PHYSIOLOGICAL PARAMETERS AS CORRELATED RESPONSE TO SELECTION FOR PHYTATE PHOSPHORUS BIOAVAILABILITY IN CHICKEN

by

PANKAJ KUMAR SETHI

(Under the Direction of SAMUEL E. AGGREY)

ABSTRACT

A study was conducted to study the hormonal dynamics in a chicken population divergently selected for phytate phosphorus bioavailability (PBA) in chickens. The utilization of phytate phosphorus in poultry is an important issue because excessive loss of phosphorus (P) in the excreta leads to environmental pollution. From the hormonal dynamics study, it was established that insulin like growth factor I (IGF I) and T_3 (triidothyronine) were higher in the high PBA line compared to low PBA line. On the other hand, glucagons levels were higher in low PBA line compared to high PBA line. Leptin levels were not different in the two lines. Increased levels of IGF I and T_3 may be responsible for the increase PBA in the high line. On the other hand high glucagon levels in the low line may have negative effect on both calcium and phosphorus bioavailability. Both IGF-I and glucagon were moderately heritable in both high and low PBA lines, whereas T_3 was heritable only in the high line. This study provides us some insight into the physiological state of chicken when selected for high and low phytate phosphorus bioavailability.

INDEX WORDS: Phytate phosphorus bioavailability, hormonal dynamics

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B.V.Sc & A. H, Punjab Agricultural University, India, 2003

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2006

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December 2006

DEDICATION

To my parents, family and friends who have supported me all the time during my studies.

ACKNOWLEDGEMENTS

First of all, I would like to thank God for his grace to help me to complete my masters program. I am also thankful to my major professor Dr. Aggrey for his guidance and advice through out my masters. I am grateful to my advisory committee members, Dr. Pesti and Dr. Edwards for their advice and encouragement. I am indebted to Dr. Remzi Bakalli for his assistance and guidance. I would also thank Ruchika and all the graduate students of the Poultry Science department, who always answered my questions and offered their assistance in all possible ways. Finally, I would like to thank my family and friends for their support and prayers.

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CHAPTER 1

INTRODUCTION

Phosphorus (P) is a second most abundant mineral in the body with 80% of the total quantity found in the skeletal system. Deficiency of P can cause rickets, retarded growth and other skeletal deformities. In poultry it is an essential element for growth and development and plays an important role in metabolism of carbohydrates, lipids and amino acids. Hence it is important that birds should receive adequate amount of available phosphorus in their diet to meet their metabolic demands. Poultry diets containing plant ingredients such as cereal grains, cereal by-products and oil seed meals, about 60-80 % of total phosphorus exists as phytate P that is P bound to phytic acid (Ravindran et al., 1994). Phytic acid (also known as inositol hexaphosphate) reduces the availability of P in monogastrics animals by forming an insoluble salts (phytate) with divalent cations (Ca, Mg, Fe, Zn and Mn) under weak acid to neutral conditions (Kratzer et al., 1959). Due to the lack of sufficient quantity of endogenous phytase activity (enzyme required to release the phosphate group from phyate to make phosphorus available to the bird or animal) in the digestive tract, poultry cannot utilize phyate phosphorus adequately (Heuser et al., 1943) and results in loss of phosphorus through the excreta. Manure containing residual phosphorus when applied to land can cause significant pollution threat to the environment (Ravindran et al., 1995). High amounts of phosphorus in the land can reach natural waterways through soil erosion and agricultural runoff leading to eutrophication (over abundance of nutrient in surface water). Excess phosphorus can cause proliferation of

undesirable aquatic plants, and lead to oxygen deprivation due to their decomposition and results in death of aquatic life. Several studies have shown that supplementation of poultry diets with exogenous phytases and its interaction with vitamin D, calcium and other nutrients can improve phytate phosphorus utilization by 20-45% (Simons et al., 1990, Edwards, 1993; Ravindran et al., 1995, Kies et al., 2001). Understanding various physiological parameters influencing phosphorus metabolism can be another approach to improve phytate phosphorus utilization in poultry.

Phosphorus metabolism is controlled by several physiological parameters. Several studies have demonstrated that phosphorus, calcium and vitamin D influence the absorption and metabolism of each other. Active metabolite of Vitamin D (1,25 dihydroxycholecatiferol) stimulate calcium uptake and its absorption. Increase in calcium absorption cause increase in calcium retention which leads to increase in phosphorus retention because calcium requires phosphorus for skeletal growth (Helander et al., 1996). Normal level of calcium and phosphorus in the blood is under the hormonal control of parathyroid, vitamin D and clacitonin. Parathyroid hormone mobilizes calcium and phosphorus from bone by stimulation of biological-active form of vitamin D with in the kidney. To prevent excessive increase of phosphate, parathyroid decreases the reabsorption of sodium, calcium and phosphate in the proximal tubules of kidney (Agus et al., 1973). High levels of calcium in the blood stimulates calcitonin secretion by the parafollicular cells in thyroid gland, Calcitonin acts on osteclast and stop bone resorption, while low levels of calcium suppresses the calcitonin secretion. Calcitonin can also prevent hypercalcaemia and hypercalciuria by slowing intestinal absorption of calcium and phosphate in rapidly growing animals (Juan et al., 1976). Laroche and Boyer (2005)

suggested that other hormones like IGF I, thyroid hormones, insulin increases tubular phosphorus reabsorption. Quigley and Baum (1991) demonstrated that IGF I microperfusion simulates the absorption of phosphate in rabbit proximal convoluted tubules. Several studies have also demonstrated the effect of thyroid hormones on phosphorus reabsoption and serum phosphorus levels, although the results are conflicting (Beisel et al., 1958; Logan et al., 1941; Kobe et al., 1999).

The objective of our study is to investigate the affect of some physiological factors in a divergent line selected for high and low phyate phosphorus bioavailability.

CHAPTER 2

LITERATURE REVIEW

Thyroid Hormones

Thyroxine (T_4) and triidothyronine (T_3) are the two principal hormones secreted by the thyroid gland, one of the largest endocrine glands in the body (Figure 2.1). Thyroid hormones are derivatives of the amino acid tyrosine bound covalently to iodine. The thyroid gland also secretes another hormone calcitonin that controls the blood calcium levels. Kendall (1919) isolated thyroxine (T_4) from thyroid extracts. Harington (1926) identified it as 3,5,3′,5′-tetraido-L-thyroxine and along with Barger (1927) described the constitution and synthesis of thyroxine. Gross and Pitt-Rivers (1954) synthesized 3,5,3′-tri-ido-L- thyroxine (T_3) and demonstrated its presence in human plasma. Thyroid hormones are poorly soluble in water, and are carried to the target organs bound mainly to thyroxine-binding globulin, a glycoprotein synthesized in the liver. Several studies have demonstrated that thyroid hormones play important roles in growth, metabolism, differentiation and development (Chatterjee et al., 1992; Beach and Jacobson, 1979).

Production of Thyroid Hormone

The secretion of thyroid hormones is under the influence of thyrotropin releasing horomones (TRH). Several studies have shown that hypothalamic extract of animals contain TRH (Bowers, et al., 1965; Schally, et al., 1966, Bower et al., 1971). In mammals

TRH stimulates thyroid stimulating hormone (TSH) from the anterior pituitary (Guillemin et al., 1963; Bowers et al., 1965) that in turn stimulate thyroid to release thyroid hormones. Guillemin et al (1963) studied the effect of sheep TRF (TSH-releasing factor) on the pituitary glands of rat and observed an increase in amount of TSH as a linear function of the log dose of added TRF. Their study demonstrated the effect of TRF on pituitary *in vitro*. Bowers et al. (1965) reported similar result of increase TSH secretion from the pituitary of rats in response to TRH releasing factor from ox, sheep, pig and human both *in vitro* and *in vivo*. They observed significant increase in the plasma levels of TSH 15 minutes after the administration of TRH from all four species into the thyroidectomized, triidothyronine-treated rats. Redding et al. (1970) reported that the release of TSH from the sheep interior pituitary tissue is greatly enhanced when porcine TRH is added to the medium of sheep pituitary tissue. Bowers et al. (1971) studied the effect of synthetic pyroglutamyl-histidyl-prolinamide of TRH on the release of TSH from the anterior pituitary of humans. He observed that intravenous injection TRH increased the serum level of TSH rapidly when TRH was added to anterior pituitary tissue.

Regulation of Synthesis and Secretion of Thyroid Hormone

Regulation of thyroid hormone synthesis and secretion is under the negative-feedback system involving hypothalamus, pituitary and thyroid gland (Shupnik et al., 1985, 1989). Diagrammatic representation of TH regulation is shown in Figure 2.2.

Levels of thyroid hormones in the blood regulate TSH production from the pituitary by a negative feed back mechanism. Shupnik et al. (1985) studied the effect of T_3 on the transcription of TSH subunit gene. They injected TtT 97 tumor cells

subcutaneously in thyroidetomized male LAF_1 mice to develop tumor with a concomitant T_3 injection. They observed decrease in mRNA synthesis of both α (28 %) and β subunits (61%) 30 minutes and by 75 % (α subunit) and >95 % (β subunit). These studies suggested the transcriptional regulation of thyrotropin genes by thyroid hormone.

Several studies have reported the inhibitory role of T_4 on the release of TSH (Larsen and Frumess, 1977; Fukuda et al., 1975; Chopra et al., 1973). Larsen and Frumess (1977) in a comparative study observed that T_4 caused same degree of suppression of TSH as done by T_3. Further, the serum T_3 levels in animals injected with T_4 increased only minimally into low to normal range while injecting T_3 in the animal caused a sharp increase in serum T_3 levels. Silva and Larsen (1977) observed that the suppression of TSH upon T_3 or T_4 injection is correlated temporally and quantitatively with the occupancy of the pituitary nuclear receptor sites by T_3. Sterling et al. (1970) investigated the conversion of T_4 to T_3 in normal human subjects and found that intravenous injection of purified thyroxine labeled with carbon 14 in ring A and in alanine side chain can be found in serum T_3 suggesting the conversion of T_4 to T_3 in normal human subjects.

Segerson et al. (1987) studied the role of hypothalamic tuberoinfundibular system in the regulation of TRH release in response to thyroid hormone. Male Sprague-Dawley rats were hypothyrodimized by intraperitoneal injection of propylthiouracil and by adding 0.02% methimazol in drinking water. Using quantitative Northern blot on hypothalamic paravetricular nucleus and *in situ* hybridization histochemistry, immunochemistry on coronal section of brain they observed two fold increase in proTRH mRNA in hypothyroid animals. This increase could be obliterated by levothyronine, indicating that

circulating thyroid hormone are inversely related to proTRH mRNA. In situ hybridization showed the paraventricular nucleus was exclusivly responsible for this response. Similar results were reported by Taylor et al. (1990) who studied regulation of TSH synthesis during hypothyroid condition in normal adult male rats hypothyroid with sham lesions in hypothalamus and hypothyroid with paraventricular nucleus lesions. They observed lesser increase in TSH levels in the rats with hypothyroid paravetricular nucleus lesions as compared to the rats hypothyroid sham group in response to decrease in levels of plasma T_4 after 2 and 4 weeks of treatment. When compared to euthyroid control rats, the pituitary content of TSHβ mRNA increased markedly in hypothyroid sham-lesion rats while there was no increase observed in paraventricular nucleus lesion rat at 2 weeks and partial increase at 4 weeks of treatment. At 4 weeks, no increase in α mRNA levels were observed in hypothyroid paraventricular nucleus rats as compared to control. Further, using in situ hybridization histochemistry they reported that TRH mRNA was not detectable in paraventicular nucleus lesioned rats at 2 weeks. These study suggested that paraventricular nucleus of hypothalamus regulate TRH biosynthesis.

Thyroid hormone metabolism: Iodothyronine deiodinases

The primary secretory product of thyroid, thyroxine T_4 is comparatively inactive and is converted to biologically active T_3 (Oppenheimer, 1979) by the enzyme thyroxine 5'-deiodinase, which causes deiodination at 5′ end. However, deiodination of T_4 on the tyrosly ring results in formation of inactive 3,3′,5′-triidothyronine (reverse T_3, rT_3) and that of T_3 on the tyrosyl ring leads to production of inactive 3,3′-diiodothyronine (T_2). T_3 has a higher affinity for the nuclear thyroid hormone receptor than T_4 (Oppenheimer,

1979). Engler and Burger (1984) reported that ~80% of secreted T_4 is deiodinated to form T_3 or rT_3 in equimolar amounts.

On the basis of the catalytic properties three major pattern of deiodination have been identified and designated as type I (D1), type II (D2), and type III (D3) iodothyronine deiodinases (Germain, 1994). D1 is predominantly found in liver, kidney and thyroid, have a key role in 5′- or 5- deiodination, that leads to production of active hormone T_3, and in clearance of metabolite reverse T_3 (Visser, 1988; Bianco et al., 2002, Leonard et al., 2000). D2 is present in pituitary, central nervous system, brown adipose tissue (BAT), skeletal muscle, aortic smooth muscle cells, osteobalst, and thyroid and perform the catalytic deiodination exclusively at 5′ position (Leonard, 1991). D2 catalyze deionation of both T_4 and rT_3. Bianco et al. (2000) reported that D2 are also present in human heart. They are widely distributed in rat brain, with relatively high levels in cerebellum, cortex, and hypothalamus (Kaplan et al., 1981). D2 is important for local production of T_3 in the brain while in skeletal muscle they contribute toward the production of plasma T_3. D3 is found in cerebral cortex, pregnant uterus, and skin of non-pregnant rats (Kaplan et al., 1983, Haunge et al., 1985), several fetal tissues, pregnant uterus and human placenta, and are induced in critical illness (Roti et al., 1981, Bianco et al., 2000; Peeters et al., 2005). D3 protect the tissue from excess of thyroid hormone by inactivating T_3 to T_4 and hence play an important role in thyroid hormone homeostasis. The metabolism of thyroid hormone is induced by D1 and D2 when T_3/T_4 is less and suppressed by D3 when T_3/T_4 ratio is more that one (Peetrs et al., 2006).

Role of Thyroid Hormones in Calcium-Phosphorus Metabolism

Phosphorus (as phosphate) is a major intracellular anion that plays an important role in growth, bone development and energy metabolism, as do the thyroid hormones and growth hormones (GH) (Palmer et al., 1987). A deficiency in dietary P in chickens is characterized by loss of appetite, rickets, and growth retardation (Scott et al., 1983). There is a significant correlation reported between serum P and thyroid function. Serum levels of total alkaline P are increased in hyperthyroidism and are decreased or normal in hypothyroidism (Mosekilde et al., 1990). Phosphate reabsorption by human kidney has been shown to increase upon administration of GH (Corvilain and Abramow, 1962). There are reports demonstrating that thyroid hormone also affect the rate of P reabsorption, although reports are conflicting (Beisel et al., 1958; Bommer et al., 1979). In chickens, Parmer et al. (1987) reported that T_3 and GH were consistently lower in P deficient chicks compared to controls. They therefore suggested that both thyroid hormone and growth hormone are altered by P deficiency.

Several studies have demonstrated the role of thyroid hormones on calcium phosphorus homeostasis in the kidney and small intestine (Cross et al., 1990). In humans, net P absorption is a linear function of dietary P, so that 60-65% is absorbed (Mosekilde et al., 1990). Borowitz et al. (1993) studied the effect of L-thryoxine on intestinal phosphorus absorption in rabbits. Using radiaolabeled phosphate they observed significant greater sodium-phosphate co-transport in the control animal as compared to treated animals. This study suggested that l-thryoxine inhibits intestinal phosphorus absorption in young rabbits. Prasad and Kumar (2005) showed that there is significant alteration of Na+-P co-transport across the brush border membrane vesicles, lipid

composition of the brush border membrane and its fluidity in response to thyroid hormone status of animals. Therefore thyroid mediated alteration of the brush border membrane may play an important role in the modulation of the intestinal absorption of P. Their studies revealed that alteration of Na+-P transport in response to thyroid hormone is quite specific for P transport since Na+ dependent glucose transport is not influenced by thyroid hormone status. Cross and Peterlik (1991) reported that T_3 facilitates the expression of genomic actions of calcitriol, and as a result play an important role in the regulation of calcium and phosphorus metabolism.

The serum P level is largely determined by the renal tubular reabsorption of P and to a lesser extent by the endogenous load and glomerular filteration rate (Mosekilde et al., 1990). Renal P excretion is increased in hyperthyroidism and correlates positively with thyroid function (Mosekilde and Christiansen, 1977). It has been suggested that the increase in maximal tubular reabsorption of P is caused by a direct effect of thyroid hormones on the kidney, but it seems more likely that it is caused by the suppressed parathyroid function found in hyperthyroidism. On the contrary, the renal excretion of P is normal or decreased in hypthyrodism (Mosekilde et al., 1990). Yusufi et al. (1985) demonstrated the modulatory effect of thyroid hormone on phosphate uptake in luminal brush border membrane of kidney cortex. They observed a dose dependent stimulatory effect of both T_3 and T_4 on brush border membrane independent to β-effects of catecholamines or altered growth hormone secretion. Inhibition of conversion from T_4 to T_3 by a potent 5′-monodeiodinase did not diminish the stimulatory effect of T_4 on brush border membrane. Further, they observed stimulatory effect of T_3 on Na^+ gradient dependent uptake in brush border membrane vesicles of outer cortical zone of kidney.

These studies suggested that both T_3 and T_4 have stimulatory effect on P uptake in renal proximal tubules.

Bommer et al. (1979) demonstrated the role of thyroid hormone in renal phosphorus handling in hyperthyroid rats. They observed increase in serum phosphorus concentration in hyperthyroid rats induced by injecting T_4 to parathyroidectomized rats and decrease in serum phosphorus in hypothyroid rats achieved by removing thyroid. Furthermore, they reported increase in phosphorus absorption in parathyroidectomized rats without change in urinary excretion of cAMP. These studies suggest that thyroxine play a regulatory role in renal phosphorus handling. In an earlier research, Logan et al. (1941) had investigated the effect of thyroid hormone on calcium and phosphorus metabolism in young male dogs. They reported that thyroid administration to normal dogs resulted in increase calcium excretion while no significant change in phosphorus excretion. Thyroid administration to thyroparathyriodectomized animals resulted in decrease phosphorus excretion. However, no change in serum phosphate levels was observed during these experiments.

Thyroid hormones stimulation of osteoblastic activity *in vivo*, and increased production of insulin-like growth factor I (IGF-I) has been documented in osteoblasts after stimulation with T_3 (Srache et al., 1986)). Therefore, it has been suggested that the effect of thyroid hormones on bone formation is caused indirectly through stimulation of growth factor production (Raisz and Kream, 1983). It is apparent that thyroid hormones and hormones from the growth hormone axis, especially IGF-I may independently or interact to affect phosphorus metabolism.

Growth hormone

Growth hormone (GH) is a protein hormone that is synthesized and secreted by somatotrophs cells of the anterior pituitary gland under the regulatory control of hypothalamus (Hall et al., 1986, Scanes et al., 1984, Theill et al., 1993). Growth hormone is the major participant in control of several complex physiological processes, including growth and development. In chicken, growth hormone is not detectable and does not effect embryonic growth. However, they are obligatory for normal postnatal growth and development (Harvey et al., 1997).

The transcription of GH is under cell type specific and hormonal control [Growth hormone releasing hormone (GHRH) and Somatostatin]. GH belongs to the family of somatogenic hormones such as prolactin (PR) and placental lactogen, also called chorionic somatomammotropins (Slater et al., 1986; Ohta et al., 1993; De Vos et al., 1992). Growth hormone is also known to have extra-pituitary origin, for example, the brain, cells of immune system and testis (Harvey et al., 1997). In chickens the GH protein is 191 amino acid peptide chains (Harvey et al., 1997). Sequencing of turkey and duck GH gene has also been successful (Chen et al., 1988; Foster et al., 1990). In these species, the pulsatile secretion is observed at the time of maximal relative body weight gain. However, it is important to know that the pattern of GH release and GH levels are not representatives of the rate of growth, but they can be related to the conversion of dietary protein into body protein (Decuypere and Buyse, 2004).

Control of growth hormone secretion

Secretion of growth hormone from anterior pituitary is regulated by several factors like basal secretion, hypothalamic control, feedback mechanism, other hormones and metabolic factors, and external factors (Hall et al., 1986). Harvey and Hull (1998) reported that growth hormone can be produced and regulated in many extra-pituitary tissues like brain, immune system, placenta, mammary glands and testis that complement the traditional regulatory mechanism of hypothalamus-pituitary axis. In birds and mammals, growth hormone releasing factor (GHRF) from hypothalamus stimulates the secretion of growth hormone from the anterior pituitary (Scanes, et al., 1984; Hall et al., 1986). Other factors that stimulate growth hormone secretion are thyroid hormones (Mulloy et al., 1992), retinoic acid (Bedo et al., 1989) and glucocorticoid (Paek et al., 1987) and ghrelin. However, somatostatin a peptide produced by hypothalamus and several other tissues in the body inhibits growth hormone release by altering the intracellular cAMP concentrations (Tuggle et al., 1996). Growth hormone secretion is also inhibited by a negative feed back mechanism of IGF I. High levels of IGF I in blood decrease the secretion of growth hormone not only by suppressing the pituitary somatotrophs, but also by stimulating the release of somatostatin from hypothalamus.

Insulin-like growth factors

Insulin like growth factors (IGF I and IGF II) are polypeptide hormones circulating in the blood and have several metabolic and anabolic properties (McMurtry et al., 1997). They are one of the most important factors required for growth (Marquardt et al., 1981) and are produced by several tissues like liver, fat, muscle, kidney, brain, skin,

heart and gonads (Han et al., 1987) in response to growth hormone. Liver is the main source of circulating IGF I (Schwander et al., 1983). The chicken IGF-1 (cIGF-1) gene sequence differs from human, bovine and porcine on at three locations. Such as Serine ^{26} replacing Asparagine, Lysine ^{41} replacing Threonine and Isoleucine ^{64} replacing Leucine (Rinderknecht and Humbel, 1978). Although infrequently, it is seen that Glutamine ^{50} has been replaced by Arginine. These four substitutions occur at four different positions in the molecule, but it does not change the net charge of the molecule. Other differences in the peptide sequence are, Leucine ^{38} replacing Alanine, Histidine ^{39} replacing Proline, Histidine ^{40} replacing Glutamine, Proline ^{67} replacing Alanine (Ballard et al., 1990).

In poultry production of insulin like growth factors are either under control of growth hormone or independent of growth hormone, and is dependent upon tissue and age of the bird (McMurtry et al., 1997). In the embryonic stage IGF I secretion is minimally controlled by growth hormone while in post-hatch birds, growth hormone is the principle regulator of IGF I production (McMurtry et al., 1997). Not much is known about the control of IGF II secretion in poultry. Burnside and Cogburn (1992) reported that the peak levels of IGF I mRNA in chicken is observed at 4 weeks of age and the levels decline to basal levels by 10 weeks of age. However, some studies have failed to demonstrate consistent and significant increase in IGF I when pituitary-derived or recombinant chicken growth hormone was administered to pituitary intact birds (Vasilatos-Younken, 1999)

Interaction between Growth hormone, Insulin-like Growth Factor I and Thyroid hormones

Several physiological and clinical studies have demonstrated that growth hormone, IGF I and thyroid hormones interact at the level of the hypothalamus and pituitary. In hypothyroidism, there is decrease in pulsatility of growth hormone and its response to number of secretagougues are altered, whereas in hyperthyroidisim there is an increase in size and number of spontaneous growth hormone bursts, but decrease in responsiveness to direct pituitary stimulation with GHRH (Valcavi et al., 1992). In poultry, thyrotropin-releasing hormone (TRH) and growth hormone releasing factor (GHRF) stimulates, while somatostatin inhibits the secretion of growth hormone (Hall et al. 1986). Growth hormone is thyrotropic in poultry (Kunh et al., 1987) and causes increase in the levels of T_3 in the circulation. The increased levels of T_3 in the circulation inhibit the release of growth hormone (Scanes and Harvery, 1989) by direct or indirect mechanism. Harvey and Baidwan (1990) studied the effect of T_3 treatment on growth hormone secretion in poultry and reported that T_3 down-regulate pituitary TRH receptor and inhibit secretion of growth hormone. This study suggested the direct mechanism of action of thyroid hormone on pituitary, to regulate growth hormone secretion. De Los Frasilies et al (1988) observed the increase in biosynthesis of somatostatin in response to thyroid hormone in rat. Leung and Taylor (1983) reported that TRH-induced GH release is suppressed by somatostatin. These studies indicated the indirect inhibition of GH by the thyroid hormone.

Darrs et al. (1992) observed increase plasma T_3 and decrease in plasma T_4, both in 18-day old chicken embryo and newly hatched chicks due to impairment of type III

deiosinase activity 2 hours after the injection of growth hormone. Similar results were reported by Vasilatos-Younken et al. (2000) in chicken while studying the effect of chronic growth hormone administration on skeletal muscle growth. They observed that growth hormone decrease the hepatic type III deiodinase activity in a dose –dependent manner. This led to increase in plasma triidothyronine (T_3) and hepatic IGF I protein content. However, no change in the levels of circulating IGF I was observed. This study suggested that growth hormone regulate thyroid hormone metabolism at pre- and post-translation level and hyper-T_3 state may impair release of hepatic IGF-I in response to growth hormone in birds. However, Ikeda et al. (1991) observed an increase secretion of IGF I levels when T_3 was infused in a perfused rat liver. Studies in humans have reported decrease in the circulatory levels of IGF I in hypothyroid patients (Valcavi et al., 1987). Miells et al. (1994) demonstrated the increase in level of insulin like growth factor binding protein II and decreased levels of IGF I, IGF II, Insulin like growth factor binding proteins I and III in hypothyroid rats. Thomas et al. (1993) suggested the decrease in levels of IGF I in rat due to suppression of IGF-I gene expression in liver. However, it is not established that whether this is due to the direct effect of thyroid hormone or due to the secondary effect of decreased growth hormone secretion.

Role of IGF I in Calcium and Phosphorus metabolism

There are limited studies on role of IGF I in phosphorus metabolism. Hittmeier et al. (2006) studied the effect of interactions between the dietary phosphorus and genetic background on the expression of genes in bone marrow. They fed pigs from two different line (heavier boned and lighter boned) fed either a phosphorus adequate, phosphorus

repletion or phosphorus deficient diets and bone marrow samples were collected after 14 days. They observed increase in the expression of IGF I gene when phosphorus deficient diets were fed in both the lines.

IGF I is a potent stimulator of brush-border membrane Na/Pi-cotransport which in turn stimulate renal tubular Pi reabsorption. Caverzasio et al. (1990) studied the effect of recombinant human IGF I on the renal phosphorus absorption in hypophysectomized rats. They observed significant increase in the phosphorus absorption in the brush border membrane vesicles isolated from renal cortex. Quigley and Baum (1991) performed in vitro microperfusion study to determine the effect of IGF I on proximal convoluted tubule (PCT) transport in rabbit. They observed the stimulatory effect of IGF I on phosphorus absorption via basolateral and apical membranes in PCT. These studies suggest that IGF I is an important mediator for growth hormone in renal Pi absorption.

Kasukawa et al. (2003) suggested that IGF I can play a role in homeostasis due to its direct effect on 1,25-dihydroxyvitamin D and parathyroid hormone levels. They fed IGF I knock out and wild type mice diet containing either low calcium (0.01%) or normal calcium (0.6%) for 2 weeks and serum samples were collected. They observed an elevated levels of parathyroid hormone and decreased levels of serum 1,25-dihydroxyvitamin D in IGF I knock out mice maintained on normal calcium diet. This study suggest that IGF I can enhance ca absorption in the gut through its effect on I,25-dihydroxyvitamin D system.

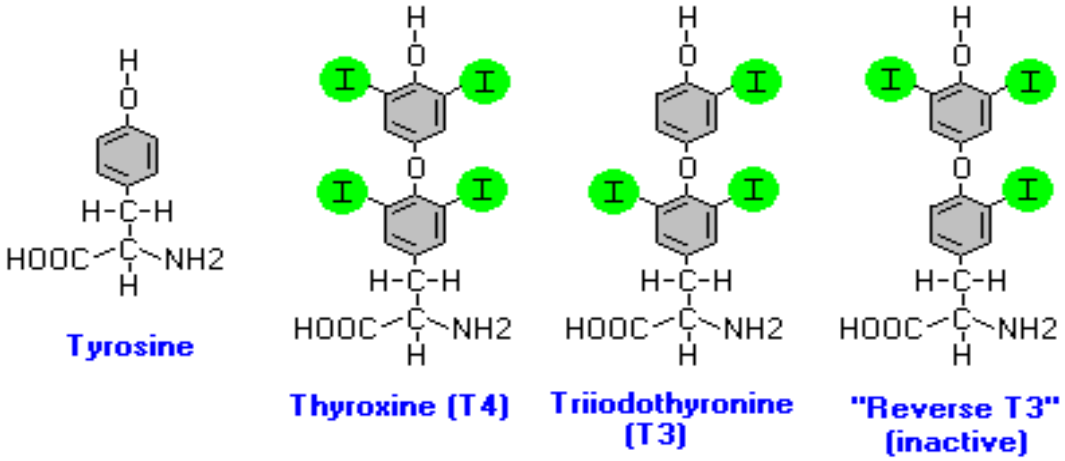

Figure 2.1: Chemical Structure of thyroid hormones

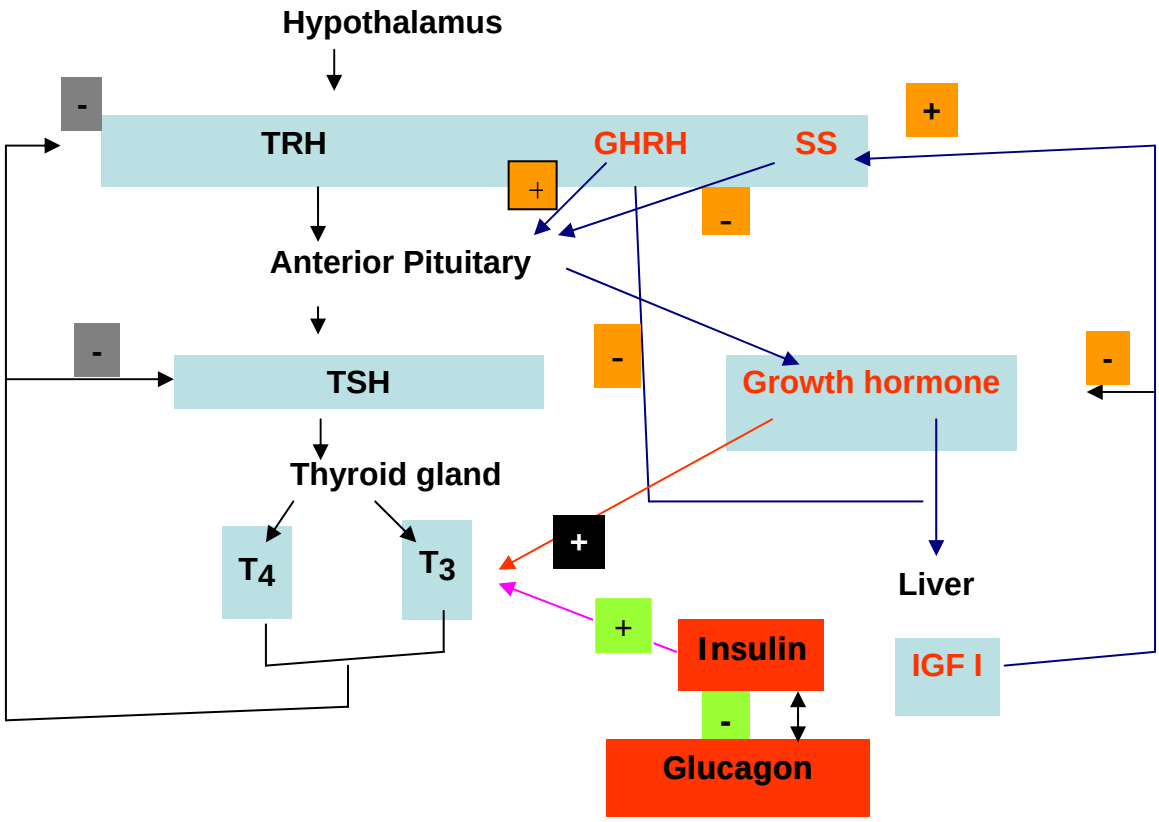

Figure 2.2 Regulation of thyroid hormones and their interaction with the growth hormone axis.

CHAPTER 3

PHYSIOLOGICAL PARAMETERS IN A RANDOM MATING CHICKEN POPULATION DIVERGENTLY SELECTED FOR PHYTATE PHOSPHORUS BIOAVAILABILITY[1]

[1] Pankaj K. Sethi, Samuel Aggrey, Gene Pesti, H.M Edwards. To be submitted to Poultry Science

Abstract

The utilization of phytate phosphorus is poultry is an important issue because excessive loss of phosphorus (P) in the excreta leads to environmental pollution. Phosphorus utilization in poultry is affected by several factors like bioavailability of calcium and phosphorus, level of vitamin D, age and genetic strain of bird and endogenous phytases. However, little is known about the effect of various physiological factors on phyate P utilization. We therefore studied the effects of hormones on phytate P bioavailability in divergently selected lines for high or low phyate P. The mean levels of IGF I, Leptin, T_3, T_4, and insulin were higher in high line compared to the low line. However, IGF II and glucagon were higher in low line compared to high line. The mean value for T_3 / T_4 was similar in both lines. There were highly significant difference ($P < 0.01$) in mean levels of IGF I, IGF II, and T_3 hormones between the high and low lines and nearly significant difference for glucagon. Further, there were no significant differences in the levels of leptin, T_4, T_3 / T_4 and insulin levels between the two lines.

Introduction

Phosphorus (P) is a second most abundant mineral in the body with 80% of the total quantity found in the skeletal system. Deficiency of P can cause rickets, retarded growth and other skeletal deformities. In poultry it is an essential element for growth and development and plays an important role in metabolism of carbohydrates, lipids and amino acids. Hence it is important that birds should receive adequate amount of available phosphorus in their diet to meet their metabolic demands. Poultry diets containing plant ingredients such as cereal grains, cereal by-products and oil seed meals, about 60-80 % of

total phosphorus exist as phytate P that is, P bound to phytic acid (Ravindran et al., 1994). Phytic acid (known as inositol hexaphosphate) reduces the availability of P in monogastrics animals by forming an insoluble salts (phytate) with divalent cations (Ca, Mg, Fe, Zn and Mn) under weak acid to neutral conditions (Kratzer et al., 1959). Due to the lack of sufficient quantity of endogenous phyates (enzyme required to release phosphate group from phyate to make phosphorus available to animal) in digestive tract, poultry cannot utilize phyate phosphorus adequately (Heuser et al., 1943) and results in loss of phosphorus through excreta. Manure containing residual phosphorus when applied to land can cause significant pollution threat to the environment (Ravindran et al., 1995). High amounts of phosphorus in the land can reach natural waterways through soil erosion and agricultural runoff leading to eutrophication (over abundance of nutrient in surface water). Excess of phosphorus can cause proliferation of undesirable aquatic plants, and lead oxygen deprivation due to their decomposition and results in death of aquatic life. Several studies have shown that supplementation of poultry diets with exogenous phytases and its interaction with vitamin D and calcium can improve phytate phosphorus utilization by 20-45% (Simons et al., 1990; Edwards, 1993; Ravindran et al., 1995; Kies et al., 2001). There is ample evidence to suggest involvement of physiological parameters in phosphorus metabolism.

Phosphorus metabolism is controlled by several physiological parameters. Several studies have demonstrated that phosphorus, calcium and vitamin D influence the absorption and metabolism of each other. Active metabolite of Vitamin D, 1,25 dihydroxycholecatiferol stimulate calcium uptake and its absorption. Increase in calcium absorption cause increase in calcium retention which leads to increase in phosphorus

retention because calcium requires phosphorus for skeletal growth (Helander et al., 1996). Normal level of calcium and phosphorus in the blood is under the hormonal control of parathyroid, vitamin D and clacitonin. Parathyroid hormone mobilizes calcium and phosphorus from bone by stimulation of biological-active for of vitamin D with in the kidney. To prevent excessive increase of phosphate, parathyroid decreases the reabsorption of sodium, calcium and phosphate in the proximal tubules of kidney (Agus et al., 1973). High levels of calcium in the blood stimulate calcitonin secretion by the cells in thyroid gland. Calcitonin acts on osteclast and stop bone resorption, while low levels of calcium suppresse the calcitonin secretion. Calcitonin can also prevent hypercalcaemia and hypercalciuria by slowing intestinal absorption of calcium and phosphate in rapidly growing animals (Juan et al., 1976). Laroche and Boyer (2005) suggested that other hormones like IGF I, thyroid hormones, insulin increases tubular phosphorus reabsorption. Quigley and baum (1991) demonstrated that IGF I microperfusion simulates the absorption of phosphate in rabbit proximal convoluted tubules. Several studies have also demonstrated the effect of thyroid hormones on phosphorus reabsoption and serum phosphorus levels, although the results are conflicting (Logan et al., 1941; Beisel et al., 1958; Kobe et al., 1999).

The objective of our study is to investigate the hormonal dynamics in a divergent chicken population selected for high or low phytate phosphorus bioavailability.

Materials and Methods

Birds

The data were collected on an unselected random mating Athens-Canadian randombred (ACRB) chicken population (Hess, 1962). Twenty-six males were pedigree mated to 71 dams (sex ratio 1:2~3) to hatch 1,004 chicks in 6 hatches at 7 d intervals. Chicks were placed in pens with litter and fed a corn and soybean meal based diet containing 23 % protein, 3.2 Kcal ME/kg, 0.90% calcium, 0.675% total P, and 0.45 % available P until 4 wk of age. At 4 wk of age, birds were transferred to individual metabolic cages and fed the same diet with the mineral source of P largely removed and calcium and total P adjusted to 1.06 and 0.35% respectively. After a 3 d period of acclimatization, excreta produced in 3 consecutive d were collected and feed consumed was (FC) measured. Individual 4-wk BW and BW gain (BWG) during the 3 d excreta collection period were also measured. Excreta were oven-dried at 80^{o}C and ground. Phytate P in the feed and dried excreta was determined by method described by Latta and Eskim (1980). Feed conversion ratio (FCR) was calculated as the ratio of FC per BW gain (BWG). The bioavailability of phytate P was computed as follows:

PBA = (A-B)/A * 100 %

where PBA= the bioavailability of the nutrient, A = content of the phytate in the feed (%) * feed intake (g), B = content of the phytate in dried excreta (%) * dried excreta weight (g). Phytate P intake bioavilabilit body weight at 4 wk, BWG at day 28-35 d, FC, feed conversion ratio (FCR) for BW were collected on 1,004 birds. The FCR was calculated as the ratio of FC per BWG during the excreta collection period.

Selection Lines

The selection of broilers for high and low PBA was described by Zhang et al. (2003). The procedure they followed were as follows: Birds were ranked according to their hatch-corrected PBA values to establish the divergent sub-populations. In the high (H line) and low (L line) PBA lines, 18 to 22 males and 40 to 46 females with the highest and lowest hatch-corrected PBA values were selected as breeder candidates. However, at generation 2 (G_2) the breeder candidates for the H line were selected on their expected breeding values rather than their phenotypic values. From the breeder candidates, 12 males and 36 females that had normal performance in meeting artificial insemination (AI) and egg collection requirements were randomly selected as the actual breeders for each line. One sire was mated to 3 dams by AI, and sibling mating was avoided whenever possible. The direct selection for PBA was performed for 3 generations. About 900 individuals in the base generation and 343 individuals per line in generations 1 to 3 were established.

Hormonal Analysis

At generation 3 of selection 4 ml of blood were collected at 8 week of age using EDTA as an anticoagulant from 299 birds in high PBA line and 274 birds in low PBA line. The blood samples were centrifuged for 10 minutes at 3,000 rpm, and the plasma was separated and stored frozen at -20^{o}C. .The frozen plasma was analyzed for IGFI, IGFII, insulin, glucagon, leptin, T_3 and T_4 levels using standard hormone assays.

Specific radioimmunoassays were used to determined plasma hormone concentrations. All samples were analyzed within 1 assay to avoid interassay variations. Double antibody radioimmunoassay were used to determine plasma concentrations of

IGF-I with an intraassay CV of 2.8% (McMurtry et al., 1994), chicken IGF-II with intraassay CV of 3.7% (McMurtry et al., 1998), insulin with intraassay CV of 2.2%, (McMurtry et al., 1983), and leptin with intraassay CV of 3.9% (Evock-Clover et al., 2002). The T3 and T4 were determined with an intrassay CV of 2.5 and 2.8%, respectively. Plasma glucagon (Linco Research Inc., St. Charles, MO) was determined using commercial kits with an intraassay CV of 1.9%. For glucagon analysis, an aliquot of plasma was stored in the presence of 1,000 KIU of aprotinin.

Results

The hormonal differences between the high and low PBA lines are presented in Table 3.1. The graphical differences between the high and low PBA lines with respect to the hormones determine are present in Figures 3.1-3.8. We observed that the mean level of IGF I hormone in high and low PBA lines were 43.06 ng/ml and 39.26 ng/ml with a SD of 0.57 and 0.60, respectively. There was a significant difference ($P < 0.001$) in level of IGF I hormone between the high and low PBA lines. Similarly the value of IGF II in high PBA line (40.57 ± 1.02 ng/ml) were significantly ($P <0.013$) different that for low PBA line (44.23 ± 1.07 ng/ml). Though leptin levels were higher in high PBA line (2.93 ± 0.102 ng/ml) than the low PBA line (2.86 ± 0.11 ng/ml) the difference was not significant ($P = 0.69$). T_3 values were significantly different ($P < 0.0019$) in high PBA line (3.01 ± 0.05 ng/ml) compared to the low PBA line (2.80 ± 0.05 ng/ml). T_4 values between the high PBA line (11.25 ± 0.18 ng/ml) and the low PBA line (10.90 ± 0.19 ng/ml) was not significantly ($P < 0.1894$) different. The TR (T_3/T_4) value was not significantly ($P < 0.3204$) differences between the high PBA line and the low PBA line. Insulin levels were higher in high PBA line (1265.60 ± 31.60 ng/ml) compared to the low

PBA line (1215.33 ± 33.12 ng/ml), however, the difference was not significant ($P < 0.2727$). There was nearly significant difference ($P < 0.0557$) between glucagon levels in the high PBA line (184.65 ± 7.50 pg/ml) compared to the low PBA line (205.30 ± 7.72 pg/ml).

Discussion

The means, standard deviation of hormones in high and low PBA lines are given in Table 1 and Figures 3.1-3.8. The divergent selection for PBA has resulted in a high IGF-I levels in the high PBA line compared to the low PBA line. Even though there is no direct evidence to demonstrate that the selection program caused an increase in endogenous phytase, it can be assumed within reason that the increase in PBA in the high line may be due to a concomitant increase in endogenous phytase and consequent increase in renal tubular reabsorption of P. IGF I significantly increase renal tubular reabsorption of inorganic phosphorus (Caverzasio et al., 1990; Laroche and Boyer, 2005). IGF I can increase phosphorus absorption through an apical as well as basolateral tubule receptors in kidney (Hirschberg et al., 1995). It has been shown that, there is a strong positive association between phosphorus intake with plasma IGF I levels (Giovannucci et al., 2003). Kanatani et al. (2002) suggest that high phosphorus concentration stimulates DNA synthesis through increased secretion of IGF I. Both GH and IGF-I increase glomerus filteration rate (Tanaka et al., 1994) and production of 1,25-$(OH)_2D_3$ *in vivo*. Additionally IGF-I has been shown to stimulates the production of 1,25-$(OH)_2D_3$ by kidney cells in vitro (Condamine et al., 1994). In rats raised on P deficient diets, Barnard et al. (1988) demonstrated that IGF-I restored the increase in serum 1,25-(OH)2D

induced by restriction of dietary P. Therefore, the high IGF-I level in the high PBA line could be due to high plasma P levels. Also, the high IGF-I level in the high line potentially increase tubular P transport and 1,25-$(OH)_2D_3$ production rate. Therefore IGF-I might play an important role in the control of P economy.

The leptin level was not different among the high and low PBA lines (Table 3.1, Figure 3.5). The existence of the chicken leptin gene has been controversial. Taouis et al. (1998) reported on the existence of the chicken leptin genes, however, there has not been any independent confirmation of the chicken leptin gene (Carre et al., 2006), even though the leptin receptor gene has been identified. The leptin levels reported in this study is the recombinant human leptin, and not the chicken leptin. McMurtry et al. (2003) observed that chicks fed low-phosphorus diets had elevated plasma leptin levels. Several studies have reported increase in the leptin secretion by human adipocyte (Kolaczynski et al., 1996), chicken liver (Ashwell et al., 1999) and human plasma (Boden et al., 1997) levels with prolonged hyperinsulinemia. However, in this study, divergent selection for PBA did not affect leptin level.

Glucagon level was higher in the low PBA line compared to the high PBA lines. The glycogen levels were 184.65 pg/ml and 205.30 pg/ml for the high and low PBA lines, respectively. Glucagon has been reported to stimulate calcitonin release and induces hypophosphatemia (Srivastav and Swarup,1983). Talmage et al.(1974) have reported that calcitonin lowers the serum calcium by preventing release of calcium by bones whereas it lowers serum phosphate by increasing its exit from circulation rather than by inhibiting its release from bones. Therefore, the high glucagon level and low IGF-I level could explain the low PBA in the low lines. Insulin level was expected to be

higher in the high PBA line compared to the low PBA line. Even though the high PBA line had a higher insulin level compared to the low PBA line, the difference was not significant. Insulin may not have any substantial role in P metabolism. In an earlier studies, Koldziejska et al. (1926) have reported that insulin play an insignificant role in total phosphorus metabolism in the blood.

The thyroid glad secrets T_3, T_4 and calcitonin, that affect numerous metabolic activities including calcium-phosphorus interactions. It is therefore possible that hormones of the thyroid glad may affect phyate phosphorus bioavailability either directly or indirectly. We observed a significantly higher T_3 (3.01 ± 0.04 ng/ml) levels in high PBA line as compared to low PBA (2.79 ± 0.04 ng/ml) line. Several studies have demonstrated the varying role of thyroid hormones upon phosphorus metabolism. Thyroid hormone enhances the action of calcitriol (1,25 dihydroxycholecalciferol) in the small intestine (Cross and Peterlik, 1991) and calcitriol increase phosphorus absorption form the intestinal tract through the stimulation of secondary active, sodium-coupled P-i-co-transport system in small intestine (Schroder et al., 1996). Parmer et al. (1987) observed that T_3 levels were lower in chicks fed a P deficient diet compared to controls. Severe P deficiency affects thyroid hormone function by decreasing serum T_3 levels. The depression of T_3 may be caused by an impairment in peripheral conversion of T_4 to T_3. Enzymes that deiodinate thyroxine are found both liver and kidney (Yamazaki and Slingerland, 1959). As kidney was enlarged by the P deficiency (Parmer et al., 1987), it is possible that damage to the kidney may have impaired the deiodination mechanism in this organ. Therefore, the inability to utilize phytate P may be responsible for the lower T_3 level in the low PBA line compared to the high PBA line. The ability of the high line

to better able to utilize phytate P may have an effect on the conversion of T_4 into T_3, hence the higher levels of T_3 in the high line compared to the low line.

It appears that divergent selection for PBA may be responsible for the correlated responses in IGF-I, glucagon and thyroid hormones. The high IGF-I, T_3 and low glucagon levels in the high PBA may be the reason for the line's ability to utilize phytate P or the selection regime has change an array of genes in both the thyroid and GH pathways. It can therefore be concluded that the physiological state of a growing bird can be used to indirectly predict its ability to utilize its ability to utilize phyate phosphorus.

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Table 3.1: Means and standard errors of insulin like growth factor I (IGF I), insulin like growth factor II (IGF II), leptin, thyronine (T_3), triidothyrionine (T_4), T_3/T_4 ratio (TR), insulin and glucagon hormones in high and low phytate phosphorus bioavailability (PBA) lines

Hormone	High PBA Line N=299	Low PBA line N=274	Pr > F
IGF I (ng/ml)	43.06 ± 0.57	39.26 ± 0.60	0.0001
IGF II (ng/ml)	40.57 ± 1.02	44.23 ± 1.07	0.0133
Leptin (ng/ml)	2.93 ± 0.10	2.86 ± 0.11	0.6913
T_3 (ng/ml)	3.01 ± 0.05	2.80 ± 0.05	0.0019
T_4 (ng/ml)	11.25 ± 0.18	10.90 ± 0.19	0.1894
TR (ng/ml)	0.28 ± 0.00	0.28 ± 0.00	0.3204
Insulin (ng/ml)	1265.60 ± 31.60	1215.33 ± 33.12	0.2727
Glucagon (pg/ml)	184.65 ± 7.50	205.30 ± 7.72	0.0557
PBA	30.76 ± 0.20	28.20± 0.20	0.0034

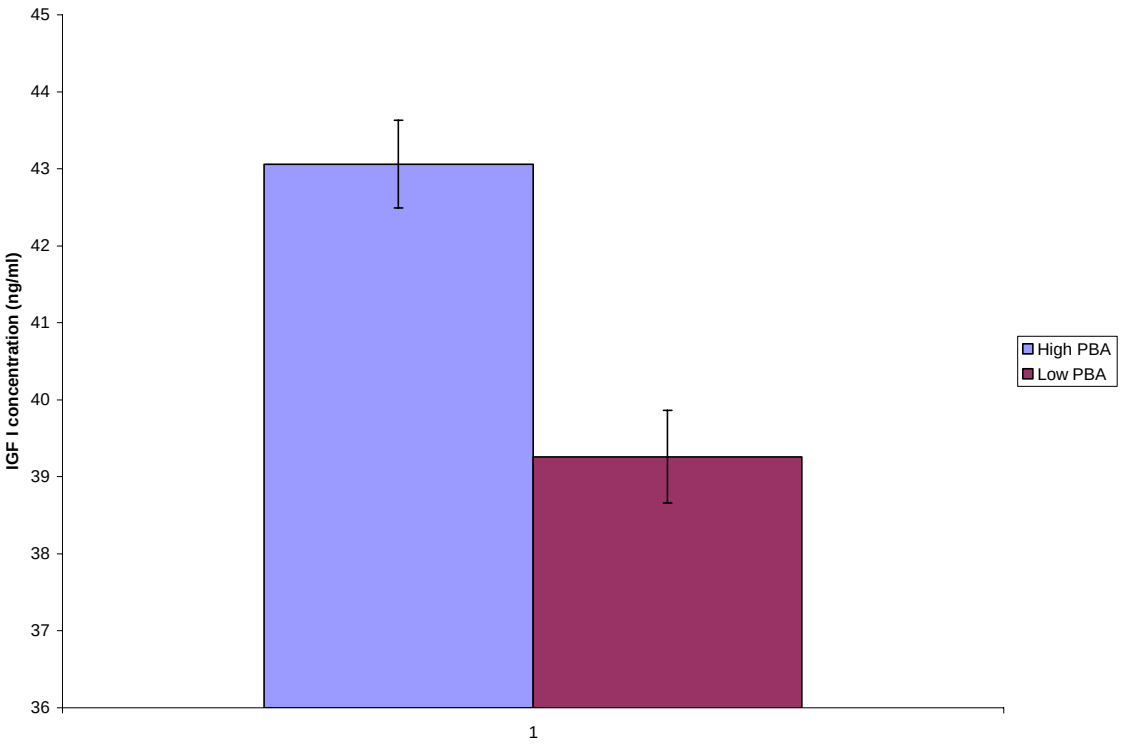

Figure 3.1: Insulin like growth factor I (IGF I) concentration (ng/ml ± S E) in high and low phytate Phosphorus bioavailability (PBA) lines

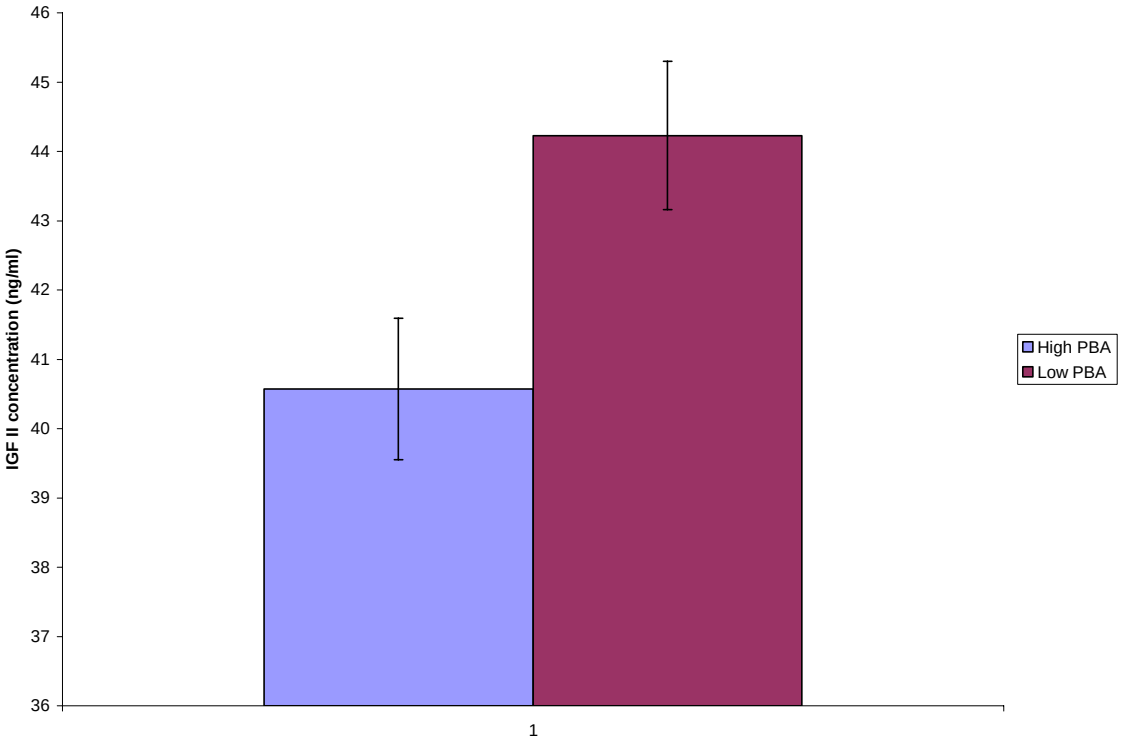

Figure 3.2: Insulin like growth factor II (IGF II) concentration (ng/ml ± S E) in high and low phytate Phosphorus bioavailability (PBA) lines

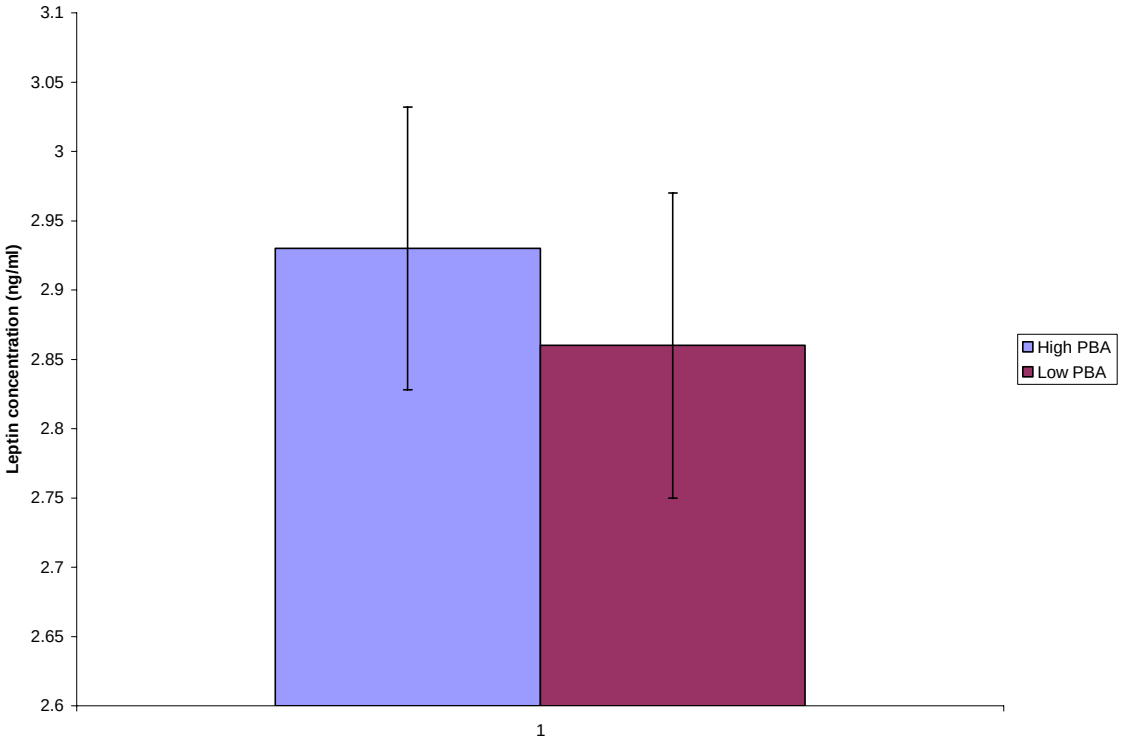

Figure 3.3: Leptin concentration (ng/ml ± S E) in high and low phytate phosphorus bioavailability(PBA) lines

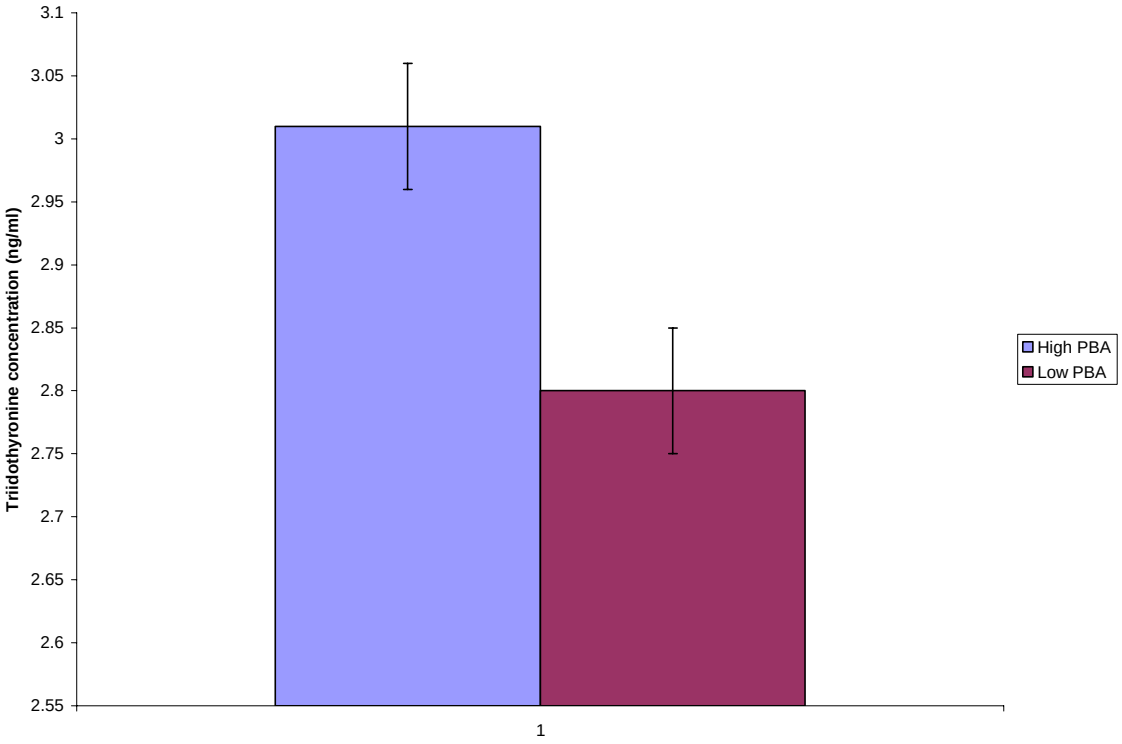

Figure 3.4: Triidothyronine (T_3) concentration (ng/ml ± S E) in high and low phytate phosphorus bioavailability (PBA) lines

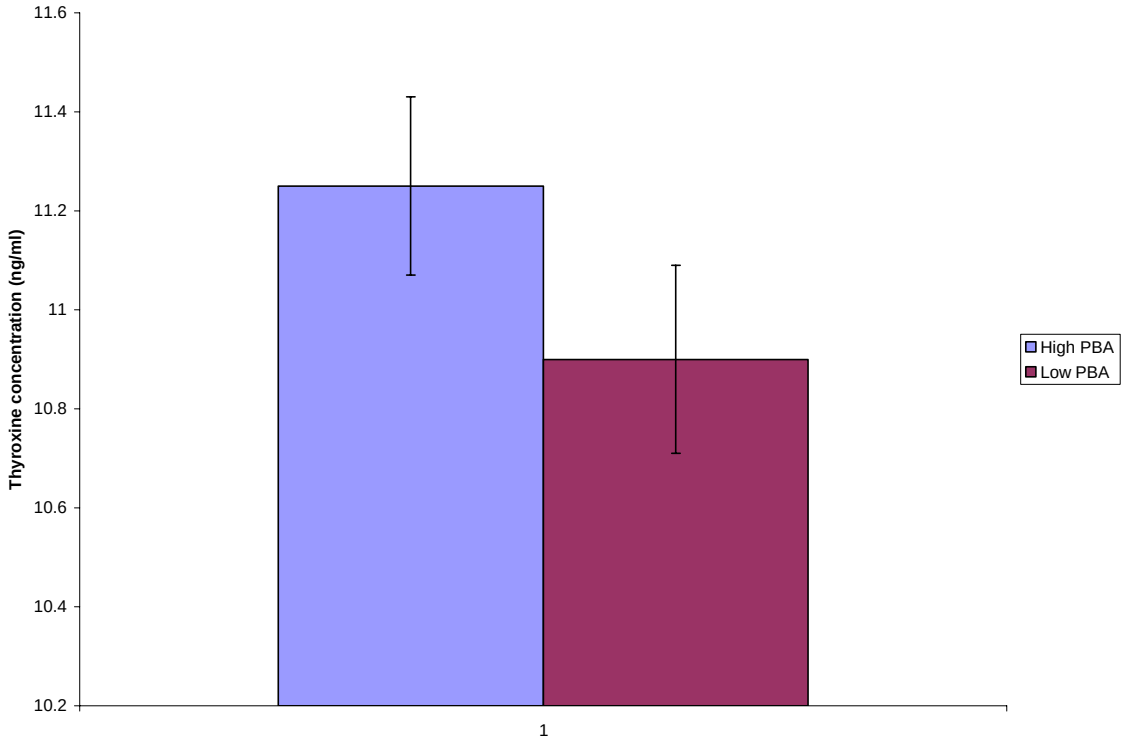

Figure 3.5: Thyroxine (T_4) concentration (ng/ml ± S E) in high and low phytate phosphorus bioavailability (PBA) lines

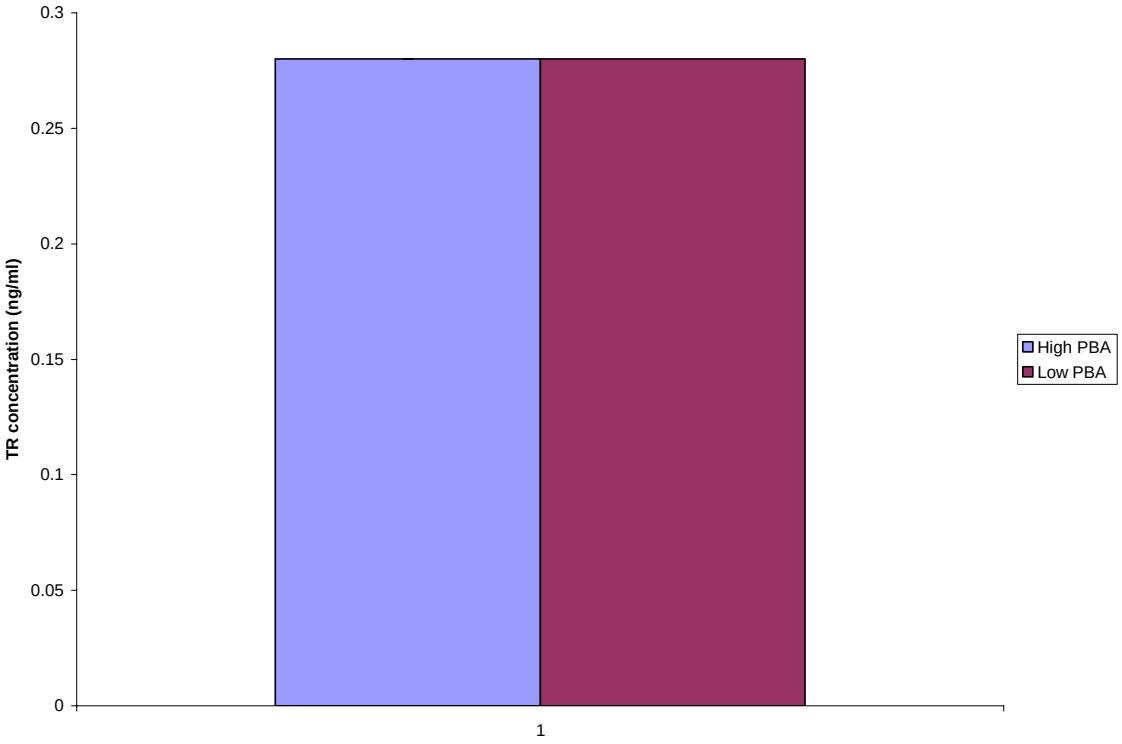

Figure 3.6: TR[Triidothyronine (T_3)/ Thyroxine (T_4)] concentration (ng/ml ± S E) in high and low phytate phosphorus bioavailability (PBA) lines

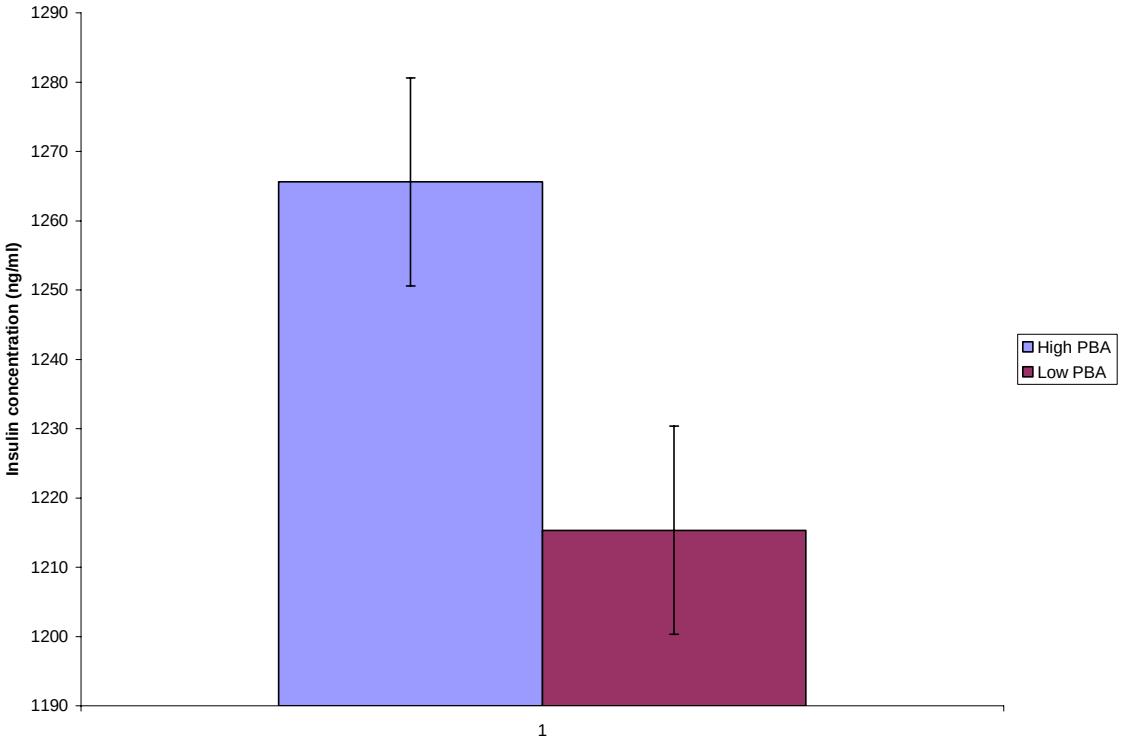

Figure 3.7: Insulin concentration (ng/ml ± S E) in high and low phytate phosphorus bioavailability(PBA) lines

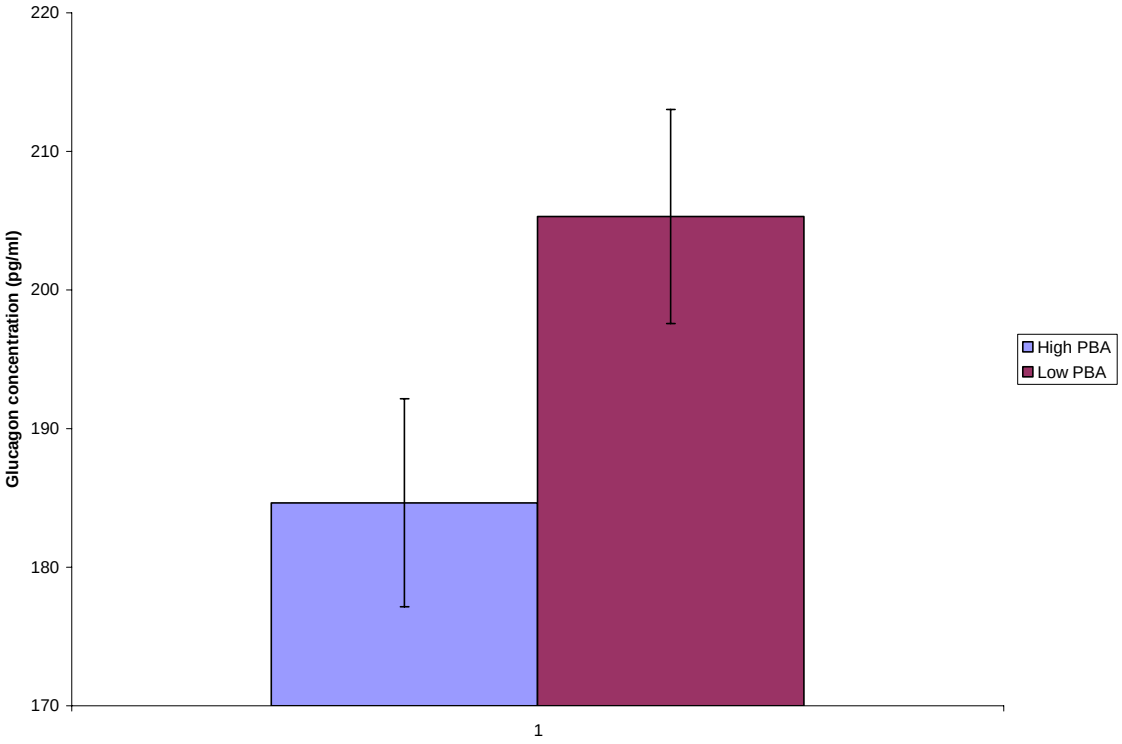

Figure 3.8: Glucagon concentration (pg/ml ± SE) in high and low phytate phosphorus bioavailability(PBA) lines

CHAPTER 4

HERITABILITY ESTIMATES OF PHYSIOLOGAL PARAMETERS IN A DIVERGENT CHICKEN POPULATION SELECTED FOR PHYTATE PHOSPHORUS BIOAVAILABILITY

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Abstract

A study was conducted to examine heritability estimates of various hormones effecting phyate phosphorus bioavailability (PBA) in divergently selected chicken lines. The heritability of PBA was very low in both high (0.07) and low (0.09) PBA lines. IGF I and glucagons was moderately heritable in both the lines, with a heritability of 0.27 in high line and 0.39 in low line for IGF I, and 0.21 in high line and 0.36 in low line for glucagon. This study suggested that IGF I, T_3, T_4 and glucagon appear to have some association with phytate phosphorus bioavailability and IGF I can be used as a biomarker to select the birds for PBA.

Introduction

Phosphorus is an important mineral required in poultry diets for normal growth and development. It plays an important role in the metabolism of carbohydrates, amino acids, lipids and energy. Poultry diets are made primarily of ingredients of plant origin, including cereal grains, cereal by-products and oil seed meals. The main sources of phosphorus in plant tissues are phosphates, phytic acid and other myo-inositol phosphates (Sebastian et al., 1996). Phytates are largely unavailable to monogastrics because they lack significant sources of intestinal phytase (Nelson, 1976; Kornegay, 1996). Phytase makes phosphorus available by removing phosphorus groups from the inositol hexaphosphate molecule (Sandberg et al., 1993) and release phosphorus for absorption and utilization. The inability of monogastrics to utilize phytate phosphorus results in a substantial loss of nutrient efficiency and creates a significant pollution threat when manure containing residual phosphorus is applied to land. The addition of microbial phytase to the diet has been found to improve phytase phosphorus utilization (Kornegay,

1996). The efficacy of microbial phytase however, is influenced by several factors including the amount of vitamin D present (Mohammed et al., 1991; Edwards, 1993), calcium to total P (Ca: tP) ratio (Liu et al., 1998), calcium bioavailability and inorganic P supplements, age of the bird (Kornegay, 1996), size of the bird (Punna and Roland, 1999) and the level of phytase in the diet (Shirley et al., 2003).

There is evidence to suggest that genetic variation exists in populations for phytate phosphorus utilization, and it has been shown to range from zero (Nelson, 1967) to over 50% (Edwards, 1983), depending on the genetic strain of bird (Edwards, 1983), age of the bird, ingredient type, dietary levels of calcium and inorganic phosphorus (Ravindran et al., 1995), and vitamin D (Edwards, 1993). Punna and Roland (1999) demonstrated that variation in phytate phosphorus utilization in the same strain of chickens is related to growth, livability and skeletal strength. Recently Zhang et al. (2003) also evaluated the potential of genetically improving poultry to utilize phytate phosphorus.

Since the utilization of phytate phosphorus is affected by calcium, vitamin D, size of the bird, variability in any of these factors would invariably affect phytate phosphorus utilization. Several studies have shown the involvement of hormones in the growth hormone and thyroid pathways in calcium and phosphorus homeostasis (Beisel et al., 1958; Quigley and Baum, 1991; Kanatani et al., 2002; Laroche and Boyer, 2005). We have established the correlated response of IGF-I, IGF-II, glucagon, insulin, leptin, T_3 and T_4 to divergent selection of phytate P bioavailability in a random mating population (Chapter 3; this thesis).

The objective of this study was to ascertain the genetic inter-relationship among IGF-I, IGF-II, glucagon, insulin, leptin, T_3 and T_4 in a random mating population divergently selected for phytate phosphorus bioavailability.

Materials and Methods

Data editing and Statistical Analysis

Data editing were done by obtaining descriptive statistics using the PROC MEANS and PROC UNIVARIATE methods (SAS Institute, 1998). Data that deviated more than 3 standard deviations from the mean were identified as outliers and removed from the data set. After data editing, 919 individuals (476 males and 443 females) with complete data with complete records of all the traits measured from 26 sires and 71 dams, and 105 (44 males and 61 females) grandparents were used for estimation of genetic parameters. Mixed animal models (Mrode, 1996) and restricted maximum likelihood (REML) (Henderson, 1985) methods were used for estimating the variance components of the traits measured. The animal model used was:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Zu} + \mathbf{e}$$

$$\text{with } \text{var}(\mathbf{u}) = \mathbf{A} \otimes \mathbf{G} \text{ and } \text{var}(\mathbf{e}) = \mathbf{I} \otimes \mathbf{R}$$

where, **y** is the vector of phenotypic observations, **X** the matrix that relates fixed effects to phenotypic records, **Z** the matrix that relates animals to phenotypic records, **β** the vector of sex and hatch fixed effects, u the vector of random animal effects, **e** the vector of residual effects, **A** the additive relationship matrix, **G** the (co)variance matrix for genetic effects, I the identity matrix, and **R** the (co)variance matrix for residual effects.

Heritability estimates, and genetic and phenotypic correlations were estimated for body weight at 4 weeks, relative growth, phytate phosphorus-, calcium-, nitrogen- and energy bioavailabilities. A multivariate model was used to estimate the genetic and phenotypic

correlations. Pedigree information of the parents was utilized and the formation of the inverse of the A- matrix (A^{-1}) was based on Henderson (1975) and Quaas (1976) methods. The estimations of variance components were accomplished with the average information algorithm for REML (Johnson and Thompson, 1995). Convergence was considered to have been reached when

$$(\hat{\theta}_t - \hat{\theta}_{t-1})'(\hat{\theta}_t - \hat{\theta}) / \hat{\theta}_t{}'\hat{\theta}_t < 5 \times 10^{-11}$$

where $\hat{\theta}_t$ is the vector of estimated parameters in the t^{th} iteration.

Results

Results from the Chapter 3 (this thesis) clearly indicate different among the different lines with respect to the hormones determined. Therefore genetic parameters were determined within lines. The heritability estimates of hormones in high and low PBA lines are presented in Table 4.1. The heritability of phytate phosphorus in high line was 0.07 and in the low line was 0.09. In the high line T_3 had the highest heritability of 0.31. However, in low PBA line its value was 0.00. In high PBA line heritability of leptin, insulin and IGF II were 0.00 while in low PBA line their values were 0.08, 0.16 and 0.01, respectively. IGFI had the heritability of 0.27 in high line and 0.39 in low PBA line. Heritability of T_4 was 0.21 and 0.10 in high and low PBA lines, respectively. The phenotypic correlations among the hormones and phytate P for the high and low PBA lines are given in Tables 4.2 and 4.3, respectively. The phenotypic correlation between PBA and T_3 in high PBA line was 0.08, however it was not significant. Phenotypic correlation between PBA and IGF I, IGF II, insulin, glucagon and T_4 were low and negative. The phenotypic correlation between PBA and T_4 was significant in high line. In

low line phenotypic correlation between PBA and IGF I, IGF II, T_3, T_4, was low and negative and between PBA and insulin, glucagons and leptin was positive. Correlation between PBA and T_3 was significant in low line.

Discussion

The heritabilities of various hormones measured in high and low PBA lines are shown in Table 4.1. The heritability of PBA was 0.07 in low line and 0.09 in high line. It indicates that it is difficult to improve this trait based upon mass selection. However, there could be other physiological indicators that could assist in pre-selecting birds for PBA. Utilization of phytate phosphorus is dependent on various factors such a bioavailability of calcium, energy and nitrogen and inorganic phosphorus intake (Ankra-Badu et al., 2004), therefore change in any of the factor can be the reason for low heritability. Additionally sources of error in estimating PBA was high and that could have affected the heritability estimate. This means that genetic parameter used here are only applicable under similar conditions of nutritional environments. Heritability of IGF I is lower (0.27) in high PBA line and higher (0.39) in low PBA line. As shown in the previous chapter we observed significant difference ($P < 0.001$) in mean level of IGF I in high and low PBA line. In both lines IGFI was moderately heritable. Since IGF I significantly increase renal tubular reabsorption of phosphorus (Caverzasio et al., 1990; Laroche and Boyer, 2005) and a strong positive association exit between phosphorus intake and plasma IGF I levels in young and adult humans (Giovannucci et al., 2003; Rogers et al., 2005). Kanatani et al. (2002), plasma levels of IGFI can be used as a biomarker for PBA.

Though we observed significant difference ($P < 0.0019$) in the mean level of T_3 in high and low line but the heritability estimates show that T_3 is only heritable ($h^2 = 0.31$) in high PBA line and not in low PBA line ($h^2 = 0.00$). This implies that thyroid metabolism is different in the two lines. They may be different array of genes responding to the selection criteria in the high and the low lines. Hence the differences in heritability estimate of T_3. The positive correlation between T_3 and PBA in high PBA line can be due to the stimulation of phosphorus absorption by T_3. Beisel et al. (1958) observed increase in serum phosphorus when a single does of T_3 was administered to normal dogs. However, this correlation is weak in the high line (r=0.08). Thyroid hormones are also reported to increase phosphorus absorption in intestine (Cross and Peterlik, 1991) and kidney (Yisufi et al., 1985). However, we observed significant ($P < 0.001$) negative correlation between levels of T_4 and PBA in high line. This can be due to conversion of T_4 into an active metabolite T_3 outside the thyroid gland (Merryman, 1998) and T_3, in turn stimulate phosphorus absorption. This is further confirmed as we observed a significant ($P < 0.0365$) positive correlation (0.1231) between the levels of T_3 and T_4 in high PBA line. However, in low line the stimulatory effect of T_3 on phosphorus absorption is counteracted by a significant ($P < 0.0008$) negative correlation (-0.2060) between the levels of T_3 and PBA. The significant positive correlation between T_3 and T_4 in low line indicates that extra thyroidal conversion of T_4 to T_3 in low PBA line further support the low PBA in low line. We observed a positive and significant correlation between the levels of T_3 and insulin in high line. These results are in consistent with Blum et al. (1977) as they observed increase of levels of blood T_3 and T_4 when insulin was administered in normal and thyroidectomized dogs. High values of insulin can cause

increase phosphorus absorption by stimulating T_3. However, in low PBA significant negative correlation between T_4 and insulin was observed. This might be due to the different in physiology effect of hormones in two lines. Glucagon has a heritability of 0.21 and 0.36 in high and low line, respectively and there is nearly significant ($P < 0.055$) difference between the mean values of glucagon in high and low PBA lines.

In this study we estimated genetic parameters for physiological traits in a population divergently selected for phytate phosphorus bioavailabilty. Among the hormones studied, IGFI, T_3, T_4 and Glucagon appear to have some association with PBA. Several studies have reported that IGF I increases the absorption of inorganic phosphorus (Caverzasio et al., 1990; Laroche and Boyer, 2005). We observed a significant negative correlation between IGF I and PBA in low line indicating that the positive effect of IGF I on phosphorus absorption is apposed by negative correlation in low line. The significant negative correlation between T_3 and IGF I in both high and low PBA lines can be due to the suppression of growth hormone secretion when chicks were exposed to T_3 (Harvey, 1990). Heritability estimate of zero for some of the hormones made it statistically impossible to estimate genetic correlations, since the additive variance of the hormone is needed in the estimation. The sample size for estimation is also small and should be considered in the interpretation of the results. Nevertheless, this study provides us with the first insight into the physiological changes in a chicken population divergently selected for phytate phosphorus bioavailability.

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Table 4.1: Heritability Estimates (h^2) of insulin like growth factor I (IGF I), insulin like growth factor II (IGF II), insulin, glucagon, leptin, triidotherionine (T_3), thyroxine (T_4), phytate phosphorus bioavailability (PBA) in divergently selected high and low PBA lines

Hormone	High PBA Line h^2	Low PBA Line h^2
IGF I	0.27	0.39
IGF II	0.00	0.01
Insulin	0.00	0.16
Glucagon	0.21	0.36
Leptin	0.00	0.08
T_3	0.31	0.00
T_4	0.21	0.10
PBA	0.07	0.09

Table 4.2: Correlation Coefficient and Probabilities of phytate phosphorus bioavailability (PBA), insulin like growth factor I (IGF I), insulin like growth factor II (IGF II), insulin, glucagons, leptin, triidothyronine (T_3), and thyroxine (T_4) in high PBA line

Hormones	IGF I	IGF II	Insulin	Glucagon	Leptin	T_3	T_4
PBA	-0.0211	-0.0197	-0.0652	-0.0398	-0.0399	0.0759	-0.2479
	0.7209	0.7387	0.2693	0.5226	0.4998	0.1983	<0.0001
IGF I		-0.1592	-0.0332	0.0983	0.0570	-0.1672	0.1764
		0.0067	0.5739	0.1139	0.3347	0.0053	0.0026
IGF II			-0.0701	0.1966	0.1472	-0.1220	0.0066
			0.2347	0.0012	0.0122	0.0382	0.9112
Insulin				0.0642	-0.2209	0.1187	-0.0150
				0.3024	0.0002	0.0438	0.8002
Glucagon					-0.0541	-0.1465	0.0457
					0.3853	0.0181	0.4631
Leptin						-0.2258	0.2000
						0.0001	0.0006
T_3							0.1231
							0.0365

Table 4.3: Correlation Coefficient and Probabilities of phytate phosphorus bioavailability (PBA), insulin like growth factor I (IGF I), insulin like growth factor II (IGF II), insulin, glucagons, leptin, triidothyronine (T_3), and thyroxine (T_4) in low PBA line

Hormones	IGF I	IGF II	Insulin	Glucagon	Leptin	T_3	T_4
PBA	-0.1210	-0.0430	0.0258	0.0652	0.0491	- 0.2060	-0.0177
	0.0495	0.4871	0.6460	0.3089	0.4979	0.0008	0.7748
IGF I		-0.1923	0.0360	-0.2977	0.1947	-0.2403	0.1411
		0.0017	0.5606	<0.0001	0.0015	<0.0001	0.0218
IGF II			-0.0798	0.0069	-0.0466	0.2727	0.4081
			0.1969	0.9142	0.4508	<0.0001	<0.0001
Insulin				-0.1848	-0.2271	0.0768	-0.2325
				0.0038	0.0002	0.2143	0.0001
Glucagon					-0.0222	-0.1516	0.1291
					0.7297	0.0176	0.0435
Leptin						-0.1952	0.1912
						0.0014	0.0018
T_3							0.2620
							<0.0001

CHAPTER 5

GENERAL CONCLUSIONS

A study was conducted to study the hormonal dynamics in a chicken population divergently selected for phytate phosphorus bioavailability (PBA). Phosphorus is an essential mineral required for normal growth and development. However, most poultry diets contain phosphorus in its phytate form which is unavailable to chicken because it lacks adequate quantities of the endogenous enzyme, phytases which is required to hydrolyze phyate phosphorus. Excessive loss of phosphorus (P) in the excreta leads to environmental pollution. From the hormonal dynamics study, it was established that, Insulin like growth factor I (IGF I) and triidothyronine (T_3) levels were higher in the high PBA line compared to the low PBA line. On the other hand, glucagon level was higher in the low PBA line compared to the high PBA line. Leptin levels were not different in the two lines.

Increased level of IGF-I and T_3 increase P resborption in the kidney, and elevated levels of IGF-I and T_3 may be responsible for the increase PBA in the high line. On the other hand high levels glucagon in the low line may lead to increase calcitonin level which has a negative effect on both calcium and phosphorus bioavailability.

Both IGF-I and glucagon were moderately heritable in both the high and low PBA lines, whereas, T_3 was heritable only in the high PBA line. The lack of additive genetic variance in some of the hormones made it statistically impossible to estimate genetic

correlations among the traits. Nevertheless, this study provides us some insight into the physiological state of chicken when selected for high or low phytate phosphorus bioavailability.

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