ABSTRACT

LIN, YUN-MEI. Effects of Rearing Feeding Programs, Phosphorous, and Phytase on Female Broiler Reproduction Performance and the Effect of Phytase on Progeny Live Performance (Under the direction of Dr. John Brake).

Previous research compared sigmoid and linear feeding programs for Ross 308 broiler breeder females to 21 wk of age. The linear treatment, where the feed allocation at 6 wk of age was 43 versus 50 g/pullet/d for the sigmoid program, exhibited increased fertility. Ross 308 and 708 were bred for different purposes. The Ross 308 had been selected to produce a high number of eggs combined with good hatchability, while the Ross 708 had been developed to produce higher breast meat yielding broilers. Therefore, the present experiments in Manuscript I and II investigated the effect of feeding programs for Ross 708 broiler breeder females that were the primary flock type in North Carolina. In Manuscript I, the cumulative fertility and hatchability of the treatment with lower feed intake during early rearing was significantly increased as compared to the other two treatments. There was no difference in fertile hatchability or egg production. Feeding Ross broiler breeder pullets lower feed intake early in rearing as compared to higher feed intake produced an improved persistency of fertility. Manuscript II compared four rearing feeding programs with Ross 708 broiler breeders. The 4 female breeder feeding programs were termed high-high (HH), high-low (HL), low-high (LH), and low-low (LL) with feed/pullet/d at 6, 15, and 21 wk of age being (HH) 52-70-94, (HL) 52-64-94, (LH) 47.5-70-94, and (LL) 47.5-64-94, respectively. The LH feeding program exhibited the best fertility and hatchability, while the HH feeding program produced the poorest results.

Manuscripts III and IV investigated the effects of dietary available phosphorus (AvP) and phytase in broiler breeders and their broiler progeny. Manuscript III studied two levels of AvP (A-POS and B-NEG) in grower and layer diets and two levels of phytase (C-NEG+250 FTU

or D-NEG+500 FTU) “on top” of the low AvP treatment. The A-POS treatment and D-NEG+500 had the best overall fertility and total egg hatchability from 25 to 64 wk of age relative to B-NEG and C-NEG+250. Total egg weight was decreased by reduced dietary AvP and addition of phytase recovered the lost egg weight. Egg component results complemented the hatchability and fertility results, where the A-POS eggs had the greatest yolk:albumen ratio and thinnest egg shell and exhibited the best hatchability from 25 to 34 wk of age. Manuscript IV studied the live performance of the broiler progeny chicks that originated from the broiler breeder study in Manuscript III. These broilers were provided one of three diets, two levels of AvP and 1000 IU phytase “on top” of the lower AvP diet (CON, NC, and NC+1000) in a 4 breeder diet X 3 broiler diet design. No interactions of breeder and broiler dietary treatments were observed. The BW at hatching as affected by breeder diet was greater in the A-POS, and D-NEG+500, followed by C-NEG+250 breeder treatment relative to the B-NEG diet, which was expected due to a heavier initial egg weight as the recovery of egg weight due to phytase was reflected in chick weight.

Effects of Rearing Feeding Programs, Phosphorous, and Phytase on Female Broiler Reproduction Performance and the Effect of Phytase on Progeny Live Performance

by
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DEDICATION

This dissertation is dedicated to my Father Jeen-kuo Lin, my mother Jui-hui Tu, and my sister Yun-hsuan Lin.

BIOGRAPHY

Yun-mei Amy Lin, first daughter of Jeen-kuo Lin and Jui-hui Tu, was born and raised in Taipei, Taiwan. She received her bachelor degree in Animal Science from National Pingtung University of Science and Technology in 2009. In her junior and senior years as an undergraduate student, she joined the animal nutrition and physiology lab and participated in research on effect of antibacterial growth promoters and antibiotic-free alternatives on growth performances of broilers under the supervision of Dr. Chi Yu. With these experiences, she changed her mind about studying veterinary medicine after graduation, and she decided to move to North Carolina and pursue a MS in Poultry Science under the direction of Dr. John Brake. Her Masters research was focused on the effect of turning, preheating temperature, and incubation, and the degree was completed in 2011. After obtaining her MS she decided to continue her PhD in Poultry Science with a minor in Nutrition in the same institute. She worked on the effect of feeding programs during early rearing stages on the performance of broiler breeders, and the effects of phosphorous and phytase on the performance of broiler breeders and their progeny. She acquired experience in the various poultry production areas including hatchery, broilers, broiler breeders, feed milling, and processing.

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LIST OF ABBREVIATIONS

AdjFCR	Adjusted feed conversion ratio, corrected for mortality
AvP	Available phosphorus
BW	Body weight
C	Celsius
Ca	Calcium
cm	Centimeter
CP	Crude protein
d	Day
EW	Egg weight
F	Fahrenheit
FTU	Phytase units
g	Gram
h	Hour
HPGA	Hypothalamic-pituitary-gonadal axis
Kcal	Kilocalories
kg	Kilogram
m	Meter
ME	Metabolizable energy
min	Minute
NPP	Non phytate phosphorus
P	Phosphorus
RH	Relative humidity
wk	Week

LITERATURE REVIEW

Development of the Reproductive System of the Broiler Breeder Hen. Oviparity has been a characteristic of both avian and reptiles. While reptiles exhibit two functional ovaries, only the left ovary has normally been fully functional in avian species. Scientists have hypothesized that loss of one ovary decreased BW and was beneficial for efficiency of flight (Johnson, 2015). Similar to other species, the chicken embryo has been reported to possess two Müllerian (paramesonephric) ducts during early embryonic development. Anti-Müllerian hormone (AMH) was found to be expressed in both ovaries and caused the Müllerian duct to undergo apoptosis (Teng, 1987). However, estrogen from the left ovary has apparently protected the left Müllerian duct from regression (Teng, 2000, 2001). Avian gonads have originated in a thickened ventromedial surface of the embryonic mesonephros. During early incubation, the primordial germ cells (PGCs), precursor of the gametes, have been observed to migrate from the yolk sac membrane through the vascular system into the primordial gonads. By 3.5 day of incubation, the embryonic mesonephros (primordial gonad) has begun to differentiate into cortex and medulla. The ultimate destination and depth of the PGC migration has determined the ultimate differentiation of the gonad. PGCs located on the cortex have formed ovaries, while PCGs located in the medulla have led to the development of a testis (Stebler *et al.*, 2004). At fertilization, the genetic sex was determined with females being the heterogametic sex (ZW) and the male the homogametic sex (ZZ). However, in a manner somewhat similar to reptiles that express temperature dependent sex determination, altering the temperature during early incubation has changed the expression of certain genes and altered hormone related organ size and breast meat growth in birds (Stebler *et al.,* 2004; Smith *et al.,* 2009; Lin, 2010; Piestun *et al.,* 2013). At the time of hatching, oocytes would be expected to be mostly in meiotic prophase I, and organized into primordial follicles. In immature birds, the left ovary was composed of a

large quantity of small ova that can be observed with the naked eye, but these remained small and undeveloped until the bird reached sexual maturity. The oviduct developed quickly as the bird approached sexual maturity and became fully functional at the onset of egg production (Breneman, 1956; Joseph *et al.*, 2002).

Onset of Sexual Maturity in Broiler Breeder Hens. The onset of sex maturity in female poultry has been reported to be affected by numerous factors, such as nutrition (Dunnington and Siegel, 1983, 1984; Katanbaf *et al.*, 1989), environment management, patterns of BW gain, chronological age, body composition (Eitan and Soller, 2001), and time of feeding (de Avila *et al.*, 2003). Renema *et al.* (1999) and Zuidhof (2014) concluded that in order for birds to achieve puberty, 4 major biological thresholds must be met: 1) age, which was related to hypothalamic hormone system maturity; 2) composition of carcass, where birds required a certain amount of fat storage; 3) body weight; and 4) light stimulation. All of these factors interacted with the gonadotropin releasing hormone (GnRH-I) producing cells of the hypothalamus, where the GnRH-I hormone signaled the anterior pituitary gland through the hypothalamic-hypophyseal portal system. When the anterior pituitary received the GnRH-I hormone signal, it stimulated the secretion of numerous hormones that passed via the blood to the gonads and body to synchronize growth, development, and functioning of the reproductive system (Dunn *et al.*, 2009).

Body weight. For many years, research has used BW at the end of rearing as one evaluator of the success of the pullet feeding program for subsequent laying bird performance (Gous and Stielau, 1976; Balnave, 1984) but there has been doubt that the birds required a minimum BW for onset of sexual maturity. Grossman and Koops (1988) described growth as being in two phases, where 82% of the total BW was reached by the age of 12 wk, and maximized at 27 wk

of age. Kwakkel *et al.* (1993) conducted a multiphasic analysis of white leghorn pullet body growth and concluded that body growth was best fit by a tetraphasic growth model that estimated total asymptotic weight to be 1,669 g. While 70% of BW was reached by approximately 14 wk, and 80% was reached by 16 wk of age, only 8% of total BW was gained after 22 wk of age with this analysis. The first 16 wk posthatch represented the growth of skeletal development, feathering, muscle development, and other maintenance (supply) organs such as the gastrointestinal tract (Lilja *et al.*, 1985). Further, Bornstein *et al.* (1984) found the correlation between BW and age at first egg to be negative.

*Body Composition. S*exual maturity has been associated with the 16 to 22 wk period with an increased fat deposition in the abdomen also observed at this age. Growth of the reproductive system has been coincident with abdominal deposition of adipose tissue that indicated a physiological relationship between sexual maturity and fat due to sex hormone production (Kwakkel *et al.*, 1993). However, in cases where excessive feeding occurred, such as providing pullets with excess nutrients at photostimulation or before sexual maturity, greatly increased the rate of yolk deposition and the production of excess large yellow ovarian follicles leading to double yolk eggs that were not settable (Hocking *et al.*, 1987; Yu *et al.*, 1992; Renema *et al.*, 1999).

Nutrition. The amount of life long cumulative protein, energy, and fat has been reported to affect the development of the reproduction system. In order to support the persistency of sexual maturity, a minimum nutrient accumulation has been thought to be required to respond to photostimulation. Walsh and Brake (1997) estimated 1,200 g of CP at 20 wk of age to be the quantity for persistency of female fertility and 23,000 kcal ME at 20 wk of age was estimated to be the quantity for egg production (Brake, 2003).

Lighting. The purpose of a lighting program has differed between broilers and broiler breeders. Lighting has been used to manipulate feed intake and feed efficiency for broilers. In broiler breeders, lighting affects feed intake slightly, but has been used primarily to control sexual maturity. Birds normally have been found to be seasonal breeders, with the reproductive cycle affected by changes in day length. Day length signaled the hypothalamus and stimulated the gonadotropin releasing hormone (GnRH-I) that controlled ovulation in hens. Lewis *et al.* (2003) investigated the effect of two constant photoperiods, 11 h and 16 h, and two treatments with changed photoperiod length (8 to 11 h or 8 to 16 h) at 140 d of age. The birds that remained constantly on 11 h photoperiods achieved sexual maturity 22 d earlier than the birds that remained on the 16 h photoperiod. Sexual maturity for birds transferred from 8 to 11 h at 140 d was 10 d earlier than for birds that were constantly on the 11 photoperiod. Birds transferred to a 16 h photoperiod matured 35 d earlier than constant 16 h. Photoreceptors have been located in the hypothalamus, where they were stimulated by wave length, light intensity, and photoperiod to release the gonadotropins follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which control sexual maturity. Sexual maturity has been thought to be induced by light exposure during the photosensitive phase of the subjective night in the circadian rhythm cycle scheme of birds.

Egg Formation and Egg Components. The reproductive tract has been described as consist of 5 different regions that included the infundibulum, magnum, isthmus, shell gland, and vagina. During ovulation, the ovum was either captured by the infundibulum or was reabsorbed within 72 h within the body cavity. After entering the infundibulum, spermatozoa stored in the secondary sperm storage glands were released to fertilize the ovum in the infundibulum after which the first layer of albumen was formed. This process normally consumed 15 min. The

contractions of oviduct then moved the ovum into the magnum, which was the largest portion of the oviduct, where most of the albumen was formed. This required the ovum to reside about 2 to 3 h in the magnum. There have been three types of cells in the magnum identified as being responsible for protein secretion. First is the tubular glands regulated by estrogen that produced ovalbumin, lysozyme, and conalbumin. Then there are the ciliate cells and the goblet cells that synthesized avidin after stimulation by progesterone and estrogen (Tuohimaa *et al.*, 1989). The egg was next forced into the isthmus where the egg remained for 75 min. The isthmus has been described as having a thicker muscular layer but a less developed glandular tissue compared to the magnum. Shell membranes were formed in the isthmus and function as a barrier that prevented bacteria from entering the eggs. However, the shell membranes have been found to thin as the flock aged. The next step of egg formation occurred happens in the uterus (shell gland) where the egg resides about 18 to 20 h during which water and salts were deposited and the egg shell was formed. Shell formation has been described as a unique structure that protected the embryo from dehydration, bacterial infection, temperature fluctuation, allowed gaseous exchange, and provided a Ca source for embryo development. The final section of the oviduct was the vagina, from which the egg would be ejected. No egg formation function per se has been observed in this region (Bell, 2002). Egg components have included the yolk, albumen, the shell organic matrix, and the crystalline shell. Under normal circumstances, the yolk to albumen density, and portion of yolk to egg weight has remained uniform within species (Hincke *et al.*, 2008; Solomon, 2010). The yolk has functioned as a nutrient source for the embryo, as it contained about 36% lipid, 17% protein, and 47% water (Anton, 2007). The albumen comprised 4 layers, the chalaziferous layer attached to the yolk, the inner thin layer, the outer thick layer, and the outer thin layer. The function of albumen has been to serve as a

barrier to prevent microorganism infection, and provide water, protein, and minerals to the embryo.

Factors that Inhibit Optimal Reproduction Performance. *Genetic.* Broiler breeders and commercial laying hens have been bred for different purposes. Commercial laying hens have been genetically selected for high egg production and low BW. On the contrary, in the commercial broiler industry, selection for high BW and/or breast muscle yield has been major genetic objectives. According to Soller and Rappaport (1970), genetic traits such as BW gain and breast meat yield were negatively related to reproductive traits. Siegel and Dunnington (1985) found that selection for BW was negatively related to sexual maturity and fertility even as a result of short term selection breeding. A single line of chickens bred for both egg and meat traits has been generally considered to be difficult to achieve in the past, but improvements in management, lighting programs, nutrition, and feed restriction have kept the broiler breeder reproductive system relatively consistent. Although genetics and breeding programs have generally achieved a balance favorable among traits of broiler breeders to support growth and meat production as well as adequate egg production, broiler breeders still may have issues with poor persistency of fertility and hatchability.

Excessive feeding. Full-fed broiler breeders have been reported to gain excessive BW and deposit too much internal fat that interfered with fertility and egg production. Birds subject to ad libitum feeding tended to have two or more follicles maturing on the same day, resulting in the production of unsettable eggs such as doubled yolk eggs, misshaped, malformed, thin shelled, or membranous eggs (Hocking, 2009). The primary objective of restricted feeding of broiler breeders has been to restrict energy intake and produce leaner pullets that were smaller in size, required less nutrients for maintenance, not fat, and properly conditioned to respond to

photostimulation. Therefore, precise restricted feeding practices have been adapted for broiler breeder management.

Feed management. The hypothalamic pituitary gonadal axis (HPGA), which controls the avian reproduction system, has typically functioned normally in commercial egg-laying type hens. On the other hand, in broiler breeder hens selected for egg production as well as meat traits, the HPGA has often functioned in an abnormal manner if not carefully managed. Siegel and During (1985) concluded that BW was negatively correlated with sexual maturity and function. Thus, the broiler industry has used feed restriction as a tool in broiler breeder management in order to control reproduction function. Specifically, the objective has been to produce an orderly ovarian hierarchical follicular pattern by altering the activity of cGnRH-1 neurons. Broiler breeder feed management has been subject to ongoing research since the 1960s. Overall, two major feed restricting programs during rearing have developed and have remained in use by the industry. The first has been a type of skip-day feeding, where the feed was available on alternate days. This method has been used to allow birds to consume enough feed at once, to achieve a uniform feed intake, to decrease the aggressive behavior of the birds in the flock, and to improve uniformity of BW. This method evolved due to equipment being unable to deliver the small amounts of feed required for daily feeding during rearing. Advanced equipment has now made daily feeding possible (Bell and Weaver, 2002).

Body weight. BW has been reported to influence the egg mass of laying birds, with different size birds having various egg production patterns, such as larger bird producing heavier eggs and lighter birds producing smaller eggs (Leeson and Summers, 1987; El Zubeir and Mohammed, 1993). Pérez-Bonilla *et al.* (2012) separated Hy-Line brown laying hens into two different groups according to the BW at 21 wk of age: light (1,606±39 g), and heavy (1,733±48

g). These birds were placed on 4 diets with different energy concentrations (2,650, 2,750, 2,850, and 2,950 kcal of AMEn/kg) at photostimulation. Higher energy feed increased egg production and egg mass. Larger birds exhibited greater feed intake, produced larger eggs, and produced more egg mass than lighter hens, but the lighter hens exhibited better feed and energy efficiency. Renema *et al.* (1999) investigated ovarian morphology and plasma hormones of the Shaver Starbro broiler breeder. The flock was individually weighed at 21 wk of age, and 30 birds that weighed near the mean were selected as the standard BW group (1,995 g), while the low BW (1,639 g) and high BW (2,394 g) groups were selected from the lower and higher 20% of the flock. At photostimulation, the oviduct and ovary were similar in weight on a percentage of BW basis. Nevertheless, on an absolute weight basis, the high BW group had a significantly greater oviduct and ovary weight compared to the low BW group, while the standard BW group had a significantly greater ovary weight than the low BW group. At sexual maturity, the relative oviduct weight was greater in birds that were in the low BW group, but the ovary weight did not differ due to BW. The high BW group had the greatest plasma estradiol-17B hormone concentration at photostimulation and sexual maturity followed by the standard and low BW groups. Plasma LH and FSH did not differ due to BW.

Female Fertility and Sperm Storage. The presence of spermatozoa in the sperm storage/host glands in combination with normal ovulation in hens was facilitated a fertile egg production. Therefore, during discussions of fertility problems most people have believed this was due to the male breeder, and female fertility was easily neglected. Female breeders can lay fertile eggs for an average of 7 d after a single mating or artificial insemination (Leeson and Summer, 2002), but some hens can store spermatozoa up to a maximum of 30 d (Birkhead and Moller, 1993). The sperm storage function was a part of the species strategy of reproduction, and was

likely due to intense evolutionary selection such as spermatozoal competition when females mated multiple times during a short period (Parker, 1970; Birkhead and Moller, 1992). Some researchers have believed the spermatozoa storage region was able to provide nutrients and keep the spermatozoa active for an extended period (Smith and Yanagimachi, 1990; Krishna, 1997) without decreasing the number of spermatozoa. In birds, spermatozoa were stored in a blind-ended sperm storage tubules (SST) located near the utero-vaginal junction (Zavaleta and Ogasawara, 1987). When the spermatozoa left the STT, it moved towards the infundibulum for fertilization (Holt, 2011). The percentage of stored spermatozoa used in fertilization was relatively low in birds. Birkhead and Moller (1993) reported that hens may receive 100 million spermatozoa during mating, but less than 1 million would be stored in the SST, and only a few thousand would actually leave the SST and reach the secondary spermatozoa storage site higher in the reproductive tract where they would be available for fertilization.

Thomas and Wishart (1991) and Ashizawa *et al.* (1993) reported that the spermatozoa and the SST epithelial cells become immotile at 41°C, which was the hen's normal body temperature. However, this temperature-induced immotility could be reversed by addition of 2 μm calcium by the phosphatidylinositol 3-kinase signaling cascade (Ashizawa *et al.*, 2009). This immotile reaction may be why spermatozoa were able to survive a prolonged period of time in the female reproductive tract. Bramwell (1996) reported the fertility decline associated with hen age shown in Table LR-1. The fertility decline was due to the number of spermatozoa that penetrated the germinal disc and the perivitelline layer. The ability of spermatozoa to penetrate was related to the spermatozoa receptors on the surface of the germinal disc that may decline in number or efficacy as the hen aged. McDaniel *et al.* (1995) also observed the fertility decline due to spermatozoal penetration when the perivitelline layer was affected by heat stress. These

investigations illustrated factors that may have affected spermatozoa storage and fertility. However, these did not clearly explain how the spermatozoa survive in the STTs nor the mechanism of fertility.

Table LR-1. Broiler breeder fertility and spermatozoa penetration holes as affected by flock age. [1]

Flock Age	Fertility	Spermatozoa penetration holes
(Wk)	(%)	(n)
27	-	113
30	86	112
33	92	108
36	93	127
45	95	117
56	84	60

[1]Adapted from Bramwell *et al.* (1996).

Relationship of Feed Intake and Ovulation Control. The body has been reported to be able to sense energy status and adjust its metabolic status according to a variety of signaling molecules including neuropeptides, hormones, nutrients, and metabolites that were produced by peripheral and central nervous system (CNS) tissues. For example, leptin has been produced in adipose tissue in proportion to the size of the adipose tissue. These signals were received by the energy sensing system available in the peripheral and CNS tissues, so that the body knew the amount of energy that was stored. A list of peptide signals associated with feed intake regulation and energy metabolism are shown in Table LR-2. The orexigenic hormones were responsible for stimulating appetite (anabolic), while the anorexigenic hormones inhibited appetite (catabolic). Mammals and birds may share the same hormonal signal, but function of each molecule may serve different purposes (Richards and Proszkowiec-Weglarz, 2007). After these signals have been released, receptors received the signals and adjusted metabolism pathways. Two well studied energy sensor systems will be discussed here. The AMP-activated

protein kinase (AMPK), which was a serine-threonine kinase, was activated when the cell depleted its energy level (ATP, adenosine triphosphate). When AMPK was activated, it promoted energy synthesis pathways (catabolic) including glucose uptake, glycolysis, fatty acid oxidation, and mitochondrial biogenesis, while the energy consuming activities (anabolic) such as protein synthesis, glycogen synthesis, gluconeogenesis, and fatty acid/ cholesterol synthesis were inhibited (Hardie *et al.*, 2006). The other energy sensor system was the mammalian target of rapamycin (mTOR), which was also a serine-threonine kinase complex that received signals from nutrients and hormones. Both mTOR and AMPK have responded to changes in energy reserve, but have opposite functions in terms of controlling metabolic activities. Unlike AMPK, mTOR was activated during leveled energy status (Richards and Proszkowiec-Weglarz, 2007).

Ovulation functions were known to be associated with the development of the ovary under the control of the hypothalamic-pituitary-gonadal axis (HPGA) at two levels. One was termed the "long-term" response, and was affected by the environment and nutritional status of birds, such as change in photoperiod and food availability. This level of response was majorly controlled by the gonadotropin-releasing-hormone neurons type 1 (GnRH-1) that originated outside the brain and migrated into developing organs but was ultimately located in the hypothalamus (Schwanzel-Fukuda and Pfaff, 1989; Wray *et al.*, 1988). The second level of response was termed the "short- term" response that occurred in the pituitary. The GnRH-1 receptors on the pituitary were activated by GnRH-1 and stimulated the release of follicle-stimulating hormone (FSH), and luteinizing hormone (LH) (Fraser and Sharp, 1978). Chronic or short term feed restriction have been used in broiler breeder management to control reproductive functions. The feed restriction decreased the secretion of GnRH-1, which resulted in producing less FSH

and LH, and ultimately produced an orderly pattern of ovarian growth (Lal *et al.*, 1990; Contijoch *et al.,* 1992). The peptide signals shown in Table LR-2 interacted with the GnRH-1 neurons in different ways, and controlled the release of GnRH-1, and neuronal activities according to the energy status of the animal (Klenke *et al.,* 2010).

Effect of Restricted Feeding on Physiological and Metabolic Changes in Female Broiler Breeders. Studies on the effect of restricted feeding on physiological and metabolic changes in the female broiler breeders have been very limited. Bruggeman *et al.* (1998) reported comparing the effect of *ad libitum* feeding, restricted feeding, and restricted feeding to maintain intermediate BW of Hybro G female broiler breeder. Ovary and oviduct development were delayed in the pullets that were restricted fed when compared to the intermediate BW and *ad libitum* feeding treatments. The GnRH-1 (the paper referred to chicken luteinizing hormone-releasing hormone-I, cLHRH-1) concentration in the median eminences was significantly greater in the *ad libitum* feeding treatment from 8 to 24 wk of age, as was the pituitary LH concentration. The pituitary FSH concentration did not show any

differences until 18 wk of age, which indicated that FSH was only partially regulated by GnRH-1. Surprisingly, the LH and FSH concentration in the plasma did not show any differences from 14 to 24 wk of age. The author believed that the result was probably due to the timing of sampling that may differ from when the bird released LH.

Table LR-2. Neuropeptides affecting feed intake in birds. [1]

Orexigenic [2]	No effect	Anorexigenic [3]
Neuropeptide Y	Melanin concentrating hormone [5]	A-Melanocyte stimulating hormone
Agouti-related peptide	Orexins [5]	Cocaine and amphetamine regulated transcript
Peptide YY [4]	Motilin [5]	Corticotropin-releasing hormone
Pancreatic polypeptide [4]		Ghrelin [5]
		Cholecystokinin
		Bombesin
		Glucagon-like peptide
		Gastrin
		Urotensin I/ Urocortin
		Neuromedin U/S

[1]From Wayne (2015). Data reported in Furuse (2002), and Richards and Proszkowiec-Weglarz (2007).
[2]The orexigenic hormones stimulates appetite.
[3]The anorexigenic hormones inhibits appetite.
[4]In mammals, the peptide decreases food intake.
[5]In mammals, the peptide increases food intake.

Function of Phosphorus in Poultry. Phosphorous (P) has been one of the most widely studied minerals in animal nutrition due to its wide range of physiological functions in the animal body. The functions enumerated in the literature have included bone formation and maintenance. About 85% of the P in the body was located in the skeleton in the form of a calcium phosphate salt called hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] (Ternouth, 1990; Heaney, 2012). The remaining 15% was either located in cells and extracellular fluid or in blood as organic phosphate compounds and the common inorganic pentavalent form of P (PO_4^{3-}). P

was involved in muscle tissue and egg formation where phospholipids were a major component of the yolk and major structural component of cell membranes. Information coding and components of nucleic acid such as DNA and RNA required P (Karn, 2001). P had acted as a major intracellular buffer in the body and was involved in acid base balance as it acted as a buffer in its salt form (Cromwell *et al.*, 1995; Leeson and Summers, 2001). All energy production and storage have been shown to be dependent on phosphorylated compounds, such as adenosine triphosphate (ATP) and phosphocreatine (Karn, 2001). P was also involved as both a component and activator of enzyme systems. P regulated enzyme systems by phosphorylation of catalytic proteins. A number of enzymes, hormones, and cell-signaling molecules have been reported to depend upon phosphorylation for their activation.

Source of Phosphorus and Bioavailability in Poultry. Typical P sources in animal feeds originate from plants that become animal feed ingredients and inorganic P that can be supplemented when the feed ingredient P level does not meet the requirement of the birds (Van der Klis and Versteegh, 1996; Waldroup, 1999). Plant source P in feed ingredients have been organized into two categories, the organically bounded P that were in the form of phytic acid (PP) and other forms (nPP) (Waldroup 1999). Phytic acid, also known as inositol hexakisphosphate (IP6), inositol polyphosphate, or myo-inositol 1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate, and phytate when in its salt form. In the past, one-third of the P in plant source feed ingredients was assumed to be available to monogastric animals due to the fact that two thirds of the P in plant source feed ingredients was in the form of phytate. Phytate was the main storage form of P in most grain ingredients used in feed, possessing approximately 28.2% of bound P (Ravindran *et al.*, 1995). However, the bioavailability of P could be affected by various factors. For example, with feed ingredients the P bioavailability for poultry in

cereals such as corn, barley, rye, wheat, rice, and sorghum ranged from 48 to 73%, 48 to 79% in rice and wheat, and 40 to 65% for oil-seed meals such as soybean meal, cottonseed meal, and rapeseed meal (Soares, 1995). The presence of minerals and vitamins in excess may have affected the absorption of P. High calcium interacted with PO_4 forming insoluble complexes, and magnesium interacted with P to form complexes that reduced the absorption of both P and magnesium. The presence of vitamin D increased blood absorption and bone reabsorption of P (Edwards and Veltmann, 1983). In order to compensate for the low P level in plant source ingredients, inorganic P sources have been added to the diet to meet the nutritional requirement of poultry. The common supplemental P sources in animal feed have been dicalcium phosphate (22% Ca and 18.5% P), monocalcium phosphate (16% Ca and 21% P), and defluorinated phosphate or tricalcium phosphate [$Ca_3(PO_4)_2$] (33% Ca and 18% P). Dicalcium and monocalcium phosphate have been the two most frequently used sources of inorganic P in poultry feed (Newman and Leeson, 1997).

Issues of Phytate and Excess Phosphorous. Phytate, the major form of P stored in plants has acted like a chelating agent, where it interacted with other minerals such as calcium, magnesium, zinc, iron, copper, (Cheryan and Rackis, 1980; Maenz *et al.*, 1999; Maenz, 2001), amino acids (De Rham and Jost, 1979), and starch (Ravindran *et al.*, 1999). Phytate also constrained the activities of other digestive enzymes for example, protease, amylase, and trypsin (Liu 1998; Mate 2002; Lin, 2005; Isaksen, 2006). Numerous investigations have shown that phytate negatively affected growth and performance of animals. The anti-nutritional effect of phytate was demonstrated in broilers, where energy and amino acid digestibility declined with phytate inclusion level in the diet (Ravindran *et al.*, 2006). The lack of sufficient endogenous phytase combined with low absorption of P in animals has led to the excess P

being excreted in the feces. Chicken manure normally contained feces, urine, and bedding materials that were rich in nitrogen and P that can be used as fertilizer after decomposition. However, high density animal production has created a number of areas of high concentration of manure. Poultry manures on average supplied two to three times more P than plants require, which has raised concerns about the accumulation of P in soil, and pollution of surface water during runoff (Sharpley, 1996; Mikkelsen, 2000; Sims *et al.*, 2000). P has supported the growth of algae and aquatic plants that were the food source and habitat for a number of aquatic organisms. However, presence of excess P in water systems may cause over population of algae (toxic and non-toxic) and aquatic plants, which may result in water toxification and eutrification that significantly reduced or eliminated oxygen in the water. Lack of oxygen in the water has caused illness or death of fish and aquatic organisms in large quantity. Due to these environmental concerns about excess P pollution, and the fact that poultry and other animal production industries were considered to be one of the major P contributors to the environment, research has investigated methods to reduce the amount of P released into the environment. The United States, China, Morocco, and Western Sahara have remained the major phosphate rock production regions around the world, and the U. S. has imported 2.0 to 3.4 million metric tons of phosphate rocks annually since 2009 (Jasinski, 2015). The Morocco and Western Sahara region has been the major phosphate rock export region in the world, and the pricing of their phosphate rock from 2005 to present is presented in Figure LR-1. From 2006 to 2007, the phosphate rock cost increased by 68%, which was about $51/MT. The greatest price fluctuation within the decade happened in 2008, when the price went up to about $430/MT. The fluctuation of price was affected by increased agriculture demand and tight supplies of phosphate rock. Due to the limited quantity of P resources, P has become one of

the most expensive feed ingredients in animal feed (Summers, 1997). In poultry production, feed accounts for 60 to 75 % of the total production cost from hatching to the processing plant (Donohue and Cunningham, 2009). Thus, researchers had been focused on ways to increase the utilization of nutrients in the birds to minimize cost of production while finding a solution to prevent P pollution of the environment.

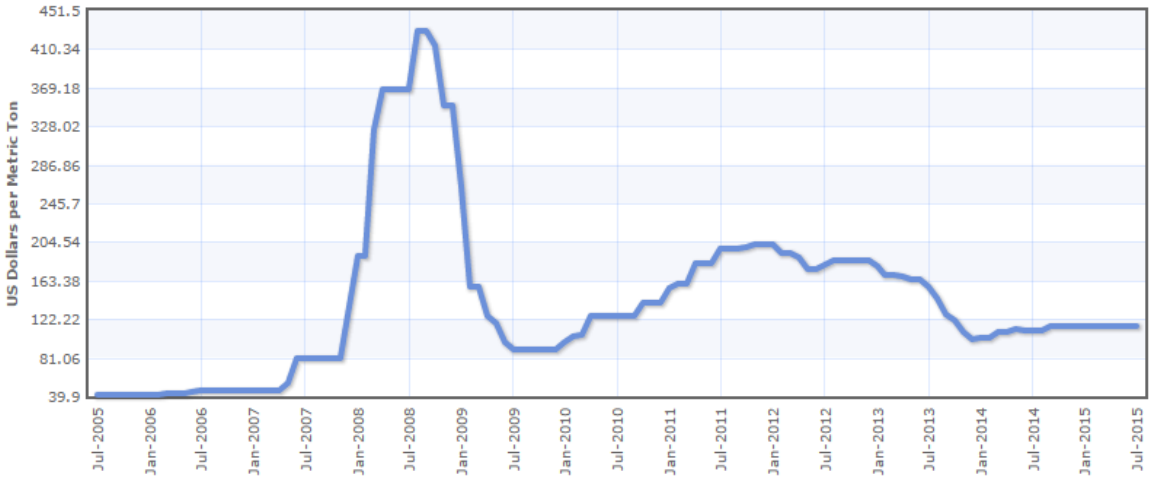

Figure LR-1. Price of phosphate rock ($ /MT) from Jul 2005 to Jul 2015.

Phytase Functions, Source, Nomenclature, and Categorization. Humans have been reported to be relatively efficient in utilizing phytate-P and can absorb about 50% of phytase-P. On the other hand, poultry and swine have been found to be relatively poor in utilizing phytate-P due to a lack of efficient endogenous phytase secretion required to hydrolyze the carbon-phosphate bonds on the myo-inositol ring (McCuaig *et al.,* 1972). Further, these animals were typically fed corn-soy based diets containing over 60% of P in the form of phytate that was not available to the animal. There were four major sources of phytase that we needed to consider when investigating the effect of additional phytase in the diet. Phytase from plant source feed

ingredients or phytase from both gut microfloral and mucosa in the animal that were normally referred to as endogenous enzyme, and addition of phytase products that were normally of microbial or fungal origin that were termed exogenous microbial phytase (Greiner *et al.*, 1997; Sandberg and Andlid, 2002). The plant source feed ingredients such as wheat, wheat bran, rye, and barley have high quantities of phytase (Eeckhout and de Paepe, 1994). Phytic acid was the major storage form of P in many seeds and pollen and was formed during seed and pollen maturation and represented 60–90% of total P in dormant seeds and served to prevent germination during unfavorable conditions (Maga, 1982; Reddy *et al.*, 1982). During germination of seeds, phytate has reported to be hydrolyzed so that stored P and other minerals were released for germination and development of the seedling. Therefore, phytase was required for mobilizing these P reserves for the growing seedling (Mandal and Biswas, 1970; Walker, 1974) as well as pollen germination (Helsper *et al.*, 1984; Jackson and Linskens, 1982). The phytase secreted by intestinal microbes and mucosa in the animal was mainly present in the large intestine (Selle and Ravindran, 2007). Although mucosal phytase activity in poultry was low, there have been studies that reported broilers seemed to have an adaptive capacity to increase mucosal phytase activity in the intestine when P was not adequate in their diets (McCuaig *et al.*, 1972). Tamim *et al.* (2004) found a similar result where 69.2% of ileal phytate were hydrolyzed in 22-d-old broilers fed a non-phytase supplemented corn-soybean meal diet. According to Zhang *et al.* (2005), the capacity of chicks to degrade phytate can be inherited. The gut microfloral phytase had been studied in swine and large amounts of phytate were found in the digesta in the lower part of the intestine (Seynaeve *et al.*, 2000; Schlemmer *et al.*, 2001). In contrast to pigs, fewer investigations have been performed concerning gut

microfloral phytase in poultry. However, Kerr *et al.* (2000) reported that microorganisms in the poultry crop were very likely to contribute to phytate hydrolysis.

In order to reduce the environmental impact of animal production operations, reduce feed cost, and better utilize P in the feed ingredients, products containing microbial phytase of microbial or fungal origin have been developed, investigated, and commonly included in diets of poultry and swine (Angel *et al.,* 2002; Applegate *et al.,* 2003). By adding these exogenous microbial phytase products, there should be a theoretical reduction in the use of inorganic P level in the diet, which ultimately decreased the mineral content in the feces (Kerr *et al.*, 2010). The function of these exogenous microbial phytases has been to hydrolyze phytate and increase the digestion of P, which consequently decreased the excretion of P and reduced environment pollution, as has been well documented. Due to the phytate characteristics that interacted with cations, protein, and energy, the function of phytase were not focus only on Ca and P. The use of phytase as a tool to improve amino acid and energy utilization by diminishing the anti-nutritional effects of phytate has been recognized but has required extensive investigation.

Phytase has been categorized by the position of first hydrolysis on the inositol ring and according to its most favorable pH (Kumar *et al.*, 2010). The International Committee of Biochemical Nomenclature has classified phytase according to the location that started the hydrolysis of the phytate molecule, the most common classification were 3-phytases (EC 3.1.3.8) or 6-phytases (EC 3.1.3.26) (Selle and Ravindran, 2007). Phytase that initiated dephosphorylation of phytic acid at position 3‘ of the inositol ring were largely from microbes. A phytase that initiated the dephosphorylation of phytic acid at position 6‘ of the inositol ring were generally from plant sources (Cosgrove, 1966). Exceptions such as phytase in soybean that were 3-phytases and E. coli phytase that were 6-phytases have been identified (Sandberg

and Andlid, 2002). Another categorization has been based on the most favorable pH of the phytase with acidic phytase having an optimal pH range between 3.0 and 5.5, and alkaline phytase having a range between 7.0 and 8.0 (Yin *et al.*, 2007; Vijayaraghavan *et al.*, 2013).

Phosphorus Terms and Requirement in Broiler Breeders. In order to understand the requirements of P for animals, one must be able to distinguish terms and forms of P. Angel (2011) developed a list of P forms with their definitions and descriptions. Total P (tP) was the broadest term of all and included all forms of analyzed elemental P, and the availability of P to the animal was not enumerated. Available P (AvP), also known as relative bioavailable P, was the term used when referring to the P that originated from feed and was absorbed and available at tissue level for the animal. This term was the second most commonly used term to express the quantity of P in feed ingredients as well as nutritional requirements for poultry. Inorganic P (iP), was the P that was not bound to an organic molecule. Phytate P (PP or Phytate P) referred to the organic form of P that was attached to the six carbon ring phosphorylated cyclic sugar alcohol form called myo-inositol 1,2,3,4,5,6-hexakis dihydrogen phosphate, commonly known as phytate or phytic acid. Non-phytate P (NPP), has referred to the P that was not bound to phytate (NPP = tP - PP). This was the most commonly used term for expressing the requirements in poultry nutrition (NRC, 1994). Since Ca and P have been demonstrated to be highly interactive, the requirement of P for poultry had to be considered in conjunction with the requirements of Ca. The standard recommendations for P in broiler breeder starter and grower diets have been 0.9 to 1.0% Ca and 0.45 to 0.50% NPP at a 2:1 Ca:P ratio, and 2.5% Ca with 0.23% NPP at a 11:1 Ca:P ratio at peak egg production (NRC, 1994). However, this suggested requirement was established two decades earlier than the present research. The lines of birds used at present were different from those of the past. Due to

variations in management recommendations, feeding programs, and production goals most genetic companies have established their own Ca and NPP recommendations for specific genetic lines of birds as shown in Table LR-3.

Table LR-3. Recommended Ca and NPP levels for different broiler breeder female strains based on a 2,800 kcal metabolizable energy/kg diet[1].

Strain	Phase				
	Starter	Grower	Pre-Breeder	Breeder I	Breeder II
	Ca, NPP (%)				
Arbor Acres Plus	1.00, 0.45	0.90, 0.42	1.20, 0.35	3.00, 0.35	3.20, 0.32
Ross 308 / 708	1.00, 0.45	0.90, 0.42	-	3.00, 0.35	3.20, 0.32
Cobb 500 / 700	1.00, 0.45	1.07, 0.44	1.47, 0.44	2.93, 0.44	3.13, 0.39

[1] Aviagen, Inc., 2013abc; Cobb-Vantress 2013abc.

Effect of Dietary Phosphorous and Phytase on Broiler Breeder Performance. Wilson *et al.* (1980) reported that diets containing 0.41% total dietary P exhibited the best hatchability and egg production rate during 28 wk of production, while 0.36% and 0.31% total P were evidenced by lower performance. Berry *et al.* (2003) reported that egg production was maintained in a diet without any inorganic P with 0.1% AvP with supplementation of phytase at 300 FTU/kg. Plumstead *et al.* (2007) also suggested that 0.1 % NPP with phytase to achieve 0.22% AvP maintained egg production. Ekmay and Coon (2009, 2010) reported that egg production and egg quality were not affected when dietary NPP was reduced to 0.2%. However, when NPP was reduced to 0.15%, production was impaired to 40 wk of age. The research further suggested the NPP requirement for egg production was above 0.15% NPP. The same research group produced different data later when they compared breeder diet NPP levels of 0.15% versus 0.40%, and found that egg weight and egg production were decreased in conjunction with increased female breeder mortality (Ekmay *et al.*, 2012).

Effect of Dietary Phosphorous and Phytase in Broiler Breeder Diets on Broiler Development. Harms *et al.* (1964) suggested that maternal P supplementation had limited effect on progeny tibia ash. Ekmay and Coon (2010) found varying the NPP level from 0.4 % to 0.2% did not affect progeny BW or tibia ash. These results were repeated later, which no differences being observed for tibia ash when comparing breeder diet NPP levels of 0.15% versus 0.40% (Ekmay *et al.*, 2012). Triyuwanta *et al.* (1992), on the other hand, reported that the progeny of dwarf broiler breeder hens fed 0.2, 0.6, or 1.0% AvP exhibited an initial tibia breaking strength that was strongly positive correlated with the AvP level of the maternal diet, but the effect did not persist as the progeny aged.

Effect of Dietary Phosphorous on Egg Shell Formation. Egg quality can be easily affected by feed composition, in that dietary P and Ca have been found to have direct effects on eggshell quality. P and Ca were stored in the hydroxyapatite crystal form in bones. When insufficient Ca was provided in the feed to meet eggshell formation needs, Ca was mobilized from the bone also released P into the blood. A high P intake has increased the P concentration of the blood, which inhibited bone Ca release and ultimately decreased eggshell quality (Miles *et al.*, 1983). However, Panda *et al.* (2005) investigated the effect of dietary NPP levels of 0.15, 0.18, 0.21, 0.24, 0.27, and 0.30% on egg shell quality in white leghorn layers, and found no effect of NPP on egg shell weight, shell thickness, shell strength, or specific gravity.

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MANUSCRIPT I. Effect of Female Feeding Program during Rearing on Ross 708 Broiler Breeder Reproductive Performance

ABSTRACT

Previous research has compared sigmoid and linear feeding programs for Ross 308 broiler breeder females to 21 wk of age. In this previous research, female BW at 4, 6, 8, 10, and 12 wk of age was increased by the sigmoid program while the line feeding program increased female BW at 40, 48, and 56 wk of age. The sigmoid feeding program decreased female mortality during hot weather, which resulted in increased hen-housed egg production without differences in hen-day production. However, the linear treatment, where the feed allocation at 6 wk of age was 43 versus 50 g /pullet/d for the sigmoid program, exhibited increased persistency of fertility. In the present research, day-old chicks were fed a single starter diet with 17.5% crude protein (CP) and 2.9 kcal ME/g to 6 wk of age. This was followed by a 14.5% CP, 2.795 kcal ME/g crumbled growing diet to 26 wk of age. The layer diet was fed from 27 to 64 wk of age and the diet was formulated to contain 2.8 kcal ME/g and 14.5 % CP. The experiment involved two feeding programs with feed/pullet/d at 6 wk being 50, and 42.2 g. All birds were fed in a similar manner after 19 wk of age. The cumulative fertility and hatchability of the 42.2 g group was significantly increased as compared to the 50 g other treatment. There was no difference in fertile hatchability or egg production. Feeding Ross broiler breeder pullets approximately 42 g/pullet/d at 6 wk of age produced an improved persistency of fertility.

Key words: broiler breeders, pullet feeding program, sigmoid feeding, hatchability, fertility

INTRODUCTION

Although broiler breeders have been selected for both meat and egg traits, they have become unable to self-regulate their feed intake to closely match their energy requirements for maintenance, growth, and reproduction (Richards *et al.*, 2010). It has been well documented that selection for BW and muscle traits was negatively correlated with the onset of sexual maturity and reproductive performances of broiler breeders as evidenced by negative effects on age at sexual maturity, body composition, egg size, egg production, ovarian morphology, and multiple ovarian hierarchies (Hocking *et al.*, 1987, 1993; Joseph *et al.*, 2002; deBeer and Coon, 2007). Although genetics and breeding programs have widely improved favorable reproductive traits of broiler breeders, broiler breeders still have issues of being unable to maintain the persistency of fertility and hatchability. The hypothalamic-pituitary-gonadal axis (HPGA) that controls the reproductive system in egg type birds has been reported to exhibit less frequent abnormal functions when compared to meat type birds such as broiler breeders. For broiler breeders, the tools that have been available at this point to sustain the flock reproductive performance were management of environmental factors such as lighting, nutrition, feeder space, and feed program management. Researchers have found that female broiler breeder fed *ad libitum* exhibited high mortality, lower production, and decreased hatchability when compared to birds that were restricted fed (Hocking, 2002). Appropriate broiler breeder hen BW control has been demonstrated to improve control of ovarian function. Birds that were fed on an *ad libitum* basis frequently exhibit two or more follicles maturing on the same day, resulting in the production of unsettable eggs such as doubled yolk, misshaped, malformed, thin shelled, or membranous eggs (Hocking, 2009). Therefore, feed restriction has become an obligatory practice for the commercial poultry industry. Leksrisompong (2010)

investigated two feeding programs, a sigmoid and a line with Ross 308 pullets. The cumulative nutrient intake to 21 wk (147 d) for these two treatments was the same (25.7 Kcal of ME and 1,337 g of CP). However, small but significant differences were found in BW, where the sigmoid treatment had a greater BW from 4 to 12 wk of age. Hen-day production was the same (67.7%) for both treatments but fertility was greater for the linear treatment (94.0 vs 92.5%). The results indicated that the birds that consumed less feed early in the rearing period were more fertile that their full-fed counterparts. The degree to which differential feeding programs affected fertility in different genetic lines has not been fully investigated. Therefore, the objective of this study was to investigate the effects of feeding programs on Ross 708 females since this was the primary female line used in commercial production in N.C. and the southeastern U.S.

MATERIALS AND METHODS

Broiler Breeder Rearing Management. The experimental protocol used in this study was approved by the North Carolina State University Institutional Animal Care and Use Committee. A total of 1840 1-d-old female Ross 708 and 200 male Ross 344 broiler breeders (Aviagen, Huntsville, AL) were raised in an enclosed fan-ventilated 16-pen litter floor rearing house with 8 (40.1 m^2 area) pens for females and 8 (6.1 m^2 area) pens for males. At placement, there were 230 females and 28 males placed in each female and male pen. All females and males were permanently identified with a neck tag at placement. The litter temperature was 35°C (95° F) for the first 2 d and the ambient temperature was 29.4~32.2 °C (85~90° F) through 7 d of brooding. Each female rearing pen was initially equipped with 12 tube feeders (DH-4; Kuhl, Flemington, New Jersey). Each feeder pan had a circumference of 132 cm. At placement, the brooding area of all female pens had 7 feeder lids along the barrier and pen wire, and 4

tube feeders, while all male pens had 4 feeder lids along the barrier and pen wire, and 1 tube feeders. At 14 d of age, all feeder lids were removed and 6 tube feeders were employed in the female pens at this time. Then the number of feeders in the female pens were adjusted to 8 feeders at 8 wk of age, 10 feeders at 10 wk of age, and 12 feeders at 15 wk of age. Two tube feeders were used in the male pens at all times. Water was typically available for 6 h daily and limited by a time clock and solenoid system to allow the birds to have unlimited access to water while feed was present and a similar amount on non-feed days during rearing. The floor was covered with new wood shavings. After 6 wk of age, grower diets were fed. The house was ventilated to maintain ammonia levels below 25 ppm but not totally absent.

Pullet and Breeder Diets. Day-old chicks (females and males) were fed a single crumbled starter diet with 17.0% crude protein (CP), and 2.91 kcal/g to 6 wk of age. This was followed by a 15.55% CP, 2.91 kcal ME/g crumbled growing diet to 27 wk of age. The layer diet was fed from 28 to 64 wk of age. The diet was formulated to contain 2.91 kcal ME/g and 14.5 % CP. Table I-1 shows the formulation of the starter, grower, and layer diets. All diets were manufactured by Southern States (Cleveland, NC).

Feeding Programs. Two feeding programs were applied in this experiment, with the treatment code reflecting the 6 wk feed intake. The A50.0 pullets were fed 50.0 g of feed per bird per day at 6 wk of age. The B42.5 pullets were fed 42.5 g of feed per bird per day at 6 wk of age. The female feeding programs during rearing are shown in Figure I-1. Females were fed 4/3 to 12 wk, 5/2 to 21 wk, and daily thereafter.

Broiler Breeder Laying Management. At 21 wk of age (148 d), all males and females were moved on the same day and mixed in an 8-pen laying house (40.1 m^2 total area/pen, 13.4 m^2 litter area/pen). There were 20 males and 190 females mixed per pen. There were 10 tube

feeders with special grills for females (sixteen 4.8 x 5.8 cm holes per pan) and 2 tube feeders for males in each pen, each with a diameter of 29 cm. The special grill was used to prevent the non-dubbed males from accessing the feed. Water was limited to 8 h per day during the laying period. Eggs were collected and recorded twice daily before being stored in an egg cooler prior to incubation. Birds were photostimulated with 14 h of light when moved at 21 wk of age. The day length was subsequently increased to 15 h 10 d later, to 15.5 h at 5% production, and finally to 16 h at 50% production. Natural light entered the slat-litter house through open or translucent curtains during normal daylight hours. Supplemental light provided an average intensity of 35 lux at bird head level using 100 W fluorescent and 50 W HPS light bulbs when natural light was not present.

Body Weight. All birds were group weighed by pen at placement. The females were group weighed in a random group of 25 females per pen at 2, 3, 4, 5, 7, 8, 12, 16, 20, and 22 wk of age.

Egg Production and Hatchability. Nest eggs were collected twice daily and identified by pen, separated from floor eggs, and stored in an egg cooler at 16-18° C and 60% RH until set for incubation purposes. A minimum of 180 eggs per pen were set biweekly from 28 wk to 64 wk to determine hatchability. After hatching was completed, eggs that did not hatch were examined macroscopically to determine fertility and/or time of embryonic mortality. Embryos that died between 1d and 7d of incubation were termed early dead and the embryos that died after 7 d were termed as late dead. Cracked eggs that occurred during handling and incubation were not included in the calculations. The eggs were set in a Natureform model I-14 setter and hatched in Nature form H-10 (Natureform International, Jacksonville, FL 32202).

Statistical Analysis. A one-way ANOVA using a completely randomized design with 4 replicate pens per feeding program treatment was employed. Variables such as egg production and fertility were analyzed on both a quartile age period and overall basis from 24 to 64 wk of age. There were four 10-wk quartile age periods. Data pertaining to egg variables were analyzed by one-way ANOVA within each sample age. Data were examined for normality of distributions and homogeneity of variance, and percentage data were subjected to arcsine transformation to normalize distribution. The general linear model of SAS (SAS Institute, 2011) was used to analyze variables and differences among means were partitioned by LSMEANS. Differences among means were considered statistically different when $P<0.05$, while $P<0.10$ were considered to approach statistical significance.

RESULTS

Broiler breeder female BW, as affected by feeding program during rearing, and as sampled at 2, 3, 4, 5, 6, 7, 8, 12, 16, 20, and 22 wk of age is shown in Table I-2. Female BW at 7, 8, 12, and 20 wk of age was significantly increased by the A 50.0 feed allocation program as compared to the B 42.5 treatment.

Table I-3 shows the cumulative feed, crude protein (CP), and metabolizable energy (ME) intake of the pullets at various ages. The BW effect exhibited at 7, 8, 12, and 20 wk of age reflected a delayed effect of the nutrients consumed by the birds.

The effects of rearing feeding programs on broiler breeder egg production and mortality from 25 to 64 wk of age are shown in Table I-4. Hen-housed egg production did not exhibit any differences. However, hen-day egg production approached statistical significance at 25 to 34 wk and throughout the overall 25 to 64 wk production period where A 50.0 treatment had about

2% greater production. A significant difference at peak production (35 to 44 wk) was also observed where the A 50.0 had a greater production than the B 42.5 hens.

No difference was found in female mortality. However, a difference that approached significance was observed where A 50.0 the birds that had more feed during rearing had a higher mortality compared to B 42.5 birds that received less feed from 25 to 34 wk of age.

The effect of feeding programs on broiler breeder fertility, hatchability, and embryonic mortality are shown in Table I-5. With regards to the overall production period (25 to 64 wk of age) fertility was significantly greater in the B 42.5 treatment. A difference in fertility that approached significance from 45 to 54 wk of age was observed where the B42.5 treatment had a greater fertility. There were no differences found in hatchability of fertile eggs. However, the hatchability of total eggs in the B 42.5 treatment was significantly greater during the early production period (25 to 34 wk of age) and during the overall production period (25 to 64 wk of age), but approached statistical significance at 45 to 54 wk of age. The B 42.5 treatment significantly improved overall production period (25 to 64 wk of age) for early embryonic mortality. No difference in late embryonic mortality was observed.

DISCUSSION

Pullets on the A 50.5 feeding program received more feed during the rearing period up to 15 wk of age than did the birds on the B 42.5 feeding program. After 15 wk of age, the feed allocation was the same. Although the B 42.5 feeding program birds received less feed, the cumulative nutrition at photostimulation (Table I-3) met the minimum requirement of 1300 g of CP and 23,000 kcal ME (Brake, 2003). The two feeding programs did not affect the hen-housed egg production (164 vs 161), probably due to female mortality masking the effect (Table I-4). Hen-day egg production, on the other hand, exhibited either statistical or numerical

(P<0.10) differences at 25 to 34, 35 to 44, and 25 to 64 wk of age, where the A 50.0 treatment exhibited about 2% more production than the B 42.5 treatment. The body was able to sense energy status and adjust its metabolic status according to a variety of signaling molecules including neuropeptides, hormones, nutrients and metabolites such as leptin that have been shown to be produced by peripheral and central nerve system (CNS) tissues. These are received by the receptors such as AMP-activated protein kinase (AMPK) or the mammalian target of rapamycin (mTOR) and trigger numerous energy metabolism pathways controlling the metabolic activities (Richards and Proszkowiec-Weglarz, 2007). The ovulation function was known to be associated with the development of the ovary under the control of the hypothalamic-pituitary-gonadal axis (HPGA). Chronic or short term feed restriction were used in broiler breeder management to control reproductive functions. Feed restriction decreased the secretion of GnRH-1 that resulted in less FSH and LH being produced, and ultimately produced an orderly pattern of ovarian growth (Lal *et al.*, 1990; Contijoch *et al.*, 1992). In this study, the amount of cumulative ME was more in the birds that were reared on the A 50.0 feeding program. These birds were evidently able to utilize the energy and produced an average of 2% more eggs throughout the production period than the birds that were reared on the B 42.5 feeding program.

We also observed fertility differences between the A 50.0 and B 42.5 treatments. The B 42.5 birds that were fed with less feed exhibited a better fertility. Fertility and hatchability performance issues normally become apparent following peak egg production in broiler breeders (Leeson and Summers, 2000). The typical issue with fertility has been persistency. Walsh and Brake (1997) demonstrated that fertility persistency was related to crude protein (CP) intake during rearing. In this experiment both treatments consumed adequate CP before

photostimulation and were fed the exact same amount of feed after 15 wk of age. Nevertheless, the B 42.5 treatment wherein the birds received less feed during rearing exhibited improved fertility. The reason may be related to how the birds were conditioned to utilize CP. Lilja (1983) developed the concept of supply and demand organs. The supply organ such as the intestines and liver were responsible for processing the energy, versus demand organs such as muscles and feathers that use the energy. It should be noted that broiler breeders were bred foremost to produce broilers, which were subsequently bred and selected to produce more meat efficiently, which represented a significant nutrient demand. Broilers tend to utilize CP mainly for breast meat, and feeding these birds less feed in early rearing phases seems to change this. Although oviduct development occurred within a short time period after photostimulation, and increased dramatically from 22 to 32 wk of age, the growth of oviduct then reached a plateau (Breneman, 1956; Joseph *et al.*, 2002). Zuidhof *et al.* (2007) reported that the growth and development of the oviduct was very responsive to feed allocation during sexual maturation. The differences shown in production, fertility and hatchability in this study were solely due to the feeding during early rearing. The birds on B 42.5 feeding program were able to utilize the CP for development of the oviducts, instead of growing more breast meat. Therefore we observed the increased fertility in the B42.5 treatment birds, along with a better hatchability, and less early embryonic mortality.

Figure I-1. Female Feeding program from rearing to peak period for Ross 708 pullets. The A 50.0 pullets were fed 50.0 g of feed per bird per day at 6 wk of age. The B 42.5 pullets were fed 42.5 g of feed per bird per day at 6 wk of age.

Table I-1. Composition of starter, grower, and layer diets.

Ingredients	Starter Diet[6]	Grower Diet[7]	Layer Diet[8]
	(%)		
Corn meal	61.94	64.46	67.67
Soybean meal (48% CP)	20.31	14.46	17.22
Wheat midds	13.72	13.21	5.00
DDGS	-	3.88	-
Lard	0.50	-	0.97
Limestone	1.12	1.25	6.38
Mono/D Phosphate	1.31	1.46	1.49
Salt	0.49	0.50	0.53
DL-Methionine	0.05	0.11	0.11
L-Lysine	0.01	0.09	0.02
Choline chloride (60%)	0.05	0.07	0.11
Vitamin premix[1]	0.10	0.10	0.10
Mineral premix[2]	0.25	0.25	0.25
Selenium premix[3]	0.05	0.05	0.05
Pellet binder[4]	0.10	0.10	0.05
Coccidiostat[5]	-	-	0.05
Total	100.00	100.00	100.00
Calculated nutrient content			
Crude protein (%)	17.00	15.55	14.5
Calcium (%)	0.84	0.90	2.90
Available phosphorus (%)	0.42	0.45	0.42
Lysine (%)	0.85	0.77	0.73
Methionine (%)	0.33	0.37	0.35
Methionine + cysteine (%)	0.62	0.63	0.60
Sodium (%)	0.24	0.27	0.24
Metabolizable energy (kcal/g)	2.91	2.91	2.91

[1]Vitamin premix supplied the following per kg of diet: 6,614 IU vitamin A, 2,000 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 1.98 mg menadione (K_3), 1.98 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 3.97 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.
[2]Mineral premix supplied the following per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt, 1 mg.
[3]Selenium premix provided 0.2 mg Se (as Na_2SeO_3) per kg of diet.
[4] Maxi-Mil HP was used as a pellet binder to improve pellet quality.
[5]Coccidiostat supplied monensin sodium at 99 mg/kg of feed.
[6]Starter diet was fed to 6 wk of age.
[7]Grower diet was fed from 7 to 26 wk of age.
[8]Layer diet was fed from 27 to 64 wk of age.

Table I-2. Broiler breeder female BW at various ages as affected by feeding programs during rearing.

Flock Age	Dietary Treatments		SEM [3]	P-Value
	A 50.0 [1]	B 42.5 [2]		
(Wk)	(g)			
2	195	187	3.51	0.181
3	261	254	5.88	0.408
4	347	345	6.22	0.212
5	469	454	14.27	0.463
6	602	594	5.50	0.344
7	799 [A]	722 [B]	15.92	0.014
8	897 [A]	808 [B]	14.64	0.005
12	1451 [A]	1334 [B]	19.82	0.006
16	1910	1857	22.53	0.147
20	2359 [A]	2277 [B]	14.06	0.006
22	2504	2519	9.91	0.333

[A,B] Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1] A50.0: Female broiler breeders were fed with 50.0 g of feed per bird per day at 6 wk of age.
[2] B 42.5: Female broiler breeders were fed with 42.5 g of feed per bird per day at 6 wk of age.
[3] Standard error of mean (SEM) for n=4 pens with 25 birds per pen weighed.

Table I-3. Cumulative intake of feed, crude protein, and metabolizable energy of female broiler breeders.

	Feed		Crude Protein		Metabolizable Energy	
Flock Age	A 50.0 [1]	B 42.5 [2]	A 50.0 [1]	B 42.5 [2]	A 50.0 [1]	B 42.5 [2]
(wk)	(g)		(g)		(kcal)	
2	425	396	72	67	1,236	1,153
3	684	634	116	108	1,990	1,846
4	964	892	164	152	2,805	2,595
5	1,279	1,168	217	199	3,722	3,400
6	1,629	1,464	277	249	4,740	4,259
7	2,007	1,775	336	297	5,840	5,166
8	2,399	2,106	397	349	6,981	6,127
12	4,107	3,622	662	584	11,951	10,539
16	6,046	5,494	964	875	17,594	15,986
20	8,402	7,843	1,330	1,241	24,450	22,823

[1] A50.0: Female broiler breeders were fed 50.0 g of feed per bird per day at 6 wk of age.
[2] B 42.5: Female broiler breeders were fed 42.5 g of feed per bird per day at 6 wk of age.

Table I-4. Broiler breeder egg production and female mortality as affected by flock age, and feeding programs during rearing.

Feeding Programs	Flock Age	Egg Production		Female Mortality
		Eggs per Hen Housed	Hen-day Production	
	(wk)	(n)	(%)	(%)
A 50.0 [1]	25 - 34	56.5	74.2 [x]	2.8 [x]
B 42.5 [2]		55.4	72.2 [y]	1.3 [y]
SEM [3]		0.36	0.01	0.01
P-Value		0.166	0.059	0.065
A 50.0 [1]	35 - 44	47.1	70.0 [a]	1.8
B 42.5 [2]		47.0	68.8 [b]	2.0
SEM [3]		0.25	0.01	0.01
P-Value		0.717	0.034	0.835
A 50.0 [1]	45 - 54	37.2	56.1	1.8
B 42.5 [2]		36.6	54.6	2.1
SEM [3]		0.51	0.01	0.01
P-Value		0.580	0.246	0.670
A 50.0 [1]	55 - 64	23.1	44.7	2.5
B 42.5 [2]		21.9	42.0	2.8
SEM [3]		0.55	0.01	0.01
P-Value		0.325	0.190	0.802
A 50.0 [1]	25 - 64	163.9	62.7 [x]	9.0
B 42.5 [2]		160.8	60.9 [y]	8.2
SEM [3]		1.38	0.01	0.01
P-Value		0.305	0.075	0.537

[a,b] Means in a column within each time period that possess different superscripts differ significantly ($P \leq 0.05$).

[x,y] Means in a column within each time period that possess different superscripts differ significantly ($P \leq 0.10$).

[1] A50.0: Female broiler breeders were fed 50.0 g of feed per bird per day at 6 wk of age.

[2] B 42.5: Female broiler breeders were fed 42.5 g of feed per bird per day at 6 wk of age.

[3] Standard error of mean (SEM) for n=4 pens with initially 190 birds per pen weighed.

Table I-5. Broiler breeder fertility, hatchability, and embryonic mortality as affected by flock age, and feeding programs during rearing.

Feeding Programs	Flock Age	Fertility	Hatchability of Fertile Eggs	Hatchability of Total Eggs	Early Dead	Late Dead
	(wk)	(%)				
A 50.0 [1]	25 - 34	94.6 [y]	93.1 [y]	88.1 [B]	4.6	1.8
B 42.5 [2]		96.2 [x]	94.2 [x]	90.6 [A]	4.0	1.5
SEM [3]		0.41	0.28	0.36	0.25	0.29
P-Value		0.100	0.091	0.012	0.246	0.666
A 50.0 [1]	35 - 44	94.1	91.9	86.5	3.9	3.5
B 42.5 [2]		94.7	92.5	87.6	3.7	3.1
SEM [3]		0.90	0.49	0.99	0.26	0.18
P-Value		0.189	0.473	0.159	0.164	0.686
A 50.0 [1]	45 - 54	85.0 [y]	91.5	77.8 [y]	5.8	2.2
B 42.5 [2]		90.3 [x]	92.2	83.2 [x]	4.7	2.2
SEM [3]		1.34	0.36	1.25	0.29	0.33
P-Value		0.100	0.374	0.073	0.110	0.985
A 50.0 [1]	55 - 64	81.1	88.1	71.4	7.5	4.1
B 42.5 [2]		84.2	89.5	75.4	6.4	3.4
SEM [3]		1.73	0.46	1.73	0.36	0.25
P-Value		0.405	0.182	0.294	0.201	0.240
A 50.0 [1]	25 - 64	88.5 [B]	91.2	80.8 [B]	5.6 [A]	2.8
B 42.5 [2]		91.6 [A]	92.2	84.5 [A]	4.7 [B]	2.5
SEM [3]		2.32	1.40	2.80	0.80	0.80
P-Value		0.003	0.106	0.003	0.013	0.443

[x,y] Means in a column within each flock age that possess different superscripts differ significantly ($P \leq 0.10$).
[a,b] Means in a column within each flock age that possess different superscripts differ significantly ($P \leq 0.05$).
[A,B] Means in a column within each flock age that possess different superscripts differ significantly ($P \leq 0.01$).
[1] A50.0: Female broiler breeders were fed 50.0 g of feed per bird per day at 6 wk of age.
[2] B 42.5: Female broiler breeders were fed 42.5 g of feed per bird per day at 6 wk of age.
[3] Standard error of mean (SEM) for n=4 pens of initially 190 hens per pen.

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MANUSCRIPT II. Effect of Feeding Program during Rearing on Body Weight, Egg Production, and Fertility of Ross 708 Broiler Breeder Females.

ABSTRACT

Four female breeder feed allocation programs were compared during the starter (17.5% CP, 2.9 kcal/g ME, 0~6 wk) and grower (14.5% CP, 2.85 kcal/g ME, 7~21 wk) phases. The 4 female breeder feeding programs were termed high-high (HH), high-low (HL), low-high (LH), and low-low (LL) with feed/pullet/d at 6, 15, and 21 wk being (HH) 52 g- 70 g- 94 g, (HL) 52 g- 64 g- 94g, (LH) 47.5 g- 70 g- 94 g, and (LL) 47.5 g- 64 g-94 g, respectively. The cumulative feed to photostimulation was 9128 g, 8808 g, 8943 g, and 8616 g for HH, HL, LH, and LL, respectively. The females were fed similarly thereafter. Hatchability and fertility were determined weekly from 28 to 63 wk of age. Egg production was recorded daily from 24 to 64 wk of age. There were no effects of the different feeding programs on feed conversion, hen-housed, hen-day egg production, or female mortality. With regards to hatchability and fertility, there were no differences from 25 to 35 wk of age. However, from 36 to 45 wk of age the LH feeding program exhibited greater hatchability (94.1%) than HL (92.1%) and LL (92.25%) feeding programs with HH (93.06%) being intermediate ($P \leq 0.05$). From 46 to 55 wk early embryonic mortality was greater (4.35%) on the HH feeding program compared with the LH (3.58%) and LL (3.37%) feeding programs with HL (3.82%) being intermediate ($P \leq 0.01$). From 56 to 64 wk the LH and LL feeding programs produced greater fertility ($P \leq 0.05$) and hatchability ($P = 0.10$) than the HH feeding program with HL being intermediate. Overall, the LH feeding program exhibited the best fertility (94.9%) and hatchability (89.3%), while the HH feeding program produced the poorest fertility (90.5%) and hatchability (84.7%).

Key words: broiler breeder, feeding program, hatchability, fertility

INTRODUCTION

The purpose of feed restriction of broiler breeders during rearing has been to control BW and prevent obesity related reproduction issues such as defective eggs, reduced fertility, and low hatchability (Robinson and Robinson, 1991; Robinson *et al.*, 1995; Renema *et al.*, 2008). Although broiler performance had increased by 400% over 5 decades (Zuidhof *et al.*, 2014), the standard BW target for broiler breeder flock rearing had not been updated (Renema *et al.*, 2007). This resulted in under feeding of breeder flocks, which created later stage persistency problems with regards to fertility and hatchability. Leksrisompong (2010) investigated two feeding patterns, a sigmoid and a linear with Ross 308 females. The cumulative nutrient intake to 21 wk (147 d) for these two treatments were the same (25.7 Mcal of ME and 1,337 g of CP). However, differences were found in BW, where the sigmoid treatment had a greater BW from 4 to 12 wk of age. The hen-day production was the same (67.7%) for both treatments. Fertility trended to be greater in the linear treatment (94.0 vs 92.5%; $P \leq 0.10$). Therefore, the birds that were provided less feed early in the rearing period exhibited an increased fertility later in production. The Ross 708 has been the primary Ross female line In North Carolina where greater breast meat yield has been sought and it suffered from poor persistency of fertility as did the Ross 308. Therefore our previous experiment (manuscript I) was conducted to investigate how differential feeding programs during early growth and development would affect Ross 708 fertility. Two feeding programs with 42.5 g and 50.0 g at 6 wk of age were evaluated. The results from that study (manuscript I) demonstrated that reduced feeding early in the hen's development numerically reduced hen-day egg production ($P \leq 0.10$), increased fertility ($P \leq 0.05$), increased hatchability ($P \leq 0.05$), and reduced early embryonic mortality ($P \leq 0.05$). The present study was designed to be more global in nature, and two additional

pullet feeding programs were included to determine whether additional benefits in egg production and fertility could be achieved via nutritional manipulation. Therefore, our experimental approach was to determine the effects of four different starter and grower feeding programs that differed in feed intake at 6 and 15 wk of age on BW and reproductive performance of Ross 708 broiler breeder females.

MATERIALS AND METHODS

Broiler Breeder Rearing Management. The experimental protocol used in this study was approved by the North Carolina State University Institutional Animal Care and Use Committee. A total of 1280 1-d-old female Ross 708 and 288 male Ross 344 broiler breeders (Aviagen, Huntsville, AL) were raised in 16 female pens (14.3 m^2 area; 80 females per pen) and 16 male pens (4.6 m^2 area; 18 males per pen) on new pine wood shavings floor pens in an enclosed fan-ventilated house. The house was completely enclosed except for the perimeter inlets where fresh air entered each pen and two 24-inch and two 36-inch exhaust fans. Five space heaters and 6 upward directed fans were placed in the central walkway with pens on either side to insure temperature uniformity. All females and males were permanently identified with a neck tag at placement. The litter temperature was 35°C (95° F) for the first 2 d and the ambient temperature was 29.4~32.2 °C (85~90° F) through 7 d of brooding. Each female pen was initially equipped with 5 tube feeders (DH-4; Kuhl, Flemington, New Jersey). Each feeder pan had a circumference of 132 cm. From placement to 14 d of age all female pens had 4 tube feeders followed by 3 tube feeders to 10 wk of age. From 11-15 wk, 4 tube feeders were again used, and from 16 wk until birds were moved to the laying house there were 5 tube feeders used for females. Males had one tube feeder per pen at all times. Two bell-type drinkers were used per female pen during both rearing and laying, while males had one bell-type drinker

per pen during rearing and shared the female drinkers during laying. During the first week of brooding, an additional 6 feeder lids per female pen and 3 feeder lids per male pen were used. An additional 2 font drinkers per female pen and one font per male pen were used during the first week. The general litter management in both of the pullet and breeder houses focused on developing natural immunity against coccidia by lightly spraying water on the wood shavings 4 times per wk (on feed days) during the period from 1-6 wk of age. After 6 wk of age, grower diets were fed and shavings were sprayed 3 times weekly. The house was ventilated to maintain ammonia levels below 25 ppm but not totally absent. The lighting program utilized 23 h of light per day to 7 d of age, and 8 h of light per day at an average intensity of 15 lux by using 12W florescent light bulbs to 22 wk (155 d) of rearing. Water was typically available for 6 h daily and limited by a time clock and solenoid system to allow the birds to have unlimited access to water while feed was present and a similar amount on non-feed days during rearing. All the male breeders were managed on the same feeding program during rearing, as shown in Figure II-1. The female feeding programs from 1 to 64 wk of age are shown in Figure II-2. The females were assigned to 4 feeding programs that were termed High-High (HH), High-Low (HL), Low-High (LH), and Low-Low (LL) from 3 to 21 wk of age (Figure II-3). Each treatment was applied to 4 pens each. The study was designed by using some key feed reference points. At 6 wk of age, the high treatments were fed 52 g per bird per day while the low treatments were fed 47.5 g of feed per bird per day. At 15 wk of age, the high treatments were fed 70 g of feed and the low treatments were fed 64 g of feed per day per bird. At 21 wk of age, all 4 treatments were fed the same 94 g of feed per day. Females were fed 4/3 to 12 wk, 5/2 to 21 wk, and daily thereafter.

***Pullet and Breeder Diets*.** Day-old chicks (females and males) were fed a single mash starter diet with 17.5% crude protein (CP), and 2.9 kcal/g ME to 6 wk of age with corn ground using a roller mill to 800 μm. This was followed by a 14.5% CP, 2.85 kcal ME/g mash growing diet to 26 wk of age containing corn ground to 1200 μm with a roller mill. The layer diet was fed from 27 to 64 wk of age. The corn was ground by roller mill to 1200 μm and the diet was formulated to contain 2.85 kcal ME/g and 15.0 % CP. All feed formulas are shown in Table II-1.

Feed Manufacture. The corn was ground with a two-pair roller mill (Model C128829, RMS, Tea, SD) with a gap setting of 0% opening (0.15-0.18 mm) on the top pair of rollers and 100% opening (7.16 mm) on the bottom pair of rollers. Dry ingredients were blended for 180 sec in a twin shaft counterpoise mixer (Model TRDB126-0604, Hayes and Stolz, Fort Worth, TX), followed by addition of fat and mixing for an additional 90 sec.

Broiler Breeder Laying Management. At 22 wk of age, 8 males and 64 females were selected according to BW as being in the middle range of the flock from each pen (both sexes received the same dietary treatment during growing), and moved to a 16-pen laying house. Each of the 16 laying pens (15.9 m^2) were equipped with two-third slats and one-third pine shavings litter (5.9 m^2 litter area/pen). There were 4 tube feeders (DH-4; Kuhl, Flemington, New Jersey) for females with 16-hole grills that excluded males and 1 smaller tube feeder for males in each pen. Separation of sexes was ensured by special grills (sixteen 4.8 x 5.8 cm holes) on each female feeder that prevented the non-dubbed males from accessing the feed. Water was limited to 8 h per day during the laying period beginning at the time of feeding. Each pen had a conventional nest box comprised of two double (50.8 cm wide per nest space) and four single nests (25.5 cm wide per nest space). Eggs were collected and recorded twice daily before being

stored in an egg cooler prior to incubation. Birds were photostimulated with 14 h of light when moved at 22 wk of age. The day length was subsequently increased to 15 h 10 d later, to 15.5 h at 5% production, and finally to 16 h at 50% production. Natural light entered the slat-litter house through open or translucent curtains during normal daylight hours. Supplemental light provided an average intensity of 35 lux at bird head level using 18W fluorescent light bulbs when natural light was not present.

Body Weight. All females were group weighed at placement, 2, 3, 4, and 5 wk of age and individually weighed at 15 of age. After moving into the laying house, the females were group weighed in a random group of 25 females per pen at 32, 41, 52, and 64 wk of age.

Egg Production and Hatchability. Nest and floor eggs were collected and recorded twice daily. Nest eggs were collected and identified by pen, separated from floor eggs, and stored in an egg cooler at 16.7°C (62° F) and 70% RH until set for incubation purposes. A minimum of sixty eggs per pen were set at least every other week from 28 to 64 wk. After hatching was completed, chicks were counted and eggs that did not hatch were examined macroscopically to determine fertility and/or time of embryonic mortality. Embryos that died between 1d and 7d of incubation were termed early dead and the embryos that died after 7 d were termed late dead. Cracked eggs that occurred during handling and incubation were not included in the calculations.

Total Egg Weight. Egg weight of all eggs produced in one day were measured three times per week from 27 to 35 wk, and at 41, 45, 50, 53, 57, 61, and 65 wk of age.

Egg Sample. All morning eggs from one pen per treatment were collected at 30, 41, 53, and 61 wk of age for additional egg quality measures. Weights of yolk, shell, and albumen, shell thickness, and percentage yolk, albumen, and shell were determined.

Statistical Analysis. A one-way ANOVA using a completely randomized design with 4 replicate pens per dietary treatment was employed for BW, egg production, hatchability, total egg weight. Variables such as egg production and fertility data were analyzed on a 10-wk quartile age period and overall basis from 24 to 64 wk of age. There were four 10-wk quartile age periods. Data pertaining to egg variables were analyzed by one-way ANOVA within each sample age. Data were examined for normality of distributions and homogeneity of variance, and percentage data were subjected to arcsin transformation to normalize distribution. The general linear model of SAS (SAS Institute, 2011) was used to analyze variables and differences among means were partitioned by LSMEANS. Differences among means were considered statistically different when $P<0.05$, while $P<0.10$ were considered to approach statistical significance.

RESULTS

Broiler breeder female BW as affected by different feeding programs during rearing is shown in Table II-2. The only BW difference due to the treatments was observed at 15 wk of age, where HH was significantly heavier than LL while HL and LH groups were intermediate. Table II-3 showed the cumulative feed, crude protein (CP), and metabolizable energy (ME) of the female pullets at various ages. The BW matched the cumulative feed and nutrients, where HH pullets consumed the greatest amount of feed consumed, and LL the least feed, while HL and LH were intermediate.

Effect of feeding program on egg production and mortality from 25 to 64 wk of age as well as the 4 quartiles are shown in Table II-4. Hen housed egg production and hen-day egg production did not differ due to the pullet feeding program. However, the HL treatment approached statistical significance in terms of decreased egg production on a hen-day basis, as well as on

eggs per hen housed basis. This could have been due to having a photostimulation age of 22 wk, which allowed more metabolizable energy and crude protein to accumulate prior to the start of egg production. There was also no difference in female mortality among the treatments. Broiler breeder fertility, hatchability, and embryonic mortality from 25 to 64 wk of age as well as the 4 quartiles are shown in Table II-5. Feeding program affected persistency of fertility. From 55 to 64 wk, and 25 to 64 wk of age, the LH and LL treatments that consumed less feed at 6 wk of age exhibited improved fertility when compared to HH, while HL was intermediate. There was no difference found for hatchability of fertile eggs. The hatchability of total eggs set from 25 to 64 wk of age for the four feeding treatments generally reflected fertility, where LH had the best hatchability, LL and HL were intermediate, and HH had the poorest hatchability. From 35 to 44 wk of age, LH had the best hatchability, HH was intermediate, while HL and LL programs exhibited the lowest hatchability. Early embryo mortality was different from 45 to 54 wk of age, where the HH had greater early embryo mortality than the two treatments that started with a lower feeding allocation (LH and LL) at 6 wk of age, while HL was intermediate. No differences were observed in late embryo mortality.

Table II-6 displays the egg weight at various ages as affected by female feeding program during rearing. At 31 wk of age, egg weight of the HH feeding program was significantly greater than that of the LL feeding program, with the other two programs intermediate. This reflected the single difference in BW that was observed at 15 wk of age. At 41 wk of age, egg weight of HH approached statistical significance greater than LL.

Effects of female feeding program during rearing on egg components at 30, 41, 53, and 61 wk of age are shown in Table II-7. At 30 wk of age, HL had the smallest yolk weight when compared to the other three feeding program treatments. The albumen component was greatest

in HH treatment, HL and LH were intermediate, and the LL feeding program was least at 30 wk of age. At 53 wk of age, the LL treatment had the least albumen component. Also at 61 wk of age, the albumen component was greater in HL and LH, HL was intermediate, and LL had the least albumen. There was no difference observed in egg shell weight at any age. The yolk to albumen ratio at 30 wk of age was greater in the two treatments LH and LL that consumed less feed at 6 wk of age. Shell thickness was greatest in treatment HL, HH was intermediate, and LL and LH was thinnest at 30 wk of age. At 61 wk of age a similar response for shell thickness was observed with the HH and HL treatments having thicker shells than the LH and LL treatments. Albumen height was greater in the HL and LH treatments at 30 wk of age.

DISCUSSION

Birds on the HH dietary treatment consumed the largest amount of feed during the rearing period to 21 wk of age, while the LH and HL were intermediate, and LL consumed the least feed (Table II-3). Therefore the difference in BW shown at 15 wk of age basically reflected the cumulative nutrients consumed (Table II-2). No difference was found earlier than 15 wk of age, which was probably due to the time of sampling as well as there being less differences in cumulative nutrients at earlier ages. The birds were fed the same amount of feed after 21 wk of age, therefore no BW differences were observed during the production period.

Though, Lilburn and Myers-Miller (1990) found a difference in egg production in birds that had been given different patterns of feed allocation during their rearing period, there were no significant differences observed in hen housed and hen-day production in the present study (Table II-4). This may be affected by the different line of birds used. However, the HL dietary treatment, where the birds consumed more feed to 6 wk of age, and less feed from 6 to 15 wk of age, exhibited a lower number of hen-housed eggs produced (153.5), as well as a lower hen-

day production (58.1%) throughout the entire production period related to the other treatments. These birds that consumed more feed during early rearing, but less feed during the later rearing period exhibited a decreased egg production of 2%. Bruggeman *et al.* (1998) demonstrated that feed allocation from 2 to 24 wk of age affected the hypothalamic-pituitary-gonadal axis (HPGA) that was associated with the development of the ovary and oviduct. The GnRH-1 (the paper referred to cLHRH-1) concentrations in the median eminence were significantly higher in the *ad libitum* feeding treatment from 8 to 24 wk of age, as was the pituitary LH concentration, which resulted in a greater ovary and oviduct weight. In the current study, the small difference we observed in egg production may or may not be due to such an effect, as cumulative nutrient intake at photostimulation was adequate.

Persistence of fertility has remained a major issue as fertility has often been observed to decline dramatically during the later laying period (Kirk *et al.,* 1980). Hatchability would be expected to decrease along with fertility. The fertility issues has been thought to be primarily a male broiler breeder problem because female fertility was acceptable when artificial insemination was used (Brillard and McDaniel, 1986). Large male broiler breeders at the end of the laying period have been observed to encounter difficulties of cloacal contact with the hens during mating (Soller *et al.,* 1965; Hocking and Duff, 1989). All the male broiler breeders in the present experiment were treated the same, so that the differences observed were simply the effects of the female feeding programs during rearing.

In this experiment, fertility and hatchability during the early production phase were the same among the treatments. The fertility differences were obvious in the later laying period from 55 to 64 wk of age. The LH and LL dietary treatments where the birds consumed less feed to 6 wk of age displayed better fertility, the HL treatment was intermediate, and HH had the poorest

fertility (Table II-5). Total hatchability reflected fertility in this study. This suggested that Ross 708 female pullets that were fed less feed to 6 wk did not experience the typical late stage decline in fertility. Although no BW differences were observed until 15 wk of age, fertility results somewhat reflected those of VanKrey and Siegel (1974) who found that female broiler breeders selected for greater BW at 8 wk of age exhibited decreased fertility and poorer spermatozoa storage in the host glands located in the oviduct. Walsh and Brake (1997) suggested that decreased fertility was due to feeding programs that were designed to target a standard BW without considering cumulative nutrient intakes at photostimulation. These authors suggested that a cumulative CP intake of at least 1,180 g per pullet at 20 wk of age, before photostimulation was required to maintain fertility at later laying ages. Walsh and Brake (1997) also demonstrated that fertility persistency was related to crude protein content of the feed. The birds in the present study were nutrient replete, but may have utilized nutrients differently due to differences in their feeding programs. The results of the present study suggested that the pattern of feed allocation during the rearing period can influence the amount of feed used, and was also important to achieving maximum female fertility. The results of the present study suggested that early rearing feeding allocation to 6 wk of age affected persistency of fertility. It appeared to be important to start the Ross 708 female slowly to 6 wk of age, as long as the birds achieved the minimum cumulative quantity of CP suggested before the time of photostimulation, in order to achieve satisfactory persistency of fertility.

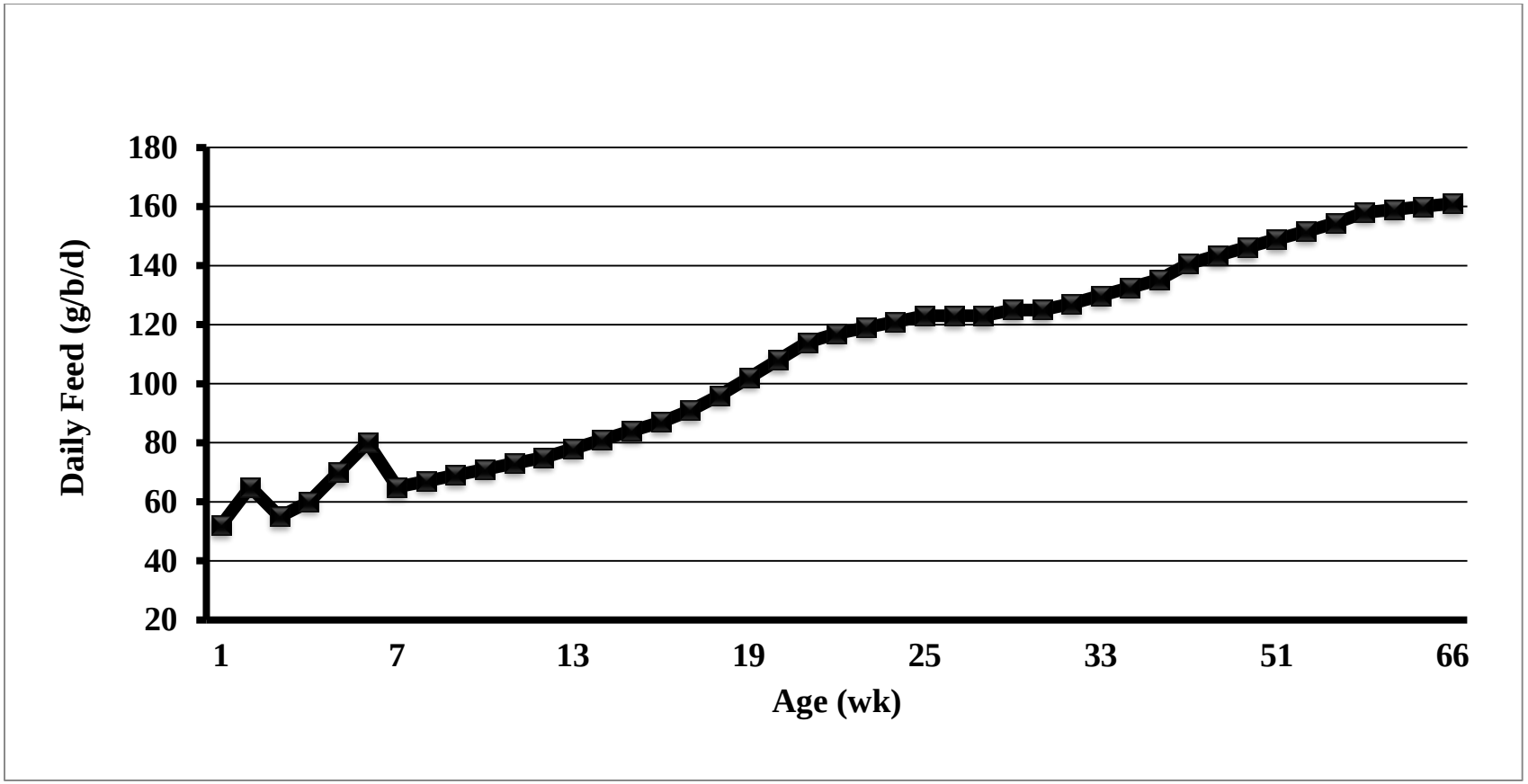

Figure II-1. Feeding program for Ross 344 male broiler breeders during rearing and laying periods.

Figure II-2. Overall feeding program for Ross 708 female broiler breeders during rearing and laying periods. The programs were High-High (HH) that provided a daily feed amount of 52 g of feed per bird per day at 6 wk of age, and 70 g of feed per bird per day at 15 wk of age. The High-Low (HL) program provided a daily feed amount of 52 g of feed per bird per day at 6 wks of age, and 64 g of feed per bird per day at 15 wk of age. The Low-High (LH) provided a daily feed amount of 47.5 g of feed per bird per day at 6 wk of age, and 70 g of feed per bird per day at 15 wk of age. The Low-Low (LL) program provided a daily feed amount of 47.5 g of feed per bird per day at 6 wk of age, and 64 g of feed per bird per day at 15 wk of age. All programs achieved 94 g of feed per bird per day at 21 wk of age.

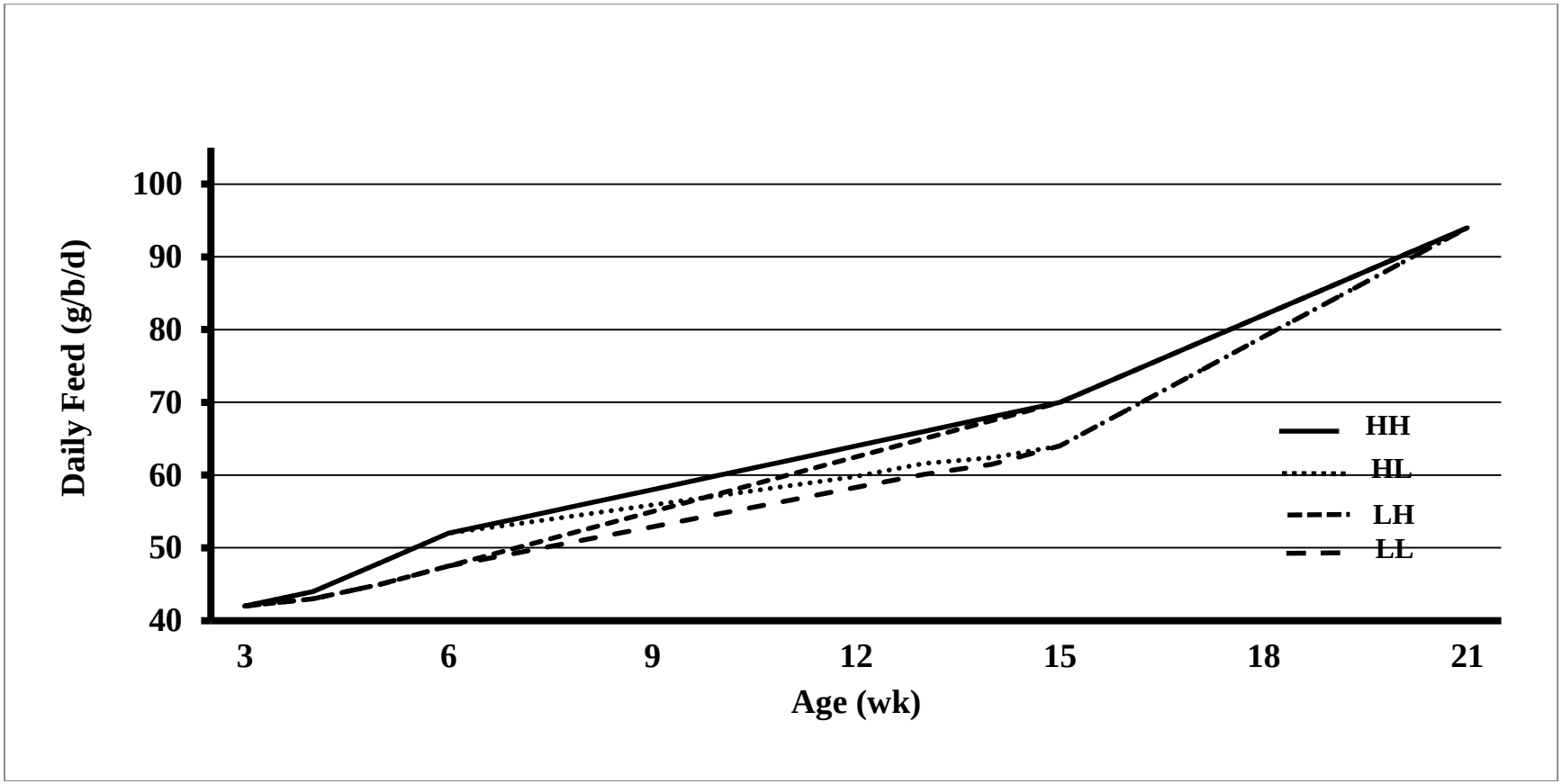

Figure II-3. Female feeding program treatments from 3 wk to 21 wk. The programs were High-High (HH) that provided a daily feed amount of 52 g of feed per bird per day at 6 wk of age, and 70 g of feed per bird per day at 15 wk of age. The High-Low (HL) program provided a daily feed amount of 52 g of feed per bird per day at 6 wks of age, and 64 g of feed per bird per day at 15 wk of age. The Low-High (LH) provided a daily feed amount of 47.5 g of feed per bird per day at 6 wk of age, and 70 g of feed per bird per day at 15 wk of age. The Low-Low (LL) program provided a daily feed amount of 47.5 g of feed per bird per day at 6 wk of age, and 64 g of feed per bird per day at 15 wk of age. All programs achieved 94 g of feed per bird per day at 21 wk of age.

Table II-1. Composition of starter, grower, and layer diets.

Ingredients	Starter Diet[4]	Grower Diet[5]	Layer Diet[6]
	(%)	(%)	(%)
Corn	67.96	63.58	63.67
Soybean meal (48% CP)	25.31	13.45	17.81
Dicalcium phosphate (18.5% P)	2.05	1.89	2.08
Wheat bran	1.50	15.85	6.56
Poultry fat	1.00	3.00	3.00
Limestone	0.96	1.04	5.70
Salt	0.50	0.50	0.50
Choline chloride (60%)	0.20	0.20	0.20
Vitamin premix[1]	0.10	0.10	0.10
Mineral premix[2]	0.20	0.20	0.20
Selenium premix[3]	0.10	0.03	0.03
DL-Methionine	0.11	0.08	0.11
L-Lysine	-	-	0.03
L-Threonine	0.01	0.08	0.01
Total	100.00	100.00	100.00
Calculated nutrient content			
Crude protein (%)	17.5	14.5	15.0
Calcium (%)	0.90	0.90	2.70
Available phosphorus (%)	0.45	0.45	0.45
Lysine (%)	0.90	0.73	0.75
Methionine (%)	0.40	0.33	0.36
Threonine (%)	0.60	0.50	0.53
Methionine + cysteine (%)	0.69	0.60	0.62
Sodium (%)	0.20	0.20	0.20
Metabolizable energy (kcal/g)	2.90	2.85	2.85

[1]Vitamin premix supplied the following per kg of diet: 6,614 IU vitamin A, 2,000 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 1.98 mg menadione (K_3), 1.98 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 3.97 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.

[2]Mineral premix supplied the following per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt, 1 mg.

[3]Selenium premix provided 0.2 mg Se (as Na_2SeO_3) per kg of diet.

[4] Starter diet was fed to 6 wk of age.

[5]Grower diet was fed from 7 to 26 wk of age.

[6]Layer diet was fed from 27 to 64 wk of age.

Table II-2. Broiler breeder female BW as affected by flock age and feeding programs during rearing.

Flock Age	Dietary Treatments				SEM	P-Value
	HH [1]	HL [2]	LH [3]	LL [4]		
(wk)	(g)					
1	42	42	42	42	0.23 [5]	0.465
2	194	201	203	201	4.32 [5]	0.489
3	341	353	349	358	5.31 [5]	0.211
4	541	555	543	567	10.12 [5]	0.267
5	791	807	797	794	10.97 [5]	0.748
15	1743 [A]	1641 [B]	1652 [B]	1589 [C]	12.59 [6]	0.001
32	3408	3410	3390	3425	38.20 [6]	0.933
41	3813	3812	3726	3734	43.31 [6]	0.349
52	3781	3769	3677	3792	48.82 [6]	0.363
64	3874	3863	3854	3861	72.88 [6]	0.998

[A-C] Means in a row within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1] HH: 52 g of feed per bird per day at 6 wk of age, and 70 g of feed per bird per day at 15 wk of age.
[2] HL: 52 g of feed per bird per day at 6 wk of age, and 64 g of feed per bird per day at 15 wk of age.
[3] LH: 47.5 g of feed per bird per day at 6 wk of age, and 70 g of feed per bird per day at 15 wk of age.
[4] LL: 47.5 g of feed per bird per day at 6 wk of age, and 64 g of feed per bird per day at 15 wk of age.
[5] Standard error of mean (SEM) for n=4 pens with 80 birds per pen weighed.
[6] Standard error of mean (SEM) for n=4 pens with 25 birds per pen weighed.

Table II-3. Cumulative intake of feed, crude protein, and metabolizable energy of female broiler breeders.

	Feed				Crude Protein				Metabolizable Energy			
Flock Age	HH [1]	HL [2]	LH [3]	LL [4]	HH [1]	HL [2]	LH [3]	LL [4]	HH [1]	HL [2]	LH [3]	LL [4]
(wk)	(g)				(g)				(kcal)			
1	147	147	147	147	26	26	26	26	426	426	426	426
2	392	392	392	392	69	69	69	69	1,137	1,137	1,137	1,137
3	686	686	686	686	120	120	120	120	1,989	1,989	1,989	1,989
4	994	994	987	987	174	174	173	173	2,883	2,883	2,862	2,862
5	1,330	1,330	1,302	1,302	233	233	228	228	3,857	3,857	3,776	3,776
15	5,600	5,385	5,415	5,193	863	832	834	802	16,045	15,432	15,513	14,883
20	8,470	8,150	8,285	7,958	1,279	1,233	1,250	1,203	24,224	23,312	23,693	22,763
21	9,128	8,808	8,943	8,616	1,374	1,328	1,346	1,298	26,100	25,188	25,568	24,638
22	9,814	9,501	9,629	9,309	1,474	1,428	1,445	1,399	28,055	27,163	27,523	26,613

[1] HH: 52 g of feed per bird per day at 6 wk of age, and 70 g of feed per bird per day at 15 wk of age.
[2] HL: 52 g of feed per bird per day at 6 wk of age, and 64 g of feed per bird per day at 15 wk of age.
[3] LH: 47.5 g of feed per bird per day at 6 wk of age, and 70 g of feed per bird per day at 15 wk of age.
[4] LL: 47.5 g of feed per bird per day at 6 wk of age, and 64 g of feed per bird per day at 15 wk of age.

Table II-4. Broiler breeder egg production and female mortality as affected by flock age and different feeding programs during rearing.

Feeding Programs	Flock Age	Egg Production		Female Mortality
		Eggs per Hen Housed	Hen-day Production	
	(wk)	(n)	(%)	(%)
HH [1]	25 - 34	48.0	62.7	0.8
HL [2]		45.4	59.7	1.2
LH [3]		47.1	61.2	0.0
LL [4]		47.3	61.7	1.6
SEM[5]		1.01	0.02	0.01
P-Value		0.343	0.498	0.606
HH [1]	35 - 44	47.7	69.3	1.2
HL [2]		46.1	67.8	2.0
LH [3]		48.7	70.3	2.0
LL [4]		48.2	70.4	1.2
SEM[5]		0.98	0.02	0.01
P-Value		0.337	0.405	0.865
HH [1]	45 - 54	39.5	58.1	1.6
HL [2]		37.8	56.4	0.8
LH [3]		40.5	59.1	1.2
LL [4]		40.5	59.8	0.8
SEM[5]		1.13	0.02	0.01
P-Value		0.323	0.492	0.438
HH [1]	55 - 64	25.5	47.5	1.2
HL [2]		24.2	45.5	0.4
LH [3]		25.6	47.1	0.0
LL [4]		26.5	49.1	0.8
SEM[5]		0.82	0.02	0.01
P-Value		0.325	0.349	0.539
HH [1]	25 - 64	160.7	60.1	4.7
HL [2]		153.5	58.1	4.4
LH [3]		161.8	60.2	3.1
LL [4]		162.5	60.9	4.3
SEM[5]		2.76	0.01	0.01
P-Value		0.133	0.168	0.635

[1] HH: 52 g of feed per bird per day at 6 wk of age, and 70 g of feed per bird per day at 15 wk of age.
[2] HL: 52 g of feed per bird per day at 6 wk of age, and 64 g of feed per bird per day at 15 wk of age.
[3] LH: 47.5 g of feed per bird per day at 6 wk of age, and 70 g of feed per bird per day at 15 wk of age.
[4] LL: 47.5 g of feed per bird per day at 6 wk of age, and 64 g of feed per bird per day at 15 wk of age.
[5] Standard error of mean (SEM) for n=4 pens of initially 64 hens each.

Table II-5. Broiler breeder fertility, hatchability, and embryonic mortality as affected by flock age and different feeding programs during rearing.

Feeding Programs	Flock Age	Fertility	Hatchability of		Early Deads	Late Deads
			Fertile Eggs	Total Eggs		
	(wk)	(%)				
HH [1]	25 - 34	96.9	91.2	88.4	3.8	3.1
HL [2]		96.7	93.9	90.8	3.5	1.7
LH [3]		96.6	92.4	89.2	3.6	2.2
LL [4]		97.8	92.2	90.2	3.8	2.7
SEM[5]		0.55	1.33	1.20	0.33	0.53
P-Value		0.393	0.554	0.511	0.945	0.318
HH [1]	35 - 44	97.2	95.7	93.1 [ab]	2.3	1.6
HL [2]		96.9	95.0	92.1[b]	3.1	1.6
LH [3]		97.8	96.2	94.1 [a]	2.7	0.8
LL [4]		96.7	95.4	92.3 [b]	2.8	1.5
SEM[5]		0.31	0.43	0.50	0.32	0.25
P-Value		0.091	0.296	0.048	0.364	0.084
HH [1]	45 - 54	90.3	94.3	85.1	4.4 [a]	1.2
HL [2]		92.3	94.3	87.1	3.8 [ab]	1.5
LH [3]		95.1	94.9	90.3	3.6 [b]	1.3
LL [4]		93.3	94.9	88.6	3.4 [b]	1.5
SEM[5]		1.47	0.39	1.58	0.19	0.27
P-Value		0.182	0.533	0.174	0.017	0.755
HH [1]	55 - 64	74.6 [b]	92.9	69.4	4.6	2.0
HL [2]		81.4 [ab]	91.4	74.4	5.4	2.9
LH [3]		87.7 [a]	90.9	79.8	5.5	2.8
LL [4]		86.3 [a]	91.6	79.0	5.3	2.5
SEM[5]		3.20	0.70	3.04	0.59	0.33
P-Value		0.052	0.273	0.104	0.701	0.331
HH [1]	25 - 64	90.5 [C]	93.7	84.7 [C]	3.6	1.9
HL [2]		92.5 [B]	93.8	86.9 [BC]	3.9	1.8
LH [3]		94.9 [A]	94.0	89.3 [A]	3.6	1.6
LL [4]		94.0 [AB]	93.8	88.2 [AB]	3.6	1.9
SEM[5]		3.73	2.84	4.54	1.41	1.07
P-Value		0.001	0.979	0.001	0.866	0.507

[a,b] Means in a column within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).

[A-C] Means in a column within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).

[1] HH: 52 g of feed per bird per day at 6 wk of age, and 70 g of feed per bird per day at 15 wk of age.
[2] HL: 52 g of feed per bird per day at 6 wk of age, and 64 g of feed per bird per day at 15 wk of age.
[3] LH: 47.5 g of feed per bird per day at 6 wk of age, and 70 g of feed per bird per day at 15 wk of age.
[4] LL: 47.5 g of feed per bird per day at 6 wk of age, and 64 g of feed per bird per day at 15 wk of age.
[5] Standard error of mean (SEM) for n=4 pens of initially 64 hens per pen.

Table II-6. Egg weight (EW) at various ages as affected by female feeding programs during rearing.

Flock Age	Feeding Programs				SEM [5]	P-Value
	HH [1]	HL [2]	LH [3]	LL [4]		
(wk)	(g)					
27	49.72	48.99	49.80	49.00	0.400	0.340
28	52.08	52.04	50.55	51.37	0.817	0.534
29	54.85	54.88	53.73	53.22	0.679	0.266
30	55.82	55.71	55.42	55.78	0.612	0.965
31	57.13 [a]	57.04 [a]	56.82 [a]	56.07 [b]	0.247	0.042
32	57.92	57.96	58.22	57.49	0.280	0.372
33	58.88	57.87	58.02	57.21	0.304	0.589
34	59.44	59.58	59.33	59.02	0.289	0.202
35	60.02	60.47	60.15	59.67	0.365	0.503
41	63.98 [x]	63.29 [xy]	63.31 [xy]	62.49 [y]	0.346	0.068
45	64.82	64.35	64.00	63.97	0.239	0.090
50	65.21	64.89	65.37	64.72	0.192	0.119
53	66.34	66.19	66.52	65.53	0.265	0.097
57	68.02	67.90	67.88	67.36	0.269	0.356
61	71.44	69.62	70.08	68.97	1.005	0.395
65	71.13	70.81	70.87	69.79	0.510	0.312

[a,b] Means in a column within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).

[x,y] Means in a column within each replicate that possess different superscripts differ significantly ($P \leq 0.08$).

[1] HH: 52 g of feed per bird per day at 6 wk of age, and 70 g of feed per bird per day at 15 wk of age.

[2] HL: 52 g of feed per bird per day at 6 wk of age, and 64 g of feed per bird per day at 15 wk of age.

[3] LH: 47.5 g of feed per bird per day at 6 wk of age, and 70 g of feed per bird per day at 15 wk of age.

[4] LL: 47.5 g of feed per bird per day at 6 wk of age, and 64 g of feed per bird per day at 15 wk of age.

[5] Standard error of mean (SEM) for n=4 pens of initially 64 hens per pen.

Table II-7. Egg components at 30, 41, 53, and 61 wk of age as affected by female feeding programs during rearing in manuscript II.

Feeding Programs	Flock Age	Yolk Weight	Albumen	Egg Shell weight	Yolk : Albumen	Shell Thickness	Albumen height
	(wk)	(g)			(g:g)	(µm)	(mm)
HH [1]	30	15.49 [a]	39.16 [A]	5.26	0.40 [B]	368.9 [ab]	8.60 [B]
HL [2]		14.54 [b]	36.62 [B]	5.27	0.40 [B]	378.5 [a]	9.19 [A]
LH [3]		15.41 [a]	36.12 [B]	5.11	0.43 [A]	362.0 [b]	9.20 [A]
LL [4]		15.18 [a]	34.99 [C]	5.04	0.44 [A]	367.1 [b]	8.32 [B]
SEM[5]		0.22	0.24	0.08	0.01	3.89	0.17
P-Value		0.017	0.001	0.126	0.001	0.046	0.001
HH [1]	41	19.21	38.98	5.73	0.50	380.0	7.40
HL [2]		19.41	38.67	5.71	0.49	382.9	7.40
LH [3]		19.81	40.26	5.71	0.47	378.5	7.46
LL [4]		19.66	39.28	5.73	0.50	379.1	6.55
SEM[6]		0.37	0.87	0.11	0.03	5.55	0.34
P-Value		0.688	0.611	0.999	0.747	0.949	0.187
HH [1]	53	21.26	40.12 [a]	5.68	0.53	362.7	7.92
HL [2]		20.93	39.98 [a]	5.71	0.53	360.0	7.38
LH [3]		21.13	39.79 [a]	5.84	0.53	364.3	7.65
LL [4]		21.08	38.29 [b]	5.72	0.55	376.06	7.17
SEM[7]		0.42	0.43	0.12	0.02	5.71	0.37
P-Value		0.965	0.009	0.823	0.687	0.178	0.548
HH [1]	61	22.26	41.53 [ab]	6.12	0.54	381.9 [a]	6.83
HL [2]		23.11	40.79 [bc]	5.98	0.57	339.2 [a]	8.24
LH [3]		22.50	42.59 [a]	6.12	0.53	374.3 [b]	7.49
LL [4]		21.62	39.37 [c]	5.80	0.55	325.7 [b]	7.74
SEM[8]		0.55	0.56	0.19	0.02	10.09	0.52
P-Value		0.323	0.001	0.510	0.598	0.001	0.349

[a-c] Means in a column within each flock age that possess different superscripts differ significantly ($P \leq 0.05$).

[A-C] Means in a column within each flock age that possess different superscripts differ significantly ($P \leq 0.01$).

[1] HH: 52 g of feed per bird per day at 6 wk of age, and 70 g of feed per bird per day at 15 wk of age.

[2] HL: 52 g of feed per bird per day at 6 wk of age, and 64 g of feed per bird per day at 15 wk of age.

[3] LH: 47.5 g of feed per bird per day at 6 wk of age, and 70 g of feed per bird per day at 15 wk of age.

[4] LL: 47.5 g of feed per bird per day at 6 wk of age, and 64 g of feed per bird per day at 15 wk of age.

[5] Standard error of mean (SEM) for n= 30 to 44 samples (all morning eggs per pen).

[6] Standard error of mean (SEM) for n= 16 to 26 samples (all morning eggs per pen).

[7] Standard error of mean (SEM) for n= 11 to 18 samples (all morning eggs per pen).

[8] Standard error of mean (SEM) for n= 6 to 23 samples (all morning eggs per pen).

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MANUSCRIPT III. Phosphorus and phytase application to broiler breeder grower and layer diets affect egg weight and body weight during production

ABSTRACT

The effects on egg weight, egg components, and broiler breeder female BW of a 6-phytase derived from *Buttiauxella spp*. expressed in *Trichoderma reesei* in corn-soy-wheat bran grower (7-26 wk of age) and layer (27-64 wk of age) diets that varied in available phosphorus (AvP) were determined. Dietary treatments for the grower and layer diets, respectively, were: (A-POS) 0.45% AvP and 0.42% AvP, (B-NEG) 0.3% AvP and 0.15 AvP, (C-NEG+250) 0.3% AvP and 0.15 AvP, each with 250 FTU/kg phytase, and (D-NEG-500) 0.3% AvP and 0.15 AvP, each with 500 FTU/kg phytase. Dietary Ca was provided on a 2:1 basis with AvP in grower diets while dietary Ca was 2.7% in the layer diets as adjusted for phytase contribution. Weight of all eggs laid on a single day was determined weekly from 29 to 36 wk of age, and at 41, 53, 64, and 65 wk of age. Shell thickness, and percentage yolk, albumen, and shell were determined at 31, 41, 50, and 60 wk of age. Egg weight was reduced ($P<0.05$) by the (B) 0.3% AvP grower + 0.15 AvP layer treatment relative to the (A-POS) 0.45% AvP grower + 0.42% AvP layer treatment while phytase in C-NEG+250 and D-NEG-500 treatments restored ($P<0.05$) egg weight at 30, 34, 41, 64, and 65 wk of age, with remaining sampling times approaching statistical significance. Cumulatively, average egg weight was 63.2, 62.0, 63.2, and 63.1 for treatments A-POS, B-NEG, C-NEG+250, and D-NEG-500, respectively. This demonstrated that AvP and phytase significantly influenced egg weight. Female BW did not differ between treatments.

Key words: broiler breeders, phytase, egg weight, available phosphorus, albumen

INTRODUCTION

Poultry have been described as being poor in utilizing phytate-P due to lack of sufficient endogenous phytase secretion required to hydrolyze the carbon-phosphate bonds on the myo-inositol ring of phytate (McCuaig *et al.*, 1972). Yet these animals have typically been fed corn-soy based diets containing over 60% of P in the form of phytate that was not available to the animal. In order to reduce the environmental impact of animal production operations, reduce feed cost, and better utilize P in the feed, an accurate evaluation of the quantity of P required for broiler breeders was needed, as were products of exogenous phytase enzymes developed from microbial or fungal origin that have been developed, studied, and commonly included in diets of poultry (Angel *et al.,* 2002; Applegate *et al.,* 2003). Phytase has been shown to hydrolyze phytate and increase the digestion of P, consequently reducing the excretion of P and resultant environment pollution. Phytate, the major form of P stored in plants has acted like a chelating agent, and interacted with minerals such as calcium, magnesium, zinc, iron, copper (Cheryan and Rackis, 1980; Maenz *et al.*, 1999; Maenz, 2001), amino acids (De Rham and Jost, 1979), and starch (Ravindran *et al.*, 1999). Theoretically, phytase digestion of phytate should also benefit the release of the other nutrients that were chelated by phytate. Due to an increased cost of feed, poultry production companies have been eager to find solutions to lower feed price and may sometimes utilize enzyme levels that were lower than those recommended by the enzyme company. The purpose of the following investigation was to determine the phosphoric effects of phytase under commercial conditions and in situations where phytase and phosphorus were supplemented at a lower inclusion for broiler breeders.

MATERIALS AND METHODS

Pullet and Breeder Diets. The experimental protocol used in this study was approved by the North Carolina State University Institutional Animal Care and Use Committee. Day-old chicks (females and males) were fed a single starter crumbled feed (Table III-1) with 17.0% crude protein (CP) and 2.9 kcal/g ME to 6 wk of age with corn ground to 800 μm using a roller mill. This was followed by a 14.5% CP, 2.8 kcal ME/g crumbled growing diet manufactured with 1200 μm roller mill ground corn. The 4 grower dietary treatments are shown in Table III-2. The A-POS grower diet had 0.90% calcium (Ca), and 0.45% dietary available phosphorus (AvP) and the B-NEG contained 0.60% Ca and 0.30% AvP while the two phytase treatments (C-NEG+250 and D-NEG+500) were added "on top" of the negative control diet. Phytase (Danisco Animal Nutrition, Marlborough, UK) was a Buttiauxella spp. derived phytase enzyme that provided 0.83 FTU activity per g of product and was added at 275 g/ MT (C-NEG+250) or 550 g/MT (D-NEG+500) to achieve the final two dietary treatments. The grower diets were fed from 6 to 26 wk of age which was approximately 50% rate of lay. A total of 4 dietary treatments for layer breeder diets (Table III-3) were employed. The control layer diet had 2.7% Ca and 0.42% AvP, while the negative control was 2.7% Ca and 0.15% AvP. The two phytase treatments were added "on top" of the negative control diet at 275 g/MT (C-NEG+250), and 550 g/MT (D-NEG+500). Corn was ground by roller mill to 1200 μm and diets were formulated to contain 14.5% CP and 2.8 kcal ME/g of feed. The corn was ground with a two-pair roller mill (Model C128829, RMS, Tea, SD) with a gap setting of 0% opening (0.15-0.18 mm) on the top pair of rollers and 100% opening (7.16 mm) on the bottom pair of rollers. Dry ingredients were blended for 180 sec in a twin shaft counterpoise mixer (Model TRDB126-0604, Hayes and Stolz, Fort Worth, TX), followed by addition of fat and mixing

for an additional 90 sec. Feed sample were collected and analyzed individually. All of the treatment diets were corn-soybean based with wheat bran as the by-product source. Starter, grower, and layer diets were pelleted and crumbled to destroy endogenous phytase that might be present in the ingredients. The pelleting conditions were targeted to be 17-18% moisture and 80.5 to 82.0°C (177 to 180°F).

Broiler Breeder Rearing Management. There were 1,568 1-d-old female Ross 308SF and 288 male Ross 344 broiler breeder placed in an enclosed fan-ventilated house with 16 female pens (14.3 m^2 area; 80 females per pen) and 16 male pens (4.6 m^2 area; 18 males per pen). All females and males were permanently identified with a neck tag at placement. The litter temperature was 35.0°C (95.0°F) for the first 2 d and the ambient temperature was 30.5-32.2°C (87.0-90.0°F) through 7 d of brooding. From placement to 14 d of age all female pens had 4 tube feeders (DH-4; Kuhl, Flemington, NJ), followed by 3 tube feeders to 10 wk of age. From 11-15 wk, 4 tube feeders were again used, and from 16 wk until pullets were moved to the laying house there were 5 tube feeders used. This maintained appropriate feeder space relative to size of bird. Males had one tube feeder per pen at all times. Two bell drinkers per female pen were used, while males had one bell drinker per pen. During the first week of brooding, an additional 6 feeder lids per female pen and 3 feeder lids per male pen were used. An additional 2 font drinkers per female pen and one font drinker per male pen in addition to the automatic bell drinkers were used. The floor was covered with new wood shavings and general litter management in both of the pullet and breeder houses focused on developing natural immunity against coccidia by spraying water on the wood shavings 2 times per wk on feed days. The house was ventilated to maintain ammonia levels below 25 ppm but not totally absent. The lighting program consisted of 23 h of light per day to 7 d of age, then 8 h to 20 wk

of age when the flock was moved to the laying house. Both sexes received the same dietary treatment during growing. The male and female feeding programs during rearing are outlined in Table III-4.

Broiler Breeder Laying Management. At 20 wk (141 d) of age, 8 males and 64 females were selected according to BW as being in the middle range of the flock (not extremes) from each pen, and moved to a 16-pen laying house. Each of the 16 laying pens (15.9 m^2) was equipped with two-third slats and one-third pine shavings litter (5.9 m^2 litter area/pen). There were 4 tube feeders for females with grills that excluded males and 1 smaller tube feeder for males in each pen. Separation of sexes was insured by special grills (sixteen 4.8 x 5.8 cm holes) on each female feeder that prevented the non-dubbed males from accessing the feed. Water was limited to 8 h per day during the laying period beginning at the time of feeding. Each pen had a conventional nest box comprised of 4 double (50.8 cm wide per nest space) and 4 single nests (25.5 cm wide per nest space). Nest eggs were collected twice daily and identified by pen, separated from floor eggs, and stored in an egg cooler at 16.7°C (62°F) and 70% RH until set for incubation purposes. Birds were photostimulated with 14 h of light when moved at 20 wk of age (141 d). The day length was subsequently increased to 15 h 10 d later, to 15.5 h at 5% production, and finally 16 h at 50% production. Natural light entered the slat-litter house through open or translucent curtains during normal daylight hours. Supplemental light provided an average intensity of 35 lux at bird head level using 18W fluorescent lamps when natural light was not present. The female feeding to peak from 5% lay program is outlined in Table III-5. The male feeding program during lay is outlined in Table III-6.

Body Weight. All males were group weighed at placement, 2, 3, and 4 wk of age, and individually weighed at 5, 6, 13, and 19 wk of age. At 26, 31, 41, 53, and 64 wk of age all

males were weighed individually and 20 randomly selected females per pen were weighed in groups of 4. All females were group weighed at placement, 2, 3, 4, 5, and 6 wk of age and individually weighed at 15 and 19 wk of age.

Egg Production and Hatchability. A minimum of sixty eggs per pen were set at least every other week. After hatching was completed, eggs that did not hatch were examined macroscopically to determine fertility and/or time of embryonic mortality. Embryos that died prior to 8 d of incubation were termed early dead and the embryos that died after 7 d were termed late dead. Cracked eggs that occurred during handling and incubation were not included in the calculations. The eggs were set in a Jamesway model 252B incubator (Butler Manufacturing Co., Ft. Atkinson, WI 53538).

Total Egg weight and Egg Sampling. Total egg weight was determined 3 times per week on all the eggs that were produced within the given day from 29 to 36 wk of age, and at 41, 53, 64, and 65 wk of age. Egg samples (all morning eggs from one pen per treatment) were collected at 31, 41, 50, and 60 wk of age for egg quality assessment. Weights of egg, yolk, shell, and albumen, shell thickness, and percentage yolk, albumen, and shell were determined.

Statistical Analysis. A one-way ANOVA using a completely randomized design with 4 replicate pens per dietary treatment was employed. Variables such as egg production and fertility data were analyzed on both an age quartile and a cumulative basis from 25 to 64 wk of age. There were four 10-wk age quartiles. Data pertaining to egg variables were analyzed by one-way ANOVA within each flock age sample. Data were examined for normality of distributions and homogeneity of variance, and percentage data were subjected to arcsin transformation to normalize distribution prior to analysis. The general linear model of SAS (SAS Institute, 2011) was used to analyze variables, and differences among means were

partitioned by LSMEANS. Differences among means were considered statistically different when $P<0.05$, while $P<0.10$ were considered to approach statistical significance.

RESULTS AND DISCUSSION

Enzyme recovery results for the grower and layer feeds is shown in Table III-7. The A-POS and B-NEG diets had no phytase added. However, the recovery analysis showed that the A-POS diets had 149 FTU/kg in the grower and 64 FTU/kg in the layer, while the B-NEG diets had 110 FTU/kg in the grower and 64 FTU/kg in the layer. This was due to the endogenous phytase that was in the grains (primarily wheat bran). Khan (1986, 1991) demonstrated that heat processing with different methods can reduce up to 57.4% of phytate in wheat and corn. In comparison with other grain feed ingredients, wheat has exhibited a greater phytase activity (915 to 1561 FTU/kg) as well as greater amount of phytate in whole wheat and wheat bran where it ranged from 0.39 to 1.35 g/100 g and 2.1 to 7.3 g/100 g, respectively (Eeckhout and Depaepe, 1994; Schlemmer *et al.*, 2009). Although the ingredients were steam conditioned and feed was pelleted, only a limited amount of endogenous grain phytase was removed. This was most obvious in the grower feed where 14% wheat bran was used in the formula. Wheat bran also had high fiber content that slowed the pelleting throughput and created production rate problems, as fiber was hard to compress into a pellet in this particular feed. Therefore, the endogenous enzymes were not denatured completely. The A and B diets were manufactured prior to the C and D diets to preclude carryover from those diets. The C diet had 278 FTU/kg in the grower and 280 FTU/kg in the layer and D diet had 415 FTU/kg in the grower and 430 FTU/kg. These were reasonable recoveries.

The BW data for males and females are presented in Table III-8 and III-9. All birds were fed the same starter diet to 6 wk of age. After 6 wk of age, the birds were provided different diets

but the same weekly amounts within the same sex. The only formulated differences between the diets were the levels of AvP and Ca, which were lower in the B-NEG treatment. Therefore, BW differences were not expected due to the birds having the same daily and cumulative ME and CP. However, male BW was observed to be different ($P \leq 0.05$) at 41 wk of age where the B-NEG exhibited a lower BW as compared to the A-POS and two enzyme addition treatments C-NEG+250 and D-NEG+500. Overall, the males and females BW of B-NEG exhibited a lower BW. Female BW at 31 wk of age approached statistical significance ($P \leq 0.10$) where the A-POS and B-NEG females weighed less than the C-NEG+250 phytase added treatment, while D-NEG+500 was intermediate. The BW results indicated that both male and female BW was slightly depressed when AvP was reduced in the feed.

Table III-10 depicts the hen-housed egg production, hen-day egg production, and female mortality. The treatment effect for eggs per hen housed approached significance ($P \leq 0.10$) when B-NEG diet with the lowest AvP compared against the A-POS diet during the first quartile of production. The C-NEG+250 and D-NEG+500 treatments with addition of phytase were intermediate. Hen-day production significant differences ($P \leq 0.05$) were observed from 25 to 34 wk of age, where the A-POS exhibited greater egg production than the B-NEG, C-NEG+250, and D-NEG+500 treatments. There were no female mortality differences observed at any point of production. Liu *et al.* (2007) demonstrated that three types of phytase added at 300 FTU/kg in a low P diet recovered overall hen-day production in Hy-Line Brown hens. The AvP level was similar to that of the present study, where grower and layer AvP levels were 0.28% and 0.15%, while the present study was 0.30% and 0.15%. When Ekmay *et al.* (2012) placed Cobb 500 breeder pullets on two different NPP level diets (0.15% and 0.40%), hen-housed egg production was significantly ($P < 0.01$) reduced in hens fed 0.15% NPP by more

than 8 eggs by 40 wk of age. The differences in hen-day production observed by Liu *et al.* (2007), and hen-housed production observed by Ekmay *et al.* (2012) may be due to the bird line differences where Liu *et al.* (2007) utilized Hy-Line Brown layers, Ekmay *et al.* (2012) utilized Cobb 500 breeders, and Ross 308 breeders were used in the current study. Previous studies reported that broiler breeder egg production was not affected negatively when AvP was lowered to 0.20% (Ekmay and Coon, 2010), and that egg production was maintained by 0.09% NPP when the diet was supplemented with phytase (Plumstead *et al.*, 2007).

The effects of dietary phosphorus and addition of phytase to grower and layer diets on fertility and hatchability are shown in Table III-11. The A-POS treatment had the best overall fertility from 25 to 64 wk of age when compared to the C-NEG+250 that exhibited the poorest fertility with B-NEG and D-NEG+500 intermediate ($P \leq 0.05$). In a similar manner, during the first ten wk of production (25 to 34 wk), the A-POS diet produced the best hatchability of fertile eggs as compared to the other three treatments ($P \leq 0.05$). Although there was no difference in the overall fertile egg hatchability, the overall total egg hatchability result showed the A-POS diet to produce the best hatchability while the C-NEG+250 and B-NEG had the worst hatchability, and the C-500 hatchability was intermediate *(*$P \leq 0.01$). These data reflected the differences in fertility. Therefore, adding phytase at 500 FTU in the diet improved total egg hatchability. Early dead differences were observed during 25 to 34 wk of age, where A-POS had the least early embryonic mortality, the C-NEG+250 had the most early embryonic mortality, and B-NEG and D-NEG+500 were intermediate ($P \leq 0.01$). The increase in late embryonic mortality from 55 to 64 wk of age in the A-POS approached statistical significance ($P \leq 0.10$), but no differences were observed during any other production periods. A similar result was reported

by Ekmay *et al.* (2012), where no statistical differences in fertile hatchability to 40 wk of age was observed. However, no specific fertility nor embryonic mortality was reported.

The effects of dietary phosphorus and addition of phytase to grower and layer diets on total egg weight data are shown Table III-12. The greater AvP A-POS diet generally produced a greater egg weight when compared to the B-NEG diet that had the least AvP. The egg weight differences approached statistical significance ($P \leq 0.10$) at 32, and 65 wk of age, and were statistically different ($P \leq 0.05$ or $P \leq 0.01$) at 34, 41, and 64 wk of age, which indicated that egg weight was affected by AvP. The addition of enzyme in treatments C-NEG+250 and D-NEG+500 prevented the egg weight from decreasing. This suggested that the enzyme at both dosages was releasing enough AvP to ameliorate the low AvP effect of the B-NEG diet. Ekmay *et al.* (2012) demonstrated that hens fed diets with 0.15% NPP from 21 to 45 wk of age produced a 1 g significantly ($P \leq 0.05$) smaller egg as compared with the hens that were fed 0.40% NPP.

The effect of dietary phosphorus and addition of phytase to grower and layer diets on egg composition are presented in Table III-13. These eggs sampled in the mornings were independent of the eggs that were sampled for total egg weight above. Normally ovulation happens in the morning. The afternoon eggs have spent more hours in the oviduct, and may have a different egg composition than the eggs that were laid in the morning (Roland, 1974). Yolk weight did not differ at 31, 41, and 50 wk of age. However, the decreased yolk weight at 60 wk of age in hens fed the A-POS diet approached statistical significance ($P \leq 0.10$).

Hens fed the B-NEG and D-NEG+500 exhibited the greatest albumen weight, C-NEG+250 was intermediate, and A-POS was lowest at 31 wk of age. At 41 wk of age, the pattern of albumen weight was different with the D-NEG+500 enzyme added treatment having the

greatest albumen weight, C-NEG+250 intermediate, and A-POS and B-NEG having the lowest albumen weight. No difference in albumen weight was observed at 50 and 60 wk of age. The A-POS eggs had a greater yolk to albumen ratio at 31 wk of age ($P \leq 0.01$), indicating a relatively larger yolk and relatively less albumen weight ($P \leq 0.01$) as a result of the higher AvP in the diet, as compared to all other diets. Although the two enzyme added treatments restored total egg weight, they apparently did not affect yolk to albumen ratio as the C-NEG+250 and D-NEG+500 diets had a similar yolk to albumen ratio as the B-NEG diet. Egg shell weight was significantly different ($P \leq 0.05$) at 41 wk of age, where the B-NEG birds produced the least shell weight compared to all other diets. Shell thickness basically reflected the shell weight, but the only significant difference ($P \leq 0.05$) was observed at 31 wk of age, where the shell thickness was greater from B-NEG and D-NEG+500 eggs while the A-POS and C-NEG+250 demonstrated the thinnest shell within the treatments ($P \leq 0.01$). Previous research demonstrated that 0.15% low dietary NPP would affect egg shell quality, measured by breaking strength, where laying hens fed 0.28% dietary NPP in the layer diet exhibited increased quality of the shell. However, harder shells were not necessarily thicker shells (Liu *et al.*, 2007). In our study, shell breaking strength was not measured, and the difference that was observed in the shell thickness may have been due to enough AvP being present during egg formation. In such a case the bird would not have had to draw AvP from the bones, which meant that there was less Ca available for shell formation. Another possibility could be a thinner eggshell membrane, which was not measured. The two enzyme added treatments had a similar shell thickness to the B-NEG eggs.

Comparing the egg component data (Table III-13) along with the hatchability data (Table III-11) it was observed that during the first quartile of production, all four treatments had excellent

hatchability. However, the A-POS exhibited improved hatchability probably due to relatively less albumen and a thinner shell. Thick albumen and shell has been a common issue in early flock hatchability, and may be partially the result of low AvP.

According to Ekmay *et al.* (2012), dietary NPP needed to be at least 0.20% in order for the Cobb breeders to maintain their egg production, meet physiological requirements, and maintain egg weight. The present study showed similar results, where egg weight was decreased when the birds were fed lower AvP and suggested that phytase may be an economical way to increase AvP for broiler breeders. The enzyme dosage of 250 and 500 FTU in a low phosphorus feed maintained egg weight and BW in male birds. However, egg components were not affected at these enzyme levels.

Table III-1. Breeder starter diet fed from 1-6 wk of age.

Ingredients	(%)
Corn	64.94
Soybean meal (48% CP)	22.10
Poultry fat	1.46
Wheat bran	7.00
Limestone	0.92
Dicalcium phosphate (18.5% P)	1.82
Salt	0.50
DL-Methionine	0.07
Choline chloride (60%)	0.20
Vitamin premix[1]	0.10
Mineral premix[2]	0.20
Selenium premix[3]	0.05
Inert filler[4]	0.63
Total	100.00
Calculated nutrient content	
Crude protein	17.00
Calcium	0.84
Available phosphorus	0.42
Lysine	0.85
Methionine	0.36
Threonine	0.58
Methionine + cysteine	0.65
Sodium	0.20
Metabolizable energy (kcal/g)	2.90

[1]Vitamin premix supplied the following per kg of diet: 6,614 IU vitamin A, 2,000 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 1.98 mg menadione (K_3), 1.98 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 3.97 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.
[2]Mineral premix supplied the following per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt, 1 mg.
[3]Selenium premix provided 0.2 mg Se (as Na_2SeO_3) per kg of diet.
[4]Vermiculite was used as inert filler.

Table III-2. Breeder grower diet fed from 7-26 wk of age.

Ingredients	Dietary Treatments [8]			
	A-POS	B-NEG	C-NEG+250	D-NEG+500
	(%)			
Corn	65.92	65.92	65.92	65.92
Soybean meal (48% CP)	14.62	14.59	14.59	14.59
Poultry fat	0.50	0.50	0.50	0.50
Wheat bran	14.00	14.00	14.00	14.00
Limestone	1.03	0.78	0.78	0.78
Dicalcium phosphate (18.5% P)	1.91	0.98	0.98	0.98
Salt	0.50	0.50	0.50	0.50
DL-Methionine	0.08	0.09	0.09	0.09
L-Lysine	0.06	0.08	0.08	0.08
Choline chloride (60%)	0.20	0.20	0.20	0.20
Vitamin premix[1]	0.10	0.10	0.10	0.10
Mineral premix[2]	0.10	0.10	0.10	0.10
Selenium premix[3]	0.05	0.05	0.05	0.05
Phytase[4]	-	-	0.03	0.06
Inert filler[5]	0.93	0.92	0.92	0.92
Sand[6]	-	1.19	1.16	1.13
Total	100.00	100.00	100.00	100.00
Calculated nutrient content				
Crude protein	14.50	14.50	14.50	14.50
Calcium [7]	0.90	0.60	0.70	0.75
Available phosphorus [7]	0.45	0.30	0.40	0.45
Lysine	0.72	0.73	0.73	0.73
Methionine	0.34	0.34	0.34	0.34
Threonine	0.50	0.50	0.50	0.50
Methionine + cysteine	0.60	0.60	0.60	0.60
Sodium	0.20	0.20	0.20	0.20
Metabolizable energy (kcal/g)	2.80	2.80	2.80	2.80

[1]Vitamin premix supplied the following per kg of diet: 6,614 IU vitamin A, 2,000 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 1.98 mg menadione (K_3), 1.98 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 3.97 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.

[2]Mineral premix supplied the following per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt, 1 mg.

[3]Selenium premix provided 0.2 mg Se (as Na_2SeO_3) per kg of diet.

[4]Phytase was added at 250 FTU/kg for C-NEG+250, and 500 FTU/kg for D-NEG+500.

[5]Vermiculite was used as inert filler.

[6]Sand was used as filler at similar density as dicalcium phosphate and limestone.

[7]Avp and Ca of C-NEG+250 and D-NEG+500 were adjusted according to the phytase matrix value of 0.10 for 250 FTU/kg, 0.15 for 500FTU/kg above the B-NEG.

[8] A-POS was positive control and B-NEG was negative control in breeder diet.

Table III-3. Breeder layer diets fed from 27-64 wk of age.

Ingredients	Dietary Treatments [7]			
	A-POS	B-NEG	C-NEG+250	D-NEG+500
	(%)			
Corn	68.56	68.16	68.16	68.16
Soybean meal (48% CP)	16.74	16.36	16.36	16.36
Poultry fat	0.50	0.50	0.50	0.50
Wheat bran	5.38	6.81	6.81	6.81
Limestone	5.82	6.80	6.80	6.80
Dicalcium phosphate (18.5% P)	1.91	0.20	0.20	0.20
Salt	0.50	0.50	0.50	0.50
DL-Methionine	0.10	0.10	0.10	0.10
L-Lysine	0.04	0.06	0.06	0.06
Choline chloride (60%)	0.20	0.20	0.20	0.20
Vitamin premix[1]	0.10	0.10	0.10	0.10
Mineral premix[2]	0.10	0.10	0.10	0.10
Selenium premix[3]	0.05	0.05	0.05	0.05
Phytase[4]	-	-	0.03	0.06
Inert filler[5]	-	0.06	0.03	-
Total	100.00	100.00	100.00	100.00
Calculated nutrient content				
Crude protein	14.5	14.5	14.5	14.5
Calcium [6]	2.70	2.70	2.80	2.85
Available phosphorus [6]	0.42	0.15	0.25	0.30
Lysine	0.72	0.72	0.72	0.72
Methionine	0.35	0.35	0.35	0.35
Threonine	0.49	0.49	0.49	0.49
Methionine + cysteine	0.60	0.60	0.60	0.60
Sodium	0.20	0.20	0.20	0.20
Metabolizable energy (kcal/g)	2.80	2.80	2.80	2.80

[1]Vitamin premix supplied the following per kg of diet: 6,614 IU vitamin A, 2,000 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 1.98 mg menadione (K_3), 1.98 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 3.97 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.

[2]Mineral premix supplied the following per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; cobalt, 1 mg.

[3]Selenium premix provided 0.2 mg Se (as Na_2SeO_3) per kg of diet.

[4]Phytase was added at 250 FTU/kg for C-NEG+250, and 500 FTU/kg for D-NEG+500.

[5]Vermiculite was used as inert filler.

[6] AvP and Ca of C-NEG+250 and D-NEG+500 were adjusted according to the phytase matrix value of 0.10 for 250 FTU/kg, 0.15 for 500FTU/kg above the B-NEG.

[7] A-POS was positive control and B-NEG was negative control in breeder diet.

Table III-4. Feeding program from initial placement to sexual maturity.

Flock Age	Feed Allocation[1]	
	Female	Male
(wk)	(g/bird/d)	
1	32.4	38.9
2	32.4	50.0
3	34.0	55.0
4	37.0	60.0
5	40.0	70.0
6	43.0	80.0
7	45.0	65.0
8	47.0	67.0
9	49.0	69.0
10	51.0	71.0
11	53.0	73.0
12	55.0	75.0
13	58.0	78.0
14	61.0	81.0
15	64.0	84.0
16	68.0	87.0
17	72.0	91.0
18	78.0	96.0
19	84.0	102.0
20	90.0	108.0
21	94.0	114.1
22	98.0	116.9

[1] Females and males were fed on a daily basis to 28 d of age followed by 4/3 feeding to 13 wk of age. A 5/2 feeding program was therefore employed to photostimulation and housing at 20 wk of age. Daily feeding was utilized beginning at photostimulation at 141 d of age.

Table III-5. Female feeding to peak program from 5% lay.

Daily increase	Feed Allocation[1]
(d)	(g/bird/d)
1	110.8
2	111.6
3	112.4
4	113.2
5	114.0
6	115.0
7	116.0
8	118.0
9	121.0
10	124.0
11	127.0
12	131.0
13	135.0
14	140.0
15	146.0
16	152.0
17	155.0

[1]Females received daily feed amounts as shown above beginning at 5% rate of lay.

Table III-6. Male broiler breeder feeding program from photostimulation at 20 wk of age.

Flock Age	Feed Allocation[1]
(wk)	(g/bird/d)
20	108.0
21	114.1
22	116.9
23	119.2
24	121.5
25	123.2
26	123.0
27	123.0
28	125.0
29	125.0
30	127.1
33	127.1
36	129.8
39	132.6
42	135.3
45	138.0
48	140.7
51	143.5
54	146.2
57	148.9
60	151.6

[1] Males were fed amounts as shown above on a daily basis in a separate male feeder.

Table III-7. Dietary enzyme recovery analysis results. [1]

Feed Type	Treatment	Enzyme Added (g/MT)	Formula Enzyme (FTU/kg)	Enzyme Recovery (FTU/kg)
Grower	A-POS[2]	0	0	149.6
	B-NEG[3]	0	0	110.0
	C-NEG+250[4]	275	250	278.2
	D-NEG+500[5]	550	500	415.0
Layer	A-POS	0	0	64.0
	B-NEG	0	0	64.0
	C-NEG+250	275	250	280.3
	D-NEG+500	550	500	429.8

[1] Enzyme recovery analysis by DuPont Nutrition Biosciences ApS, Denmark.
[2] A-POS: Grower with 0.90% Ca and 0.45% AvP, and layer with 2.7% Ca and 0.42% AvP.
[3] B-NEG: Grower with 0.60% Ca and 0.30% AvP, and layer with 2.7% Ca and 0.15% AvP.
[4] C-NEG+250: Grower with 0.60% Ca and 0.30% AvP +250 FTU phytase, and layer with 2.7% Ca and 0.15% AvP +250 FTU phytase.
[5] D-NEG+500: Grower with 0.60% Ca and 0.30% AvP +500 FTU phytase, and layer with 2.7% Ca and 0.15% AvP +500 FTU phytase.

Table III-8. Body weight of male broiler breeder as affected by flock age, different levels of available phosphorous (AvP), calcium (Ca), and addition of phytase in grower and layer diets of broiler breeder feed.

	Dietary Treatments [1]					
Flock Age	A-POS [2]	B-NEG [3]	C-NEG+250 [4]	D-NEG+500 [5]	SEM	P-Value
(wk)			(g)			
0	40	39	40	40	0.31[6]	0.736
2	259	264	264	246	4.26[6]	0.039
3	509	522	506	473	8.72[6]	0.012
4	756	794	771	690	24.21[7]	0.054
5	1089	1098	1130	1173	20.03[7]	0.046
6	1234	1290	1276	1333	22.11[7]	0.053
13	2142	2127	2148	2149	37.78[7]	0.826
19	3107	3118	3099	3132	13.46[7]	0.374
24	3672	3573	3652	3678	94.79[7]	0.850
26	3723	3676	3779	3728	76.07[7]	0.822
31	3915	3767	3886	4008	102.66[7]	0.451
41	4069 [a]	3723 [b]	4117 [a]	4179 [a]	85.32[7]	0.011
53	4511	4411	4624	4590	82.00[7]	0.310
64	4913	4891	5066	5004	102.11[7]	0.604

[a,b] Means in a row within each flock age that possess different superscripts differ significantly ($P \leq 0.05$).
[1] A-POS was positive control and B-NEG was negative control in breeder diet.
[2] A-POS: Grower with 0.90% Ca and 0.45% AvP, and layer with 2.7% Ca and 0.42% AvP.
[3] B-NEG: Grower with 0.60% Ca and 0.30% AvP, and layer with 2.7% Ca and 0.15% AvP.
[4] C-NEG+250: Grower with 0.60% Ca and 0.30% AvP+250 FTU phytase, and layer with 2.7% Ca and 0.15% AvP+250 FTU phytase.
[5] D-NEG+500: Grower with 0.60% Ca and 0.30% AvP+500 FTU phytase, and layer with 2.7% Ca and 0.15% AvP+500 FTU phytase.
[6] Standard error of mean (SEM) for n=4 pens with 18 birds per pen weighed.
[7] Standard error of mean (SEM) for n=4 pens with 8 birds per pen weighed.

Table III-9. Body weight of female broiler breeder as affected by flock age, different levels of available phosphorous (AvP), calcium (Ca), and addition of phytase in grower and layer diets of broiler breeder feed.

Flock Age	Dietary Treatments [1]				SEM	P-Value
	A-POS [2]	B-NEG [3]	C-NEG+250 [4]	D-NEG+500 [5]		
(wk)	(g)					
0	41	40	41	41	0.18 [6]	0.617
2	224	226	226	231	2.54 [6]	0.294
3	397	399	404	408	5.16 [6]	0.492
4	520	515	526	525	3.26 [6]	0.109
5	623	621	627	622	5.84 [6]	0.862
6	723	724	725	720	4.98 [6]	0.905
15	1531	1544	1519	1548	13.46 [6]	0.437
19	1996	2012	2007	1998	10.31 [6]	0.687
26	3028	2982	3065	3050	39.47 [7]	0.505
31	3411 [y]	3409 [y]	3501 [x]	3454 [xy]	24.01 [7]	0.060
41	3718 [x]	3616 [y]	3652 [xy]	3667 [xy]	26.24 [7]	0.101
53	3807	3906	3963	3879	45.25 [7]	0.162
64	4048	3964	4048	3944	36.38 [8]	0.132

[x,y] Means in a row within each replicate that possess different superscripts approached statistical significance ($P \leq 0.10$).
[1] A-POS was positive control and B-NEG was negative control in breeder diet.
[2] A-POS: Grower with 0.90% Ca and 0.45% AvP, and layer with 2.7% Ca and 0.42% AvP.
[3] B-NEG: Grower with 0.60% Ca and 0.30% AvP, and layer with 2.7% Ca and 0.15% AvP.
[4] C-NEG+250: Grower with 0.60% Ca and 0.30% AvP+250 FTU phytase, and layer with 2.7% Ca and 0.15% AvP+250 FTU phytase.
[5] D-NEG+500: Grower with 0.60% Ca and 0.30% AvP+500 FTU phytase, and layer with 2.7% Ca and 0.15% AvP+500 FTU phytase.
[6] Standard error of mean (SEM) for n=4 pens with 80 birds per pen weighed.
[7] Standard error of mean (SEM) for n=4 pens with 20 birds per pen weighed.
[8] Standard error of mean (SEM) for n=4 pens with 64 birds per pen weighed.

Table III-10. Broiler breeder egg production and female mortality as affected by flock age, different levels of available phosphorous (AvP), calcium (Ca), and addition of phytase in grower and layer diets of broiler breeder feed.

Dietary Treatments[1]	Flock Age	Egg Production		Male Mortality	Female Mortality
		Eggs per Hen Housed	Hen-day Production		
	(wk)	(n)	(%)	(%)	(%)
A-POS[2]	25 - 34	53.1 [x]	69.7 [a]	9.4	2.3
B-NEG[3]		50.4 [y]	65.9 [b]	9.4	1.6
C-NEG+250[4]		51.0 [xy]	66.6 [b]	3.6	1.2
D-NEG+500[5]		51.2 [xy]	67.1 [b]	9.8	1.6
SEM[6]		0.65	0.01	0.04	0.01
P-Value		0.059	0.019	0.681	0.882
A-POS[2]	35 - 44	49.8	72.2	0.0	0.8
B-NEG[3]		48.7	71.0	9.4	1.2
C-NEG+250[4]		50.0	72.7	6.3	1.2
D-NEG+500[5]		49.1	70.6	3.1	0.8
SEM[6]		0.96	0.02	0.04	0.01
P-Value		0.744	0.672	0.383	0.938
A-POS[2]	45 - 54	42.9	62.8	0.0	0.8
B-NEG[3]		41.1	60.3	3.1	0.8
C-NEG+250[4]		42.1	61.8	0.0	0.8
D-NEG+500[5]		41.6	60.4	0.0	0.4
SEM[6]		1.23	0.02	0.02	0.01
P-Value		0.768	0.587	0.426	0.893
A-POS[2]	55 - 64	28.4	53.0	0.0	1.2
B-NEG[3]		27.4	51.5	0.0	2.7
C-NEG+250[4]		25.7	48.1	0.0	2.7
D-NEG+500[5]		27.2	50.5	6.3	3.1
SEM[6]		0.09	0.02	0.03	0.01
P-Value		0.280	0.206	0.426	0.680
A-POS[2]	25 - 64	174.3	65.7	9.4	5.1
B-NEG[3]		167.6	63.0	21.9	6.3
C-NEG+250[4]		168.8	63.2	9.8	5.9
D-NEG+500[5]		169.3	63.4	19.2	5.9
SEM[6]		3.13	0.02	0.06	0.02
P-Value		0.472	0.257	0.375	0.947

[x,y] Means in a column within each flock age that possess different superscripts approached statistical significance ($P \leq 0.10$).

[a,b] Means in a column within each flock age that possess different superscripts differ significantly ($P \leq 0.05$).

[1] A-POS was positive control and B-NEG was negative control in breeder diet.

[2] A-POS: Grower with 0.90% Ca and 0.45% AvP, and layer with 2.7% Ca and 0.42% AvP.

[3] B-NEG: Grower with 0.60% Ca and 0.30% AvP, and layer with 2.7% Ca and 0.15% AvP.

[4] C-NEG+250: Grower with 0.60% Ca and 0.30% AvP+250 FTU phytase, and layer with 2.7% Ca and 0.15% AvP+250 FTU phytase.

[5] D-NEG+500: Grower with 0.60% Ca and 0.30% AvP+500 FTU phytase, and layer with 2.7% Ca and 0.15% AvP+500 FTU phytase.

[6] Standard error of mean (SEM) for n=4 pens of approximately 64 hens per pen.

Table III-11. Broiler breeder fertility, hatchability, and embryonic mortality as affected by flock age, different levels of available phosphorous (AvP), calcium (Ca), and addition of phytase in grower and layer diets of broiler breeder feed.

Dietary Treatments[1]	Flock Age	Fertility	Hatchability of		Early Deads	Late Deads
			Fertile Eggs	Total Eggs		
	(wk)			(%)		
A-POS[2]	25 - 34	96.8	94.5 ^{a}	91.5	2.0 ^{C}	2.5
B-NEG[3]		97.6	91.4 ^{b}	89.3	3.1 ^{B}	4.1
C-NEG+250[4]		96.7	90.2 ^{b}	87.3	5.3 ^{A}	3.8
D-NEG+500[5]		97.7	91.8 ^{b}	89.7	4.2 ^{B}	3.5
SEM[6]		0.03	0.02	0.03	0.01	0.01
P-Value		0.631	0.031	0.116	0.001	0.423
A-POS[2]	35 - 44	95.1	94.2	89.6	3.9	1.4
B-NEG[3]		92.5	92.9	86.0	4.2	2.5
C-NEG+250[4]		94.7	94.4	89.4	3.5	1.9
D-NEG+500[5]		95.3	93.5	89.2	4.1	2.0
SEM[6]		0.06	0.03	0.05	0.01	0.01
P-Value		0.789	0.595	0.658	0.872	0.362
A-POS[2]	45 - 54	95.4	94.0	89.6	4.3	1.4
B-NEG[3]		91.2	93.7	85.4	4.1	1.9
C-NEG+250[4]		91.0	92.5	84.2	5.2	1.7
D-NEG+500[5]		94.2	92.7	87.3	4.5	2.2
SEM[6]		0.05	0.02	0.04	0.01	0.01
P-Value		0.294	0.454	0.336	0.730	0.386
A-POS[2]	55 - 64	94.1	89.2	83.9	4.8	5.0 ^{x}
B-NEG[3]		94.3	91.9	86.7	4.7	2.8 ^{y}
C-NEG+250[4]		91.0	90.6	82.5	5.2	3.0 ^{y}
D-NEG+500[5]		91.9	91.1	83.8	5.0	3.2 ^{y}
SEM[6]		0.04	0.03	0.03	0.01	0.01
P-Value		0.325	0.473	0.438	0.955	0.063
A-POS[2]	25 - 64	95.5 ^{a}	93.1	88.9 ^{A}	4.0	2.5
B-NEG[3]		93.9 ^{bc}	92.7	87.1 ^{BC}	4.1	2.7
C-NEG+250[4]		93.3 ^{c}	91.9	85.8 ^{C}	4.8	2.6
D-NEG+500[5]		94.9 ^{ab}	92.3	87.7 ^{AB}	4.4	2.7
SEM[6]		0.08	0.07	0.03	0.02	0.02
P-Value		0.014	0.294	0.007	0.125	0.943

^{x,y} Means in a column within each flock age that possess different superscripts differ significantly ($P \leq 0.10$).

$^{a-c}$ Means in a column within each flock age that possess different superscripts differ significantly ($P \leq 0.05$).

$^{A-C}$ Means in a column within each flock age that possess different superscripts differ significantly ($P \leq 0.01$).

[1] A-POS was positive control and B-NEG was negative control in breeder diet.

[2] A-POS: Grower with 0.90% Ca and 0.45% AvP, and layer with 2.7% Ca and 0.42% AvP.

[3] B-NEG: Grower with 0.60% Ca and 0.30% AvP, and layer with 2.7% Ca and 0.15% AvP.

[4] C-NEG+250: Grower with 0.60% Ca and 0.30% AvP+250 FTU phytase, and layer with 2.7% Ca and 0.15% AvP+250 FTU phytase.

[5] D-NEG+500: Grower with 0.60% Ca and 0.30% AvP+500 FTU phytase, and layer with 2.7% Ca and 0.15% AvP+500 FTU phytase.

[6] Standard error of mean (SEM) for n=4 pens of initially 64 hens per pen.

Table III-12. Total egg weight as affected by flock age, different levels of available phosphorous (AvP), calcium (Ca), and addition of phytase in grower and layer diets of broiler breeder feed.

Flock Age	Dietary Treatments[1]				SEM [6]	P-Value
	A-POS [2]	B-NEG [3]	C-NEG+250 [4]	D-NEG+500 [5]		
(wk)	(g)					
29	55.5	55.6	55.4	55.4	0.44	0.990
30	58.0	56.9	57.4	57.5	0.35	0.217
31	58.6	57.6	60.0	58.2	0.99	0.401
32	59.0	58.1	58.3	58.3	0.24	0.101
33	60.2	57.9	60.2	60.1	0.85	0.184
34	60.8 [a]	60.1 [b]	60.7 [a]	61.1 [a]	0.18	0.012
35	61.4	61.3	61.6	61.4	0.29	0.913
36	62.6	61.7	62.2	62.6	0.45	0.535
41	66.6 [a]	66.4 [b]	66.5 [a]	66.6 [a]	0.28	0.042
53	70.2	68.6	70.4	72.1	1.01	0.181
64	72.1 [a]	69. 9 [b]	72.2 [a]	72.2 [a]	0.40	0.004
65	72.8 [a]	70.9 [b]	72.8 [a]	72.6 [a]	0.48	0.057

[a,b] Means in a row within each flock age that possess different superscripts differ significantly ($P \leq 0.05$).
[1] A-POS was positive control and B-NEG was negative control in breeder diet.
[2] A-POS: Grower with 0.90% Ca and 0.45% AvP, and layer with 2.7% Ca and 0.42% AvP.
[3] B-NEG: Grower with 0.60% Ca and 0.30% AvP, and layer with 2.7% Ca and 0.15% AvP.
[4] C-NEG+250: Grower with 0.60% Ca and 0.30% AvP+250 FTU phytase, and layer with 2.7% Ca and 0.15% AvP+250 FTU phytase.
[5] D-NEG+500: Grower with 0.60% Ca and 0.30% AvP+500 FTU phytase, and layer with 2.7% Ca and 0.15% AvP+500 FTU phytase.
[6] Standard error of mean (SEM) for n=4 pens with 3 sample collections per week. All eggs laid on a given day weighed as a pen group.

Table III-13. Egg weight and egg components at 31, 41, 50, and 60 wk of age as affected by flock age, different levels of available phosphorous (AvP), calcium (Ca), and addition of phytase in grower and layer diets of broiler breeder feed.

Dietary Treatments[1]	Flock Age	Yolk Weight	Albumen	Egg Shell weight	Yolk : Albumen	Shell Thickness	Albumen height
	(wk)		(g)		(g:g)	(µm)	(mm)
A-POS[2]	31	17.11	33.81 ^{C}	5.42	0.51 ^{A}	373.0 ^{B}	7.83 ^{a}
B-NEG[3]		16.53	37.19 ^{A}	5.73	0.45 ^{B}	405.3 ^{A}	8.33 ^{a}
C-NEG+250[4]		16.96	36.52 ^{B}	5.63	0.47 ^{B}	385.0 ^{B}	7.55 ^{b}
D-NEG+500[5]		17.11	36.78 ^{AB}	5.68	0.47 ^{B}	406.3 ^{A}	8.05 ^{a}
SEM [6]		1.417	1.494	0.617	0.058	0.21	1.105
P-Value		0.318	0.001	0.207	0.001	0.002	0.020
A-POS[2]	41	20.94	39.64 ^{b}	5.96 ^{x}	0.53	385.4	6.53 ^{y}
B-NEG[3]		20.38	39.37 ^{b}	5.73 ^{y}	0.52	380.9	7.19 ^{xy}
C-NEG+250[4]		20.45	40.18 ^{ab}	6.30 ^{x}	0.51	394.4	7.37 ^{x}
D-NEG+500[5]		20.80	40.62 ^{a}	6.04 ^{x}	0.51	383.6	7.39 ^{x}
SEM[7]		1.432	1.493	0.609	0.053	2.67	1.214
P-Value		0.561	0.032	0.058	0.722	0.485	0.096
A-POS[2]	50	22.16	41.72	5.95	0.53	392.9	7.09
B-NEG[3]		22.78	43.24	6.05	0.53	392.9	6.77
C-NEG+250[4]		22.26	42.98	6.05	0.52	393.1	6.31
D-NEG+500[5]		22.13	40.89	5.82	0.54	385.2	6.85
SEM[8]		4.266	8.097	0.630	0.106	3.11	1.601
P-Value		0.720	0.269	0.780	0.350	0.905	0.548
A-POS[2]	60	22.34 ^{y}	42.32	5.86	0.53 ^{y}	33.28	6.29
B-NEG[3]		22.98 ^{x}	41.80	6.06	0.55 ^{x}	35.19	5.82
C-NEG+250[4]		22.85 ^{x}	42.96	6.07	0.54 ^{x}	34.05	5.31
D-NEG+500[5]		22.92 ^{x}	42.18	5.89	0.55 ^{x}	34.17	5.03
SEM[9]		3.356	6.688	0.684	0.090	3.50	1.846
P-Value		0.061	0.174	0.890	0.098	0.671	0.426

[x,y] Means in a column within each flock age that possess different superscripts approached statistical significance ($P \leq 0.10$).

[a-c] Means in a column within each flock age that possess different superscripts differ significantly ($P \leq 0.05$).

[A-C] Means in a column within each flock age that possess different superscripts differ significantly ($P \leq 0.01$).

[1] A-POS was positive control and B-NEG was negative control in breeder diet.

[2] A-POS: Grower with 0.90% Ca and 0.45% AvP, and layer with 2.7% Ca and 0.42% AvP.

[3] B-NEG: Grower with 0.60% Ca and 0.30% AvP, and layer with 2.7% Ca and 0.15% AvP.

[4] C-NEG+250: Grower with 0.60% Ca and 0.30% AvP+250 FTU phytase, and layer with 2.7% Ca and 0.15% AvP+250 FTU phytase.

[5] D-NEG+500: Grower with 0.60% Ca and 0.30% AvP+500 FTU phytase, and layer with 2.7% Ca and 0.15% AvP+500 FTU phytase.

[6] Standard error of mean (SEM) for 30 to 44 samples (all morning eggs per pen).

[7] Standard error of mean (SEM) for 16 to 26 samples (all morning eggs per pen).

[8] Standard error of mean (SEM) for 11 to 18 samples (all morning eggs per pen).

[9] Standard error of mean (SEM) for 6 to 23 samples (all morning eggs per pen).

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MANUSCRIPT IV. Effect of Dietary Available Phosphorous and Phytase in Broiler Breeder and Broiler Progeny Diets on the Live Performance of the Broilers.

ABSTRACT

In previous research it had been suggested that broiler breeders fed different levels of AvP and 6-phytase derived from *Buttiauxella spp*. expressed in *Trichoderma reesei* exhibited altered egg components and egg weight. Thus, this study was conducted to evaluate the effect of similar diets on broiler progeny performance. Broiler hatching eggs were collected from a 36-wk-old breeder flock that was fed four dietary treatments in their grower and layer diets, respectively: (A-POS) 0.45% AvP and 0.42% AvP, (B-NEG) 0.3% AvP and 0.15 AvP, (C-NEG+250) 0.3% AvP and 0.15 AvP, each with 250 FTU/kg phytase, (D-NEG+500) 0.3% AvP and 0.15 AvP, each with 500 FTU/kg phytase. Broiler BW and feed intake were measured at 0, 14, 28, 35, and 42 d of age, and any mortality was collected twice daily and weighed. Chick weight at hatching was significantly greater ($P \leq 0.05$) in the A-POS treatment where breeders consumed feed with higher AvP, the two phytase added treatments C-NEG+250 and D-NEG+500 were intermediate, and the B-NEG chicks exhibited the lowest BW at hatching. The broilers were placed on three dietary treatments, Control (CON) with higher AvP, Negative Control (NC) with lower AvP, and (NC+1000), the NC with addition of 1000 FTU/kg phytase (NC+1000). The broilers fed the NC+1000 diet had significantly greater BW at 14, 28, 35, and 42 d of age when compared to the other two treatments. The BW gain was significantly greater in the NC+1000 treatment to 35 d of age, as was feed intake at 0-14 d, 15-28 d, and cumulative feed intake at 28 and 35 d of age. There was lack of interaction of breeder diet effect on overall

broiler performance observed in this study, indicating that there was no maternal effect of parental treatment on progeny response to the dietary phytase evaluated in this study.

Key words: broiler breeder, phosphorous, phytase, broiler, maternal effect

INTRODUCTION

It has been well documented that poultry were poor in utilizing phytate-P, especially young birds, due to lack of sufficient endogenous phytase secretion required to hydrolyze the carbon-phosphate bonds on the myo-inositol ring of phytate (McCuaig *et al.*, 1972). These animals have typically been fed a corn-soy based diet that contained over 60% of P in phytate that was not available to the animals. Phytase products have been developed as feed additives to reduce the environmental impact of animal production operations, reduce feed cost, and better utilize P in the feed. Phytase has been shown to hydrolyze phytate and increase the digestion of P, consequently reducing the excretion of P with a benefit towards reduced environment pollution. Phytate has been shown to be a chelating agent that interacted with minerals, amino acids (De Rham and Jost, 1979), and starch (Ravindran *et al.,* 1999). The addition of phytase was thought to benefit the release of the other nutrients that were chelated by phytate. Depending on the enzyme product and the feed formulation, the combination of reduced dietary AvP and added phytase has demonstrated little to no effect on egg production (Berry *et al.,* 2003; Li *et al.,* 2002; Brake *et al.,* 2003). Research concerning the maternal effects of enzymes was very limited. However, some research has suggested that there may be negative maternal effects of multi-enzyme cocktails fed to broiler breeders on broiler progeny performance when the progeny also received the same enzyme(s) in the feed (Arguelles-Ramos, 2011). However, new versions of phytase have been developed over the years and the effect of the phytase on broiler breeders and their progeny may have changed. Previously it was suggested that broiler breeders fed diets with a 6-phytase derived from *Buttiauxella spp*. expressed in *Trichoderma reesei* in the diet exhibited altered egg components and egg weight. Due to the differences in egg components, we expected to observe differences in progeny

growth and development. The objective for this study was to study the effect of phosphorus and phytase application to broiler breeder grower and layer diets on the live performance of their progeny when the progeny received similar diets.

MATERIALS AND METHODS

Pullet and Breeder Diets. The experimental protocol used in this study was approved by the North Carolina State University Institutional Animal Care and Use Committee. Day-old chicks (females and males) were fed a single starter crumbled feed (Table III-1) with 17.0% crude protein (CP) and 2.9 kcal/g ME to 6 wk of age with corn ground to 800 μm using a roller mill. This was followed by a 14.5% CP, 2.8 kcal ME/g crumbled grower diet manufactured with 1200 μm roller mill ground corn. The 4 grower dietary treatments are shown in Table III-2. The A-CON grower diet had 0.90% calcium (Ca), and 0.45% dietary available phosphorus (AvP) and the B-NEG contained 0.60% Ca and 0.30% AvP while the two phytase treatments (C-NEG+250 and D-NEG+500) were added "on top" of the negative control diet. Axtra® PHY 2500 TPT (Danisco Animal Nutrition, Marlborough, UK) was a Buttiauxella spp. Expressed in *Trichoderma reesei* derived phytase enzyme that provided 0.83 FTU activity per g of product and was added at 275 g/MT (C-NEG+250) or 550 g/MT (D-NEG+500) to achieve the final two breeder dietary treatments. The grower diets were fed from 6 to 26 wk of age, which was approximately 50% rate of lay. A total of 4 dietary treatments for breeder layer diets (Table III-3) were employed. The control layer diet (A-CON) had 2.7% Ca and 0.42% AvP, while the negative control was 2.7% Ca and 0.15% AvP. The two phytase treatments were added "on top" of the negative control (B-NEG) diet at 275 g/MT (C-NEG+250), and 550 g/MT (D-NEG+500). Corn was ground by roller mill to 1200 μm and diets were formulated to contain 14.5% CP and 2.8 kcal ME/g of feed. The corn was ground with a two-pair roller mill (Model

C128829, RMS, Tea, SD) with a gap setting of 0% opening (0.15-0.18 mm) on the top pair of rollers and 100% opening (7.16 mm) on the bottom pair of rollers. Dry ingredients were blended for 180 sec in a twin shaft counterpoise mixer (Model TRDB126-0604, Hayes and Stolz, Fort Worth, TX) followed by addition of fat and mixing for an additional 90 sec. Samples from each batch of feed were collected and analyzed individually. All of the treatment diets were corn-soybean meal based with wheat bran as the by-product source. Starter, grower, and layer diets were pelleted and crumbled to destroy endogenous phytase that might be present in the ingredients. The pelleting conditions were targeted to be 17-18% moisture and 80.5 to 82.0°C (177 to 180°F).

Broiler Breeder Rearing Management. There were 1,568 1-d-old female Ross 308SF and 288 male Ross 344 broiler breeders placed in an enclosed fan-ventilated house with 16 female pens (14.3 m^2 area; 80 females per pen) and 16 male pens (4.6 m^2 area; 18 males per pen). All females and males were permanently identified with a neck tag at placement. The litter temperature was 35.0°C (95.0°F) for the first 2 d and the ambient temperature was 30.5-32.2°C (87.0-90.0°F) through 7 d of brooding. From placement to 14 d of age all female pens had 4 tube feeders (DH-4; Kuhl, Flemington, NJ), followed by 3 tube feeders to 10 wk of age. From 11-15 wk, 4 tube feeders were again used, and from 16 wk until pullets were moved to the laying house there were 5 tube feeders used. This variation of number of feeders maintained appropriate feeder space relative to size of bird. Males had one tube feeder per pen at all times. Two bell drinkers per female pen were used, while males had one bell drinker per pen. During the first week of brooding, an additional 6 feeder lids per female pen and 3 feeder lids per male pen were used. An additional 2 font drinkers per female pen and one font drinker per male pen in addition to the automatic bell drinkers were used. The floor was covered with new wood

shavings and general litter management in both of the pullet and breeder houses focused on developing natural immunity against coccidia by spraying water on the wood shavings 2 times per wk on feed days. The house was ventilated to maintain ammonia levels below 25 ppm but not totally absent. The lighting program consisted of 23 h of light per day to 7 d of age, then 8 h to 20 wk of age when the flock was moved to the laying house. Both sexes received the same dietary treatment during growing. The male and female feeding programs during rearing are outlined in Table III-4.

Broiler Breeder Laying Management. At 20 wk (141 d) of age, 8 males and 64 females were selected according to BW as being in the middle range of the flock (not extremes) from each pen, and moved to a 16-pen laying house. Each of the 16 laying pens (15.9 m^2) was equipped with two-third slats and one-third pine shavings litter (5.9 m^2 litter area/pen). There were 4 tube feeders for females with grills that excluded males and 1 smaller tube feeder for males in each pen. Separation of sexes was insured by special grills (sixteen 4.8 x 5.8 cm holes) on each female feeder that prevented the non-dubbed males from accessing the feed. Water was limited to 8 h per day during the laying period beginning at the time of feeding. Each pen had a conventional nest box comprised of 4 double (50.8 cm wide per nest space) and 4 single nests (25.5 cm wide per nest space). Nest eggs were collected twice daily and identified by pen, separated from floor eggs, and stored in an egg cooler at 16.7°C (62°F) and 70% RH until set for incubation purposes. Birds were photostimulated with 14 h of light when moved at 20 wk of age (141 d). The day length was subsequently increased to 15 h 10 d later, to 15.5 h at 5% egg production, and finally to 16 h at 50% egg production. Natural light entered the slat-litter house through open or translucent curtains during normal daylight hours. Supplemental light provided an average intensity of 35 lux at bird head level using 18W fluorescent lamps when

natural light was not present. The female feeding to peak from 5% lay program is outlined in Table III-5. The male feeding program during lay is outlined in Table III-6.

Egg collection and Incubation. To evaluate the possible maternal effects of broiler breeder dietary phytase and AvP level, a broiler progeny trial was conducted. Broiler hatching eggs were collected at 36 wk of age and the eggs were identified by breeder pen. A total of 5,760 eggs, 360 eggs per breeder pen (16 pens), were set in 3 Jamesway model 252B incubators (Butler Manufacturing Co., Ft. Atkinson, WI 53538). The eggs were initially incubated at 37.5 ºC (99.5 ºF) dry bulb. Then the temperature was adjusted gradually to 36.7 ºC (98 ºF). The wet bulb temperature was 28.9 ºC (84 ºF). At 18 d of incubation, the trays were transferred into 4 Jamesway model 252B incubators. At 21.5 d of incubation the chicks that had completed the hatching process were removed from the trays, counted, group weighed, and sexed using the feather-sexing method.

Placement and Pen Set Up. After the hatching and sexing process was completed, the chicks were permanently identified with neck tags and then distributed among 72 single-sex floor pens with wood litter shavings. There were 28 male chicks from the same broiler breeder treatment placed in each pen with the area of each pen being 1.2 m width by 3.8 m length, with a stocking density of 6.1 chicks/m^2. The litter temperature was 35 ºC (95° F) for the first 2 d and the ambient temperature was 32.2ºC (90° F) through 7 d of brooding. Each pen contained one bell-type drinker and two tube feeders (DH-4; Kuhl, Flemington, NJ). An additional 3 supplemental feeder flats were used until 5 d of age, 2 flats to 10 d, and 1 flat to 14 d of age. An additional front drinker was used to 10 d of age. The birds were raised on used litter that was top-dressed with fresh litter.

Lighting Program. The lighting program during the first 7 d was 23 d of light, then 21 h to 21 d. After 21 d of age only natural light was used to control excessive growth and improve livability.

Broiler Dietary Treatments. Each broiler pen was assigned to one of three broiler diet treatments within the 4 breeder treatments with 6 replicates per interaction. A total of 3 broiler dietary treatments were tested with 2 levels of AvP: high and low, and low AvP with enzyme addition. The control (CON) treatment was prestarter with 1.0% Ca, and 0.50 AvP, starter with 0.9% Ca and 0.45 AvP, grower with 0.7% Ca and 0.35% AvP, and finisher with 0.6% Ca, and 0.30% AvP. The negative control (NC) was prestarter with 0.85% Ca and 0.35 AvP, starter with 0.75% Ca and 0.30% AvP, grower with 0.55% Ca and 0.20% AvP, and finisher with 0.45% Ca and 0.15% AvP. The negative control with 1000FTU/kg enzyme added (NC+1000) was prestarter with 0.85% Ca, and 0.35 AvP, starter with 0.75% Ca and 0.30% AvP, grower with 0.55% Ca and 0.20% AvP, and finisher with 0.45% Ca and 0.15% AvP + 1000 FTU phytase. The chicks were given a budget of 0.22, 0.45, 2.26, and 3.62 kg (0.5, 1, 5, and 8 lbs.) prestarter, starter, grower, and finisher feeds, respectively. Broilers were provided feed for *ad libitum* consumption with prestarter and starter feed offered as crumbles, while grower and finisher feed were offered in pellet form. All the treatment diets were corn-soybean meal based, and the formulas are shown in Tables IV-1 to IV-4.

Feed Manufacture. The corn was ground with a two-pair roller mill (Model C128829, RMS, Tea, SD) with a gap setting of 0% opening (0.15-0.18 mm) on the top pair of rollers and 100% opening (7.16 mm) on the bottom pair of rollers. Basal diets were made for prestarter and starter diets to decrease the variation between diets. After the basal diet was made, the appropriate enzyme dosage, dicalcium phosphate, limestone, vermiculite, sand, and fat were

added to the basal feed to create the final feed product. Grower and finisher diets were made independently due to feed mill equipment capacity and time limitation. Dry ingredients were blended for 180 sec in a twin shaft counterpoise mixer (Model TRDB126-0604, Hayes and Stolz, Fort Worth, TX), followed by addition of fat and mixing for an additional 90 sec. All the diets were pelleted to destroy endogenous phytase that might be present in the ingredients. Prestarter and starter feeds were crumbled to reduce the feed size for young birds. The pelleting conditions were targeted to be 17-18% moisture and 80.5 to 82.0°C (177 to 180°F). Feed sample were collected and analyzed individually.

Data Collection. Chicks were group weighed at placement, 14, 28, 35, and 42 d of age. Feed consumption was determined at 14, 28, 35, and 42 d of age and adjusted feed conversion ratio (AdjFCR) was calculated. All dead birds were weighed twice daily and recorded and their BW was used in the AdjFCR calculation.

Statistical Analysis. A randomized complete block design with a 4 x 3 factorial design was used (4 broiler breeder diets x 3 broiler diets). The general linear model of SAS (SAS Institute, 2011) was used to analyze broiler live performance and differences among means were partitioned by LSMEANS. Differences among means were considered statistically different when $P<0.05$, while $P<0.10$ were considered to approach statistical significance.

RESULTS AND DISCUSSION

Enzyme recovery results for all diets are shown in Table IV-5. The CON and NC diets had no phytase added. However, the recovery analysis showed that the CON treatment had 144, 70, 73, 72 FTU/kg in the prestarter, starter, grower, and finisher diets respectively. The NC phytase levels were generally lower than 55 FTU/kg. These phytase levels were produced by endogenous phytase in the grains. These broiler diets were exposed to the same pelleting

process as the breeder diets, but only a limited amount of endogenous grain phytase was denatured. However, comparing these recovery enzyme numbers with the previous breeder diet shown in III-7, the phytase levels recovered from the non phytase added treatments (CON and NC) were relatively low. This was probably due to the fact that these broiler diets had no wheat bran inclusion in the diet, as it was well known that wheat bran had high levels of endogenous phytase as compared to other grain feed ingredients (Eeckhout and Depaepe, 1994; Schlemmer *et al.*, 2009). The NC diet was manufactured prior to the CON and NC+1000 diets to preclude carryover from those diets. The NC+1000 diet was expected to recover enzymes at approximately 1000 FTU/kg, but only the prestarter was close to the targeted level. The enzyme recovery levels for the enzyme added treatments were 969, 1502, 1575, and 1628 in prestarter, starter, grower, and finisher diets respectively. This could be due to various reasons, such as the mixing process in the feed mill, the sample collection, the consistency of the enzyme product, or the accuracy of the enzyme recovery test. It was surmised that the dosage of the enzyme was understated.

The BW of male broiler chickens as affected by levels of AvP and phytase in broiler breeders and broiler feeds is shown in Table IV-6. Of the main effects, breeder dietary treatments affected the 0 d BW significantly ($P \leq 0.01$) where the A-POS and D-NEG+500 had the greatest BW at 0 d (hatching), C-NEG+250 was intermediate, and B-NEG exhibited the lowest BW. The BW differences reflected egg weight shown in Table III-12, where the egg weight was greater in A-POS and D-NEG+500, which had the larger chicks. This result was expected due to a strong positive correlation between egg weight and chick weight (McNaughton *et al.*, 1978). At 28 d, there was a BW difference that approached statistical significance ($P \leq 0.10$), where the A-POS still remained larger, but the B-NEG became intermediate, while the two

enzyme added treatments C-NEG+250 and D-NEG+500 exhibited the lowest BW. Pinchasov (1991) suggested that the weight of hatching eggs and hatching chick BW were highly correlated, but the initial close correlation diminished and was insignificant after 5 d of hatching. On the other hand, Morris *et al.* (1986) suggested otherwise and stated that broiler BW was strongly correlated with hatching egg weight up to 84 d after hatching, but the correlation decreased with age. In our study, we observed the same results, where the BW effect due to breeder diets did not carry on through later ages.

With regards to the main effects of broiler diets, all treatments weighed the same at 0 d (placement). However, the NC+1000 treatment with lower AvP addition plus 1000 FTU/kg phytase resulted in a significantly ($P \leq 0.01$) greater BW at 14, 28, 35, and 42 d when compared to the CON and NC treatments. There were no significant two-way interactions observed at any age for BW.

The BW gain of male broiler chickens as affected by levels of AvP and phytase in broiler breeder and broiler feeds is shown in Table IV-7. There was no effect of breeder dietary treatment on BW gain observed at any age. With regards to broiler dietary treatments, the NC+1000 treatment produced a significantly ($P \leq 0.01$) greater BW gain at 0-14, 15-28, and 29-35 d when compared to the CON and NC treatments. There were no significant two-way interactions observed at any age for BW gain.

Feed intake of male broiler chickens as affected by levels of AvP and phytase in broiler breeder and broiler feeds is shown in Table IV-8. There was no effect of breeder dietary treatment on BW gain observed at any age. However, the NC+1000 broiler treatment exhibited a significantly greater feed intake at 0-14 d ($P \leq 0.01$), 15-28 d ($P \leq 0.05$), 0-28 d ($P \leq 0.01$), and 0-

35 d ($P \leq 0.01$) as compared to the CON and NC treatments. There were no significant two-way interactions observed at any age for feed intake.

Adjusted FCR (AdjFCR) of male broiler chickens as affected by levels of AvP and phytase in broiler breeder and broiler feeds is shown in Table IV-9. The effect of breeder dietary treatment on AdjFCR approached statistical significance ($P \leq 0.10$) during the 15 to 28 d period, where the A-POS and B-NEG exhibited an improved AdjFCR relative to the two enzyme added treatments C-NEG+250 and D-NEG+500. The NC+1000 broiler treatment produced a significantly ($P \leq 0.01$) improved AdjFCR at 0-14 d and 0-35 d of age, and the increase approached statistical significance ($P \leq 0.10$) at 0-42 d when compared to the CON and NC treatments. There were no significant two-way interactions observed at any age for adjusted FCR. Mortality of male broiler chickens as affected by levels of AvP and phytase in broiler breeder and broiler feeds is shown in Table IV-10. The increased mortality in the C-NEG+250 treatment approached statistical significance during the 0-14 d period, while A-POS and D-NEG+500 treatments were intermediate. The NC broiler treatment exhibited significantly lower mortality when compared to the CON and NC+1000 treatments from 29-35 d of age. The same trend was observed during the 0-35 d and 0-42 d periods where mortality approached statistical significance was less ($P \leq 0.10$) for the NC treatment. Two-way interactions were exhibited during 15-28 d, and 0-42 d periods where D-NEG+500 with NC+1000 and B-NEG with NC exhibited the least mortality at 0-14 d period, and the D-NEG+500 with NC+1000 exhibited the least mortality during the 0-42 d period. Greater mortality was exhibited by the A-POS with NC+1000 during the 15-28 d period, and B-NEG with NC+1000 during the 0-42 d period.

In our study, the broiler feed with lower AvP and addition of phytase (NC+1000) exhibited improved BW, BW gain, feed intake, and FCR. This was similar to other studies that showed the benefit of phytase added to adequate or marginally deficient diets due to increased digestibility of phosphorus, calcium, amino acids, and metabolizable energy which ultimately improved broiler BW gain, feed intake, and feed efficiency (Persia and Saylor, 2006; Selle and Ravindran, 2007). The birds on the NC+1000 phytase treatment exhibited an improved performance to 36 of age but no BW gain difference was observed from 36 to 42 d of age, which suggested that the enzyme effect had diminished. The slowed BW gain also reflected feed intake, where after 29 d of age, the greater feed intake of the NC+1000 treatment diminished while the AdjFCR during the 36-42 d period exhibited a large increase. This growth plateau was probably due to several reasons. Barasch (2015) suggested the effect of enzymes was significantly increased when added to a higher fat level diet (5.7%) comparing to a lower fat inclusion diet (2%). This may be related to increased micelle formation during digestion in the higher fat diet that would also be expected to slow gut passage, allowing more time for the phytase to work on substrates. In the present study, the fat inclusion level was relatively low in all diets and ranged from 1.26 to 2.02%. The birds were on the finisher feed from 34 to 42 d of age, which was the same time period during which BW gain slowed. The fat inclusion in the finisher feed was the lowest among all the diets at 1.26%. These two studies somewhat demonstrated the importance of fat presence when enzymes were added to a diet. Another possible explanation would be related to the acid-base balance. The acid-base balance has been reported to be affected by numerous cations including Na^{+}, K^{+}, Ca^{+2}, and Mg^{+2}, and the anions Cl^{-}, HPO_4^{-2} , $H_2PO_4^{-}$, and SO_4^{-2} (Mongin, 1981). The monovalent minerals (Na+ , K+ , Cl-) were also referred to as the “strong ions” due to the greater influence on acid-base balance of

the body fluid, while the remaining ions to a lesser extent still impacted performance of the animal (Patience, 1990). As the bird aged in the present study, the AvP in the diet decreased and the soybean meal inclusion decreased. Soybean meal was a primary potassium source, so the dietary potassium level decreased with age.

It was well know that maternal nutrition could significantly affect progeny intestinal function (Rebel *et al.,* 2006) as well as affect the endocrine system that controlled feed intake and influenced of metabolic pathways (Bautista *et al.,* 2008; Vickers *et al.,* 2000), which ultimately affected progeny livability (Virden *et al.,* 2003) and well-being. Research concerning the maternal effects of enzymes has been very limited but has suggested that there may be negative maternal effects of multi-enzymes fed to broiler breeders on broiler progeny live performance when the progeny also received the same enzyme(s) in the feed. In our present study, there were overall no interaction effects observed for any broiler variables other than mortality, which suggested that the effects observed were only due to either breeder diets or broiler diets alone. There were no maternal effects of the phytase observed on the broiler progeny. This may be because the levels of phytase used in the breeder feed were not potent enough to interfere with the hormonal signals that were stored in the yolk, utilized by the embryo, and influenced the chick's metabolic pathways.

Table IV-1. Broiler prestarter diet fed from 1-7 d of age.

	Dietary Treatments[9]		
Ingredients	CON	NC	NC+1000
	(%)		
Corn	53.84	53.84	53.84
Soybean meal (48% CP)	40.15	40.15	40.15
Poultry fat	1.32	1.32	1.32
Limestone	0.85	0.99	0.99
Dicalcium phosphate (18.5% P)	2.30	1.37	1.37
Salt	0.50	0.50	0.50
DL-Methionine	0.26	0.26	0.26
L-Lysine	0.05	0.05	0.05
L-Threonine	0.13	0.13	0.13
Choline chloride (60%)	0.10	0.10	0.10
Coban	0.05	0.05	0.05
Vitamin premix[1]	0.05	0.05	0.05
Mineral premix[2]	0.20	0.20	0.20
Selenium premix[3]	0.05	0.05	0.05
Phytase[4]	-	-	0.04
Inert filler[5]	0.15	0.15	0.11
Sand[6]	-	0.79	0.79
Total	100.00	100.00	100.00
Calculated nutrient content			
Crude protein	24.00	24.00	24.00
Calcium [7]	1.00	0.85	1.02
Available phosphorus [8]	0.50	0.35	0.52
Lysine	1.37	1.37	1.37
Methionine	0.62	0.62	0.62
Threonine	0.93	0.93	0.93
Methionine + cysteine	0.98	0.98	0.98
Sodium	0.21	0.21	0.21
Metabolizable energy (kcal/g)	2.80	2.80	2.80

[1]Vitamin premix supplied the following per kg of diet: 6,614 IU vitamin A, 2,000 IU vitamin D3, 33 IU vitamin E, 0.02 mg vitamin B12, 0.13 mg biotin, 1.98 mg menadione (K_3), 1.98 mg thiamine, 6.6 mg riboflavin, 11 mg d-pantothenic acid, 3.97 mg vitamin B6, 55 mg niacin, and 1.1 mg folic acid.

[2]Mineral premix supplied the following per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt, 1 mg.

[3]Selenium premix provided 0.2 mg Se (as Na_2SeO_3) per kg of diet.

[4]Phytase enzyme (Axtra® PHY 2500 TPT) was added at 1000 FTU/kg to create NC+1000.

[5]Vermiculite was used as inert filler.

[6]Sand was used as filler at similar density as dicalcium phosphate and limestone.

[7]Calcium level was adjusted according to the phytase matrix value of 0.17 above the NC.

[8]AvP level was adjusted according to the phytase matrix value of 0.17 above the NC.

[9] CON was positive control and NC was negative control in broiler diet.

Table IV-2. Broiler starter diet fed from 8 - 14 d of age.

	Dietary Treatments[9]		
Ingredients	CON	NC	NC+1000
		(%)	
Corn	58.31	58.31	58.31
Soybean meal (48% CP)	35.34	35.34	35.34
Poultry fat	2.02	2.02	2.02
Limestone	0.78	0.93	0.93
Dicalcium phosphate (18.5% P)	2.03	1.09	1.09
Salt	0.50	0.50	0.50
DL-Methionine	0.22	0.22	0.22
L-Lysine	0.08	0.08	0.08
L-Threonine	0.12	0.12	0.12
Choline chloride (60%)	0.10	0.10	0.10
Coban	0.05	0.05	0.05
Vitamin premix[1]	0.05	0.05	0.05
Mineral premix[2]	0.20	0.20	0.20
Selenium premix[3]	0.05	0.05	0.05
Phytase[4]	-	-	0.04
Inert filler[5]	0.15	0.15	0.11
Sand[6]	-	0.79	0.79
Total	100.00	100.00	100.00
Calculated nutrient content			
Crude protein	24.00	24.00	24.00
Calcium [7]	0.90	0.75	0.92
Available phosphorus [8]	0.45	0.30	0.47
Lysine	1.25	1.25	1.25
Methionine	0.56	0.56	0.56
Threonine	0.85	0.85	0.85
Methionine + cysteine	0.90	0.90	0.90
Sodium	0.21	0.21	0.21
Metabolizable energy (kcal/g)	2.90	2.90	2.90

[1]Vitamin premix supplied the following per kg of diet: 13,200 IU vitamin A, 4,000 IU vitamin D3, 66 IU vitamin E, 0.04 mg vitamin B12, 0.26 mg biotin, 4 mg menadione (K_3), 4 mg thiamine, 13.2 mg riboflavin, 22 mg d-pantothenic acid, 8 mg vitamin B6, 110 mg niacin, and 2.2 mg folic acid.

[2]Mineral premix supplied the following per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt, 1 mg.

[3]Selenium premix provided 0.2 mg Se (as Na_2SeO_3) per kg of diet.

[4]Phytase enzyme (Axtra® PHY 2500 TPT) was added at 1000 FTU/kg to create NC+1000.

[5]Vermiculite was used as an inert filler.

[6]Sand was used as a filler at similar density as dicalcium phosphate and limestone.

[7]Calcium level was adjusted according to the phytase matrix value of 0.17 above the NC.

[8]AvP level was adjusted according to the phytase matrix value of 0.17 above the NC.

[9] CON was positive control and NC was negative control in broiler diet.

Table IV-3. Broiler grower diet fed from 15 - 33 d of age.

Ingredients	Dietary Treatments[9]		
	CON	NC	NC+1000
		(%)	
Corn	64.88	64.88	64.88
Soybean meal (48% CP)	30.18	30.18	30.18
Poultry fat	1.35	1.35	1.35
Limestone	0.63	0.78	0.78
Dicalcium phosphate (18.5% P)	1.44	0.51	0.51
Salt	0.50	0.50	0.50
DL-Methionine	0.19	0.19	0.19
L-Lysine	0.12	0.12	0.12
L-Threonine	0.11	0.11	0.11
Choline chloride (60%)	0.10	0.10	0.10
Coban	0.05	0.05	0.05
Vitamin premix[1]	0.05	0.05	0.05
Mineral premix[2]	0.20	0.20	0.20
Selenium premix[3]	0.05	0.05	0.05
Phytase[4]	-	-	0.04
Inert filler[5]	0.15	0.15	0.11
Sand[6]	-	0.78	0.78
Total	100.00	100.00	100.00
Calculated nutrient content			
Crude protein	20.00	20.00	20.00
Calcium [7]	0.70	0.55	0.72
Available phosphorus [8]	0.35	0.20	0.37
Lysine	1.14	1.14	1.14
Methionine	0.50	0.50	0.50
Threonine	0.78	0.78	0.78
Methionine + cysteine	0.82	0.82	0.82
Sodium	0.20	0.20	0.20
Metabolizable energy (kcal/g)	2.95	2.95	2.95

[1]Vitamin premix supplied the following per kg of diet: 13,200 IU vitamin A, 4,000 IU vitamin D3, 66 IU vitamin E, 0.04 mg vitamin B12, 0.26 mg biotin, 4 mg menadione (K_3), 4 mg thiamine, 13.2 mg riboflavin, 22 mg d-pantothenic acid, 8 mg vitamin B6, 110 mg niacin, and 2.2 mg folic acid.

[2]Mineral premix supplied the following per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt, 1 mg.

[3]Selenium premix provided 0.2 mg Se (as Na_2SeO_3) per kg of diet.

[4]Phytase enzyme (Axtra® PHY 2500 TPT) was added at 1000 FTU/kg to create NC+1000.

[5]Vermiculite was used as inert filler.

[6]Sand was used as filler at similar density as dicalcium phosphate and limestone.

[7]Calcium level was adjusted according to the phytase matrix value of 0.17 above the NC.

[8]AvP level was adjusted according to the phytase matrix value of 0.17 above the NC.

[9] CON was positive control and NC was negative control in broiler diet.

Table IV-4. Broiler finisher diet fed from 34 - 42 d of age.

Ingredients	Dietary Treatments[9]		
	CON	NC	NC+1000
	(%)		
Corn	69.08	69.08	69.08
Soybean meal (48% CP)	26.44	26.44	26.44
Poultry fat	1.26	1.26	1.26
Limestone	0.56	0.71	0.71
Dicalcium phosphate (18.5% P)	1.15	0.22	0.22
Salt	0.50	0.50	0.50
DL-Methionine	0.17	0.17	0.17
L-Lysine	0.14	0.14	0.14
L-Threonine	0.10	0.10	0.10
Choline chloride (60%)	0.10	0.10	0.10
Coban	0.05	0.05	0.05
Vitamin premix[1]	0.05	0.05	0.05
Mineral premix[2]	0.20	0.20	0.20
Selenium premix[3]	0.05	0.05	0.05
Phytase[4]	-	-	0.04
Inert filler[5]	0.15	0.15	0.11
Sand[6]	-	0.79	0.79
Total	100.00	100.00	100.00
Calculated nutrient content			
Crude protein	18.50	18.50	18.50
Calcium [7]	0.60	0.45	0.62
Available phosphorus [8]	0.30	0.15	0.32
Lysine	1.06	1.06	1.06
Methionine	0.46	0.46	0.46
Threonine	0.72	0.72	0.72
Methionine + cysteine	0.76	0.76	0.76
Sodium	0.20	0.20	0.20
Metabolizable energy (kcal/g)	3.00	3.00	3.00

[1]Vitamin premix supplied the following per kg of diet: 13,200 IU vitamin A, 4,000 IU vitamin D3, 66 IU vitamin E, 0.04 mg vitamin B12, 0.26 mg biotin, 4 mg menadione (K_3), 4 mg thiamine, 13.2 mg riboflavin, 22 mg d-pantothenic acid, 8 mg vitamin B6, 110 mg niacin, and 2.2 mg folic acid.

[2]Mineral premix supplied the following per kg of diet: manganese, 120 mg; zinc, 120 mg; iron, 80 mg; copper, 10 mg; iodine, 2.5 mg; and cobalt, 1 mg.

[3]Selenium premix provided 0.2 mg Se (as Na_2SeO_3) per kg of diet.

[4]Phytase enzyme (Axtra® PHY 2500 TPT) was added at 1000 FTU/kg to create NC+1000.

[5]Vermiculite was used as inert filler.

[6]Sand was used as filler at similar density as dicalcium phosphate and limestone.

[7]Calcium level was adjusted according to the phytase matrix value of 0.17 above the NC.

[8]AvP level was adjusted according to the phytase matrix value of 0.17 above the NC.

[9] CON was positive control and NC was negative control in broiler diet.

Table IV-5. Dietary enzyme recovery results. [1]

Feed Type	Treatment [2]	Enzyme Added (g/MT)	Formula Enzyme (FTU/kg)	Enzyme Recovery (FTU/kg)
Pre-Starter	CON [3]	0	0	144
	NC [4]	0	0	53
	NC+1000 [5]	400	1000	969
Starter	CON [3]	0	0	70
	NC [4]	0	0	<50
	NC+1000 [5]	400	1000	1502
Grower	CON [3]	0	0	73
	NC [4]	0	0	<50
	NC+1000 [5]	400	1000	1575
Finisher	CON [3]	0	0	72
	NC [4]	0	0	<50
	NC+1000 [5]	400	1000	1628

[1] Enzyme recovery analysis by DuPont Nutrition Biosciences ApS, Denmark.
[2] CON was positive control and NC was negative control in broiler diet.
[3] CON: Prestarter with1.0% Ca and 0.50 AvP, starter with 0.9% Ca and 0.45 AvP, grower with 0.7% Ca and 0.35% AvP, and finisher with 0.6% Ca, and 0.30% AvP.
[4] NC: Prestarter with 0.85% Ca and 0.35 AvP, starter with 0.75% Ca and 0.30%AvP, grower with 0.55% Ca and 0.20% AvP, and finisher with 0.45% Ca and 0.15% AvP.
[5] NC+1000: Prestarter with 0.85% Ca, and 0.35 AvP, starter with 0.75% Ca and 0.30% AvP, grower with 0.55% Ca and 0.20% AvP, and finisher with 0.45% Ca and 0.15% AvP +1000 FTU phytase.

Table IV-6. Body weight of male broiler chickens as affected by different levels of available phosphorous (AvP), calcium (Ca), and addition of phytase in grower and layer diets of broiler breeders, and levels of AvP, Ca, and addition of phytase to broiler feed.

Breeder Treatment [1]	Broiler Treatment [2]	Body Weight For Ages Shown				
		0 d	14 d	28 d	35 d	42 d
		(g)				
A-POS [3]		42.9 [A]	519	1823 [x]	2655	3557
B-NEG [4]		42.1 [C]	512	1807 [xy]	2642	3529
C-NEG+250 [5]		42.3 [BC]	516	1783 [y]	2631	3551
D-NEG+500 [6]		42.6 [AB]	518	1778 [y]	2630	3556
SEM [7]		0.25	6.79	23.11	26.26	40.11
Probability		0.001	0.626	0.070	0.612	0.806
	CON [8]	42.4	503.9 [B]	1782 [B]	2605 [B]	3511 [B]
	NC [9]	42.6	505.7 [B]	1755 [B]	2583 [B]	3502 [B]
	NC+1000 [10]	42.5	539.4 [A]	1856 [A]	2729 [A]	3631 [A]
	SEM [11]	0.12	3.39	11.56	13.13	20.06
	Probability	0.512	0.001	0.001	0.001	0.001

[x,y] Means in a column within each replicate that possess different superscripts approached statistical significance ($P \leq 0.10$).
[A-C] Means in a column within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1] A-POS was positive control and B-NEG was negative control in breeder diet.
[2] CON was positive control and NC was negative control in broiler diet.
[3] A-POS: Grower with 0.90% Ca and 0.45% AvP, and layer with 2.7% Ca and 0.42% AvP.
[4] B-NEG: Grower with 0.60% Ca and 0.30% AvP, and layer with 2.7% Ca and 0.15% AvP.
[5] C-NEG+250: Grower with 0.60% Ca and 0.30% AvP +250 FTU phytase, and layer with 2.7% Ca and 0.15% AvP +250 FTU phytase.
[6] D-NEG+500: Grower with 0.60% Ca and 0.30% AvP +500 FTU phytase, and layer with 2.7% Ca and 0.15% AvP +500 FTU phytase.
[7] Standard error of mean (SEM) for n=18 pens.
[8] CON: Prestarter with1.0% Ca and 0.50 AvP, starter with 0.9% Ca and 0.45 AvP, grower with 0.7% Ca and 0.35% AvP, and finisher with 0.6% Ca, and 0.30% AvP.
[9] NC: Prestarter with 0.85% Ca and 0.35 AvP, starter with 0.75% Ca and 0.30%AvP, grower with 0.55% Ca and 0.20% AvP, and finisher with 0.45% Ca and 0.15% AvP.
[10] NC+1000: Prestarter with 0.85% Ca, and 0.35 AvP, starter with 0.75% Ca and 0.30% AvP, grower with 0.55% Ca and 0.20% AvP, and finisher with 0.45% Ca and 0.15% AvP + 1000 FTU phytase.
[11] Standard error of mean (SEM) for n=24 pens.

Table IV-6 (continued). Body weight of male broiler chickens as affected by different levels of available phosphorous (AvP), calcium (Ca), and addition of phytase in grower and layer diets of broiler breeders, and levels of AvP, Ca, and addition of phytase to broiler feed.

Breeder Treatment [1]	Broiler Treatment [2]	Body Weight For Ages Shown				
		0 d	14 d	28 d	35 d	42 d
		(g)				
A-POS [3]	CON [7]	42.8	506	1789	2581	3463
	NC [8]	42.9	510	1778	2603	3495
	NC+1000 [9]	43.1	540	1901	2781	3713
B-NEG [4]	CON [7]	42.0	501	1819	2631	3511
	NC [8]	42.1	501	1754	2585	3513
	NC+1000 [9]	42.2	534	1848	2711	3561
C-NEG+250 [5]	CON [7]	42.2	502	1756	2591	3518
	NC [8]	42.4	506	1746	2574	3498
	NC+1000 [9]	42.4	541	1846	2725	3638
D-NEG+500 [6]	CON [7]	42.5	507	1764	2618	3553
	NC [8]	42.8	506	1740	2571	3503
	NC+1000 [9]	42.5	542	1830	2700	3613
SEM [10]		0.14	3.92	13.34	15.16	23.16
Probability		0.957	0.998	0.673	0.346	0.186

[1] A-POS was positive control and B-NEG was negative control in breeder diet.
[2] CON was positive control and NC was negative control in broiler diet.
[3] A-POS: Grower with 0.90% Ca and 0.45% AvP, and layer with 2.7% Ca and 0.42% AvP.
[4] B-NEG: Grower with 0.60% Ca and 0.30% AvP, and layer with 2.7% Ca and 0.15% AvP.
[5] C-NEG+250: Grower with 0.60% Ca and 0.30% AvP +250 FTU phytase, and layer with 2.7% Ca and 0.15% AvP +250 FTU phytase.
[6] D-NEG+500: Grower with 0.60% Ca and 0.30% AvP +500 FTU phytase, and layer with 2.7% Ca and 0.15% AvP +500 FTU phytase.
[7] CON: Prestarter with1.0% Ca and 0.50 AvP, starter with 0.9% Ca and 0.45 AvP, grower with 0.7% Ca and 0.35% AvP, and finisher with 0.6% Ca, and 0.30% AvP.
[8] NC: Prestarter with 0.85% Ca and 0.35 AvP, starter with 0.75% Ca and 0.30%AvP, grower with 0.55% Ca and 0.20% AvP, and finisher with 0.45% Ca and 0.15% AvP.
[9] NC+1000: Prestarter with 0.85% Ca, and 0.35 AvP, starter with 0.75% Ca and 0.30% AvP, grower with 0.55% Ca and 0.20% AvP, and finisher with 0.45% Ca and 0.15% AvP + 1000 FTU phytase.
[10] Standard error of mean (SEM) for n=6 pens.

Table IV-7. BW gain of male broiler chickens as affected by different levels of available phosphorous (AvP), calcium (Ca), and addition of phytase in grower and layer diets of broiler breeders, and levels of AvP, Ca, and addition of phytase to broiler feed.

Breeder Treatment [1]	Broiler Treatment [2]	BW Gain For Ages Shown			
		1-14 d	15-28 d	29-35 d	36-42 d
		(g)			
A-POS [3]		476	1304	832	902
B-NEG [4]		470	1295	836	886
C-NEG+250 [5]		474	1266	848	921
D-NEG+500 [6]		476	1260	852	926
SEM [5]		3.90	11.31	12.90	15.35
Probability		0.698	0.018	0.657	0.242
	CON [8]	462[B]	1278 [B]	823 [B]	906
	NC [9]	463 [B]	1249 [C]	829 [B]	919
	NC+1000 [10]	497 [A]	1317 [A]	873 [A]	902
	SEM [11]	3.38	9.79	11.17	13.29
	Probability	0.001	0.001	0.004	0.643

[A-C] Means in a column within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1] A-POS was positive control and B-NEG was negative control in breeder diet.
[2] CON was positive control and NC was negative control in broiler diet.
[3] A-POS: Grower with 0.90% Ca and 0.45% AvP, and layer with 2.7% Ca and 0.42% AvP.
[4] B-NEG: Grower with 0.60% Ca and 0.30% AvP, and layer with 2.7% Ca and 0.15% AvP.
[5] C-NEG+250: Grower with 0.60% Ca and 0.30% AvP +250 FTU phytase, and layer with 2.7% Ca and 0.15% AvP +250 FTU phytase.
[6] D-NEG+500: Grower with 0.60% Ca and 0.30% AvP +500 FTU phytase, and layer with 2.7% Ca and 0.15% AvP +500 FTU phytase.
[7] Standard error of mean (SEM) for n=18 pens.
[8] CON: Prestarter with1.0% Ca and 0.50 AvP, starter with 0.9% Ca and 0.45 AvP, grower with 0.7% Ca and 0.35% AvP, and finisher with 0.6% Ca, and 0.30% AvP.
[9] NC: Prestarter with 0.85% Ca and 0.35 AvP, starter with 0.75% Ca and 0.30%AvP, grower with 0.55% Ca and 0.20% AvP, and finisher with 0.45% Ca and 0.15% AvP.
[10] NC+1000: Prestarter with 0.85% Ca, and 0.35 AvP, starter with 0.75% Ca and 0.30% AvP, grower with 0.55% Ca and 0.20% AvP, and finisher with 0.45% Ca and 0.15% AvP + 1000 FTU phytase.
[11] Standard error of mean (SEM) for n=24 pens.

Table IV-7 (continued). BW gain of male broiler chickens as affected by different levels of available phosphorous (AvP), calcium (Ca), and addition of phytase in grower and layer diets of broiler breeders, and levels of AvP, Ca, and addition of phytase to broiler feed.

Breeder Treatment [1]	Broiler Treatment [2]	BW Gain For Ages Shown			
		1-14 d	15-28 d	29-35 d	36-42 d
		(g)			
A-POS [3]	CON [7]	463	1283	792	882
	NC [8]	467	1268	824	892
	NC+1000 [9]	497	1360	880	932
B-NEG [4]	CON [7]	459	1318	811	881
	NC [8]	459	1253	831	928
	NC+1000 [9]	492	1314	864	850
C-NEG+250 [5]	CON [7]	460	1254	835	927
	NC [8]	464	1240	828	924
	NC+1000 [9]	499	1305	880	912
D-NEG+500 [6]	CON [7]	464	1257	854	935
	NC [8]	463	1234	832	931
	NC+1000 [9]	500	1288	870	913
SEM [10]		6.75	19.59	22.35	26.59
Probability		0.998	0.508	0.773	0.422

[1] A-POS was positive control and B-NEG was negative control in breeder diet.
[2] CON was positive control and NC was negative control in broiler diet.
[3] A-POS: Grower with 0.90% Ca and 0.45% AvP, and layer with 2.7% Ca and 0.42% AvP.
[4] B-NEG: Grower with 0.60% Ca and 0.30% AvP, and layer with 2.7% Ca and 0.15% AvP.
[5] C-NEG+250: Grower with 0.60% Ca and 0.30% AvP +250 FTU phytase, and layer with 2.7% Ca and 0.15% AvP +250 FTU phytase.
[6] D-NEG+500: Grower with 0.60% Ca and 0.30% AvP +500 FTU phytase, and layer with 2.7% Ca and 0.15% AvP +500 FTU phytase.
[7] CON: Prestarter with1.0% Ca and 0.50 AvP, starter with 0.9% Ca and 0.45 AvP, grower with 0.7% Ca and 0.35% AvP, and finisher with 0.6% Ca, and 0.30% AvP.
[8] NC: Prestarter with 0.85% Ca and 0.35 AvP, starter with 0.75% Ca and 0.30%AvP, grower with 0.55% Ca and 0.20% AvP, and finisher with 0.45% Ca and 0.15% AvP.
[9] NC+1000: Prestarter with 0.85% Ca, and 0.35 AvP, starter with 0.75% Ca and 0.30% AvP, grower with 0.55% Ca and 0.20% AvP, and finisher with 0.45% Ca and 0.15% AvP + 1000 FTU phytase.
[10] Standard error of mean (SEM) for n=6 pens.

Table IV-8. Feed intake of male broiler chickens as affected by different levels of available phosphorous (AvP), calcium (Ca), and addition of phytase in grower and layer diets of broiler breeders, and levels of AvP, Ca, and addition of phytase to broiler feed.

Breeder Treatment [1]	Broiler Treatment [2]	Feed Intake For Ages Shown						
		0-14 d	15-28 d	29-35 d	36-42 d	0-28 d	0-35 d	0-42 d
		(g)						
A-POS [3]		655	1955	1435	1680	2610	4045	5724.8
B-NEG [4]		647	1959	1446	1664	2606	4052	5715.7
C-NEG+250 [5]		649	1964	1430	1701	2612	4042	5743.0
D-NEG+500 [6]		656	1950	1445	1731	2606	4051	5781.8
SEM [5]		5.17	18.56	14.19	19.75	21.81	26.74	38.25
Probability		0.512	0.958	0.816	0.105	0.996	0.992	0.626
	CON [8]	643 [B]	1948 [b]	1428	1697	2591 [B]	4019 [B]	5716
	NC [9]	639 [B]	1927 [b]	1433	1706	2566 [B]	4000 [B]	5706
	NC+1000 [10]	673 [A]	1995 [a]	1456	1679	2668 [A]	4124 [A]	5803
	SEM [11]	4.48	16.07	12.29	17.10	18.89	23.16	33.13
	Probability	0.001	0.012	0.252	0.525	0.001	0.001	0.083

[a-b] Means in a column within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).

[A-B] Means in a column within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).

[1] A-POS was positive control and B-NEG was negative control in breeder diet.

[2] CON was positive control and NC was negative control in broiler diet.

[3] A-POS: Grower with 0.90% Ca and 0.45% AvP, and layer with 2.7% Ca and 0.42% AvP.

[4] B-NEG: Grower with 0.60% Ca and 0.30% AvP, and layer with 2.7% Ca and 0.15% AvP.

[5] C-NEG+250: Grower with 0.60% Ca and 0.30% AvP +250 FTU phytase, and layer with 2.7% Ca and 0.15% AvP +250 FTU phytase.

[6] D-NEG+500: Grower with 0.60% Ca and 0.30% AvP +500 FTU phytase, and layer with 2.7% Ca and 0.15% AvP +500 FTU phytase.

[7] Standard error of mean (SEM) for n=18 pens.

[8] CON: Prestarter with1.0% Ca and 0.50 AvP, starter with 0.9% Ca and 0.45 AvP, grower with 0.7% Ca and 0.35% AvP, and finisher with 0.6% Ca, and 0.30% AvP.

[9] NC: Prestarter with 0.85% Ca and 0.35 AvP, starter with 0.75% Ca and 0.30%AvP, grower with 0.55% Ca and 0.20% AvP, and finisher with 0.45% Ca and 0.15% AvP.

[10] NC+1000: Prestarter with 0.85% Ca, and 0.35 AvP, starter with 0.75% Ca and 0.30% AvP, grower with 0.55% Ca and 0.20% AvP, and finisher with 0.45% Ca and 0.15% AvP + 1000 FTU phytase.

[11] Standard error of mean (SEM) for n=24 pens.

Table IV-8 (continued). Feed intake of male broiler chickens as affected by different levels of available phosphorous (AvP), calcium (Ca), and addition of phytase in grower and layer diets of broiler breeders, and levels of AvP, Ca, and addition of phytase to broiler feed.

Breeder Treatment [1]	Broiler Treatment [2]	Feed Intake For Ages Shown						
		0-14 d	15-28 d	29-35 d	36-42 d	0-28 d	0-35 d	0-42 d
		(g)						
A-POS [3]	CON [7]	642	1944	1397	1671	2586	3983	5654
	NC [8]	643	1945	1415	1673	2588	4003	5676
	NC+1000 [9]	680	1976	1493	1696	2656	4149	5845
B-NEG [4]	CON [7]	641	1986	1443	1672	2627	4069	5741
	NC [8]	636	1933	1459	1726	2570	4028	5754
	NC+1000 [9]	662	1959	1436	1595	2622	4057	5652
C-NEG+250 [5]	CON [7]	637	1911	1422	1679	2548	3970	5649
	NC [8]	636	1938	1429	1721	2574	4003	5724
	NC+1000 [9]	674	2041	1438	1703	2715	4153	5856
D-NEG+500 [6]	CON [7]	651	1952	1450	1765	2603	4053	5818
	NC [8]	642	1891	1431	1706	2533	3964	5669
	NC+1000 [9]	675	2006	1455	1722	2681	4136	5859
SEM [10]		8.96	32.14	24.57	34.21	37.78	46.32	66.25
Probability		0.947	0.268	0.336	0.207	0.362	0.323	0.119

[1] A-POS was positive control and B-NEG was negative control in breeder diet.
[2] CON was positive control and NC was negative control in broiler diet.
[3] A-POS: Grower with 0.90% Ca and 0.45% AvP, and layer with 2.7% Ca and 0.42% AvP.
[4] B-NEG: Grower with 0.60% Ca and 0.30% AvP, and layer with 2.7% Ca and 0.15% AvP.
[5] C-NEG+250: Grower with 0.60% Ca and 0.30% AvP +250 FTU phytase, and layer with 2.7% Ca and 0.15% AvP +250 FTU phytase.
[6] D-NEG+500: Grower with 0.60% Ca and 0.30% AvP +500 FTU phytase, and layer with 2.7% Ca and 0.15% AvP +500 FTU phytase.
[7] CON: Prestarter with1.0% Ca and 0.50 AvP, starter with 0.9% Ca and 0.45 AvP, grower with 0.7% Ca and 0.35% AvP, and finisher with 0.6% Ca, and 0.30% AvP.
[8] NC: Prestarter with 0.85% Ca and 0.35 AvP, starter with 0.75% Ca and 0.30%AvP, grower with 0.55% Ca and 0.20% AvP, and finisher with 0.45% Ca and 0.15% AvP.
[9] NC+1000: Prestarter with 0.85% Ca, and 0.35 AvP, starter with 0.75% Ca and 0.30% AvP, grower with 0.55% Ca and 0.20% AvP, and finisher with 0.45% Ca and 0.15% AvP + 1000 FTU phytase.
[10] Standard error of mean (SEM) for n=6 pens.

Table IV-9. Adjusted FCR (AdjFCR) of male broiler chickens as affected by different levels of available phosphorous (AvP), calcium (Ca), and addition of phytase in grower and layer diets of broiler breeders, and levels of AvP, Ca, and addition of phytase to broiler feed.

Breeder Treatment [1]	Broiler Treatment [2]	Adjusted FCR For Ages Shown						
		0-14 d	15-28 d	29-35 d	36-42 d	0-28 d	0-35 d	0-42 d
		(g:g)						
A-POS [3]		1.38	1.53	1.79	1.96	1.49	1.58	1.66
B-NEG [4]		1.38	1.53	1.82	1.95	1.49	1.58	1.67
C-NEG+250 [5]		1.37	1.57	1.75	1.90	1.51	1.58	1.66
D-NEG+500 [6]		1.38	1.57	1.78	1.90	1.51	1.59	1.67
SEM [5]		0.01	0.02	0.03	0.04	0.01	0.01	0.01
Probability		0.653	0.087	0.456	0.529	0.162	0.446	0.779
	CON [8]	1.39 [A]	1.55	1.81	1.92	1.50	1.59 [A]	1.67
	NC [9]	1.38 [A]	1.56	1.77	1.91	1.51	1.59 [A]	1.67
	NC+1000 [10]	1.36 [B]	1.54	1.77	1.95	1.48	1.56 [B]	1.65
	SEM [11]	0.01	0.01	0.03	0.03	0.01	0.01	0.01
	Probability	0.001	0.456	0.537	0.592	0.150	0.001	0.081

[A,B] Means in a column within each replicate that possess different superscripts differ significantly ($P \leq 0.01$).
[1] A-POS was positive control and B-NEG was negative control in breeder diet.
[2] CON was positive control and NC was negative control in broiler diet.
[3] A-POS: Grower with 0.90% Ca and 0.45% AvP, and layer with 2.7% Ca and 0.42% AvP.
[4] B-NEG: Grower with 0.60% Ca and 0.30% AvP, and layer with 2.7% Ca and 0.15% AvP.
[5] C-NEG+250: Grower with 0.60% Ca and 0.30% AvP +250 FTU phytase, and layer with 2.7% Ca and 0.15% AvP +250 FTU phytase.
[6] D-NEG+500: Grower with 0.60% Ca and 0.30% AvP +500 FTU phytase, and layer with 2.7% Ca and 0.15% AvP +500 FTU phytase.
[7] Standard error of mean (SEM) for n=18 pens.
[8] CON: Prestarter with1.0% Ca and 0.50 AvP, starter with 0.9% Ca and 0.45 AvP, grower with 0.7% Ca and 0.35% AvP, and finisher with 0.6% Ca, and 0.30% AvP.
[9] NC: Prestarter with 0.85% Ca and 0.35 AvP, starter with 0.75% Ca and 0.30%AvP, grower with 0.55% Ca and 0.20% AvP, and finisher with 0.45% Ca and 0.15% AvP.
[10] NC+1000: Prestarter with 0.85% Ca, and 0.35 AvP, starter with 0.75% Ca and 0.30% AvP, grower with 0.55% Ca and 0.20% AvP, and finisher with 0.45% Ca and 0.15% AvP + 1000 FTU phytase.
[11] Standard error of mean (SEM) for n=24 pens.

Table IV-9 (continued). Adjusted FCR (AdjFCR) of male broiler chickens as affected by different levels of available phosphorous (AvP), calcium (Ca), and addition of phytase in grower and layer diets of broiler breeders, and levels of AvP, Ca, and addition of phytase to broiler feed.

Breeder Treatment [1]	Broiler Treatment [2]	Adjusted FCR For Ages Shown						
		0-14 d	15-28 d	29-35 d	36-42 d	0-28 d	0-35 d	0-42 d
		(g:g)						
A-POS [3]	CON [7]	1.39	1.53	1.83	1.99	1.50	1.59	1.68
	NC [8]	1.38	1.56	1.76	1.89	1.51	1.59	1.66
	NC+1000 [9]	1.37	1.49	1.78	2.00	1.46	1.55	1.64
B-NEG [4]	CON [7]	1.40	1.52	1.83	1.91	1.49	1.58	1.66
	NC [8]	1.39	1.55	1.78	1.95	1.50	1.59	1.68
	NC+1000 [9]	1.35	1.52	1.84	1.98	1.47	1.57	1.66
C-NEG+250 [5]	CON [7]	1.39	1.55	1.79	1.84	1.50	1.59	1.65
	NC [8]	1.38	1.58	1.75	1.93	1.52	1.59	1.67
	NC+1000 [9]	1.36	1.58	1.70	1.94	1.51	1.57	1.65
D-NEG+500 [6]	CON [7]	1.41	1.58	1.77	1.95	1.53	1.61	1.69
	NC [8]	1.39	1.55	1.81	1.87	1.50	1.60	1.66
	NC+1000 [9]	1.35	1.57	1.75	1.89	1.50	1.57	1.65
SEM [10]		0.01	0.03	0.05	0.06	0.02	0.01	0.01
Probability		0.758	0.480	0.825	0.631	0.573	0.885	0.360

[1] A-POS was positive control and B-NEG was negative control in breeder diet.
[2] CON was positive control and NC was negative control in broiler diet.
[3] A-POS: Grower with 0.90% Ca and 0.45% AvP, and layer with 2.7% Ca and 0.42% AvP.
[4] B-NEG: Grower with 0.60% Ca and 0.30% AvP, and layer with 2.7% Ca and 0.15% AvP.
[5] C-NEG+250: Grower with 0.60% Ca and 0.30% AvP +250 FTU phytase, and layer with 2.7% Ca and 0.15% AvP +250 FTU phytase.
[6] D-NEG+500: Grower with 0.60% Ca and 0.30% AvP +500 FTU phytase, and layer with 2.7% Ca and 0.15% AvP +500 FTU phytase.
[7] CON: Prestarter with1.0% Ca and 0.50 AvP, starter with 0.9% Ca and 0.45 AvP, grower with 0.7% Ca and 0.35% AvP, and finisher with 0.6% Ca, and 0.30% AvP.
[8] NC: Prestarter with 0.85% Ca and 0.35 AvP, starter with 0.75% Ca and 0.30%AvP, grower with 0.55% Ca and 0.20% AvP, and finisher with 0.45% Ca and 0.15% AvP.
[9] NC+1000: Prestarter with 0.85% Ca, and 0.35 AvP, starter with 0.75% Ca and 0.30% AvP, grower with 0.55% Ca and 0.20% AvP, and finisher with 0.45% Ca and 0.15% AvP + 1000 FTU phytase.
[10] Standard error of mean (SEM) for n=6 pens.

Table IV-10. Mortality of male broiler chickens as affected by different levels of available phosphorous (AvP), calcium (Ca), and addition of phytase in grower and layer diets of broiler breeders, and levels of AvP, Ca, and addition of phytase to broiler feed.

Breeder Treatment [1]	Broiler Treatment [2]	Mortality For Ages Shown						
		0-14 d	15-28 d	29-35 d	36-42 d	0-28 d	0-35 d	0-42 d
		(%)						
A-POS [3]		0.6	5.6	4.4	6.5	6.2	10.3	16.1
B-NEG [4]		0.0	3.4	3.5	6.8	3.4	6.8	13.1
C-NEG+250 [5]		1.2	4.2	3.9	4.7	5.4	9.1	13.5
D-NEG+500 [6]		0.8	3.8	4.0	3.6	4.6	8.3	11.7
SEM [5]		0.33	0.91	1.02	1.04	0.94	1.34	1.56
Probability		0.089	0.361	0.938	0.114	0.203	0.301	0.262
	CON [8]	0.6	4.2	4.1 [b]	4.7	4.8	8.6	13.0
	NC [9]	0.9	4.2	1.9 [a]	5.2	5.1	6.8	11.8
	NC+1000 [10]	0.5	4.3	5.9 [b]	6.4	4.8	10.4	16.1
	SEM [11]	0.29	0.79	0.88	0.90	0.82	1.16	1.35
	Probability	0.533	0.990	0.008	0.408	0.957	0.103	0.074

[a-b] Means in a column within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
[1] A-POS was positive control and B-NEG was negative control in breeder diet.
[2] CON was positive control and NC was negative control in broiler diet.
[3] A-POS: Grower with 0.90% Ca and 0.45% AvP, and layer with 2.7% Ca and 0.42% AvP.
[4] B-NEG: Grower with 0.60% Ca and 0.30% AvP, and layer with 2.7% Ca and 0.15% AvP.
[5] C-NEG+250: Grower with 0.60% Ca and 0.30% AvP +250 FTU phytase, and layer with 2.7% Ca and 0.15% AvP +250 FTU phytase.
[6] D-NEG+500: Grower with 0.60% Ca and 0.30% AvP +500 FTU phytase, and layer with 2.7% Ca and 0.15% AvP +500 FTU phytase.
[7] Standard error of mean (SEM) for n=18 pens.
[8] CON: Prestarter with1.0% Ca and 0.50 AvP, starter with 0.9% Ca and 0.45 AvP, grower with 0.7% Ca and 0.35% AvP, and finisher with 0.6% Ca, and 0.30% AvP.
[9] NC: Prestarter with 0.85% Ca and 0.35 AvP, starter with 0.75% Ca and 0.30%AvP, grower with 0.55% Ca and 0.20% AvP, and finisher with 0.45% Ca and 0.15% AvP.
[10] NC+1000: Prestarter with 0.85% Ca, and 0.35 AvP, starter with 0.75% Ca and 0.30% AvP, grower with 0.55% Ca and 0.20% AvP, and finisher with 0.45% Ca and 0.15% AvP + 1000 FTU phytase.
[11] Standard error of mean (SEM) for n=24 pens

Table IV-10 (continued). Mortality of male broiler chickens as affected by different levels of available phosphorous (AvP), calcium (Ca), and addition of phytase in grower and layer diets of broiler breeders, and levels of AvP, Ca, and addition of phytase to broiler feed.

Breeder Treatment [1]	Broiler Treatment [2]	Mortality For Ages Shown						
		0-14 d	15-28 d	29-35 d	36-42 d	0-28 d	0-35 d	0-42 d
		(%)						
A-POS [3]	CON [7]	0.6	4.2 ^{abc}	4.9	3.2	4.8 ^{wxyz}	9.5	12.5 ^{abc}
	NC [8]	1.2	4.8 ^{abc}	1.2	6.4	6.0 ^{xyz}	7.1	13.1 ^{abc}
	NC+1000 [9]	0.0	7.7 ^{c}	7.1	9.8	7.7 ^{z}	14.3	22.6 ^{abc}
B-NEG [4]	CON [7]	0.0	2.4 ^{ab}	3.1	5.1	2.4 ^{wx}	5.4	10.1 ^{ab}
	NC [8]	0.0	1.8 ^{a}	1.2	6.7	1.8 ^{w}	3.0	9.5 ^{ab}
	NC+1000 [9]	0.0	6.0 ^{abc}	6.1	8.7	6.0 ^{xyz}	11.9	19.6 ^{c}
C-NEG+250 [5]	CON [7]	1.2	4.2 ^{abc}	3.1	7.1	5.4 ^{wxyz}	8.3	14.9 ^{bc}
	NC [8]	1.2	6.0 ^{b}	1.9	2.6	7.1 ^{yz}	8.9	11.3 ^{abc}
	NC+1000 [9]	1.2	2.4 ^{ab}	6.8	4.5	3.6 ^{wxy}	10.1	14.3 ^{abc}
D-NEG+500 [6]	CON [7]	0.6	6.0 ^{b}	5.2	3.3	6.6 ^{yz}	11.3	14.3 ^{abc}
	NC [8]	1.2	4.2 ^{abc}	3.1	5.1	5.4 ^{wxyz}	8.3	13.1 ^{abc}
	NC+1000 [9]	0.6	1.2 ^{a}	3.6	2.5	1.8 ^{w}	5.4	7.7 ^{a}
SEM [10]		0.57	1.58	1.77	1.81	1.63	2.32	2.79
Probability		0.948	0.040	0.683	0.107	0.070	0.088	0.019

$^{a-c}$ Means in a column within each replicate that possess different superscripts differ significantly ($P \leq 0.05$).
$^{w-z}$ Means in a column within each replicate that possess different superscripts differ significantly ($P \leq 0.10$).
[1] A-POS was positive control and B-NEG was negative control in breeder diet.
[2] CON was positive control and NC was negative control in broiler diet.
[3] A-POS: Grower with 0.90% Ca and 0.45% AvP, and layer with 2.7% Ca and 0.42% AvP.
[4] B-NEG: Grower with 0.60% Ca and 0.30% AvP, and layer with 2.7% Ca and 0.15% AvP.
[5] C-NEG+250: Grower with 0.60% Ca and 0.30% AvP +250 FTU phytase, and layer with 2.7% Ca and 0.15% AvP +250 FTU phytase.
[6] D-NEG+500: Grower with 0.60% Ca and 0.30% AvP +500 FTU phytase, and layer with 2.7% Ca and 0.15% AvP +500 FTU phytase.
[7] CON: Prestarter with1.0% Ca and 0.50 AvP, starter with 0.9% Ca and 0.45 AvP, grower with 0.7% Ca and 0.35% AvP, and finisher with 0.6% Ca, and 0.30% AvP.
[8] NC: Prestarter with 0.85% Ca and 0.35 AvP, starter with 0.75% Ca and 0.30%AvP, grower with 0.55% Ca and 0.20% AvP, and finisher with 0.45% Ca and 0.15% AvP.
[9] NC+1000: Prestarter with 0.85% Ca, and 0.35 AvP, starter with 0.75% Ca and 0.30% AvP, grower with 0.55% Ca and 0.20% AvP, and finisher with 0.45% Ca and 0.15% AvP + 1000 FTU phytase.
[10] Standard error of mean (SEM) for n=6 pens.

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SUMMARY AND CONCLUSIONS

This research investigated the effects of feed regimen during the rearing period on Ross 708 broiler breeder reproductive performance and established a better understanding of the effect of the inclusion of phytase enzyme on the reproductive performance of broiler breeders and their progeny.

Manuscripts I and II. Effect of Female Feeding Program during Rearing on Ross 708 Broiler Breeder Reproductive Performance.

Although restricted feeding has been a common practice to control BW and enhance reproduction in heavy line broiler parents, it has been determined that it was crucial for pullets to receive a minimum allocation of 23,000 kcal ME and 1,200 g crude protein before photostimulation, irrespective of BW, in order to achieve sexual maturity followed by persistent egg production and fertility. In Manuscript I birds that consumed less feed, but still adequate cumulative nutrition, from 3 to 15 wk of age exhibited improved persistency of fertility. This suggested that a relatively greater degree of feed restriction during early rearing was beneficial in terms of persistency of fertility during subsequent reproduction. Manuscript II further investigated the time frame during which feed intake control was most important in terms of affecting persistency of fertility. It was observed that the feeding regimen from 3 to 6 wk of age did not affect egg production but affected persistency of fertility and associated hatchability as evidenced by improved fertility during the second half of the egg production cycle. However, neither study produced any egg production differences due to feed intake, in conflict with some literature. This may be due to genetic line differences, but most likely was due to the absence of a commercially impractical *ad libitum* treatment as was used in other published studies. The treatments chosen for the current studies had a similar cumulative

consumption of crude protein and ME to that which might be used commercially. Given that egg production was primarily an ovary effect, the fertility differences we observed due to reduced feed intake during early rearing had to be related to altered oviduct function. It has been well documented that the expression of broiler genetic traits such as breast meat development and rapid BW gain associated with rapid feed intake was negatively associated with reproduction. These data suggested that if rapid early growth of broiler breeder pullets was relatively more limited this genetic expression must be limited to a degree such that persistency of fertility was improved. The oviduct normally has been reported to develop immediately after photostimulation during a coincident time of typically rapid feed increase intended to support the onset of sexual maturity in feed-restricted broiler breeder females. It was surmised that a pullet exposed to reduced feed intake during early rearing diverted more of the available nutrients during sexual maturation towards reproduction than towards expression of broiler traits such as breast meat, which would ultimately affect the function of spermatozoal storage glands and perhaps overall oviduct function. Unfortunately, research concerning how feed intake could affect the development of the oviduct relative to overall body metabolism, and the mechanistic pathways, hormones, and molecules that may be involved was scarce. It was surprising that such small differences early in life had such a repeatable long term influence on fertility. Nevertheless, there has been numerous investigations that have indicated a long term effect of nutritional imprinting at early stages of life. In conclusion, in the first part of this dissertation it was demonstrated that restricted-fed broiler breeder females can apparently be programed by relatively low feed allocations at early stages of rearing such that the partitioning of the nutrients directed towards reproductive traits, specifically fertility, during sexual maturation was somehow altered sufficiently to positively

influence fertility a year later. This was thought to be due to the suppression of the early expression of "broiler genetics" by a less aggressive feeding program during the time when broiler genetics would be normally robustly expressed, i.e. the first 6 wk of rearing.

This also suggested that we may be able to program other limiting nutrients such as P in the broiler breeder if we find the right time frame. Broiler breeders have been found to be very efficient metabolically due to always being in a physiological state of semi-starvation, but they may also be very sensitive to poorly programmed and abrupt changes in management and nutrition.

Manuscripts III and IV. Effect of Dietary Available Phosphorous and Phytase in Broiler Breeder and Broiler Progeny Diets on the Reproductive Performance of Broiler Breeders and Live Performance of the Broiler Progeny.

The second part of the dissertation investigated the effects of dietary AvP and phytase in broiler breeders and their broiler progeny. Manuscript III studied two levels of AvP (A-POS and B-NEG) in grower and layer diets and two levels of phytase (C-NEG+250 FTU or D-NEG+500 FTU) "on top" of the low AvP treatment. The A-POS treatment and D-NEG+500 had the best overall fertility and total egg hatchability from 25 to 64 wk of age relative to B-NEG and C-NEG+250. Total egg weight was greater in A-POS, C-NEG+250, and D-NEG+500 treatments than in the B-NEG treatment at 34, 41, 64, and 65 wk of age. This suggested that egg weight was decreased by reduced dietary AvP and that addition of phytase recovered the lost egg weight. However, yolk weight did not differ statistically at 31, 41, 50, or 60 wk of age but, at 31 wk of age, A-POS exhibited the least albumen when compared with B-NEG, C-NEG+250, and D-NEG+500 treatments. The yolk:albumen ratio was therefore greatest in A-POS at 31

wk of age. Egg component results complemented the hatchability and fertility results, where the A-POS eggs had greatest yolk:albumen ratio and thinnest egg shell and exhibited the best hatchability from 25 to 34 wk of age. Egg production was not significantly different, but A-POS had an overall 2% increase in hen-day production as compared to the B-NEG, C-NEG+250, and D-NEG+500 treatments. The decrease in egg production of B-NEG, C-NEG+250, and D-NEG+500 treatments indicated that the hens had a slower rate of yolk deposition that may have been due to insufficient AvP. This suggested that the P from phytase hydrolysis required additional time for the hens to utilize relative to inorganic P. These circumstances could have ultimately resulted in eggs with relatively smaller yolks and greater albumen.

Manuscript IV studied the live performance of the broiler progeny chicks that originated from the broiler breeder study in Manuscript III. These broilers were provided one of three diets, two levels of AvP and 1000 IU phytase "on top" of the lower AvP diet (CON, NC, and NC+1000) in a 4 breeder diet X 3 broiler diet design. No interactions of breeder and broiler dietary treatments were observed. The BW at hatching as affected by breeder diet was greater in the A-POS, and D-NEG+500, followed by C-NEG+250 breeder treatment relative to the B-NEG diet, which was expected due to a heavier initial egg weight as the recovery of egg weight due to phytase was reflected in chick weight.

We were unable to demonstrate a maternal effect of breeder phytase on broiler progeny probably due to the low dosage of phytase consumed by the breeders. According to other investigations from our laboratory, there could be negative maternal effects on broiler progeny live performance when normally recommended dosages of phytase were consumed by broiler breeders. The reason for the prior negative maternal effect was possibly due to the details of

the feeding program and the changes in the feed formulation of broiler breeders. The feeding program used in the prior studies was similar to that of the present studies with the birds reared on a slow weekly feed increase to approximately 15 wk of age where the feed intake was approximately 65 g/bird/d. This was then followed by a more dramatic weekly feed increase to approximately 95 g/bird/d prior to photostimulation at approximately 21 wk of age. The typical dietary change from grower diet to layer diet then occurred at about 26 wk of age. This change typically decreased the inclusion of wheat byproducts and SBM, which would have decreased dietary phytate content at the time that total phytase consumption was increasing in conjunction with feed intake. Therefore, while the phytase was maintained at the same concentration in the feed, the main substrate would had been reduced as the birds came into lay. The relative excess of phytase and phytate may have caused some negative effects in the broiler breeders of previous studies, influenced the signals and content of the egg, and ultimately influenced the broiler progeny live performance. Therefore, in order to prevent any negative effects of phytase, a lower dosage in broiler breeders seemed to be reasonable. In retrospect, it would probably have been beneficial to adjust the enzyme dosage according to the feed intake, ingredients, and the available substrates that were provided in each formula.

As for broilers, the same effects may have been present but due to the short life span of the broilers, the effects were not as obvious. Nevertheless, in Manuscript IV where the broilers were provided feed with greater than 1000 FTU phytase the benefit of phytase diminished after 28 d of age in concert with greater feed intake and reduced SBM inclusion. The enzyme recovery analysis of the NC+1000 diet was at least 50% greater than expected in starter, grower, and finisher diets. Therefore, the enzyme added treatment may have had an effectively greater AvP and Ca level than planned and may have been somewhat of an example of “super

dosing." There has been suggestions that "super dosing" of phytase enzyme had additional live performance benefits in broilers. The suggestion was based upon the concept that destruction of all phytate in the GIT would release nutrients and enhance absorption. However, the use of phytase with broiler diets containing low phytate SBM reduced broiler live performance in our laboratory, and personal communications indicated a similar response observed by others, specifically phytase manufacturers. These data suggested that a certain amount of phytate in the GIT was beneficial and possibly required, given that these animals had been genetically selected on high phytate feeds for 60 years. There was a noticeable absence of papers at the recent PSA meetings concerning super dosing and personal communications concerning another research project indicated that even with super dosing there was a great deal of intact phytate still remaining in the GIT.

One common observation concerning the use of phytase has been wet litter. In our own laboratory we have used potassium carbonate to correct wet litter problems in low P breeder diets. One must remember that the lower GIT functions somewhat as the reabsorption site that completes kidney function in chickens. Reverse peristalsis has to be active in order for this to function. Fiber, fat, and feed particle size all work to control digesta retention time and peristalsis in the chicken. How these might work together and their relative contributions to digesta passage have not been fully delineated. However, as breeder diets have been generally low energy (low fat), the removal of fiber could accelerate digesta passage and disturb acid-base and water balance if dietary P and K were low. Our laboratory has intermittently observed negative effects on broiler progeny of phytase use in the broiler breeder parent. Given the absence of effect in the current study it was surmised that our dosages were sufficiently low to avoid severe effects on the progeny. However, subtle changes in egg composition and weight

did produce reduced hatchability of fertile eggs and smaller chicks at hatching. These data may reflect the process of adaption as dietary substrate changed at the onset of lay. Although smaller chicks have been frequently related to poorer broiler live performance, that effect was not observed in the present research. That, again, could have been dosage related in that chicks that were well managed might have been able to eat their way out of an initial disadvantage in BW if there were no longer term metabolic disadvantages.