Lee *et al.*, 1971; Wilson and Harms, 1986; Katanbaf *et al.*, 1989b). Successful feed restriction programs for broiler breeders require severe control of feed intake, which has led to the development of management practices to maximize flock uniformity. Programs include daily restriction of feed to alternate or every third day feeding (Bartov *et al.*, 1988; Katanbaf *et al.*, 1989a;b;c) during rearing and daily restriction after the onset of lay. An increase in production of normal eggs due to lowered rates of erratic ovulation and defective egg syndrome (EODES) is also achieved by feed restriction (Christmas and Harms, 1982; Hocking *et al.*, 1989; Katanbaf *et al.*, 1989b). Part of the reduction in EODES may be due to

prevention of more than one developmental series of ova in the ovarian follicular hierarchy (Hocking *et al.*, 1987; 1989).

Hatchability studies utilizing broiler breeders show reduced hatchability of fertile eggs during the period following sexual maturity (Yafei and Noble, 1988). These observations are consistent with studies that shown an influence of parental age upon the hatchability of fertile eggs from meat-type parent stocks (Smith and Bohren, 1975; Shanawany, 1984) and layer stocks (Garwood and Lowe, 1982). Anthony *et al.* (1989a) detailed effects of selection for body weight on correlated changes in egg components. Yolk:albumen ratios in the selected lines bracketed the  $F_1$  crosses of parental lines. Noble (1987) hypothesized that embryos from hens of recent sexual maturity may have poorer ability to mobilize and metabolize lipids in the yolk sac than embryos from older hens. Although results from electron microscopy studies provide evidence supporting this explanation (Yafei and Noble, 1988; 1990), additional data are needed.

Feed restriction of autoimmune-prone mice led to a marked inhibition of immunological involution associated with aging and an enhancement of immunological vigor (Ogura *et al.*, 1989). Although improved livability of broiler breeders, when feed is restricted, has been demonstrated (Katanbaf *et al.*, 1989c), data are needed to evaluate the effects of feed restriction on immunocompetence of meat-type stocks. Livability of growth selected lines and two relaxed sublines were studied (Dunnington *et al.*, 1989) to 188 weeks of age in female chickens fed *ad libitum*. There were no significant differences between survivors and



non-survivors within populations for antibody production to sheep, swine or horse erythrocytes.

The relationship between feed intake and efficiency of egg production has been studied extensively in commercial layer hens (see review by Pirchner, 1985). Genetic components of feed efficiency of laying chickens are associated with genetic components involved with egg mass produced, body weight, body weight changes and residual food consumption (Bordas and Mérat, 1974; 1976). The latter, defined as the difference between predicted (from production traits) and observed food intake, has a moderate heritability (Wing and Nordskog, 1982a,b; Wing *et al.*, 1983; Luiting and Urff, 1991b). The phenotypic variance of residual feed consumption is also influenced by alleles at particular loci such as those for plumage color (Mérat *et al.*, 1979). Bordas and Mérat (1981) modified Byerly's (1941) model for prediction of feed intake from an individual's production record. The difference between the predicted and realized feed intake allows residual feed intake to be estimated from traits including body weight, body weight changes and egg mass production.

Genetic improvement in residual feed intake can result in improvements in layer performance (Wing *et al.*, 1983; Pirchner, 1985). When genetic correlations of residual feed intake with other variables of economic interest are in the same direction (i.e. improved indirectly by selection for the primary economic traits), studies of the phenotypic correlations may lead to additional advantages for commercial layers (Bordas and Mérat, 1981; Luiting and Urff, 1991a;b;c) as well as for broiler breeders.

### *Feed Intake in Broilers*

Interest in feed restriction of broilers has focused on attempts to improve feed efficiency and reduce carcass fat (Washburn and Bondari, 1978; Beane *et al.*, 1979; Plavnik and Hurwitz, 1985; Plavnik *et al.*, 1986; Pinchasov and Jensen, 1989; Summers *et al.*, 1990). Accelerated growth when chicks are returned to *ad libitum* feeding has been demonstrated, but it has varied due to differences in time, length and severity of restriction and the length of time after restriction. Sexual dimorphism has been reported for accelerated growth (Wilson, 1954), response to length of restriction, ability to achieve a complete accelerated growth to weight of *ad libitum* fed controls, feed efficiency and amount of carcass (Plavnik and Hurwitz, 1988; 1991).

Growth curves of chickens of different breeds (Knížetová *et al.*, 1985) and bidirectionally selected normal and dwarf lines (Zelenka *et al.*, 1986b) show large differences for the length of the autoacceleration phase of growth (i.e., that rapid growth period up to the point of inflection in the growth curve). The length of autoaccelerated growth is dependent on the inflection point of the growth curve which is amenable to manipulation by selection (McCarthy and Bakker, 1979). Selection for body weight in various gallinaceous species has resulted in changes in both the point of inflection and the asymptote (Anthony *et al.*, 1991a).

Synchronization of growth, first of supply organs (i.e., heart, lungs, gastrointestinal tract, pancreas, liver and kidneys) and then of demand organs (i.e., skeletal muscles, feathers, adipose tissue) is essential to maximize genetic potential (Lilja, 1981). Katanbaf *et al.*

(1988a,b) suggests a biological basis for the development of production systems where a feed restriction period is used to allow better synchrony of growth between supply and demand organs for broilers marketed at heavier weights. Initial studies by Katanbaf *et al.* (1989c) demonstrated a feed restriction program for the heavier ( > 3.5 kg ) type of broiler other than the arresting of growth proposed by Plavnik and Hurwitz (1985).

Digestive enzyme levels in pancreas and small intestinal chyme were influenced by genetic stocks (Nitsan *et al.*, 1989; 1991b), feed composition and level of feed intake (Corring, 1980; Nitsan, 1985). Pancreatic adaptations to diet by regulation of synthesis and secretion of digestive enzymes have been reviewed (Brannon, 1990). Studies of the interactions among feed intake, genetic background, and age have revealed a highly adaptive response mechanism in meat-type chickens (Nir *et al.*, 1987; Pinchasov *et al.*, 1990; Nitsan *et al.*, 1991a) and for turkeys (Krogdahl and Sell, 1989; Sell *et al.*, 1991). Recent studies have indicated age as a factor influencing digestive enzyme activity (Sell *et al.*, 1991; Nitsan *et al.*, 1991a). Because reduced age to market weight increases the importance of the first weeks in a broiler's life, further data on early ontogeny of digestive enzyme levels in relationship to feed intake in diverse genetic stocks are required.

#### *Form of Feed*

Advantages of feeding diets in pelleted or crumble form to broilers, commercial layers and turkeys have been demonstrated in numerous studies including those of Stewart and Upp (1951), Larson and Smyth (1955), Reddy

*et al.* (1962), Jenson *et al.* (1962; 1965), Summers *et al.* (1968), Pepper *et al.* (1969) and Choi *et al.* (1986). Variation in performance when feeding pelleted or crumble diets has been due to increasing nutrient density, physiological modifications of the gastrointestinal tract, feeding behavior, age of bird and management practices such as debeaking (Deaton *et al.*, 1987). Inconsistency of performance when crumbles are fed may be due to the habituation in feeding behavior. Whether this habituation influences production performance needs further study.

### *Feathering Genotypes*

Certain matings allow for autosexing of chicks at hatch by plumage color or feather growth (Hutt, 1949). One example of autosexing which has gained popularity in the broiler industry utilizes the sex-linked locus for early (*k+*) and late (*K*) feathering. The dominant allele for late feathering is associated on the Z chromosome with the endogenous virus gene *ev21* that encodes for infectious endogenous virus EV21 (Bacon *et al.*, 1988). The provirus, *ev21* is integrated into a specific site on the Z chromosome and its deletion results in reversion to wild phenotype (Levin and Smith, 1990). In Leghorn strains, *K* chickens are more susceptible than *k+* individuals to exogenous infection with avian leukosis virus (ALV) subgroup E (Crittenden *et al.*, 1984). The linkage of allele *K* to the *ev21* locus is critical, but in the presence or absence of *ev21*, congenital infection with ALV confers infection-induced tolerance to pathogenetic avian leukosis virus infections (Smith and Fadly, 1988). Both *k+* and *K* progeny of *K*/*-* dams have a higher incidence

of vertically transmitted ALV than progeny of  $k+/-$  dams which may be associated with lowered immunotolerance to infection by exogenous ALV (Crittenden *et al.*, 1987).

Among breeders of commercial white egg producing stocks, allele  $K$  has not found favor because of the higher incidence of lymphoid leukosis associated with it (Harris *et al.*, 1984). Broiler dams do not routinely remain in lay for periods as long as those for commercial egg layers. Hence, the rate of lymphoid leukosis infection that leads to noticeable losses in production may not be as evident in broiler breeder flocks. As management techniques for the broiler breeders improve with use of feed restriction and separate feeding systems for males and females, length of time in production may increase. A longer period of lay would allow production losses associated with  $K$  to be expressed, if indeed the syndrome exists, in the background genome of broiler-breeder.

In addition to the higher incidence of lymphoid leukosis in vertically infected progeny, Havenstein *et al.* (1989) observed poorer performance of commercial layer crosses of  $k+/-$  daughters from  $K/-$  than  $k+/-$  dams. Although they did not measure levels of ALV viremia, there was evidence suggesting vertical transmission of ALV viremia occurred in the absence of the *ev21* gene. These results suggest that segregation at this locus in grandparents may have a detrimental effect on the broiler breeder hen's performance due to ALV viremia even if the incidence of lymphoid leukosis is not a significant factor in determining rates of production. Katanbaf *et al.* (1989b) presented data for hen-housed production of broiler breeders. During the latter part of lay (age 350

to 450 days of age), ovulation rates were higher in *k+/-* than *K/-* hens. Additional data are needed on performance of *k+/-* and *K/-* broiler breeders kept for extended periods of production.

Slower growth to market weight and reduced productivity of broiler stocks infected with ALV were reported by Gavora et al. (1982). Crittenden et al. (1983) presented evidence for reduced growth rates in broiler chicks testing positive for ALV infection, as well as chicks from dams with positive tests for group-specific (g-s) antigen in the egg albumen to ALV. Additional information is needed on the production performance and immunoresponsiveness of *K* versus *k+* broilers.

Males infected with ALV contribute to the horizontal spread of the disease by shedding ALV into the environment (Spencer, 1984). Incidence of ALV shedding males was lower in lines selected for increased egg production than in unselected random bred control lines (Spencer *et al.*, 1979; Gavora and Spencer, 1985). It was hypothesized that this difference was because ALV non-shedders had higher egg production than shedders and were preferred as breeders in lines artificially selected for higher egg production. In the unselected control population only natural selection discriminated against shedders.

CHAPTER I

GROWTH AND CARCASS CHARACTERISTICS OF EARLY- AND LATE-FEATHERING  
BROILERS REARED UNDER DIFFERENT FEEDING REGIMENS

## SUMMARY

Growth, immunological characteristics and carcass traits were measured in early- and late-feathering chickens maintained under three feeding regimens. The regimens were 1) AL, *ad libitum*, 2) RL, alternate day feed restriction from 6 to 27 days of age (DOA) and then released to *ad libitum*, 3) SOD, alternate day feeding restriction from 6 DOA to the end of the experiment. Sexual dimorphism was observed for accelerated growth with convergence in BW of RL with AL chickens occurring at a younger age in males than females. Absolute BW, feather, and gastrointestinal tract (GIT) weights were lower for SOD, after 45 DOA, than for AL and RL chickens. Relative to BW, however, GIT and liver weights, at 45 DOA but not 64 DOA, were heavier for SOD than AL and RL chickens. Percentage of carcass lipid was lower for SOD than for AL and RL chickens. Generally carcass traits and components of the GIT were similar for Regimens AL and RL at 45 and 64 DOA. Exceptions were lower percentages for carcass lipid and liver lipid for RL than AL chickens at 64 DOA. There was a significant feeding regime by age interaction for heterophil to lymphocyte ratios caused by a greater increase in ratios with age for AL than for RL and SOD chickens. At 37 DOA, AL chickens had lower antibody titers to SRBC than SOD or RL chickens that did not differ. The AL chickens had reduced bursa and thymus weights relative to BW; indicative of immunological involution, along with reduced livability compared with RL and SOD chickens which did not differ in livability. Early- and late-feathering chickens were similar for most of the traits



measured. One exception was higher antibody titers to SRBC in early- than late-feathering chickens.

## INTRODUCTION

Broilers are marketed as value-added, further processed products, cut-up parts, and whole birds with further processed products gaining an increasing share of the retail market. Heavier body weights at marketing are desirable for further processed broilers, hence, age at marketing is increased. Concomitant with these developments is an interest in feed restriction programs designed to improve feed efficiency and to reduce both abdominal and carcass fat (Washburn and Bondari, 1978; Beane *et al.*, 1979; Plavnik and Hurwitz, 1985; Plavnik *et al.*, 1986; Pinchasov and Jensen, 1989). Although accelerated growth occurs when feed-restricted chicks are returned to *ad libitum* feeding (AL), results are inconsistent. There is sexual dimorphism for accelerated growth (Wilson, 1954) as well as differences in the ability to achieve a complete compensation of growth to the weight, feed efficiency, and amount of carcass lipid of AL fed chickens (Plavnik and Hurwitz, 1985; 1988).

Postnatal growth of birds is determined by distribution of growth among organs (Lilja, 1981; Prescott *et al.*, 1985). Synchronization of growth, first of supply organs (i.e., lungs, gastrointestinal tract, pancreas, liver) and then of demand organs (i.e., skeletal muscles, feathers, adipose tissue), is essential to maximize genetic potential. Katanbaf *et al.* (1988a;b) discussed a biological basis for a feed

restriction program used to enhance synchrony of growth between supply and demand organs for broilers marketed at heavier weights. Studies by Katanbaf *et al.* (1989b) demonstrated a feed restriction program with potential for producing a heavier broiler.

Negative correlations between antibody titers to SRBC and BW have been reported for chickens (Siegel *et al.*, 1982; 1984; van der Zijpp *et al.*, 1987; Martin *et al.*, 1988). Correlations between response to SRBC antigen and weights of bursa and thymus have been equivocal (Yamamoto and Glick, 1982; Ubosi *et al.*, 1985; Martin *et al.*, 1988). Viability and productivity of chickens improved when feed intake was restricted (Han and Smyth, 1972; Katanbaf *et al.*, 1987; 1989a). These results are consistent with the observation that feed restriction of autoimmunity-prone mice enhances immunological vigor (Ogura *et al.*, 1989) and contributes to a marked inhibition of immunological involution associated with aging.

The objective of the research reported here was to examine the relationships of feeding regime with growth, production traits, and immunological characteristics in broilers segregating at the sex-linked feathering locus.

## MATERIALS AND METHODS

### *Stocks and Husbandry*

Chicks from a broiler-breeder parent stock segregating at the sex-linked feathering locus were used in this experiment. Matings were

made to produce genotypes  $K/K$ ,  $K/-$ ,  $k^+/k^+$ , and  $k^+/-$ . On Day 22 of incubation, chicks were removed from the hatcher, classified as either early- or late-feathering, vaccinated for Marek's disease, wingbanded, and weighed to the nearest g.

Chicks were reared as sex-intermingled flocks in six floor pens with wood shavings as litter. There were three early- and three late-feathering flocks of approximately 40 individuals per flock. Feed was available AL to six days of age (doa). Thereafter, two flocks of each genotype were placed on an alternate day (SOD) feed restriction program, which followed a broiler-breeder recommendation for sex-intermingled flocks, to achieve target BW of 460 and 1600 g at 27 and 64 doa, respectively. At 27 doa one pen of restricted chicks from each genotype was released to *ad libitum* feeding (RL) in a step-up fashion over a 2-day period. Chicks were fed a corn-soybean diet containing 3146 cal of ME/g of feed and 24% crude protein in mash form. Lighting was continuous. Room temperature was maintained by hot air brooding at  $35 \pm 1$  C during the first 7 days posthatch. Temperature was then gradually reduced to  $20 \pm 1$  C by 35 doa. Water was available at all times.

#### *Traits Measured*

Individual body weights (g) were obtained at hatch, 6, 21, 35, 42, 56, and 63 doa. On Day 27, length of the right tarsometatarsus (shank) and diameter at the distal end of the metatarsus were measured (mm) on each individual. Feed consumption was measured on a flock basis and feed

efficiency (BW/divided by the weight of feed consumed) was determined from hatch to fixed ages.

Carcass and organ data were obtained at two ages: when BW of RL males was converging with that of AL males (45 doa) and when the average BW of the RL males reached 3.5 Kg (64 doa). At each age, data were obtained for 4 males from each genotype-feeding regime (n = 24). Prior to killing by cervical dislocation, feed and water were withdrawn for 12 h. Length of the right shank and diameter of the metatarsus were measured in millimeters. Feather weight was determined as the difference in BW before and after feather removal. The *Pectoralis major* and *P. minor* were removed, by hot deboning and weighed (.1 g). Each carcass was then eviscerated, contents of the gastrointestinal tract (GIT) removed, and weights (.01 g) of the esophagus and crop, proventriculus, gizzard, liver, small intestine, and abdominal (retroperitoneal) fat pad obtained. Lengths (mm) of esophagus and crop and of the small intestine were also measured. The right leg with femur, fibula, and tibiotarsus was removed at the femoral-acetabulum joint, the shank was removed from the leg at the intertarsal joint and each was weighed (.01 g).

Livers and abdominal fat pads were split along the median line and a 16 g subsample to the right of the median cut was removed and stored at -20 C. Subsequently, two 4 g subsamples were homogenized by a polytron homogenizer (Brinkmann Polytron, Kinematica GmbH, Luzern, Switzerland) and lipid content analyzed by chloroform-methanol extraction (Folch *et al.*, 1957). Each carcass (including all dissected elements but excluding GIT contents, feathers, and the subsamples of liver and abdominal fat pad)

was sealed in a plastic bag and stored at -20 C. Subsequently, each carcass was cut into pieces while frozen and passed 3 times through a meat grinder (Hobart Corp., Chicago, Il 60673) to homogenize. Two 4 g subsamples of each ground carcass were analyzed for total lipid content by chloroform-methanol extraction (Folch *et al.*, 1957). If duplicate samples agreed within 1.5%, the mean was used for statistical analysis of total lipid content; otherwise two additional samples were analyzed (Murphy and Boag, 1989). Repeatabilities of lipid extractions were .75 for carcass, .82 for liver, and .98 for fat pad.

At 36 and 50 doa, different samples of birds from each subclass were chosen at random for the following measurements. Five males and five females from each genotype-feeding regimen subclass were injected i.v. with .1 ml of a .25% suspension of SRBC. Antibody production in response to SRBC antigen was measured 5 days postinjection by the microtiter hemagglutination procedure of Wegmann and Smithies (1966) and expressed as  $\log_2$  of the reciprocal of the last dilution for which agglutination was detected. After blood was removed for antigen measurements, males were returned to their flock. Females were placed in pens without feed or water for 12 h after which each was weighed, electrocuted, and spleens, bursae of Fabricius, and thymi were removed and weighed (.01 g).

At the times of injection with SRBC, blood samples were obtained from each chicken injected and placed in tubes with EDTA as the anticoagulant. Subsequently, a one-drop sample was placed on a slide, spin-prepared, and stained with May-Grünwald-Giemsa stain. Sixty cells per slide were classified as either heterophils (H) or lymphocytes (L)

and H:L ratios calculated (Gross and Siegel, 1983). This procedure was repeated on a third sample of chickens at 63 doa.

### *Statistical Analyses*

The model for analysis of variance was:

$$Y_{ijkl} = \mu + G_i + S_j + F_k + (GS)_{ij} + (GF)_{ik} + (SF)_{jk} + (GSF)_{ijk} + e_{ijkl}$$

where  $i = 1,2$  feathering genotypes,  $j = 1,2$  sexes,  $k = 1,2,3$  feeding regimens, and  $l = 1,2,\dots,n$  individuals. Sex was omitted from the model where data were obtained for only one sex. Age was included in the model for analysis of H:L ratios, absolute and relative weights of bursa, thymus, and spleen. Age was not included in the model for SRBC antibody titers as confounding occurred between time and the source of SRBC.

Prior to statistical analyses, absolute weights and lengths were transformed to natural logarithms and percentages to arc sine square roots. Carcass data were expressed relative to body weight minus GIT contents. Where differences in feeding regimens were significant, comparisons among means were made by Duncan's multiple range test. Statistical significance was taken at  $P \leq .05$ , unless otherwise stated.

Livability data were analyzed by Bonferroni's chi-squared statistic using the simultaneous test procedure (Jensen *et al.*, 1968). Because data were used three times (early vs. late, AL vs. RL, AL vs. SOD, and RL vs. SOD), tau was set at three.

## RESULTS

### *Body Weight, Shank Measurements, and Feed Efficiency*

First- and second-order interactions involving genotype, feeding regime, and sex were not significant for BW or for length, diameter, and weight of shank. Late-feathering chicks were heavier than early-feathering chicks from hatch to 21 doa, after which BWs were similar; differences reappeared at 63 doa when the early-feathering chickens were heavier than the late-feathering ones (2606 vs. 2499 g). Shank length, diameter, and weight were similar for both genotypes.

Effect of feeding regimen on body weight was evident by 21 doa with SOD chickens weighing about 20% less than those fed *ad libitum* (Table 1). Although 8 days after release (35 doa) there was no difference between SOD and RL chickens, by 15 days after release (42 doa), RL chickens were heavier than SOD chickens. Accelerated growth of RL chickens (sexes pooled) continued so that at 56 doa they were 78% heavier than SOD chickens and not different from those fed *ad libitum*. At 27 doa, shanks were longer and larger in diameter for AL than for SOD chicks (Table 1). At 45 doa (18 days after release), shanks of AL and RL chickens were equivalent in length and diameter and were larger in both dimensions than those of SOD chicks. Convergence of BW for the RL with AL feeding regimen occurred at an earlier age in early feathering (51 doa) than in late feathering (61 doa) chickens and at older ages but similar BW in females (59 doa) as in males (53 doa). Figure 1 shows growth curves for these feeding regimens for males.

Confounding of treatment and pen precluded statistical analysis of feed consumption and feed efficiency data (Table 2). Descriptively, cumulated feed intake was about 20% lower in the SOD regime than in the AL regime at the time of release and remained lower throughout the experiment (Figure 2). By 64 doa, feed consumption of AL chickens was twice that of SOD chickens.

#### *Carcass Traits and the Gastrointestinal Tract*

Genotype by feeding regime interactions were not significant for carcass and GIT traits with the exception of the relative weight and the percentage lipid in the abdominal fat pad at 64 doa. These interactions were due to late-feathering SOD males having smaller abdominal fat depots that contained less lipid than early-feathering males on the SOD regimen; there were no differences between genotypes on the AL or RL regimens (Table 3).

There were no differences between genotypes for carcass traits with the exception of relative crop and esophagus weight, which was heavier in early- (.72) than late-feathering males (.64) at 64 doa. The general lack of differences between the genotypes allowed for their pooling for presentation of feeding regimen data in Table 4. Carcass traits were similar for Regimens AL and RL at both ages, except for percentages of carcass lipid at both ages and of liver lipid at 64 doa where values for AL were greater than for RL chickens. At 45 doa, relative weights of feathers, *P. major*, thigh and drumstick, liver, percentage carcass, and percentage liver lipid was less for SOD than AL and RL chickens. This



relationship among feeding regimens at 64 doa was observed for *P. major* and *P. minor* and for percentage carcass lipid.

The AL and RL chickens had similar weights of empty GIT at both ages and they were heavier than that for SOD chickens (Table 5). The weight of the GIT contents was similar for AL and RL chickens at both ages, but GIT contents of the SOD chickens were heavier than those of AL and RL chickens at 45 doa. By 64 doa, differences in GIT contents between Regimens AL, RL, and SOD were no longer present. When the contents of the GIT were expressed relative to BW, SOD chickens had consistently heavier GIT contents at both ages than AL and RL chickens. When weights of the segments of the GIT were expressed relative to BW, those for SOD chickens were heavier than those for AL and RL chickens at both ages. The AL and RL chickens did not differ at either age. A different pattern emerged for length of GIT. At 45 doa, all regimens were similar; at 64 doa, GIT were longer for Regimens AL and RL than for SOD. The GIT length of SOD chickens did not change from 45 to 64 doa. Length of the small intestine as a percentage of GIT was longer at 64 doa for AL and RL chickens than for the SOD chickens. Crop and esophagus length as a percentage of GIT length was similar among the regimens at 45 doa but by 64 doa was longer for SOD chickens than AL and RL chickens.

#### *Heterophil:Lymphocyte Ratios and Sheep Red Blood Cell Antibody Titers*

Ratios of H:L were similar for genotypes and sexes at 37, 50, and 63 doa. Interactions of age by feeding regimen for H:L ratios were significant due to elevated H:L ratios at 63 doa for AL chickens (Figure

3). The H:L ratios were lower for SOD than for AL and RL chickens at 50 doa.

First- and second-order interactions involving genotypes, feeding regimens, and sexes were not significant for SRBC antibody at either 37 or 50 doa. There were also no differences between the sexes for this trait at either age. Antibody titers were higher for early- than late-feathering chickens at both ages (Table 6). Although at 37 doa, RL and SOD chickens had higher titers than AL chickens, there were no differences among feeding regimens at 50 doa.

#### *Immunological Organs*

Significant interactions for weights of immunological organs included age by feeding regimen for relative weights of bursa, absolute and relative weights of thymus, and absolute weights of spleen (Table 7). The age by feeding regimen interaction for relative bursa weight was due to involution occurring in AL chickens but not among RL chickens, but relative weight of bursa increased among SOD chickens. Involution of the thymus (relative and absolute) occurred among the AL chickens but not RL and SOD chickens, causing the age by feeding regimen interaction for these traits. For absolute spleen weight the interaction was due to an increase in spleen weight of AL and RL but not SOD chickens.

Following are summaries of weights where there were no interactions among main variables genotype, feed regimen, and age. Bursal weights were heavier for early- than late-feathering chickens (absolute: 3.29 vs. 2.75 g; relative: .20 vs. .16). Absolute bursal weights did not differ for

AL (3.43 g) and RL (3.11 g) chickens, but in both cases, they were heavier than for SOD chickens (2.54 g). Relative spleen weights were heavier for AL (.17) than SOD chickens (.13) with RL weights (.15) intermediate and not different from the other regimens. Absolute bursa weights were lighter at 36 doa (2.71 g) than at 50 doa (3.35 g) while relative spleen weights were heavier at 36 doa (.16 g) than 50 doa (.14 g).

### *Livability*

Livability for all flocks combined was 96.2% with ascites and flip-over deaths accounting for more than half of the total mortalities. Although there was no difference between the genotypes, livability was lower for AL (90.9%) than for RL (98.8%) and SOD (98.7%) chickens.

## DISCUSSION

Restriction of feed intake for a 5- to 7-day period at a young age to retard growth to a maintenance level has met with varied responses (Washburn and Bondari, 1978; Plavnik and Hurwitz, 1985; 1988; Plavnik *et al.*, 1986; Pinchasov and Jensen, 1989). This experiment was designed to restrict but not arrest growth. Patterns of accelerated growth shortly after release followed that described by Beane *et al.* (1979); however, in our case, the chickens were maintained to older ages. Accelerated growth was observed with full compensation of growth by 56 doa for straight-run chickens. Although feed intake increased rapidly during the period of accelerated growth, cumulative feed intake of RL chickens never

exceeded that of AL chickens. Bone measurements were similar to patterns observed for BW. Sexual dimorphism has been reported for response to restriction in terms of accelerated growth (Wilson, 1954; Plavnik *et al.*, 1986; Plavnik and Hurwitz, 1988; Pinchasov and Jensen 1989). The convergence in BW of females occurred at older ages than for males, however, convergence occurred at the same BW in the both sexes.

Chickens for which feed was restricted (RL and SOD), had a consistently lower percentage of carcass lipid than those fed *ad libitum*. These results support those of Cartwright *et al.* (1986) who reported an inhibition of adiposite hyperplasia in broilers after a period of accelerated growth following feed restriction early in life. Relative to BW, *P. major*, drumstick and thigh, but not *P. minor* were heavier for AL and RL than SOD chickens at 45 doa. At 64 doa this difference also included *P. minor*, showing differential growth occurred among muscles with these feeding regimens.

These data are consistent with increased hepatic lipogenesis, lipolysis and hepatic hypertrophy associated with intermittent feeding (Simon and Brisson, 1972; Nir and Nitsan, 1979). The higher levels of carcass and liver lipid for AL than RL and SOD chickens observed in these data were consistent with observations of Calabotta *et al.* (1985) that lipid metabolism was greater in growing chickens fed *ad libitum* than in those where feed intake was restricted. Also, greater increases in relative weight and length of the crop and esophagus of SOD than AL and RL chickens suggested ability to retain food and to control food evacuation from the crop (Nir and Nitsan, 1979; Nir *et al.*, 1987; Katanbaf

*et al.*, 1989c). Food retention was greater for SOD than for RL and AL chickens. This observation agreed with results of Cherry and Siegel (1978) who reported slower GIT clearance in chickens with heavier relative gut weights.

Higher mortality and higher H:L ratios suggested that AL chickens were stressed more than RL and SOD chickens at later ages. Behavioral modifications in feed intake occurred concomitantly with selection for increased BW (Siegel *et al.*, 1984) and in this experiment percentage carcass fat in the AL chickens was essentially double that of those fed on alternate days. Under AL feeding, individuals became obese more rapidly and their health subsequently declined (Han and Smyth, 1972; Pym and Dillon, 1974; Katanbaf *et al.*, 1987; van Niekerk *et al.*, 1988; Katanbaf *et al.*, 1989a). Bursal and thymic involution among AL chickens was evident by 50 doa, and they also had lower SRBC antibody titers, at a younger age (37 doa), during the time of T-cell dependent immune response maturation (McCorkle and Glick, 1980). These findings support work with mice where Ogura *et al.* (1989) noted an increase in life span and enhanced immunological vigor with feed restriction of autoimmunity-prone mice.

These results provide a general procedure for accelerated growth for broilers marketed at heavier BW. Broilers reared under this procedure achieved similar BW at the same age as AL-fed broilers. Moreover, they had lower carcass fat and improved livability.

TABLE 1. Means for BW and length, diameter and weight of shank

Trait <sup>2</sup>	Age (d)	Feeding regimen <sup>1</sup>		
		AL	RL	SOD
BW, g	0	45a	...	45a
	6	110a	...	106a
	21	503a	...	402b
	35	895a	677b	682b
	42	1799a	1593b	1051c
	56	2601a	2525a	1415b
	63	3065a	3053a	1603b
Shank Length, mm	27	7.26a	...	6.65b
	45	10.18a	10.01a	8.56b
	63	12.02a	12.19a	10.06b
Diameter, mm	27	1.47a	...	1.21b
	45	1.79a	1.84a	1.34b
	63	2.11a	2.16a	1.61b
Weight, g	45	46.4a	43.3a	29.4b
	63	65.7a	68.2a	40.6b

a,b Means within a row with no common superscript are significantly different ( $P \leq .05$ ).

<sup>1</sup>AL = ad libitum;

RL = restricted as SOD from Day 6 to 27, then released to ad libitum;

SOD = alternate day feed restriction.

<sup>2</sup>Sexes pooled for each trait.

TABLE 2. Feed efficiency measured<sup>1</sup> on a flock basis from hatch to various ages

Period (hatch to d)	Feathering genotype		Feeding regimen <sup>2</sup>		
	Early	Late	AL	RL	SOD
28	.64	.61	.71	...	.58
41	.60	.57	.60	.59	.56
62	.52	.49	.49	.51	.51

<sup>1</sup>No statistical analyses were conducted as treatment was confounded with pen.

<sup>2</sup>AL = ad libitum;

RL = restricted as SOD from Day 6 to 27, then released to ad libitum;

SOD = alternate day feed restriction.

TABLE 3. Means for relative weight of abdominal fat pad and percentage of lipid of the fat pad at 64 days of age

Abdominal fat	Feathering genotype	Feeding Regimen <sup>1</sup>		
		AL	RL	SOD
Grams of tissue/100g BW	Early	1.05 <sup>a</sup>	1.06 <sup>a</sup>	.47 <sup>b</sup>
	Late	NS	NS	*
Lipid, %	Early	.99 <sup>a</sup>	1.12 <sup>a</sup>	.12 <sup>b</sup>
	Late	78.1 <sup>b</sup>	76.9 <sup>b</sup>	89.2 <sup>a</sup>
		NS	NS	*
		78.8 <sup>a</sup>	75.2 <sup>a</sup>	52.3 <sup>b</sup>

<sup>a, b</sup>Means within a row with no common superscript are significantly different (P ≤ .05).

\* Adjacent means within a column are significantly different (P < .05).

NS Adjacent means within a column were not significantly different (P > .05).

<sup>1</sup>AL = ad libitum;

RL = restricted as SOD from Day 6 to 27, then released to ad libitum;

SOD = alternate day feed restriction.



TABLE 4. Means for carcass traits at 45 and 64 days of age for males

Trait	45 doa			64 doa		
	AL <sup>1</sup>	RL	SOD	AL	RL	SOD
<u>BW<sup>2</sup>, g/100g</u>						
Feather	5.6 <sup>ab</sup>	6.6 <sup>a</sup>	5.1 <sup>b</sup>	5.7 <sup>a</sup>	5.7 <sup>a</sup>	5.1 <sup>a</sup>
Pectoralis						
Major	11.0 <sup>a</sup>	11.5 <sup>a</sup>	9.7 <sup>b</sup>	13.6 <sup>a</sup>	13.2 <sup>a</sup>	11.1 <sup>b</sup>
Minor	3.7 <sup>a</sup>	3.5 <sup>a</sup>	4.3 <sup>a</sup>	4.4 <sup>a</sup>	4.2 <sup>a</sup>	3.9 <sup>b</sup>
TD <sup>3</sup>	11.3 <sup>a</sup>	11.1 <sup>a</sup>	10.4 <sup>b</sup>	11.3 <sup>a</sup>	11.3 <sup>a</sup>	11.5 <sup>a</sup>
Liver	2.0 <sup>a</sup>	2.1 <sup>a</sup>	3.1 <sup>b</sup>	1.9 <sup>a</sup>	1.8 <sup>a</sup>	2.0 <sup>a</sup>
<u>Lipid % in</u>						
Carcass	13.6 <sup>a</sup>	10.1 <sup>b</sup>	7.2 <sup>c</sup>	13.3 <sup>a</sup>	11.2 <sup>b</sup>	7.3 <sup>c</sup>
Liver	4.6 <sup>ab</sup>	3.4 <sup>a</sup>	6.1 <sup>b</sup>	6.1 <sup>a</sup>	2.9 <sup>b</sup>	4.9 <sup>ab</sup>

<sup>a,b,c</sup>Means within a row with no common superscript are significantly different ( $P \leq .05$ ).

<sup>1</sup>AL = ad libitum;

RL = restricted as SOD from Day 6 to 27, then released to ad libitum;

SOD = alternate day feed restriction.

<sup>2</sup>Weight of gastrointestinal tract contents was subtracted from BW prior to analyses.

<sup>3</sup>TD = Thigh and drumstick muscles plus femur, fibula, and tibiotarsus.

TABLE 5. Means for gastrointestinal tract (GIT) and its segments at 45 and 64 days of age (don) for males

Trait	45 don			64 don		
	AL <sup>1</sup>	RL	SOD	AL	RL	SOD
GIT, g						
Empty Contents	105.0 <sup>a</sup> 48.1 <sup>b</sup>	95.9 <sup>a</sup> 41.1 <sup>b</sup>	84.6 <sup>b</sup> 76.5 <sup>a</sup>	132.6 <sup>a</sup> 66.6 <sup>a</sup>	138.4 <sup>a</sup> 66.7 <sup>a</sup>	106.3 <sup>b</sup> 57.0 <sup>a</sup>
BW <sup>2</sup> , g/100g						
GIT Contents	2.5 <sup>b</sup>	2.3 <sup>b</sup>	7.2 <sup>a</sup>	2.1 <sup>b</sup>	2.1 <sup>b</sup>	3.4 <sup>a</sup>
Crop and esophagus	.5 <sup>b</sup>	.6 <sup>b</sup>	1.1 <sup>a</sup>	.5 <sup>b</sup>	.6 <sup>b</sup>	1.0 <sup>a</sup>
Proventriculus	.4 <sup>b</sup>	.4 <sup>b</sup>	.5 <sup>a</sup>	.3 <sup>b</sup>	.3 <sup>b</sup>	.4 <sup>a</sup>
Gizzard	2.0 <sup>b</sup>	1.9 <sup>b</sup>	3.4 <sup>a</sup>	1.5 <sup>b</sup>	1.6 <sup>b</sup>	3.1 <sup>a</sup>
Small intestine	2.5 <sup>b</sup>	2.4 <sup>b</sup>	3.0 <sup>a</sup>	1.9 <sup>b</sup>	1.9 <sup>b</sup>	2.2 <sup>a</sup>
Length, mm						
GIT	69.3 <sup>a</sup>	68.7 <sup>a</sup>	66.2 <sup>a</sup>	79.7 <sup>a</sup>	81.4 <sup>a</sup>	66.9 <sup>b</sup>
Length of GIT, %						
Small intestine	82.3 <sup>a</sup>	83.7 <sup>a</sup>	81.2 <sup>a</sup>	82.0 <sup>a</sup>	81.9 <sup>a</sup>	80.0 <sup>b</sup>
Crop and esophagus	10.1 <sup>a</sup>	10.4 <sup>a</sup>	10.9 <sup>a</sup>	11.2 <sup>a</sup>	11.4 <sup>a</sup>	12.3 <sup>b</sup>

a, b Means within a row with no common superscript are significantly different (P ≤ .05).

<sup>1</sup>AL = ad libitum;

RL = restricted as SOD from Day 6 to 27, then released to ad libitum;

SOD = alternate day feed restriction.

<sup>2</sup>Weight of GIT contents was subtracted from BW prior to analyses.

TABLE 6. Means of sheep red blood cells antibody titers<sup>1</sup>

Age (d) <sup>2</sup>	Feathering genotype		Feed regimen <sup>3</sup>		
	Early	Late	AL	RL	SOD
37	5.5 <sup>a</sup>	3.9 <sup>b</sup>	3.7 <sup>b</sup>	5.3 <sup>a</sup>	5.2 <sup>a</sup>
50	7.4 <sup>a</sup>	5.2 <sup>b</sup>	6.0 <sup>a</sup>	6.0 <sup>a</sup>	7.0 <sup>a</sup>

a,b Means within a row with no common superscript are significantly different ( $P \leq .05$ ).

<sup>1</sup>Sexes were pooled, as the sexes did not differ for this trait.

<sup>2</sup>Age is the day of inoculation with SRBC, antibody titers were measured 5 days post injection on different samples of chicks at each age and expressed as  $\log_2$  of the reciprocal of the last dilution for which agglutination was detected.

<sup>3</sup>AL = ad libitum;

RL = restricted as SOD from Day 6 to 27, then released to ad libitum;

SOD = alternate day feed restriction.

TABLE 7. Means ( $\pm$  SEM) for age by feeding regime<sup>1</sup> interactions for absolute (g) and relative weights of bursa, thymus, and spleen at 42 and 56 days of age

Organ	Age (d)	Feed regime <sup>2</sup>		
		AL	RL	SOD
<u>Bursa</u>				
Relative	42	.20 <sup>a</sup>	.18 <sup>a</sup>	.20 <sup>a</sup>
	56	* .15 <sup>b</sup>	NS .17 <sup>b</sup>	* .23 <sup>a</sup>
<u>Thymus</u>				
Absolute	42	8.25 <sup>a</sup>	5.94 <sup>b</sup>	2.83 <sup>c</sup>
	56	* 7.62 <sup>a</sup>	NS 8.57 <sup>a</sup>	NS 2.94 <sup>b</sup>
Relative	42	.49 <sup>a</sup>	.40 <sup>a</sup>	.27 <sup>b</sup>
	56	* .32 <sup>a</sup>	NS .39 <sup>a</sup>	NS .23 <sup>b</sup>
<u>Spleen</u>				
Absolute	42	2.90 <sup>a</sup>	2.43 <sup>a</sup>	1.58 <sup>b</sup>
	56	* 3.82 <sup>a</sup>	* 3.08 <sup>b</sup>	NS 1.51 <sup>c</sup>

a, b, c Means within a row with no common superscript are significantly different ( $P \leq .05$ ).  
\* Adjacent means within a column are significantly different ( $P \leq .05$ ).  
NS Adjacent means within a column were not significantly different ( $P > .05$ ).  
<sup>1</sup>AL = ad libitum;  
RL = restricted as SOD from Day 6 to 27, then released to ad libitum;  
SOD = alternate day feed restriction.

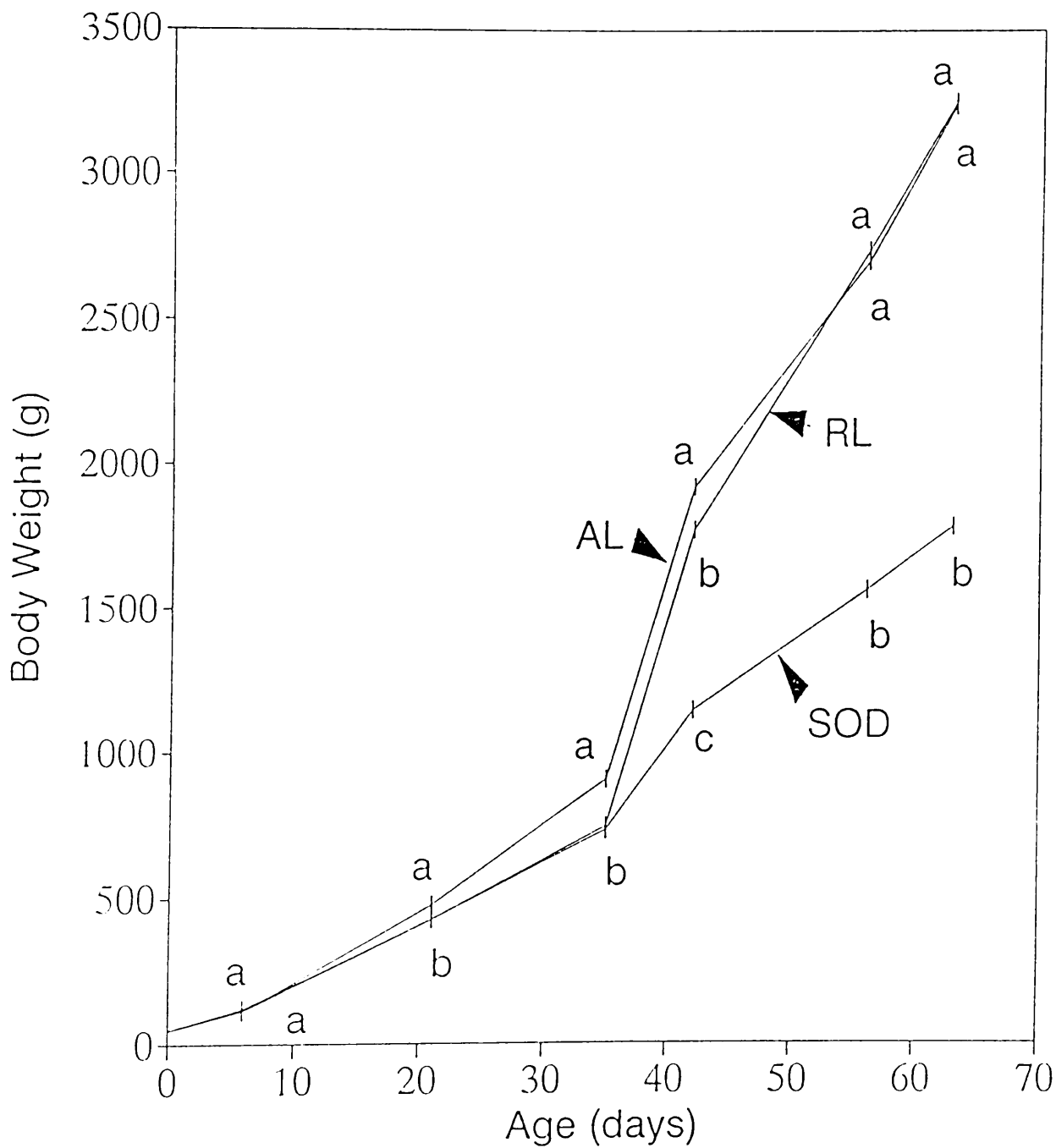


FIGURE 1. Growth curves of early-feathering males reared under three feeding regimens, 1) AL, *ad libitum*, 2) SOD, alternate day feed restriction, 3) RL, restricted as SOD from day 6 to day 27, then released to *ad libitum*. a,b,c Means within an age with no common letter are significantly different ( $P \leq .05$ ).

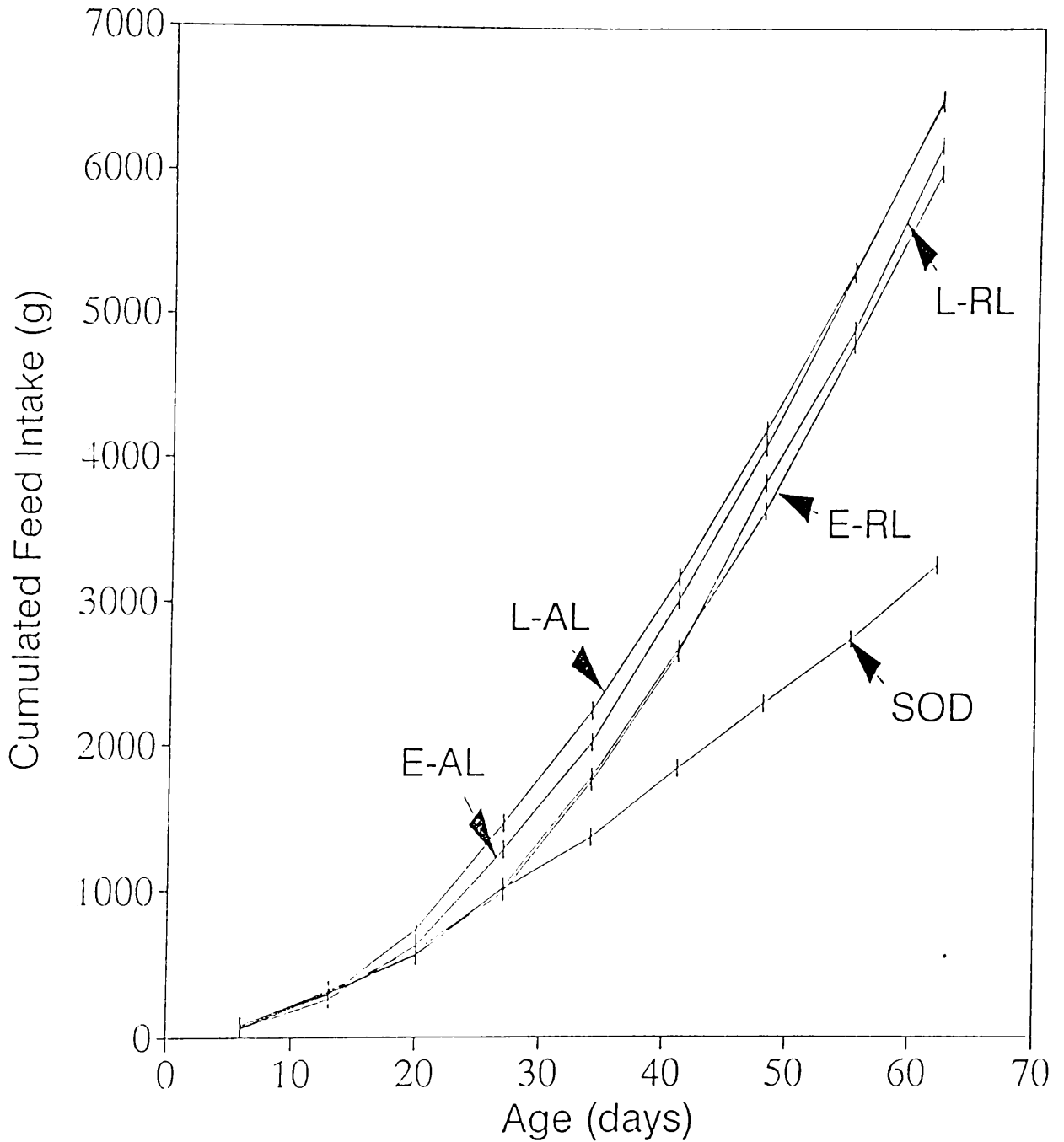


FIGURE 2. Cumulated feed intake per bird, sexes pooled, for early (E) and late (L) feathering broilers reared under three feeding regimens. 1) AL, *ad libitum*, 2) SOD, alternate day feed restriction, 3) RL, restricted as SOD from day 6 to day 27, then released to *ad libitum*. The SOD is for both E and L chickens because feed intake was the same for both genotypes. Confounding of treatment and pen precluded statistical analysis of data.

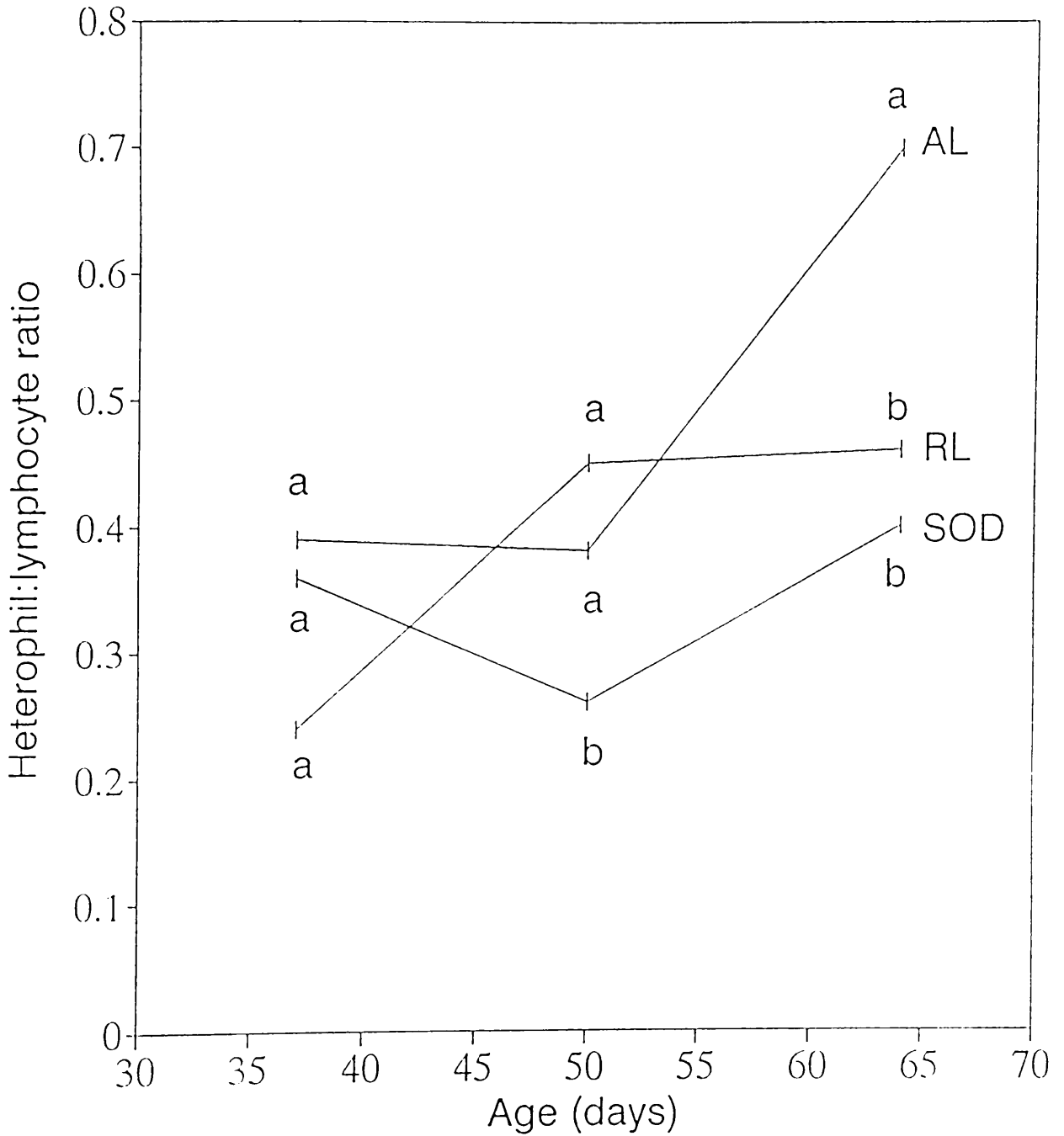


FIGURE 3. Heterophil:lymphocyte ratios for age by feeding regime. 1) AL, *ad libitum*, 2) SOD, alternate day restriction, 3) RL, restricted as SOD from day 6 to day 27, then released to *ad libitum*. <sup>a,b</sup>Means within an age with no common letter are significantly different ( $P < .05$ ).

## CHAPTER II

### PERFORMANCE OF EARLY- AND LATE-FEATHERING BROILER BREEDER FEMALES WITH DIFFERENT FEED REGIMENS



## SUMMARY

Growth, reproductive characteristics, and immunocompetence were evaluated in a line of broiler breeder females segregating at the sex-linked feathering locus when maintained on three feeding regimens with two forms of feed in a factorial design. Long term obesity, but not short term weight gain, had deleterious consequences for reproductive characteristics, response to sheep red blood cell antigen, resistance to *Escherichia coli*, and livability. Reproductive performance of early-feathering females was superior to that of late-feathering ones. Differences were attributed to an association between the allele for late-feathering and an endogenous viral locus *ev21*, which encodes for avian leucosis virus. Reproductive performance of breeders fed crumbles was superior to that of breeders fed a combination of crumbles and fines.

## INTRODUCTION

Long-term selection for rapid growth to market age alters brain and peripheral mechanisms influencing feed intake (Burkhart *et al.*, 1983; Lacy *et al.*, 1985; Denbow, 1989). Meat-type chickens, when fed *ad libitum*, eat at near gut capacity leading to obesity (McCarthy and Siegel, 1983; Katanbaf *et al.*, 1989c), which contributes to reproductive complications and deterioration of health (e.g., McDaniel *et al.*, 1981; Siegel and Dunnington, 1985; Wilson and Harms, 1986). These correlated responses may be circumvented partially by control of body weight through

restricting feed intake. Restriction of feed intake during rearing delays sexual maturity (e.g., Pym and Dillon, 1974; Bornstein *et al.*, 1984; Soller *et al.*, 1984; Robbins *et al.*, 1986) and increases egg size (Katanbaf *et al.*, 1989b). After the onset of lay, production of defective eggs can be reduced by controlling feed intake (Hocking *et al.*, 1987; 1989; Katanbaf *et al.*, 1989b). Improvements in resistance to neoplasms and in immunocompetence were associated with feed intake restriction of chickens (Han and Smyth, 1972; Katanbaf *et al.*, 1987; 1989a), observations consistent with those in mice (Ogura *et al.*, 1989).

Numerous feeding programs have been developed for controlling body weight of meat-type breeders. Concomitantly, a considerable body of literature has appeared on the feeding of diets in mash, pellet or crumble form to broilers, layers, and turkeys (e.g., Stewart and Upp, 1951; Reddy *et al.*, 1962; Jensen *et al.*, 1962; Choi *et al.*, 1986). Variation in performance when feeding diets in various forms may be due to nutrient density, physiological modification of gastrointestinal tract, and to changes in feeding behavior, age, or husbandry practices.

Phenotypic expression of alleles  $k^+$  and  $K$  at the sex-linked feathering locus is used routinely in broiler production to differentiate between male and female chicks at hatching. The late-feathering allele  $K$  is associated on the Z chromosome with endogenous virus (*ev 21*) that encodes for infectious lymphoid leucosis virus (Bacon *et al.*, 1988). Production losses among commercial Leghorn stocks have been associated with this allele (e.g., Harris *et al.*, 1984; Havenstein *et al.*, 1989),

however, information on broiler breeders kept for similar periods of lay is lacking.

This chapter contains a report of the results of a factorial experiment to study the effects of three feeding regimes and two forms of feed in a line of broiler breeders segregating at the sex-linked feathering locus. Criteria of evaluation include growth, onset of lay, egg production, egg weights, duration of fertility, antibody response to sheep red blood cells (SRBC), resistance to *Escherichia coli* (*E. coli*) and livability. Other data will be presented in Chapters III and IV.

## MATERIALS AND METHODS

### *Stocks*

Pullets used in this experiment were from a commercial broiler breeder line segregating at the sex-linked locus for early ( $k^+$ ) and late ( $K$ ) feathering. Eggs from age-contemporary parents were incubated and chicks were removed from the hatcher on the 22<sup>nd</sup> day of incubation, classified according to feathering phenotype, vaccinated for Marek's disease, vent sexed, wingbanded, and the females placed in floor pens for rearing.

### *Husbandry*

Females were randomly assigned to either *ad libitum* (AL) or alternate-day feeding programs, the latter designed to control body weight. There were two feed restricted pens and one AL pen of each genotype. Females were fed AL until 6 days of age (doa) and then fed

either AL or restricted from 7 to 240 doa. Flocks on the restricted regimen were given twice their daily feed allowance every other day.

Females were fed a mash diet containing 20 % crude protein and 2685 Kcal of ME/kg of feed from 1 to 70 doa and a diet of 14 % crude protein and 2827 Kcal of ME/kg of feed from 71 to 105 doa. Lighting was continuous to day 6 and from 0700 to 1800 hr daily from 7 to 55 doa. All females were then exposed to increasing natural light from 56 to 105 doa (mid-April to mid-June). Water was continuously available throughout the experiment. Rearing was in floor pens with wood shavings to 105 doa, after which females were transferred to individual cages (46 cm high, 30 cm wide and 46 cm deep) in a windowless room with a photoperiod from 0600 to 2000 hr. Feed was provided to the individually caged females from troughs which provided feed for five individuals.

A mash diet, containing 16 % crude protein and 2752 Kcal of ME/kg of feed, was offered daily commencing at 105 doa. At 147 doa the form of feed was changed from mash to crumbles or to a mix of crumbles and fines. Half of the females were offered feed as crumbles only (CO) with fines removed by sifting (mesh, Sieve No. 16, 1.18 mm) for the duration of the experimental period. The remaining half (CF) were fed sifted crumbles for 14 days, followed by sifted crumbles plus 40% fines (by weight) for 14 days. This cycle was repeated 13 times (to 511 doa). Samples of crumbles and crumble plus fines mix were analyzed for protein, ash, fiber, and energy at 250, 350, and 450 doa and no differences were found among them.

The transition to lay began at 105 doa with half of the restricted females placed on a daily feed restriction program (DR) designed to meet the target body weights of commercial breeder hens. The other half of the restricted females (RL) followed the same program except that they were released to AL at 238 doa when egg production was estimated at 10 % past peak. The release was for six days after which feed was again restricted.

There were twelve subclasses consisting of two feathering genotypes:  $k^{+/-}$  and  $K^{-/}$ , three feeding regimens: AL, DR and RL and two forms in which the feed was offered: CO and CF.

#### *Traits Measured*

Body weights of each individual were obtained every 14 days from hatch to 500 doa. Body weights of a subsample ( $n = 25$ ) of restricted birds were also obtained on a weekly basis to monitor weight changes and allow for adjustments in feed consumption to meet target body weights.

A random subsample ( $n = 20$ ) of the adults were tested for presence or absence of *ev21* (Smith and Crittenden, 1986). DNA was obtained from blood samples, which were digested with *Hae III*. A  $^{32}P$  labelled insert of a 1.6 kb fragment flanked by two *Hae III* sites, *ev21*-Int, was used as the hybridization probe on blotted nitrocellulose membranes. DNA samples obtained from a second random subsample of females ( $n = 30$ ) were digested with restriction endonuclease *SacI* to give characteristic DNA fragments (Bacon et al., 1988). Major *ev* bands and any random exogenous proviral integration were detected using a  $^{32}P$  labeled *pRAV-2* probe.

At 175 doa ten females from each of four ( $n = 40$ ) subclasses, namely  $k+/-$  AL,  $k+/-$  DR,  $K/-$  AL, and  $K/-$  DR, were chosen randomly and given a 0.1 ml intravenous injection of .25% SRBC. Antibody production to SRBC antigen was measured five days post-immunization from a 1 ml blood sample obtained from the brachial vein and placed in tubes with EDTA. Blood was centrifuged to obtain plasma in which antibodies to SRBC antigen were measured by the microtiter hemagglutination procedure of Wegmann and Smithies (1966). Antibodies were expressed as the  $\log_2$  of the reciprocal of the last dilution for which agglutination was detected. This procedure was repeated at 455 doa for 12 subclasses ( $n = 108$ ) with two genotypes, three feed regimens and two forms of feed.

Age and body weight at sexual maturity (day of first oviposition) were recorded for each female. Egg production was recorded daily and each egg classified as normal (also called hatching), double yolk, extra-calcified, compressed, broken, soft shelled, membrane, yolk, or other defectives (van Middelkoop and Siegel, 1976). Egg production was quantified as % normal, % double yolk, % extra-calcified and compressed, % broken, % other defective eggs and total ovulations from onset of lay to 500 doa. Data were expressed on a % hen-day basis, and as proportions of type of egg to total ovulations. Hatching eggs were expressed on a hen-housed basis. The first normal egg laid and each egg laid from 210 to 212, 360 to 362 and 460 to 462 doa were weighed. For each 3-day period the average egg weight for each hen was used for statistical analysis.

Beginning at 250 doa and again at 480 doa each hen, from RL and DR feeding regimens only, was inseminated twice on successive days with 0.025

ml of pooled semen collected from *k+/k+* males of an unrelated *F*<sub>1</sub> cross between two divergently selected body weight lines of White Plymouth Rock chickens (Siegel and Dunnington, 1987). Duration of fertility was measured as the number of days from last insemination to the day the last fertile egg was produced. A hen was considered infertile when two consecutive eggs were infertile. Eggs were broken-out daily and macroscopically classified as fertile or infertile (Kosin, 1944).

Resistance to *E. coli* was measured for the entire flock with each treatment subclass represented. At 507 doa 63 hens were inoculated with 0.1 ml containing 10<sup>6</sup> *E. coli* (serotype 01:K1 incubated for 24 h in tryptose broth) into the posterior thoracic air sac. Sixty-two hens were similarly inoculated with 10<sup>4</sup> *E. coli*. Twelve birds were not inoculated as a check on possible horizontal transmission of *E. coli*. Hens which died were examined to determine if cause of mortality was pericarditis. Five days after inoculation (511 doa) survivors were killed by cervical dislocation and scored by the same individual (WBG) for pericardial and air sac lesions. Scoring was as follows: 1 - none, 2 - mild air sac, 3 - moderate air sac, 4 - mild to moderate heart, 5 - extensive heart, and 6 - death between 507 and 511 doa of pericardial lesions.

Throughout the experiment the following data were obtained for each hen on the day of her death: body weight at death, age at death, days from last oviposition to death, and cause of mortality. Causes of mortality were determined by gross postmortem examination and grouped into four categories, 1) cardiac associated disease - aortic rupture, ascites, and hydropericardium, 2) bacterial infections - pericarditis, airsacculitis,

and peritonitis, 3) lymphoid leucosis, and 4) miscellaneous - splenomegaly, internal layer, kidney failure, fatty liver syndrome, and unknown etiology. At the end of the experiment all hens were examined for lymphoid leucosis tumors .

### *Statistical Analyses*

All data reported in this paper were obtained and analyzed on an individual bird basis. The statistical model for an analysis of variance was:

$$Y_{ijkl} = \mu + G_i + F_j + D_k + (GF)_{ij} + (GD)_{ik} + (FD)_{jk} + (GFD)_{ijk} + e_{ijkl}$$

where  $i = 1, 2$  feathering genotypes,  $j = 1, 2, 3$  feeding regimens,  $k = 1, 2$  forms of feed and  $l = 1, 2 \dots n$  individuals. Age was included in the model for the analysis of duration of fertility. Dose was included in the model for the analysis of *E. coli* lesions. Because body weights of some chickens were obtained weekly and others fortnightly, frequency of handling was included as a fifth main variable in analysis of the *E. coli* data. The AL hens were not included in this model.

Prior to analyses, body weight, age at first egg, egg number, egg weights, age at death, and days from last oviposition to death were transformed to natural logarithms, and percentages to arc sine square roots and *E. coli* lesion scores to square roots. Where significant differences were found among feed regimens, means were separated by



Tukey's pairwise comparisons (SAS, 1982). A probability level of  $P \leq .05$  was taken as significant. Percentage hen-day ovulations were calculated and curves were constructed using rolling average calculations for time series data (Broom, 1979).

Categorical data were analyzed by Bonferroni's chi-squared statistic using the simultaneous test procedure (Jensen *et al.*, 1968). Tau was set at 3, as data was used in 4 simultaneous tests. Subdivision of contingency tables to isolate cause of significant chi-squared statistic was determined by a graphical method (Snee, 1974) utilizing hypotheses testing procedures described in Zar (1984).

## RESULTS

### *Body Weight*

Body weights of AL females increased rapidly until about 4200 g after which there was a gradual increase to 4600 g at 500 doa (Figure 1). The AL females maintained heavier body weights than those on regimens DR or RL for the duration of the experiment. The effect of a brief release to *ad libitum* feeding on body weight of RL females was dramatic. Females on RL regimen gained 515 g during the six day released period while respective increases for regimens AL and DR were 27 g and 27 g. When the feed intake for regimen RL was returned to that of the DR regimen, there was initially a short, rapid decrease in body weight followed by a gradual decline and leveling. Significant differences in body weight between DR

and RL regimens were observed from release at 238 doa and persisted to 274 doa.

*Genotype ev21:K.*

All chicks phenotypically classified as late-feathering at hatch also tested positive for *ev21*. Those chicks which were classified as early-feathering at hatch tested negative for *ev21*. Southern blots probed with <sup>32</sup>P- labeled *pRAV-2* did not show smearing which is indicative of random exogenous proviral integrations in the genome. These results suggest that the ALV detected was of endogenous origin.

*Onset of Lay*

Age at first egg. Among feeding regimens, AL females came into egg production at a younger age (165 doa) than those whose intake was restricted (210 doa). Interactions involving feeding regimen with other main variables were not significant. There was, however, an interaction between genotype and form of feed. Early-feathering females matured at younger ages than late-feathering females when provided feed in CO form, while there was no difference between genotypes fed feed in CF form (Table 1).

Body weight at first egg. At onset of egg production, females fed AL were heavier (4402 g) than those on restricted feed (3563 g) and late-feathering females were heavier (3941 g) than early feathering ones (3731 g). Form of feed did not influence body weight at first egg nor

were there any interactions between genotypes, feeding regimens, or form of feed for this trait.

### *Egg Production*

Hen-day ovulations. Females fed AL commenced lay at a younger age than those on regimens DR and RL, however, their peak production and persistency of lay were lower (Figure 2). These patterns were reflected in differences in % hen-day ovulations over the course of the study which were lower for AL (21) than for DR (48) and RL (48) females which did not differ. Early-feathering chickens consistently had higher % hen-day ovulations, than late-feathering ones (Figure 3) resulting in an overall higher % hen-day ovulations (44 vs 36). These means included the low production values for AL females. Comparisons between two forms of feed (Figure 4) showed slightly higher % hen-day ovulations before, during and after peak production for CO- than CF-fed females with the overall means of 42 for CO and 39 for CF (these means include AL values) being different. Interactions between genotypes, feeding regimens, and form of feed were not significant for % hen-day ovulations.

Normal and defective eggs. Because each category of defective eggs followed the same trend, categories were pooled and presented as % defective eggs of total ovulations. Among the feeding regimens, AL females produced a higher % defective eggs (32.7 %) than either DR (3.2 %) or RL (6.2 %) females which did not differ from each other. There were no differences between the genotypes or forms of feed for % defective eggs, nor were interactions significant among genotypes, feeding regimes,

and form of feed. A somewhat different picture emerged when analyses were made for % normal ovulations. While there was still no difference between genotypes, the feeding regimen by form of feed interaction was significant (Table 2). Although AL females laid more normal eggs when feed was provided in CO than in CF form, there was no effect of form of feed in DR and RL regimens.

Number of hatching eggs. When expressed as number of hatching eggs to 500 doa, values were lower for AL (36.0) than for DR (130.7) or RL (126.1) females which did not differ. More hatching eggs were produced by early (113.7) than late (88.1) feathering females and by those fed CO (106.3) than CF (100.2).

### *Egg Weights*

First egg. Weight of the first egg laid was heavier for females whose feed intake had been restricted than for those fed AL (Table 3). Late-feathering females laid a heavier first egg than early-feathering ones. There were no differences between the two forms of feed for this trait nor were interactions between the main variables significant. The patterns observed among main variables for egg weight at 210 doa were the same as for weight of first egg.

By 360 and 460 days there was a reversal among feeding regimens for egg weight with the heavier eggs being laid by AL than either DR or RL females which did not differ (Table 3). Late-feathering females continued to lay heavier eggs than early-feathering ones and the lack of form of

feed effect and of interactions observed between the main variables persisted.

#### *Duration of Fertility*

Duration of fertility was not measured in AL females as their egg production was so erratic as to preclude reliable measurement. Duration of fertility was not different between genotypes or between restricted (DR) or short term released (RL) females. Females offered feed in CO form had a shorter duration of fertility (11.9 days) than those offered feed in CF form (13.2 days). Duration of fertility was longer at 250 doa (14.2 days) than at 480 days (10.8 days).

#### *Response to SRBC*

Regimen AL females had consistently lower SRBC titers at both ages than those for which feed intake was restricted (Table 4). There were no differences between DR and RL females in their ability to produce antibodies to SRBC. No differences were found in antibody titers between genotypes or birds fed different forms of feed. There were no significant interactions between the main variables for response to SRBC antigen.

#### *Resistance to *E. coli**

Absence of lesions in all the uninoculated controls implied that there was no horizontal transmission of *E. coli* and these hens were dropped from further analysis of *E. coli* inoculation. Resistance to *E. coli*, as indicated by lesion scores, was lower for AL (4.9) than for

regimens DR (2.2) and RL (2.2) which did not differ from each other. Genotype, form of feed, dose of etiologic agent, and frequency of human handling did not differ in resistance to *E. coli*. There were no interactions between the main variables for lesion scores.

### *Livability*

Mortality to 507 doa was greater for AL (70.8 %) than either DR (6.5 %) or RL (8.2 %) hens which did not differ. For the AL regimen mortality due to cardiac disease was 39 %, bacterial infection was 11 %, lymphoid leucosis was 28 %, and miscellaneous causes was 22 %. There was no difference in mortality between feathering genotypes (27.3 % vs. 32.0 %, for early and late, respectively) or between form of feed (27.1 % vs. 31.1 %, for CO and CF, respectively). Although there were no differences between feeding regimens or forms of feed for cause of death, mortality from lymphoid leucosis was higher in late- (17.9%) than early-feathering (.9%) females. No differences between the main variables were found for mortality from cardiac disease, bacterial infection, or miscellaneous causes.

Age at death was not different among feeding regimens, form of feed, or genotypes. Death occurred at younger ages in chickens that died from cardiac disease than from other causes (Table 5). Moreover, those that died from cardiac disease were also heavier than those which died from lymphoid leucosis or miscellaneous causes (Table 5). Body weight at death was higher for early- (3311 g) than late- (3232 g) feathering hens.

Hens which died from bacterial infections stopped laying 4 days before death which was the shortest time out of lay (Table 5). Heart disease and lymphoid leucosis and miscellaneous causes were not different in time spent out of lay before death.

## DISCUSSION

Short term *ad libitum* feeding after sexual maturity and the associated rapid weight gain observed under RL regimen had little, if any, effect on reproduction, response to SRBC, resistance to *E. coli*, or livability. Ovulation patterns were the same for these individuals as for those on regimen DR. These data suggest that, while the AL regimen caused a disruption of normal ovarian hierarchical development associated with long term obesity (Hocking *et al.*, 1987; 1989; Katanbaf *et al.*, 1989b), no such disruption in the ovarian hierarchy was apparent for the RL regimen which induced rapid short term gains in body weight near peak egg production. No change in % hen-day ovulations was observed under regimen RL which suggests that broiler breeders can withstand a short term weight gain without adversely affecting production, an observation consistent with that of Robbins *et al.* (1986). Also, because regimen RL had no negative effect on livability, egg production on a hen-housed basis followed the same trend as hen-day egg production for RL and DR regimens. The long term obesity observed under AL regimen was debilitating as evidenced by low egg production, low titers to SRBC antigen, reduced resistance to *E. coli* inoculation, and increased morbidity and mortality.

The lower weights of eggs laid by AL females during the early period of lay reflected their reaching sexual maturity at younger ages combined with a greater number of rapidly developing ova. These results agreed with those of Katanbaf *et al.* (1989c). Lower ovulation rates and reduced number of ova in development at later ages were associated with higher egg weights at later ages for the AL than for RL and DR regimens, agreeing with results of Hocking *et al.* (1987; 1989) and Katanbaf *et al.* (1989c).

Form of feed influenced egg production, with chickens fed a consistent high quality crumble having higher egg production than those fed in cycles which included a fortnight of high quality crumble followed by a fortnight of a mix of crumbles and fines. Because proximate analyses were the same for CO and CF feeds and all feed was consumed each day, it is unlikely that nutrient density was the basis of the differences in egg production between these treatments. An absence of interactions between form of feed and the other main variables for egg production indicated that similar responses to feed consistency were observed for feeding regimens and feathering genotypes.

Immune responses to SRBC were similar for early- and late-feathering females, a result consistent for this line (Dunnington *et al.*, 1987) and with those reported for other populations (Bacon *et al.*, 1987; Dunnington and Siegel, 1987). Differences between the genotypes for traits measured at the onset of lay were minimal, confirming observations by Dunnington and Siegel (1987). Early-feathering hens, however, had a substantial advantage in intensity and persistence of egg production, which resulted in their producing 25 more eggs than late-feathering hens



by 505 do. Absence of interactions between genotypes and the other main variables implied similar responses in egg production of hens of the two genotypes to feeding regimens and forms of feed. The difference in egg production between genotypes was consistent with earlier observations in White Leghorn stocks (Harris *et al.*, 1984). While hens of early-feathering genotypes were more resistant to lymphoid leucosis, those of late-feathering genotypes did not suffer from an increased morbidity in restricted regimens nor were there longer pauses out of lay prior to death from lymphoid leucosis. It appeared that lymphomas developed in the absence of exogenous avian leucosis virus infection. The late-feathering females fed *ad libitum* were more susceptible to endogenous avian leucosis virus infection than the other groups. Differences in egg production may have been due to pleiotropy of *K* allele in association with expression of *ev21* viral genes. Because these effects were not evident early in life, maintenance of flocks in production for an extended period of lay was critical to their demonstration.

The consistently heavier egg weights of late than early-feathering females corroborate differences reported between these alleles in other genetic backgrounds (Lowe *et al.*, 1965; Brake, 1988). Differences in egg weights shortly after onset of lay, when egg production was not different between the alleles, suggested that they were due to pleiotropic effects and not associated directly with the overall lower ovulations of late-feathering females.

TABLE 1. Means for age (days) at onset of lay with a genotype by form of feed interaction<sup>1</sup>

Genotype	Form of feed		Crumble Fines
	Crumble Only		
Early	187.4	NS	197.2
	***		NS
Late	209.6	NS	207.3

\*\*\* Means in a column were significantly different ( $P \leq .01$ ). Means are based on actual data, comparisons were made with transformed data.

NS Means adjacent in a column or row were not significantly different ( $P \geq .05$ ).

<sup>1</sup> Feeding regimens were pooled.

TABLE 2. Means for percent normal eggs<sup>1</sup> with a feeding regimen<sup>2</sup> by form of feed interaction

Feeding regimen	Form of feed	
	Crumble only	Crumble Fines
AL	73.3 <sup>b</sup>	53.8 <sup>b</sup>
DR	97.1 <sup>a</sup>	96.5 <sup>a</sup>
RL	93.6 <sup>a</sup>	94.2 <sup>a</sup>

a, b Means in a column with no common superscript were significantly different ( $P \leq .05$ ). Means were based on actual data, comparison were made with transformed data.

\*\* Means in a row were significantly different ( $P \leq .01$ ).

NS Means in a row were not significantly different ( $P > .05$ ).

<sup>1</sup>Percent normal eggs = (normal ovulations / total ovulation) X 100.

<sup>2</sup>Feeding regimens:

AL = *ad libitum*.

DR = restricted to control body weight.

RL = restricted, released for a brief period to *ad libitum*, and returned to restriction.

TABLE 3. Means by feeding regimen<sup>1</sup>, genotype, and form of feed for egg weight (g) at four ages

	Age (days)			
	1st egg	210	360	460
<u>Feeding regimen</u>				
AL	37b	43b	64a	64a
DR	47a	60a	62b	61b
RL	. .	. .	62b	62b
<u>Genotype</u>				
Early	43b	53b	61b	60b
Late	48a	55a	64a	64a
<u>Form of feed</u>				
Crumble only	45a	54a	62a	62a
Crumble fines	46a	54a	63a	62a

a, b Means within each main heading in a column with no common superscript were significantly different ( $P \leq .05$ ). Means were based on actual data, comparisons were made with transformed data.

<sup>1</sup>Feeding regimens:

AL = *ad libitum*.

DR = restricted to control body weight.

RL = restricted, released for a brief period to *ad libitum*, and returned to restriction.

TABLE 4. Means for sheep red blood cell (SRBC) antibody titers at two ages by feeding regimen

Feeding regimen <sup>2</sup>	Age <sup>1</sup>	
	175	455
AL	2.9 <sup>b</sup>	1.7 <sup>b</sup>
DR	3.8 <sup>a</sup>	3.0 <sup>a</sup>
RL	.....	3.1 <sup>a</sup>

a, b Means within a column with no common superscript are significantly different ( $P \leq .01$ ). Means were based on actual data, comparisons were made with transformed data.

<sup>1</sup> SRBC antibody titers were not analyzed with age as a main effect due to confounding of age with SRBC antigen source. All titers were measured 5 days after a primary inoculation with SRBC antigen.

<sup>2</sup> AL = *ad libitum*.

DR = restricted to control body weight.

RL = restricted as DR except for a brief release to *ad libitum* and then returned to DR regimen.

TABLE 5. Means for age at death, body weight at death and days from last oviposition to death by cause of death

Cause of death	Age at death (days)	Body weight at death (g)	Last oviposition to death
Cardiac disease <sup>1</sup>	255b	3891a	42ab
Bacterial <sup>2</sup>	320a	3506ab	4b
Lymphoid Leucosis <sup>3</sup>	295a	2730b	66a
Miscellaneous <sup>4</sup>	296a	2925b	79a

a, b Means within a column with no common superscript are significantly different ( $P \leq .05$ ). Means were based on actual data, comparisons were made on transformed data.

<sup>1</sup>Cardiac disease = aortic rupture (7), ascites (5), and hydropericardium (7)

<sup>2</sup>Bacterial infection (n = 8).

<sup>3</sup>Lymphoid leucosis (n = 15).

<sup>4</sup>Miscellaneous = splenomegaly (3), internal layer (1), kidney failure (2) fatty liver syndrome (4), and unknown etiology (3).

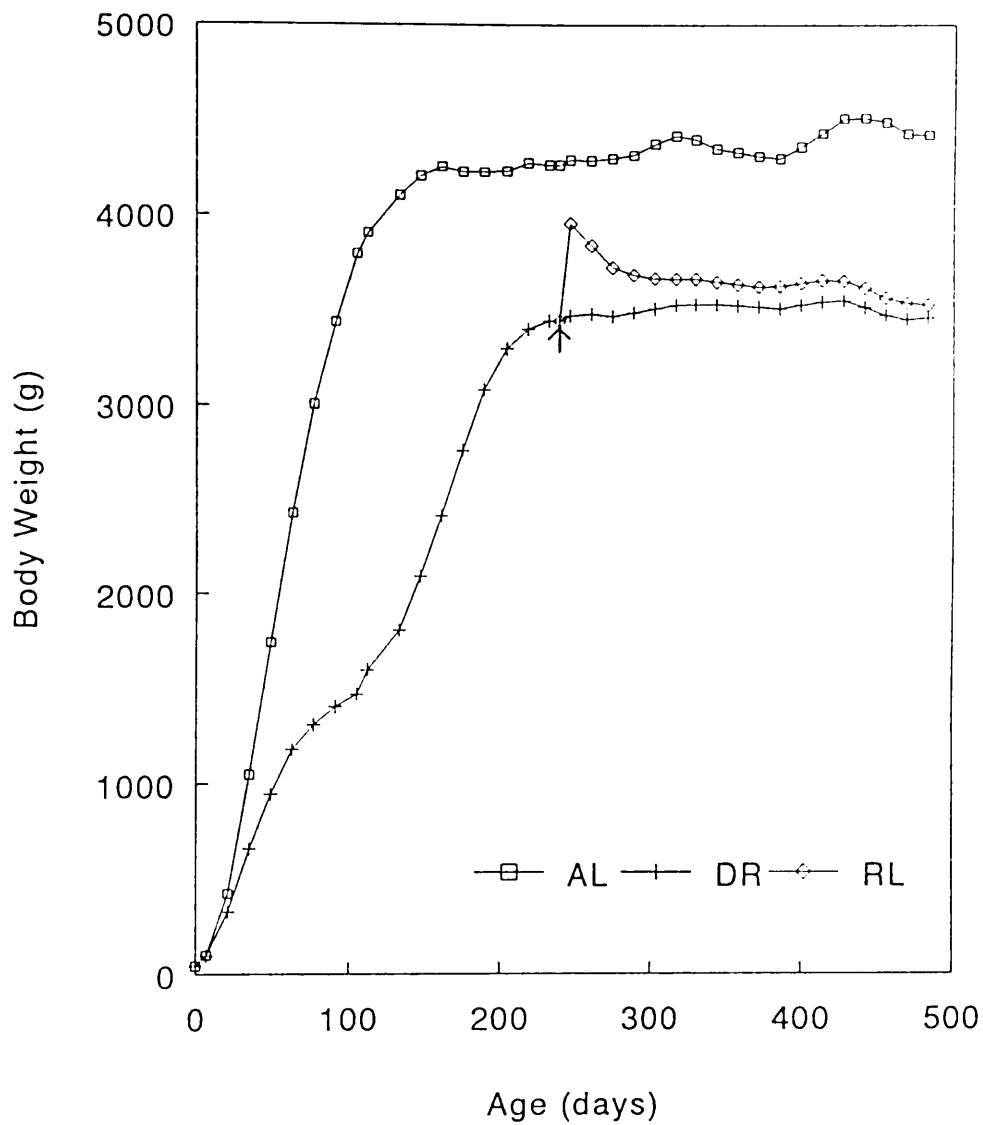


FIGURE 1. Body weights for female chickens fed *ad libitum* (AL), restricted feeding (DR), and restricted then released for six days to *ad libitum* and returned to restriction (RL). ↑ indicates the day RL females were released to AL.

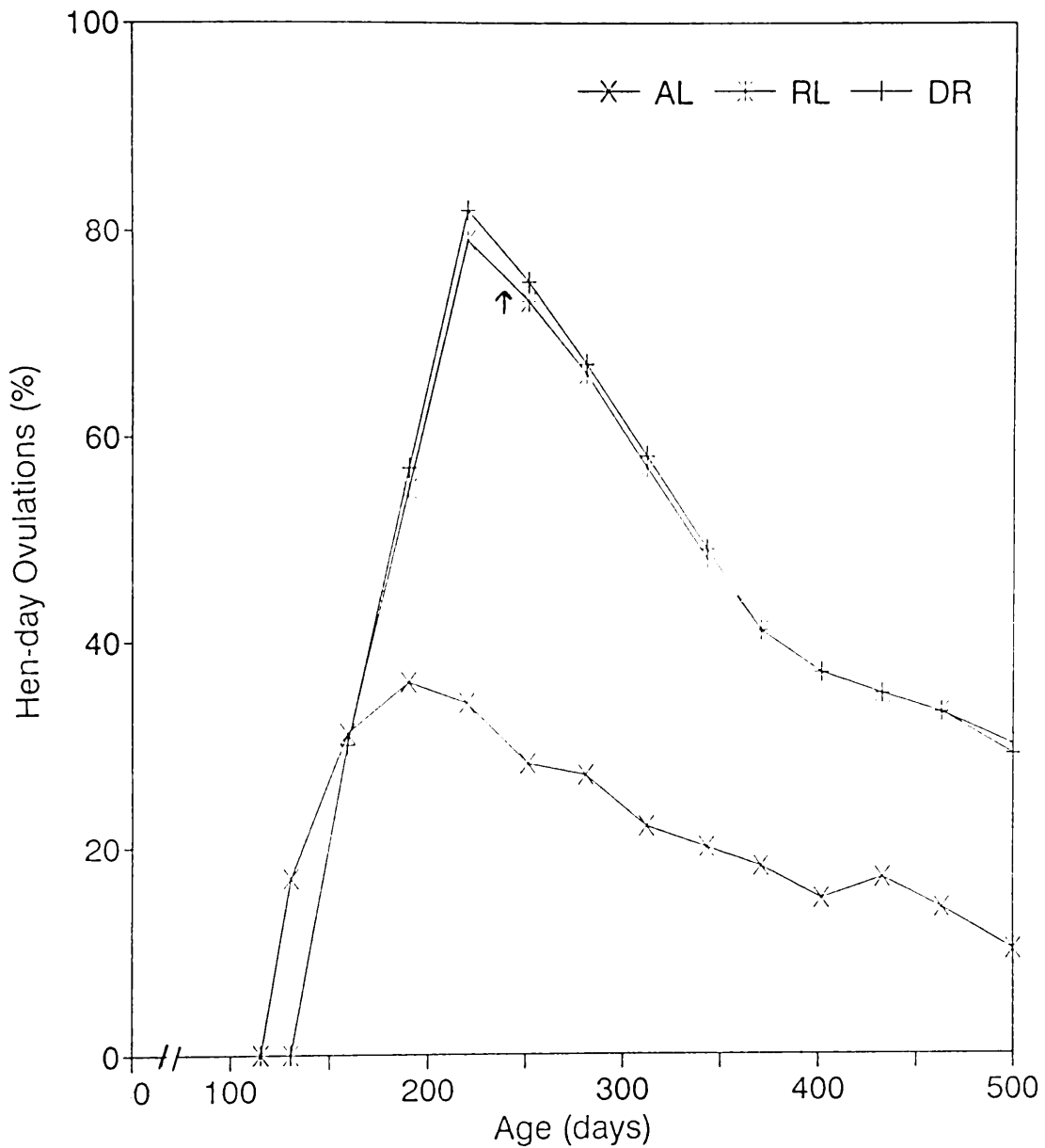


FIGURE 2. Percentage hen-day ovulation curves for chickens fed *ad libitum* (AL), restricted feeding (DR), and restricted then released for six days to *ad libitum* and returned to restriction (RL). Hen-day ovulations were lower for AL than either DR or RL regimens which did not differ ( $P \leq .05$ ). † indicates the day RL females were released to AL.



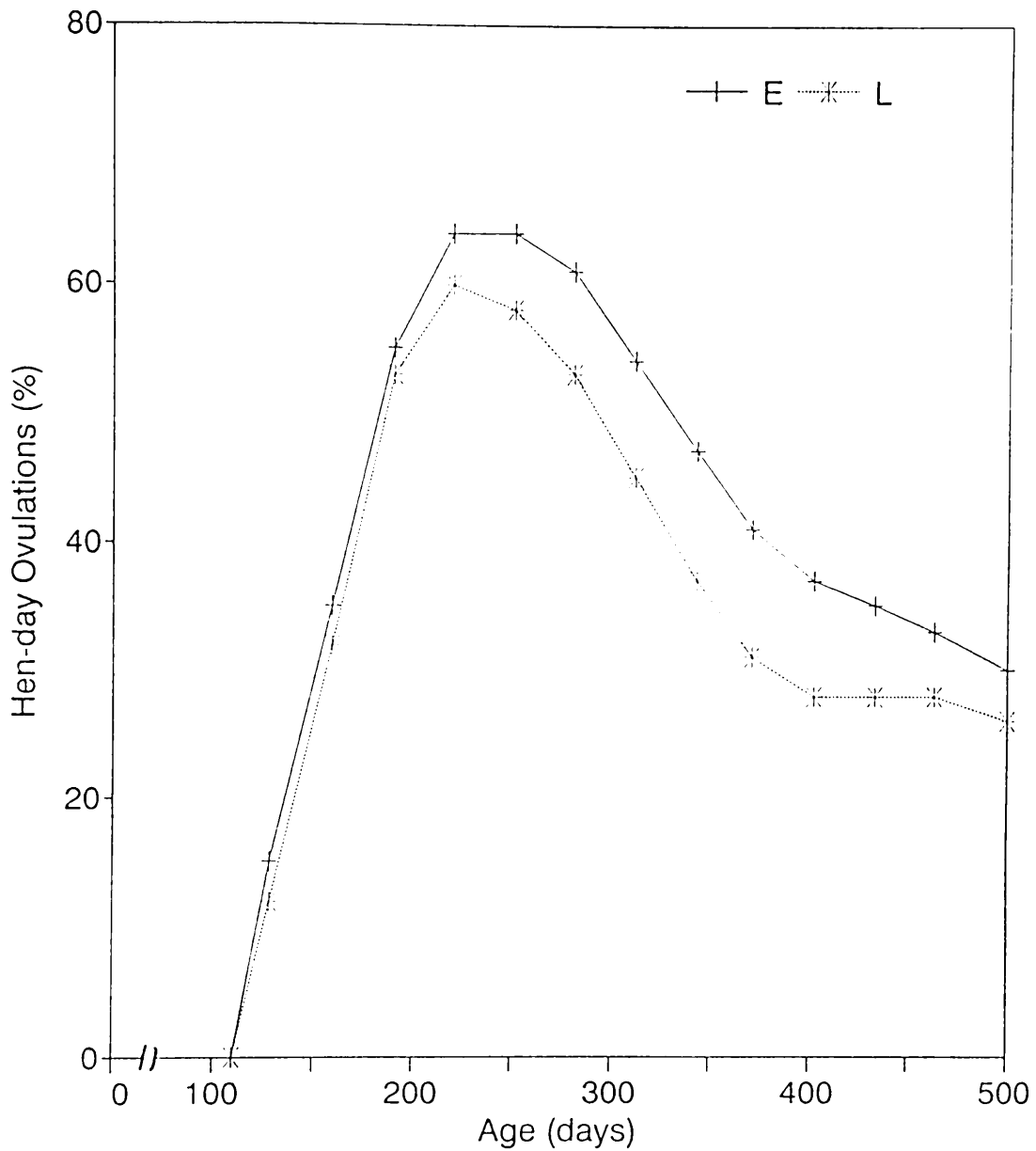


FIGURE 3. Percentage hen-day ovulation curves for early (E) and late (L) feathering chickens. Hen-day ovulations were greater for early ( $k+/-$ ) than late ( $K/-$ ) genotypes ( $P \leq .05$ ).

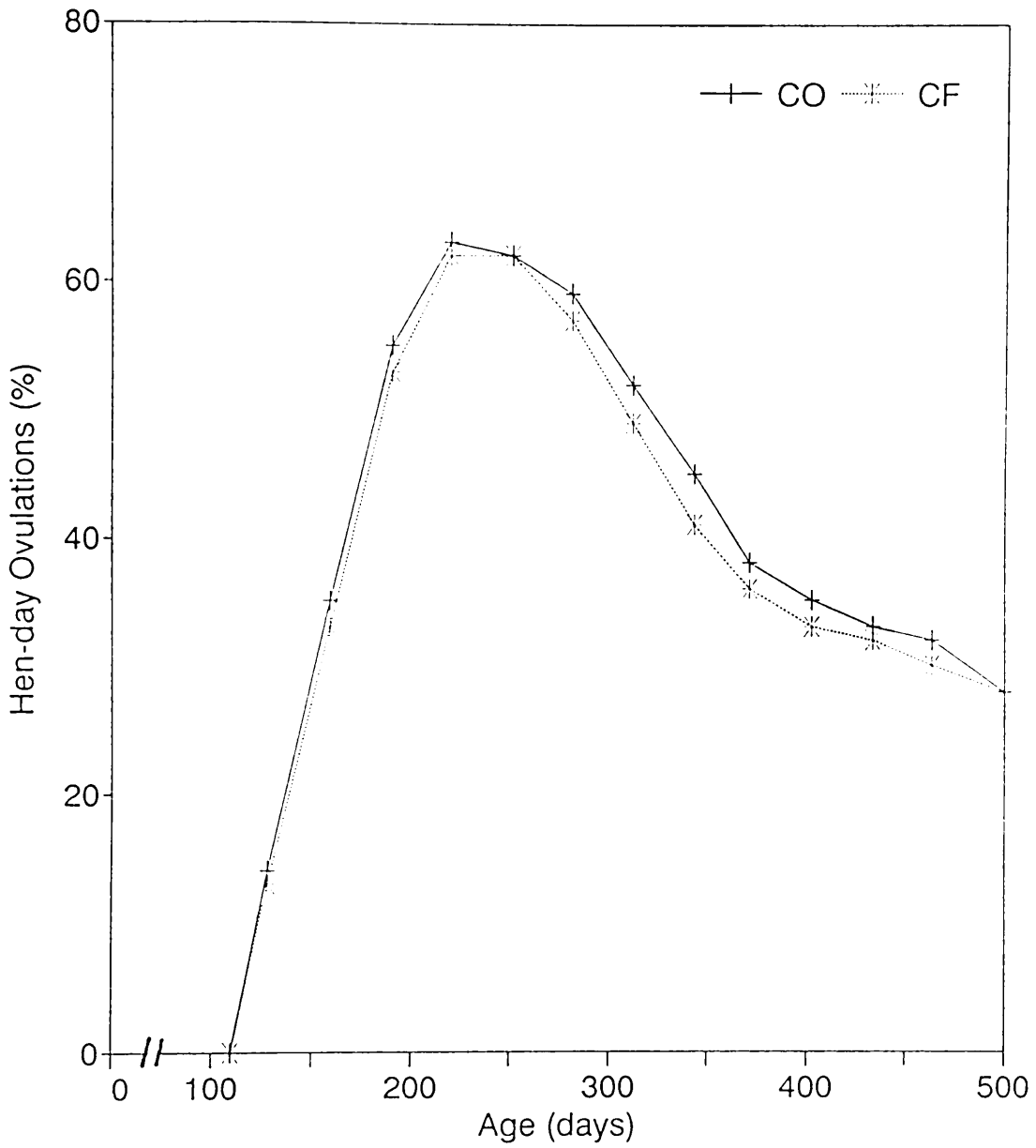


FIGURE 4. Percentage hen-day ovulation curves for diets provided as crumble only (CO) or as crumbles for 14 days followed by a crumble fines mix (60/40, w/w) for 14 days (CF) in repeated cycles. Hen-day ovulations were greater for CO than CF females ( $P \leq .05$ ).

## CHAPTER III

RELATIONSHIPS AMONG AGE OF DAM, EGG COMPONENTS, EMBRYO  
LIPID TRANSFER, AND HATCHABILITY OF BROILER BREEDER EGGS

## SUMMARY

Fertility, hatchability and their relationships to age of dam, egg components, embryo growth, and embryo lipid transfer were studied in a broiler breeder line segregating at the sex-linked feathering locus. Early season increases in hatchability of eggs were due to a reduction in embryo deaths between day 1 and 7 of incubation. No late seasonal declines in either fertility or hatchability were observed. While less proficient in young dams, embryo lipid transfer was not directly associated with the lower hatchability of their eggs. Egg weight, weights of 18-day embryos, and embryo:egg ratios increased with parental age. Also with age, there were increases in shell, albumen, and yolk weights. Heavier albumen weight was due to increased moisture content, and that for shell and yolk was due to dry matter accumulation. Changes in yolk:albumen ratios reflected large increases in relative yolk weight, on a wet and dry weight basis, that were associated with age of dam. Eggs from late-feathering dams were heavier than those from early-feathering ones with heavier embryos, also, by 18 days of incubation which was attributed to the transfer of albumen from the egg to the embryo.

## INTRODUCTION

A negative genetic correlation exists between body weight and egg number in turkeys (Nestor, 1971), Japanese quail (Marks, 1979), and meat-type chickens (Siegel and Dunnington, 1985). With genetic changes

in growth approximating 3% per year (McCarthy and Siegel, 1983), the importance of maximizing hatchability of settable eggs from broiler breeders has increased. The magnitude of genetic changes in growth, means that producers must avail themselves of genetically superior replacement breeders, rather than recycling existing breeding flocks. Yet, it is well recognized that hatchability is lower in pullets entering lay than later in life (Smith and Bohren, 1975; Shanawany, 1984; Mauldin, 1989). Egg component weights and their ratios change with age of dam (Anthony et al., 1989a), as does 18-day embryo weight (Shanawany, 1984). Moreover, lipid transfer from the yolk sac to the embryo may be less efficient in embryos from young breeders (Noble, 1987; Yafei and Noble, 1988). Additional data on these traits and their relationships with hatchability of eggs from broiler dams during various periods in their reproductive lives are required. This chapter contains a summary of relationships among age of dam, egg components, embryo growth, embryo lipid transfer, and age-related hatchability failures, in a broiler breeder line segregating at the sex-linked feathering locus. Other data are presented in Chapters II and IV.

## MATERIALS AND METHODS

### *Husbandry*

Pullets used in this experiment were obtained from a commercial broiler breeder line segregating at the sex-linked locus for early ( $k^+$ ) and late ( $K$ ) feathering. At hatching chicks were wingbanded, vaccinated

for Marek's disease and placed in light-controlled pens with wood shavings as litter. Pullets were fed a mash diet containing 20 % crude protein and 2685 cal of ME/g of feed from 1 to 70 days of age (doa). From 71 to 105 doa the diet consisted of 14 % crude protein and 2827 cal of ME/g of feed. Feed was offered on alternate days in a broiler breeder feed restriction program designed to control body weight. Lighting was continuous to 6 doa, and from 0700 to 1800 hr daily from 7 to 55 doa. At 56 doa pullets were placed in pens with windows allowing exposure to increasing natural light (mid-April to mid-June). At 105 doa, pullets were transferred to individual cages (46 cm high, 30 cm wide and 46 cm deep) in a windowless room and exposed to a photoperiod from 0600 to 2000 hr. A mash diet, containing 16 % crude protein and 2752 cal of ME/g of feed, was offered daily commencing at 105 doa. Water was continuously available throughout the experiment.

At 147 doa the feed consistency was changed from mash to crumbles or to a mix of crumbles and fines. Half of the pullets were offered feed as crumbles only (CO) with fines removed by sifting (mesh, Sieve No. 16, 1.18 mm) for the duration of the experimental period. The remaining half (CF) were fed sifted crumbles for 14 days, followed by sifted crumbles plus 40% fines (by weight) for 14 days. This cycle was repeated 13 times (to 511 doa). Samples of CO and CF were analyzed for protein, ash, fiber, and energy at 250, 350, and 450 doa. No differences occurred between diets for these measures. At 105 doa pullets were placed on a daily feed restriction program (DR). At 238 doa when egg production was estimated at 10 % past peak, half of the pullets were released to *ad libitum* feeding

for six days after which feed was again restricted (RL). Thus there were two feathering genotypes ( $K/-$ , or  $k^+/-$ ), two feeding regimens (DR or RL), and two feed consistencies (CO or CF).

### *Traits Measured*

Body weight, egg production, immunocompetence, and livability data have been reported (Chapter II). Fertility and hatchability of each hen was measured for eight weeks in weekly settings commencing at 50% lay (early season) and again for five weekly setting commencing at 64 weeks of age (late season). Each hen was inseminated twice before egg collection commenced and twice during each subsequent week. Each insemination consisted of 0.025 ml of pooled semen from an unrelated  $F_1$  cross between two early feathering lines of White Plymouth Rocks that have been divergently selected for juvenile body weight (Siegel and Dunnington, 1987).

Eggs, individually identified by hen and day laid, were collected daily, and stored at 13 C and 65 % relative humidity for each weekly setting. Eggs were incubated in the same machine for 18 days, then candled and live embryos were transferred to a hatcher. The remaining eggs were broken-out and macroscopically examined to determine fertility (Kosin, 1944). Those considered fertile were hatchability failures (Mauldin, 1989) and classified as early dead (0 to 6 days of development) or advanced dead (7 to 17 days of development). The latter were grouped with early dead for statistical analysis because they never exceeded .3%. On the 22<sup>nd</sup> day of incubation chicks were removed from the hatcher and

counted. The late deads (18 to 21 days of development) and pips (i.e. pipped through the shell, but not free of the shell) were also recorded. Hatchability was calculated for total eggs set (hatchability) and hatchability of all fertile eggs set (hatch of fertiles).

For weeks 2, 4, 6, and 8 random subsamples of embryos taken from all subclasses were removed from incubation on Day 18 and their weights, free of membranes, obtained. Liver and yolk sac were removed and weighed. All weights were to the nearest .01 g and the embryo, liver, and yolk sacs were stored at -20 C for subsequent homogenization and extraction of total lipid with chloroform:methanol 2:1 (Folch et al., 1957). Each embryo was reweighed and dried to a constant weight, to determine total dry matter content (AOAC, 1975), prior to lipid extraction.

During weeks 2, 4, 6, 8, 35, 39, and 41, nonincubated eggs were weighed to the nearest .01 g, hard-boiled (Lee, 1985) and then cooled for 5 min in cold running water. From each egg, samples of shell without membrane from the top, middle, and bottom were measured (.01 mm) and their average considered a shell thickness for that egg. Shell with membranes, albumen, and yolk were separated, and weighed (.01 g). Samples were then dried to a constant weight (AOAC, 1975) and reweighed to obtain shell and membrane, albumen, and yolk dry matter. Moisture of components was calculated by subtraction.

### *Statistical Analyses*

Fertility, hatchability, and hatchability failures were subjected to regression analysis using the following model.



$$Y_{ijkl} = \beta_1 a_{ijk} + g_i + f_j + c_k + e_{ijkl}$$

where  $\beta_1$  = the regression coefficient of Y on age of dam,  $i = 1, 2$  feathering genotypes,  $j = 1, 2$  feeding regimens,  $k = 1, 2$  feed consistencies,  $l = 1, 2 \dots n$  observations. Homogeneity of variances among genotypes, feeding regimens, and feed consistencies was tested by a folded F-test (Zar, 1984) avoiding any assumptions regarding homogeneity of variance (Satterwaite, 1946). Heterogeneity of slopes among genotypes, feeding regimens and feed consistencies was tested with age of dam as the independent variable (Freund and Littell, 1981). A linear or quadratic effect of dam age was fitted and tested for goodness of fit.

Egg components and 18-day embryo data were subjected to ANOVA with age of dam, feathering genotype, feeding regimen, and feed consistency as main effects and their first and second order interactions fitted to the model. Weights were transformed to natural logarithms. Percentages were divided by 100 and transformed with other ratios to arc sine square roots prior to statistical analysis. Untransformed means are presented in tables. Means for dam age were separated by Tukey's studentized range test (SAS, 1982). Unless otherwise stated statistical significance was taken at  $P \leq .05$ .

## RESULTS

### *Fertility and hatchability*

Fertility of eggs during early egg production ranged from 93 to 98 percent of eggs set with no significant effects due to genotype, feeding regimen, feed consistency, or age of dam ( $\beta = .004 \pm .005$ ). The % hatchability of all eggs and of fertiles increased with age of dam ( $\beta = .020 \pm .008$ ,  $\beta = .017 \pm .007$ , respectively) from wk 1 to 8 (Table 1). There was no relationship between % hatchability or % hatch of fertiles and genotype, feeding regimen, and feed consistency. There was a reduction in the incidence of hatchability failures (Table 1) due to a decrease in early dead embryos associated with age of dam ( $\beta = -.008 \pm .002$ ). No changes in early or late dead embryos and pips were associated with genotypes, feeding regimen, or feed consistency. Regressions were not different from each other and variances associated with fixed effects were homogeneous.

During the week RL dams were fed *ad libitum* there were more late dead embryos (10.8 %) than for restricted (DR) dams (5.9 %). No differences due to feeding regimen were observed when RL dams were returned to the restricted feeding program. The % fertility, hatchability, and hatchability failures during late season egg production (Table 2) showed no changes associated with age of dam, genotype, feeding regimen, or feed consistency (Table 2).

### *Eighteen-day embryo*

Embryo, yolk sac, and liver weights on an absolute basis and relative to egg weight increased during early season production (Table 3). Although % lipid also increased in both the embryo and yolk sac with age of dam, this relationship was not evident for liver lipid. Embryo dry matter increased over time (Table 3), as did embryo:egg ratios. Embryo weights were similar for feeding regimens and feed consistencies. Embryos from late-feathering dams were heavier and had more dry matter than those from early feathering ones (embryo wt: 22.8 vs. 21.8 g, dry matter: 18.9 vs. 18.5 %). Embryos from early-feathering dams had heavier yolk sacs (14.7 vs. 13.7 g) with a similar lipid content than those from late-feathering ones.

### *Egg components*

Egg weight increased with age of dam (Table 4), however, there was no change in shell thickness, even though shell weight increased and shell moisture decreased with age. An increase in albumen weight with age was due to greater moisture content of the albumen. The increase in yolk weight with age of dam was not due to moisture, but to an accumulation of dry matter. Yolk:albumen ratios on a wet and dry weight basis indicate a large increase in yolk relative to albumen as dams matured.

While feeding regimen and feed consistencies were similar for egg components, late-feathering dams laid heavier eggs with thinner shells than early-feathering ones (59.9 vs. 57.0 g, .32 vs. .33 mm). Albumens were heavier both in dry matter and moisture for eggs from late- than

early-feathering dams ( wet wt: 35.6 vs. 33.3 g, and dry matter: 5.4 vs. 5.2 g).

## DISCUSSION

The results showing that the incidence of early embryonic mortality decreased over time are consistent with Leghorn stocks (Sunde and Bird, 1959) and with field observations of broiler breeders (Mauldin, 1989). Delays in embryo growth which were persistent to 18 days of incubation also corroborated data reported by Shanawany (1984). Disturbances in lipid metabolism of embryos from young dams were consistent with observations by Noble and Conner (1984), Noble (1987) and Yafei and Noble (1990). These results may reflect an overall retardation of development reported by Smith and Bohren (1975) and Mather and Laughlin (1979).

Egg size and 18-day embryo weight increased with age of dam, observations consistent with those reported by Shanawany (1984). The relative distribution of egg components was also a function of age of dam, a result consistent with those of Cunningham *et al.* (1960) and Anthony *et al.* (1989a). The accumulation of dry matter in yolk which continued throughout the production cycle suggests that increases in embryo weight and hatchability from older dams are a function of changes in embryo metabolism as dams mature and/or of greater availability of resources from the egg.

Fertility via artificial insemination was maintained above 93% even among older hens in this study, a result in contrast with field data for

natural matings where there is a late seasonal decline (Mauldin, 1989). These results obtained with artificial insemination suggest that late seasonal declines in fertility in natural mating situations may reflect reduced libido among older males.

Late-feathering dams laid heavier eggs than early-feathering ones, corroborating reports by Lowe *et al.* (1965), Brake (1988), and Katanbaf *et al.* (1989b). The differences in egg weight were due to heavier albumen among eggs from late-feathering than early-feathering dams. The presence of a larger yolk sac in the eggs of early-feathering than late-feathering dams may account in part for the observation Chapter I that the former had the ability to surmount hatch weight differences post hatch.

TABLE 1. Mean % hatchability, fertility, hatch of fertile eggs and hatchability failures for setting weeks 1 to 8 and slope of their regression line ( $\beta$ )  $\pm$  S.E.

Trait	Setting week <sup>1</sup>								$\beta \pm$ S.E.
	1	2	3	4	5	6	7	8	
Fertility	93	93	95	98	98	96	93	95	.004 $\pm$ .005†
Hatchability	74	75	82	85	85	83	82	83	.020 $\pm$ .008*
Hatch of fertiles	79	76	85	91	88	85	85	85	.017 $\pm$ .007*
<u>Hatchability Failures</u>									
Early dead <sup>2</sup>	9	8	5	5	6	3	3	3	-.008 $\pm$ .002*
Late dead <sup>3</sup>	9	9	7	7	6	9	8	8	.002 $\pm$ .002†
Pips	1	1	1	1	1	1	.2	1	-.001 $\pm$ .001†

<sup>1</sup>Dams were 28 weeks of age in 50% lay at the start of setting week 1.

<sup>2</sup>Early embryo deaths were 1 to 17 days of incubation.

<sup>3</sup>Late embryo deaths were 18 to 21 days of incubation.

\* Regression was significant ( $P \leq .01$ )

† Regression was not significant ( $P \geq .05$ )

TABLE 2. Mean % hatchability, fertility, hatch of fertile eggs and hatchability failures for setting weeks 35 to 39 and slope of regression line ( $\beta$ )  $\pm$  S.E.

Trait	Setting week <sup>1</sup>				$\beta \pm$ S.E.	
	35	36	37	38		
Fertility	97	96	94	98	98	.010 $\pm$ .010†
Hatchability	84	84	86	87	84	.012 $\pm$ .018†
Hatch of fertiles	86	85	89	89	85	.009 $\pm$ .017†
<u>Hatchability Failures</u>						
Early dead <sup>2</sup>	3	3	2	5	5	.005 $\pm$ .005†
Late dead <sup>3</sup>	9	8	6	6	8	-.002 $\pm$ .007†
Pips	1	1	.1	.1	1	.000 $\pm$ .003†

<sup>1</sup>Dams were 64 weeks of age at the start of setting week 35.

<sup>2</sup>Early embryo deaths were 1 to 17 days of incubation.

<sup>3</sup>Late embryo deaths were 18 to 21 days of incubation.

† Regression was not significant ( $P \geq .05$ )

TABLE 3. Means of embryo, yolk sac, embryo liver weights, fat contents and their ratio to egg weight at 18 days of incubation

Trait	Setting week <sup>1</sup>			
	2	4	6	8
<u>Weight, g</u>				
Embryo	21.2 <sup>b</sup>	21.8 <sup>b</sup>	23.0 <sup>a</sup>	23.4 <sup>a</sup>
Yolk sac	11.9 <sup>b</sup>	14.6 <sup>b</sup>	15.2 <sup>a</sup>	15.4 <sup>a</sup>
Liver	.37 <sup>d</sup>	.46 <sup>c</sup>	.56 <sup>b</sup>	.69 <sup>a</sup>
<u>% Lipid in</u>				
Embryo	3.5 <sup>b</sup>	3.7 <sup>ab</sup>	3.7 <sup>ab</sup>	3.9 <sup>a</sup>
Yolk sac	20.1 <sup>b</sup>	21.0 <sup>ab</sup>	20.8 <sup>ab</sup>	21.3 <sup>a</sup>
Liver	9.9 <sup>a</sup>	5.1 <sup>b</sup>	9.1 <sup>a</sup>	5.6 <sup>b</sup>
<u>Percentage of embryo</u>				
Dry matter	18.2 <sup>b</sup>	18.7 <sup>ab</sup>	19.1 <sup>a</sup>	18.9 <sup>a</sup>
<u>Ratios</u>				
Embryo:egg	.43 <sup>b</sup>	.43 <sup>b</sup>	.44 <sup>ab</sup>	.46 <sup>a</sup>
Yolk sac:egg	.25 <sup>b</sup>	.29 <sup>ab</sup>	.29 <sup>ab</sup>	.30 <sup>a</sup>

<sup>1</sup>Dams were 28 weeks of age and in 50% lay at the start of setting week 1.

<sup>ab</sup>Means within a row with no common superscripts are significantly different (P ≤ .05).



TABLE 4. Means of egg weight, shell thickness, egg component weights and yolk:albumen ratios

Trait	Setting week <sup>1</sup>									
	2	4	6	8	9	35	39	41		
Egg wt (g)	53.3 <sup>d</sup>	55.5 <sup>cd</sup>	56.5 <sup>bc</sup>	57.5 <sup>bc</sup>	57.8 <sup>bc</sup>	62.5 <sup>a</sup>	60.8 <sup>a</sup>	61.4 <sup>a</sup>		
<u>Shell</u>										
Thickness (mm)	.33 <sup>a</sup>	.33 <sup>a</sup>	.34 <sup>a</sup>	.33 <sup>a</sup>	.33 <sup>a</sup>	.33 <sup>a</sup>	.33 <sup>a</sup>	.33 <sup>a</sup>		
Wt (g)	6.5 <sup>c</sup>	6.6 <sup>bc</sup>	6.8 <sup>ab</sup>	7.0 <sup>a</sup>	6.7 <sup>bc</sup>	7.2 <sup>a</sup>	7.0 <sup>a</sup>	7.1 <sup>a</sup>		
Moisture (%)	17.4 <sup>c</sup>	17.0 <sup>bc</sup>	16.6 <sup>b</sup>	16.2 <sup>a</sup>	16.8 <sup>b</sup>	15.9 <sup>a</sup>	16.0 <sup>a</sup>	16.0 <sup>a</sup>		
<u>Albumen</u>										
Wt (g)	31.8 <sup>d</sup>	32.9 <sup>d</sup>	33.3 <sup>c</sup>	33.7 <sup>b</sup>	33.9 <sup>b</sup>	37.1 <sup>a</sup>	34.8 <sup>ab</sup>	36.2 <sup>a</sup>		
Moisture (%)	83.9 <sup>b</sup>	84.0 <sup>b</sup>	84.0 <sup>b</sup>	84.1 <sup>b</sup>	84.2 <sup>b</sup>	85.7 <sup>a</sup>	85.1 <sup>a</sup>	85.7 <sup>a</sup>		
<u>Yolk</u>										
Wt (g)	16.3 <sup>d</sup>	17.4 <sup>c</sup>	17.8 <sup>bc</sup>	18.4 <sup>b</sup>	18.9 <sup>ab</sup>	20.9 <sup>a</sup>	20.1 <sup>a</sup>	20.4 <sup>a</sup>		
Moisture (%)	47.9 <sup>ab</sup>	48.3 <sup>a</sup>	47.8 <sup>ab</sup>	47.8 <sup>ab</sup>	48.1 <sup>a</sup>	47.4 <sup>ab</sup>	46.3 <sup>b</sup>	46.6 <sup>b</sup>		
<u>Yolk:albumen ratios</u>										
Wet	.51 <sup>d</sup>	.53 <sup>dc</sup>	.53 <sup>dc</sup>	.55 <sup>bc</sup>	.56 <sup>bc</sup>	.56 <sup>bc</sup>	.58 <sup>ab</sup>	.56 <sup>bc</sup>		
Dry	1.67 <sup>e</sup>	1.73 <sup>de</sup>	1.75 <sup>d</sup>	1.78 <sup>d</sup>	1.85 <sup>c</sup>	2.08 <sup>b</sup>	2.08 <sup>b</sup>	2.14 <sup>a</sup>		

<sup>1</sup>Setting week 1 dams were 28 weeks of age and in 50 % lay.

a,b,c,d,e means within a row with no common superscript are significantly different (P ≤ .05).

## CHAPTER IV

### FEEED INTAKE, UTILIZATION, AND THE RESIDUAL COMPONENT IN EARLY- AND LATE-FEATHERING BROILER BREEDER DAMS

## SUMMARY

Feed intake, feed efficiency, and residual feed consumption (RFC) were measured in broiler breeder females for 12 production periods of 28 days each. Experimental variables included 3 feeding regimens, 2 feed consistencies, and 2 sex-linked feathering genotypes. The habituation of broiler breeders to feed restriction did not inhibit expression of hyperphagia when released to *ad libitum* feed. Long term obesity, but not short term weight gain was detrimental to broiler breeder performance. The late-feathering allele negatively influenced reproductive responses and feed utilization. An inconsistent quality of crumbles was associated with poorer performance both under *ad libitum* and restricted feeding regimens. There was considerable variation among hens for RFC, with variation associated with different genotypes and husbandry practices, suggesting that measurement of RFC may facilitate development of improved management guidelines.

## INTRODUCTION

The relationship between feed intake and efficiency of egg production has been studied extensively in commercial layer hens (see review by Pirchner, 1985). Genetic components of feed efficiency of egg-type females are associated with genetic components involved in egg mass produced, body weight, body weight changes and residual feed consumption (Bordas and Mérat, 1974; 1976; 1981). The heritability of

residual feed consumption (RFC), defined as the difference between predicted (from production records) and observed food intake, was moderate to high (Wing and Nordskog, 1982a; Wing *et al.*, 1983; Luiting and Urff, 1991b). There was systematic as well as permanent variation for RFC (Luiting and Urff, 1991a) with differences due to maintenance being the major component of the systematic variation.

Lacking are data for RFC and its relationships with different feeding programs, feed consistencies, and alleles at the sex-linked feathering locus ( $K$ ,  $k^+$ ) in the broiler breeder. In this paper feed intake, feed efficiency, and RFC are reported for early- and late-feathering broiler breeder hens under 3 feeding regimens, of 2 feed consistencies. Previous chapters contained reports of growth, egg production, immunocompetence, and livability (Chapter II) and fertility, hatchability, egg and embryo components (Chapter III) for these stocks and dietary regimens.

## MATERIALS AND METHODS

### *Stocks and Husbandry*

Pullets used in this experiment were from a commercial broiler breeder line segregating at the sex-linked locus for early ( $k^+$ ) and late ( $K$ ) feathering. Eggs from age-contemporary parents were incubated in the same machine and chicks were removed from the hatcher on the 22nd day of incubation, classified according to feathering phenotype, vaccinated for Marek's Disease, vent sexed, wingbanded, and the females placed in floor

pens for rearing. Females were randomly assigned to either *ad libitum* (AL) or alternate-day feeding programs, the latter designed to control body weight. There were 2 feed restricted pens and one AL pen of each genotype.

Females were fed a mash diet containing 20% crude protein and 2685 Kcal of ME/Kg of feed for the first 10 weeks and a diet of 14% crude protein and 2827 Kcal of ME/Kg of feed from weeks 10 to 15. Lighting was continuous for the first week and from 0700 to 1800 h daily from weeks 1 to 8. All females were then exposed to increasing natural light from weeks 8 to 15 (mid-April to mid-June). Water was continuously available. Rearing was in floor pens with wood shavings to week 15, after which females were transferred to individual cages (46 cm high, 30 cm wide and 46 cm deep) in a windowless room with a photoperiod from 0600 to 2000 h. Feed was provided to the individually caged females from troughs which provided feed for 5 individuals.

A mash diet, containing 16% crude protein and 2752 Kcal of ME/Kg of feed, was offered daily commencing with week 15. At week 21 the form of feed was changed from mash to either crumbles or to a mix of crumbles and fines. Half of the females were offered feed as crumbles only (CO) with fines removed by sifting (mesh, Sieve No. 16, 1.18 mm) for the duration of the experiment. The remaining half (CF) were fed sifted crumbles for 14 days, followed by sifted crumbles plus 40% fines (by weight) for 14 days. This cycle was repeated every 28 days, with each cycle considered a production period. There were 12 production periods in all. Samples of crumbles and the crumble plus fines mix were analyzed

for protein, ash, fiber, and energy during periods 4, 8, and 11. No differences were found between feeds of different consistencies.

At the end of the 4th production period, half of the restricted females remained on a daily feed restriction program (DR) designed to meet the target body weights of commercial breeder hens. The other half of the restricted females (RL) followed the same program except that they were allowed feed at all times for 6 consecutive days which included days 26, 27, and 28 of period 4 and days 1, 2, and 3 of period 5. This release to AL feeding was designed to occur when egg production was estimated at 10 % past peak. After the release, feed was again restricted to that provided to DR females. There were 12 subclasses consisting of 2 feathering genotypes ( $k^{+/-}$  and  $K^{-}$ ), 3 feeding regimens (AL, DR and RL) and 2 forms in which the feed was offered (CO and CF).

#### *Traits Measured*

Feed consumption was measured daily for groups of 5 hens ( $n = 40$ ) which formed the experimental units. Daily feed intake and intake for 28-day periods were calculated for each group. Records of daily mortality allowed for adjustment of group size to obtain estimates of average intake per hen. Individual body weights were obtained every 14 days and egg production was recorded daily. Each egg was classified as normal, defective (membrane, soft shelled, compressed, and extra calcified) or double yolked as described by van Middelkoop and Siegel (1976). Weight of the first normal egg laid by each hen was obtained, as were weights of eggs laid during 3 consecutive days during production periods 4, 8,

and 12. Group records from individual production records were calculated for each of the 12 production periods. Efficiency of egg production was calculated for each group on a egg mass per hen-day basis.

A modified feed intake prediction model (Byerly, 1941; Byerly *et al.*, 1980) was used with information on growth, metabolic body weight, and egg mass production included in the equation [1]. Residual feed consumption was calculated as the difference between predicted and observed feed intake, equation [2].

$$Y_{Ti} = b_0 + b_1W^{\alpha}_i + b_2\Delta W_i + b_3Ec_i \quad [1]$$

$$Y_{Ri} = Y_{O_i} - Y_{Ti} \quad [2]$$

In [1] predicted food intake was estimated by a multiple regression model including growth, metabolic body weight, and egg mass production where,  $i = 1 \dots n$ ,  $n =$  number of groups,  $Y_T$  is the predicted feed intake,  $b_0$  is the intercept,  $W^{\alpha}$  is the mean body weight corrected by  $\alpha$  to give an estimate of metabolic body weight ( $\alpha = .75$ ),  $\Delta W$  is the change in body weight,  $Ec$  is egg mass production corrected for defective egg production (Luiting and Urff, 1991c), and  $b_1$ ,  $b_2$  and  $b_3$  are the partial regression coefficients of metabolic body weight, growth and corrected egg mass production respectively. In [2],  $Y_O$  is the observed (recorded) feed intake and  $Y_R$  is the residual feed component.

### *Statistical Analyses*

Feed intake, feed efficiency and RFC were subjected to ANOVA (SAS Institute, 1982) using the following statistical model:

$$Y_{ijkl} = \mu + G_i + F_j + D_k + (GF)_{ij} + (GD)_{ik} + (FD)_{jk} + (GFD)_{ijk} + e_{ijkl}$$

where  $i = 1, 2$  feathering genotypes,  $j = 1, 2, 3$  feeding regimens,  $k = 1, 2$  forms of feed and  $l = 1, 2 \dots n$  individuals. Feed intake was transformed to common logarithms and RFC data were transformed to a modified square root transformation (Bartlett, 1936) where  $X' = \sqrt{X + 3000}$  (Zar, 1984). Homogeneity of variances were tested in pairwise comparisons with genotypes, feeding regimens, and forms of feed with a folded F'test (Freund and Littell, 1981).

## RESULTS AND DISCUSSION

### *Feed Intake*

Second order interactions for feed intake during production periods 1, 2, 3, and 6 (when feed intake to DR and RL hens was restricted), were artifacts due to genotype by form of feed interactions. Feed intake of AL hens with this interaction are presented (Table 1). For early-feathering hens, more feed was consumed during period 6 when offered as CO than CF while similar amounts were consumed in periods 1 and 2. Although late-feathering hens consumed more CF than CO feed during periods 1 and 2, this trend was reversed for periods 3 and 6. Late-feathering hens consumed more feed than early-feathering ones on both forms of feed during period 1, less CO during periods 2 and 6 and more during period 3. High obesity-related mortality of AL hens (Chapter II) precluded inclusion of this group in the analysis for production periods 7 through 12. Over these periods AL hens consumed more CO than CF feed. From daily



records (data not shown) a tendency for all AL hens to indulge in compulsive consumption was evident.

To meet target body weights, DR hens were provided with 3360 and 3290 g of feed during periods 4 and 5, respectively. To maintain target body weights, feed allotments from period 5 onward were less for late- than early-feathering hens, because the latter had higher egg production and therefore more feed was required to maintain body weight (Chapter II). That is, feed was directed to reproduction rather than to body weight gain. Genotype by feeding regimen interactions was present for feed intake during periods 4 and 5 (Table 2). These interactions were due to restricted feed intake of DR hens and continuous availability of feed to AL and RL females. During these periods intake was greater for RL than AL hens regardless of genotype.

A genotype by form of feed interaction was observed for feed intake during period 4, due to reduced feed intake among early- but not late-feathering hens (3209 vs 3410 g) for CF feed. No differences between genotypes were present when feeding CO (3446 vs 3406 g). Within genotypes, only early-feathering hens ate less CF than CO feed (3209 vs 3446 g). The feeding regimen by form of feed interaction during period 4 was caused by AL hens consuming less CF than CO feed (2692 vs 3309 g). There were no differences between forms of feed for the other feeding regimens. Within forms of feed, intakes of CF hens were RL > DR > AL, while for CO hens RL > AL and DR which were similar to each other.

### *Feed Efficiency*

During period 1 the only significant effect was among feeding regimens when AL hens were less efficient than DR hens (.14 vs .33). Interactions for periods 2 and 3 involved genotypes and forms of feed (Table 3) and were due in period 2 to late-feathering hens being more efficient than early-feathering ones with CO but not with CF feed. The genotype by form of feed interaction in period 3 was due to superior efficiency on CF feed of early- than late-feathering hens, and no differences between genotypes for CO. Within genotypes, early-feathering hens were more efficient on CF than CO feed while there was no difference between form of feed for late-feathering ones. A genotype by feeding regimen interaction in period 4 was caused by differences between early- and late-feathering hens fed AL (.16 vs .08, respectively) while there were no differences among DR or RL hens regardless of genotype.

Main effects were significant for feed efficiency during periods 1, 5, 6, 7, 10, and 11. Feed efficiency was about a 50% lower for AL than RL and DR regimens throughout the experiment. Hens of different genotypes differed in feed efficiency during periods 5, 6, 7, 10, and 11 with early-feathering hens being more efficient than late-feathering ones. For period 8, 9, and 12, neither main effects nor interactions between them were significant for feed efficiency.

### *Residual Feed Consumption*

Coefficients of multiple determination ( $R^2$ ) obtained within each production period for equation 1 were low to moderate (Table 4). Partial

regression coefficients of metabolic body weight ( $W^\alpha$ ) were significant in 10 out of 12 production periods, the exception being periods 4 and 5, i.e., the periods that included the time when RL hens were released to *ad libitum* feeding. The partial regression coefficients of change in body weight ( $\Delta W$ ) were significant during period 1, 2, 4, 6, and 12. Egg production, corrected for defective eggs, had significant partial regression coefficients, which were concentrated during peak egg production periods 2 through 5 as well as for period 10.

While first order interactions for RFC were significant for all periods except 7, 11, and 12, second order interactions were not significant during any period. Variances for RFC were unequal among feeding regimens because of larger variance for AL. Regimens DR and RL were homogeneous. Thus RFC are presented separately for AL hens. RFC of AL hens was lower for early- than late-feathering genotypes in periods 1 (-179 vs 295 g), 3 (-137 vs 31 g), 4 (-307 vs 215 g), and 5 (-146 vs 92 g). There was no difference during period 2 (-6 vs 72 g) when hens of both genotypes were at peak egg production. The difference in RFC for period 1 was probably due to differences in onset of lay and in periods 3 through 5 to differences in intensity of lay and weight gain (Chapter II).

Among AL hens, differences in RFC between forms of feed were observed during periods 3 and 4. RFC was lower for CO than CF feed (-123 vs 28 g) during period 3. This pattern reversed in period 4 (154 vs -422 g). This reversal was due to much greater feed intake decline from period 3 to 4 in CF (3385 vs 2692 g) than in CO feed (3314 vs 3309 g).

RFC were higher for early- than late-feathering hens during periods 6, through 12 (Table 5). In regimen RL, feed intake differences between genotypes during period 5 resulted in differences for RFC with higher values for early- than late-feathering hens (218 vs 42 g). During periods 6, 7, 11, and 12 feeding regimen, form of feed as well as interactions involving them were not significant. For periods 8, 9, and 10 there were feeding regimen by form of feed interactions (Table 6), which were due to lower RFC for CF than CO hens under RL but not DR feeding. RFC was higher for RL than DR hens for CO but not CF feed.

#### *General Discussion*

The habituation of hens to restriction of feed intake did not inhibit the expression of hyperphagia when they were released to AL feeding. While egg production was similar for RL and DR hens (Chapter II) the extra body weight of RL hens resulted in a higher RFC, which was not reflected in corresponding changes in feed efficiency or egg production. This pattern highlights the importance of groups being of similar body weights at the onset of test periods for RFC. While results are consistent with those of Robbins *et al.* (1988) for feed efficiency, the release of restricted females to AL was of a much shorter duration than that of Robbins *et al.*. The poor performance of AL hens corroborates results reported by McDaniel *et al.* (1981) and Katanbaf *et al.* (1989a,b). The traits measured in this study were independent of those affected by mortality (Chapter II) and were effected by long term obesity (AL hens), but not short term weight gain (RL hens).

Limitations in performance were observed when late-feathering hens were fed a poorer quality of crumble (CF). Moreover, under AL reduced performance was more pronounced for late- than early-feathering hens. The lower egg production of the late- than early-feathering hens (Chapter II), despite their more severe feed restriction, was associated with lower feed efficiency. The association of K allele with *ev21* (Bacon *et al.*, 1988) was confirmed for these stocks (Chapter II) and suggests that the arrangement of the *ev21* late-feathering complex (Levin and Smith, 1990) has an impact on reproduction responses and feed utilization of broiler breeders.

Feeding inconsistent quality crumbles reduces intake with *ad libitum* feeding, which corroborated reports in growing chickens and turkeys (Steward and Upp, 1951; Jenson *et al.*, 1962; 1965; Choi *et al.*, 1986). The results suggest that poorer quality crumbles were more likely to be associated with lower RFC, but such that intake, by weight, was limiting and, thus, eventually associated with lower egg production (Chapter II). Interactions associated with genotypes and forms of feed for feed efficiency during early egg production suggest that both late-feathering and poorer quality crumble were first limiting factors in performance.

The range of  $R^2$  values obtained in this experiment was considerably lower than those obtained on individual White Leghorn hens (Luiting and Urff, 1991a). Partial regression coefficients reflected the lower demands made on resources devoted to egg production outside the periods of peak egg production. Changes in body weight had significant

coefficients when there were rapid weight gains such as those associated with onset of lay and release of restricted hens to AL feeding. Coefficients associated with metabolic body weight were most often significant, which parallels the importance of maintenance costs (Luiting, 1990). The lower RFC of late- than early-feathering hens was consistent for all periods after period 5 when their feed allocations was reduced. Thus, the allocation of feed above requirements to sustain egg production was evident. Lower variation in RFC associated with feed restriction supports those those by Luiting and Urff (1991a) when energy intake was low. This supports a hypothesis that reduced energy intake also reduced systematic variation in RFC, which was due to lower maintenance cost of the hen. It appears that although RFC in broiler breeder hens should not be a target for direct improvement in female parent lines, it may be a useful in devising suitable management guidelines for commercial stocks.

Differences in RFC due to major genes *C-* and *cc* (Mérat, 1968), *I-* and *ii* (Mérat and Bordas, 1972), *Na-* and *na<sup>+</sup>na<sup>+</sup>* (Mérat and Bordas, 1974), and *P-* and *pp* (Mérat and Bordas, 1979; Mérat *et al.* 1979) appears to have been due to their influence on heat conductance and maintenance costs. The influence of the *K* allele in its study was complex due to the association with low egg production and feed restriction for maintenance of target body weights. The broiler breeder background genome with its large growth and appetite potential, may have influenced resources associated with egg production, causing a diversion of "surplus" food

resources into body weight. Thus, lower RFC was observed for late- than early-feathering hens when the former were restricted more severely.

TABLE 1. Mean feed intake (g) per hen for each of 4 production periods<sup>1</sup> for hens fed *ad libitum* when the genotype<sup>2</sup> by form of feed<sup>3</sup> interaction was significant

Genotype	Production periods							
	1		2		3		6	
	CF	CO	CF	CO	CF	CO	CF	CO
Early	2700	ns 2900	3066	ns 3182	3436	* 3133	3237	* 3720
	*	*	ns	*	ns	*	*	*
Late	3490	* 3194	3099	* 2667	3289	* 3481	2485	* 3363

<sup>1</sup>Production periods were 28 days in duration, period 1 began when pullets were 22 weeks of age.

<sup>2</sup>Genotypes; early-feathering (K<sup>+</sup>/-) and late-feathering (K/-).

<sup>3</sup>Form of feed; CF = 14-day cycles each of, crumble:fines mix (60:40) followed by crumble only and CO = crumble only.

\* Adjacent means are significantly different ( $P \leq .05$ ).

ns Adjacent means are not significantly different ( $P > .05$ ).



TABLE 2. Mean feed intake (g) per hen for production periods<sup>1</sup> 4 and 5 when the genotype<sup>2</sup> by feeding regimen<sup>3</sup> interaction was significant.

Genotype	Production periods					
	4			5		
	AL	RL	DR	AL	RL	DR
Early	2803 c	3677 a	3360 b	3142 c	3612 a	3360 b
	*	*	ns	*	*	ns
Late	3425 b	3558 a	3360 c	3351 b	3420 a	3220 c

<sup>1</sup>Production periods were 28 days in duration, period 1 began when pullets were 22 weeks of age.

<sup>2</sup>Genotypes; early-feathering (K<sup>+</sup>/-) and late-feathering (K/-).

<sup>3</sup>Feeding regimens; AL, *ad libitum* access to feed, RL, restricted as DR except for a 6-day release to AL during end of period 4 and early period 5, DR, restricted to commercial broiler breeder guidelines.

\* Adjacent means are significantly different (P ≤ .05).

a,b,c, means with different superscripts in a row are significantly different (P ≤ .05).

TABLE 3. Mean feed efficiency<sup>1</sup> for production periods<sup>2</sup> 2 and 3 when the genotype<sup>3</sup> by form of feed<sup>4</sup> interaction was significant

Genotype	Production periods					
	2			3		
	CF	CO		CF	CO	
Early	.24	*	.21	.27	*	.24
	ns		*	*		ns
Late	.22	*	.25	.24	ns	.24

<sup>1</sup>Feed efficiency of egg mass production, total egg mass:feed Intake.

<sup>2</sup>Production periods were 28 days in duration, period 1 began when pullets were 22 weeks of age.

<sup>3</sup>Genotypes; early-feathering (*k*<sup>+</sup>/-) and late-feathering (*K*/-).

<sup>4</sup>Form of feed; CF = 14-day cycles each of, crumble:fines mix (60:40) followed by crumble only and CO = crumble only.

\* Adjacent means are significantly different ( $P \leq .05$ ).

ns Adjacent means are not significantly different ( $P > .05$ ).

TABLE 4. Coefficient of multiple determination ( $R^2$ ) and the partial regression coefficients of the feed intake prediction equation

Parameters	Production period											
	1	2	3	4	5	6	7	8	9	10	11	12
$R^2$	.07	.33	.08	.26	.09	.05	.03	.13	.13	.20	.29	.12
$W^\alpha$	1.18*	-1.49*	1.52*	.16	.15	.85*	1.44*	2.17*	2.03*	2.18*	2.64*	.96*
$\Delta W$	.17*	.40*	.10	.42*	-.04	.14*	-.04	.06	.17	.21	.01	.31*
Ec	-.06	.19*	.15*	.24*	.16*	.06	.05	-.07	.02	.18*	-.07	-.03

<sup>1</sup>Production periods were 28 days in duration, period 1 began when pullets were 22 weeks of age.

<sup>2</sup>Parameters;  $R^2$  = coefficient of multiple determination.

$W^\alpha$  = partial regression coefficient of metabolic body weight,  $\alpha = .75$ .

$\Delta W$  = partial regression coefficient of change in body weight.

Ec = partial regression coefficient of egg mass production, corrected for defective egg production.

\* Partial regression coefficient was significantly different from zero.

TABLE 5. Mean residual feed consumption<sup>1</sup> (g) of broiler breeder hens for production periods<sup>2</sup> 6 to 12 by genotype<sup>3</sup> for DR and RL feeding regimens

Genotype	Production periods						
	6	7	8	9	10	11	12
Early	130	95	81	80	76	26	34
	*	*	*	*	*	*	*
Late	-182	-194	-223	-191	-148	-78	-51

<sup>1</sup>Residual feed consumption was the difference between predicted and observed feed intake.

<sup>2</sup>Production periods were 28 days in duration, period 1 began when pullets were 22 weeks of age.

<sup>3</sup>Genotypes; early-feathering ( $k^+/-$ ) and late-feathering ( $K/-$ ).

\* Adjacent means in a column are significantly different ( $P \leq .05$ ).

TABLE 6. Mean residual feed consumption<sup>1</sup> (g) in production periods<sup>2</sup> 8 to 10 of broiler breeder hens with a feeding regimen<sup>3</sup> by form of feed<sup>4</sup> interaction

Form of feed	Production periods						
	8		9		10		
	RL	DR	RL	DR	RL	DR	
CF	-144	ns	-110	ns	-10	ns	-65
	*	ns	*	ns	*	ns	ns
CO	93	*	83	*	90	*	-95

<sup>1</sup>Residual feed consumption was the difference between predicted and observed feed intake.

<sup>2</sup>Production periods were 28 days in duration, period 1 began when pullets were 22 weeks of age.

<sup>3</sup>Feeding regimens, DR hens were on a commercial broiler breeder restriction program, RL hens had been restricted as DR except for 6 day release to AL feed at the end of period 4.

<sup>4</sup>Form of feed; CF = 14-day cycles each of, crumble:fines mix (60:40) followed by crumble only and CO = crumble only.

\* Adjacent means are significantly different ( $P \leq .05$ ).

ns Adjacent means are not significantly different ( $P > .05$ ).

## CHAPTER V

CORRELATED RESPONSES IN LINES OF CHICKENS DIVERGENTLY SELECTED FOR  
56-DAY BODY WEIGHT: GROWTH, FEED INTAKE, AND FEED UTILIZATION

## SUMMARY

Correlated responses for growth, feed intake, and feed utilization were measured in female chickens from lines after 32 generations of divergent selection for 56-day body weight, and in sublines where selection had been relaxed for 5 generations. The diet used in this experiment was that under which selection had been practiced (20 % crude protein and 2685 Kcal of ME/Kg). Feed intake between each selected line and its relaxed subline was equalized by paired-feeding. *Ad libitum* fed controls were also maintained. At 21 days of age, differences between the selected lines for *ad libitum* fed chickens were 404 % for body weight, 279 % for feed intake, and 138 % for feed conversion ratio (FCR). Corresponding percentages for the high and low relaxed lines under *ad libitum* feeding were 267, 223, and 121. When pair-fed, growth of the high-weight selected line was unimpeded and FCR improved. When low-weight relaxed chicks were pair-fed, FCR also improved, but growth was reduced and appetite development inhibited. Hyperphagic behavior was observed in the high-weight selected line. In the low-weight selected line the chicks exhibited hypophagia. Although residual feed consumption was unchanged by selection for 56-day body weight, variation was present for this trait with lower values under restricted than *ad libitum* feeding.

## INTRODUCTION

Studies with mice (e.g., Fowler, 1962; Sutherland *et al.*, 1970; Jara-Almonte and White, 1973; Eisen, 1977; Roberts, 1981), chickens (Lepore, 1965; Siegel and Wisman, 1966; Proudman *et al.*, 1970), and quail (Marks, 1980; 1991) have demonstrated positive correlated responses in feed efficiency when selecting for body weight at a fixed age, or for weight gain under *ad libitum* feeding. Realized genetic correlations between growth rate and feed conversion ratio (the inverse of feed efficiency) of  $-.51$  and  $-.50$  were reported for chickens by Wilson (1969) and by Pym and Nicholls (1979), respectively.

There is a large correlated increase in feed consumption with selection for increased body weight or decreased age at market weight in meat-type chickens (McCarthy and Siegel, 1983). Mice (McCarthy, 1982) and chickens (Siegel and Dunnington, 1987) from lines selected for rapid growth under *ad libitum* feeding show consistent increases in food consumption. Changes in feed utilization in such growth-selected lines have been attributed mainly to availability of more energy for growth over maintenance requirements (Hayes and McCarthy, 1976; Yüksel, 1979). Studies with force-fed chickens during the growing period (Nir *et al.*, 1978) showed that meat-type stocks selected for age to market body weight eat to near gut capacity. This ability, to maximize consumption of food relative to body weight, results in relatively lower maintenance costs.

Experiments in which selection was for growth under restricted feeding regimens in mice (Falconer and Latyszewski, 1952; Falconer, 1960;



McPhee and Trappett, 1987) were designed to evaluate growth responses when differences in food intake were minimal. This approach attempted to exploit genetic variation associated with efficiency and not that associated with feed intake, as occurred when selection was under *ad libitum* feeding (Roberts, 1979; 1981). An impediment to this approach was the presence of genotype by environment interactions that occurred when lines selected on one regimen were placed on other feeding regimens (Hetzl and Nicholas, 1986; McCarthy and Roberts, 1989).

Evaluation of feed utilization, measured as a correlated response to selection for growth rate or body weight, is confounded by correlated changes in feed intake (Pym, 1982). Siegel and Wisman (1966) circumvented this confounding by pair-feeding chicks from a line selected for high body weight (HS) to the feed intake level of the low body weight (LS) selected line. They found an advantage in feed efficiency for HS chicks relative to LS chicks when pair-fed, while no advantage was observed under *ad libitum* feeding, demonstrating that the superior feed efficiency was masked by intake. A number of generations later, comparisons with these lines demonstrated an advantage in feed efficiency in line HS relative to line LS under *ad libitum* feeding (Owens *et al.*, 1971). This chapter is a report on growth, feed conversion ratio (FCR), feed intake, and residual feed consumption in these weight-selected lines and in their sublines where selection had been relaxed. Pair-feeding was used to equalize feed intake and *ad libitum* fed chicks were also maintained. Companion chapters include data on organ growth and cellular content (Chapter VI) and digestive enzyme activity (Chapter VII).

## MATERIALS AND METHODS

### *Stocks and Husbandry*

Chicks used in this experiment were from 4 lines. Lines HS and LS have been divergently selected for high or low 56-day body weight respectively (Siegel, 1978; Dunnington and Siegel, 1985). These lines had been studied in generations 5, by Siegel and Wisman (1966) and 11 and 12 by Owens *et al.* (1971). Chicks for the present experiment were from the 32nd generation of selection (S<sub>32</sub>). The relaxed lines, HR and LR were sublines from lines HS and LS, respectively, where selection was relaxed in generation 28.

Chicks from age-contemporary parents were hatched on the same day, vent-sexed, wingbanded, vaccinated for Marek's Disease, and females placed in individual cages with continuous light. The diet under which selection was practiced was fed in a mash form and formulated to contain 20% crude protein and 2685 Kcal of ME/Kg of feed. Hot-air brooding maintained environmental temperatures of 35, 33, and 29°C, from 0 to 7, 8 to 14, and 15 to 21 days posthatch, respectively. Temperature fluctuation in the room was  $\pm 1^\circ$  C.

The design of the experiment consisted of two groupings; one with HS and HR chicks fed *ad libitum* (A) or pair-fed (P), and the other with LS and LR chicks fed A or P. In the first grouping, 20 HS chicks were individually assigned to 20 HR chicks which were their pair-feeding

partners (HS-P vs HR-A). An additional 15 HS chicks were fed *ad libitum* (HS-A). In the second grouping 30 LR chicks (LR-P) were assigned to 30 LS chicks which were their pair-feeding partners (LS-A). An additional 30 LR chicks (LR-A) were fed *ad libitum*. Greater numbers of low-weight chicks than high-weight chicks were placed because the former were prone to starve-out. Relaxation of selection for body weight did not alter weights of chicks at hatch; however, appetites of the relaxed lines were intermediate to those of the selected lines. Assignment of chicks to groupings was at random within the high-weight and within the low-weight lines. Feed allocation to each pair-fed individual was the intake for the previous day of its pair-fed partner.

#### *Traits Measured*

Body weight and feed intake were measured daily for each chick. Feed utilization (feed intake/body weight) was calculated as feed conversion ratio (FCR). Residual feed consumption was estimated for two growth periods, hatch to Day 8 and Day 9 to 21. This delineation was based on *a priori* information for organ and total body weight relationships of these lines (Katanbaf *et al.*, 1988a,b). A modified Byerly's feed intake prediction model (Byerly, 1941; Byerly *et al.*, 1980) was utilized with information on growth and metabolic body weight included in the equation [1]. Residual feed consumption was calculated as the difference between predicted and observed feed intake, equation [2].

$$Y_T = b_0 + b_1 W^\alpha + b_2 \Delta W \quad [1]$$

$$Y_R = Y_O - Y_T \quad [2]$$

In [1] predicted food intake was estimated by a multiple regression model including growth and metabolic body weight where  $Y_T$  was the predicted feed intake,  $b_0$  was the intercept,  $W^\alpha$  was the average body weight corrected by  $x$  to give an estimate of metabolic body weight ( $\alpha = .75$ ),  $\Delta W$  was the change in body weight, and  $b_1$  and  $b_2$  were the partial regression coefficients of metabolic body weight and growth respectively. In [2],  $Y_0$  was the observed feed intake and  $Y_R$  was the residual feed component.

### *Statistical Analyses*

Several analysis were used. One involved a paired t-test between the pair-fed individuals within a pairing (HS-P vs HR-A, and LS-A vs LR-P). A second paired t-test was utilized between pair-fed and *ad libitum* fed chicks within a line (HS-P vs HS-A, and LR-P vs LR-A). In addition, analysis of variance were performed comparing the 6 line-feeding regimens in a completely randomized design. Where appropriate, significant differences between line-feeding regimens were compared by Tukey's studentized paired comparisons (SAS, 1982). The statistical model for an analysis of variance was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where  $i = 1, 2 \dots 6$  line-feeding regimens and  $j = 1, 2 \dots n$  individuals. Prior to analyses, body weights and feed intake data were transformed to common logarithms. Residual feed data were transformed to a modified square root transformation (Bartlett, 1936) where  $X' = \sqrt{X + 100}$  (Zar,

1984). Untransformed means are presented in text, table, and figure. Mortality data were expressed as percentages and actual numbers subjected to a Chi-square goodness of fit test ( $v = 3$ ,  $\alpha = .05$ ). Unless otherwise stated statistical significance was taken at  $P \leq .05$ . Since data were subjected to more than one statistical test, each test was based on a .05 alpha rejection region. Reuse of data increased probability of an occurrence of a Type 1 error to greater than the alpha level.

## RESULTS

### *Mortality*

More LS-A chicks died (13 %) than among the low-weight relaxed groups (LR-A and LR-P) where mortality was 3 %. Deaths of LS-A and LR-P chicks occurred on Days 7 and 8. The feed consumption of the LS-A chicks which died was below 6g at Day 7. Feed consumption to Day 7 for LS-A survivors was 37g with a range of 10 to 80g, such that a bimodal distribution was observed for feed intake to Day 7 between survivors and nonsurvivors. No patterns were observed for mortality among high-weight groups, where % mortality was 5, 0 and 5 for HS-A, HS-P, and HR-A, respectively.

### *Body weight*

Regardless of feeding regimen, chicks from the high-weight lines were heavier than those from the low-weight ones at all ages. Comparisons between the selected lines fed *ad libitum* showed that while at hatch high

line chicks were 157 % heavier than those from the low lines, the difference increased to 404 % by Day 21.

To 9 days of age, body weights were similar for HS-A, HS-P, and HR-A chicks, thereafter HS-A and HS-P were heavier than HR-A chicks (Figure 1). For the low-weight populations, body weights of the line-feeding regimens were similar to Day 3, after which LR-A chicks were heavier than LS-A ones. The LR-P chicks were heavier than LS-A chicks from Day 15 onwards.

#### *Feed intake*

By Day 5 posthatch, consumption of feed by HS-A chicks was 122 % that of LS-A chicks with these differences becoming greater by Days 10 and 21, becoming 186 % and 279 %, respectively. Feed consumption in the high-weight lines increased rapidly with age. By Day 12 consumption by HS-A chicks was greater than by either HS-P or HR-A chicks, which did not differ. A consistent, but not significant, pattern of slightly greater feed consumption for HR-A than HS-P chicks was due to the one day delay of the pair-feeding protocol. Feed intake among low-weight chicks was similar through Day 9. Thereafter LR-A chicks consumed more feed than LR-P ones. Less feed was consumed by LS-A than LR-A chicks from Day 11 onward, and from Day 14 onward LS-A consumed more feed than LR-P chicks. While LR-P chicks were given access to the same quantities of feed as consumed by LS-A chicks, to which they were pair-fed, the LR-P chicks did not consume their full daily feed allocations. The result was that differences accumulated and this pattern persisted for the duration of the experiment.

### *Feed utilization*

Until Day 6, the FCR was similar for the high-weight groups. On this day and thereafter, FCR was lower for HS-P than HS-A or HR-A chicks, which did not differ. In the low-weight groups by Day 5 the FCR for LS-A chicks was greater than for LR-P or LR-A chicks, which by Day 6 also differed (LR-A > LR-P) in FCR.

Table 1 summarizes FCR from hatching to Days 10 and 21 for the line-feeding regimens. Although the difference of .47 in FCR between the selected lines (LS-A vs HS-A) fed *ad libitum* present at Day 10 increased to .62 by Day 21, on a relative basis the difference between them was essentially constant (i.e. ~ 38 %). Restriction of feed intake of LR-P chicks to that of LS-A improved their FCR to that of HS-P chicks to Day 10, with both groups having FCR ratios superior to those of HS-A and HPA chicks. By Day 21, however, the FCR of LR-P chicks was similar to that of the HS-A and HR-A groups, which were inferior to that of HS-P chicks.

### *Residual feed consumption*

During the early growth period (from Day 0 to 8), residual feed consumption was similar for HS-A and HR-A chicks both of which had greater values than HS-P chicks (12.4 and 4.0 vs -5.9 g, respectively). During the second growth period from Day 9 to 21, values for HS-A and HS-P chicks were similar (-9.8 and -5.9 g,) but smaller than for HR-A chicks (16.6 g). In the low-weight groups, LR-P had the smallest residual feed

consumption during both growth periods (-13.5 and -11.6 g). During early growth LR-A chicks had lower residual feed consumption than LS-A chicks (2.0 vs. 6.1 g). There were no differences between HS-A and LS-A chicks for residual feed components for either growth period. Restriction of feed to chicks from HS and LR lines reduced residual feed components.

During the early period posthatch, metabolic body weight and line-feeding regimen, but not growth accounted for significant variation in residual feed consumption ( $R^2 = .72$ ). During the second growth period, however, metabolic body weight, growth and line-feeding regimens were all significant sources of variation in residual feed consumption ( $R^2 = .98$ ).

## DISCUSSION

Selection for 56-day body weight has resulted in a 404 % difference in 21-day body weight, 279 % difference in feed consumption and 138 % difference in FCR between selected lines fed *ad libitum*. No differences in residual feed consumption were observed between the selected lines. Body weight differences are consistent with earlier reports for these lines at selection age and as adults (Dunnington and Siegel, 1985), and with reports of concomitant changes in growth curves of these lines (Zelenka *et al.*, 1986b; Anthony *et al.*, 1991a). Hyperphagic behavior of the high-weight lines (Barbato *et al.*, 1983) was evident immediately posthatch as was hypophagia among low-weight lines. Relaxation of selection resulted in values for these traits which were less divergent than those for the selected lines. Differences on Day 21 between the



relaxed lines were 267, 223 and 121 % for body weight, feed intake, and FCR, respectively.

Consistent with reported high correlations between body weight and feed consumption (McCarthy and Siegel, 1983) was the large increase in feed consumption associated with the large increase in body weight to Day 21. The smaller changes in FCR to this age are consistent with lower reported correlations between body weight or weight gain and feed efficiency in chickens (Pym, 1982), Japanese quail (Marks, 1991) and mice (McCarthy and Roberts, 1989). Thus, selection for 56-day body weight exploited additive genetic variation associated with feed intake more than that associated with feed utilization. These correlated responses to selection have, in terms of fitness, been contrary to natural selection for an intermediate weight, causing a slowing (high-weights) or limiting (low-weights) of selection progress in these lines of chickens (Siegel and Dunnington, 1985; Dunnington *et al.*, 1986; Dunnington, 1990) and are similar to those reported in mice (McCarthy, 1982). The development of anorexia during the first week posthatch by some LS chicks in this experiment was consistent with the extreme degree of anorexia associated with adult females from this line (Zelenka *et al.*, 1988) and the absence of this trait in the relaxed low-weight line (Dunnington, 1990).

The response to feed restriction of the high-weight lines was consistent with that observed in commercial meat-type chickens (Siegel and Dunnington, 1987; Boa-Amponsen *et al.*, 1991a) where a mild feed restriction resulted in a minimal impediment to growth, and improved feed utilization considerably. Genotype by environment interactions have been

demonstrated in mice, when weight selected lines were placed on different nutritional environments (Falconer and Latyszewski, 1952; Hetzel and Nicholas, 1986; McCarthy and Roberts, 1989) and in Japanese quail (Marks, 1991). The response of the low-weight relaxed line to feed restriction observed in this experiment is unique because feed consumption, when exposed to restriction, was less than allocated. This may indicate a role for early life experience in appetite development of this line.

As divergent selection for 56-day body weight was not associated with changes in residual feed consumption, no phenotypic correlation between body weight and residual feed consumption appears to have been expressed. Variation in this trait was evident, however, as feed restriction resulted in lower residual feed consumption which was associated with superior FCR. Perhaps residual feed consumption, forms a source of variation for feed efficiency which is not correlated with body weight. Due to the nature of the iterative equation used to predict residual feed consumption, which includes expressions to account for differences in metabolic body weight and growth, it seems logical that a correlation between body weight and residual feed consumption would be minimal. In laying hens, Luiting (1990) concluded that variation associated with residual feed consumption was largely due to differences in maintenance requirements between strains and among individuals within a strain. In growing chicks, little evidence was found to support a change in maintenance in fast and slow growing stocks (Ross *et al.*, 1954), in lines divergently selected for oxygen consumption (Steward *et al.*, 1980) or between the divergently selected lines used in this experiment

(Owens *et al.*, 1971). Thus, Luiting's thesis may be consistent for both growing chickens and for laying hens.

TABLE 1 Mean feed conversion ratios to Days 10 and 21 by line-feeding regimen<sup>1</sup>

Line-feeding regimen	Day	
	0 to 10	0 to 21
HS-A	1.21 b	1.65 c
HR-A	1.12 b	1.69 c
HS-P	1.00 c	1.51 d
LS-A	1.68 a	2.27 a
LR-A	1.63 a	2.04 b
LR-P	.94 c	1.68 c

<sup>1</sup>Regimens: HS-A = high selected line fed *ad libitum*, HR-A = high relaxed line fed *ad libitum*, HS-P = high selected line pair-fed to feed intake of HR-A, LS-A = low selected line fed *ad libitum*, LR-P = low relaxed line pair-fed to feed intake of LS-A, LR-A = low relaxed line fed *ad libitum*.

abcdMeans in a column with different superscripts are significantly different (P ≤ .05).

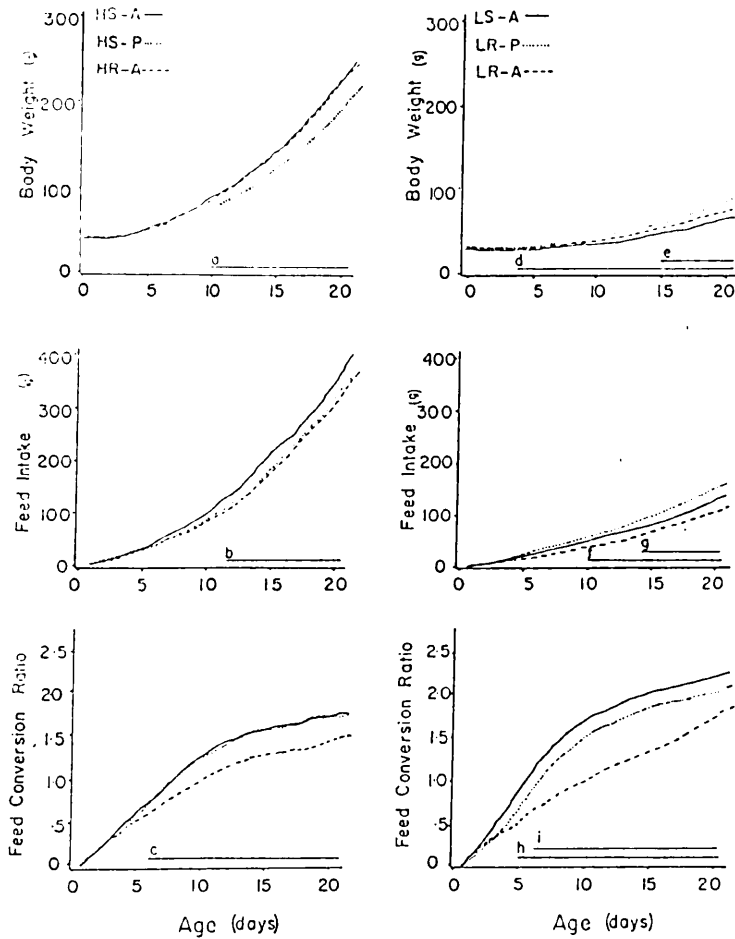


FIGURE 1. Body weight, feed intake, and feed conversion ratio in high-weight lines, left side of page, and low-weight lines, right side of page. HS-A, high select line fed *ad libitum*; HS-P, high select line pair-fed to HR-A chicks feed intake; HR-A, high relaxed line fed *ad libitum*; LS-A, low select line fed *ad libitum*; LR-P, low relaxed line pair-fed to LS-A chicks feed intake; LR-A, low relaxed line fed *ad libitum*. In the high-weight lines differences between means were indicated as a = (HS-A = HS-P)  $\neq$  HR-A, b = HS-A  $\neq$  (HS-P = HR-A), c = (HS-A = HR-A)  $\neq$  HS-P. In the low-weight lines differences between means were indicated d = LS-A  $\neq$  (LR-A = LR-P), e = LR-A > LR-P > LS-A, f = LR-A  $\neq$  (LR-P = LS-A), g = LR-A > LS-A > LR-P, h = LS-A  $\neq$  (LR-A = LR-P), i = LS-A > LR-A > LR-P. Significance was taken at  $P \leq .05$ .

CHAPTER VI

CORRELATED RESPONSES IN LINES OF CHICKENS DIVERGENTLY SELECTED  
FOR 56-DAY BODY WEIGHT: ORGAN GROWTH, DNA, RNA,  
AND PROTEIN CONTENT

## SUMMARY

Growth of organs relative to body weight, and cellular protein, RNA, DNA, and cell unit size of breast muscle, liver, and small intestinal tissue, were measured in females from 4 lines of chickens. Two lines had undergone 32 generations of divergent selection for 56-day body weight, and the other 2 lines were derived by sampling the first 2 lines at generation 28 and relaxing selection for the next 5 generation. The diet used in this experiment was the same diet under which selection was practiced (20% crude protein and 2685 Kcal of ME/Kg). Comparisons at common chronological ages and a common body weight revealed that supply organ weights, especially small intestine, were associated with subsequent growth of demand organs. While the upper gastrointestinal tract was also important in this respect, it was more susceptible to influences such as feed intake. Selection for juvenile body weight resulted in correlated changes in cell size of breast muscle, but not liver and small intestine. Muscle increased posthatch as cells underwent hypertrophy while liver and small intestine grew chiefly by hyperplasia.

## INTRODUCTION

Selection for high (HS) and low (LS) 56-day body weight (Siegel, 1978; Dunnington and Siegel, 1985) resulted in correlated responses in synchrony of growth (Zelenka *et al.*, 1986b; Katanbaf *et al.*, 1988a). Differences between altricial and precocial birds in their early life

history (Ricklefs, 1968; 1973; 1979; Ricklefs and Marks, 1985) and among diverse chicken breeds (Knížetová *et al.*, 1985), suggest similar underlying biological phenomena. Anthony *et al.* (1991a) reported divergent selection for body weight in various gallinaceous species had changed the length of autoaccelerated growth phase by changes in the points of inflection and the asymptotes.

Postnatal growth of birds was determined by distribution of growth among organs (Lilja, 1981; Prescott *et al.*, 1985). Characterizations of the chicken lines HS and LS and F<sub>1</sub> crosses between them (Katanbaf *et al.*, 1988b) showed that growth of organs was not proportional to body weight, varied with age, and was amenable to alteration by selection. Differential posthatch growth among organs of supply (e.g., gastrointestinal tract, liver, pancreas) and demand (e.g., muscles, feathers, adipose tissue) was indicative of a synchronization of growth (Knutsson *et al.*, 1980; Lilja, 1983; Katanbaf *et al.*, 1988a). Similarly, relationships among cell size, number and DNA, RNA content were not independent of genetic background, age, or organ (Smith, 1963; Lepore *et al.*, 1965; Moyer *et al.*, 1967; Anthony *et al.*, 1989b; 1991b). This chapter contains a reports of relationships among organ weights, DNA, RNA, protein content and cell unit size in breast, liver, and small intestinal tissues studied in lines of chickens that have undergone different modes of selection for juvenile body weight. Companion chapters included data on growth, feed intake, and feed utilization (Chapter V) and digestive enzyme activity (Chapter VII).



## MATERIALS AND METHODS

### *Stocks and Husbandry*

Chicks from lines HS and LS, which have been divergently selected for high or low 56-day body weight (Siegel, 1978; Dunnington and Siegel, 1985) for 32 generations, and their respective sublines, HR and LR, where selection has been relaxed for 5 generations were utilized in this experiment. Eggs from age-contemporary parents were incubated in the same machine. On the 22nd day of incubation chicks were removed from the hatcher, vent-sexed, wingbanded, vaccinated for Marek's Disease, and females were placed in individual cages with continuous light. The diet under which selection had been practiced was fed in a mash form and formulated to contain 20% crude protein and 2685 Kcal of ME/Kg of feed. Hot air brooding maintained environmental temperatures of 35°C during the first week, 33°C the second week, and 29°C the third week after hatch. Temperature fluctuation in the room was  $\pm 1^\circ\text{C}$ .

Pairings were made between a selected line and its respective relaxed line in both high- and low-weight lines. The first pairing was of 20 HR chicks fed *ad libitum* (HR-A) assigned to 20 HS chicks as pair-fed partners (HS-P). An additional 15 HS chicks (HS-A) were fed *ad libitum*. The second set consisted of 30 LS chicks fed *ad libitum* to which were assigned 30 LR chicks as pair-fed partners (LR-P). An additional 30 LR chicks were fed *ad libitum* (LR-A). Chick assignments were made at random within lines. The rationale for this design was presented in a companion paper (Chapter V).

### *Organ Growth*

Organ data were obtained at hatch and on Days 8 and 21, from random samples of 4 chicks from each line-feeding regimen (n = 64). These ages were chosen from *a priori* information for these lines (Katanbaf *et al.*, 1988b). In the case of pairs, the sample included both members of the pair. Chicks were killed by cervical dislocation just prior to the time daily feed allocations were normally provided. Hot deboning was used to remove the 4 breast muscles (right and left pectoralis major and minor) and the leg muscles (gastrocnemius) without skin. Heart, lungs, liver, kidneys, crop and esophagus, proventriculus, small intestine (duodenum, ileum and jejunum), and large intestine (ceaca included) were removed and weighed (.01 g). Pancreas, yolk sac, crop contents and small intestine chyme were obtained on Days 8 and 21 only. Second weight of each gastrointestinal segment was obtained after contents were removed.

### *Organ DNA, RNA, and Protein*

Tissue samples for analysis of DNA, RNA, and protein content of breast muscle, liver, and small intestine were frozen immediately in liquid nitrogen and stored at -70°C. Each sample of 500 mg of tissue was homogenized with a polytron homogenizer (Brinkmann Polytron, Kinematica GmBh, Luzern, Switzerland) in 4 ml of sucrose buffer. DNA, RNA, and protein were liberated using the sequential procedure of Shibko *et al.* (1967). DNA concentration was assayed with diphenylamine (Burton, 1968)

with calf thymus DNA (Sigma Chemical Company, St Louis, MO.) as a working standard. The RNA content was analyzed by the orcinol procedure (Schneider, 1957), using baker's yeast RNA (Sigma Chemical Company, St Louis, MO.) as a working standard. All absorbances were measured with a spectrophotometer (Spectronic 1001, Bausch and Lomb, Rochester, New York). Total protein was determined through gravimetric methodology after RNA, DNA and lipid extractions were complete (Shibko *et al.*, 1967) and each pellet had been dried to a constant weight (A.O.A.C., 1975). Duplicate samples were measured and their means used for subsequent analysis, except for breast tissue of low-weight line chicks at hatch because the volume of tissue was sufficient for only one extraction.

### *Statistical Analyses*

Analysis of variance was performed for all traits with age and line-feeding regimens as main effects and their interaction using the following model:

$$Y_{ijk} = \mu + G_i + A_j + (GA)_{ij} + e_{ijk}$$

where  $i = 1, 2 \dots 6$  line-feeding regimen,  $j = 1, 8, 21$  days and  $k = 1, 2 \dots n$  individuals. Prior to analyses, weights were transformed to common logarithms, organ weights were expressed relative to body weight and transformed to arc sine square root, and cell size to square roots. Where appropriate, significant differences between line-feeding regimen were compared by Tukey's studentized paired comparisons (SAS, 1982). When

line-feeding regimen by age interactions were significant, line-feeding regimens were analyzed within ages and ages were analyzed within line-feeding regimens. Comparisons were also made when chicks had similar body weight but different ages with appropriate contrasts to compare means. Unless otherwise stated statistical significance was taken at  $P \leq .05$ .

## RESULTS

### *Weight Comparisons at the Same Chronological Age*

There was a significant interaction of line-feeding regimen by age for body weight. This interaction was expected from the reported (Anthony *et al.*, 1991a) differences in growth of these lines where divergence increased with age (Table 1). Because of this pattern for total body weight, organ weights were analyzed relative to body weight. Line-feeding regimen by age interactions were significant for weights of the breast and leg muscles, heart, pancreas, and all segments of the gastrointestinal (GIT) tract. The interaction was not significant for lung, liver, kidney, yolk sac, and contents of the crop and small intestine.

Line-feeding regimen by age interactions present. At hatch breast weights were similar for all lines except HR-A chicks which were heavier (Table 1). During the next 21 days, breast weights increased much faster in the high- than low-weight lines regardless of feeding regimen. Weights of leg muscles were similar across lines at hatch and line-feeding

regimens on Day 8. By Day 21, however, weights increased in the high- but not in the low-weight lines.

At hatch, heart weights for LR-A chicks were greater than those of LS-A chicks with HR-A and HS-A intermediate (Table 2). By Day 8, however, differential growth was occurring according to feeding regimen and genetic background. Then, by Day 21 responses among line-feeding regimens had equalized. Although pancreas weights declined between Days 8 and 21 in the high-weight lines, weights in the low-weight lines were similar at both ages.

Genetic background had no effect on weights of any segment of the GIT at hatch (Table 3). Although weights of crop and esophagus were similar for all of line-feeding regimens at hatch and on Day 8, by Day 21 weights were lower for the high- than low-weight lines. Across ages, the low-weight lines had similar crop and esophagus weights, while for the high-weight lines there was a decline between Days 8 and 21.

Genetic background had no effect on proventriculus weight at hatch. Differences among line-feeding regimens developed with age and by Day 21 the proventriculus was smaller for HR-A and HS-A than LS-A chicks with LR-A, LR-P, and HS-P chicks intermediate and not different from the other line-feeding regimens. Proventriculus weights for the high-weight lines were greater on Day 8 than at hatch and Day 21. For the low-weight lines proventriculus weights were similar at all ages. Gizzard weights were similar among line-feeding regimens at hatch and Day 8. By Day 21, however, gizzards were heavier for LS-A and LR-P than HS-A and HS-P chicks with LR-A and HR-A chicks intermediate. Among ages, various patterns of

gizzard growth were evident. Weights increased from hatch to Day 8 for HS-A and LS-A chicks, but not for HR-A and LR-A chicks. Between Day 8 and 21 gizzard weights declined in all line-feeding regimens except LS-A, with HS-A chicks having the largest decrease.

Weights of the small intestine were similar among line-feeding regimens at hatch and Day 8. On Day 21, however, they were greater for LS-A and LR-P than for HS-A, HS-P, and HR-A chicks with LR-A chicks intermediate. Generally weights of the small intestine increased from hatch to Day 8 in the low-weight lines with little change from Day 8 to Day 21. While the age pattern in the high-weight lines was similar to that in the low-weight lines to Day 8, by Day 21 a decline had occurred. Weights of large intestine were similar for all lines at hatch. On Day 8, these weights were greater for HS-A than LS-A chicks with weights for the other line-feeding regimens being intermediate. By Day 21, means for high-weight lines were lower than for LS-A with LR-A and LR-P chicks being intermediate. Within the high-weight lines large intestine weights were similar across ages. In the low-weight lines weights of large intestine for LS-A and LR-P chicks increased between Days 8 and 21 while there were no changes across the ages for LR-A chicks.

Line-feeding regimen by age interactions absent. Weights of lungs relative to body weight were lower for HS-P, HR-A, and HS-A chicks than for LR-A ones, with LS-A and LR-P chicks being intermediate (Table 4). Across ages weights of lungs were lower on Day 21, than earlier ages. Liver weight for LS-A and LR-A chicks were heavier than for HS-A and HR-A chicks. Means for LR-P and HS-P chicks were intermediate and not different

from the other line-feeding regimens. Age effects for liver weight were due to an increase from hatch to Day 8 and then a marked decline, relative to body weight, so that by Day 21 they were smaller than at hatch. Kidney size was similar among line-feeding regimens and at the three ages measured.

No differences were found between Days 8 and 21 or among line-feeding regimens for crop content (.05g/100g BW), or for small intestine chyme (.68g/100g BW). The yolk sac regressed rapidly in all line-feeding regimens and weighed less than .2 g by Day 8.

#### *Cellular Content at the Same Chronological Age*

Protein Content. There was no line-feeding regimen by age interaction for protein content of breast tissue, nor were there differences among line-feeding regimens (Figure 1). Protein content of the breast muscles increased between Days 8 and 21, having been similar at hatch and Day 8.

The significant line-feeding regimen by age interaction for protein content of liver tissue was due mainly to the LS-A chicks. At hatch, liver protein content of the LS-A chicks was almost two-fold that of the other lines. By Day 8, however, the mean for LS-A chicks was similar to that of the other low-weight lines, but greater than that for any of the high-weight lines. Among line-feeding regimens, liver protein was similar on Day 21. Across ages only LS-A chicks decreased in protein levels between hatch and Day 8, while none of the other groups changed with age.

There was no line-feeding regimen by age interaction for protein content of small intestine. At hatch, HS-A chicks had higher protein levels than HR-A chicks. Although HR-A levels were lower than for HS-A and HS-P chicks on Day 8, these differences were absent by Day 21. LR-P chicks had higher levels of protein only on Day 8 than LS-A and LR-A chicks. An age effect was observed with small intestine protein being higher at hatch than on Days 8 or 21 for LS-A and LR-A chicks.

RNA Content. Line-feeding regimen by age interactions were significant for RNA levels of breast muscle, liver, and small intestine tissue (Figure 2). The interaction for breast muscle resulted from decreases in RNA in all line-feeding regimens between hatch and Day 8, and a further decrease from Day 8 to 21 in the low- but not high-weight lines. By Day 21 low-weight lines had less breast muscle RNA than HS-A chicks with HS-P and HR-A chicks being intermediate.

The RNA levels in the liver tissue were higher in LR-A chicks than the other lines at hatch. On Day 8, there was a large difference between high- and low-weight lines due to an increase among low- and a decrease among high-weight lines. On Day 21, RNA levels were greater for LR-A than LS-A chicks with the other line-feeding regimens were intermediate. Among ages, there was a decrease in the high-weight lines from hatch to Day 8 followed by a sharp increase on Day 21. In contrast, there was a small decline from hatch to Day 8, then increasing to Day 21, in the low-weight lines.

The line-feeding regimen by age interaction for RNA levels in the small intestine was due to a sharp posthatch decline for all line-feeding



regimens except LS-A by Day 8. On Day 21, levels were higher for HS-P and HS-A than LR-A chicks with the other groups being intermediate. Among ages, LS-A chicks exhibited a slower decline in RNA levels than that for the other line-feeding regimens.

DNA Content. There was no line-feeding regimen by age interaction for DNA content of breast muscle tissue (Figure 3). There was a steady decline with age from hatch to Days 8 and 21. In general, DNA levels were higher for low- than high-weight lines. The LS-A chicks had higher DNA levels than the LR-A ones at at hatch and Day 21.

A line-feeding regimen by age interaction was present for DNA content of liver tissue. While values for all lines were similar at hatch, by Day 8 that for HS-A chicks was greater than those for LR-A, LR-P, and HR-A chicks which in turn were greater than for LS-A, HS-P chicks. A different pattern had emerged by Day 21 in which DNA content was greater for LR-P than for LR-A, LS-A, HR-A, and HS-A chicks which in turn were greater than that for HS-P chicks. Across ages, there was a rapid increase from hatch to Day 8 followed by a decline by Day 21, in HS-A chicks. In contrast, for the other line-feeding regimens contents were similar at hatch and Day 8 and then increased by Day 21.

The line-feeding regimen by age interaction was absent for DNA levels of the small intestine. Line-feeding regimen had similar DNA levels in the small intestine except for the LS-A chicks which had lower levels than HR-A and HS-A chicks at hatch. There was an age effect as DNA levels increased between Days 8 and 21.

Cell Unit Size. There were significant line-feeding regimen by age interactions for cell unit size in breast muscles and liver (Table 5). The interaction for breast muscle tissue was due to the very dramatic increase in cell unit size posthatch among high-weight lines compared to the low-weight lines. Although similar at hatch, the differences were about 4-fold on Day 8 and more than 20-fold on Day 21.

The interaction for liver tissue cell unit size was not as dramatic. It resulted from LS-A chicks having larger cell unit sizes at hatch and on Day 8 than the other line-feeding regimens. By Day 21, line-feeding regimen differences had disappeared. Among ages, a decrease with age in liver cell unit size was common for all line-feeding regimens, but due to the large initial cell unit size of LS-A chicks it was more pronounced for this group than the others.

Cell unit size of small intestine followed similar patterns with age in all line-feeding regimens. It declined from hatch to Day 8 and remained at this size to Day 21.

#### *Comparisons at a Common Body Weight*

Organ Weights. Comparisons of organ growth to a common body weight ( $80 \pm 5g$ ) did not include the LS-A chicks because they had not reached this weight on Day 21 when the experiment was terminated. At a common body weight the demand organs, breast and leg muscles, had similar weights across groups as did the supply organs, heart, lung, liver, kidney, pancreas and large intestine (Table 6). In contrast, differences were found for the weights of the upper GIT and small intestine. Crop and

esophagus were heavier for HS-P and LR-P than HS-A and LR-A chicks with HR-A chicks intermediate and not different from the first two groups. Proventriculus weight was greater for HS-P and HS-A than LR-A and LR-P with HR-A chicks intermediate, and larger than LR-A chicks. Gizzard weight was greater for HS-A and HR-A than for LR-A chicks with HS-P and LR-P chicks intermediate. HR-A chicks had heavier small intestines than LR-A and LR-P chicks with HS-A and HS-P chicks intermediate.

Cellular Data. Protein content at a common body weight was similar among the line-feeding regimens in breast muscle while both RNA and DNA were lower for low- than high-weight lines (Table 7). Thus, it followed that cell unit size was greater in low- than high-weight lines. Protein content of liver was higher for LR-A than HS-P chicks with LR-P, HS-A, and HR-A chicks being intermediate. RNA content was generally lower in high- than low-weight lines with a similar pattern observed for DNA content while cell unit size was similar among the line-feeding regimens. Protein content, RNA, DNA, and cell unit size were all similar among line-feeding regimens for small intestine tissue.

## DISCUSSION

Selection for high and low 56-day body weight has altered the growth patterns of both supply and demand organs at common chronological ages. At a common body weight, however, allomorphy was similar among lines with the exception of the GIT. These results suggest that changes in body weight are preceded in early life development by the ability of the GIT

segments to develop at rates that are largely genetically determined, corroborating allometric growth relationships of organs in these divergently selected lines of chickens (Katanbaf *et al.*, 1988b), in meat-type chickens (Nitsan *et al.*, 1991a), and in turkeys (Sell *et al.*, 1991). The importance of similar patterns of development was distinguishable between altricial and precocial chicks (Ricklefs, 1979; Lilja, 1983) and was to our knowledge first reported for a domestic avian species in geese (Knutsson *et al.*, 1980; Lilja, 1981).

Comparisons made at a common body weight demonstrate an early emphasis on development of the GIT, and in particular the small intestine. These results are consistent with those that show that feed restriction, resulting in meal feeding, mediates increased size of the crop and esophagus, proventriculus, and gizzard but not the small intestine (Pinchasov *et al.*, 1985). Large intestine growth mirrors that of other non-gastrointestinal tract supply organs. It appears that divergent selection for juvenile body weight has resulted in correlated responses in development of the small intestine during early life. The development of the upper GIT is influenced by hyperphagia. Thus development was greater in the high- than low-weight lines because of their feed intake behavior (Dunnington *et al.*, 1987), with LR-P and HS-P chicks having the largest means for these traits for the low- and high-weight lines, respectively, because of the meal feeding associated with feed restriction.

The influence of selection for 56-day body weight on cell unit size suggests that a demand organ like breast muscle has little posthatch

hyperphasic growth and, therefore, size increases are primarily due to hypertrophy of the cells of these organs. A different pattern was apparent for both supply organs in terms of cell unit size with liver and small intestine growth being due primarily to cell hyperplasia. Some posthatch hypotrophy noted in liver cells has been attributed to the reduction of liver lipemia (Nitsan *et al.*, 1991a) as the liver developed for posthatch growth.

Different developmental potentials exist for organs of supply, which are derived from embryo endodermal layers and demand organs which are mesodermal in origin (Patten, 1951). The endodermally derived gastrointestinal tract, with its hyperplastic growth was influenced by environmental factors such as feed allocation. Muscles, which were mesodermally derived, had hypertrophic growth which was a function of accumulating intercellular protein.

TABLE 1. Mean body weight<sup>1</sup> and weights of breast and leg muscles (demand organs) per 100g of body weight with a line-feeding regimen<sup>2</sup> by age interaction

Trait	Age (days)	Line-feeding regimen					
		HS-P	HR-A	HS-A	LS-A	LR-A	LR-P
Body weight g	0	—	36.5 Δ c	35.8 A c	23.0 B c	24.7 B b	—
	8	82.3 A b	73.3 Δ b	75.5 A b	31.9 B c	30.9 B b	33.8 B b
	21	238.5 A a	216.0 Δ a	249.2 Δ a	48.6 C a	87.6 B a	61.9 BC a
Breast muscle	0	—	2.02 A c	1.44 B c	1.36 B c	1.82 B b	—
	8	5.17 A b	5.60 A b	4.49 A b	2.34 B b	2.18 B b	2.33 B b
	21	8.56 Δ a	8.70 Δ a	8.19 Δ a	4.73 B a	5.85 B a	5.07 B a
Leg muscle	0	—	4.30 A b	4.00 A b	3.85 A a	4.80 A a	—
	8	4.17 A b	4.10 A b	3.90 A b	3.17 A a	3.41 A b	3.40 A b
	21	4.96 A a	4.86 Δ a	5.45 A a	3.48 B a	3.91 B a	3.59 B a

<sup>1</sup> Body weight was corrected for gastrointestinal content weight.

<sup>2</sup> Line-feeding regimen: HS-A = high selected line fed *ad libitum*, HR-A = high relaxed line fed *ad libitum*, HS-P = high selected line pair-fed to feed intake of HR-A, LS-A = low selected line fed *ad libitum*, LR-P = low relaxed line pair-fed to feed intake of LS-A, LR-A = low relaxed line fed *ad libitum*.

ABC Means within a row with different superscripts are significantly different ( $P \leq .05$ ).

abc Means in a column within a trait with different superscripts are significantly different ( $P \leq .05$ ).

TABLE 2. Mean weights of heart and pancreas (supply organs) per 100g of body weight<sup>1</sup> with a line-feeding regimen<sup>2</sup> by age interaction

Trait	Age (days)	Line-feeding regimen					
		HS-P	HR-A	HS-A	LS-A	LR-A	LR-P
Heart	0	—	.70 AB a	.77 AB a	.64 B b	.83 A a	—
	8	.69 B a	.62 B ab	.73 AB a	.86 A a	.76 AB a	.66 B a
	21	.53 A b	.52 A b	.52 A b	.55 A b	.62 A b	.60 A a
Pancreas	8	.89 A a	.64 A a	.71 A a	.54 A a	.62 A a	.61 A a
	21	.52 B b	.51 B b	.52 B b	.66 AB a	.65 AB a	.72 A a

<sup>1</sup> Body weight was corrected for gastrointestinal content weight.

<sup>2</sup> Line-feeding regimen: HS-A = high selected line fed *ad libitum*, HR-A = high relaxed line fed *ad libitum*, HS-P = high selected line pair-fed to feed intake of HR-A, LS-A = low selected line fed *ad libitum*, LR-P = low relaxed line pair-fed to feed intake of LS-A, LR-A = low relaxed line fed *ad libitum*.

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abc Means in a column within a trait with different superscripts are significantly different ( $P \leq .05$ ).

TABLE 3. Mean weights of gastrointestinal tract segments (supply organs) per 100g of body weight<sup>1</sup> with a line-feeding regimen<sup>2</sup> by age interaction

Trait	Age (days)	Line-feeding regimen					
		HS-P	HR-A	HS-A	LS-A	LR-A	LR-P
Crop & esophagus	0		1.54 A a	1.51 A a	1.18 A a	1.08 A a	a
	8	1.61 A a	1.54 A a	1.30 A ab	1.35 A a	1.15 A a	1.60 A a
	21	.97 CD b	.91 D b	.85 D b	1.38 B a	1.24 BC a	1.89 A a
Proventriculus	0		.88 A ab	.78 A b	.88 A a	1.01 A a	a
	8	1.20 A a	1.10 A a	1.35 A a	1.09 A a	1.02 A a	1.06 A a
	21	.76 AB b	.67 B b	.70 B b	.88 A a	.79 AB a	.82 AB a
Gizzard	0		6.62 A a	4.96 A b	4.90 A b	6.21 A ab	ab
	8	5.99 A a	6.21 A a	6.21 A a	6.38 A a	6.93 A a	6.98 A a
	21	3.80 CD b	4.19 BC b	3.25 D c	5.10 Ab ab	4.69 ABC b	5.29 A b
Small intestine	0		3.09 A ab	3.00 A b	2.91 A b	3.34 A b	b
	8	5.83 A a	6.62 A a	5.86 A a	5.13 A a	5.11 A a	5.26 A a
	21	3.94 C b	3.89 C ab	4.00 C ab	5.30 A a	4.60 ABC a	4.90 AB a
Large intestine	0		1.30 A a	.90 A a	.83 A b	.89 A a	a
	8	.97 AB a	.88 AB a	1.03 A a	.81 B b	.93 AB a	.89 AB b
	21	.86 B a	.91 B a	.92 B a	1.32 A a	1.05 AB a	1.09 AB a

<sup>1</sup> Body weight was corrected for gastrointestinal content weight.

<sup>2</sup> Line-feeding regimen: HS-A = high selected line fed *ad libitum*, HR-A = high relaxed line fed *ad libitum*, HS-P = high selected line pair-fed to feed intake of HR-A, LS-A = low selected line fed *ad libitum*, LR-P = low relaxed line pair-fed to feed intake of LS-A, LR-A = low relaxed line fed *ad libitum*.

ABCDEFMeans within a row with different superscripts are significantly different ( $P \leq .05$ ).

abcdefMeans in a column within a trait with different superscripts are significantly different ( $P \leq .05$ ).



TABLE 4. Mean weights of lung, liver and kidney (supply organs) per 100g of body weight<sup>1</sup> with line-feeding regimen<sup>2</sup> and age as main effects

Trait	Line-feeding regimen					
	HS-P	HR-A	HS-A	LS-A	LR-A	LR-P
Lung	.64 c	.65 c	.69 bc	.85 ab	.89 a	.72 abc
Liver	2.97 ab	2.80 b	2.73 b	3.22 a	3.47 a	3.10 ab
Kidney	.64 a	.69 a	.71 a	.61 a	.83 a	.74 a
	Age (days)					
	0		8		21	
Lung	.84 a		.77 a		.65 b	
Liver	3.02 b		3.52 a		2.58 c	
Kidney	.67 a		.76 a		.67 a	

<sup>1</sup>Body weight was corrected for gastrointestinal content weight.

<sup>2</sup>Line-feeding regimen: HS-A = high selected line fed *ad libitum*, HR-A = high relaxed line fed *ad libitum*, HS-P = high selected line pair-fed to feed intake of HR-A, LS-A = low selected line fed *ad libitum*, LR-P = low relaxed line pair-fed to feed intake of LS-A, LR-A = low relaxed line fed *ad libitum*.

abcMeans in a row with different superscripts are significantly different ( $P \leq .05$ ).

TABLE 5. Means of cell unit size (protein/DNA) for breast, liver, and small intestine tissue with a line-feeding regimen<sup>1</sup> by age interaction

Trait	Age (days)	Line-feeding regimen					
		HS-P	HR-A	HS-A	LS-A	LR-A	LR-P
Breast	0	—	55 A c	60 A c	70 A b	71 A b	—
	8	336 A b	244 A b	223 A b	78 B b	62 B b	85 B b
	21	41,770 A a	24,858 A a	28,849 A a	680 B a	2,254 B a	1,153 B a
Liver	0	—	57 B a	59 B a	116 A a	59 B a	—
	8	32 BC a	29 BC b	21 C b	52 A b	43 AB ab	43 AB a
	21	25 A a	20 A b	23 A b	26 A c	27 A b	22 A b
Small intestine	0	—	20 A a	22 A a	31 A a	29 A a	—
	8	17 A a	13 A b	19 A ab	12 A b	12 A b	15 A a
	21	19 A a	15 A b	16 A b	14 A b	15 A b	13 A a

<sup>1</sup>Line-feeding regimen: HS-A = high selected line fed *ad libitum*, HR-A = high relaxed line fed *ad libitum*, HS-P = high selected line pair-fed to feed intake of HR-A, LS-A = low selected line fed *ad libitum*, LR-P = low relaxed line pair-fed to feed intake of LS-A, LR-A = low relaxed line fed *ad libitum*.

ABC Means within a row with different superscripts are significantly different ( $P \leq .05$ ).

abc Means in a column within a trait with different superscripts are significantly different ( $P \leq .05$ ).

TABLE 6. Mean weights of organs per 100g of body weight<sup>1</sup> by line-feeding regimen<sup>2</sup> at a common body weight (80 ± 5g)

Trait	Line-feeding regimen			
	HS-P	HR-A	HS-A	LR-P
Breast	5.17 a	5.60 a	4.49 a	5.85 a
Legs	4.17 a	4.10 a	3.90 a	3.91 a
Heart	.69 a	.62 a	.73 a	.62 a
Lung	.65 a	.59 a	.68 a	.71 a
Liver	3.25 a	3.27 a	3.09 a	2.68 a
Kidney	.67 a	.75 a	.91 a	.77 a
Crop & esophagus	1.61 a	1.54 ab	1.30 b	1.24 b
Proventriculus	1.20 a	1.10 ab	1.35 a	.79 c
Gizzard	5.99 ab	6.21 a	6.21 a	4.69 b
Small intestine	5.83 ab	6.62 a	5.86 ab	4.60 b
Pancreas	.89 a	.64 a	.71 a	.65 a
Large intestine	.97 a	.88 a	1.03 a	1.05 a

<sup>1</sup>Body weight was corrected for gastrointestinal content weight.

<sup>2</sup>Line-feeding regimen: HS-A = high selected line fed *ad libitum*, HR-A = high relaxed line fed *ad libitum*, HS-P = high selected line pair-fed to feed intake of HR-A, LS-A = low selected line fed *ad libitum*, LR-P = low relaxed line pair-fed to feed intake of LS-A, LR-A = low relaxed line fed *ad libitum*.

abcMeans within a row with different superscripts are significantly different ( $P \leq .05$ ).

TABLE 7. Means of protein, RNA, and DNA content (mg/g) and cell unit size (protein/RNA) in breast, liver, and small intestinal tissue at common body weight by line-feeding regimen<sup>1</sup>

Trait	Line-feeding regimen				
	HS-P	HR-A	HS-A	LR-A	LR-P
<i>Breast</i>					
Protein	158 a	146 a	157 a	191 a	203 a
RNA	9.70 ab	11.03 a	9.35 ab	7.43 b	7.01 b
DNA	.61 a	.63 a	.88 a	.09 b	.24 b
Cell unit size	336 b	244 b	244 b	2,254 a	1,153 a
<i>Liver</i>					
Protein	68 b	72 ab	79 ab	101 a	87 ab
RNA	6.69 c	6.57 c	7.28 bc	9.60 ab	10.54 a
DNA	2.30 b	2.64 ab	3.83 a	3.69 ab	4.06 a
Cell unit size	32 a	29 a	21 a	27 a	22 a
<i>Small intestine</i>					
Protein	69 a	54 a	73 a	69 a	63 a
RNA	7.15 a	7.80 a	7.30 a	6.90 a	7.50 a
DNA	4.08 a	4.22 a	3.87 a	4.50 a	4.78 a
Cell unit size	17 a	13 a	19 a	15 a	13 a

<sup>1</sup>Line-feeding regimen: HS-A = high selected line fed *ad libitum*, HR-A = high relaxed line fed *ad libitum*, HS-P = high selected line pair-fed to feed intake of HR-A, LS-A = low selected line fed *ad libitum*, LR-P = low relaxed line pair-fed to feed intake of LS-A, LR-A = low relaxed line fed *ad libitum*.

abc means within a row with different superscripts are significantly different ( $P \leq .05$ ).

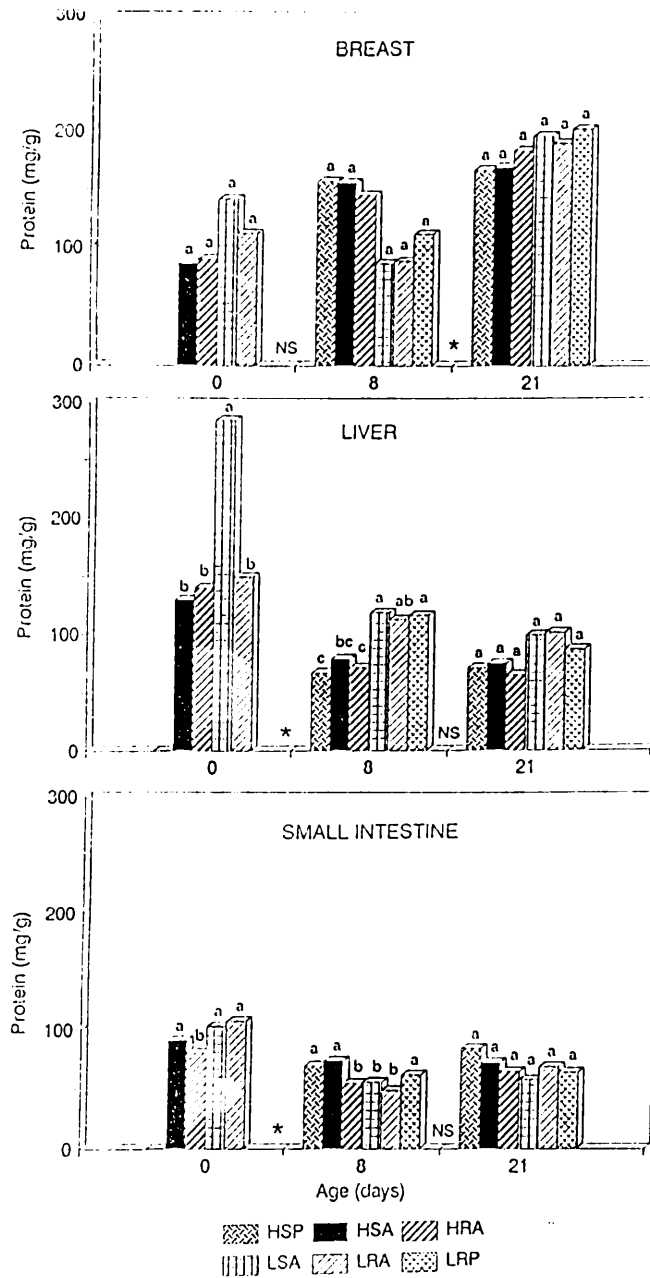


FIGURE 1. Protein content (mg/g of tissue) of breast muscle, liver, and small intestine for age and line-feeding regimens. HS-A = high selected line fed *ad libitum*, HS-P = high selected line pair-fed to feed intake level of HR-A, HR-A = high relaxed line fed *ad libitum*, LS-A = low selected line fed *ad libitum*, LR-A = low relaxed line fed *ad libitum*, LR-P = low relaxed line pair-fed to feed intake level of LS-A. <sup>abc</sup>Means within an age with different superscripts are significantly different ( $P \leq .05$ ). \* Means between adjacent ages are significantly different ( $P \leq .05$ ). ns Means between adjacent ages are not significantly different ( $P \geq .05$ ).

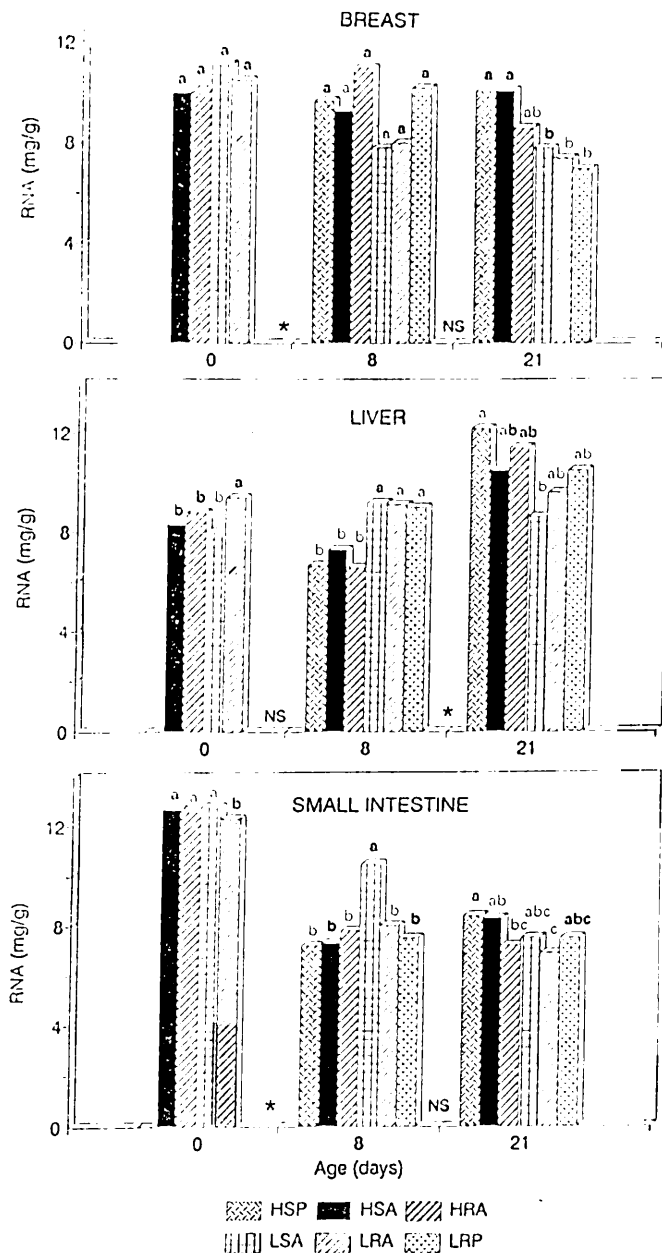


FIGURE 2. RNA content (mg/g of tissue) of breast muscle, liver, and small intestine for age and line-feeding regimens. HS-A = high selected line fed *ad libitum*, HS-P = high selected line pair-fed to feed intake level of HR-A, HR-A = high relaxed line fed *ad libitum*, LS-A = low selected line fed *ad libitum*, LR-A = low relaxed line fed *ad libitum*, LR-P = low relaxed line pair-fed to feed intake level of LS-A. <sup>abc</sup>Means within an age with different superscripts are significantly different ( $P \leq .05$ ). \* Means between adjacent ages are significantly different ( $P \leq .05$ ). ns Means between adjacent ages are not significantly different ( $P \geq .05$ ).

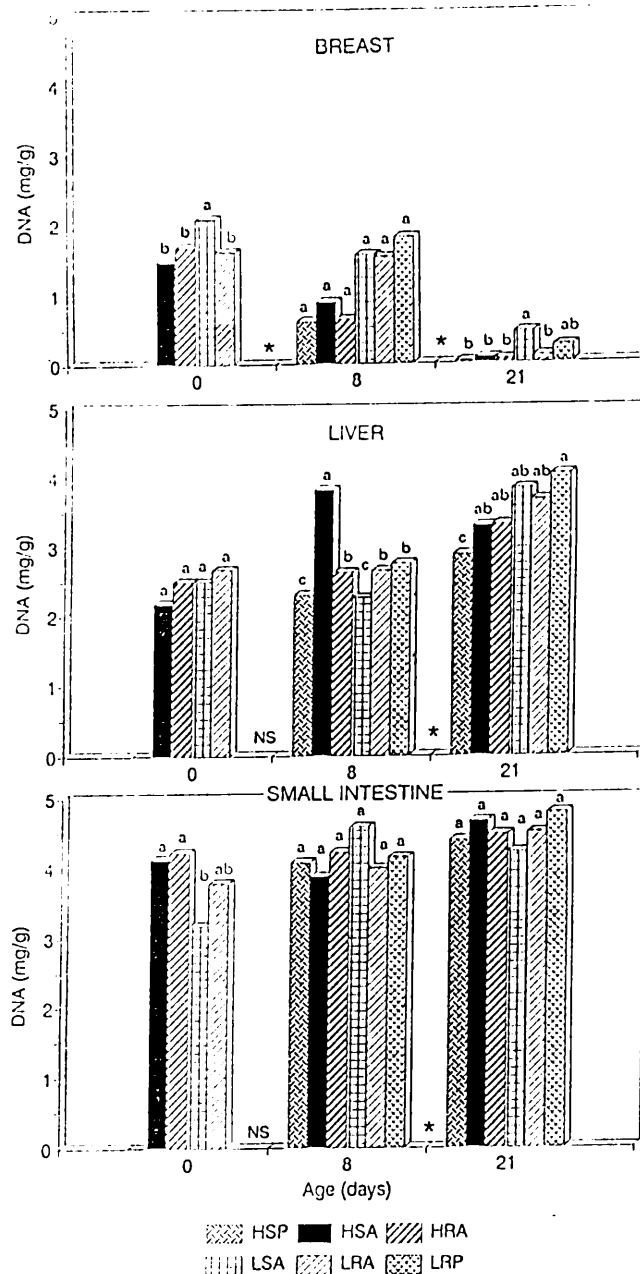


FIGURE 3. DNA content (mg/g of tissue) of breast muscle, liver, and small intestine for age and line-feeding regimens. HS-A = high selected line fed *ad libitum*, HS-P = high selected line pair-fed to feed intake level of HR-A, HR-A = high relaxed line fed *ad libitum*, LS-A = low selected line fed *ad libitum*, LR-A = low relaxed line fed *ad libitum*, LR-P = low relaxed line pair-fed to feed intake level of LS-A. <sup>abc</sup>Means within an age with different superscripts are significantly different ( $P \leq .05$ ). \* Means between adjacent ages are significantly different ( $P \leq .05$ ). ns Means between adjacent ages are not significantly different ( $P \geq .05$ ).

CHAPTER VII

CORRELATED RESPONSES IN LINES OF CHICKENS DIVERGENTLY SELECTED  
FOR 56-DAY BODY WEIGHT: DIGESTIVE ENZYMES



## SUMMARY

Levels of amylase, trypsin, chymotrypsin, and lipase in the pancreas and small intestinal chyme were measured in females from 4 lines of chickens. Two of the lines had undergone 32 generations of divergent selection for 56-day body weight, while in the other 2 lines selection for high- or low-weight had been relaxed for 5 generations. The diet used in this experiment was that under which selection had been practiced (20% crude protein and 2685 Kcal of ME/Kg). Comparisons between divergently selected lines at common ages revealed higher enzyme levels for high- than low-weight lines. When comparisons were made at a common body weight ( $80 \pm 5$  g) there were no differences between lines. These results suggested that correlated responses in feed intake were mediating the regulation of digestive enzyme levels in the pancreas and in intestinal chyme of growth selected lines of chickens. Chicks from high-weight lines had elevated enzyme levels after a mild feed restriction compared to those fed *ad libitum*. It was hypothesized that hyperphagia associated with the high-weight lines, in combination with a mild feed restriction and the associated meal feeding, stimulated synthesis and secretion of digestive enzymes.

## INTRODUCTION

Levels of digestive enzymes in organs and contents of gastrointestinal tract were influenced by genetic stock (Nitsan *et al*,

1989; 1991b), feed composition and level of feed intake (Corring, 1980; Nitsan, 1985). Pancreatic adaptation to diet by regulation of synthesis and secretion of digestive enzymes in domestic mammals and birds has been reviewed (see Brannon 1990). The regulation of proteolytic enzymes was influenced by a synergistic action between cholecystokinin and amino acids (Yang *et al.*, 1988), while that for amylase and lipase has not been elucidated. Ratios of digestive enzymes produced by the pancreas of turkeys (Krogdahl and Sell, 1989) and chickens (Pubols, 1991) have been profiled, and age has been clearly demonstrated as an influencing factor ( Sell *et al.*, 1991; Nitsan *et al.*, 1991a).

Studies of the interactions among feed intake, genetic background, and age have revealed a highly adaptive response mechanism in meat-type chickens (Nir *et al.*, 1987; Pinchasov *et al.*, 1990; Nitsan *et al.*, 1991a) and turkeys (Krogdahl and Sell, 1989; Sell *et al.*, 1991). Selection for high and for low 56-day body weight (Siegel, 1978; Dunnington and Siegel, 1985) has resulted in correlated changes in appetite. High-weight line chickens are hyperphagic (Barbato *et al.*, 1983) and unresponsive to lesioning of the ventromedial hypothalamus (Burkhart *et al.*, 1983). Chickens from the low-weight line exhibit hyperphagic responses to lesioning (Burkhart *et al.*, 1983) and anorexia is not uncommon (Zelenka *et al.*, 1988). This chapter provides a report of digestive enzyme activity at 2 ages and at a common body weight as a correlated response in lines of chickens which have been divergently selected for juvenile body weight, and in their sublimes where selection was relaxed, utilizing a paired feeding technique to equalize feed intakes and maintaining *ad libitum* fed

controls. Companion chapters included data on growth, feed intake, and feed utilization (Chapter V), organ growth and cellular content (Chapter VI).

## MATERIALS AND METHODS

### *Stocks and Husbandry*

Chicks used in this experiment were from the S<sub>32</sub> generation of lines divergently selected for high (HS) or low (LS), 56-day body weight (Siegel, 1978; Dunnington and Siegel, 1985) and sublines of the selected populations in which selection was relaxed for 5 generations. The relaxed lines are designated as HR and LR for the high and low-weight lines, respectively.

Chicks from age-contemporary parents were hatched on the same day, vent-sexed, wingbanded, vaccinated for Marek's Disease, and females placed in individual cages with continuous light. The diet under which selection was practiced was a mash diet formulated to contain 20% crude protein and 2685 Kcal of ME/Kg of feed. Hot-air brooding maintained environmental temperatures of 35°C during the first week, 33°C the second week, and 29°C the third week. Temperature fluctuation in the room was  $\pm 1^\circ$  C.

The design of the experiment consisted of two sets. The first set consisted of 20 HR chicks (HR-A) fed *ad libitum* to which 20 HS chicks were assigned as pair-fed partners (HS-P). An additional 15 HS chicks (HS-A) were fed *ad libitum*. The second consisted of 30 LS chicks (LS-A) fed *ad*

*libitum* to which 30 LR chicks were assigned as pair-fed partners (HS-P). An additional 30 LR chicks (LR-A) were fed *ad libitum*. All assignments of chicks were made at random within a pairing. The rationale for this design was presented in a companion paper (Chapter V).

### *Enzyme Assays*

At 8 and 21 days of age, 4 chicks from each line-feeding regimen subclass (n = 48) were weighed and then killed by cervical dislocation just prior to the time daily feeding allocations were normally provided. In the case of pairs, both members were included. The pancreas and contents of small intestine (duodenum, ileum and jejunum), i.e. the chyme, were removed immediately, weighed, and then stored at -70°C for later analysis. Levels of  $\alpha$  amylase (EC 3.2.1.1), trypsin (EC 3.4.21.4), chymotrypsin A (EC 3.4.21.1), and lipase (EC 3.1.1.3) were assayed. All samples were homogenized with a polytron (Brinkmann Polytron, Kinematica GmBH, Luzern, Switzerland) in 10 vol of ice cold distilled water and centrifuged at 40,000g for 20 min. Amylase activity was determined by the method of Bernfeld (1955) as modified by Gertler and Nitsan (1970). Amylase activity unit was defined spectrophotometrically (Spectronic 1001, Bausch and Lomb, Rockester, New York) as a change of  $10^{-5}$  absorbance at 540 nm due to liberation of reducing groups from a solution of 1% starch after 3 min incubation at 37°C at pH 6.9 in excess 3,5 dinitro salicylic acid. Trypsinogen and chymotrypsinogen from pancreas samples were activated by incubation in .1% enterokinase (EC 3.4.21.9) solution 1:1 (v:v) for 1h at 37°C. This incubation yielded trypsin and chymotrypsin

which were then assayed for activity. Endogenous activation levels were assayed in small intestinal chyme. Activity was determined spectrophotometrically (Gertler and Nitsan, 1970), with N benzoyl DL arginine p nitroanilide HCl as substrate for trypsin and N actyl L tyrosine p nitroanilide as substrate for chymotrypsin with final concentrations being 1.25 mM in 3.7 mM Tris buffer, pH 7.8, .6 mM CaCl<sub>2</sub> and 25mg/ml dimethyl sulfoxide. The reactions proceeded at 37°C for 60 min and were stopped with acetic acid. Activity units were defined as substrate hydrolysis yielding a change of 10<sup>-3</sup> absorbance at 410 nm. Lipase activity was determined by modified procedure of Nitsan *et al.* (1974). Briefly, substrate was .3 mM β Naphthyl laurate in acetone with final concentrations being 8.8 mM phosphate buffer, .01 mM O-dianizidine tetrazotized and .04 mM taurocholic acid. The reaction was stopped after 30 min at 37°C with 40% trichloroacetic acid. Ethyl acetate was added (5 ml) and samples centrifuged for 10 min at 550g and the upper fraction read at 540 nm, lipase activity unit was defined as a change of 10<sup>-3</sup> absorbance.

Enzyme units were defined such that a micromole of substrate was hydrolyzed per min for concentrations, pH, and conditions stated. A dilution for each enzyme to be assayed was determined by constructing activity curves of each enzyme at assay concentrations of substrate. All dilutions were measured only at absorbance readings between 1 x 10<sup>-1</sup> and 9 x 10<sup>-1</sup>. Total activity was defined as the product of sample activity and total sample weight (pancreas, small intestinal chyme), and relative

activity was the total activity expressed per 100 g body weight (Pinchasov *et al.*, 1990).

### *Statistical Analyses*

Data were analyzed several ways. One involved a paired t-test between pair-fed individuals within a pairing (HS-P vs HR-A, and LS-A vs LR-P). A further paired t-test was used between pair-fed and *ad libitum* fed chicks within a line (HS-P vs HS-A, and LR-P vs LR-A). In addition, analysis of variance was performed for all data with age and line-feeding regimen as main effects and the first order interaction involving them. The statistical model for analysis of variance was.

$$Y_{ijk} = \mu + G_i + A_j + (GA)_{ij} + e_{ijk}$$

where  $i = 1, 2 \dots 6$  line-feeding regimen,  $j = 8, 21$  days and  $k = 1, 2 \dots n$  individuals. Prior to analysis of variance, enzyme activities were transformed to square roots. Where appropriate, significant differences among line-feeding regimens were compared by Tukey's studentized paired comparisons (SAS, 1982). Enzyme data were also compared at a common body weight but different ages with appropriate contrasts to compare means. Means presented here are untransformed. Unless otherwise stated statistical significance was taken at  $P \leq .05$ . Because data were subjected to more than one statistical test, each test having a .05 alpha rejection region, there was an increased probability of an occurrence of a Type 1 error to a level greater than alpha.

## RESULTS

Interactions between line-feeding regimens and ages were not significant, with the exceptions of total pancreatic amylase, and relative activities of trypsin in the pancreas and chyme. Total pancreatic amylase activity increased with age in all line-feeding regimens except HR-A, with the relative magnitude of change varying considerably among regimens (Table 1). Comparisons of total pancreatic amylase within ages showed higher values for the high than low-weight lines on Day 8. This pattern was evident on Day 21, with the exception of HR-A chicks which were intermediate. The interaction for relative pancreatic trypsin was due to higher activities on Day 8 than 21 for HR-A chicks. There was a reversal of this pattern for LR-P chicks, and no age effect for the other line-feeding regimens. Generally, on Day 8 relative pancreatic trypsin levels were greater for the high- than low-weight lines; by Day 21 these differences had disappeared. Relative levels of trypsin in the chyme were generally greater for the high- than low-weight lines on Day 8, with this difference absent on Day 21. A large decrease in chyme levels of relative trypsin was observed only among HS-A chicks from Day 8 to 21.

Line-feeding regimen differences in pancreatic enzyme activities were frequently due to higher activity levels in HS-A than LS-A chicks, i.e., relative amylase, total trypsin, and total lipase (Table 2). Differences between HS-P and LS-A chicks were present in activities of total chymotrypsin. While the low-weight lines were similar for most

enzymes measured, LS-A chicks had lower total pancreatic lipase activity than LR-A or LR-P chicks which were similar. Enzyme activity levels in the pancreas increased with age for relative amylase, total trypsin, total and relative chymotrypsin, and total and relative lipase.

Differences in line-feeding regimens were found for activities of total amylase, total trypsin, and total chymotrypsin in chyme (Table 3). These differences, while generally due to lower activities among low- than high-weight line chicks, were most evident for the HS-A vs LS-A comparison. No differences among line-feeding regimens were found for levels of the other enzymes measured in the chyme. Comparisons between Days 8 and 21 showed a decrease with age for relative amylase, an increase for total chymotrypsin, and no changes for the other enzymes measured in chyme.

Paired t-test analysis showed several differences in enzyme activity between high-weight pairs (Table 4). On Day 8, total and relative chymotrypsin activities in the pancreas were higher for HS-P than for HR-A and HS-A chicks. Differences in total amylase activity in the pancreas were present on Day 21 with HS-A > HS-P > HR-A. On a relative basis, however, activity for HR-A was less than HS-P and HS-A, which were similar. Also on Day 21, total and relative trypsin activities in the pancreas, and total and relative chymotrypsin activity in the chyme were higher for HS-P than HS-A chicks. Although at Day 21, total and relative trypsin activities in the pancreas were higher for HS-P than HR-A chicks, there were no differences between these groups for total and relative chymotrypsin in the chyme.



Comparisons of enzyme activities at a common body weight ( $80 \pm 5$  g) were made among HR-A, HS-P, HS-A, LR-P and LR-A chicks. The LS-A chicks were excluded from this analysis because they never reached this weight. At the common body weight, total and relative activities of amylase, chymotrypsin, and lipase in the pancreas and small intestinal chyme were similar. Respective means for these enzymes were 53, .72, 104, 1.41, 1,568, 18.4, in the pancreas and 3.65, .05, 958, 13.03, 13.10, .16, in the small intestine. A different pattern emerged for trypsin where both total and relative activities in pancreas and in chyme were greater for the high- than low-weight lines (Table 5).

#### DISCUSSION

The differences in enzyme activity between high- and low-weight lines of chick at the same chronological age were consistent with previous reports for these lines (Cherry *et al.*, 1987; Nitsan *et al.*, 1989; 1991b). When comparisons were made at a similar body weights, however, line differences were noted for trypsin only. The delayed rate of digestive enzyme synthesis in a population-dependent manner was observed by Nir *et al.* (1987), and suggests that resource allocations were varied primarily according to life cycle demands and biological function rather than chronological age. This pattern was similar to that reported for reproductive traits (Reddy and Siegel, 1977b; Siegel and Dunnington, 1985; 1987), where chickens from the low-weight line proceeded at a slower

pace through their early life cycle than those from the high-weight line (Zelenka *et al.*, 1986b; Katanbaf *et al.*, 1988a).

Mechanical stimulation of the intestinal wall stimulates digestive enzyme synthesis (Nitsan *et al.*, 1974; Brannon, 1990). Because of hypophagic behavior (Barbato *et al.*, 1983; Chapter V), less stimulation may have occurred in low-weight line chicks with resulting reductions in synthesis and secretion of digestive enzymes. Trypsin activity was lower in low than high-weight lines, even at similar body weights, suggesting the importance of the presence of amino acid intake and cholecystokinin synergistic action (Yang *et al.*, 1988), in addition to mechanical stimulation of the gut for this enzyme.

The elevated enzyme synthesis and secretion of the HS-P than HS-A chicks may be attributed, in part, to restricted feeding resulting in meal eating, which was similar to that reported for chickens which eat to near gut capacity (Nir *et al.*, 1978). Growth was enhanced more in HS-P than HS-A and HR-A chicks when feed intake was equalized (Chapter V). The unique combination of meal feeding, which occurs with either a mild restriction or intermittent feeding in the hyperphagic chick, resulted in an increase in mechanical stimulation of the gastrointestinal tract and contributed to increased synthesis of digestive enzymes. The absence of hyperphagia in the LR-P chicks suggest that meal feeding to gut capacity, and not mild feed restriction itself, was eliciting elevated digestive enzyme synthesis and/or storage in the pancreas.

TABLE 1. Mean digestive enzyme activities<sup>1</sup> with a line-feeding regimen<sup>2</sup> by age interaction

Age (days)	Line-feeding regimen					
	HS-P	HR-A	HS-A	LS-A	LR-A	LR-P
<i>Total pancreatic amylase</i>						
8	53 a **	56 a ns	59 a **	8 b **	10 b **	11 b **
21	219 ab	99 bc	225 a	25 c	66 c	59 c
<i>Relative pancreatic trypsin</i>						
8	5.85 ab ns	6.26 a *	5.15 abc ns	1.85 cd ns	2.10b cd ns	1.40 d *
21	4.44 a	4.09 a	3.81 a	2.71 a	3.29 ba	2.96 a
<i>Relative intestinal trypsin</i>						
8	1.01 ab ns	1.07 ab ns	1.76 a *	.51 b ns	.36 b ns	.24 b ns
21	0.19 a	.28 a	.34 a	.53 a	.53 a	.66 a

<sup>1</sup> Units of activity were a change in absorbance of 10<sup>-3</sup> for trypsin, chymotrypsin, and lipase and 10<sup>-5</sup> for amylase. Total activity was units by organ or chyme weight and relative activity was total activity per 100 g body weight.

<sup>2</sup> Line-feeding regimen: HS-A = high selected line fed *ad libitum*, HR-A = high relaxed line fed *ad libitum*, HS-P = high selected line pair-fed to feed intake of HR-A, LS-A = low selected line fed *ad libitum*, LR-P = low relaxed line pair-fed to feed intake of LS-A, LR-A = low relaxed line fed *ad libitum*.

abcdMeans in a row with different superscripts are significantly different (P ≤ .05).  
\* Means adjacent in a column are significantly different (P ≤ .05).  
\*\* Means adjacent in a column are significantly different (P ≤ .01).  
ns Means adjacent in a column are not significantly different (P > .05).

TABLE 2. Means and SEM of digestive enzyme activities<sup>1</sup> in the pancreas by line-feeding regimen<sup>2</sup> and age

Line-feeding regimen	Amylase		Trypsin		Chymotrypsin		Lipase	
	total <sup>3</sup>	relative	total	relative <sup>3</sup>	total	relative	total	relative
HS-P	—	.77 a	775 a	—	334 a	2.09 a	1,919 a	10.05 a
HR-A	—	.61 ab	676 a	—	272 a	1.60 a	367 b	2.97 a
HSA	—	.83 a	671 a	—	173 ab	.98 a	2,434 a	13.98 a
LS-A	—	.39 b	100 b	—	52 b	1.26 a	87 c	1.97 a
LR-A	—	.53 ab	176 b	—	78 b	1.08 a	398 b	46.16 a
LR-P	—	.62 ab	119 b	—	65 b	1.24 a	264 b	5.59 a
Age (days)								
8	—	.51 b	254 b	—	66 b	1.09 b	257 b	4.51 b
21	—	.74 a	585 a	—	264 a	1.67 a	2,761 a	22.40 a
SEM	—	.05	55	—	32	.15	615	5.89

<sup>1</sup> Units of activity were a change in absorbance of 10<sup>-3</sup> for trypsin, chymotrypsin, and lipase and 10<sup>-5</sup> for amylase. Total activity was units by organ weight and relative activity was total activity per 100 g body weight.

<sup>2</sup> Line-feeding regimen: HS-A = high selected line fed *ad libitum*, HR-A = high relaxed line fed *ad libitum*, HS-P = high selected line pair-fed to feed intake of HR-A, LS-A = low selected line fed *ad libitum*, LR-P = low relaxed line pair-fed to feed intake of LS-A, LR-A = low relaxed line fed *ad libitum*.

<sup>3</sup> See Table 1 for line-feeding regimen by age interaction.

abc Means in a column within a main effect with different superscripts are significantly different ( $P \leq .05$ ).

TABLE 3. Means and SEM of digestive enzyme activities<sup>1</sup> in the small intestinal chyme by line-feeding regimen<sup>2</sup> and age

Line-feeding regimen	Amylase		Trypsin		Chymotrypsin		Lipase	
	total	relative	total	relative <sup>3</sup>	total	relative	total	relative
HS-P	4.38	ab 3.85 a	65	ab	1,023	ab 6.99 a	10.70	a .06 a
HR-A	3.58	ab 3.11 a	72	ab	1,124	ab 9.58 a	12.86	a .11 a
HS-A	6.81	a 4.76 a	112	a	2,172	a 14.82 a	9.30	a .06 a
LS-A	1.65	b 4.21 a	23	b	484	b 11.71 a	5.18	a .11 a
LR-A	3.74	ab 8.34 a	29	b	642	b 10.07 a	24.11	a .32 a
LR-P	2.08	b 4.58 a	24	b	554	b 10.47 a	2.10	a .05 a
Age (days)								
8	3.3	a .06 a	56	a	670	b 11.28 a	5.22	a .10 a
21	4.2	a .03 b	52	a	1,364	a 9.91 a	16.81	a .14 a
SEM	.48	>.01	7		131	.96	3.24	.03

<sup>1</sup> Units of activity were a change in absorbance of 10-3 for trypsin, chymotrypsin, and lipase and 10-5 for amylase. Total activity was units by chyme weight and relative activity was total activity per 100 g body weight.

<sup>2</sup> Line-feeding regimen: HS-A = high selected line fed *ad libitum*, HR-A = high relaxed line fed *ad libitum*, HS-P = high selected line pair-fed to feed intake of HR-A, LS-A = low selected line fed *ad libitum*, LR-P = low relaxed line pair-fed to feed intake of LS-A, LR-A = low relaxed line fed *ad libitum*.

<sup>3</sup> See Table 1 for line-feeding regimen by age interaction.

abcMeans in a column within a main effect with different superscripts are significantly different (P ≤ .05).

TABLE 4. Mean digestive enzyme activities<sup>1</sup> among high weight line-feeding regimens<sup>2</sup> analysed by paired t-tests.

	Day 8			Day 21				
	Pancreas			Chyme				
	Chymotrypsin total relative	Amylase total relative	Trypsin total relative	Chymotrypsin total relative	Amylase total relative	Trypsin total relative		
<i>Line-feeding regimen</i>								
HR-A	77.8	1.05	98.5	.45	890	4.09	465.9	2.15
	**	**	**	**	**	**	ns	ns
HS-P	171.8	2.07	218.6	.90	1,064	4.44	516.8	2.11
	**	**	**	ns	**	**	*	*
HS-A	60.6	.80	224.8	.92	937	3.81	284.8	1.17

<sup>1</sup> Units of activity were a change in absorbance of 10<sup>-3</sup> for trypsin, chymotrypsin, and lipase and 10<sup>-5</sup> for amylase. Total activity was units by organ or chyme weight and relative activity was total activity per 100 g body weight.

<sup>2</sup> Line-feeding regimen: HS-A = high selected line fed *ad Libitum*, HR-A = high relaxed line fed *ad Libitum*, HS-P = high selected line pair-fed to feed intake of HR-A,

\* Means adjacent in a column are significantly different ( $P \leq .05$ ).

\*\* Means adjacent in a column are significantly different ( $P \leq .01$ ).

ns Means adjacent in a column are not significantly different ( $P > .05$ ).

TABLE 5. Mean trypsin activity<sup>1</sup> at a common body weight (80 ± 5g.) by line-feeding regimen<sup>2</sup>

Contrasts	Pancreas		Chyme	
	total	relative	total	relative
<i>Line</i>				
High	450 a	5.75 a	86 a	1.29 a
Low	238 b	3.18 b	45 b	.60 b
<i>High weights</i>				
HS-P	485 a	5.85 a	45 a	1.01 a
HR-A	459 a	6.26 a	80 a	1.10 a
HS-A	405 a	5.15 a	134 a	1.76 a
<i>Low weights</i>				
LR-A	289 a	3.39 a	47 a	.53 a
LR-P	187 a	2.96 a	43 a	.66 a

<sup>1</sup> Units of activity were an change in absorbance of 10<sup>-3</sup>. Total activity was units by organ or chyme weight and relative activity was total activity per 100 g body weight.

<sup>2</sup> Line-feeding regimen: HS-A = high selected line fed *ad libitum*, HR-A = high relaxed line fed *ad libitum*, HS-P = high selected line pair-fed to feed intake of HR-A, LS-A = low selected line fed *ad libitum*, LR-P = low relaxed line pair-fed to feed intake of LS-A, LR-A = low relaxed line fed *ad libitum*.

abcMeans in a column with in a contrast with different superscripts are significantly different (P ≤ .05).

## GENERAL SYNTHESIS

Within each stage of the life history of an individual, a compromise of strategies is determined by an interplay of its genotype and previous environmental experiences. Just as the individual's phenotype is an expression of an interplay of genetics and environments, so too are each of the components which make up the whole. Experimental and commercial criteria have provided selected populations of chickens which vary across a continuum of extremes for growth. The nutritional environment provides an easily manipulated, highly repeatable environment which influences growth potential. The experimental paradigms in this dissertation involved populations of various growth potentials exposed to feeding regimens which quantitatively regulated feed intake at or below the genetic potential.

Interest has been generated by studies of the synchrony of growth, in particular as it applies to the organs of supply and demand (Lilja, 1981; Katanbaf *et al.*, 1988b). The implications of this research range from an understanding of the fundamental differences posthatch of the ontogeny of growth between precocial and altricial birds and the application of growth manipulation, by changing nutritional feeding programs, for poultry meat production (Plavnik and Hurwitz, 1991) especially the broiler (Beane *et al.*, 1979; Plavnik and Hurwitz, 1985; 1988; Chapter I). A further benefit of this research may be to develop reliable prediction of correlated changes from cellular to whole organ



when selection has been practiced for body weight (Anthony *et al.*, 1989b; Chapter VI).

Selection for divergent extremes in a trait such as body weight is expected to lead to a disruption of genetic homeostasis (Lerner, 1954; Eisen, 1980; Dunnington, 1990). However, theory does not predict the nature of such limits, only their presence after long-term artificial selection in populations of small to moderate size, and especially for traits maintained at intermediate optimums by natural selection. Resource allocation to functions other than growth has emerged as a serious deficiency among growth selected stocks (Dunnington, 1990). When such stocks are undergoing rapid growth, their resistance to infectious agents and ability to mount an immune response when challenged by an antigen are reduced (Katanbaf *et al.*, 1988c; Boa-Amponsem *et al.*, 1991b; Chapter I and II). However, certain benefits to the immune system were maintained during accelerated growth, resulting in improved livability of broilers (Chapter I) whereas long term obesity was deleterious to adult females (Chapter II).

Reproductive complications associated with growth-selected chickens (Reddy and Siegel, 1977a,b; Siegel and Dunnington, 1985) have been moderated to a substantial degree by restriction of feed consumption (Katanbaf *et al.*, 1989a,b,c; Chapter II). Long term obesity seriously impeded resistance to *E coli*, lymphoid leucosis, and chronic heart disease, as well as reducing titers to SRBC. Susceptibility to lymphoid leucosis had a large genetic influence when the population consisted of early- and late-feathering hens (Chapter II). Control of endogenous viral

infections and its associated increased susceptibility to exogenous avian leucosis virus have been achieved in White Leghorns (Smith and Crittenden, 1986). Continued exploitation of late-feathering for autosexing in broilers will require screening of commercial lines for the *tvb<sup>r</sup>* allele and not dependence on *ev6* interference of congenital transmission of *ev21* products, as the latter appears counterproductive (Smith *et al.*, 1991). At present, a serious impediment to progress in screening stocks for the *tvb<sup>r</sup>* allele has been the highly labor-intensive nature of the chorioallantoic membrane pock mark assay. A seriological assay utilizing *tvb<sup>r</sup>* antisera would greatly facilitate selection of EV21 resistant stocks (Bacon *et al.*, 1988; Smith *et al.*, 1991). If differences in egg production between early- and late-feathering hens homozygous for *tvb<sup>r</sup>* persisted then it could be concluded that *ev21* itself when present in the genome, directly effects egg production independent of viremia associated with *ev21* products.

Hatchability failures associated with eggs from dams initially after onset of lay were due to embryonic deaths during the first week of incubation (Chapter III). A role for defective lipid metabolism could not be demonstrated in these hatchability failures, as had been previously proposed (Noble, 1987). While lipid metabolism was less proficient for younger dams, the effects of moisture content of eggs and embryos may warrant further investigation.

The theory and application of residual feed consumption (RFC) records for selection of the egg-type hen (Wing and Nordskog, 1982a,b; Wing *et al.*, 1983) are independent of egg production and body weight

(Luiting and Urff, 1991a,b). Benefits of RFC selection may be feasible in broilers if RFC is also independent of body weight. Improvements in feed efficiency of broilers from selection for body weight are largely due to lower maintenance costs as market weight occurs at younger ages. Improvements of feed efficiency by selection for RFC would exploit additive genetic variation which is independent of body weight (Chapter V). Broiler breeder dams may be provided with feed which is surplus to egg production demands and is expended via higher maintenance costs and body weight (Chapter IV).

Selection for juvenile body weight results in a large correlated response in feed intake and a moderate change in feed efficiency (Chapter V). While a large amount of additive genetic variance is common to body weight and feed intake, much less additive variance is shared by body weight and feed efficiency (Wilson, 1969; Pym and Nicholls, 1979). Thus, while improvements in body weight and feed efficiency are desirable selection goals, selection on body weight and residual feed component could result in a successful selection program with benefits in both body weight and feed efficiency. If the additive genetic variance in both body weight and RFC are uncorrelated, but the latter forms a part of feed efficiency then improvement in both body weight and feed efficiency, could be achieved economically via genetics.

Growth of demand organs is highly correlated to initial posthatch growth of the small intestine, and to a lesser extent the upper GIT. Feed intake had a large effect on the upper GIT, with small intestine growth essentially independent of feed intake (Chapter VI). Feed intake mediated

the synthesis and secretion of digestive enzymes (Chapter VII). The relationship of feed restriction on levels of digestive enzymes in production systems for broilers requires further study. The biological basis of accelerated growth in broilers may be related to two phenomena : (1) growth of supply organs to full potential without reallocation of resources to demand organs prior to supply organs being fully mature, and (2) to stimulation of high levels of pancreatic digestive enzymes by meal feeding during restriction which enhances digestive enzyme synthesis and later secretion when returned to full feed.

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## APPENDIX I

### AMYLASE ASSAY

Amylase (EC 3.2.1.1) assay modified from Gertler and Nitsan (1970).

1. Bring the water bath to 37°C. Heat another water bath to 100°C. Warm up the spectrophotometer.
2. Prepare the following reagents:
  - a. PHOSPHATE BUFFERS (STOCK)
    - 1) X = 0.2M NaH<sub>2</sub>PO<sub>4</sub> (28.4g NaH<sub>2</sub>PO<sub>4</sub> in 1000ml dH<sub>2</sub>O)
    - 2) Y = 0.2M Na<sub>2</sub>HPO<sub>4</sub> • 7H<sub>2</sub>O (53.6g Na<sub>2</sub>HPO<sub>4</sub> • 7H<sub>2</sub>O in 1000ml dH<sub>2</sub>O)
  - b. PHOSPHATE BUFFER (WORKING)
    - 1) 45ml X + 55ml Y.
    - 2) Add .3915g NaCl in 1000ml dH<sub>2</sub>O.
    - 3) Adjust pH to 6.9.
  - c. SUMNER REAGENT
    - 1) Dissolve 10g dinitrosalicylic acid (C<sub>7</sub>H<sub>4</sub>N<sub>2</sub>O<sub>7</sub> • H<sub>2</sub>O), 16g NaOH, and 300g K-Na tartarate (C<sub>4</sub>H<sub>4</sub>KNaO<sub>6</sub> • 4H<sub>2</sub>O) in 800ml dH<sub>2</sub>O.
    - 2) Stir and heat.
    - 3) Once dissolved, allow to cool and bring volume up to 1000ml with dH<sub>2</sub>O.
  - d. STARCH SOLUTION (Prepare fresh daily)
    - 1) This is a 1% starch solution in working buffer. Prepare as much as is needed for the day. (3 ml/ sample plus 2 ml/ general blank).
    - 2) Bring half of the required amount of buffer to a boil.
    - 3) Add the starch to the remaining half.
    - 4) Slowly add the cold solution to the boiling buffer (away from the heat).
    - 5) Bring entire solution back to a boil. This solution should be almost clear and should not separate.
3. Sample preparation (note: keep samples cold at all times).
  - a. Homogenize in 10 vol. of cold dH<sub>2</sub>O (ex - 1g sample:9ml dH<sub>2</sub>O ).
    - 1) Cut samples into small pieces prior to homogenization.
    - 2) Place the sample in dH<sub>2</sub>O.
    - 3) Homogenize on ice, using short times; 20-30 sec. total. Clean all tissue from blades.
  - b. Centrifuge for 20 min. at 30000 G (16000 rpm).
  - c. Dilute supernatant<sup>1</sup> - 10X for pancreas, 5X for intestine cont. (1 - after storage if stored)

- d. Store undiluted supernatant at -20 C for other enzyme assays.
- e. Dilution series must be calculated for each new sample group for each enzyme assay. See procedures for serial dilution.

#### 4. Procedure

##### a. Test tube setup

- 1) Label 3 glass tubes (2 sample and 1 blank) per sample. Do no more tubes than can be done in 3 min. (Allow about 15 sec per set of 3 to add solutions - max of 12 sets).
- 2) Label 1 general blank (for spec).

##### b. Steps

- 1) Add 1ml of working buffer and sample (vol. determined by dilution series) to each test tube (except the general blank).
- 2) Place rack of tubes in the water bath (37°C).
- 3) Add 1ml substrate (starch solution) to sample tubes only. Vortex well.
- 4) Time carefully, allowing 3 min incubation per sample.
- 5) After 3 min add 2ml of Sumner (stop) solution. Vortex well.
- 6) To each blank tube add 2ml of Sumner solution followed by 1ml of substrate. Vortex well.
- 7) To the general blank add 2ml of working buffer, dH<sub>2</sub>O (in twice the amount of the sample volume), 4ml Sumner and 2ml of substrate. Vortex well.
- 8) Place rack in 100°C. water for 5 min.
- 9) Allow tubes to cool.
- 10) Read absorbance at 540nm.

##### c. Calculations

- 1) Activity = O.D. reading x dilution factor (units/g of tissue)
- 2) O.D. reading = (sample A + sample B)/2 - sample blank
- 3) Dilution factor = dil. when homogenized x dil. of supernatant x dil. when sample is added to buffer

APPENDIX II

TRYPSIN ASSAY

Trypsin (EC 3.4.21.4) assay modified from Gertler and Nitsan (1970).

1. Bring the water bath to 37°C. Warm up the spectrophotometer.
2. Prepare the following reagents:
  - a. TRIS BUFFERS
    - 1) .05M Tris (6.05g) + .02M CaCl<sub>2</sub> • 2H<sub>2</sub>O (2.94g) dissolved in 900ml H<sub>2</sub>O. Adjust pH to 8.2. Bring buffer volume up to 1000ml with dH<sub>2</sub>O.
    - 2) .05M Tris (0.61g) + .01M CaCl<sub>2</sub> • H<sub>2</sub>O (1.47g). Adjust pH to 7.2. Bring volume to 100ml. Used only to make a 1% enterokinase solution. See step 2d.
  - b. SUBSTRATE (prepare fresh daily)
    - 1) Dissolve 20mg BAPNA (N-Benzoyl-DL-Arginine pnitroanilide) in 1ml DMSO (Dimethyl sulfoxide). Mix in a small glass vial. Add to 50 ml of buffer 1 when fully dissolved.
  - c. STOP SOLUTION - 30% acetic acid.
  - d. .1% ENTEROKINASE (prepare fresh daily) - dissolve .1g Enterokinase in four drops of dH<sub>2</sub>O and add to 100ml of buffer 2. Used to activate pancreas samples only.
3. Sample preparation (note: keep samples cold at all times).
  - a. Homogenize in 10 vol. of cold dH<sub>2</sub>O (ex - 1g sample:9ml dH<sub>2</sub>O ).
    - 1) Cut samples into small pieces prior to homogenization.
    - 2) Place the sample in dH<sub>2</sub>O.
    - 3) Homogenize on ice, using short times; 20-30 sec. total. Clean all tissue from blades.
  - b. Centrifuge for 20 min. at 30000 G (16000 rpm).
  - c. Dilute supernatant<sup>1</sup> - 10X for pancreas, 5X for intestine cont. (1 - after storage if stored)
  - d. Store undiluted supernatant at -20 C for other enzyme assays.
  - e. Dilution series must be calculated for each new sample group for each enzyme assay. See procedures for serial dilution.
  - f. Activation (pancreas only)
    - 1) Mix equal volumes of diluted sample and .1% enterokinase solution.
    - 2) Incubate for 60 min. at 37°C.

#### 4. Procedure

##### a. Test tube setup

- 1) Label 3 glass tubes (2 sample and 1 blank) per sample. Do no more tubes than can be done in 30 min.
- 2) Label 1 general blank (for spec).

##### b. Steps

- 1) Add 0.5ml of Tris buffer to sample tubes.
- 2) Add 1ml of Tris buffer to sample blanks.(Add 2ml to general blank).
- 3) Bring tubes and activated samples to 37°C in water bath.
- 4) Add sample to all tubes except the general blank ( vol. determined by the dilution series).
- 5) Add 0.5ml substrate to sample tubes only (not blanks).
- 6) Vortex well and incubate for exactly 30 min. If activity is low incubate 60 min.
- 7) Add 0.5ml of acetic acid to all tubes.
- 8) To the general blank add 2ml of acetic acid followed by 2ml of substrate and dH<sub>2</sub>O: 4x the amount of the original sample used (ex - 4x50ul).

c. Vortex all tubes well.

d. Read absorbance at 410nm.

#### 5. Calculations

a. Activity = O.D. reading x dilution factor (units/g of tissue)

b. O.D. reading = (sample A + sample B)/2 - sample blank

c. Dilution factor = dil. when homogenized x dil. of supernatant x dil. when sample is added to buffer (Note: for pancreas samples there is also a 2x dilution due to activation)

APPENDIX III

CHYMOTRYPSIN ASSAY

Chymotrypsin A (EC 3.4.21.1) assay modified from Gertler and Nitsan (1970).

1. Bring the water bath to 37°C. Warm up the spectrophotometer.
2. Prepare the following reagents:
  - a. TRIS BUFFERS
    - 1) .05M Tris (6.05g) + .02M CaCl<sub>2</sub> • 2H<sub>2</sub>O (2.94g) dissolved in 900ml H<sub>2</sub>O. Adjust pH to 8.2. Bring buffer volume up to 1000ml with dH<sub>2</sub>O.
    - 2) .05M Tris (0.61g) + .01M CaCl<sub>2</sub> • H<sub>2</sub>O (1.47g). Adjust pH to 7.2. Bring volume to 100ml. Used only to make a 1% enterokinase solution. See step 2d.
  - b. SUBSTRATE (prepare fresh daily)
    - 1) Dissolve 20mg N-Acetyl L-tyrosin-4-nitroanilide in 1ml DMSO (Dimethyl sulfoxide). Mix in a small glass vial. Add to 50ml buffer 1 when fully dissolved.
  - c. STOP SOLUTION - 30% acetic acid
  - d. .1% ENTEROKINASE (prepare fresh daily) - dissolve .1g Enterokinase in four drops of dH<sub>2</sub>O and add to 100ml of buffer 2. Used to activate pancreas samples only.
3. Sample preparation (note: keep samples cold at all times).
  - a. Homogenize in 10 vol. of cold dH<sub>2</sub>O (ex - 1g sample:9ml dH<sub>2</sub>O ).
    - 1) Cut samples into small pieces prior to homogenization.
    - 2) Place the sample in dH<sub>2</sub>O.
    - 3) Homogenize on ice, using short times; 20-30 sec. total. Clean all tissue from blades.
  - b. Centrifuge for 20 min. at 30000 G (16000 rpm).
  - c. Dilute supernatant<sup>1</sup> - 10X for pancreas, 5X for intestine cont. (1 - after storage if stored)
  - d. Store undiluted supernatant at -20 C for other enzyme assays.
  - e. Dilution series must be calculated for each new sample group for each enzyme assay. See procedures for serial dilution.
  - f. Activation (pancreas only)
    - 1) Mix equal volumes of diluted sample and .1% enterokinase solution.
    - 2) Incubate for 60 min. at 37°C.



#### 4. Procedure

##### a. Test tube setup

- 1) Label 3 glass tubes (2 sample and 1 blank) per sample. Do no more tubes than can be done in 30 min.
- 2) Label 1 general blank (for spec).

##### b. Steps

- 1) Add 0.5ml of Tris buffer to sample tubes.
- 2) Add 1ml of Tris buffer to sample blanks.(Add 2ml to general blank).
- 3) Bring tubes and activated samples to 37°C in water bath.
- 4) Add sample to all tubes but the general blank (vol. determined from the dilution series).
- 5) Add .5ml substrate to sample tubes only (not blanks).
- 6) Vortex well and incubate for exactly 30 min. If activity is low incubate for 60 min.
- 7) Add 0.5ml of acetic acid to all tubes.
- 8) To the general blank add 2ml of acetic acid followed by 2ml of substrate and dH<sub>2</sub>O: 4x the amount of the original sample used (ex - 4x50ul).

c. Vortex all tubes well.

d. Read absorbance at 410nm.

#### 5. Calculations

a. Activity = O.D. reading x dilution factor (units/g of tissue)

b. O.D. reading = (sample A + sample B)/2 - sample blank

c. Dilution factor = dil. when homogenized x dil. of supernatant x dil. when sample is added to buffer (Note: for pancreas sample there is a 2x dilution due to activation)

## APPENDIX IV

### LIPASE ASSAY

Lipase (EC 3.1.1.3) assay modified from Nitsan *et al.* (1974).

1. Bring the water bath to 37°C. Warm up the spectrophotometer and cool the centrifuge rotor (size J14).
2. Prepare the following reagents:
  - a. PHOSPHATE BUFFERS (STOCK)
    - 1) X = 0.2M NaH<sub>2</sub>PO<sub>4</sub> (28.4g NaH<sub>2</sub>PO<sub>4</sub> in 1000ml dH<sub>2</sub>O)
    - 2) Y = 0.2M Na<sub>2</sub>HPO<sub>4</sub> • 7H<sub>2</sub>O (53.6g Na<sub>2</sub>HPO<sub>4</sub> • 7H<sub>2</sub>O in 1000ml dH<sub>2</sub>O)
  - b. PHOSPHATE BUFFER (WORKING) - 39X + 61Y + 100ml dH<sub>2</sub>O
  - c. TAUROCHOLIC ACID - 890mg / 100ml dH<sub>2</sub>O (store at 4°C)
  - d. SUBSTRATE (keep at 4°C at all times)
    - 1) Stock - 100mg 2-Naphthyl laurate / 100ml acetone (store in freezer)
    - 2) Working substrate - (make fresh daily)
      - a) 35ml dH<sub>2</sub>O + 10ml phosphate buffer.
      - b) Add 5ml stock 2-Naphthyl laurate very slowly to the above, using a burette. Keep the burette tip under the surface.
      - c) Add slowly enough for the cloudiness to dissipate once it appears. If 2-Naphthyl laurate comes out of solution (milky) begin again!
      - d) Only make enough standard for 12 samples. Remake throughout the day as necessary for all the samples being assayed.
  - e. COLOR REAGENT - 40mg O-dianizidine tetrazotized in 10ml of cold dH<sub>2</sub>O. (make fresh daily). Keep at 4°C at all times.
  - f. STANDARD - 10mg 2-Naphthol / 100ml dH<sub>2</sub>O (store at 4°C).
    - 1) Dissolve naphthol in 100 ml dH<sub>2</sub>O using a magnetic stirrer.
    - 2) Add up to 5 drops of 5N NaOH to help it dissolve.
    - 3) Be patient. It takes about 10 min. to fully dissolve. DO NOT HEAT.
  - g. 40% TCA (Trichloroacetic acid).
3. Sample preparation (note: keep samples cold at all times).
  - a. Homogenize in 10 vol. of cold dH<sub>2</sub>O (ex - 1g sample:9ml dH<sub>2</sub>O ).
    - 1) Cut samples into small pieces prior to homogenization.
    - 2) Place the sample in dH<sub>2</sub>O.

- 3) Homogenize on ice, using short times; 20-30 sec. total. Clean all tissue from blades.
- b. Centrifuge for 20 min. at 30000 G (16000 rpm).
- c. Dilute supernatant<sup>1</sup> - 10X for pancreas, 5X for intestine cont. (1 - after storage if stored)
- d. Store undiluted supernatant at -20 C for other enzyme assays.
- e. Dilution series must be calculated for each new sample group for each enzyme assay. See procedures for serial dilution.

#### 4. Procedure

##### a. Test tube setup

- 1) Label 3 centrifuge tubes (plastic) per sample; one sample, one standard and one blank.
- 2) Do not do more than 30 samples at a time.

##### b. Steps

- 1) Add .5ml Taurocholate to all test tubes.
- 2) Add sample to sample and standard tubes.(vol. determined by the dilution series)
- 3) Add .5ml buffer to standard tubes.
- 4) Add 1.3ml dH<sub>2</sub>O to standard and dH<sub>2</sub>O (in the same amount as sample used) to the blank tubes.
- 5) Add .2ml of 2-Naphthol to the standards only.
- 6) Bring to temperature in 37°C water bath. (Takes about 6 min).
- 7) Add 2ml substrate to sample and blank tubes. Vortex and then incubate for 10 min.
- 8) Add .5ml of color reagent to all tubes. Let sit for 2 min.
- 9) Add .5ml of TCA to all tubes.
- 10) Add 5ml of ethyl acetate (DO THIS UNDER THE HOOD). Invert each tube to fully mix the layers. Taking precautions not to cross contaminate from tube to tube.
- 11) Centrifuge 2000rpm for 10min
- 12) Read upper fraction at 540nm

##### c. Calculations

- 1) Activity = (O.D. reading x 20) / (standard O.D. x dilution factor)
- 2) Dilution factor = dil. when homogenized x dil. of supernatant x dil. when sample is added to buffer

APPENDIX V

ENZYME DILUTION DETERMINATION

### Enzyme dilution determination series.

It is essential to first determine the activity range (curve) of the of the enzyme at assay concentrations of substrate. From this data determine a dilution at which the enzymes activity will be measured for all samples collected during an sample day for an experiment. The dilution chosen should allow spectrophotometer absorbance readings in the .200 to .900 range. Use more then one sample to test range, try using one of each treatment or one from each of the most extreme treatments.

1. Samples will have 3 dilution steps. If an activation of sample occurs (ex. pancreas samples of proteolytic enzyme activated with enterokinase) this then increases the dilution steps to four.
2. Construct an initial dilution series with as wide a dilution factor (df) as possible eg 100, 500, 1,000, 5,000 and 10,000 df when the level of activity is unknown. If the approx. range of activity is known proceed to step 3.
3. Construct a dilution range within 1,000 df of the activity level expected. (eg expect activity at about 500 df, use a 100, 250, 500, 750 and 1,000 df).
4. First dilution occurs due to a homogenization of the samples in 10 vol. of cold dH<sub>2</sub>O. Stored supernatant is therefore 10 df.
  - a. Second dilution occurs when sample is either diluted 10 X for pancreas or 5 X for intestinal samples in dH<sub>2</sub>O.
  - b. Enterokinase activated samples have an additional 2 X dilution of the supernatant sample, activation prior to the normal second dilution step on 5.a.
5. Addition of diluted sample from step 4 a to test tubes results in a dilution due to addition of sample to buffer in test tube.

## VITA

Neil (Cornelius) P. O'Sullivan, the son of the late Eamonn O'Sullivan and H. Patricia O'Sullivan, was born January 18, 1963 in Cork, Ireland. He completed his secondary education at Coperwood College, Palliskenry Co Limerick, Ireland, in June of 1981. He graduated with a Bachelor of Agricultural Science Degree from University College Dublin, National University of Ireland, in 1986, and continued his studies at that institution under the direction of Prof. John McCarthy in Quantitative Genetics, receiving a Master of Agricultural Science Degree in January of 1989. In August of 1988 he was admitted to Virginia Polytechnic Institute and State University as a candidate for the Doctorate of Philosophy in Genetics from the Department of Poultry Science. Neil completing his studies under the direction of Dr. Paul B. Siegel in September 1991.

*Neil O'Sullivan*