GENES IN THE IGF PATHWAY AND THEIR ASSOCIATION WITH FEED EFFICIENCY

by

AARTI PRAKASH SANGLIKAR

(Under the Direction of SAMUEL E.AGGREY)

ABSTRACT

A study was conducted to examine the molecular basis of feed utilization efficiency. Feed costs constitute about 70% of the production cost. Improvement in feed efficiency will produce efficient birds, increase profitability and reduce manure output. From a global gene expression study using microarray methodology, it was established that genes in the Insulin like growth factor (IGF) pathway including IGF binding protein 5 (IGFBP5), platelet-derived growth factor B (PDGFB), interferon regulatory factor 1 (IRF1), melanocortin 5 receptor (MC5R) and tumor necrosis factor receptor (TNF) super family (TNFSF1B) affected feed efficiency. In a divergent line selected for phytate phosphorus bioavailability, insulin was found to be associated with feed consumption and feed conversion ratio in both lines. These genes can be used in further development of genetic markers which can be used in marker assisted selection.

INDEX WORDS: IGF pathway, Feed Efficiency
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by

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DEDICATION

I offer my dedication to my God who has given me all the strength to pursue my goal. To my Dad, Mom, sisters Namita and Minal, Shirish, Little Mrinmayee and of course my adorable dogs who have been a continuous source of inspiration to me. And, to wonderful and loving Obi, my boyfriend, who has read my thesis more than me and has given me fathomless support and love. I hope to someday find a way to give as unselfishly and unconditionally as you.
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CHAPTER 1
INTRODUCTION

Insulin-like Growth Factors (IGFs) consist of a family of polypeptide hormones structurally associated with insulin with multiple metabolic and anabolic functions (McMurtry et al., 1997). Positive and negative stimulation to the hypothalamus by growth hormone releasing hormone (GHRH) and somatostatin respectively, leads to the pulsatile secretion of Growth hormone (GH) by the anterior pituitary into the circulatory system. GH is known to have direct and indirect effects on the body (Nicol et al., 1986). Direct effects include the action of GH on the adipocytes. Growth hormone helps in the breakdown of adipocytes into triglycerides which lowers the circulating lipid molecules. Though called as indirect effects, they are some of the most vital effects for normal functioning of the cells and thus the entire physiology of the animal. Growth Hormone binds with the growth hormone receptors (GHR) which are present on the hepatocytes and non-parenchymal cells such as hepatocyte stellate cells (HSCs) in the liver. This activates the GHR and prompts these cells for the production of IGF-1.

Besides liver many other organs like kidney, brain, skin and gonads also produce IGFs (Han et al., 1987). This leads to the activation of their secretory mechanism and IGF is produced. Insulin-like growth factors are also called as somatomedin (mediator of growth) (Marquart et al., 1981). Circulating half lives of IGFs are very short and thus IGF binding proteins (IGFBPs) are responsible for longer half-life of IGFs in the circulation. These proteins are secreted by various tissues in the body and found in the biological fluids (Ferry et al., 1999). There are six IGFBPs found in the circulation. Their concentration in the plasma is nutrition dependent (Kita et al., 1996). It improves the half-life from a few minutes up to 15 hours (Daughaday et al., 1989). IGFBPs can inhibit or potentiate IGF actions (Allan et al., 2004). Any mutation, truncation or modification in the N- or the C- termini of IGF binding site leads to altered
biological activity of IGF (Brinkman et al., 1991). Insulin-like growth factor is carried in complex with IGFBP and acid labile subunit (ALS) and binds to the IGF receptors (IGF-1R) (LeRoith et al., 2001; Figure 3). These receptors are present on almost every cell in the body except for liver cells and adipose tissues.

Upon binding with IGF, the IGF-1 receptor (IGF-1R) initiates the signaling cascade which incorporates proliferation, differentiation, cell survival and many other vital metabolic effects. Insulin-like growth factor-I is important in post-natal development and IGF-II is vital in pre-natal development (Kikuchi et al., 1991). High IGF-1 levels in genetically selected chickens have shown to be directly related to high growth rate (Froesch et al., 1985). Insulin-like growth factor has its positive effect on glucose metabolism, organ homeostasis and immune and nervous system (Powell-Braxton et al., 1993). It has been shown that the most prominent effects of IGFs are on the muscle and bone cell cartilage growth and nutrient partitioning (Hart et al., 1987). In poultry, IGFs affect proliferation, differentiation and metabolism of myogenic cell lines (Schmid et al., 1991). IGF-1 reduces protein degradation and improves protein synthesis (Duclos et al., 1999), governs differentiation of mesodermal cells (Schmid et al., 1991) and controls total bone density and total bone mass including strength also. Other than mediating GH effects, IGFs are responsible for delayed apoptosis during normal development and also in stressed or diseased condition (Vincent et al., 2002).

According to Yakar and associates (2001), IGF-1 maintains a balance between GH and insulin. These effects are primarily responsible for the growth and development. Variation in IGF gene can lead to growth disorders in relation with their feed intake and their body composition, skeleton integrity and metabolic traits (Zhou et al., 2004). Insulin-like growth factor-1 has a very strong effect on metabolic diseases like diabetes disorders, reproductive, immune and bone cartilage systems. In Layers, IGF-1 has shown to have a great effect on egg production (McMurtry et al., 1997). In chickens, carcass fat content is increased by IGF-II (Spencer et al., 1996) and decreased by IGF-1 (Huybrechts et al., 1992).

Due to the effects of IGF-1 on the average daily gain and feed efficiency, it has been selected as the biological candidate gene to investigate growth, body composition, metabolic, and skeletal traits in
chickens (Amills et al., 2003; Zhou et al., 2004). In the fasting state IGF-1 levels were lower than in the feed state. Thus it was concluded that IGF-1 is responsible for alteration in protein metabolism and energy expenditure. Variation in plasma IGF-1 levels is mainly due to dietary manipulation (Rosebrough and McMurtry, 1993). Experiments by Thissen (1994) showed that methionine deficiency leads to depressed IGF-1 levels. This condition prompted chickens to eat more. In these chickens calories were converted to fat and which subsequently replaced water in the body (Carew and Hill, 1961). A single injection of recombinant human IGF-1 (rhIGF-1) was given to 7 day old chicks (Conlon et al., 2002). The catabolic process showed improvement, net protein loss was reduced by 15% and thus it was indicated that rhIGF-1 infusion can be used to prevent unnecessary protein loss in animals as well as humans. Kim et al (2004) observed a positive correlation between serum IGF-1 with feed efficiency and suggested that IGF-1 directly promotes this trait in Korean Native Ogol Chicken. In experiment by Pym et al (1989) they used two groups of chickens to measure IGF-1 in fed (IGF-1_f) and in starved (IGF-1_s) condition. The goal was to determine the inheritance and genetic as well as phenotypic correlation between IGF-1 and body weight gain (BWG), feed consumption (FC), food conversion ratio (FCR) and abdominal fatness (AF). The results were indicative of a fairly large negative correlation estimates between IGF-1_f and all other performance trait mentioned above but the estimates for IGF-1_s were variable. The final result of studies by Pym suggests that a possible negative genetic correlation between IGF-1_f and the growth rate suggests that selection for increased growth rate might result in a decreased plasma IGF-1 concentration and thus the heritability ($h^2$) of IGF-1 is generally low in chicken and mice. According to Boyd and Bauman (1989), IGF-1 is responsible for improved feed efficiency however; these findings are contrary to the findings by Huybrechts et al (1992) who reported that there was no change in the feed efficiency in the chicken models they used for IGF-1 infusion.

The objective was to study genes and hormones in the IGF pathway and their association with feed related traits, growth and body composition in a random breed meat type chicken population.
CHAPTER 2

REVIEW OF LITERATURE

2.1 Growth Hormone (GH)

Growth Hormone (GH) is a 23 KDa protein expressed by the somatotroph cells of the anterior pituitary gland with pulsatile secretion (Baumann et al., 1991; Scanes et al., 1995; Theill et al., 1993; Figure 1). Its transcription 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., 1993; Arkins et al., 1993). Growth hormone is also known to have extrapituitary origin, for example, the brain, cells of immune system, 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 et al., 2004).

2.2 GH gene and protein structure

GH gene appears to be conserved across the species (Karin et al., 1990; Rousseau et al., 1992; Theill et al., 1993; Andersen et al., 1994; Lemaigre et al., 1989). It is 2 to 3 Kilobase pairs (Kbp) in size in mammals (Tang et al., 1993). The chicken growth hormone gene (cGH) is 3.5 to 4.5 Kbp comprising of 4,101 base pairs with five exons and four introns and this arrangement is maintained in the mammalian GH gene as well (Tanaka et al., 1992; Nei et al., 2005). The only difference between the mammalian and the avian GH gene is that the introns of the cGH gene are larger than that of mammals (Stumph et al.,...
1983). At the 5’ flanking region, there are two TATA boxes however, the proximal box is known to be the functional TATA box for cGH (Tanaka et al., 1992). In mammals, there are two transcription factors in the promoter region named ‘pituitary specific transcription factor’ or GHF-1 / Pit-1. These factors account for the pituitary specific GH expression (Bodner et al., 1988; Ingraham et al., 1988). Pit-1 is specific to pituitary cells that bound elements that were required for the pituitary specific expression of the GH and PR genes (Nelson et al., 1988). Pit-1 protein is found in only three cell types producing somatotrophs, lactotrophs and thyrotrophs (Simmons et al., 1990). The sequences of these promoter region are well conserved in mammals (Vize and Wells, 1987; Kioka et al., 1989). A section of the promoter region of the chicken cGH gene is highly homologous to the antisense strand of the rat GH gene which suggests that pituitary specific expression of cGH is regulated by factors similar to GHF-1/ Pit-1 in mammals (Tanaka et al., 1992).

2.3. Control of GH expression in Pituitary

Growth hormone regulation has been divided into three levels: Basal, cell-type specific and hormonally induced expression. In basal, which is a low-level regulation, the expression is controlled by several tissue-general factors which are capable of positive as well as negative regulation (Theill et al., 1993). In higher-level regulation (cell-type specific and hormonally induced) it is GHF-1/ PIT-1 that directs the expression and secretion. There are many other factors like environmental and other hormonal regulation that acts on the GH locus. Cyclic-adenosine mono phosphate (cAMP) levels are known to affect the proliferation of the pituitary cells and ultimately the GH synthesis (Harvey et al., 1995).

Positive Regulation of GH

1) **GHRH** (Growth Hormone-Releasing Hormone): GHRH is also referred to as growth hormone-releasing factor. GHRH increases intracellular cAMP levels, which activates the protein kinase A [PKA] (Brent et al., 1988; Dana et al., 1993). Cyclic adenosine mono phosphate levels are known to affect the proliferation of the pituitary cells positively as well as negatively (Harvey et al.,
In Rats, protein kinase C (PKC) activates the GH promoters but the mechanism is not well known (Gutierrez-Hartmann, 1994).

2) **TH** (Thyroid Hormone): Thyroidectomized rodents showed significant reduction in GH-mRNA expression, but upon exogenous administration, the levels were restored (Mulloy et al., 1992). GH expression induced by thyroid receptor (TR) is independent of PIT-1 as TR acts directly on the transcriptional factor to activate the GH expression as seen in in-vitro experiments (Suen et al., 1993). Whereas *in vivo*, PIT-1 and TR act synergistically (Schaufele et al., 1992).

3) **RA** (Retinoic Acid): it is a derivative of vitamin A and is very important element in the embryonic development, reproduction and growth of many cell types in vertebrates (Giguere et al., 1994). Specific RA receptors bind to GH promoter to induce the GH production (Bedo et al., 1989).

4) **GC** (Glucocorticoid): GH expression is elevated because of GC. In humans, there was a marked increase in the GH-mRNA levels and GC also showed to stabilize the GH-mRNA levels thus controlling the over expression of the hormone (Paek et al., 1987).

**Negative regulation of GH**

1) **Somatostatin**: A hypothalamic peptide, represses the GH expression from the anterior pituitary. It is not well documented if somatostatin is capable of inducing the direct negative actions on GH synthesis but it is believed to do so by altering the intracellular cAMP concentrations (Tuggle et al., 1996, Figure.1).

2) **Lack of PIT-1**: This is one of the major mechanisms that cause negative effect on the GH expression. It is achieved by the lack of expression of PIT-1 protein. PIT-1 is involved in GH activation and pituitary development (Tripputi et al., 1988).
2.4 Growth Hormone Receptor

Growth hormone Receptor (GHR) gets activated upon binding with GH and stimulates the growth and developmental processes in the body (Nicol et al., 1986). Endocrine hormone receptors are found on various cells and are responsible for the cell growth and differentiation. There is marked similarity observed in extracellular domain of the endocrine hormone receptors and the cytokine receptors. These receptors are interleukins, granulocyte and granulocyte-macrophage stimulating factors and erythropoietin (Cosman et al., 1990). The extracellular domain of human Growth hormone (hGH) has 246 residues and has seen in the form of a binding protein whose affinity for the GH is equivalent as the intact protein (Fuh et al., 1990). This may play an important role in the clearance of the hormone. One hGH molecule binds to two GHR molecules (Cunningham et al., 1991). De Vos et al (1992) raised the possibility that the binding surface of the receptor could reorganize itself to have the second set of hormone binding determinants. The extracellular part of the receptor has two domains (residue 1 to 123 and from 128 to 238). There are seven beta (β) strands in each domain and together they form a sandwich of two antiparallel β sheets one with four strands and one with three (Bazan et al., 1990). Once the complex is formed, the receptors are activated and leads to activation of liver cells for the further step that is the production IGF-1.

2.5 Insulin-like Growth Factors system

Insulin-like growth factors, IGFs are the circulating growth factors / polypeptides which belong to a family of soluble peptides known as somatomedins (mediators of growth) which makes them one of the most important factors required for growth (Marquardt et al., 1981). They are capable of performing various important functions like regeneration, metabolism and proliferation in a variety of cells. They are produced by a variety of cells such as liver, fat, muscle, kidney, brain, skin, gonads, heart (Han et al., 1987). There are two types of IGFs; IGF-1 and IGF-II. The IGFs are always tightly bound to one of six IGF Binding Proteins [IGFBPs] to ensure prolonged circulating half life (Daughaday et al., 1989; Jones et al., 1995; McMurtry et al., 1997). The IGFBPs also regulate interactions of IGFs with their respective
receptors (Flyvbjerg et al., 1995). Though IGFs mimic many actions of insulin, the latter is more potent in producing metabolic effects. This entire system in the body plays much more important role that just mediating GH effects. It has potent ability to prevent programmed cell death (apoptosis) during normal development and also during stress or disease (Vincent et al., 2002).

2.6. Insulin-like Growth Factor-I (IGF-1)

Many of the actions of GH have been mediated by IGF-1 (Froesch et al., 1985; Figure 1). Although GH production is pulsatile and episodic in nature (Johnson et al., 1987), plasma IGF-1 is stable and is independent of this nature. IGF-1 is a pleiotropic hormone reported to affect growth, glucose metabolism, organ homeostasis, and the immune and nervous systems (Powell-Braxton et al., 1993). Hart in 1987 observed that IGF-1 is imperative in cell division, cartilage growth and nutrient partitioning (Figure 1). IGF-1 has been identified as a 70-residues single chain peptide that is mostly produced in the liver of birds. Therefore, IGF-1 gene expression in liver tissues is detectable throughout the post-hatch growth (Mark et al., 2005). Hepatocytes and non-parenchymal cells such as hepatocyte stellate cells (HSCs) are IGF-1 producers in the liver. The circulating IGF-1 has minimal effect on hepatocytes as they express very few IGF-1 receptors (Sanz et al., 2005). The circulating IGF-1 increases with age (Kajimoto et al., 1991). IGF-1 plays a vital role in cellular growth by mediating many of the actions of GH (Kajimoto et al., 1991; Figure 1). A single-copy gene is transcribed and then processed into multiple mRNAs, where its size ranges from 1 to 7.5 kb, encoding at least two peptide precursors. As observed by Duclos and his team (2005), there is a positive relation between the IGF-1 mRNA and growth rate and it also determined the paracrine IGF-1 levels and post-hatch muscles growth. Studies with cDNA has confirmed the fact that the IGF-1 chicken gene is far more compact than its mammalian homologue (Daughaday et al., 1989). In the chromosomal DNA of 50 kb, there are 4 exons which are transcribed and processed into mRNAs of 1.9 to 2.6 kb (Kajimoto et al., 1989). Structural heterogeneity is observed at both the ends of the gene. On the IGF-1-mRNA there are two regions of polyadenylation mapped to exon-4 and separated by approximately 0.7 kb. At the 5′ end of the mRNA, many transcription initiation sites
were located within 74 nucleotide portion of the exon-1 which is a very highly conserved area in chicken and mammalian IGF-1 gene. In chickens selected for high or low growth rate show related high or low levels of IGF-1-mRNA (Beccavin et al., 2001; Beccavin et al., 2001).

The chicken IGF-1 (cIGF-1) gene sequence differs from human, bovine and porcine on at three locations. Such as Serine \textsuperscript{26} replacing Asparagine, Lysine \textsuperscript{41} replacing Threonine and Isoleucine \textsuperscript{64} replacing Leucine (Rinderknecht et al., 1978). Although infrequently, it is seen that Glutamine \textsuperscript{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 \textsuperscript{38} replacing Alanine, Histidine \textsuperscript{39} replacing Proline, Histidine \textsuperscript{40} replacing Glutamine, Proline \textsuperscript{67} replacing Alanine (Ballard et al., 1989). There are marked low concentration levels in the IGF-1 plasma levels on starvation in birds as well as in humans compared to their non-starved contemporaries. This suggests that the mechanism that carries out the IGF-1 regulation must be highly conserved in vertebrates (Van Wyk, 1984; Ballard et al., 1989). Yakar et al. (2002) showed that in order to obtain longer circulating half life, IGF-1 binds to IGFBP as well as acid labile subunit (ALS). ALS is required for IGFBP stability in circulation and also promotes their endocrine actions (Binoux et al., 1988). The biological activity of IGF-1 is regulated by ALS as it controls the passage of IGF-1 from the circulation into the extravascular chamber. Liver (hepatocytes) is the chief organ that produces ALS which is, GH dependent (LeRoith et al., 2001).

IGF-1 gene expression can be detected soon after fertilization through day 8 in chick embryos (DePablo et al., 1990), but the same is not detectable in liver until after hatching, suggesting that circulating IGF-1 is extra hepatic in origin in the developing chick embryo (Kikuchi et al., 1991). External stimulus of IGF-1 to the developing embryo exhibits multiple and diverse effects on the growth and development of the embryo (McMurtry et al., 1997). There is stimulation of amino acid and glucose uptake, increased DNA synthesis and protein synthesis and stimulation of tissues growth. This explains that the effects of IGF-1 are overall and very significant in all events of embryogenesis (McMurtry et al., 1998).
Liver IGF-1 was low throughout the embryonic development, but increased more than 8-folds by 3 weeks post hatch (Richard et al., 2005). On the contrary, IGF-1 expression in brains was higher than the liver expression throughout the embryonic development reaching its peak point at 3 weeks post hatch (Richard et al., 2005). Armstrong et al. (1989) observed that, in chickens, 70% of the total IGF-1 is bound to the binding protein (150 kd), 20-25% is bound to acid-stable (45 kd) protein and a very high percentage (6%) circulates as free or unbound. Chickens have been known to produce IGFs but before secreting them into the blood IGF-1 and IGF-II are purified (Dawe et al., 1988). Strains of chicken that are selected for high endogenous concentration of IGF-1, showed high growth rate (Froesch et al., 1985).

2.7. IGF-1 Receptor (IGF-1R)

The IGF-1 receptor (IGF-1R) is ubiquitous in most tissues. The IGF-1 and the insulin receptors are structurally similar molecules encoded by discrete genes (Armstrong et al., 1992). Because IGF-1 shows configurational homology with the tertiary structure of insulin, cross reactivity of IGF-1 with insulin receptors can occur (Darling et al., 1996; McMurtry et al., 1997; Figure 2). These receptors are members of tyrosine kinase (TK) family that includes the epidermal growth factor (EGF) and the platelet derived growth factor (PDGF). Their mode of action involves binding of polypeptide ligands to cell surface receptors that possess tyrosine kinase catalytic activity. Thereafter, the receptor tyrosine kinases (RTKs), which are transmembrane glycoproteins, are activated by binding to their cognate ligands. They carry the signal from the extra cellular by phosphorylating tyrosine residues on the receptors themselves (Stevan et al., 2000). RTK has numerous significant functions such as cell proliferation, differentiation, migration and metabolic changes (Schlessinger et al., 1992). However, they are different in a way that they occur in heterotetrameric structure and are predimerized; whereas, the other receptors of this family require the ligand binding for dimerization and activation. The process of dimerization causes structural changes in the receptor and makes it apt for the ligand binding (Steve et al., 2000). The structure shows two extra cellular alpha (α) chains disulfide-linked to two membrane-spanning β chains. The α chains are also disulfide-linked to one another, forming a α₂β₂ hetero-tetramer (Flanagan et al., 1998; Stevan et al.,
These regions in IGF-1 receptor are responsible for the IGF-1 binding whereas the ligand binding to the insulin receptors requires regions flanking the cysteine-rich domain (Ward et al., 2001).

### 2.8. Insulin-like Growth Factor-II (IGF-II)

Though mammalian IGF-1 and IGF-II proteins genes have been sequenced, the avian genomic sequence encoding for the IGF-II genes and its proteins has not been successfully identified. Mature chicken IGF-II protein has been purified and sequenced but the amino acid sequence of prepro-IGF-II is not known (Kallincos et al., 1990; Upton et al., 1995). Using IGF-II-cDNA and genomic clones, there have been successful isolation and characterization. IGF-II appears to be important during the embryonic development and growth while having little or no effect on differentiation and morphogenesis (DeChiara et al., 1990). Chicken IGF-II gene contains three coding exons and two introns. There is an open reading frame of 561 nucleotides with the capacity to encoded protein of 187 amino acids corresponding to prepro-IGF-II. Comparing the chicken IGF-II gene with the prepro-IGF-II of human and rat it provides enough evidence of 82% amino acid sequence homology. It also implies that chicken and mammalian IGF-II have the similar mitogenic properties (Upton et al., 1995). An intron of 5 Kbp has been identified to interrupt the chicken IGF-II-peptide-coding sequence. Similarly in human and rat IGF-II genes there is presence of an intron (varying between 600 to 1000 bp) at exactly the same position. However, the avian and the mammalian genes have undergone mutation in the core region that encodes the mature IGF-II peptide (Darling et al., 1996). The actions of IGF-II is mediated by type I receptors because the Mannose-6-phosphate receptor does not bind the IGF-II.

Richards et al. (2005), after working with avian IGF-II, showed that there is more that 95% homology between the sequences of the IGF-II turkey and the chicken IGF-II. He and his team, they showed that the turkey IGF-II gene codes for 187 amino acid precursor proteins. Primer directed reverse transcriptase polymerase chain reaction (RT-PCR) was used to obtain cDNA of 1300bp from IGF-II mRNA of turkey liver. IGF-II levels in blood are higher during prenatal life and decreases with age. IGF-II in layers birds participates in the progression that occurs in the theca-interstitial androgen producing
compartment (Giudice, 1992). It also determines the fate of the follicles (Armstrong and Hogg, 1996). The probable reason for the IGF-II being so ineffective for promoting growth could be its higher rate of clearance. Due to this there is less sustainability of IGF-II compared to IGF-1 (Tomas et al., 1998).

2.9. IGF-II receptor (IGF-IIR)

IGF-II-R is also called as Mannose-6-phosptate receptors (M-6-PR). It is different from the IGF-1 and insulin receptors because it has a long extra cellular domain consisting of numerous repeats of a short amino acid sequence, a transmembrane domain and a short cytoplasmic tail and weighs 250 kDa (Kasuga et al., 1981). The extra cellular domain contains the binding sites for the IGF-II and the Mannose-6-phosphate. Ligand binding at one site influences binding at the other site. Since this receptor contains the lysosomal enzymes it is considered to be important in controlling the IGF-II activity (D’Ercole et al., 1996). Detailed structure of IGF-II receptors has not been identified in chickens (Weiland et al., 1987). Type II receptors are absent in chick embryo (Bassas et al., 1988). However, IGF-II plasma concentration is similar to their mammalian counterpart (Czech et al., 1989). It was observed by Kasuga et al. (1982) that biological activity of IGF-II in chicken is exhibited by IGF-II binding to IGF-1 receptor. IGF-II competes with IGF-1 both; for its receptor as well as IGFBPs. It is also know to be an important modulator of free IGF-1 levels in IGF-1 infusion (Tomas et al., 1998).

2.10. Insulin-like Growth Factor Binding Proteins

Table.1 shows the relationship between insulin-like growth factor binding proteins and IGFs. The family of six binding proteins - Insulin-like growth factor binding proteins (IGFBPs)-is the carriers and regulators of IGF-1 and IGF-II (Table.1). They are secreted by various tissues and are found in most biological fluids (Ferry et al., 1999). These six binding proteins are structurally related to each other and on binding the IGFs, they help their rapid uptake into the cell (Jones et al., 1995; Yao et al., 2004). These proteins are highly conserved and are expressed in all the stages of development. However, their plasma concentration is dependent on the nutrition status especially in meat type chicken (Kita et al., 1996;
Bruggeman et al., 1997). The quantity and quality of the dietary protein also seems to regulate the plasma IGFBP concentration in mammals (Noguchi, 2001). In fed state IGF-1 concentration in plasma increases and thus IGF-1+IGFBP complex is required in the tissues where the anabolic actions are prevalent (Clemmons et al., 1981). They have the ability to either inhibit or enhance the actions of IGF-1 and may show their effect in important cell processes like apoptosis (Allan et al., 2004). However IGFBPs are dependent on a protein, ALS for their longevity and stability. In case of ALS degradation, there is marked reduction of circulating IGFBPs. Which ultimately leads to reduction in circulating IGF-1 (LeRoith et al., 2001). They have molecular mass between 24 to 45 Kda and each of them contains 216-289 amino acids (Firth et al., 2002; Bach et al., 1995). All IGFBPS show highly conserved N-terminal with 12 cysteine residues, C-terminal with 6 cysteine residues but the linker domain is variable. This linker domain is conserved across this gene family and the species (Duan et al., 1999; Maures et al., 2002). In spite of showing sequence homology, each IGFBP shows diverse structural and functional features the reasons for which are not quite well known (Clemmons, 2001; Firth and Baxter, 2002). The globular structure of N- and the C-terminal is the result of the intra domain disulphide bonds; whereas the linker domain has sites for post-translational regulation, including glycosylation, phosphorylation, and proteolysis (Forbes et al., 1998; Neumann et al., 1999). Glycosylation affects the IGF stability, its cell association and circulating half life as well (Firth et al., 1999, 2002; Marinaro et al., 2000). The N-terminal has the major IGF-binding site where as C-terminal provides ligand binding sites and also mediates interaction between the IGFBP and other proteins. The C-terminal or C-domain has wide variety of actions and interacts with various proteins, which may have IGF dependent, or IGF independent actions (Firth et al., 2000). The actions include growth inhibition, promotion of apoptosis and modulation of cell adhesion and migration (Bach et al., 2005). Any kind of mutation, truncation or modification in N- or C-termini residues of IGF binding site altered the binding activity of IGF. This can be a natural or induced mutation (Brinkman et al., 1991). There are discrepancies between the N- and the C-termini binding ability which are likely to be the factor differentiating the six IGFBPs. Importantly it appears that neither the N- nor the C- termini are alone responsible for the IGF binding activity; while
their actions are highly coordinated and regulated by the linker region (Vorwerk et al., 1998; Brinkman et al., 1991). The linker region is not considered to be a part of the IGF binding domain. It is the site for proteolysis, post-transitional modification and holds the controls of IGF independent activities of IGFBP (Rosenzweig, 2004).

The primary function of all the IGFBPs is to bind to the IGFs as their carrier protein. It helps the regulation and turnover of the IGFs which decides the physiological concentration in blood. The complex of IGFBP-IGF has two important functions such as it acts as a reservoir for IGF and also increases the half life of circulating IGF. For instance, free IGF has half life of 20-30 minutes whereas its complex with IGFBP has half life over 15 hours (Guler et al., 1987). The ability of IGFBP to form complex with IGF helps the transportation of IGF from blood to the target tissues and also prevents the cross binding of IGFs to insulin receptors (Rajaram et al., 1997). All IGFBPs modulate the IGF activities in the target cells. With an exception of IGFBP-1 all other IGFBPs are expressed in peripheral tissues. IGFBPs have a very high affinity for IGF and are subject to a variety of post-translational regulations; they regulate IGF actions (Table.1). Important cellular functions like cell proliferation, differentiation, survival and migration have been the core of many research laboratories (Figure.1). Tissues like, fibroblasts, osteoblasts, myoblasts, smooth muscle cells, breast, and prostate cancer cells have been studied extensively to know the role of IGFBP and its complex with IGF (Firth et al., 2002; Jones et al., 1997). There are many evidences that IGFBPs have many ligand-independent biological activities but the molecular basis for this is not yet known (Jones et al., 1993). Insulin-like growth factor binding proteins have the abilities to boost or inhibit the bioavailability and the actions of IGFs. These specific actions of IGFBPs suggest that they have hormonal activity (Duan 2004).

IGFBP-1 binds to IGFs via its C-domain Arg-Gly-Asp sequence which mediates the IGF-independent cell migration (Jones et al., 1993, Table.1). It has also been shown to have effects on the regulation of cell growth. IGFBP-2 is the most abundant IGFBP species in humans and is also known to cause apoptosis in human mesangial cells. Conditions like extreme starvation, diabetes mellitus and inflammation causes IGFBP-3 induced apoptosis by intracellular signal transduction (Verzola et al., 2001;
Grellier et al., 1996). It is the most abundantly found binding protein in blood stream which is a 45 kDa protein that circulates in a ternary structure with IGF and ALS (Firth et al., 2002). This ternary structure provides longer half life to IGFs so that there is no free IGF available to cause hypoglycemic effects of high concentration of IGF. On the cellular demand, proteolysis of IGFBP-3 gives away free IGF and it is moved across the cell wall (Lee et al., 1996). IGFBP-5 is well known for its IGF-Independent activity. It is a growth factor in its own rights (Mohan et al., 1995). It stimulates the osteoblast proliferation and its activity (Kling et al., 1996). IGFBP-5 shows extra- and intra-cellular presence. It binds to a putative receptor on the cell surface and stimulates downstream signaling (Andress et al., 1998; Mohan et al., 1999). Gene transcription in the nucleus is affected as the protein enters the nucleus with the help of nuclear localization sequence. Kuemmerle (2002) showed that IGFBP-5 can also induce proliferation of normal intestinal cells and also secrete IGF-1.

IGFBP-6 is a unique kind of IGFBP as it prefers IGF-II over IGF-1 and is also know to be an inhibitory IGFBP (Bach 1999).

2.11. IGFBP-Related Proteins (IGFBP-RP)

IGFBP-related proteins (IGFBP-RPs) are the superfamily of the IGFBPs. There are nine such related proteins found to date. They all are structurally similar at their N-terminal domain and at least three of them have been shown to bind to IGF-1 and IGF-II (Hwa et al., 1999). Not much is known about them; however, it is likely that some of them regulate cell growth (Murphy et al., 1993). It also has a tumor suppressing activity. Its overproduction seems to have effects such as apoptosis and decreased tumor formation (Sprenger et al., 1999).

2.12. Effect on Bones and Muscles

Cell proliferation, differentiation and metabolism of myogenic cell lines are the most important effects of IGFs in various species including poultry (Schmid et al., 1991; Florini et al., 1996; Duclos et al., 1999; Conlon and Kita, 2002; Figure 1). Regulation of body and muscle growth in poultry is
dominated by IGFs. It encourages the differentiation of the myogenic precursors independent of any stimulation of growth (Ewton et al., 1981). IGF-1 stimulates DNA synthesis in the chicken muscle satellite cells. It also improves glucose and amino acid uptake and also protein synthesis / protein accretion. On the other hand it reduces the protein degradation in the muscle cells (Duclos et al., 1999). IGF-1 and –II are found equipotent pertaining to replication of chicken embryo fibroblast. Differentiation of cells belonging to mesodermal origin i.e. erythrocytes, skeletal muscle cells and chondrocytes is promoted by IGF and not insulin (Schmid et al., 1991; Figure 1).

Experimental in-ovo administration of recombinant human IGF-1 (rhIGF-1) by Kocamis et al, (1998) has shown to have positive effect such as improved feed efficiency, growth and tissue development such as proliferation, differentiation and protein accretion. In this particular experiment it was observed that live weight, leg weight, breast weight and heart weight in male broiler chicken was increased due to rhIGF-1 administration. It is important to know that there was no adverse effect on the meat tenderness which is a very important economic trait in meat type chicken. IGF-1 influences many life supporting processes, such as, myocytes differentiation and cell multiplication, chondrocyte colony formation and alkaline phosphatase activity including osteoblast division and proliferation (Zapf and Froesch, 1999; Figure 1) In meat-producing animals, IGFs cover the prenatal events of development (myogenesis) including the rate of proliferation, the rate and extent of fusion, and the differentiation of three myoblast populations, giving rise to primary fibers, secondary fibers, and a satellite cell population, respectively (Figure 1). IGFs also have a postnatal effect such as myofiber hypertrophy leading to satellite cell proliferation and differentiation, and protein turnover. Production in these muscle cells depend on species of the animal, muscle type, stage of development of that particular muscle type. These studies suggest that there are autocrine and paracrine mode of action of IGF-related factors (Oksbjar et al., 2004; Lelbach et al., 2005). IGF-1 deficiency and overproduction in humans has shown to have serious damaging effects on heart muscles. It is shown to prevent or delay the death of cardio myocytes (Wang 2001) because the IGF-1 has been an important stimulus for the regeneration of the myocytes in an adult heart (Anversa et al., 2002). IGF-1 has shown to prevent apoptosis thus increasing the life span of the cell.
(Muta et al., 1993) and thus IGF-1R is called as the central regulator of mammalian lifespan (Holzenberger et al., 2003). These life saving actions of IGF-1 are achieved by, expression of growth related genes, DNA replication, myocytes nuclear mitotic division and cell division (Anversa et al., 2002). Patients with hypopituitarism shows predilection for congestive heart failure; similarly over production of IGF-1 leads to hypertrophic cardiomyopathy (Ren et al., 1999).

Tomas et al (1998) injected rhIGF-1 into chicken and studied growth and feed efficiency. Carcass fat content was seen to be decreased by IGF-1 and increased by IGF-II (Spencer et al., 1996; Tomas et al., 1998). Decreased fatness after IGF-1 infusion is due to lower insulin levels thereby causing lipogenic activity. After treating a group of chickens with rhIGF-1 the results were compared with controlled group and the findings were very distinctive of the fact that abdominal fat pad mass and total carcass fat was inversely related with the plasma IGF-1 levels. Another study by Huybrechts et al (1992) showed that infusion with rhIGF-1 had no effect on the body weight but it surely showed significant reduction in abdominal fat. The results varied as the dose parameters varied. Kita et al (1996) observed that IGF-II plasma concentration is not nutrition dependent. He also observed that IGF-II does not play any significant role in metabolic regulation. But IGF-1 has been associated with improved feed efficiency and reduced carcass fat content. Reduction in fat levels by IGF-1 is probably due to modulation of protein breakdown rate (Tomas et al., 1998). Yakar et al (2002) showed that there was 30% reduction in total body length of LID + acid labile subunit knock out (ALSKO) mice when compared with normal / control mice. Also femur length of LID+ALSKO mice was 20% shorter than the controls. These effects on bone are contributed to decrease in the ratio of bone volume / total volume which is called as trabecular bone density. This can be contributed to not only to IGF-1 total amount but also its bioavailability. This experiment demonstrated that IGF-1 plays an important role in acquiring peak bone mass and strength. Experiments by Douglas et al (1991) showed that, IGF-1 treated sheep showed reduced net loss of protein in starving conditions by stimulating protein synthesis as well as inhibiting protein degradation. These sheep also exhibited heavier long bones compared to untreated sheep which was the evidence that the
osteoblast differentiation and the collagen formation by the osteoblast were stimulated (Schmid et al., 1991; Figure 1). However such studies can be used in the osteoporosis.

### 2.13. Intermediate metabolism and growth

Exogenous administration of IGF in the poultry showed that IGF may be more tightly coupled to intermediary metabolism rather than growth rate. McGuiness and Cogburn (1991) showed no effect on the growth rate of the growing chickens when IGF-1 was administered. Yakar et al (2001) observed liver IGF-1 deficient (LID) mice to determine the metabolic consequences of IGF-1 deficiency. IGF-1 has been known to be a mitogenic hormone and has a vital role in glucose homeostasis, lipolysis, proteolysis and protein oxidation (Froesch et al., 1996). There are a few receptors of IGF-1 on liver and adipose tissues. This leads to the direct effect of IGF-1 on either pancreatic insulin secretion or on the glucose uptake by muscles. Findings from the LID mice by Yakar and associates (2001) suggest that IGF-1 plays an important role in the hormone balance between GH and insulin. There was peripheral insulin insensitivity but the reasons are not known. Very low levels of hepatic IGF-1 was responded by abnormally high levels of GH. It is well documented that GH and insulin have antagonistic effects on each other; such as, insulin increases lipogenesis and glucose uptake whereas GH promotes lipolysis and decreases the glucose metabolism. In mice, IGFBP-3 is a carrier protein for IGF-1 and they are positively correlated. LID mice showed six-fold reduction in serum IGFBP-1 and -3. Therefore it can be concluded that normal glucose metabolism is dependent on interrelation between IGF-1, GH and insulin.

Experiments by Kim et al (2004) showed that high and low IGF-1 levels were directly related to higher and lower body weights respectively. Weight gain was also directed by IGF-1. Comparatively, female chickens have lower growth rate than the male and thus it is apparent from their IGF-1 levels (Bacon et al., 1993). Variation in IGF gene and its associates can lead to growth disorders in relation with their feed intake and their body composition, skeleton integrity and metabolic traits (Zhou et al., 2004). It also has a very strong effect on metabolic diseases like diabetes disorders in nervous, reproductive, immune and bone cartilage systems. In Layers, IGF-1 has shown a great effect on their egg production.
In chickens, the carcass fat content is increased by IGF-II (Spencer et al., 1996) and decreased by IGF-1 (Huybrechts et al., 1992). IGF-1 exhibits insulin-like hypoglycemic activity in many species such as, rat, mice, dog, pig, monkey and humans. Its action on glucose appearance and disappearance is similar to insulin but low in potency. For the IGF-1 to produce effects like insulin, its concentration must exceed than insulin by about 100-fold and only then it will be able to bind to the insulin receptors in hepatic and adipose tissues (Tomas et al., 1997). However, IGF-1 also differs from insulin in counter-regulatory response that is direct or indirect suppression of pancreatic secretion of glucagon by IGF-1 (Laager et al., 1993). It is evident that IGF-1 manipulates growth rate in chickens (Goddard et al., 1988; Scanes et al., 1989; Ballard et al., 1990). On comparison a chicken line selected for increased breast meat and decrease fatness compared with an unselected control line. Insulin-like growth factor-I concentration was lower in unselected line of chickens (Tesseraud et al., 2003). Administration of rhIGF-1 in chicken caused enhanced growth and low carcass fat content in chickens (Tomas et al., 1998). Average daily gain (ADG) and feed efficiency was strongly related to subject and thus, it is selected as a candidate gene (Zhou et al., 2004). But such exogenous administration of recombinant IGFs has different effects on various species. For e.g. rats show relative lack of IGF-II in plasma which thus alters the interactive dynamics between the IGF-1, IGFBP and the receptors. Consequently it also modifies its efficiency of growth promotion (Tomas et al., 1998).

**2.14. Effects on Feed Efficiency**

Due to the effects of IGF-1 on the average daily gain and feed efficiency, it is selected as the biological candidate gene to investigate growth, body composition, metabolic, and skeletal traits in chickens (Amills et al., 2003; Zhou et al., 2004). Surprisingly higher levels of IGFs are present in the blood of poultry compared to mammals (Duclos et al., 1999). Research on energy expenditure in rats by Ling et al. (1995) showed that IGF-1 is primarily responsible for alteration in protein metabolism and ultimately energy expenditure. Deficiency of any particular amino acid has not shown any effect on the IGF concentration, but low calorie and protein percentage in diet changes the IGF concentration. These
low levels were restored on the protein supplement to these chickens (Rosebrough and McMurtry, 1993). IGF-1 mRNA levels in cells of protein deficient chickens were unaffected (Kita et al., 1996). IGF-1 has been observed to be more sensitive to dietary restrictions and it is also affected by another variable, IGFBP. These binding proteins are also sensitive to dietary manipulation (Beccavin et al., 1999. Kita et al., 2002). Experiments by Thissen (1994), showed that amino acid methionine is imperative when it comes to maintaining the normal levels of IGF-1. Methionine deficiency leads to IGF-1 depressed levels. But hepatic IGFBP-1 mRNA levels were unaffected so were plasma IGFBP-1 levels (Takenaka et al., 2000). However chickens fed methionine deficient diet tend to eat slightly more to compensate the deficiency. This increased feed intake does not cause weight gain because the calories that are converted into fat replace water in the body (Carew and Hill, 1961). Carew et al. (2005), experimented by feeding diet deficient in lysine

Conlon et al (2002) conducted an experiment to clarify the role of IGF-1 in early post hatching in birds. A single injection of rhIGF-1 was given to 7 day old chicks. Net protein loss was reduced by 15% and thus it was indicated that rhIGF-1 infusion can be used to prevent unnecessary protein loss in animals as well as humans. This research led to results such as IGF-1 levels are highly nutrition dependent. Response of protein metabolism to IGF-1 between fasting and fed condition was diverse. In fasting state the protein metabolism / turnover was increased whereas in fed state (when IGF-1 levels were high) the protein metabolism was reduced. Kim et al (2004) observed a positive correlation between serum IGF-1 and feed efficiency and suggested that IGF-1 directly promotes this trait in Korean Native Ogol Chicken. Pym et al (1989) used two groups of chickens to measure IGF-1 in fed (IGF-1_f) and in starved (IGF-1_s) condition. They used sib analysis for the determination of inheritance and genetic as well as phenotypic correlation between IGF-1 and body weight gain (BWG), feed intake (FI), food conversion ratio (FCR) and abdominal fatness (AF). The results were indicative of a fairly large negative correlation estimates between IGF-1_f and all other performance trait mentioned above but the estimates for IGF-1_s were variable. The phenotypic correlation was very low between IGF-1_f and all performance traits. Between the two IGF-1 measures there was a low to moderate phenotypic correlation. Statistical analysis showed
that plasma IGF-1 concentration varies in birds but it was re-emphasized that reduction in plasma IGF-1 levels was due to starvation (Ballard et al., 1990). The final result of studies by Pym suggests that a possible negative genetic correlation between IGF-1, and the growth rate suggests that selection for increased growth rate might result in a decreased plasma IGF-1 concentration and thus the heritability ($h^2$) of IGF-1 is generally low in chicken and mice.

In this experiment by Tomas and team (1998), it was evident that continuous infusion of IGF-1 into broiler chicken increased feed utilization by 10-15% compared to untreated birds. This experiment also showed that IGF-1 modulates the rate of protein breakdown. When the birds where treated with IGF-1, they showed better feed utilization. But this was not associated with changes in feed intake and there was net weight gain per food intake. When the birds where were infused with IGF-1 and IGF-II together, again there was improved feed efficiency. These effects were combined by increased rate of protein gain, decreased rate of fat deposition without increased feed intake and improved feed efficiency. Improved weight gain per feed intake is a result of increase in lean tissue and decrease in fat deposition. This provides indirect evidence of the involvement of IGF-1 with feed efficiency (Boyd and Bauman, 1989). These findings are contrary to the findings by Huybrechts et al (1992) who reported that there was no change in the feed efficiency in the chicken models they used for IGF-1 infusion. However the chickens in this experiment showed a significantly reduced abdominal fat pad mass. Efficiency of feed utilization is inversely proportional to the rate of muscle protein breakdown (Tomas and Pym, 1995). And this has been successfully associated with the results shown by Tomas et al. (1998). Although, a 25% variation in feed utilization was observed to be due to decreased rate of muscle protein breakdown, this relationship was nevertheless considered to be an indirect or secondary effect of IGF-1. Conversely, reduced fat deposition and reduced muscle protein breakdown were noted and observed to be a direct or primary effect of IGF-1 by Tomas et al. (1998). These results were consistent across sexes and several lines with diverse feed efficiency and body composition characteristics. When meat type male broiler chickens were continuously infused with recombinant chicken IGF-1 (rcIGF-1), (Czerwinski et al., 1998) they could not establish any relationship between IGF-1 and the weight gain and muscle protein synthesis.
Table 2.1 Relationship between the IGFBP and IGFs:

<table>
<thead>
<tr>
<th>Name</th>
<th>Specific Features</th>
<th>IGF Affinity</th>
<th>Effects on IGF Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGFBP-1</td>
<td>Arg-Gly-Asp* (RGD Sequence)</td>
<td>IGF-1 = IGF-II</td>
<td>Inhibit / Potentiate</td>
</tr>
<tr>
<td>IGFBP-2</td>
<td>RGD Sequence</td>
<td>IGF-1 &lt; IGF-II</td>
<td>Inhibition</td>
</tr>
<tr>
<td>IGFBP-3</td>
<td>N-Glycosylation</td>
<td>IGF-1 = IGF-II</td>
<td>Potentiate</td>
</tr>
<tr>
<td>IGFBP-4</td>
<td>Extra Cysteines</td>
<td>IGF-1 = IGF-II</td>
<td>Inhibition</td>
</tr>
<tr>
<td>IGFBP-5</td>
<td>Independent activity</td>
<td>IGF-1 &lt; IGF-II</td>
<td>Potentiate</td>
</tr>
<tr>
<td>IGFBP-6</td>
<td>O-Glycosylation</td>
<td>IGF-1 &lt; IGF-II</td>
<td>Inhibition</td>
</tr>
</tbody>
</table>

*Arginine-Arg-R; Glycine-Gly-G; Aspartic acid-Asp-D.*
Figure 2.1: Mechanism of action of Growth Hormone and Insulin-like Growth Factor-1 on growth and development.
IGF-1 and IGF-1R complex and their intracellular and intranuclear consequences. IGFBP is set free after the IGF-1-IGF-1R complex has settled on the cellular surface.
Cysteine–rich region
Transmembrane Domain
Tyrosine Kinase Domain

Figure 2.3: IGF-1R showing alpha and beta chains across the cell membrane
CHAPTER 3

MICROARRAY ANALYSIS OF HIGH AND LOW FEED EFFICIENCY GROUPS OF CHICKEN

1 Sanglikar, A.P. and S. E. Aggrey. To be submitted to Poultry Science.
ABSTRACT

Feed cost contributes major part of poultry operations. Reduction in the amount of feed consumed per unit gain will increase profitability and reduce poultry manure. cDNA microarray methodology was used to study differential gene expression in two groups of chickens that differ in feed efficiency. Duodenum RNA for high and low efficiency group at 28-35 d and 35-42 d were compared. There were some genes that were up-regulated whereas some were down-regulated. There was a putative down-regulation of gluconeogenesis in the low feed utilization efficiency (FUE) group and up-regulation of glucocorticoids in the high FUE group. This demonstrates different energetic states of the two groups of birds. Several energy and mitochondrial activity genes were up-regulated in high FUE group. Thyroid hormones (T3 and T4) have profound effects on metabolism, growth and development. In the high FUE group, type 1 deiodinase (DIO1) and THR associated protein were both upregulated. This further suggests that the metabolic state of the high FUE group was higher than the low FUE group. On the other hand, type 2 deiodinase (DIO2) was down-regulated in the low FUE group. It can be concluded that DOI2 may be an essential component in the thyroid sympathetic synergism required for thermal homeostasis. The microarray data obtained from this study can unravel genes associated with feed efficiency. These differentially expressed genes can be used to develop models that will increase our understanding of how complex gene networks contribute towards feed efficiency in meat-type birds.

INTRODUCTION

Poultry is a healthier alternative to red meat in diets. Domestic consumption of poultry meat in the USA rose from 8.9 billion kilograms (kg) in 1991 to 14 billion kg in 2001 suggest that American preference for white meat is gaining over other non-white meat varieties. Like most business, poultry farmers are preoccupied with reducing production cost, increasing profitability and controlling waste such as manure produced by poultry. However, the cost of feed comprises about 70% of the total production.
costs and could impact profit. A typical broiler chicken eats about 4.0 kg. of feed during the growth period of 42 days. Improvement in feed efficiency could have significant economic bottom-line effect both for the farmer and the industry at large. However, most research in broiler chicken continuously expands on the growth potential of poultry birds at the expense of feed utilization efficiency (FUE). There is a genetic variation in FUE that needs to be exploited for genetic gain (Zhang and Aggrey 2003). The first step in understanding the molecular basis of FUE is by undertaking genome profiling. Genes affect the cells that facilitate nutrient transport, stores nutrients and manipulate their availability to the cell and the body. Cellular differentiation, maintenance of cell cycle and apoptosis and cell reconstruction also occur during these processes. The objective of this study was to understand the molecular basis of the feed intake, nutrient retention and their balance, and related growth parameters using cDNA microarray methodology.

MATERIALS AND METHODS

3.1. Experimental Population

The Arkansas randombred population (ARB) was used for this experiment. The ARB population is a composite of 6 female and 7 male elite commercial parent lines representing the 1996 poultry broiler breeding industry. Growth is consistent with industry performance at 1996, and thus represents a modern commercial broiler control population. Twenty-four males were pedigree mated to 72 dams (sex ratio 1:3) to hatch 650 chicks in 3 hatches. Chicks were placed in pens with litter and fed a broiler starter diet of corn and soybean containing, 22.5% protein, 3,080 kcal ME/kg, 0.95% Ca, and 0.72% total P (0.45% available P) until 18 d of age. At 18 d of age, birds were fed a diet containing 20.5% protein, 3,150 kcal ME/kg, 0.90% Ca, and 0.67% total P (0.41% available P) At 28 d of age, birds were transferred to individual metabolic cages fasted for 12 hours before providing them with feed ad-libitum. At 35 d of age, the birds were provided with the same feed but mixed with 0.1% chromic oxide. At 43 d the birds were sacrificed and carcass traits were measured.
3.2. Data

Weekly body weight at 0, 7, 14, 21, 28, 35 and 42 d were collected, feed consumption (FC) between 28-35 d and 35-42 d. For each hatch, feed conversion ratio (FCR) was calculated for each individual at 28-35 d and 35-42 d, and duodenum samples of 8 extreme individuals for FCR were taken dipped into liquid nitrogen and stored at –80°C. Statistics of the top 50 and bottom 50 individuals for FCR are shown in Table 3.1.

3.3. Design for Microarray studies

For the comparison of global gene expression between the high (H) and low (L)- FCR groups within each of the two developmental stages (28-34d, and 35-42d), the replicated circle loop design (Kerr and Churchill 2001) was employed. The design consists of direct comparison of four groups (11=H28-35 [FCR=1.58]; 12=H35-42 [FCR=1.58]; 21=L28-35 [FCR=3.68]; 22=L35-42 [FCR=3.54]) [11 vs 12; 11 vs 21; 22 vs 12; and 22 vs 21] (Fig.4). The experiment was conducted with 4 biological replicates and 4 technical replicates to compare differential gene expression among the four experimental groups. The DEL-MAR integrated systems microarray (Cogburn et al. 2004) from liver, fat, muscle, pituitary and reproductive tissues ([http://www.ncbi.nlm.nih.gov/projects/geo/query/acc.cgi?acc/GPL1731](http://www.ncbi.nlm.nih.gov/projects/geo/query/acc.cgi?acc/GPL1731) (Fig. 3.1) was used. The CAP3 assembly of the gene index on the DEL-MAR array has been reported by Carre et al. (2005). Total RNA was extracted from the duodenum samples using Trizol reagents and Qiagen kits. Extraction was performed according to the manufacturer’s protocol. Chicken cDNA targets were generated from total RNA samples (25μg) and labeled with AA-dUTP using reverse transcriptase Superscript II (Invitrogen) in 20 μl volume. After labeling, the RNA template was destroyed by incubation with sodium hydroxide at 65°C. Labeled cDNA was purified in Micron filter (Millipore) followed by Cy3 or Cy5 (Amersham) incorporation. Dye-incorporated cDNA was purified again and its quality was checked. The targets were then re-suspended in 50 μl DIG Easy Hyb buffer (Roche) supplemented with 25 μg salmon sperm DNA and 25 μg yeast tRNA. After prehybridization of the microarray slides in blocking reagents (1% BSA, 5X SSC, 0.2% SDS), each slide was hybridized with
two targets incorporated with Cy3 and Cy5 independently. Slides were hybridized overnight at 42°C. Slides were washed with 1X SSC and 0.2% SDS once at 55°C for 10 min followed by 0.1X SSC and 0.2% SDS for 10 min at room temperature, subsequently rinsed in ddH₂O and dried by centrifugation. The dried slides were scanned using ScanArray 5000 Laser scanner with ArrayVision software (Amersham). Spotfinder software (http://www.tigr.org) was used for visualization of overlays from double dye visualizations. The result of the image analysis was automatically transferred to Microsoft Excel files containing the clone identification numbers/plate address, gene name/function and chromosomal location as well as location on the sequence map. (www.ensembl.org/Gallus_gallus). The data was log transformed and normalized according to Yang et al. (2002). Assessment of the contribution of biological and experimental sources of error to variation in the expression of each individual gene was done using the two-stage procedure described by Wolfinger et al. (2001). A global normalization model that includes the main effects of arrays and technical effects was undertaken. The output (residuals) of the normalized data was analyzed using mixed model methodology. The FCR-group and dye were considered as fixed effects. Biological and technical effects (array, spot, individual bird) were assumed as random and drawn from a normal distribution. The array and dye effects were fitted in the gene-specific mixed models, despite the fact that they were also fitted in the global normalization model.

RESULTS

Least square means and standard deviations for growth, feed traits and carcass characteristics are presented in Table 3.1. There were significant differences between the two FCR groups for body weight at 42 d, body weight gain, feed consumption and feed conversion ratio. Abdominal fat, Pectoralis major yield and drum yield were also significantly different. Metabolic body weight, carcass yield, wing yield and thigh yield, on the other hand were not significantly different.

Fold change and p-values of 22 differentially regulated genes in two different FCR groups are shown in Table 3.2. Some of these genes were down regulated while others were up-regulated. For the
down-regulated genes, insulin-like growth factor binding protein-5, had the highest fold change and was the most significant with a p-value of 0.03 (P ≤ 0.05). Sialyltransferase-4B (*SIAT5B*) was seen to have the second highest fold change value of 5.46 and was also highly significant. *RAD52B*, sialyltransferase-2 and Capase-8 had fold change values that ranged between 2.00 -3.00. *RAD52B* and Capase-8 had lower p-values of zero while sialyltransferase-2 had a p-value of 0.04. serine/threonine kinase 10, lamin A (*LMNA*), type 2 idothyronine 5’deiodinase (*DIO2*), very low-density lipoprotein receptor precursor (*VLDLR*), glucose-6-phosphate transporter protein 1 (*G6PT1*) and pyruvate carboxylase (*PCX*) had low but significant fold change values of less than 2. Genes that were up-regulated are shown in the second section of the table. Histidine triad protein member 5 (*HINT5*) was the highest up-regulated gene with the fold change value of 2.47 although; its significance level was not very high. Dictyostelium discoideum Mod A, platelet-derived growth factor B (*PDGFB*), interferon regulatory factor 1 (*IRF1*), melanocortin 5 receptor (*MC5R*) and tumor necrosis factor receptor (*TNF*) super family (*TNFSF1B*) had fold change values ranging from 1.58-2.00. However, tumor necrosis factor receptor (*TNF*) super family (*TNFSF1B*) had a p-value of 0.04 whereas; the others had highly significant p-values of almost zero. A fold change of less than 2-fold was observed for TNF receptor-associated factor 2 (*TRAF2*), adipocyte fatty acid binding protein (*AFABP*), calpin (*CAPNS1*) calcium protease, tropomysin (*TM1*) and chymotrypsinogen B1 precursor (*CTRB1*). Calpin (*CAPNS1*) calcium protease had the most significant p-value (P ≤0.05).

**DISCUSSION**

Table 3.1 shows that there was a significant difference in the body weight at 42 d between the two FUE groups. Although the difference in FC in both groups were not very significant, the variation in BWG and BW between the two groups was highly significant. A similar trend was observed for abdominal fat yield as well as drum yield. However no significant differences were observed for metabolic body weight, carcass yield, wing yield and thigh yield. According to Veerkamp (1998), there is some ambiguity about the relationship between body weight and feed efficiency as well as other
production traits. This could account for the fact that the effect of FCR was not consistent for all the growth and production traits.

Differentially expressed cDNA clones between the high and low feed utilization efficiency (FUE) groups from duodenum tissue are presented in Table 3.2. The results suggest that metabolic activity was high in the high FUE group compared to the low FUE group. The function of genes differentially expressed between the two groups point to the fact that FUE, nutrient utilization, growth and body fat should be studied simultaneously. The down regulation of glucose-6-phosphate transport protein-1 (G6PT1), pyruvate carboxylase (PYX) in the low FUE group is an indication of reduced gluconeogenesis. PYX plays an important role in intermediary metabolism, catalyzing the formation of oxaloacetate from pyruvate with concomitant ATP cleavage. It provides oxaloacetate for gluconeogenesis and replenishes TCA intermediates for fatty acids, amino acids and neurotransmitter synthesis (Attwood, 1956). The G6PT gene is involved in the control of hepatic production and blood glucose homeostasis. The G6PT promoter contains glucocorticoid respond unit consisting of binding sites for glucocorticoid receptor (Kallwellis-Opara et al., 2003). The G6PT protein translocates G6P from the cytoplasm to the lumen of the endoplasmic reticulum, where G6Pase metabolizes it to glucose and phosphate. The down regulation of glyconeogenesis in the low FUE group and upregulation of glucocorticoids in the high FUE group demonstrate the energetic states of the two groups of birds. Several energy and mitochondrial activity genes were up-regulated in high FUE group. Cytochrome C oxidase polypeptide III, glucocorticoid regulated kinase 2 (SGK2), ATP synthase, H+ transporting, mitochondrial F0 complex, inner mitochondrial membrane protein (IMMT) also known as mitofilin, ATPase protein-9 and translocase of outer mitochondrial membrane 20 were all upregulated in the high FUE group.

Thyroid hormones (T3 and T4) have profound effects on metabolism, growth and development. The effects of TH are mediated by thyroid hormone receptors (THR) that belong to the nuclear hormone receptor super family (Feng et al., 2000). In the high FUE group, type 1 deiodinase (DIO1) and THR associated protein were both upregulated. The DIO1 gene metabolizes different forms of thyroid hormones to control the levels of T3 (Amma et al., 2001) Physiologically THR-β regulates TH status.
through DIO1 mediation metabolism. This further suggests that the metabolic state of the high FUE group was higher than the low FUE group. This is in concordance with T3 levels in the high group. On the other hand, type 2 deiodinase (DIO2) was down-regulated in the low FUE group. DOI2 is essential for adaptive thermogenesis in brown adipose tissue of rats (de Jesus et al., 2001). It is possible that DOI2 may be an essential component in the thyroid sympathetic synergism required for thermal homeostasis.

Several nutrient transporters, digestive enzymes, muscle protein, and fatty acid/cholesterol related genes were differentially expressed. Melanocortin 5 receptor (MC5R) was upregulated in the high FUE group. The melanocortins are involved in the regulation of various cognitive and physiological processes including feeding. The MCR belong to the superfamily of G-protein-coupled receptors that participate in both peripheral and central functions including energy balance (Schioth et al., 2003). In humans an MC3R polymorphism is associated with the level of adiposity and with body fat (Boucher et al., 2002). A mutation in the porcine MC4R is associated with feed consumption, growth and fatness (Kim et al., 2000). The high FUE group had significantly lower abdominal fat yield compared to the low FUE group. Adipocyte lipid-binding protein (ALBP), a major intracellular lipid binding protein was expressed higher in the FUE group. Shen et al. (2001) demonstrated that ALBP increases the hydrolytic activity of hormone-sensitive lipase (HSL) through its ability to bind and sequester fatty acids and via specific protein-protein interaction. Thus, HSL and ALBP constitute a functionally important lipolytic complex. The upward expression of ALBP in the high FUE group is in concordance with the group’s abdominal fat yield. The regulation of voluntary feed intake is very complex, with multi-factorial mechanisms, with several underlying levels of control. Mechanisms that match energy and nutrient balance with feed intake and energy expenditure to maintain body homeostasis would delineate factors that affect FUE and body composition in broiler type birds. The microarray data obtained from this study demonstrates that using duodenum tissue of individuals from extreme tails of a population that differ in FUE can unravel genes associated with feed efficiency.
The differentially expressed genes can be used to develop models that will increase our understanding of how complex gene networks contribute towards feed efficiency in meat-type birds. Genetic markers can be developed in promising candidate genes and used in marker assisted selection.
Table 3.1. Descriptive statistics of traits for high and low feed conversion ratio (FCR) groups

<table>
<thead>
<tr>
<th>Traits</th>
<th>Low FCR (N=50)</th>
<th>High FCR (N=50)</th>
<th>Pr&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>BW0.75, 35 d (g)</td>
<td>214.42 ± 2.83</td>
<td>216.39 ± 2.83</td>
<td>0.6261</td>
</tr>
<tr>
<td>BW 42 d, (g)</td>
<td>1645.40 ± 173.89</td>
<td>1888.42 ± 212.94</td>
<td>0.0001</td>
</tr>
<tr>
<td>BWG, 35-42 d, (g)</td>
<td>353.06 ± 9.12</td>
<td>551.18 ± 9.10</td>
<td>0.0001</td>
</tr>
<tr>
<td>FC, 35-42 d,(g)</td>
<td>893.28 ± 150.09</td>
<td>951.82 ± 110.49</td>
<td>0.0287</td>
</tr>
<tr>
<td>FCR, 35-42 d, (g/g)</td>
<td>2.56 ± 0.33</td>
<td>1.73 ± 0.11</td>
<td>0.0001</td>
</tr>
<tr>
<td>Carcass yield (%)</td>
<td>69.90 ± 1.99</td>
<td>68.77 ± 2.55</td>
<td>0.8126</td>
</tr>
<tr>
<td>Abd. fat yield (%)</td>
<td>2.66 ± 1.31</td>
<td>1.72 ± 0.84</td>
<td>0.0002</td>
</tr>
<tr>
<td>P. major yield (%)</td>
<td>17.80 ± 1.42</td>
<td>18.53 ± 1.73</td>
<td>0.0429</td>
</tr>
<tr>
<td>Wing yield (%)</td>
<td>12.19 ± 1.13</td>
<td>12.08 ± 0.95</td>
<td>0.6216</td>
</tr>
<tr>
<td>Thigh yield (%)</td>
<td>17.61 ± 1.63</td>
<td>17.34 ± 1.55</td>
<td>0.4461</td>
</tr>
<tr>
<td>Drum yield (%)</td>
<td>13.31 ± 1.27</td>
<td>14.01 ± 0.86</td>
<td>0.0044</td>
</tr>
</tbody>
</table>

BW=body weight; BWG=BW gain; FC=feed consumption; Abd fat yield= Abdominal fat yield; P.major yield= Pectoralis major yield.
Table 3.2. Differential regulation of selected cDNA clones of High versus Low feed utilization efficiency groups revealed by microarray analysis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Fold change</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Down-regulated</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin-like growth factor binding protein 5 (<em>IGFBP5</em>)</td>
<td>11.10</td>
<td>0.03</td>
</tr>
<tr>
<td>Sialyltransferase 4B (<em>SIAT4B</em>)</td>
<td>5.46</td>
<td>0.00</td>
</tr>
<tr>
<td>RAD52B</td>
<td>2.68</td>
<td>0.00</td>
</tr>
<tr>
<td>Sialyltransferase 1 (<em>SIAT1</em>)</td>
<td>2.24</td>
<td>0.04</td>
</tr>
<tr>
<td>Capase-8 (<em>CASP8</em>)</td>
<td>2.17</td>
<td>0.00</td>
</tr>
<tr>
<td>Serine/threonine kinase 10</td>
<td>1.73</td>
<td>0.00</td>
</tr>
<tr>
<td>Lamin A (<em>LMNA</em>)</td>
<td>1.51</td>
<td>0.01</td>
</tr>
<tr>
<td>Type 2 idothyronine 5’deiodinase (<em>DIO2</em>)</td>
<td>1.48</td>
<td>0.02</td>
</tr>
<tr>
<td>Very low-density lipoprotein receptor precursor (<em>VLDLR</em>)</td>
<td>1.39</td>
<td>0.01</td>
</tr>
<tr>
<td>Glucose-6-phosphate transporter protein 1 (<em>G6PT1</em>)</td>
<td>1.36</td>
<td>0.02</td>
</tr>
<tr>
<td>Pyruvate carboxylase (<em>PCX</em>)</td>
<td>1.34</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Up-regulated</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histidine triad protein member 5 (<em>HINT5</em>)</td>
<td>2.47</td>
<td>0.04</td>
</tr>
<tr>
<td>Dictyostelium discoideum Mod A</td>
<td>1.97</td>
<td>0.00</td>
</tr>
<tr>
<td>Melanocortin 5 receptor (<em>MC5R</em>)</td>
<td>1.91</td>
<td>0.02</td>
</tr>
<tr>
<td>Interferon regulatory factor 1 (<em>IRF1</em>)</td>
<td>1.78</td>
<td>0.02</td>
</tr>
<tr>
<td>Platelet-derived growth factor B (<em>PDGFB</em>)</td>
<td>1.71</td>
<td>0.00</td>
</tr>
<tr>
<td>Tumor necrosis factor receptor (<em>TNF</em>) super family (<em>TNFSF1B</em>)</td>
<td>1.58</td>
<td>0.04</td>
</tr>
<tr>
<td>TNF receptor-associated factor 2 (<em>TRAF2</em>)</td>
<td>1.58</td>
<td>0.03</td>
</tr>
<tr>
<td>Chymotrypsinogen B1 precursor (<em>CTRB1</em>)</td>
<td>1.55</td>
<td>0.02</td>
</tr>
<tr>
<td>Calpin (<em>CAPNS1</em>) Calcium protease</td>
<td>1.38</td>
<td>0.00</td>
</tr>
<tr>
<td>Tropomysin (<em>TM1</em>)</td>
<td>1.34</td>
<td>0.03</td>
</tr>
<tr>
<td>Adipocyte fatty acid binding protein (<em>AFABP</em>)</td>
<td>1.29</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Figure 3.1. DEL-MAR chicken integrated microarray
Figure 3.2: Circle loop design (Kerr and Churchill 2001) for the comparison of global gene expression
CHAPTER 4

RELATIONSHIP BETWEEN PHYSIOLOGICAL PARAMETERS AND FEED EFFICIENCY IN
AN EXPERIMENTAL POPULATION DIVEREGENTLY SELECTED FOR PHYTATE
PHOSPHORUS BIOAVAILABILITY

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1 Sanglikar, A. P. and S. E. Aggrey. To be submitted to Poultry Science.
ABSTRACT

This study was conducted to determine the relationship between the physiological parameters and feed efficiency. Both positive and negative relationships between insulin like growth factor I (IGF-1) and feed conversion ratio (FCR) have been reported in literature. Our objective was to study the relationship between IGFI, IGF2, insulin, glucagons, thyroxine (T4) and triiodothyronine (T3) and feed related traits in a divergent line of chicken selected for high (HPP) or low (LPP) phytate phosphorus bioavailability.

HPP lines had lower FC values and appeared to utilize feed less efficiently than LPP lines whereas LPP lines showed higher body weight gains than the HPP lines.

There was a significant positive correlation between body weight gain and IGF-2 levels for both lines. However, IGF-2 exhibited a marked negative correlation with FCR in the HPP line and a low negative correlation in the LPP lines. In both HPP and LPP lines, Insulin showed a very significant correlation with FC and FCR. In HPP, there was a positive relationship between T3 and feed consumption (FC) and feed conversion ratio (FCR), T4 and body weight gain at 4 weeks (BW4) as well as FC but T3/T4 ratio had a negative relationship with BW4. In LPP, a positive relationship between T3 and FC and gain was observed whereas T4 had a positive correlation with body weight gain. Insulin and IGF-2 appears to be the physiological parameters associated with feed efficiency in the chicken line divergently selected for phytate phosphorus.

INTRODUCTION

Insulin-like Growth Factors (IGFs) consists of a family of polypeptide hormones produced by liver, is structurally associated with insulin with multiple metabolic and anabolic functions. Besides liver many other organs like kidney, brain, skin and gonads also produce IGFs. IGF-2 is predominant in pre-hatch development whereas IGF-1 is in post-hatch. Circulating half lives of IGFs are very short and thus IGF binding proteins (IGFBPs) binds them to increase their circulating half-life. Out of six IGFBPs, IGFBP-5 is known to have an independent hormone activity. It has the potential to stimulate proliferation
of intestinal cells as well as secret IGF-1. Effects of IGF are displayed only when they are attached with IGF receptors present on the surface of the cell. IGF-1 initiates proliferation, differentiation, cell survival and many other vital metabolic effects in the birds. The most prominent effect of IGF-1 is on the muscle and bone cell cartilage growth and is also shown to reduce protein degradation and improves protein synthesis. These studies suggest that there are autocrine and paracrine mode of action of IGF-related factors. IGF-1 has shown to prevent apoptosis thus increasing the life span of the cell and thus IGF-1R is called as the central regulator of mammalian lifespan.

Variation in IGF-1 gene can lead to growth disorders in relation with their feed intake and their body composition, skeleton integrity and metabolic traits. In-ovo administration of recombinant human IGF-1 (rhIGF-1) gave results such as improved feed efficiency, growth and tissue development such as proliferation, differentiation and protein accretion (Kocamis et al., 1998). Upon this there was improvement in live weight, leg weight, breast weight and heart weight. In chickens, carcass fat content is increased by IGF-2 and decreased by IGF-1. Variation in plasma IGF-1 levels is mainly due to dietary manipulation especially changes in the calorie and protein percentage. Experiments by Pym et al (1998) showed that IGF-1 in fed condition was fairly negatively correlated to BWG, FC, FCR and abdominal fatness (AF). These findings are contrary to the findings by Huybrechts et al (1992) who reported that there was no change in the feed efficiency in the chicken models they used for IGF-1 infusion. Due to the effects of IGF-1 on the average daily gain and feed efficiency, it has been selected as the biological candidate gene to investigate growth, body composition, metabolic, and skeletal traits in chickens. The chicken line selected for increased breast meat had higher levels of IGF-1 in their serum compared to the chicken line not selected for increased breast meat (Tesseraud et al., 2003). IGF-1 has been associated with improved feed efficiency and reduced carcass fat content (Kita et al., 1996). Studies on IGF-1 knock out mice revealed that normal glucose metabolism depends upon interrelation between IGF-1, GH and insulin (Yakar et al., 2001). There have been discrepancies in an understanding of the role some physiological parameters in feed related traits. The objective of this study was to determine the
relationship between IGF-1, IGF-2, insulin, glucagons, thyroxine and triiodothyronine and feed related traits in a chicken line divergently selected for phytate phosphorus bioavailability.

MATERIALS AND METHODS

4.1. 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 body weight (BW) and BW gain (BWG) during the 3 d excreta collection period were also measured. Excreta were oven-dried at 80°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:

\[
PBA = \frac{(A-B)}{A} \times 100\%
\]

where PBA= the bioavailability of the phytate phosphorus, 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 bioavailability body weight at 4 wk, BWG at day 28-35 d, FC, feed conversion ratio (FCR) for BWG were collected on 1,004 birds. The FCR was calculated as the ratio of FC per BWG during the excreta collection period.
4.2. Selection Lines

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 (G2) 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.

4.3. Hormonal Analysis

At generation 3 of selection 1 ml of blood was collected from each bird. Plasma was collected for the determination of IGFI, IGFII, insulin, glucagons, thyroxine and triiodothyronine levels using standard hormone assays. Correlation analysis of hormones with feed traits were performed by using PROC CORR (SAS Institute, 1996).

RESULTS

Tables 4.1 and 4.5 show means and standard deviations of feed and growth traits for high and low phytate phosphorus lines, respectively. High phytate phosphorus (HPP) line had lower mean body weight at 4 weeks compared to low phytate phosphorus (LPP) line. HPP lines had lower FC values and appeared to utilize feed less efficiently than LPP lines. The FCR value for the HPP lines was 2.49 compared to 2.39 in LPP lines. Also, LPP lines had higher body weight gains than the HPP lines. There were notable differences in the levels of IGF-1 and IGF-2 for the two divergent lines. The IGF-1 levels in HPP were much higher compared to their levels in LPP lines (42.99 compared to 38.94). On the other hand, IGF-2
levels were higher in the LPP lines. Insulin levels in the two lines were considerably different. The HPP lines had 1269.99 units of Insulin compared to 1205.21 LPP lines. By contrast, glucagon levels in LPP lines were much higher than those in HPP lines. There were higher levels of both thyroxine (T₄) and triiodothyronine (T₃) in HPP lines compared to LPP lines. The TR value which is the ratio of T₃ to T₄ was higher in HPP lines as well.

Correlation estimates of 11 feed and growth traits for the HPP and LPP lines are presented in Tables 4.6 and 4.7 and figure 4.4-4.56. Correlation between BW₄ and FC, and BW₄ and BWG in both lines was significant. Correlation between BW₄ and FCR was zero for HPP lines was negative for the LPP population. A negative relationship was observed for BWG and FCR in the two lines. There was no association between BWG and FCR in HPP lines. However, BWG was negatively related to FCR in the LPP population. There was a significant positive correlation between BWG and IGF-2 levels for both lines. However, IGF-2 exhibited a marked negative correlation with FCR in the HPP line and a low correlation in the LPP lines. The correlation between FCR and IGF-1 was low and positive in the HPP lines and low and negative in the LPP lines. IGF-1 had a negative relationship with IGF-2 in the two lines. In both HPP and LPP lines, Insulin showed a very significant correlation with FC and FCR.

**DISCUSSION**

Tables 4.6 and 4.7 represent the correlation between the feed and the growth traits. Tomas et al (1998) in a study on chicken observed that IGF-1 infusion increased their feed utilization by slowing down the protein breakdown and decreasing fat deposition but there was no evidence of increased feed intake. On the other hand, Huybrechts et al (1991) found that there was no effect of IGF-1 on feed efficiency. Our studies showed that there was a negative relationship between IGF-1 levels and feed efficiency. When the IGF-1 levels were high, the FCR was low conversely when the IGF-1 levels were low such as in LPP lines, the FCR was relatively good (Tables 4.4 and 4.5). The IGF-II levels are always very high in pre-hatch stage but not in post-hatch (DeChiara et al., 1990). In our studies it was seen that
IGF-1 and IGF-II levels are highly negatively correlated. This could be due to the competition between IGF-1 and IGF-II for IGF-1 receptor as well as faster clearance rate of IGF-2. High levels of Insulin were associated with low levels of IGF-II. This could be due to the fact that IGF-1 and Insulin cross react with the Insulin receptor. Huybrechts et al. (1991) indicated that high levels of IGF-1 are associated with high levels of T3. There is evidence that IGF-1 interact with thyroid metabolism by increasing the 5’-monodeiodinase. In contrast, the current study showed that there was a negative correlation between IGF-1 and T3. Tables 4.6 and 4.7 showed that IGF-1 and Insulin are negatively correlated. However, physiologically elevation in IGF-1 levels enhances glucose production and utilization. In response to that, the insulin level in blood also rises to facilitate the glucose uptake by cells for energy metabolism (Johansson and Arnqvist, 2005).
**Table 4.1.** Means and standard deviation of feed and growth traits in high phytate phosphorus line

<table>
<thead>
<tr>
<th>Traits</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW4 (g)</td>
<td>360</td>
<td>291.67</td>
<td>53.70</td>
<td>18.41</td>
<td>134.60</td>
<td>443.30</td>
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<tr>
<td>FC, (g)</td>
<td>360</td>
<td>116.55</td>
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<td>17.59</td>
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<tr>
<td>Gain, (g)</td>
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<td>86.50</td>
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<tr>
<td>FCR, g/g</td>
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<td>17.49</td>
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<td>3.73</td>
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<td>IGF1, (ng/ml)</td>
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<td>10.07</td>
<td>23.43</td>
<td>20.50</td>
<td>75.20</td>
</tr>
<tr>
<td>IGF2, (ng/ml)</td>
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<td>40.49</td>
<td>18.89</td>
<td>46.64</td>
<td>4.10</td>
<td>150.50</td>
</tr>
<tr>
<td>Insulin, (pg/ml)</td>
<td>290</td>
<td>1269.99</td>
<td>533.08</td>
<td>41.97</td>
<td>455.00</td>
<td>5395.00</td>
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<td>Glucagon,(pg/ml)</td>
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<td>185.56</td>
<td>117.54</td>
<td>63.34</td>
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<td>1020.00</td>
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<td>T₃, (pg/ml)</td>
<td>287</td>
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<td>6.24</td>
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<td>T₄, (pg/ml)</td>
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<td>T₃/T₄ ratio (ng/ml)</td>
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<td>0.28</td>
<td>0.09</td>
<td>33.05</td>
<td>0.07</td>
<td>0.58</td>
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BW4= Body Weight at 4 weeks; FC=Feed Consumption; Gain= Body Weight Gain; FCR= Feed Conversion Ratio; IGF-1= Insulin-like Growth Factor-1; IGF-2= Insulin-like Growth Factor-2; T₃= Triiodothyronine; T₄= Thyroxine

**Table 4.2.** Means and standard deviation of feed and growth traits in low phytate phosphorus line

<table>
<thead>
<tr>
<th>Traits</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
<th>Min</th>
<th>Max</th>
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<tr>
<td>BW4 (g)</td>
<td>334</td>
<td>318.78</td>
<td>52.36</td>
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<td>FC, (g)</td>
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<td>120.84</td>
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<td>16.14</td>
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<td>Gain, (g)</td>
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<td>51.78</td>
<td>10.97</td>
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</tr>
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<td>T₃/T₄ ratio (ng/ml)</td>
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<td>0.27</td>
<td>0.10</td>
<td>37.95</td>
<td>0.05</td>
<td>0.66</td>
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</table>

BW4= Body Weight at 4 weeks; FC=Feed Consumption; Gain= Body Weight Gain; FCR= Feed Conversion Ratio; IGF-1= Insulin-like Growth Factor-1; IGF-2= Insulin-like Growth Factor-2; T₃= Triiodothyronine; T₄= Thyroxine
Table 4.3. Correlation estimates among feed related traits and physiological parameters in a chicken line selected for high phytate phosphorus bioavailability

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<tr>
<th></th>
<th>BW4</th>
<th>FC</th>
<th>GAIN</th>
<th>FCR</th>
<th>IGF1</th>
<th>IGF2</th>
<th>INSUL</th>
<th>GLUC</th>
<th>T₃</th>
<th>T₄</th>
<th>T₃/T₄</th>
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<td>0.4700</td>
<td>0.0000</td>
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<tr>
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</table>

BW4= Body Weight at 4 weeks; FC=Feed Consumption; Gain= Body Weight Gain; FCR= Feed Conversion Ratio; IGF-1= Insulin-like Growth Factor-1; IGF-2= Insulin-like Growth Factor-2; T₃= Triiodothyronine; T₄= Thyroxine.
Table 4.4 Correlation estimates among feed related traits and physiological parameters in a chicken line selected for low phytate phosphorus bioavailability

<table>
<thead>
<tr>
<th></th>
<th>BW4</th>
<th>FC</th>
<th>Gain</th>
<th>FCR</th>
<th>IGF1</th>
<th>IGF2</th>
<th>INSUL</th>
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<td>0.2280</td>
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<td>Gain</td>
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<tr>
<td>FCR</td>
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<td>-0.0414</td>
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</table>

BW4= Body Weight at 4 weeks; FC=Feed Consumption; Gain= Body Weight Gain; FCR= Feed Conversion Ratio; IGF-1= Insulin-like Growth Factor-1; IGF-2= Insulin-like Growth Factor-2; T3= Triiodothyronine; T4= Thyroxine.
IGF-1 = 0.0159BW + 38.317

$R^2 = 0.0071$

Figure 4.1 The relationship between serum IGF-1 levels and body weight at 4 weeks in a high phytate phosphorus bioavailability line.

IGF-2 = 0.0462BW + 26.9

$R^2 = 0.0169$

Figure 4.2 The relationship between serum IGF-2 levels and body weight at 4 weeks in a high phytate phosphorus bioavailability line.
Insulin = 0.289BW + 1184.9  
$R^2 = 0.0008$

Figure 4.3 The relationship between serum insulin levels and body weight at 4 weeks in a high phytate phosphorus bioavailability line.

Glucagon = 0.1588BW + 139.11  
$R^2 = 0.0054$

Figure 4.4 The relationship between serum glucagon levels and body weight at 4 weeks in a high phytate phosphorus bioavailability line.
Figure 4.5 The relationship between serum T\textsubscript{3} levels and body weight at 4 weeks in a high phytate phosphorus bioavailability line.

Figure 4.6 The relationship between serum T\textsubscript{4} levels and body weight at 4 weeks in a high phytate phosphorus bioavailability line.
Figure 4.7 The relationship between serum T₃/T₄ levels and body weight at 4 weeks in a high phytate phosphorus bioavailability line.

Figure 4.8 The relationship between serum IGF-1 levels and feed consumption in a high phytate phosphorus bioavailability line.
Figure 4.9 The relationship between serum IGF-2 levels and feed consumption in a high phytate phosphorus bioavailability line.

Insulin = 3.852FC + 806.63
\[ R^2 = 0.0194 \]

Figure 4.10 The relationship between serum insulin levels and feed consumption in a high phytate phosphorus bioavailability line.
**Figure 4.11** The relationship between serum glucagon levels and feed consumption in a high phytate phosphorus bioavailability line.

Glucagon = 0.6674FC + 104.8

R² = 0.0122

**Figure 4.12** The relationship between serum T₃ levels and feed consumption in a high phytate phosphorus bioavailability line.

T₃ = 0.0054FC + 2.3646

R² = 0.0207
Figure 4.13 The relationship between serum T₄ levels and feed consumption in a high phytate phosphorus bioavailability line.

Figure 4.14 The relationship between serum T₃/T₄ ratio levels and feed consumption in a high phytate phosphorus bioavailability line.
Figure 4.15 The relationship between serum IGF-1 levels and body weight gain in a high phytate phosphorus bioavailability line.

Figure 4.16 The relationship between serum IGF-2 levels and body weight gain in a high phytate phosphorus bioavailability line.
Figure 4.17 The relationship between serum insulin levels and body weight gain in a high phytate phosphorus bioavailability line.

Figure 4.18 The relationship between serum glucagon levels and body weight gain in a high phytate phosphorus bioavailability line.
\[ T_3 = -0.0019 \text{Gain} + 3.1095 \]
\[ R^2 = 0.0009 \]

Figure 4.19 The relationship between serum T₃ levels and body weight gain in a high phytate phosphorus bioavailability line.

\[ T_4 = 0.0117 \text{Gain} + 10.625 \]
\[ R^2 = 0.0021 \]

Figure 4.20 The relationship between serum T₄ levels and body weight gain in a high phytate phosphorus bioavailability line.
Figure 4.21 The relationship between serum T₃/T₄ ratio levels and body weight gain in a high phytate phosphorus bioavailability line.

Figure 4.22 The relationship between serum IGF-1 levels and feed consumption ratio in a high phytate phosphorus bioavailability line.
Figure 4.23 The relationship between serum IGF-2 levels and feed consumption ratio in a high phytate phosphorus bioavailability line.

Figure 4.24 The relationship between serum insulin levels and feed consumption ratio in a high phytate phosphorus bioavailability line.
Figure 4.25 The relationship between serum glucagon levels and feed consumption ratio in a high phytate phosphorus bioavailability line.

\[ \text{Glucagon} = -13.13 \text{FCR} + 218.99 \]
\[ R^2 = 0.0023 \]

Figure 4.26 The relationship between serum T3 levels and feed consumption ratio in a high phytate phosphorus bioavailability line.

\[ T3 = 0.258 \text{FCR} + 2.3671 \]
\[ R^2 = 0.0243 \]
Figure 4.27 The relationship between serum T₄ levels and feed consumption ratio in a high phytate phosphorus bioavailability line.

Figure 4.28 The relationship between T₃/T₄ ratio levels and feed consumption ratio in a high phytate phosphorus bioavailability line.
Figure 4.29 The relationship between serum IGF-1 levels and body weight gain in a low phytate phosphorus bioavailability line.

Figure 4.30 The relationship between serum IGF-2 levels and body weight gain in a low phytate phosphorus bioavailability line.
Insulin = 0.3543BW + 1092.3
R² = 0.0011

Figure 4.31 The relationship between serum insulin levels and body weight gain in a low phytate phosphorus bioavailability line.

Glucagon = -0.057BW + 225.96
R² = 0.0006

Figure 4.32 The relationship between serum glucagon levels and body weight gain in a low phytate phosphorus bioavailability line.
Figure 4.33 The relationship between serum T₃ levels and body weight gain in a low phytate phosphorus bioavailability line.

Figure 4.34 The relationship between serum T₄ levels and body weight gain in a low phytate phosphorus bioavailability line.
Figure 4.35 The relationship between serum T₃/T₄ ratio levels and body weight gain in a low phytate phosphorus bioavailability line.

T₃/T₄ = -0.00001BW + 0.2797
R² = 0.00005

Figure 4.36 The relationship between serum IGF-1 levels and feed consumption in a low phytate phosphorus bioavailability line.

IGF-1 = 0.009FC + 37.825
R² = 0.0003
Figure 4.37 The relationship between serum IGF-2 levels and feed consumption in a low phytate phosphorus bioavailability line.

\[ IGF-2 = 0.1483FC + 26.013 \]
\[ R^2 = 0.0347 \]

Figure 4.38 The relationship between serum insulin levels and feed consumption in a low phytate phosphorus bioavailability line.

\[ Insulin = 3.1232FC + 820.69 \]
\[ R^2 = 0.0117 \]
Figure 4.39 The relationship between serum glucagon levels and feed consumption in a low phytate phosphorus bioavailability line.

Figure 4.40 The relationship between serum T₃ levels and feed consumption in a low phytate phosphorus bioavailability line.
Figure 4.41 The relationship between serum T4 levels and feed consumption in a low phytate phosphorus bioavailability line.

Figure 4.42 The relationship between serum T3/T4 ratio levels and feed consumption in a low phytate phosphorus bioavailability line.
Figure 4.43 The relationship between serum IGF-1 levels and body weight gain in a low phytate phosphorus bioavailability line.

Figure 4.44 The relationship between serum IGF-2 levels and body weight gain in a low phytate phosphorus bioavailability line.
Figure 4.45 The relationship between serum insulin levels and body weight gain in a low phytate phosphorus bioavailability line.

**Equation:**

\[ \text{Insulin} = 1.5691 \times \text{Gain} + 1123.2 \]

\[ R^2 = 0.001 \]

Figure 4.46 The relationship between serum glucagon levels and body weight gain in a low phytate phosphorus bioavailability line.

**Equation:**

\[ \text{Glucagon} = -0.2669 \times \text{Gain} + 221.78 \]

\[ R^2 = 0.0006 \]
Figure 4.47 The relationship between serum T₃ levels and body weight gain in a low phytate phosphorus bioavailability line.

Figure 4.48 The relationship between serum T₄ levels and body weight gain in a low phytate phosphorus bioavailability line.
Figure 4.49 The relationship between serum $T_3/T_4$ ratio levels and body weight gain in a low phytate phosphorus bioavailability line.

$$T_3/T_4 = 0.0002 \text{Gain} + 0.2667$$

$R^2 = 0.0003$

Figure 4.50 The relationship between serum IGF-1 levels and feed consumption ratio in a low phytate phosphorus bioavailability line.

$$IGF-1 = -0.936 \text{FCR} + 41.201$$

$R^2 = 0.0015$
Figure 4.51 The relationship between serum IGF-2 levels and feed consumption ratio in a low phytate phosphorus bioavailability line.

Figure 4.52 The relationship between serum insulin levels and feed consumption ratio in a low phytate phosphorus bioavailability line.
Figure 4.53 The relationship between serum glucagon levels and feed consumption ratio in a low phytate phosphorus bioavailability line.

Figure 4.54 The relationship between serum T₃ levels and feed consumption ratio in a low phytate phosphorus bioavailability line.
Figure 4.55 The relationship between serum T4 levels and feed consumption ratio in a low phytate phosphorus bioavailability line.

\[ T4 = -0.4604FCR + 11.977 \]
\[ R^2 = 0.003 \]

Figure 4.56 The relationship between serum T3/T4 ratio and feed consumption ratio in a low phytate phosphorus bioavailability line.

\[ T3/T4 = 0.0075FCR + 0.257 \]
\[ R^2 = 0.0008 \]
GENERAL CONCLUSION

A study was conducted to examine the molecular basis of feed utilization efficiency. Feed costs constitute about 70% of the production cost. Improvement in feed efficiency will produce efficient birds, increase profitability and reduce manure output. From a global gene expression study using microarray methodology, it was established that genes in the Insulin like growth factor (IGF) pathway including IGF binding protein 5 (IGFBP5), Platelet-derived growth factor B (PDGFB), Interferon regulatory factor 1 (IRF1), Melanocortin 5 receptor (MC5R) and Tumor necrosis factor receptor (TNF) super family (TNFSF1B) affected feed efficiency. In a divergent line selected for phytate phosphorus bioavailability, insulin was found to be associated with feed consumption and feed conversion ratio in both lines.

These genes can be used in further development of genetic markers which can be used in marker assisted selection.
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