INFLUENCE OF DIETARY PROTEIN ON FATTY ACID SYNTHESIS IN CHICKS

by

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(Under the Direction of ADAM DAVIS)

ABSTRACT

Body fat accumulation is a major concern in the poultry industry. Therefore, knowledge about the molecular mechanisms that control *de novo* fatty acid synthesis and applied nutritional research that limits fat production in chickens is of critical value to the poultry industry.

The ability for increased dietary protein concentrations to decrease *de novo* fatty acid synthesis in birds is correlated with a decrease in malic enzyme activity that is preceded by a decrease in malic enzyme mRNA expression (Adams and Davis, 2001). More importantly, the changes in malic enzyme mRNA expression occur with in 3 h of a change in the dietary protein level (Adams and Davis, 2001). The current research established that the changes in malic enzyme mRNA expression are proceeded by changes in the mRNA expression of the transcription factor spot 14. The current research also indicates that dietary protein's influence on spot 14 mRNA expression may be mediated by glucagon and/or thyroid hormone.

In conclusion, it is well established that increasing dietary protein concentrations will reduce *de novo* fatty acid synthesis in birds and subsequently body fat content. As researchers continue to delineate the biochemical mechanisms by which protein causes this effect, practical dietary manipulations may be identified to control the body fat content of broilers and the

identification of critical biochemical mediators in the process may lead to their use as genetic markers in the selection of broilers for leanness.

INDEX WORDS: Lipogenesis, Spot 14, dietary protein, chicks. mRNA expression.

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DEDICATION

This 3.5 year work is dedicate to my wife Valeria Himmel.

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It would be impossible to list all the people that marked the difference in this 3.5 year journey. You all know who you are. Gracias totales.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1. LIPOGENESIS

Biological Importance of Fat

Adipose tissue plays an essential role in the survival of animals, as a energy store for periods of fasting. Body fat is mainly composed of lipids, which are molecules that are largely hydrocarbon in nature, are highly reduced. In addition, lipids are highly hydrophobic. All of these characteristics contribute to their high energy density relative to proteins and carbohydrates. Thus, when insufficient calories are taken into the body, stored body fat is hydrolyzed into nonesterified fatty acids (NEFA) and glycerol, which are released into the circulation to be utilized by other tissues as an energy source. Subcutaneous fat also provides protection against mechanical injury and functions as thermal insulation.

The process of fat deposition is under hormonal and neuroendocrine control. When the energy requirements for maintenance and growth are fulfilled, the animal will take any extra available energy and convert it to body fat. The process of fat storage can be accomplished from utilizing lipids obtained from the diet and depositing them into fat cells or from utilizing fatty acids made from excess carbons not utilized for growth or maintenance.

The *de novo* formation of depot fat is accomplished by fatty acid synthesis, which consists of a cytosolic metabolic pathway by which acetyl CoA is converted into palmitate. The overall reaction for fatty acid synthesis is:

$$8 \text{ Acetyl CoA} + 14 \text{ NADPH} + 14 \text{ H}^+ + 7 \text{ ATP} \rightarrow \text{Palmitate} + 8 \text{ CoA} + 14 \text{ NADP} + 7 (\text{ADP} + \text{Pi}) + 6 \text{ H}_2\text{O}$$

Fatty acid synthesis occurs mainly in the adipose tissue or liver. The adipocyte is the primary site of fatty acid synthesis in ruminants and rats (Ballard et al., 1969). In humans, other primates and avian species, however, the primary site of fatty acid synthesis is the liver (Shargo et al., 1971).

Fatty Acid Synthesis Pathway

There are two enzymes required for fatty acid synthesis. The first enzyme is acetyl CoA carboxylase that catalyzes the energy dependent conversion of acetyl CoA to malonyl CoA. Acetyl CoA carboxylase uses biotin as a cofactor in this reaction. The second enzyme used for fatty acid synthesis is the multifunctional fatty acid synthetase. This enzyme catalyzes all the conversions of malonyl CoA to palmitate. Fatty acid synthetase consists of two protein subunits and has seven active catalytic sites. The palmitate made in fatty acid synthesis is constructed two carbons at a time. The first two carbons come from acetyl CoA and the rest come from malonyl CoA which result from the carboxylation of acetyl CoA catalyzed by acetyl CoA carboxylase. The addition of two carbons from malonyl CoA to the growing fatty acid molecule is catalyzed seven times until a sixteen carbon fatty acid, palmitate, is formed. Each addition of carbon atoms from malonyl CoA to the growing fatty acid requires two molecules of NADPH as a cofactor.

The acetyl CoA derived from amino acid degradation is normally insufficient for fatty acid biosynthesis. Therefore, fatty acid synthesis is dependent on acetyl CoA production from glucose. Pyruvate, the product of glycolysis is converted in the mitochondria to acetyl CoA by pyruvate dehydrogenase. If the energy demands for

cellular respiration have been met, the acetyl CoA will not enter the Krebs cycle, instead it will have to be translocated to the cytoplasm for fatty acid synthesis. Because the inner mitochondrial cell membrane is impermeable to acetyl CoA it combines with oxaloacetate (OAA) to form the tricarboxylate, citrate. Citrate is able to cross the mitochondrial membrane using a specific exchange transporter. Once in the cytosol, citrate lyase cleaves citrate to acetyl CoA and OAA. The acetyl CoA can serve as the substrate for cytosolic *de novo* fatty acid synthesis.

The excess carbons of the regenerated cytosolic OAA need to be returned to the mitochondria. The mitochondrial membrane is also impermeable to OAA and there are no transport systems to move it across the membrane. The OAA is therefore reduced to malate by the cytoplasmic enzyme NAD malate dehydrogenase. This reaction requires NADH, which is produced when pyruvate is generated from glucose in glycolysis. The malate is then oxidized to pyruvate, which can enter the mitochondria. This oxidation reaction is catalyzed by NADP malate dehydrogenase, also known as malic enzyme. The activity of malic enzyme generates one NADPH molecule for every molecule of pyruvate produced. The NADPH produced in this enzymatic process is important because they help meet the NADPH required for fatty acid synthesis. The series of reactions that result in the translocation of acetyl CoA from the mitochondria to the cytosol are summarized in **Figure 1**.

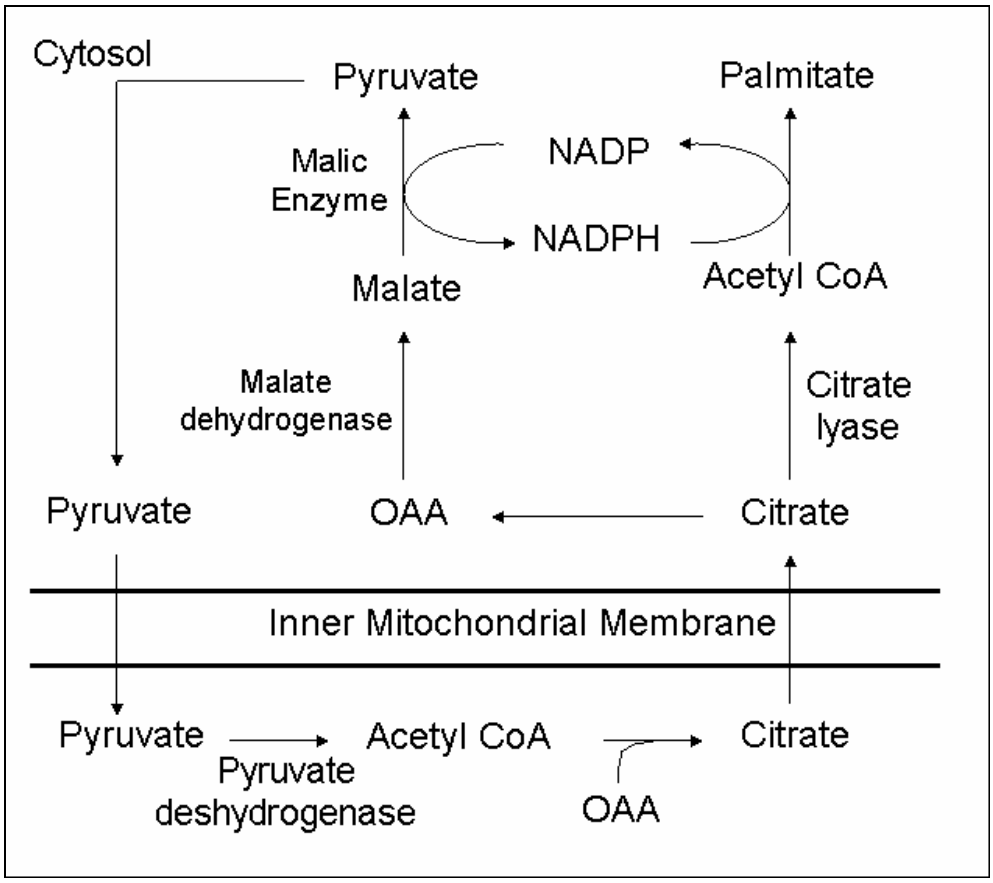

Figure 1.1 Translocation of acetyl CoA across the mitochondrial membrane for participation in fatty acid synthesis.

Abbreviations: OAA, oxyloacetate, NADP nicotin amide adenine dinucleotide phosphate, NADPH, reduced nicotinamide.

Site of Fatty Acid Synthesis in Avian Species

Goodridge and Ball (1966) studied the lipogenic capacity of avian liver and adipose tissue. Acetyl CoA carboxylase levels were compared in pigeon liver and adipose tissue, and the activity of this enzyme was found to be 20 times higher in the liver compared to adipose tissue. The activity of citrate lyase in the pigeon was also 14 times greater in the liver than in adipose tissue. In addition, the activity of malic enzyme was 12 times greater in pigeon liver than adipose tissue.

Additional evidence that fatty acid synthesis takes place in the liver rather than the adipose tissue of the bird was provided by subsequent research. The blood glucose level of pigeons injected with glucose-U-^{14}C, increased from the initial 8.1 μmoles/ml to 12 μmoles/ml in the first 30 seconds after injection and then began to decrease (Goodridge and Ball, 1967). As the blood glucose levels began to drop, the amount of labeled glucose in the liver and adipose tissue increased. The liver converted the glucose into fatty acids 25 times faster than adipose tissue (Goodridge and Ball, 1967). In plasma samples taken 15 minutes after injection, 81% of the total radioactive counts of plasma fatty acids were in the form of plasma triglycerides and in samples taken at 120 minutes post injection, 91 % of the plasma fatty acids were in triglyceride form (Goodridge and Ball, 1967). Additionally, the level of lipoprotein lipase in pigeons was twice that found in rats (Goodridge and Ball, 1967). This finding was important since lipoprotein lipase breaks triglycerides into fatty acids and glycerol prior to their entrance into adipocytes. Thus, the triglycerides incorporated into the adipose tissue from 15 to 120 minutes were transported there after being synthesized in the liver. The synthesis of fatty acids in the liver and their subsequent transport to fat cells, was also reflected by the

delayed (compared to liver and plasma samples) incorporation of the labeled glucose into fatty acids in the adipose tissue (Goodridge and Ball, 1967). From this study Goodridge and Ball (1967) concluded that the liver was responsible for 96% of fatty acid synthesis in the pigeon. A similar conclusion was reached by Leveille et al., (1968) using chickens.

Summary

De novo fatty acid synthesis is the process by which acetyl CoA is utilized to make palmitate. In avian species this metabolic process takes place in the liver, and the fatty acids that are made in the liver are then transported to fat cells for storage. Stored fat serves as an energy source when an animal does not meet its energy requirements through caloric intake. In addition, subcutaneous body fat provides protection against mechanical injury and functions as thermal insulation.

2. DIETARY INFLUENCES ON FAT DEPOSITION

Starvation and Refeeding Regulate Fatty Acid Synthesis

In vitro experiments conducted by Goodridge (1968b) demonstrated that fatty acid synthesis and malic enzyme activity were both depressed in fasted chicks and quickly returned to prefasted levels once chicks were refed. Yeh and Leveille (1971a) conducted both *in vitro* and *in vivo* studies with chicks and reported that when chicks were fasted the level of fatty acid synthesis decreased. Even short term fasts of two or more hours result in a depression of fatty acid synthesis in chicks, and refeeding the

chicks for only 1 hour completely restores hepatic fatty acid synthesis (Yeh and Leveille, 1970).

Dietary Carbohydrate and Fat Deposition

Increasing dietary carbohydrate intake will result in an increased level of glycolysis (Compe et al., 2001) and thus result in an increased production of acetyl CoA. If the energy demands of the animal have been met, the acetyl CoA will not go through the Krebs cycle of metabolic reactions, but rather be used for fatty acid synthesis. Clarke et al. (1979) reported that increasing the level of dietary carbohydrate increased fatty acid synthesis and the activities of the enzymes involved in fat synthesis in the chick. Tanaka et al. (1983a) reported a linear relationship between the level of carbohydrate consumed and hepatic fatty acid synthesis in the chick. Although the oxidation of ^{14}C labeled acetate in liver slices was not affected by increasing dietary carbohydrate levels, the incorporation of ^{14}C labeled acetate into fatty acids was increased as dietary carbohydrates were increased (Tanaka et al., 1983a). The triglyceride content of the liver was also significantly increased as the level of dietary carbohydrate increased (Tanaka et al., 1983a).

The activities of glycolytic enzymes and the activities of fatty acid synthesis enzymes (malic enzyme and citrate lyase) increased as the concentrations of dietary carbohydrate increased (Tanaka et al., 1983a). The increase in the glycolytic enzymes was due to an increased flux of glucose through glycolysis, which resulted in an increased level of acetyl CoA available for fatty acid synthesis. The activities of citrate

lyase and malic enzyme were in turn increased to support the increased flux of acetyl CoA available for fatty acid synthesis.

The actual effects of carbohydrates on fatty acid synthesis are still uncertain to some extent since alterations in dietary carbohydrate have been at the expense of dietary fat or protein in most of the research that has been conducted. But, obviously dietary carbohydrates provide substrate for fatty acid synthesis, especially when there is an excess of energy intake.

Dietary Fat and Body Fat Deposition

At an enzymatic level, consuming dietary fat inhibits *de novo* lipogenesis in part because the bird incorporates the lipids obtained from the diet into its fat depots. Yeh and Leveille (1969) reported that an increase in dietary fat at the expense of dietary carbohydrate, while maintaining a constant calorie to protein ratio, significantly impaired fatty acid synthesis. Of course the question that arises from this report was if the alteration in fatty acid synthesis was due to a change in dietary fat or due to a decrease in dietary carbohydrate content. The slopes for dietary fat and dietary carbohydrate's affect on fatty acid synthesis were different, which Yeh and Leveille (1969) interpreted to indicate that dietary fat had a specific effect on fatty acid synthesis. To further support this point, Yeh and Leveille (1970) force fed corn oil to chicks and found that it reduced fatty acid synthesis within 1 h.

Tanaka et al. (1983b), reported that an increase in dietary fat resulted in a decreased incorporation in chick livers of ^{14}C labeled acetate into fatty acids. The increase in dietary fat had no effect on the activity of malic enzyme in the liver of chicks

(Tanaka et al., 1983b), which agreed with other reports (Yeh et al., 1970, Goodridge 1972). In contrast, the activity of citrate lyase is significantly depressed when dietary concentrations of fat are increased (Tanaka et al., 1983b). Yeh et al. (1970) proposed that an increase in circulating free fatty acids would result in an increased conversion rate of fatty acids to fatty acid CoA derivatives which would in turn deprive citrate lyase of its free CoA substrate. Therefore, an increase in the level of dietary fat could reduce the availability of free CoA, and in turn limit fatty acid synthesis. Indeed chicks fed a high fat diet for 2 hour had an increased level of hepatic long chain acyl-CoA fatty acids and a decreased level of free CoA and fatty acid synthesis (Yeh and Leveille, 1971b).

Dietary Protein and Fatty Acid Synthesis

Yeh and Leveille (1969) reported that fatty acid synthesis was depressed when chicks were fed increasing levels of dietary protein. They demonstrated the rate of incorporation of glucose-U-^{14}C, pyruvate-2-^{14}C and acetate-1-^{14}C into liver fatty acids was depressed in chicks consuming diets with a higher dietary protein level. Later, Leveille and Yeh (1972) fed chicks on a three day cycle consisting of a protein adequate diet for 2 days followed by a protein free diet on the third day and malic enzyme activity and fatty acid synthesis were markedly stimulated on the day the protein free diet was fed.

As explained previously, alterations in dietary carbohydrate appear to have an effect on fatty acid synthesis in the liver. In the above studies by Yeh and Leveille, in order to alter the level of dietary protein, the level of dietary carbohydrate was also altered to keep the diets isocaloric. Therefore, it was important to determine if the

alterations seen in fatty acid synthesis were really due to changes in dietary protein or reflected changes in the dietary carbohydrate concentrations. Tanaka et al. (1983b) demonstrated that when the level of dietary protein was increased while maintaining a constant level of dietary carbohydrate, the levels of fatty acid synthesis and malic enzyme activity decreased.

Subsequent research by Rosebrough et al. (1985a, 1985b, 1986a, 1988, 1990, 1996, 1999 and 2002) and others (Bartov et al., 1974, Skinner et al., 1991, Kouba et al., 1992, Eits et al., 2002, Sklan and Plavnik, 2002 and Wylie et al., 2003) has confirmed and strengthened the original finding that the activity of malic enzyme and the synthesis of liver fatty acids is decreased by increasing dietary levels of protein.

Nutrient Density and Fat Deposition

In commercial poultry production, rations are typically formulated to have a fixed relationship between the energy and crude protein components of the diet. This fixed ratio between these two components implies that an increase in the energy will be accompanied with an increase in crude protein and the other nutrients in the diet. Maintaining this ratio helps to prevent imbalances in the intake of any nutrient, especially energy and protein. Widening the calorie:crude protein ratio can result in an over consumption of energy to meet the protein requirement (Griffiths et al., 1977). Diets containing a low energy:crude protein ratio decrease carcass fat accumulation because broilers consume less energy in meeting their protein requirement (Griffiths et al., 1977, (Mabray and Waldroup), 1981, Summers and Leeson, 1985). In contrast, increasing the dietary energy:crude protein ratio increases total carcass fat (Sizemore and Siegel, 1993).

In a series of experiments Bartov et al. (1974) investigated the effect of feeding broiler chicks with a different calorie to protein (C:P) ratio on the degree of fatness. The fat supplemented diets resulted in greater body weight gains and skin fat in both males and females. Transferring birds from a diet with a low C:P ratio to one with a high C:P ratio resulted in rapid decrease in fat deposition primarily in the skin and the adipose tissue, with the less marked changes occurring in muscle tissue.

Feeding Whole Grains and the Deposition of Abdominal Fat

Grains are the largest component of poultry diets. Even though most of the grains used in poultry diets are ground, it has been recognized that the inclusion of whole grains in poultry diets could have some benefits in gastrointestinal development and function and therefore total nutrient utilization. The use of whole grains, especially wheat and barley, in poultry production has gained favor in many European countries as way to reduce feed costs (Svihus et al., 2002). Typically the whole grains are not incorporated into the pellet but simply mixed with the pellets prior to being delivered to the flock. Research indicates that feeding whole grains can improve starch digestibility (Hetland et al., 2002, Svihus and Hetland, 2001) and is not detrimental to feed utilization, when the whole grains are incorporated into broiler diets at low levels (Kiiskinen, 1996, Svihus et al., 1997, Jones and Taylor, 2001, Hetland et al., 2002).

The influence of feeding whole grains on body fat content in broilers has not been examined extensively. Jones and Taylor (2001) reported that abdominal fat pad weights relative to total body weight were significantly increased in broilers fed diets containing whole wheat versus ground wheat.

Summary

When chicks are starved, the level of fatty acid synthesis and the activity of malic enzyme decrease. In contrast, when chicks are well fed, the levels of malic enzyme activity and fatty acid synthesis are high. Increasing the concentration of dietary fat decreases the level of fatty acid synthesis in chicks, but does not alter malic enzyme activity. On the other hand, as dietary concentrations of carbohydrate increase the levels of fatty acid synthesis and malic enzyme increase. Dietary protein has been shown to have a profound effect on fatty acid synthesis. As the level of dietary protein increases the rate of fatty acid synthesis decreases.

3. DIETARY PROTEIN REGULATES MALIC ENZYME ACTIVITY

Malic Enzyme is Essential for Fatty Acid Synthesis in the Chick

In contrast to rats, de novo fatty acid synthesis in avian species occurs in the liver. Another aspect of de novo fatty acid synthesis that is unique to birds is the source of NADPH. Rats use the hexose monophasphate shunt dehydrogenase to provide the remaining 6 molecules of NADPH required for fatty acid synthesis, that are not provided by malic enzyme in the translocation of acetyl CoA from the mitochondria to the cytosol (Tepperman and Tepperman, 1964). The activity of the hexose monophosphate shunt dehydrogenase in pigeon adipose tissue, however, is only 4 percent of that found in rat adipose tissue (Ball and Merrill, 1961). The capacity for NADPH production by malic enzyme is 20 times greater in the pigeon liver when compared to rat liver (Goodridge and Ball, 1966). Therefore, for avian species it has been concluded that the activity of malic

enzyme provides all of the reducing equivalents necessary for fatty acid synthesis (Goodridge and Ball, 1967, Goodridge, 1968a, Tanaka et al., 1983a).

Given the importance of malic enzyme activity in de novo fatty acid synthesis in birds, it is not surprising that the activity of malic enzyme in the liver is highly, positively correlated with the rate of fatty acid synthesis, percent body fat and percent abdominal fat in chicks (Yeh and Leveille, 1969; Pfaff, 1977; Tanaka et. al., 1979a,b,c, 1983a,b; Grisoni et al., 1991). Tanaka et al. (1983 a,b) even suggested that the supply of NADPH regulated de novo fatty acid synthesis in chickens.

It is interesting to note that it remains unclear how the activity of malic enzyme in producing the extra 6 molecules of NADPH for fatty acid synthesis can be uncoupled from the fatty acid synthesis reaction. If malic enzyme activity produced all of the 14 NADPH molecules required for fatty acid synthesis, that would lead to a surplus of 6 cytosolic acetyl CoA molecules, since only 8 would be used to form a molecule of palmitate. To resolve this issue, more research is needed.

The Regulation of Malic Enzyme by Dietary Protein Intake

In addition to being regulated by feeding status, dietary protein level has a marked regulatory effect on malic enzyme activity. In force-feeding experiments with chicks, when dietary carbohydrate and fat were kept constant while dietary protein was increased, the activity of malic enzyme and fatty acid synthesis decreased (Tanaka et al., 1983b). Subsequent reports have strengthened this original finding that dietary protein is an intrinsic regulator of malic enzyme activity and the synthesis of liver fatty acids in

chicks (Rosebrough and Steele, 1985a, b, c; 1990; Rosebrough et al., 1986a, 1987, 1988, 1996, 1999, 2002).

Adams and Davis (2001) conducted an experiment in broiler chicks to determine whether changes in chicken hepatic malic enzyme activity and liver lipid concentrations related to dietary protein intake, were preceded by changes in the concentration of malic enzyme mRNA. Broiler chicks were fed semi-purified diets containing 13, 22 or 40% crude protein. Switching chicks from the basal (22%) protein diet to the low or high protein diet resulted in a rapid (3 h) change in the expression of the mRNA for malic enzyme. A switch to a low protein diet increased the level of malic enzyme mRNA, whereas feeding a high protein diet decreased its level. The changes in malic enzyme mRNA were associated with subsequent changes in malic enzyme activity (at 6 h) and total liver lipid concentration (at 24 h). Rosebrough et al. (2002) reported similar results in an experiment done in birds fed three different protein levels (12, 21 and 30%).

The Potential Role of Specific Amino Acids Regulating Malic Enzyme

The modification of malic enzyme due to dietary protein levels may be due to the levels of dietary amino acids rather than total protein content. Several reports demonstrate that dietary sulfur amino acids modify lipid metabolism (Rukaj and Serougne, 1983, Yagasaki et al., 1986, Sugiyama et al., 1986, Serougne and Rukaj, 1988) and decrease the activity of malic enzyme in rats (Ayala et al., 1991, Ide et al., 1992). Medonca and Jensen (1989) reported in chicks that the percent of the abdominal fat pad relative to total body weight declined linearly as sulfur amino acids were added in increasing amounts to a low protein diet. Pesti et al. (1996) reported similar results with

sulfur amino acids and abdominal fat accruement in experiments with normal and naked-neck chickens. Additionally, Takahashi and Akiba (1996) measured the activity of malic enzyme in chickens fed a 20 % crude protein diet supplemented with 0.60, 0.70, 0.80 or 0.90 % sulfur amino acids and observed that malic enzyme activity was decreased in the birds fed the 3 higher sulfur amino acid supplemented diets when compared to the birds fed the lowest sulfur amino acid supplemented diet.

In addition to the reports with sulfur amino acids, Tanaka et al. (1992) reported lower malic enzyme activity levels in chicks fed a diet based on either soybean or corn gluten protein than those fed diets based on casein or a fish based protein diet. Finally, Yeh and Leveille (1969) and Rosebrough et al. (1986b) reported that the addition of lysine to a low protein diet depressed fatty acid synthesis in chicks.

Hormonal Regulation of Malic Enzyme

Thyroid Hormone

When liver cells from chick embryos were incubated with T_3 a 23-fold increase in malic enzyme activity was seen (Goodridge and Adelman, 1976). Malic enzyme activity returned to baseline levels when T_3 was removed from the cell culture medium. Chandrabose and Bensadoun (1971) reported that a hypophysectomy depressed hepatic malic enzyme activity in chicks and that T_4 injections increased malic enzyme activity in both hypophysectomized and intact chicks.

The effect of thyroid hormone on malic enzyme activity appears to be mediated through changes in mRNA concentrations. After a 48 hour incubation with T_3, malic

enzyme mRNA levels were 21 times higher than the level before the addition of T_3, and 14 times higher than the level found in cells incubated for 48 hours without T_3 (Back et al., 1986). In a subsequent report, the transcription of the malic enzyme gene was elevated within 1 h of adding T_3 to cultured chick hepatocytes and maximal transcriptional rates were seen 6 h after the addition of T_3 (Salati et al., 1991).

More recently, considerable research has been done on the promoter region of the chicken malic enzyme gene. The increase in malic enzyme transcription due to T_3 is mediated by at least 6 T_3 response elements (Hodnett et al., 1996, Thurmond and Goodridge, 1998, Fang and Hillgartner, 2000) that bind the T_3/receptor complex. Each of the response elements confers T_3 responsiveness by inhibiting transcription in the absence of T_3 and activating transcription in its presence (Hodnett et al., 1996, Thurmond and Goodridge, 1998). Additionally, the T_3 receptor appears to be capable of recruiting co-repressor and co-activator complexes that either enhance or depress malic enzyme transcription (Wang et al., 2002). Furthermore, additional response elements have been identified in the malic enzyme promoter sequence (Wang et al., 2002) that would be capable of binding other unidentified transcriptional factors.

Glucagon

The typical stimulation of malic enzyme activity that results from incubating chick hepatocytes with T_3, is inhibited by 97% when glucagon is added to the cell culture medium with T_3 (Goodridge and Adelman, 1976). Back et al. (1986) conducted a similar experiment and determined that glucagon inhibited the T_3 response by 93%. Lefevre et al. (1999) reported that the addition of glucagon to cultured chick hepatocytes

significantly depressed malic enzyme mRNA levels. The effect of glucagon appears to be mediated by cyclic AMP (cAMP). Cramb et al. (1982), reported that glucagon increased cAMP levels up to 60-fold within 2-10 min of administration to cultured chicken hepatocytes. Furthermore, the 5 prime region of the chicken malic enzyme gene has been shown to contain response elements for cAMP, which mediates the effect of glucagon on malic enzyme mRNA synthesis (Mounier et al., 1997).

Insulin

The addition of insulin to chick hepatocyte cultures has only a slight, but significant, stimulatory effect on malic enzyme synthesis, but no effect on malic enzyme mRNA levels (Back et al., 1986). When insulin was supplemented to chick hepatocyte cell cultures along with T_3 ,the activity of malic enzyme was 77 times greater than the activity level found in untreated cells, 40 times greater than the activity in cells treated with only insulin and 3.3 times greater than the activity found in cells treated with only T_3 (Goodridge and Adelman, 1976).

Transciptional Factors Regulating Malic Enzyme

Two transcription factors involved in the regulation of malic enzyme expression have been identified, spot 14 and sterol regulatory element binding protein 1 (SREBP-1). Spot 14, also called thyroid hormone responsive spot 14 (THRSP), was discovered in studies of thyroid hormone action in hepatocytes (Seelig et al., 1982). Spot 14 is a nuclear protein that is abundant only in lipogenic tissues, its expression is rapidly regulated by hormones and dietary constituents (Cunningham et al., 1997).

The exact molecular mechanism by which spot 14 regulates lipogenic enzyme expression as a transcription factor, remains unclear. Kinlaw et al. (1995) investigated the relationship between the expression of spot 14 and malic enzyme in hepatocyte cultures. Hepatocytes transfected with a spot 14 antisense oligonucleotide had decreased mRNA levels of malic enzyme. Spot 14 mRNA levels are increased by feeding carbohydrate rich diets or giving insulin injections and decreased by high plasma glucagon levels (Jump et al., 1993).

SREBP-1 is another transcription factor that is localized in lipogenic tissues. It binds to the sterol response element (SRE) found in the promoter region of several lipogenic enzymes such as fatty acid synthase and aceytl CoA carboxylase (Yin et al., 2002). More importantly, SREBP-1 binds to the promoter of the spot 14 gene (Mater et al., 1999, Jump et al., 2001) and is required for the T_3 induction of spot 14 gene transcription (Jump et al., 2001).

Summary

Dietary protein is a potent regulator of malic enzyme activity. As dietary protein increases the activity of malic enzyme and hence de novo fatty acid synthesis decreases. The effect of dietary protein on malic enzyme activity is mediated through a very fast reduction in the hepatic mRNA concentration of malic enzyme when dietary protein consumption increases. It is unclear what triggers the decrease in the mRNA expression of malic enzyme as protein intake increases, but dietary protein effect may be mediated by changes in glucagon or T_3 production. Both of these hormones can influence the production of the two transcription factors, spot 14 and SREBP-1, which may regulate malic enzyme mRNA production.

4. STATEMENT OF PURPOSE

The problem of excessive body fat in broilers continues to be a financial liability for the poultry industry. Continuous genetic selection for improved performance characteristics has inadvertently led to strains of broilers which tend to accumulate excessive body fat, especially in the abdominal area. As an increasing proportion of poultry products are sold as cut-up and deboned products, it is no longer possible to include fat pads with the whole carcass. Thus, fat pads enter the rendering chain at a drastic decrease in value. Therefore, a better understanding of the factors that contribute to body fat synthesis in the bird is critical.

One applied research goal of the present work is to determine if feeding broilers increasing levels of dietary fat will increase fat pad weights as a percent of total body weight, if the diets also contain increasing levels of dietary protein. In commercial broiler production, a diverse array of ME values is used in formulating broiler diets (Agri Stats, 2002). In more energy dense diets containing a lot of fat it may be necessary to increase the dietary protein levels to prevent excess body fat accumulation.

Another practical aspect of the current research is to determine if feeding low levels of whole pearl millet to broilers throughout their production cycle will increase fat pad yields. Feeding whole grains to broilers is gaining in popularity, however, one initial report indicates that feeding low levels of whole wheat had the unexpected outcome of increasing fat pad yields. If a similar result is obtained with whole pearl millet, more

research will have to be focused on determining why this unwanted side effect of feeding whole grains is occurring.

At the molecular level, the goal of the current research is to determine if spot 14 mRNA expression is quickly altered by changes in dietary protein intake. By determining if spot 14 is involved in regulating dietary proteins effect on malic enzyme mRNA expression in broilers, this research could demonstrate the benefit in using spot 14 mRNA expression as a genetic selection marker for lean broilers and provide further insight into the design of practical broiler diets that inhibit *de novo* fatty acid synthesis.

REFERENCES

Adams, K. A., and A. J. Davis. 2001. Dietary protein concentration regulates the mRNA expression of chicken hepatic malic enzyme. J. Nutr. 131(9):2269-2274.

Agri Stats, Inc., (Jan.-Jun. 2002). Fort Wayne, IN 46825.

Ayala, A., E. Gordillo, M. F. Lobato and A. Machado. 1991. Involvement of diminution of glutathione, produced by deficiency of methionine in the diet, in the elevation of malic enzyme level in rat liver. Biochim. Biophys. Acta 1084(1):48-52.

Back, D. W., M. J. Goldman, J. E. Fisch, R. S. Ochs and A. G. Goodridge. 1986. The fatty acid synthase gene in avian liver. Two mRNAs are expressed and regulated in parallel by feeding, primarily at the level of transcription. J. Biol. Chem. 261(9):4190-4197.

Ball, E. G., and M. A. Merrill. 1961. A manometric assay of insulin and some results of the application of the method to sera and islet-containing tissues. Endocrinology 69:596-607.

Ballard, F. J., R. W. Hanson and D. S. Kronfeld. 1969. Gluconeogenesis and lipogenesis in tissue from ruminant and nonruminant animals. Fed. Proc. 28(1):218-231.

Bartov, I., S., Bornstein and B. Lipstein. 1974. Effect of calorie to protein ratio on the degree of fatness in broiler fed on practical diets. Br. Poult. Sci. 15:107-117.

Chandrabose, K. A., and A. Bensadoun. 1971. Effect of hypophysectomy on some enzymes involved in lipid metabolism of the domestic chicken (Gallus domesticus). Comp. Biochem. Physiol. B 39(1):45-54.

Clarke, S. D., P. A. Watkins and M. D. Lane. 1979. Acute control of fatty acid synthesis by cyclic AMP in the chick liver cell: possible site of inhibition of citrate formation. J. Lipid. Res. 20(8):974-985.

Compe, E., G. de Sousa, F. Kamel, R. Regis, R. Roger, T. Janine, R. Michel and R. Planells. 2001. Spot 14 protein interacts and co-operates with chicken ovalbumin upstream promoter-transcription factor 1 in the transcription of the L-type pyruvate kinase gene through a specificity protein 1 (Sp1) binding site. Biochem. J. 358(Pt 1):175-183.

Cramb, G., D. R. Langslow and J. H. Phillips. 1982. Hormonal effects on cyclic nucleotides and carbohydrate and lipid metabolism in isolated chicken hepatocytes. Gen. Comp. Endocrinol. 46(3):310-321.

Cunningham, B. A., M. Maloney and W. Kinlaw. 1997. Spot 14 protein-protein interactions: evidence for both homo- and heterodimer formation in vivo. Endocrinology 138(12):5184-5188.

Eits, R. M., R. P. Kwakkel, M. W. Verstegen, P. Stoutjedijk and K. H. De Greef. 2002. Protein and lipid deposition rates in male broiler chickens: Separate responses to amino acids and protein-free energy. Poul. Sci. 81:472-480.

Fang, X., and F. B. Hillgartner. 2000. Alterations in retinoid X receptor-alpha expression contribute to cell-type dependent differences in thyroid hormone regulation of malic enzyme transcription. Mol. Cell. Endocrinology 164(1-2):41-52.

Goodridge, A.G., and E. G. Ball. 1966. Lipogenesis in the pigeon: In vitro studies. Am. J. Physiol. 211:803-808.

Goodridge, A. G., and E. G. Ball. 1967. Lipogenesis in the pigeon: In vivo studies. Am. J. Physiol. 213:245-249.

Goodridge, A. G., 1968a. Citrate-cleavage enzyme, 'malic' enzyme and certain dehydrogenases in embryonic and growing chicks. Biochem. J. 108(4):663-666.

Goodridge, A. G., 1968b. The effect of starvation and starvation followed by feeding on enzyme activity and the metabolism of [U-14C]glucose in liver from growing chicks. Biochem. J. 108(4):667-673.

Goodridge, A. G., 1972. Regulation of the activity of acetyl coenzyme A carboxylase by palmitoyl coenzyme A and citrate. J. Biol. Chem. 247:6946-6952.

Goodridge, A. G., and T. G. Adelman. 1976. Regulation of malic enzyme synthesis by insulin triiodothyronine, and glucagon in liver cells in culture. J. Biol. Chem. 251(10):3027-3032.

Griffiths, L., S. Lesson and J. D. Summers. 1977. Fat deposition in broilers: Effect of dietary energy to protein balance, and early life caloric restriction on productive performance and abdominal fat pad size. Poul. Sci. 56:638-646.

Grisoni, M. L., G. Uzu, M. Larbier and P. A. Geraert. 1991. Effect of dietary lysine level on lipogenesis in broilers. Reprod. Nutr. Dev. 31(6):683-690.

Hetland, H., B. Svihus and V. Olaisen. 2002. Effect of feeding whole cereals on performance, starch digestibility and duodenal particle size distribution in broilers chickens. Br. Poult. Sci. 42:416-423.

Hodnett, D. W., D. A. Fantozzi, D. A. Thurmond, S. A. Klautky, K. G. MacPhee, S. T. Estreem, G. Xu and A. G. Goodridge. 1996. The chicken malic enzyme gene: structural organization and identification of triiodothyronine response elements in the 5'-flanking DNA. Arch. Biochem. Biophys. 334(2):309-324.

Ide, T., M. Murata and Y. Sunada. 1992. Soybean protein-dependent changes in triacylglycerol synthesis and concentration of diacylglycerol in the liver microsomes of fasted-refed rats. Ann. Nutr. Metab. 36(2):87-96.

Jones, G. P. D. and R. D. Taylor. 2001. The incorporation of whole grain into pelleted broiler chicken diets: production and physiological responses. Brit. Poult. Sci. 42:477-483.

Jump, D. B., S. D. Clarke, O. MacDougald and A. Thelen. 1993. Polyunsaturated fatty acids inhibit S14 gene transcription in rat liver and cultured hepatocytes. Proc. Natl. Acad. Sci. U S A 90(18):8454-8458.

Jump, D. B., A. P. Thelen and M. K. Mater. 2001. Functional interaction between sterol regulatory element-binding protein-1c, nuclear factor Y, and 3,5,3'-triiodothyronine nuclear receptors. J. Biol. Chem. 276(37):34419-34427.

Kinlaw, W. B., J. L. Church, J. Harmon and C. N. Mariash. 1995. Direct evidence for a role of the "spot 14" protein in the regulation of lipid synthesis. J. Biol. Chem. 270(28): 6615-6618.

Kiiskinen, T. 1996. Feeding whole grain with pelleted diets to growing broiler chickens. Agricultural and Food Science in Finland 5:167-175.

Kouba, M., D. Catheline and B. Leclercq. 1992. Lipogenesis in turkeys and chickens: a study of body composition and liver lipogenesic enzyme activity. Brit. Poul. Sci. 33:1003-1014.

Lefevre, P., C. Diot, P. Legrand and M. Douaire. 1999. Hormonal regulation of stearoyl coenzyme-A desaturase I activity and gene expression in primary culture of chicken hepatocytes. Arch. Biochem. and Biophys. 368:329-337.

Leveille, G. A., E. K. O'Hea and K. Chakrabarty. 1968. In vivo lipogenesis in the domestic chicken. P.S.E.M.B. 128:398-401.

Leveille, G. A., and Y. Y. Yeh. 1972. Influence of intermittent fasting or protein-free feeding on lipid metabolism in young cockerels. J. Nutr. 102(6):733-740.

Mabray, C. J. And P. W. Waldroup. 1981. The influence of dietary energy and amino acid levels on abdominal fat pad development of the broiler chicken. Poult. Sci. 60 (1):151-159.

Mater, M. K., A. P. Thelen, D. A. Pan and D. B. Jump. 1999. Sterol response element-binding protein 1c (SREBP1c) is involved in the polyunsaturated fatty acid suppression of hepatic S14 gene transcription. J. Biol. Chem. 274(46):32725-32732.

Mendonca, C. X., and L. S. Jensen. 1989. Influence of protein concentration on the sulfur amino acid requirement of broiler chickens. Brit. Poul. Sci. 30:889-898.

Mounier, C., W. Chen, S. A. Klautky and A. G. Goodridge. 1997. Cyclic AMP-mediated inhibition of transcription of the malic enzyme gene in chick embryo hepatocytes in culture characterization of a cis-acting element far upstream of the promoter. J. Biol. Chem. 272(38):23606-23615.

Pesti, G., M., B. Leclercq, A.M. Chagneau, T. Cochard. 1996. Effects of the naked neck (NA) gene on the sulfur-containing amino acid requirement of broilers. Poul. Sci. 75:375-380.

Pfaff, F. E., Jr. 1977. Effects of dietary protein and amino acids on carcass composition and lipogenesis in the growing chick. Ph.D. Thesis, Cornell University, Ithaca, NY. pp. 1-159.

Rosebrough, R. W. and N. C. Steele. 1985a. Energy and protein relations in the broiler chicken. 2. Effect of varied protein and constant carbohydrate levels on body composition and lipid metabolism. Growth 49:479-489.

Rosebrough, R. W., and N. C. Steele. 1985b. Energy and protein relationships in the broiler. 1. Effect of protein levels and feeding regimens on growth, body composition, and in vitro lipogenesis of broiler chicks. Poul. Sci. 64:119-126.

Rosebrough, R. W., and N. C. Steele. 1985c. Protein and energy relations in the broiler. 3. Growth and in vitro metabolism in male and female chickens used as parent stock. Growth 49:63-77.

Rosebrough, R. W., N. C. Steele, J. P. McMurtry, M. P. Richards, A. D. Mitchell and C. C. Calvert. 1986a. Energy and protein relations in the broiler chicken. 4. Role of sex, line and substrate on in vitro lipogenesis. Growth 50:461-471.

Rosebrough, R. W., A. D. Mitchell and N. C. Steele. 1986b. Lysine status and in vivo lipid metabolism in chicks. Poul. Sci. 65 (Sup 1), 115.

Rosebrough, R. W., P. McMurtry and N. C. Steele. 1987. Protein and energy relations in the broiler. 5. Lipogenesis, glucose production and metabolic hormone levels as functions of age and dietary protein levels. Growth 51:309-320.

Rosebrough, R. W., J. P. McMurtry, A. D. Mitchell and N. C. Steele. 1988. Chicken hepatic metabolism in vitro. Protein and energy relations in the broiler chicken-VI. Effect of dietary protein and energy restrictions on in vitro carbohydrate and lipid metabolism and metabolic hormone profiles. Comp. Biochem. Physiol. 90B:311-316.

Rosebrough, R. W., A. D. Mitchell and J. P. McMurtry. 1996. Dietary crude protein changes rapidly alter metabolism and plasma insulin–like growth factor I concentration in broiler chickens. J. Nutr. 126:2888-2898.

Rosebrough, R. W. and N. C. Steele. 1990. Dietary crude protein levels and the effect of isoproterenol on in vitro lipogenesis in the chicken. J. Nutr. 120:1684-1691.

Rosebrough, R. W., J. P. McMurtry and R. Vasilatos-Younken. 1999. Dietary fat and protein interactions in the broiler. Poul. Sci. 78:992-998.

Rosebrough, R. W., S. M. Poch, B. A. Rusell and M. P. Richards. 2002. Dietary protein regulates in vitro lipogenesis and lipogenic gene expression in broilers. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 132(2):423-431.

Rukaj, A. and C. Serougne. 1983. Effect of excess dietary cystine on the biodynamics of cholesterol in the rat. Biochem. Biophys. Acta. 753(1):1-5.

Salati, L. M., X. J. Ma, C. C. McCormick, S. R. Stapleton and A. G. Goodridge. 1991. Triiodothyronine stimulates and cyclic AMP inhibits transcription of the gene for malic enzyme in chick embryo hepatocytes in culture. J. Biol. Chem. 266(6):4010-4016.

Seelig, S., D. B. Jump, H. C. Towle, C. Liaw, C. N. Mariash H. L. Schwartz and J. R. Openheimer. 1982. Paradoxical effects of cycloheximide on the ultra-rapid induction of two hepatic mRNA sequences by triiodothyronine (T3). Endocrinology 110(2):671-6713.

Serougne, C. and A. Rukaj. 1988. Induction of long-lasting hypercholesterolemia in the rat fed cystine-enriched diet. Lipids 23:930-936.

Shargo, E., J. A. Glennon and E. S. Gordon. 1971. Comparative aspects of lipogenesis in mammalian tissues. Metabolism 20(1):54-62.

Sizemore, F.G. and H. S. Siegel. 1993. Growth, feed conversion, and carcass composition in females of 4 broiler crosses fed starter diets with different energy-levels and energy to protein ratios. Poul. Sci. 72 (12):2216-2228.

Skinner, J., A. Izat and P. Waldroup. 1991. Effect of dietary amino acid levels on performance and carcass composition of broilers 42 to 49 days of age. Poul. Sci. 70:1223-1230.

Sklan, D. and I. Plavnik. 2002. Interactions between dietary crude protein and essential amino acid intake on performance in broilers. Brit. Poul. Sci. 43:442-449.

Sugiyama, K., M. Mizuno and K. Mizumo 1986. Effect of individual amino acids on plasma cholesterol level in rats fed a high cholesterol diet. J. Nutr. Sci. Vitaminol. (Tokyo) 32(6):623-633.

Summers, J. and S. Lesson. 1985. Broiler carcass composition as affected by amino acid supplementation. Can. J. Anim. Sci. 65:717-723.

Svihus, B., O. Herstad, C. W. Newman and R. K. Newman. 1997. Comparison of performance and intestinal characteristics of broiler chickens fed on diets containing whole, rolled or ground barley. Bri. Poul. Sci. 38:524-529.

Svihus, B. and H. Hetland. 2001. Ileal starch digestibility in growing broiler chickens fed on wheat-based diet is improved by mash feeding, dilution with cellulose or wheat inclusion. Br. Poul. Sci. 42:633-637.

Svihus, B., H. Hetland, M. Choct and F. Sundby. 2002. Passage through the anterior digestive tract of broiler chickens fed on diets with ground and whole wheat. Brit. Poul. Sci. 43:662-668.

Takahashi, K. and Y. Akiba. 1996. Influence of dietary protein, sulfur amino acid content and their ratio on activities of malic enzyme, fatty acid synthetase and hormone sensitive lipase in male broilers. Anim. Sci. Technol. 67:305-309.

Tanaka, K., K. Kitahara and K. Shigeno.1979a. Effect of dietary protein level on lipid metabolism in growing chicks. Jap. J. Zootech. Sci. 50:44-45.

Tanaka, K., N. Akazaki, C. M. Collado, S. Ohtani and K. Shigeno. 1979b. Effect of dietary essential fatty acid on accumulation of lipids in the liver of growing chicks. Jap. J. Zootech. Sci. 50:563-573.

Tanaka, K., K. Kitahara and K. Shigeno. 1979c. Effect of dietary protein level on lipid metabolism in growing chicks. Jap. J. Zootech. Sci. 50:100-107.

Tanaka, K., S. Ohtani and K. Shigeno. 1983a. Effect of increasing dietary energy on hepatic lipogenesis in growing chicks. I. Increasing energy by carbohydrate supplementation. Poul. Sci. 62:445-451.

Tanaka, K., S. Ohtani and K. Shigeno. 1983b. Effect of increasing dietary energy on hepatic lipogenesis in growing chicks. II increasing energy by fat or protein supplementation. Poul. Sci. 62:452-458.

Tanaka, K., Okamoto and S. Ohtani. 1992. Effects of dietary energy on lipogenic enzymes in the liver of growing chicks. Asian-Australas. J. Anim. Sci. 5:123-128.

Tepperman, H. M. and J. Tepperman. 1964. Patterns of dietary and hormonal induction of certain NADP-linked liver enzymes. Am. J. Physiol. 206:357-361.

Thurmond, D. C. and A. G. Goodridge. 1998. Characterization of thyroid hormone response elements in the gene for chicken malic enzyme. Factors that influence triiodothyronine responsiveness. J. Biol. Chem. 273(3):1613-1622.

Wang, Y., Y. Zhang and F. B. Hillgartner. 2002. Chicken ovoalbumin upstream-promoter transcription factor and E-box binding proteins enhance thyroid-hormone responsiveness of the malic enzyme gene in avian hepatocytes. Biochem. J. 361:391-400.

Wylie, L. M., G. W. Robertson and P. M. Hocking. 2003. Effects of dietary protein concentration and specific amino acids on body weight, body composition and feather growth in young turkeys. Brit. Poul. Sci. 44:75-87.

Yagasaki, K., M. Machida and R. Funabiki. 1986. Effects of dietary methionine, cystine, and glycine on endogenous hypercholesterolemia in hepatoma-bearing rats. J. Nutr. Sci. Vitaminol. (Tokyo) 32(6):643-651.

Yeh, Y. Y. and G. A. Leveille. 1969. Effect of dietary protein on hepatic lipogenesis in the growing chick. J. Nutr. 98:356-366.

Yeh, Y. Y. and G. A. Leveille. 1970. Hepatic fatty acid synthesis and plasma free fatty acid levels in chicks subjected to short periods of food restriction and refeeding. J. Nutr. 100:1389-1398.

Yeh, Y. Y. and G. A. Leveille. 1971a. In vitro and in vivo restoration of hepatic lipogenesis in fasted chicks. J. Nutr. 101(6):803-809.

Yeh, Y. Y. and G. A. Leveille. 1971b. Studies on the relationship between lipogenesis and the level of coenzyme A derivatives, lactate and pyruvate in chick liver. J. Nutr. 101(7):911-918.

Yin, L., Y. Zhang and F. B. Hillgartner. 2002. Sterol regulatory element-binding protein-1 interacts with the nuclear thyroid hormone receptor to enhance acetyl-CoA carboxylase-alpha transcription in hepatocytes. J Biol. Chem. 277(22):19554-19565.

CHAPTER 2

THE USE OF WHOLE PEARL MILLET IN BROILER DIETS[1]

[1] Hidalgo M. A., A. J. Davis, N. M. Dale and W. A. Dozier, III. Published in Journal of Applied Poultry Science 2004 13:229-234.

ABSTRACT

Previous research indicates that performance and carcass yields of broilers fed diets containing up to 50% of a rust resistant hybrid pearl millet (TifGrain 102) were equivalent or better than those of broilers fed typical corn-soybean diets. As production of new cultivars of pearl millet increases, availability of grain may be limited and seasonal. Therefore, inclusion of lower levels of millet as whole seeds in broiler diets may be more suitable. To determine the feasibility of including whole millet seeds into broiler diets, three experiments were conducted. In two battery studies broilers were fed diets containing 0, 5, 10, 15 and 20% whole millet. Disappearance of whole millet was greater than 95% for all treatments as determined by the general absence of millet seed in the excreta. Performance of broilers from 1 to 15 d was unaffected by the inclusion of whole millet in the diets but gizzard size increased in birds fed diets containing 10% or more pearl millet. In a final experiment, the performance and carcass yields of broilers fed diets containing 5 or 10% whole millet from 1 to 42 d of age were equivalent to those fed typical corn-soybean meal diets. These results indicate that whole seeds of millet can be successfully incorporated into broiler diets without compromising bird performance.

KEY WORDS: pearl millet, whole seeds, broiler, carcass yield.

INTRODUCTION

Pearl Millet (*Pennisetum glaucum*) is native to the western edges of the Sahara desert and is commonly grown as a forage and grain crop in arid areas of Africa and India. It grows well under conditions of erratic rain, high temperatures, and poor soil conditions. These agronomical characteristics make pearl millet an appealing grain option for regions such as the coastal plain of the southern United States where soils are also acidic and low in natural fertility and droughts are common. Additionally, pearl millet is a fast growing crop with an extended planting season in the southern United States.

A practical problem in growing pearl millet is that it is susceptible to rust disease which can cause significant losses of yield and grain weight [1]. TifGrain 102, developed at the Coastal Plain Experimental Station at Tifton, Georgia, however, is resistant to this fungal disease. Previous research with this hybrid indicates that pearl millet had a comparable TME_N, value (3,300 to 3,448 kcal/kg) and higher protein content (12 to 14 %) than corn [2]. In addition, the performance and carcass yield of broilers fed diets containing up to 50 % ground pearl millet were equivalent or better than those of broilers fed typical corn-soybean meal diets [2].

The use of whole grains, especially wheat and barley, in poultry production has gained favor in many European countries as a way to reduce feed costs. Typically the whole grains are not incorporated into the pellet but simply mixed with the pellets prior to being delivered to the flock. Research indicates that feeding whole grains can improve starch digestibility [3, 4] and is not detrimental to feed utilization when the whole grains are incorporated into broiler diets at low levels [3, 5, 6, 7].

Given that millet is relatively small (2-3 mm) in size, and that supplies of this grain will be limited and seasonal as cultivation of this pearl millet hybrid increases over the next few

years, it may be feasible to incorporate modest levels of this grain into broiler diets in whole form. Additionally, few feed mills have post-grinding storage capacity for more than one grain; therefore, the goal of the present research was to determine if whole pearl millet grain could be incorporated into pelleted broiler diets without adversely affecting pellet quality or broiler performance.

MATERIAL AND METHODS

The TifGrain 102 pearl millet grain used for these experiments was harvested in the fall of 2002 in Georgia. Proximate composition [8] and true metabolizable energy (TME_N) [9-11] were determined for the freshly harvested grain.

Experiment 1 (Disappearance of Whole Seeds)

Day-old Cobb x Cobb unsexed chicks from a commercial hatchery were housed in a Petersime electrically heated battery brooder. The chicks were given constant illumination and had free access to water and a standard broiler starter diet. At 14 d of age the chicks were divided into four dietary treatments each consisting of 4 replicate pens of 6 birds. The dietary treatments consisted of a mash corn/soybean diet containing either 5, 10, 15 or 20% whole pearl millet (Table 1). The birds were fasted for 12 hours to clear the digestive tract and then allowed to consume the experimental diets for 48 hours. Total feed consumption for the 48 hour period was determined for each pen. Total feces produced over a 60 hour period that commenced when access to the experimental diets was given, were collected and weighed for each pen. The excreta from each pen was mixed and then three subsamples (1, 3 and 5 g) of excreta from each pen were examined for the presence of whole millet seeds. The percent disappearance of pearl

millet was then calculated based on the number of whole pearl millet seeds found in the excreta samples divided by the number consumed, multiplied by 100, and averaged across the three subsamples.

Experiment 2 (Initial Growth Study)

This experiment utilized the same dietary treatments as in Experiment 1, plus a corn-soybean control mash diet (Table 1). Day-old Cobb x Cobb unsexed chicks were randomly allocated to 24 pens in a Petersime electrically heated battery brooder. The chicks were given constant illumination and had free access to water and food. Each dietary treatment consisted of 5 replicate pens of 7 chicks per pen except for the control treatment which only had 4 replicate pens of 7 chicks.

At the end of the 15 d experimental period, weight gain and feed consumption were measured for each pen. In addition, individual body weights were obtained for each chick in order to determine relative gizzard weight. Chicks were killed by cervical dislocation and the gizzards removed. The gizzard from each bird was then weighed after the cuticule and feed contents had been removed.

Experiment 3

One thousand and fifty six Cobb x Cobb unsexed 1-d-old chicks obtained from a commercial hatchery were randomly allocated to 24 floor pens in an environmental controlled broiler house. Each pen contained 44 birds (0.91 ft^2 bird). Eight of the pens were fed a control corn/soy diet while the other pens were fed similar diets containing either 5 or 10% whole millet (Table 2). The starter diets were fed from 1–17 d of age, grower diets from 18-36 d of age, and

the finisher diets from 37-42 d of age. The starter diets were fed in crumble form while the grower and finisher diets were fed as pellets. Weight gain and feed consumption were determined for each pen on d 17, 36 and 42 of the experiment. Mortality was monitored daily. At the end of the experiment, 10 birds (5 females, 5 males) from each replicate pen were randomly selected and processed to obtain fresh pre-chilled carcass weights. In addition, 2 female and 3 male broilers were also randomly selected from each pen for determination of fat pad weight. Duplicate samples of the grower and finisher diets were evaluated for pellet durability index [12].

RESULTS AND DISCUSION

The pearl millet was determined to have a TME_N of 3,263 kcal/kg. The crude protein, fat, and fiber content of the pearl millet were 11.7, 4.84 and 2%, respectively. These values were similar to those reported previously for this cultivar [2]. In Experiment 1, food consumption during the 48 hour period was not different among the dietary treatments (data not shown). Digestion of the whole pearl millet, as measured by the presence of whole pearl millet seed in the excreta, was (mean ± SEM) 98.5 ± 0.5, 95.3 ± 1.1, 98.3 ± 0.5 and 98.5 ± 0.6 % for diets containing 5, 10, 15 and 20% whole pearl millet, respectively. There were no statistical differences in the digestibility values between the dietary treatments. These results indicate that whole pearl millet seeds can be readily broken down by young broilers and thus be incorporated into their diets.

When whole pearl millet seeds were incorporated into broiler diets from 1 to 15 d of age, performance was equivalent to broilers fed a standard corn-soybean diet (Table 3). The relative

gizzard weight increased in the birds fed 10% or more pearl millet (Table 3). Although the pearl millet seed is relatively small, the increase in gizzard size is likely a muscular adaptation to increased grinding activity. The increase in gizzard size is consistent with previous reports that indicate feeding whole grains such as wheat, triticale, and barley increase gizzard size [5-7].

Pellet quality as measured by the pellet durability index was not significantly altered by including 5 or 10% whole pearl millet in a corn-soybean meal diet (Table 4). This finding was not surprising given that the whole pearl millet seeds are smaller than many of the corn particles used in the diets. In Experiment 3, no differences in feed conversion or weight gain were observed at the end of the starter, grower and finisher periods (Table 5). There was no difference in the mortality between the treatments (data not shown).

Percent carcass yield was not different between the pearl millet and the control birds (Table 6). Interestingly, there was a significant increase in the fat pad as a percent of live weight for the birds fed the diet containing 10% whole millet (Table 6). It is not clear why the birds fed 10% whole pearl millet had an increase in fat pad weight, but similar results have been seen when utilizing whole wheat versus ground wheat in diets [7]. One might assume that more energy would be needed to digest whole grain, leaving less for carcass fat deposition, but this effect was not observed.

CONCLUSIONS AND APPLICATIONS

1. A high percentage of whole pearl millet seeds are broken down in the gastrointestinal tract of broilers.
2. Incorporation of up to 10% whole pearl millet in diets given throughout an entire production period does not adversely affect broiler performance or pellet quality.

3. These findings improve the feasibility of using pearl millet in poultry diets since it can be utilized successfully in whole seed form. Use of whole seeds will eliminate the costs associated with grinding millet and the need for bin space to store ground millet.

REFERENCES AND NOTES

1. Wilson, J. P., W. W. Hanna, G. Gascho, and D. M. Wilson. 1995. Pearl millet grain yield loss from rust infection. Pages 54-56 in: First Natl. Grain Pearl Millet Symp. Proc., Tifton, GA . I. D. Teare, ed. University of Georgia Coastal Plain Experiment Station, Tifton, GA.
2. Davis A. J., N. M. Dale, and F. J. Ferreira. 2003. Pearl millet as an alternative feed ingredient in broiler diets. J. Appl. Poult. Res. 12:137-144.
3. Hetland H., B. Svihus, and V. Olaisen. 2002. Effect of feeding whole cereals on performance, starch digestibility and duodenal particle size distribution in broilers chickens. Br. Poult. Sci. 42:416-423.
4. Svihus B., and H. Hetland. 2001. Ileal starch digestibility in growing broiler chickens fed on wheat-based diet is improved by mash feeding, dilution with cellulose or wheat inclusion. Br. Poult. Sci. 42:633-637.
5. Kiiskinen, T. 1996. Feeding whole grain with pelleted diets to growing broiler chickens. Agricultural and Food Science in Finland 5:167-175.
6. Svihus B., O. Herstad, C. W. Newman, and R. K. Newman. 1997. Comparison of performance and intestinal characteristics of broiler chickens fed on diets containing whole, rolled or ground barley. Bri. Poult. Sci. 38:524-529.

7. Jones G. P. D., and R. D. Taylor. 2001. The incorporation of whole grain into pelleted broiler chicken diets: production and physiological responses. Bri. Poult. Sci. 42:477-483.

8. Association of Official Analytical Chemists. 1984. Official Methods of Analysis, 14th ed. Association of Official Analytical Chemists, Arlington, VA.

9. Sibbald, I.R. 1976. A bioassay for true metabolizable energy of feedingstuffs. Poult. Sci. 55:303-308.

10. Dale, N.M., and H.L. Fuller. 1984. Correlation of protein content of feedstuffs with the magnitude of nitrogen correction in true metabolizable energy determinations. Poult. Sci. 673:1008-1012.

11. Following a fasting period of 30 hours to clear the digestive tract, 30 grams of each sample were precision fed to ten White Leghorn roosters, with an additional ten serving as controls. A 48 hour collection period was used.

12. American Society of Agricultural Engineers. 1992. Cubes, pellets, and crumbles – Definitions and methods for determining density, durability, and moisture content. Pages 384-386 in American Society of Agricultural Engineers Standard S269. 4. American Society of Agricultural Engineers Yearbook of Standard. Am. Soc. Agric. Eng. St. Joseph, MI.

13. Feedstuffs Ingredient Analysis Table. 2001. Miller Publishing co., Minnetonka, MN.

14. Statistics: Analysis of variance was performed using the general linear model procedure of Minitab (Release 8.2, State College, PA). Tukey's multiple-comparison procedure was used to detect significant differences between the diets in Experiment 3.

TABLE 1. Composition of diets for Experiments 1 and 2

Item	Starter				
	0% Pearl Millet	5% Pearl Millet	10% Pearl Millet	15% Pearl Millet	20% Pearl Millet
	----------g / 100g----------				
Ingredient					
Corn	53.79	49.39	44.99	40.58	36.18
Soybean meal (48% CP)	38.33	37.90	37.47	37.05	36.62
Pearl Millet	0	5	10	15	20
Poultry fat	3.04	2.88	2.73	2.58	2.43
Dicalcium phosphate [A]	1.78	1.78	1.78	1.78	1.78
Limestone	0.74	0.74	0.74	0.74	0.74
Salt	0.29	0.29	0.28	0.28	0.28
DL-Methionine	0.19	0.19	0.18	0.17	0.16
Vitamin mix [B]	0.25	0.25	0.25	0.25	0.25
Mineral mix [C]	0.08	0.08	0.08	0.08	0.08
Insoluble ash	1.50	1.50	1.50	1.50	1.50
Calculated analysis[D]					
ME (kcal/kg)	3,025	3,025	3,025	3,025	3,025
Protein (%)	22.50	22.50	22.50	22.50	22.50
Calcium (%)	0.95	0.95	0.95	0.95	0.95
Total phosphorus(%)	0.70	0.70	0.70	0.70	0.70
Non phytate phosphorus (%)	0.45	0.45	0.45	0.45	0.45
Metionine + cystine (%)	0.92	0.92	0.92	0.92	0.92
Lysine (%)	1.27	1.27	1.27	1.27	1.27
Sodium (%)	0.21	0.21	0.21	0.21	0.21

[A]The dicalcium phosphate contained 23% calcium and 17.5% phosphorus.

[B]Vitamin mix provided the following per 100 g of diet: vitamin A, 551 IU; vitamin D_3, 110 IU; vitamin E, 1.1 IU; vitamin B_{12}, 0.001 mg; riboflavin, 0.44 mg; niacin, 4.41 mg; D-panthotenic, 1.12 mg; choline, 19.13 mg; menadione sodium bisulfate, 0.33 mg; pyridoxine HCL, 0.47 mg; thiamine, 2.2 mg; D-biotin, 0.011 mg; and ethoxyquin, 12.5 mg.

[C]Mineral mix provided the following in milligrams per 100 g of diet: Mn, 6.0; Zn, 5.0: Fe, 3.0; I, 1.5; Se, 0.5.

[D]Calculated analysis based on [13].

TABLE 2. Composition of diets for Experiment 3.

	Starter			Grower			Finisher		
Item	0% Pearl Millet	5% Pearl Millet	10% Pearl Millet	0% Pearl Millet	5% Pearl Millet	10% Pearl Millet	5% Pearl Millet	10% Pearl Millet	15% Pearl Millet
Ingredient	----------g/100 g----------								
Corn	57.84	52.62	48.02	63.27	58.67	54.08	68.49	63.49	59.30
Soybean meal (48% CP)	36.47	36.61	36.21	31.35	30.95	30.55	26.30	25.90	25.49
Pearl Millet	0	5	10	0.0	5	10	0	5	10
Poultry fat	2.35	2.45	2.47	2.2	2.21	2.22	2.14	2.15	2.16
Dicalcium phosphate [A]	1.73	1.72	1.72	1.49	1.49	1.49	1.31	1.31	1.31
Limestone	0.75	0.74	0.74	0.77	0.77	0.76	0.82	0.82	0.82
Salt	0.32	0.31	0.31	0.34	0.34	0.34	0.37	0.36	0.36
DL-Methionine	0.23	0.21	0.21	0.21	0.2	0.19	0.21	0.2	0.19
L-lysine HCL	0	0	0	0.04	0.05	0.05	0.04	0.05	0.5
Vitamin mix [B]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral mix [C]	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Calculated analysis[D]									
ME (kcal/kg)	3,050	3,050	3,050	3,050	3,050	3,050	3,050	3,050	3,050
Protein (%)	22	22	22	20	20	20	18	18	18
Calcium (%)	0.95	0.95	0.95	0.87	0.87	0.87	0.82	0.82	0.82
Total phosphorus (%)	0.68	0.68	0.68	0.62	0.62	0.62	0.57	0.57	0.57
Non phytate Phosphorus (%)	0.45	0.45	0.45	0.40	0.40	0.40	0.36	0.36	0.36
Metionine + cystine(%)	0.92	0.92	0.92	0.85	0.85	0.85	0.80	0.80	0.80
Lysine (%)	1.22	1.22	1.22	1.12	1.12	1.12	0.98	0.98	0.98
Sodium (%)	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21

[A]The dicalcium phosphate contained 23% calcium and 17.5% phosphorus.

[B]Vitamin mix provided the following per 100 g of diet: vitamin A, 551 IU; vitamin D_3, 110 IU; vitamin E, 1.1 IU; vitamin B_{12}, 0.001 mg; riboflavin, 0.44 mg; niacin, 4.41 mg; D-panthotenic, 1.12 mg; choline, 19.13 mg; menadione sodium bisulfate, 0.33 mg; folic acid, 0.55 mg; pyridoxine HCL, 0.47 mg; thiamine, 2.2 mg; D-biotin, 0.011 mg; and ethoxyquin, 12.5 mg.

[C]Mineral mix provided the following in milligrams per 100 g of diet: Mn, 6.0; Zn, 5.0: Fe, 3.0; I, 1.5; Se, 0.5.

[D]Calculated analysis based on [13].

TABLE 3. Performance of broiler chicks from 1 to 15 d of age fed different dietary levels of whole Pearl Millet (Experiment 2)[A]

Dietary Millet %	Body Weight Gain (g)	Feed/gain	Relative Gizzard Weight[B]
0	316.38 ± 6.22[ab]	1.47 ± 0.01	2.66 ± 0.02[a]
5	303.84 ± 6.94[b]	1.51 ± 0.05	2.79 ± 0.07[a]
10	299.96 ± 6.24[b]	1.64 ± 0.02	2.93 ± 0.07[b]
15	326.70 ± 13.40[ab]	1.56 ± 0.06	3.03 ± 0.08[b]
20	332.30 ± 10.00[a]	1.58 ± 0.03	2.90 ± 0.04[b]

[A]Values for feed conversion, body weight gain and relative gizzard weight are means ± SEM per chick, 4 replicate pens of 7 chicks for control and 5 replicate pens of 7 chicks per treatment for 5,10,15 and 20% millet diets. Values within a column with different superscripts differ, ($P<0.05$) [14].

[B] Gizzard weight divided by live bird weight.

TABLE 4. Pellet quality of diets containing whole pearl millet grain (Experiment 3)[A]

	Grower		Finisher	
Dietary millet	Pellets[B]	PDI[C]	Pellets[B]	PDI[C]
%	%	%	%	%
0	94.3[a]	74.9	82.6	73.3
5	75.5[b]	74.8	76.9	74.0
10	83.0[ab]	88.8	88.8	78.8

[A]Values represent the average of 3 replicate sample done in duplicates. Values within a column with different superscripts differ, ($P<0.05$) [14].
[B]Whole pellets retained on U.S. sieve 8.
[C]Pellet Durability Index, 10 minute tumble at 50 rpm.

TABLE 5. Performance of broiler chicks from 1 to 42 d of age fed different dietary levels of whole Pearl Millet (Experiment 3)[A]

Dietary millet (%)	Starter (1 to 17 d)		Grower (18 to 36 d)		Finisher (37 to 42 d)		Overall (0 to 42 d)	
	Weight gain (g)	Feed/gain	Weight Gain (g)	Feed/gain	Weight Gain (g)	Feed/gain	Weight Gain (g)	Feed/gain
0	580 ± 4.62	1.28 ± 0.01	1472 ± 16.70	1.75 ± 0.04	480 ± 11.40	2.10 ± 0.04	2533 ± 20.10	1.69 ± 0.02
5	574 ± 6.19	1.27 ± 0.01	1465 ± 17.40	1.72 ± 0.01	487 ± 12.00	2.10 ± 0.02	2528 ± 24.60	1.71 ± 0.03
10	571 ± 7.75	1.28 ± 0.01	1459 ± 14.10	1.79 ± 0.02	470 ± 12.50	2.12 ± 0.06	2500 ± 23.60	1.72 ± 0.01

[A]Values of weight gain and feed conversion are means ± SEM for 8 replicate pens of 44 chicks per pen.

TABLE 6. Carcass characteristics of broiler chickens from 1 to 42 d of age fed different dietary levels of whole pearl millet. (Experiment 3)[A]

Dietary Millet (%)	Yield (%)	Fat Pad [B]
0	72.79 ± 0.16	1.79 ± 0.07[a]
5	72.60 ± 0.16	1.86 ± 0.07[a]
10	72.68 ± 0.18	2.11 ± 0.08[b]

[A]Values for percent yield and fat pad are means ± SEM per pen at 42 d, with 8 replicates of 10 birds for yield and with 8 replicates of 5 birds for fat pad for each dietary treatment. Values within a column with different superscript differ, ($P<0.05$) [14].
[B]Fat pad weight divided by live bird weight.

CHAPTER 3

LIVE PERFORMANCE AND MEAT YIELD RESPONSES OF STRAIGHT-RUN BROILERS TO PROGRESSIVE CONCENTRATIONS OF DIETARY ENERGY MAINTAINED AT A CONSTANT METABOLIZABLE ENERGY-TO-CRUDE PROTEIN RATIO[1]

[1] Hidalgo M. A., W. A. Dozier, III, A. J. Davis and R.W. Gordon. Published in the Journal of Applied Poultry Science 2004 13:319-327.

ABSTRACT

In the U.S., a wide array of ME values ranging from deficient to adequate is being used to formulate diets for broilers to accommodate performance, meat yield, and/or economics. When formulating diets to minimize live production cost, cumulative live performance and meat recovery may be adversely affected when diets contain a sub-optimum caloric concentration. This study examined responses of Ross x Ross 308 straight-run broilers to progressive concentrations of dietary energy at a constant ME:CP ratio on live performance and processing yields during a 38-d production period. Dietary treatments consisted of six feeding regimens that provided ME concentrations from 1,350 to 1,450 kcal/lb in the starter period, from 1,370 to 1,470 kcal/lb in the grower phase and from 1,400 to 1,500 kcal/lb in the final phase of production. In general, body weight gain and feed conversion ratio suffered when broilers consumed the regimen formulated to contain the lowest ME content compared with the two regimens having the highest ME concentrations. Dietary treatments did not alter the incidence of mortality, abdominal fat percentage, chilled carcass yield or yield of carcass parts. Decreasing the energy content of diets formulated to contain a constant ME:CP ratio to sub-optimum concentrations can adversely affect performance, and this may be related to inadequate caloric and CP consumption.

Key words: live performance, meat yield, dietary energy, crude protein, metabolizable energy.

DESCRIPTION OF THE PROBLEM

In the U.S., approximately 27% of the broiler chickens are processed when achieving a weight between 1.6 and 2.2 kg [1]. The market for this smaller size bird is primarily devoted to the fast food industry. During the last several years, nutritional research has been primarily focused on optimizing breast meat yield since white meat demand dominates the domestic market, and thus less emphasis has been placed on smaller broilers marketed to the fast-food industry.

In commercial poultry production, a diverse array of ME values (1,330 to 1,500 kcal/lb) is used in formulating broiler diets [1]. Providing diets to broilers formulated to contain sub-optimum concentrations of nutrients impairs live performance and processing yields [2,3]. Because providing nutrients at either an excessive or marginal level to the bird's requirement can depress broiler growth, meat recovery, and economic returns, it is of utmost importance to determine the optimum ME value for today's high performing broiler.

Bartov et al. [4] reported that diets containing progressive ME concentrations formulated to a constant ME:CP ratio did not alter performance of heavy broilers grown to eight wk of age. This may have occurred because the birds were able to adjust their feed intake based on the ME content of the diet [5] or because of the long duration of the production period. Since broilers marketed for the fast-food industry reach their market weight approximately 12 to 18 d prior to birds grown for deboning purposes, providing smaller broilers a diet formulated to contain a sub-optimum ME concentration may adversely affect performance and processing yields more severely than broilers marketed

at 49-56 d of age. The objective of the present study was to determine the effects of dietary ME concentrations on broiler performance and processing yields during a 38-d production period while maintaining a constant ME:CP ratio.

MATERIALS AND METHODS

Bird Husbandry

A total of 2,592 Ross x Ross 308 male and female broiler chicks (vent sexed) was obtained from a commercial hatchery and randomly assigned to 6 dietary treatments in 48 floor pens (27 males and 27 females per pen; 0.07 m^2 per bird) in an environmentally controlled facility having negative-pressure ventilation with evaporative cooling pads. Each pen had fresh pine shavings and was equipped with nipple waterers and a pan feeder. All birds were offered feed and water on an *ad libitum* basis. A continuous lighting program with a lighting intensity of 20 lux was implemented from placement until 7 d of age, 16 h of lighting with an intensity of 5 lux was employed from 8 to 23 d, and 23 h of lighting with an intensity of 5 lux was used from 24 to 38 d of age. Vaccinations for Marek's disease, Newcastle disease, and infectious bronchitis were administered at the hatchery.

Dietary Treatments

The experimental treatments were six dietary regimens similar to those likely to occur in commercial practice. Each regimen used a three-phase feeding program, and the

duration of the starter, grower, and finisher periods were from placement to 17 d, 18 to 30 d, and 31 to 38 d, respectively (Tables 1, 2, and 3) to eight replicate pens of birds. These diets were formulated to contain a progressive increase in ME while maintaining a constant ME:CP ratio and the ME concentrations used were based upon values reported by [1]. Nutrient values of the feed ingredients used to formulate the diets were based upon published values [6]. In addition, these regimens also differed in lysine and methionine corresponding to the higher concentrations of CP in diets having the higher caloric content. The starter feeds were in crumble form, whereas subsequent feeds were provided in whole pellet form.

Measurements

Diets were analyzed for CP and fat content [6]. Weight gain and feed consumption were determined for each pen on d 17, 30 and 38. Mortality was recorded daily throughout the experiment. Caloric conversion was determined based upon average caloric content of the diet, feed conversion, and the estimated caloric conversion for the weight of the bird. The actual caloric conversion was adjusted to a final BW of 2.27 kg [1]. At 38 d of age, 15 males per pen were randomly selected for processing and placed in transportation coops 12 h prior to processing. Male broilers were selected for processing to remove variation due to gender effect. Birds were processed at the University of Georgia's Pilot Processing Plant. Live weight and carcass weight were determined during processing. After processing, birds were static chilled for 24 h and each carcass was allowed to drip before the chilled carcass weight was obtained. Ten carcasses from each pen were cone-deboned to obtain weights of the carcass, wings,

backhalves, fillets, and tenders. Five males per pen were also randomly selected to obtain New York dressed carcasses, so that the abdominal fat pad weight could be subsequently determined.

Statistical Analysis

Data were statistically evaluated by the analysis of variance in a one-way treatment structure in a randomized complete block design and pen location served as the blocking factor [7]. Tukey's studentized procedure was used to separate the treatment means when an *F*-test was significant $P < 0.05$ [8]. Regression analysis would have been the method of choice to statistically evaluate the dietary treatments since they contained progressive concentrations of ME, but the diets were formulated to a constant ME: CP ratio. Thus, differences were also apparent in CP, lysine, and methionine between the treatments and this allowed a multiple range test to be an appropriate alternative to test statistical differences.

RESULTS AND DISCUSION

The calculated composition of fat and CP in the experimental diets did vary with the actual analysis but relative differences of energy and protein between the treatments did occur as designed (Table 7). Variation in the absolute nutrient values compared with the calculated composition is not readily interpreted. Progressive concentrations of dietary energy increased the growth of chicks, but did not influence feed consumption, feed conversion, or the incidence of mortality from placement until 17 d of age (Table

11). Broilers consuming the diets containing the highest ME concentrations (Regimens 5 and 6) had an advantage in BW gain when compared with birds provided the diet containing the lowest ME content.

Increasing the dietary ME content improved the rate and efficiency of growth of broilers from 18 to 30 d of age (Table 8). In parallel to the starter period, providing the diet having the lowest ME content depressed BW and BW gain of broilers. In addition, feed conversion also was adversely affected with regimen 1, but feed consumption and the incidence of mortality were similar among the treatments. In the starter and grower phases birds might have had a physical limitation when trying to consume the low density diets. This finding would agree with Griffiths et al. [9] who suggested that a reduction in energy intake is due to physical limitations when feeding diets with 1330 to 1347 kcal ME/lb. As the birds become larger, the physical constraint on the amount of feed intake is lessened.

During the final phase of growth, providing progressive concentrations of ME influenced BW, feed intake, and feed conversion (Table 9). Broilers consuming a diet formulated to contain 1,440 kcal ME/lb optimized feed conversion, but the response to these measurements suffered when the diet containing 1,400 kcal/lb was provided. No treatment differences were apparent with growth rate or the incidence of mortality. Live performance, when viewed in total, indicated differences in final BW, BW gain, feed consumption, and feed conversion due to the dietary treatments (Table 10). As noted with previous periods, regimen 1 restricted bird performance when compared with the other regimens. This difference probably occurred due to an inadequate consumption of energy and CP. Even though, the birds provided regimen 1 had increased feed

consumption to compensate for the reduced growth, it did not allow for complete recovery of final BW.

Providing a diet containing a sub-optimal concentration of ME may have a more pronounced effect on performance with broilers marketed at lighter weights because the duration of the production period does not allow for compensatory growth as with heavy broilers marketed at older ages. Sizemore and Siegel [10] compared different energy concentrations while maintaining a constant ME:CP ratio. At three wk, birds receiving the higher density starter diet were significantly heavier than those fed the lower density diets, however, by seven wk of age, no treatment differences in BW were apparent.

Lesson et al. [5] evaluated performance responses of broilers provided progressive concentrations of dietary energy from 1,225 to 1,500 kcal ME/lb with CP being held constant across all the treatments. Linear regression analysis indicated feed conversion and feed consumption improved to progressive concentrations of dietary energy, but BW gain was similar among the treatments. Broilers increased feed intake in response to dietary energy dilution. This increase in feed intake for broilers fed the low caloric diets was more dramatic at six wk of age than at previous periods and indicates why these bird's obtained similar final BW when subjected to a 49-d production period.

In the present experiment, the dietary treatments maintained a constant ME:CP ratio. Live performance generally suffered when broilers were provided the dietary regimen having the lowest ME content, but dietary CP was also suboptimum for this regimen. Thus, the reduced growth may have been associated with an inadequate consumption of ME and CP. Dozier and Moran [3] reported that providing broilers with a progressive reduction in protein and essential amino acid concentrations impaired BW

gain. The dietary regimens used for that research differed in duration of days fed and the study was conducted during the summer in a curtain-sided facility [12]. In contrast, the present study was conducted in environmentally controlled rooms and temperature was maintained to optimize performance [13]; thus, temperature should not have altered feed consumption.

Progressive concentrations of ME did not alter yield of the chilled carcass or the amount of its associated abdominal fat after processing (Table 9). Deboning the carcass revealed no treatment differences in the weight and yield of carcass parts (Table 16). Similarly, Lesson et al. [5] reported that there were no differences in carcass and breast fillet weights in broilers fed gradient concentrations of ME. However, Dozier and Moran [2 and 3] reported feeding broilers diets formulated to contain sub-optimum concentrations of CP and ME impaired the amount and yield of carcass parts and the dimensions of breast fillets [3]. Additional research is warranted to evaluate the ME:CP needs of broilers differing in strain source and under environmental temperatures simulating a summer production period.

CONCLUSIONS AND APPLICATIONS

1. Growth rate and feed conversion responses of straight-run broilers were improved as the ME concentration was increased in the diet, but caloric conversion, mortality and the amount of carcass parts (males) were not affected.

2. The adverse performance of broilers provided regimen 1 (diets were formulated to conatin 1,350 kcal ME/lb and 21.2% CP from 0-17 d, 1,370 kcal ME/lb and 19.1% CP from 18 to 30 d, and 1,400 kcal ME/lb and 17.1% CP from 31 to 38 d) may be related to an inadequate consumption of energy and CP.

3. Optimum live performance for straight-run Ross x Ross 308 broilers during a 38 d production period can be obtained by formulating diets to contain 1,370, 1,390, and 1,440 kcal ME/lb and 21.5, 19.5 and 17.4% CP, in the starter, grower, and withdrawal periods, respectively.

REFERENCES AND NOTES

1. Agri Stats, Inc., (Jan.-Jun. 2002). Fort Wayne, IN 46825.

2. Dozier W. A., III and E. T. Moran, Jr. 2001. Response of early- and late-developing broilers to nutritionally adequate and restrictive feeding regimens during the summer. J. Appl. Poult. Res. 10:92-98.

3. Dozier W. A., III and E. T. Moran, Jr. 2002. Dimension and light reflectance of broiler breast fillets: Influence of strain, sex, and feeding regimen. J. Appl. Poult. Res. 11:202-208.

4. Bartov I., S Bornstein and B. Lipstein 1974. Effect of calorie to CP ratio on the degree of fatness in broilers fed on practical diets. Br. Poult. Sci. 15:107-117.

5. Lesson S., L. Caston and J. D. Summers 1996. Broiler Response to diet energy. Poult. Sci. 75:529-535.

6. AOAC. 1990. Official Methods of Analysis (15th Ed.). Association of Official Analytical Chemists, Arlington, VA.

7. SAS Institute, 1985. SAS® User's Guide. Statistics. Version 5 Edition. SAS Institute, Inc., Cary, NC.

8. Snedecor, G. W. and W. G. Cochran, 1989. Statistical Methods (8^{th} Ed.) Iowa State University Press, Ames.

9. Griffiths L., S. Leeson and J. D. Summers 1977. Influence of energy and level of various fat sources on performance and carcass composition of broilers. Poult. Sci. 56:1018-1026.

10. Sizemore F. G., and H. S. Siegel 1993. Growth, feed conversion, and carcass composition in females of four broiler crosses fed starter diets with different energy levels and energy to CP ratios. Poult. Sci. 72:2216-2228.

11. Feedstuffs Ingredient Analysis Table. 2001. Miller Publishing co., Minnetonka, MN.

12. Average temperatures during the starter, grower and finisher periods were 30.4 ± 2.4 °C, 28.0 ± 2.6 °C and 27.2 ± 3.0 °C, respectively.

13. Average temperatures during the starter, grower and withdrawal periods were 30.5 ± 1.6 °C, 25.2 ± 1.1 °C and 23.6 ± 0.5 °C, respectively.

Table 7. Composition of diets containing progressive concentrations of ME formulated to a similar ME:CP ratio provided to straight-run broilers from placement to 17 d of age

Ingredient	Regimen 1	Regimen 2	Regimen 3	Regimen 4	Regimen 5	Regimen 6
			g / 100g			
Corn	63.277	61.247	59.207	57.147	55.127	53.067
Soybean meal (48% CP)	28.560	29.550	30.540	31.550	32.540	33.530
Poultry by-product meal (60% CP)	3.000	3.000	3.000	3.000	3.000	3.000
Poultry oil	1.130	2.180	3.240	4.290	5.350	6.430
Limestone	1.090	1.080	1.080	1.080	1.070	1.070
Dicalcium phosphate	1.250	1.250	1.250	1.250	1.240	1.240
Sodium chloride	0.560	0.560	0.560	0.560	0.560	0.560
L-lysine HCl	0.080	0.080	0.070	0.050	0.040	0.030
DL-methionine	0.280	0.280	0.280	0.300	0.300	0.300
Trace-mineral premix[A]	0.080	0.080	0.080	0.080	0.080	0.080
Vitamin premix[B]	0.500	0.500	0.500	0.500	0.500	0.500
Copper sulfate	0.050	0.050	0.050	0.050	0.050	0.050
Zinc sulfate	0.003	0.003	0.003	0.003	0.003	0.003
3-Nitro[C]	0.030	0.030	0.030	0.030	0.030	0.030
BMD 50[D]	0.050	0.050	0.050	0.050	0.050	0.050
Salinomycin[E]	0.060	0.060	0.060	0.060	0.060	0.060
Total	100.000	100.000	100.000	100.000	100.000	100.000
Calculated Analyses[F]						
CP, %	21.20	21.50	21.80	22.10	22.40	22.70
ME, kcal/lb	1,350	1,370	1,390	1,410	1,430	1,450
Lysine, %	1.19	1.21	1.23	1.24	1.26	1.28
TSAA, %	0.95	0.96	0.97	0.99	1.00	1.01
Calcium, %	0.90	0.90	0.90	0.90	0.90	0.90
Available phosphorus, %	0.42	0.42	0.42	0.42	0.42	0.42
Copper, ppm	125	125	125	125	125	125
Zinc, ppm	120	120	120	120	120	120

[A]Trace-mineral premix provided the following in milligrams per kilogram of diet: selenium, 0.3; manganese, 121; iron, 75; iodine, 0.8; copper, 32; zinc, 86.

[B]Vitamin premix provided the following per kilogram of diet: vitamin A, 11,000 IU; vitamin D3, 2,200 IU; vitamin E, 22 IU; vitamin K, 2.2 mg; vitamin B12, 0.2 mg; thiamine 4.4 mg; riboflavin, 8.8 mg; vitamin B6, 4.4 mg; niacin, 88 mg; pantothenic acid, 22 mg; folic acid 1.1 mg; biotin, 2.2 mg; choline, 380 mg.

[C]3-Nitro-4-Hydroxyphenylarsonic (22.7 grams per lb), A. L. Laboratories, Inc., Ft. Lee N.J. 07024.S

[D]Bacitracin Methylene Disalicylate, (50 grams per lb), A. L. Laboratories, Inc., Ft. Lee N.J. 07024.

[E]Salinomycin sodium, (60 grams per lb), Roche Vitamins Inc., Parsippany, N.J. 07054.

[F]Calculated analysis based on [11].

Table 8. Composition of diets containing progressive concentrations of ME formulated to a similar ME:CP ratio provided to straight-run broilers from 18 to 30 d of age

Ingredient	Regimen 1	Regimen 2	Regimen 3	Regimen 4	Regimen 5	Regimen 6
			g / 100g			
Corn	69.127	67.097	65.067	63.027	61.167	59.104
Soybean meal (48% CP)	24.450	25.450	26.440	27.430	28.170	29.180
Poultry by-product meal (60% CP)	2.000	2.000	2.000	2.000	2.000	2.000
Poultry oil	0.630	1.690	2.740	3.800	4.830	5.880
Limestone	0.930	0.920	0.920	0.910	1.020	1.070
Dicalcium phosphate	1.290	1.280	1.280	1.280	1.270	1.240
Sodium chloride	0.570	0.570	0.570	0.570	0.570	0.560
L-lysine HCl	0.070	0.050	0.040	0.040	0.020	0.003
DL-methionine	0.250	0.260	0.260	0.260	0.270	0.280
Trace-mineral premix[A]	0.080	0.080	0.080	0.080	0.080	0.080
Vitamin premix[B]	0.500	0.500	0.500	0.500	0.500	0.500
Copper sulfate	0.050	0.050	0.050	0.050	0.050	0.050
Zinc sulfate	0.003	0.003	0.003	0.003	0.003	0.003
BMD 50[C]	0.050	0.050	0.050	0.050	0.050	0.050
Total	100.000	100.000	100.000	100.000	100.000	100.000
Calculated Analyses[F]						
CP, %	19.10	19.50	19.70	20.00	20.20	20.50
ME, kcal/lb	1,370	1,390	1,410	1,430	1,450	1,470
Lysine, %	1.04	1.05	1.07	1.09	1.10	1.11
TSAA, %	0.86	0.88	0.89	0.90	0.91	0.93
Calcium, %	0.80	0.80	0.80	0.80	0.80	0.80
Available phosphorus, %	0.40	0.40	0.40	0.40	0.40	0.40
Copper, ppm	125	125	125	125	125	125
Zinc, ppm	120	120	120	120	120	120

[A]Trace-mineral premix provided the following in milligrams per kilogram of diet: selenium, 0.3; manganese, 121; iron, 75; iodine, 0.8; copper, 32; zinc, 86.

[B]Vitamin premix provided the following per kilogram of diet: vitamin A, 11,000 IU; vitamin D3, 2,200 IU; vitamin E, 22 IU; vitamin K, 2.2 mg; vitamin B12, 0.2 mg; thiamine 4.4 mg; riboflavin, 8.8 mg; vitamin B6, 4.4 mg; niacin, 88 mg; pantothenic acid, 22 mg; folic acid 1.1 mg; biotin, 2.2 mg; choline, 380 mg.

[C]Bacitracin Methylene Disalicylate, (50 grams per lb), A. L. Laboratories, Inc., Ft. Lee N.J. 07024.

[F] Calculated analysis based on [11].

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Table 9. Composition of diets containing progressive concentrations of ME formulated to a similar MECP ratio provided to straight-run broilers from 31 to 38 d of age

Ingredient	Regimen 1	Regimen 2	Regimen 3	Regimen 4	Regimen 5	Regimen 6
			g / 100g			
Corn	73.797	71.747	69.997	68.267	66.217	64.507
Soybean meal (48% CP)	20.760	21.750	22.490	23.220	24.240	24.970
Poultry by-product meal (60% CP)	1.000	1.000	1.000	1.000	1.000	1.000
Poultry oil	0.760	1.820	2.850	3.850	4.910	5.910
Limestone	0.890	0.890	0.880	0.880	0.880	0.870
Dicalcium phosphate	1.320	1.320	1.320	1.320	1.310	1.310
Sodium chloride	0.580	0.580	0.580	0.580	0.580	0.580
L-lysine HCl	0.070	0.060	0.050	0.040	0.020	0.010
DL-methionine	0.190	0.200	0.200	0.210	0.210	0.210
Trace-mineral premix[A]	0.080	0.080	0.080	0.080	0.080	0.080
Vitamin premix[B]	0.500	0.500	0.500	0.500	0.500	0.500
Copper sulfate	0.050	0.050	0.050	0.050	0.050	0.050
Zinc sulfate	0.003	0.003	0.003	0.003	0.003	0.003
Total	100.000	100.000	100.000	100.000	100.000	100.000
Calculated Analyses[c]						
CP, %	17.10	17.40	17.60	17.80	18.10	18.30
ME, kcal/lb	1,400	1,420	1,440	1,460	1,480	1,500
Lysine, %	0.91	0.93	0.94	0.95	0.96	0.97
TSAA, %	0.75	0.76	0.77	0.78	0.79	0.80
Calcium, %	0.75	0.76	0.77	0.75	0.75	0.75
Available phosphorus, %	0.38	0.38	0.38	0.38	0.38	0.38
Copper, ppm	125	125	125	125	125	125
Zinc, ppm	120	120	120	120	120	120

[A]Trace-mineral premix provided the following in milligrams per kilogram of diet: selenium, 0.3; manganese, 121; iron, 75; iodine, 0.8; copper, 32; zinc, 86.

[B]Vitamin premix provided the following per kilogram of diet: vitamin A, 11,000 IU; vitamin D3, 2,200 IU; vitamin E, 22 IU; vitamin K, 2.2 mg; vitamin B12, 0.2 mg; thiamine 4.4 mg; riboflavin, 8.8 mg; vitamin B6, 4.4 mg; niacin, 88 mg; pantothenic acid, 22 mg; folic acid 1.1 mg; biotin, 2.2 mg; choline, 380 mg.

[C]Calculated analysis based on [11].

Table 10. Calculated and actual CP and fat contents of the experimental diets containing progressive concentrations of ME formulated to a similar ME:CP ratio

	Calculated (%)						Actual[A] (%)					
	Starter		Grower		Withdrawal		Starter		Grower		Withdrawal	
Treatment[B]	CP	Fat	CP	Fat	CP	Fat	CP	Fat	CP	Fat	CP	Fat
Regimen 1	21.2	4.0	19.1	3.6	17.1	3.8	20.4	3.9	18.6	3.6	16.2	3.4
Regimen 2	21.5	5.0	19.5	4.6	17.4	4.7	21.8	4.9	18.8	4.4	17.7	4.3
Regimen 3	21.8	5.9	19.7	5.5	17.6	5.7	21.7	5.6	19.3	5.2	17.1	5.1
Regimen 4	22.1	6.9	20.0	6.5	17.8	6.6	21.8	6.4	20.1	5.9	18.1	5.9
Regimen 5	22.4	7.9	20.2	7.4	18.1	7.6	21.9	7.4	20.1	6.9	18.2	6.5
Regimen 6	22.7	8.9	20.5	8.4	18.3	8.5	22.2	8.5	20.1	7.4	18.3	7.2

[A] Values are averages of duplicate samples.
[B] Treatments correspond to the following dietary specifications: regimen 1 = 0 to 17 d: 21.2% CP and 1,350 kcal ME/lb, 18 to 30 d: 19.1% CP and 1,370 kcal ME/lb, and 31 to 38 d: 17.1% CP and 1,400 kcal ME/lb; regimen 2 = 0 to 17 d: 21.5% CP and 1,370 kcal ME/lb, 18 to 30 d: 19.5% CP and 1,390 kcal ME/lb, and 31 to 38 d: 17.4% CP and 1,420 kcal ME/lb; regimen 3 = 0 to 17 d: 21.8% CP and 1,390 kcal ME/lb, 18 to 30 d: 19.7% CP and 1,410 kcal ME/lb, and 31 to 38 d:17.6% CP and 1,440 kcal ME/lb; regimen 4 = 0 to 17 d: 22.1% CP and 1,410 kcal ME/lb, 18 to 30 d: 20.0% CP and 1,430 kcal ME/lb, and 31 to 38 d: 17.8% CP and 1,460 kcal ME/lb; regimen 5 = 0 to 17 d: 22.4% CP and 1,430 kcal ME/lb, 18 to 30 d: 20.2% CP and 1,450 kcal ME/lb, and 31 to 38 d: 18.1% CP and 1,480 kcal ME/lb; regimen 6 = 0 to 17 d: 22.7% CP and 1,450 kcal ME/lb, 18 to 30 d: 20.5% CP and 1,470 kcal ME/lb, and 31 to 38 d: 18.3% CP and 1,500 kcal ME/lb.

Table 11. Live performance responses of straight-run broilers provided diets containing progressive concentrations of ME formulated to a similar ME:CP ratio from placement to 17 d of age[A]

Treatment[B]	BW (g)	Gain (g)	Feed Intake (g)	F/G[C]	Mortality (%)
Regimen 1	452[b]	411[b]	494	1.203	2.1
Regimen 2	468[ab]	428[ab]	520	1.219	1.1
Regimen 3	470[ab]	429[ab]	511	1.190	1.2
Regimen 4	464[ab]	424[ab]	506	1.196	0.7
Regimen 5	478[a]	438[a]	522	1.194	0.9
Regimen 6	473[a]	433[a]	523	1.215	0.7
SEM (35 df)	5	5	15	0.036	0.5

[a-b] Means with different superscripts within a column signify significant differences ($P < 0.05$).
[A] Values are least-square means representing 8 pens each having 54 chicks at placement. Chick weight at palcement was 41 g, and no differences were observed between the treatments ($P > 0.05$).
[B] Treatments correspond to the following dietary specifications: regimen 1 = 21.2% CP and 1,350 kcal ME/lb; regimen 2 = 21.5% CP and 1,370 kcal ME/lb; regimen 3 = 21.8% CP and 1,390 kcal ME/lb; regimen 4 = 22.1% CP and 1,410 kcal ME/lb; regimen 5 = 22.4% CP and 1,430 kcal ME/lb; regimen 6 = 22.7% CP and 1,450 kcal ME/lb.
[C] F/G = feed per gain. Values corrected for mortality.

Table 12. Live performance responses of straight-run broilers provided diets containing progressive concentrations of ME formulated to a similar ME:CP ratio from 18 to 30 d of age[A]

Treatment[B]	BW (g)	Gain (g)	Feed Intake (g)	F/G[C]	Mortality (%)
Regimen 1	1,369[b]	918[b]	1,364	1.486[a]	0.5
Regimen 2	1,401[ab]	933[ab]	1,351	1.448[ab]	0.7
Regimen 3	1,416[ab]	946[ab]	1,349	1.425[ab]	1.4
Regimen 4	1,408[ab]	944[ab]	1,317	1.396[b]	0.5
Regimen 5	1,442[a]	964[ab]	1,344	1.394[b]	0.2
Regimen 6	1,447[a]	973[a]	1,348	1.385[b]	0.2
SEM (35 df)	13	12	23	0.017	0.4

[a-b] Means with different superscripts within a column signify significant differences ($P < 0.05$) as a result of a Tukey's means comparison.
[A] Values are least-square means representing 8 pens each having 54 chicks at placement.
[B] Treatments correspond to the following dietary specifications: regimen 1 = 19.1% CP and 1,370 kcal ME/lb; regimen 2 = 19.5% CP and 1,390 kcal ME/lb; regimen 3 = 19.7% CP and 1,410 kcal ME/lb; regimen 4 = 20.0% CP and 1,430 kcal ME/lb; regimen 5 = 20.2% CP and 1,450 kcal ME/lb; regimen 6 = 20.5% CP and 1,470 kcal ME/lb.
[C] F/G = feed per gain. Values corrected for mortality.

Table 13. Live performance responses of straight-run broilers provided diets containing progressive concentrations of ME formulated to a similar ME:CP ratio from 31 to 38 d of age[A]

Treatment[B]	BW (g)	Gain (g)	Feed Intake (g)	F/G[C]	Mortality (%)
Regimen 1	1,897[b]	527	1,179[a]	2.243[a]	0.2
Regimen 2	1,934[a]	534	1,129[ab]	2.116[ab]	0.0
Regimen 3	1,955[ab]	539	1,114[ab]	2.066[b]	0.2
Regimen 4	1,921[ab]	513	1,061[b]	2.071[b]	0.0
Regimen 5	1,969[a]	527	1,104[b]	2.096[ab]	0.0
Regimen 6	1,972[a]	526	1,107[ab]	2.106[ab]	0.2
SEM (35 df)	16	7	17	0.039	0.2

[a-b] Means with different superscripts within a column signify significant differences ($P < 0.05$) as a result of a Tukey's means comparison.
[A] Values are least-square means representing 8 pens each having 54 chicks at placement.
[B] Treatments correspond to the following dietary specifications: regimen 1 = 17.1% CP and 1,400 kcal ME/lb; regimen 2 = 17.4% CP and 1,420 kcal ME/lb; regimen 3 = 17.6% CP and 1,440 kcal ME/lb; regimen 4 = 17.8% CP and 1,460 kcal ME/lb; regimen 5 = 18.1% CP and 1,480 kcal ME/lb; regimen 6 = 18.3% CP and 1,500 kcal ME/lb.
[C] F/G = feed per gain. Values corrected for mortality.

Table 14. Cumulative live performance responses of straight-run broilers provided diets containing progressive concentrations of ME formulated to a similar ME:CP ratio during a 38-d production period[A]

Treatment[B]	BW (g)	Gain (g)	Feed Intake (g)	Caloric Conversion[C]	F/G[D]	Mortality (%)
Regimen 1	1,897[b]	1,856[b]	3,029[a]	2367	1.630[a]	2.8
Regimen 2	1,934[ab]	1,894[ab]	2,985[ab]	2319	1.575[ab]	1.8
Regimen 3	1,955[ab]	1,914[ab]	2,962[ab]	2309	1.546[b]	2.7
Regimen 4	1,921[ab]	1,881[ab]	2,879[b]	2321	1.533[b]	1.1
Regimen 5	1,969[a]	1,929[a]	2,969[ab]	2361	1.538[b]	1.2
Regimen 6	1,972[a]	1,932[a]	2,976[ab]	2397	1.540[b]	1.2
SEM (35 df)	16	16	34	21	0.014	0.7

[a-b] Means with different superscripts within a column signify significant differences ($P < 0.05$) as a result of a Tukey's means comparison.
[A] Values are least-square means representing 8 pens each having 54 chicks at placement.
[B] Treatments correspond to the following dietary specifications: regimen 1 = 0 to 17 d: 21.2% CP and 1,350 kcal ME/lb, 18 to 30 d: 19.1% CP and 1,370 kcal ME/lb, and 31 to 38 d: 17.1% CP and 1,400 kcal ME/lb; regimen 2 = 0 to 17 d: 21.5% CP and 1,370 kcal ME/lb, 18 to 30 d: 19.5% CP and 1,390 kcal ME/lb, and 31 to 38 d: 17.4% CP and 1,420 kcal ME/lb; regimen 3 = 0 to 17 d: 21.8% CP and 1,390 kcal ME/lb, 18 to 30 d: 19.7% CP and 1,410 kcal ME/lb, and 31 to 38 d:17.6% CP and 1,440 kcal ME/lb; regimen 4 = 0 to 17 d: 22.1% CP and 1,410 kcal ME/lb, 18 to 30 d: 20.0% CP and 1,430 kcal ME/lb, and 31 to 38 d: 17.8% CP and 1,460 kcal ME/lb; regimen 5 = 0 to 17 d: 22.4% CP and 1,430 kcal ME/lb, 18 to 30 d: 20.2% CP and 1,450 kcal ME/lb, and 31 to 38 d: 18.1% CP and 1,480 kcal ME/lb; regimen 6 = 0 to 17 d: 22.7% CP and 1,450 kcal ME/lb, 18 to 30 d: 20.5% CP and 1,470 kcal ME/lb, and 31 to 38 d: 18.3% CP and 1,500 kcal ME/lb.
[C] Caloric cinversion adjusted to a final BW of 2.27 kg [1].
[D] F/G = feed per gain. Values corrected for mortality.

Table 15. Carcass and abdominal fat yields of male broilers provided diets containing progressive concentrations of ME formulated to a similar ME:CP ratio during a 38-d production period [A]

Treatment[B]	Carcass Wt[C] (g)	Carcass Yield[C] (%)	Abdominal fat [B] (g)	Fat Yield[D] (%)
Regimen 1	1431	75.3	45[ab]	2.32
Regimen 2	1514	76.0	47[ab]	2.42
Regimen 3	1492	74.7	52[a]	2.50
Regimen 4	1458	75.2	43[b]	2.30
Regimen 5	1520	75.5	47[ab]	2.36
Regimen 6	1527	75.7	50[ab]	2.51
SEM (35 df)	25	1.6	2	0.07

[a-b]Means with different superscripts within a column signify significant differences ($P < 0.05$) as a result of a Tukey's means comparison.

[A] Values are least-square means representing 8 pens of birds each contributing carcasses from 10 males.

[B] Treatments correspond to the following dietary specifications: regimen 1 = 0 to 17 d: 21.2% CP and 1,350 kcal ME/lb, 18 to 30 d: 19.1% CP and 1,370 kcal ME/lb, and 31 to 38 d: 17.1% CP and 1,400 kcal ME/lb; regimen 2 = 0 to 17 d: 21.5% CP and 1,370 kcal ME/lb, 18 to 30 d: 19.5% CP and 1,390 kcal ME/lb, and 31 to 38 d: 17.4% CP and 1,420 kcal ME/lb; regimen 3 = 0 to 17 d: 21.8% CP and 1,390 kcal ME/lb, 18 to 30 d: 19.7% CP and 1,410 kcal ME/lb, and 31 to 38 d:17.6% CP and 1,440 kcal ME/lb; regimen 4 = 0 to 17 d: 22.1% CP and 1,410 kcal ME/lb, 18 to 30 d: 20.0% CP and 1,430 kcal ME/lb, and 31 to 38 d: 17.8% CP and 1,460 kcal ME/lb; regimen 5 = 0 to 17 d: 22.4% CP and 1,430 kcal ME/lb, 18 to 30 d: 20.2% CP and 1,450 kcal ME/lb, and 31 to 38 d: 18.1% CP and 1,480 kcal ME/lb; regimen 6 = 0 to 17 d: 22.7% CP and 1,450 kcal ME/lb, 18 to 30 d: 20.5% CP and 1,470 kcal ME/lb, and 31 to 38 d: 18.3% CP and 1,500 kcal ME/lb.

[C] Carcasses without necks and giblets after 24 h static chilling.

[D]Values are least-square means representing 8 pens of birds each contributing New York dressed carcasses from five males.

Table 16. Carcass parts yield of male broilers provided diets containing progressive concentrations of ME formulated to a similar ME:CP ratio during a 38-d production period[A]

	Wings		Backhalve		Fillets		Tenders	
Treatment [B]	Wt (g)	Yield[C] (%)	Wt (g)	Yield[B] (%)	Wt (g)	Yield[C] (%)	Wt (g)	Yield[C] (%)
Regimen 1	163	11.4	626	43.6	242	16.9	59	4.1
Regimen 2	181	11.9	649	42.8	245	16.1	57	3.8
Regimen 3	167	11.2	637	42.7	259	17.1	62	4.1
Regimen 4	184	12.7	609	41.8	254	17.4	62	4.2
Regimen 5	196	12.9	628	41.4	257	16.9	60	3.9
Regimen 6	192	12.6	641	42.0	252	16.5	61	4.0
SEM (35 df)	13	0.9	18	0.9	7	0.4	2	0.2

[A] Values are least-square means of total of 48 pens each contributing carcasses from 10 males.
[B] Treatments correspond to the following dietary specifications: regimen 1 = 0 to 17 d: 21.2% CP and 1,350 kcal ME/lb, 18 to 30 d: 19.1% CP and 1,370 kcal ME/lb, and 31 to 38 d: 17.1% CP and 1,400 kcal ME/lb; regimen 2 = 0 to 17 d: 21.5% CP and 1,370 kcal ME/lb, 18 to 30 d: 19.5% CP and 1,390 kcal ME/lb, and 31 to 38 d: 17.4% CP and 1,420 kcal ME/lb; regimen 3 = 0 to 17 d: 21.8% CP and 1,390 kcal ME/lb, 18 to 30 d: 19.7% CP and 1,410 kcal ME/lb, and 31 to 38 d:17.6% CP and 1,440 kcal ME/lb; regimen 4 = 0 to 17 d: 22.1% CP and 1,410 kcal ME/lb, 18 to 30 d: 20.0% CP and 1,430 kcal ME/lb, and 31 to 38 d: 17.8% CP and 1,460 kcal ME/lb; regimen 5 = 0 to 17 d: 22.4% CP and 1,430 kcal ME/lb, 18 to 30 d: 20.2% CP and 1,450 kcal ME/lb, and 31 to 38 d: 18.1% CP and 1,480 kcal ME/lb; regimen 6 = 0 to 17 d: 22.7% CP and 1,450 kcal ME/lb, 18 to 30 d: 20.5% CP and 1,470 kcal ME/lb, and 31 to 38 d: 18.3% CP and 1,500 kcal ME/lb.
[C] Yield data were calculated as a percentage of chilled carcass weight.

CHAPTER 4

INCREASING DIETARY PROTEIN INTAKE ALTERS CHICKEN HEPATIC SPOT 14A AND B mRNA EXPRESSION AT DIFFERENT RATES[1]

[1] Hidalgo M., K. A. Adams and A. J. Davis. To be submitted to the Journal of Nutrition.

ABSTRACT

Changes in dietary protein consumption rapidly (3 h) alter hepatic malic enzyme mRNA expression in chicks. Spot 14 protein is a putative transcription factor involved in lipogenic enzyme expression. The purpose of this experiment was to determine the temporal relationship between changes in malic enzyme and spot 14α and β mRNA expression in chicks fed different levels of dietary crude protein. In a series of four experiments, broiler chicks were allowed free access for 1.5, 3, 6 or 24 h to a low (13 g/100 g diet), basal (22 g/100 g diet) or a high (40 g/100 g diet) protein diet. The diets were isocaloric and had equal concentrations of dietary fat. There were no differences in spot 14α mRNA expression at 1.5 h, but at 3, 6 and 24 h, spot 14α mRNA expression was significantly ($P < 0.05$) reduced in chicks fed the high protein diet compared with chicks fed the low protein or basal diet. Spot 14β mRNA expression was significantly enhanced in chicks fed the low protein diet and significantly reduced in the chicks fed the high protein diet at 1.5 h after the change in dietary protein consumption. In the final experiment, the observed differences in spot 14α and β mRNA expression were confirmed when chicks were given access to isocaloric diets with the same protein levels as the initial 4 experiments, but with the concentration of carbohydrate held constant. The results indicate that previously observed alterations in the mRNA expression of malic enzyme due to changes in dietary protein intake are preceded, by similar changes in spot 14β mRNA expression.

Key words: spot 14, protein, temporal change, lipogenesis, broilers.

INTRODUCTION

The spot 14 gene encodes for a small acidic nuclear protein that was first discovered and characterized based its response to thyroid hormone (T_3) (Seelig et al., 1981, Jump et al., 1983, Liaw and Towle, 1984 and Narayan et al., 1984). Spot 14 mRNA is mainly expressed in lipogenic tissues (Seelig et al., 1981 and Jump et al., 1983). The mRNA expression of spot 14 is influenced by factors that modulate fatty acid synthesis such as the dietary intake of carbohydrate or polyunsaturated fatty acids and the presence of glucagon and T_3 (Narayan et al., 1984, Jump et al. 1985, Mariash et al., 1986, Kinlaw et al., 1986, Towle and Mariash, 1986, Clark et al., 1990, Jump et al., 1993, Liu et al., 1994 and Koo and Towle, 2000). Transfection of hepatocytes with a spot 14 antisense oligonucleotide inhibits the normal T_3 induction of the mRNA expression of the lipogenic enzymes ATP-citrate lyase, fatty acid synthase and malic enzyme (Kinlaw et al., 1995, Brown et al., 1997).

In chickens, there is a spot 14α and a spot 14β gene that resulted from a gene duplication event (Wang et al., 2004). Furthermore, both of the chicken spot 14 genes are polymorphic. These polymorphisms result in either the insertion or deletion of nine base pairs in the spot 14α gene and the insertion or deletion of 6 base pairs in spot 14β (Wang et al., 2004). The chicken spot 14 proteins are distinct from one another and spot 14β is more similar in amino acid sequence to mammalian spot 14 (Wang et al., 2004). The mRNA expression of both forms of spot 14 is greatest in lipogenic tissues (Wang et al., 2004).

In avian species, the liver is the main site for the de novo synthesis of fatty acids (Goodridge and Ball, 1967, Goodridge, 1968a and Leveille et al., 1968) and most of the NADPH required for fatty acid synthesis is derived from the activity of malic enzyme (Goodridge and Ball, 1966, Goodridge, 1968b and Tanaka et al., 1983a). The activity of malic enzyme in avian species is regulated by dietary protein intake (Tanaka et al., 1983b, Rosebrough and Steele, 1985 a, b, c, 1990, Rosebrough et al., 1986, 1987, 1988, 1996 and 1999). Adams and Davis (2001) reported that switching chicks from a basal protein diet (22 g/100 g diet) to a low (13 g/100 g diet) or high (40 g/100 g diet) protein diet resulted in a rapid (3 h) alteration in malic enzyme mRNA expression. The switch from the basal to the low protein diet increased the mRNA expression of malic enzyme while the switch from the basal to the high protein diet decreased the mRNA expression of malic enzyme. The mechanism by which dietary protein regulates malic enzyme expression is unclear, but it may involve the putative malic enzyme gene transcription factor, spot 14. Therefore, the purpose of the present research was to determine if the changes seen previously (Adams and Davis, 2001) in malic enzyme mRNA expression were preceded by changes in spot 14α and/or β mRNA expression.

MATERIALS AND METHODS

Spot α and β cDNA clones.

A spot 14α cDNA clone (Genbank accession AW355634) contained in a pSPORT plasmid was obtained from the Delaware Biotechnology Institute, University of Delaware Chick EST Project. The complete nucleotide sequence of this cDNA clone was determined by the dideoxy chain termination method (Molecular and Genetics Instrumentation Facilities, MGIF, University of Georgia). The sequence was equivalent to the entire spot 14α sequence reported by Wang et al. (2004) that has a Genbank accession number of AY568629. For subsequent Northern analysis the entire spot 14α cDNA clone was digested from the pSPORT plasmid using EcoRI and XbaI restriction enzymes.

A spot 14β cDNA clone contained in a pBluescript II plasmid was obtained from ARK-Genomics (Roslin Institute, Midlothian, United Kingdom). This cDNA clone (Genbank accession number BU125184.1) was constructed as part of a large-scale expressed-sequence-tag project aimed at gene discovery in chickens (Boardman et al., 2002). Once obtained the cDNA clone was sequenced (MGIF, University of Georgia) and the sequence except for lacking the first 6 nucleotides, was equivalent to the spot 14β1 sequence reported by Wang et al. (2004). The spot 14α and β cDNA sequences are almost identical for the first 230 nucleotides. Therefore, for subsequent Northern analysis the spot 14β cDNA clone was digested with the Not I restriction enzyme. The spot 14β cDNA nucleotide sequence has an internal Not I site and there is a Not I site in the multiple cloning site of pBluescript. This restriction enzyme

digest generated a fragment of the spot 14β cDNA clone that contains the last 424 nucleotides of the spot 14β cDNA sequence which does not share a high degree of nucleotide sequence homology with spot 14α.

Experiments 1 – 4.

Total RNA isolated from four previous experiments (Adams and Davis, 2001) was used to determine the effect of dietary crude protein on the mRNA expression of chicken spot 14α and β. In these previous experiments, chicks were fed three semipurified diets with increasing levels of crude protein [low (13 g/100 g diet), basal (22 g/100 g diet) or high (40 g/100 g diet)] to determine the effect of dietary protein intake on hepatic malic enzyme mRNA expression and activity. All three diets were isocaloric. In experiment 1, these diets were fed for either 6 or 24 h, while in experiment 2, the diets were fed for either 1.5 or 3 h. These two initial experiments were then duplicated in experiments 3 and 4, respectively. There were 6 replicate pens of 2 chicks for each dietary treatment. For each experimental time period, food consumption was determined and liver samples were taken for RNA isolation and subsequent Northern analyses.

Experiment 5.

This experiment also utilized total RNA isolated from a previous experiment completed by Adams and Davis (2001), and was done to determine if the dietary protein induced changes in spot 14α and β mRNA expression detected in

experiments 1-4 would still occur when the concentration of dietary carbohydrate was kept constant. In experiments 1–4, the level of dietary fat was kept constant while the levels of protein and carbohydrate changed as the levels of isolated soybean protein and glucose monohydrate were adjusted to achieve isocaloric low or high protein diets. In this experiment, the amount of dietary fat was adjusted to achieve isocaloric dietary treatments while formulating the low, basal and high protein diets. Because corn oil has more than twice the energy density of glucose monohydrate, sand had to be incorporated into some of the experimental diets to maintain equal energy concentrations per gram of experimental diet. In this experiment, the decision was also made not to make any statistical comparisons between the low protein and high protein diets because to make this comparison, the low protein diet would have contained > 15 g corn oil/100 g and 15g sand/100 g to keep the dietary carbohydrate levels equal in the diets. Therefore, chicks fed the basal diet where compared with chicks fed a low protein diet containing an equivalent amount of glucose monohydrate. Similarly, chicks fed the previously tested high protein diet were compared with chicks fed a new basal diet containing a level of glucose monohydrate equivalent to that of the high protein diet. There were six replicate pens of two birds for each dietary treatment. Six hours after access was given to the experimental diets, liver samples were collected and pooled by pen for Northern analyses of spot 14α and β.

RNA extraction and Northern blot analysis.

Total RNA was extracted from liver samples pooled by pen using a guanidine isothiocyanate/phenol-chloroform method (Chomczynski and Sacchi, 1987). Forty micrograms of total RNA per sample was then run on an agarose/formaldehyde gel and then transferred to a nylon membrane as previously described (Davis and Johnson, 1998). Chicken spot 14α, spot 14β and glyceraldehydes-3-phosphate dehydrogenase (GAPDH) probes were prepared and labeled with ^{32}P for Northern blot analysis as described by Davis and Johnson (1998). The hybridization and densitometry procedures followed those described previously Davis and Johnson (1998). For each experimental duration, there were two Northern blots with the replicate samples for each dietary treatment split evenly between the two blots. The two blots were hybridized at the same time, and then exposed together on the same film. The order of the Northern analysis was spot 14α, GAPDH and spot 14β. The blots were stripped of the previously hybridized probe before being hybridized with the subsequent probe. The film exposure times for spot 14α, GAPDH and spot 14β were 2, 2 and 12 h, respectively. Relative mRNA expression of spot 14α and β was determined for the samples of each blot by calculating the signal intensity of each sample relative to the strongest spot 14α and β signal, which was assigned a value of 1. Before calculating the relative spot 14α and β mRNA levels, GAPDH mRNA expression was used to correct spot 14α and β values for equality of RNA loading and transfer for each blot.

Statistical analysis.

Data from each experiment were subjected to ANOVA according to the General Linear Model procedure. Tukey's multiple-comparison procedure (Neter et al., 1990) was used to detect significant differences among diets (experiements 1-4). All statistical procedures were done with the Minitab Statistical software package (Release 13, State College, PA), and differences were considered significant when *P*-values were < 0.05.

RESULTS

A spot 14α mRNA transcript at approximately 0.95 kb was detected by Northern analysis of total RNA derived from a liver sample of a 2-week-old broiler chick (**Fig. 2**). The detected spot 14 β mRNA transcript was just slightly smaller **(Fig. 2).**

Experiment 1-4.

Food consumption data for these four experiments has been published previously (Adams and Davis, 2001). In brief, food consumption in chicks fed either the low, basal or the high protein diet did not differ from each other at 1.5, 3 and 24 h. The only significant difference ($P < 0.05$) in food consumption was between the chicks fed the low and the high protein diet at the 6-h experimental period in experiment 1 (Adams and Davis, 2001). In this instance chicks fed the low protein diet consumed more food than the chicks fed the high protein diet.

In experiment 1, hepatic spot 14α mRNA expression was significantly different between the three dietary treatments at both 6 and 24 h with spot 14α mRNA

decreasing as dietary protein increased (**Fig. 3A**). The results for hepatic spot 14β mRNA expression (**Fig. 4A**) mirrored those obtained for spot 14α.

In experiment 2, expression of spot 14α was not significantly different among the chicks fed the three diets at 1.5 h **(Fig. 3A),** but at 3 h spot 14α mRNA expression was lower in the chicks fed the high protein diet than in chicks fed either the low or basal protein diets **(Fig. 3A).** In contrast, spot 14β mRNA expression was significantly different among the three dietary groups at both 1.5 and 3 h with expression decreasing as dietary protein increased **(Fig. 4A).**

The Northern analysis results for experiments 3 and 4 were similar to experiments 1 and 2, respectively. Spot 14α and β mRNA expression decreased as dietary protein concentrations increased and spot 14β mRNA expression was altered more quickly than spot 14α to changes in dietary protein intake (**Fig. 3B and Fig. 4B**).

Experiment 5.

Food consumption was the same for all groups over the 6 h experimental period and this data has been presented previously (Adams and Davis, 2001). When dietary carbohydrate was maintained constant as dietary protein was altered, expression of hepatic spot 14α and β mRNA was still significantly enhanced in chicks fed the low protein diet compared with those fed the basal diet (**Fig. 5**). In addition, spot 14α and β mRNA expression was significantly reduced in chicks fed the high protein diet compared with chicks fed the adjusted basal diet with an equivalent carbohydrate level **(Fig 5).**

DISCUSSION

By Northern analysis of total RNA extracted from chick liver, a spot 14α mRNA transcript of about 0.95 kb was detected. This size is similar to the 1.1 kb size reported previously for chicks by Wang et al. (2004). The spot 14β mRNA transcript had not been previously examined by Northern analysis in chicks. In the current research, the mRNA transcript for spot 14β was slightly smaller than the spot 14α transcript. In rodents and humans, the detected mRNA transcripts by Northern analysis have ranged in size from 1.1 to 1.37 kb (Liaw and Towle, 1984, Loos et al., 1991 and Ota et al., 1997).

Previously our laboratory reported that switching chicks from a basal diet (22 g/100 g diet) to a low protein diet (13 g/100 g diet) increased the level of malic enzyme mRNA, while switching chicks from the basal diet to a high protein diet (40 g/100 g diet) decreased its level (Adams and Davis, 2001). The changes in malic enzyme mRNA expression were not detected at 1.5 h, but they were at 3 h. In the current research, which utilized the stored RNA from the prior work, spot 14β mRNA expression paralleled the responses detected previously for malic enzyme, but they occurred faster (at 1.5 h). The quicker response of spot 14β mRNA expression compared to malic enzyme mRNA expression is significant since it suggests that spot 14β may be mediating the subsequent changes in malic enzyme mRNA expression. There is strong evidence in rats that spot 14 acts as a transcription factor for lipogenic enzymes including malic enzyme (Brown et al., 1997).

Previously Wang et al. (2004), reported that the protein sequence of chicken spot 14β was more similar to those of mammalian species. These researchers also reported that in chicken liver and fat, the expression of spot 14α was much greater than spot 14β, but that the expression of the mRNA for both spot 14 paralogs responded in a similar manner to fasting and re-feeding. Therefore, it is interesting to note in the current research that the mRNA expression of spot 14α in the chick did not respond as quickly as spot 14β to alterations in dietary protein intake. In fact it was not until 6 h that the relative differences in spot 14α mRNA expression paralleled those detected at 1.5 h for spot 14β. This result strongly suggests that the expression of Spot 14α and β is differentially regulated in chicks under some metabolic conditions and requires further investigation.

In the first four experiments, the level of dietary fat was held constant while the level of dietary protein was altered, but dietary carbohydrate was also changed to keep the diets isocaloric. It was essential to keep the diets isocaloric because total energy intake needed to be the same since fatty acid synthesis is very sensitive to total energy intake in chicks (Leveille, 1969 and Goodridge et al., 1989). Therefore, it could be argued that the observed changes in spot 14 mRNA expression in experiments 1-4 were due entirely to alterations in carbohydrate and not dietary protein.

To better establish the effect of dietary protein on spot 14 mRNA expression, the level of carbohydrate was held constant in experiment 5. The results were the same in experiment 5 as they had been in the first four experiments. Again, it could be argued that these results were due to changes in dietary fat content rather than dietary

protein content. However, given that the responses were similar in this experiment to those of the previous experiments, this seems unlikely. Furthermore, increasing dietary fat is associated with reduced fatty acid synthesis in chicks (Yeh et al. 1970, Romsos et al., 1976 and Tanaka et al., 1983b) and decreased spot 14 mRNA expression in rats (Clark et al., 1990 and Jump et al., 1993). In experiment 5, chicks fed the high fat, low protein diet had a higher level of hepatic spot 14 mRNA expression than the chicks fed the lower fat, higher protein basal diet which indicates that dietary protein has a very specific and strong influence on spot 14 mRNA expression.

The mechanism by which dietary protein intake rapidly modifies the mRNA expression of hepatic spot 14 in chicks is not understood, but the plasma concentrations of glucagon and/or T_3 may play a role. Plasma glucagon concentrations are elevated 1 h after feeding chicks a high protein diet (Chendrimada, 2003). Chicks fed a protein deficient diet for several days have elevated plasma T_3 concentrations (Alster and Carew, 1984, Keagy et al., 1987, Buyse et al., 1992 and Rosebrough et al., 1996). The elevated plasma T_3 concentration in chicks fed a low protein diet was independent of the carbohydrate and fat content of the diet and the deficiencies of specific amino acids seemed to play a significant role in the elevation in plasma T_3 (Carew and Alster, 1997, Carew et al., 1997, Carew et al., 2003).

In summary, switching chicks from a basal diet to a low or high protein diet resulted in a rapid (1.5 h) change in the expression of the mRNA for spot 14β. A switch to a low protein diet increased the level of spot 14β mRNA, whereas feeding a high protein diet decreased its level. The expression of the mRNA for spot 14α was

similar to that of spot 14β, except that changes in its expression due to dietary protein intake were slower to occur. The results suggest that changes in the mRNA expression of spot 14β may mediate subsequent changes in malic enzyme expression and activity when feeding chicks increasing dietary protein concentrations.

REFERENCES

Adams, K. A. and A. J. Davis. 2001. Dietary protein concentration regulates the mRNA expression of chicken hepatic malic enzyme. J. Nutr. 131:2269-2274.

Alster, F. A. and L. B. Carew. 1984. Enegy balance and thyroid function in protein deficient chicks. Nut. Rep. Intern. 30:1231-1240.

Boardman, P. E., J. Sanz-Ezquerro, I. M. Overton, D. W. Burt, E. Bosch, W. T. Fong, C. Tickle. W. R. Brown, S. A. Wilson and S. Hubbard. 2002. A comprehensive collection of chicken cDNAs. Current. Biol. 12:1965-1969.

Brown, S. B., M. Maloney and W. Kinlaw. 1997. Spot 14 protein functions at the pretranslational level in the regulation of hepatic metabolism by thyroid hormone and glucose. J. Biol. Chem. 272:2163-2166.

Buyse, J., M. A. Decuypere, E. Berghsmn, L. Kun and F. Vandesande. 1992. Effect of dietary protein content on episodic growth hormone secretion and on heat production of male broiler chickens. Brit. Poult. Sci. 33:1101-1109.

Carew, L. B. and F. A. Alster. 1997. Dietary carbohydrate and fat do not alter the thyroid response to protein deficiency in chicks. Proc. Soc. Exp. Biol. Med., Vol. 215: 82-86.

Carew, L. B., K. G. Evarts and F. A. Alster. 1997. Growth and plasma thyroid hormone concentrations of chicks fed diets deficient in essential amino acids. Poult. Sci. 76:1398-1404.

Carew. L. B., J. P. McMurtry and F. A. Alster. 2003. Effect of methionine deficiencies on plasma levels of thyroid hormones, insulin-like growth factors-I and –II, liver and body weights, and feed intake in growing chickens. Poul. Sci. 82:1932-1938.

Chendrimada, T. P. 2003. Molecular cloning of chicken hepatic histidase and protein regulation of the mRNA expression of chicken hepatic histidase and malic enzyme. Ph.D. Thesis, University of Georgia, Athens, GA. pp. 1-116.

Chomczynski, P. and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidium thiocynate-phenol-chloroform extraction. Anal. Biochem. 162:156-159.

Clarke, S. D., M. K. Armstrong and D. B. Jump. 1990. Nutritional control of rat liver fatty acid synthase and S14 mRNA abundance. J. Nutr. 120:218-224.

Davis, A. J. and P. A. Johnson. 1998. Expression pattern of messenger ribonucleic acid for follistatin and the inhibin/activin subunits during follicular and testicular development in *Gallus domesticus*. Biol. Reprod. 59:271-277.

Goodridge, A.G. and E. G. Ball. 1966. Lipogenesis in the pigeon: In vitro studies. Am. J. Physiol. 211:803-808.

Goodridge, A. G. and E. G. Ball. 1967. Lipogenesis in the pigeon: In vivo studies. Am. J. Physiol. 213:245-249.

Goodridge, A. G., 1968a. Citrate-cleavage enzyme, 'malic' enzyme and certain dehydrogenases in embryonic and growing chicks. Biochem. J. 108:663-666.

Goodridge, A. G. 1968b. The effect of starvation and starvation followed by feeding on enzyme activity and the metabolism of [U-14C]glucose in liver from growing chicks. Biochem. J. 108:667-673

Goodridge, A. G., J. F. Crish, F. B. Hillgartner and S. B. Wilson. 1989. Nutritional and hormonal regulation of the gene for avian malic enzyme. J. Nutr. 119:299-308.

Jump, D. B., P. Narayan, C. Towle and J. H. Oppenheimer. 1983. Rapid effects of triiodothyronine on hepatic gene expression: hybridization analysis of tissue-specific triiodothyronine regulation of mRNA-S14. J. Biol. Chem. 259:2789-2797.

Jump, D. B. and J. H. Oppenheimer. 1985. High basal expression and 3-5-3'-triiodothyronine regulation of messenger ribonucleic acid S14 in lipogenic tissues. Endocrinology 1117:2259-2266.

Jump, D. B., S. D. Clarke, O. MacDougald and A. Thelen. 1993. Polyunsaturated fatty acids inhibit S14 gene transcription in rat liver and cultured hepatocytes. Proc. Natl. Acad. Sci. 90:8454-8458.

Keagy, E. M., L. B. Carew, F. A. Alster and R. S. Tyzbir. 1987. Thyroid function, energy balance, body composition and organ growth in protein-deficient chicks.

J. Nutr.117:1532-40

Kinlaw, W. B., Schwartz, H. C. Towle and J. H. Oppenheimer. 1986. Opposing effects of glucagon and triiodothyronine on the hepatic levels of messenger ribinnucleic acid S14 and dependence of such effects on circadian factors. J. Clin. Invest. 78:1091-1096.

Kinlaw, W. B., J. L. Church, J. Harmon and C. N. Mariash. 1995. Direct evidence for a role of the "spot 14" protein in the regulation of lipid synthesis. J. Biol. Chem. 270:16615-8.

Koo, S. H. and H. C. Towle. 2000. Glucose regulation of mouse S(14) gene expression in hepatocytes. Involvement of a novel transcription factor complex. J. Biol. Chem. 275:5200-5207.

Leveille, G. A., E. K.O'Hea and K. Chakrabarty. 1968. In vivo lipogenesis in the domestic chicken. P.S.E.M.B. 128:398-401.

Leveille, G. A. 1969. In vivo fatty acid and cholesterol synthesis in fasted and fasted-refed chicks. J. Nutr. 98:367-372.

Liaw, C. W. and H. C. Towle. 1984. Characterization of a thyroid hormone-responsive gene from rat. J. Biol. Chem. 259:7253-7260.

Liu, H. C. and H. C. Towle. 1994. Functional synergism between multiple thyroid hormone response alements regulates hepatic expression of the rat S14 gene. Mol. Endocrinology. 8:1021-1037.

Loos, U., J. Clement, M. Behr and S. Fischer. 1991. Expression of S14-mRNA and its translational product in differentiating 3t3-l1 cells. Mol. Cell. Endocrinology. 75:R7- R11.

Mariash, C. N., S. A. Seelig, H. L. Schwartz and J. H. Oppenheimer. 1986. Rapid synergistic interaction between thyroid hormone and carbohydrate on S14 mRNA Biol. Chem. 81:4687-4691.

Narayan, P., C. W. Liaw and H. C. Towle. 1984. Rapid induction of a specific nuclear mRNA precursor by thyroid hormone. Proc. National. Acad. Sci. 81:4687-4691.

Neter, J., W. Wasserman and M. H. Kutner. 1990. Applied Linear Statistical Models, 3rd ed., p. 580. Irwin, Boston, MA.

Ota, Y., A. Mariash, J. L. Wagner and C. N. Mariash. 1997. Cloning, expression and regulation of the human S14 gene. Mol. Cell. Endoc. 126:75-81.

Romsos, D. R., S. M. Ruiz and G. A. Leveille. 1976. Influence of dietary fat source on hepatic lipogenesis in the fasted-refed chick (*Gallus domesticus*). Comp. Biochem. Physiol. 53B:19-22.

Rosebrough, R. W. and N. C. Steele. 1985a. Energy and protein relations in the broiler chicken. 2. Effect of varied protein and constant carbohydrate levels on body composition and lipid metabolism. Growth 49:479-489.

Rosebrough, R. W. and N. C. Steele. 1985b. Energy and protein relationships in the broiler. 1. Effect of protein levels and feeding regimens on growth, body composition, and in vitro lipogenesis of broiler chicks. Poult. Sci. 64:119-126.

Rosebrough, R. W. and N. C. Steele. 1985c. Protein and energy relations in the broiler. 3. Growth and in vitro metabolism in male and female chickens used as parent stock. Growth 49:63-77.

Rosebrough, R. W., N. C. Steele, J. P. McMurtry, M. P. Richards, A. D. Mitchell and C. C. Calvert. 1986. Energy and protein relations in the broiler chicken. 4. Role of sex, line and substrate on in vitro lipogenesis. Growth 50:461-471.

Rosebrough, R. W., P. McMurtry and N. C. Steele. 1987. Protein and energy relations in the broiler. 5. Lipogenesis, glucose production and metabolic hormone levels as functions of age and dietary protein levels. Growth 51:309-320.

Rosebrough, R. W., J. P. McMurtry, A. D. Mitchell and N. C. Steele. 1988. Chicken hepatic metabolism in vitro. Protein and energy relations in the broiler chicken-VI. Effect of dietary protein and energy restrictions on in vitro carbohydrate and lipid metabolism and metabolic hormone profiles. Comp. Biochem. Physiol. 90B:311-316.

Rosebrough, R. W., A. D. Mitchell and J. P. McMurtry. 1996. Dietary crude protein changes rapidly alter metabolism and plasma insulin–like growth factor I concentration in broiler chickens. J. Nutr. 126:2888-2898.

Rosebrough, R. W., J. P. McMurtry and R. Vasilatos-Younken. 1999. Dietary fat and protein interactions in the broiler. Poult. Sci. 78:992-998.

Seelig, S., C. Liaw, H. C. Towle and J. H. Oppenheimer. 1981. Thyroid hormone attenuates and augments hepatic gene expression a pretranslational level. Proc. Natl. Acad. Sci. USA. 78:4733-4737.

Tanaka, K. K. Kitahara and K. Shigeno.1979a. Effect of dietary protein level on lipid metabolism in growing chicks. Jap. J. Zootech. Sci. 50: 44-45.

Tanaka, K., S. Ohtani and K. Shigeno. 1983a. Effect of increasing dietary energy on hepatic lipogenesis in growing chicks. I. Increasing energy by carbohydrate supplementation. Poult. Sci. 62:445-451.

Tanaka, K., S. Ohtani and K. Shigeno. 1983b. Effect of increasing dietary energy on hepatic lipogenesis in growing chicks. II. Increasing energy by fat or protein supplementation. Poult. Sci. 62:452-458.

Towle, H. C. and C. N. Mariash. 1986. Regulation of hepatic gene expression by lipogenic diet and thyroid hormone. Fed. Proc. 45:2406-2411.

Wang, X., W. Carre, H. Zhou, S. Lamont and L. Cogburn. 2004. Duplicated Spot genes in the chicken: Characterization and identification of polymorphisms associated with abdominal fat traits. Gene 332:79-88.

Yeh, Y Y., G. A. Leveille and J. H. Wiley. 1970. Influence of dietary lipid on lipogenesis and on the activity of malic enzyme in the liver of the growing chick. J. Nutr. 100:917-924.

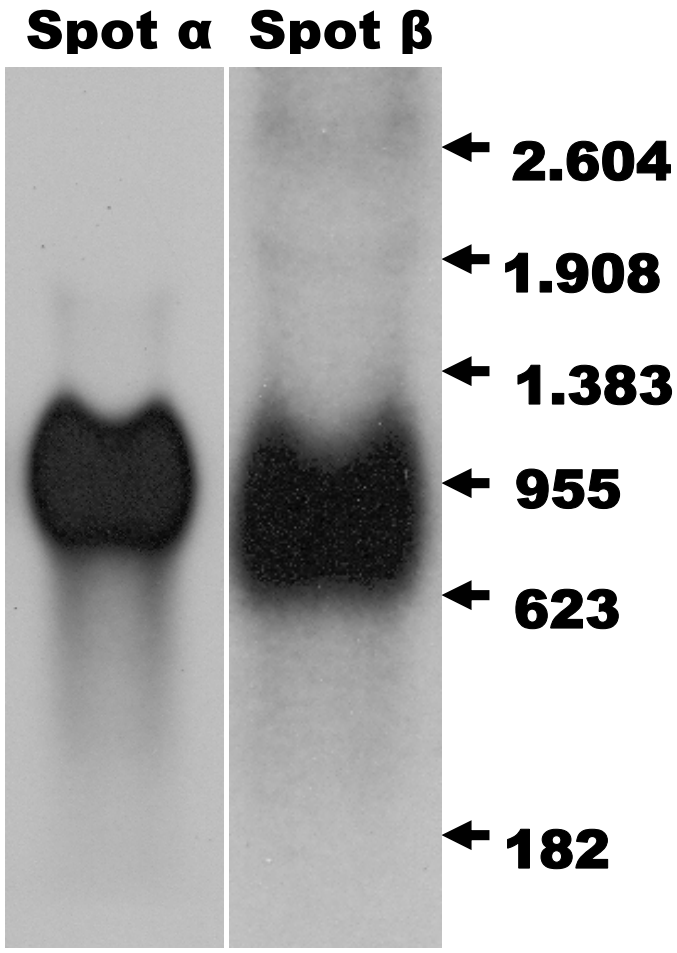

Figure 2. Autoradiograms from a Northern analysis of spot 14α and β with 40 μg of total RNA extracted from chick liver. A 0.28 to 6.58 kb RNA ladder was run in the lane adjacent to the liver sample, and kilobase sizes of the RNA bands of the ladder are shown with arrows.

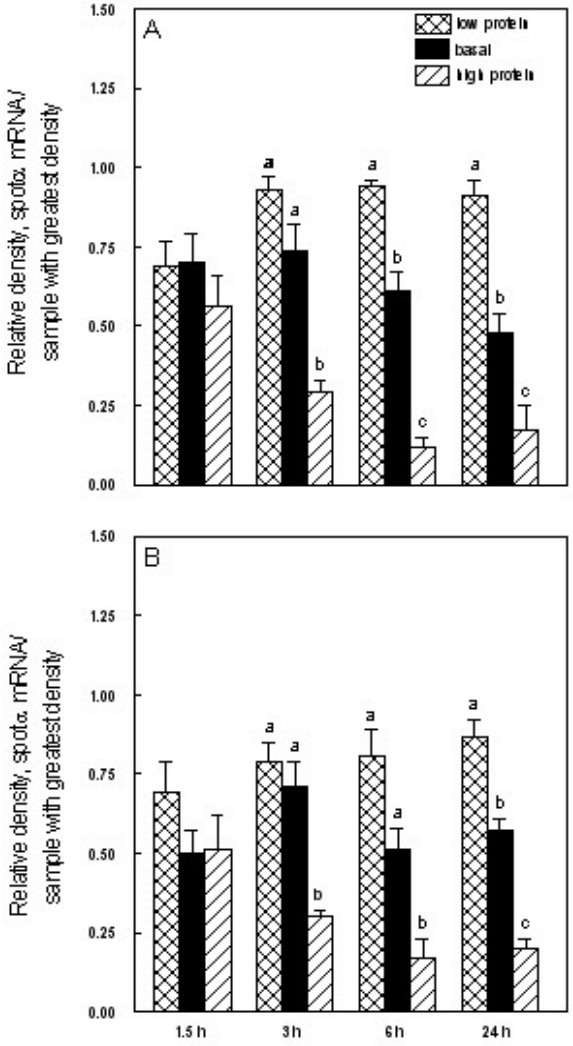

Figure 3 The relative density of hepatic spot 14α mRNA of chicks fed different dietary protein concentrations [graph A, experiments 1 (6 and 24 h) and 2 (1.5 and 3 h) and graph B, experiments 3 (6 and 24 h) and 4 (1.5 and 3 h)]. Values are means ± SEM, n = 6 replicate pens. Means at a given time with different letters differ, $P < 0.05$. Note that the relative densities of spot α mRNA to one another are specific for each time point and that all statistical comparisons are within a given time period.

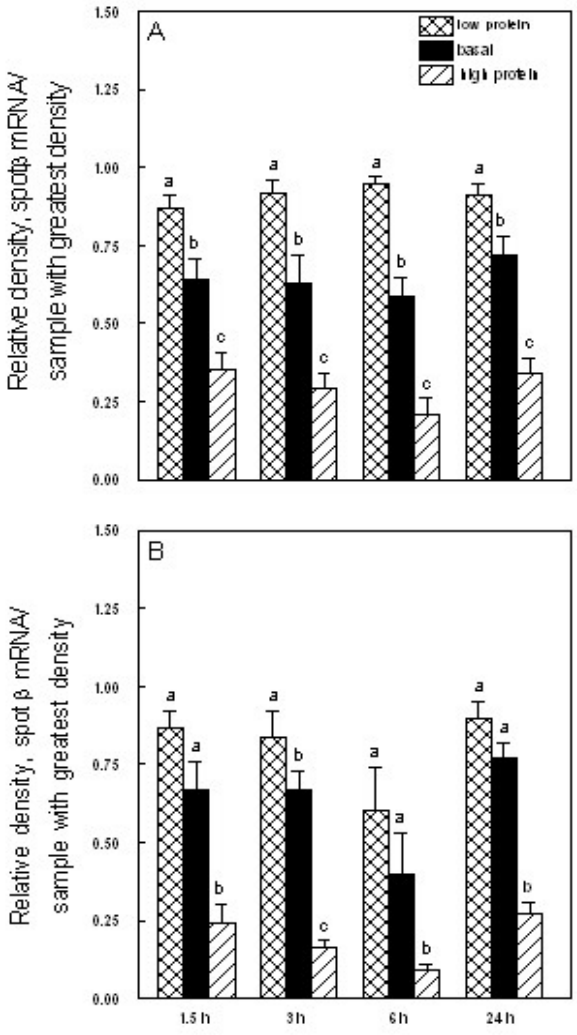

Figure 4. The relative density of hepatic spot 14β mRNA of chicks fed different dietary protein concentrations [graph A, experiments 1 (6 and 24 h) and 2 (1.5 and 3 h) and graph B, experiments 3 (6 and 24 h) and 4 (1.5 and 3 h)]. Values are means ± SEM, n = 6 replicate pens. Means at a given time with different letters differ, $P < 0.05$. Note that the relative densities of spot 14β mRNA to one another are specific for each time point and that all statistical comparisons are within a given time period.

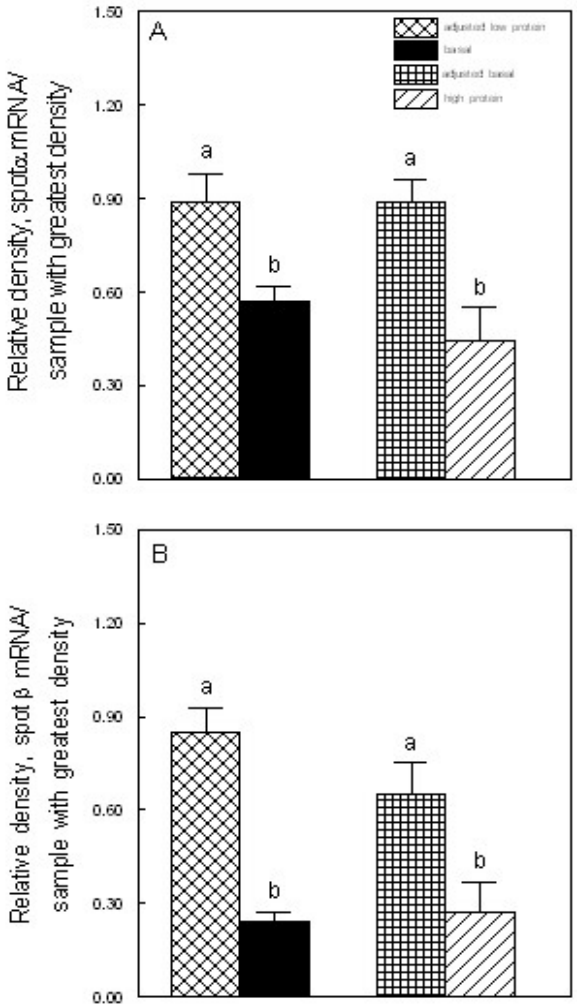

Figure 5. The relative density of hepatic spot 14α (A) and β (B) mRNA of chicks fed the four dietary treatments (experiment 5). Values are means ± SEM, n = 6 replicate pens. Means with different letters differ, $P < 0.05$. The adjusted low protein and basal diets had equal carbohydrate levels as did the adjusted basal and high protein diets.

CHAPTER 6

THE INFLUENCE OF GLUCAGON, THYROID HORMONE AND DIETARY NITROGEN ON SPOT 14A AND B mRNA EXPRESSION IN CHICKS[1]

[1] Hidalgo M., T. P. Chendrimada and A. J. Davis. To be submitted to the Journal of Nutrition.

ABSTRACT

Spot 14α and β mRNA expression is rapidly depressed when chicks consume an increased amount of dietary protein, but the mechanisms that regulate this decrease are not clear. In a series of experiments chicks were fed for 24 h a basal (22 g/100 g diet), a high (40 g/100 g diet) protein diet, or the basal diet supplemented with essential amino acids (9.16 g/100 g diet), nonessential amino acids (8.32 g/100 g diet), glycine (5 g/100 g diet), glutamic acid (9.5 g/100 g diet), ammonium bicarbonate (5.08 g/100 g diet), ammonium phosphate (4.25 g/100 g diet). The content of the essential and nonessential amino acid mixtures was such that when they were added to the basal diet the individual essential or nonessential amino acid concentrations equaled those found in the high protein diet. All of the supplements contributed 0.90 g nitrogen/100 g diet, except for the essential amino acid mixture that contributed 1.38 g nitrogen/100 g diet. In the final two experiments chicks were injected in the brachial vein with saline, glucagon or thyroid hormone. Spot 14α mRNA expression was significantly decreased ($P < 0.05$) in the chicks fed all of the supplemented diets, but the depression in expression obtained with the essential amino acid or glutamic acid supplements did not equal the depression seen when feeding the high protein diet. The mRNA expression of spot 14β was similar to that of spot 14α, except for the chicks fed the glutamic acid supplemented diet which did not have a depression in spot 14β mRNA expression. Thyroid hormone stimulated spot 14α and β mRNA expression. Glucagon depressed spot 14α mRNA expression, but did not alter spot 14β expression. The results indicate that the spot 14α and β

genes are differentially regulated, and that a simple increase in dietary nitrogen consumption is sufficient to depress spot 14 mRNA expression in the chick.

Key words: spot 14, dietary nitrogen, glucagon, thyroid hormone, chicken.

INTRODUCTION

In avian species, the liver is the main site for *de novo* fatty acid synthesis (Goodridge and Ball, 1967, Goodridge, 1968 and Leveille et al., 1968) and most of the NADPH required for fatty acid synthesis is derived from the activity of malic enzyme (Goodridge and Ball, 1966, Goodridge, 1968 and Tanaka et al., 1983a). Adams and Davis (2001) reported that switching chicks from a basal protein diet (22 g/100 g diet) to a low (13 g/100 g diet) or high (40 g/100 g diet) resulted in a rapid (3 h) alteration in malic enzyme mRNA expression. The switch from the basal to the low protein diet increased the mRNA expression of malic enzyme while the switch from the basal to the high protein diet decreased the mRNA expression of malic enzyme. In subsequent research by Hidalgo et al. (2005) the mRNA expression of spot 14, a protein that appears to act as a transcription factor for lipogenic enzyme expression, was also discovered to be rapidly altered by dietary protein intake.

A gene duplication event has resulted in chickens having two distinct forms of spot 14 (spot 14α and β, Wang et al., 2004). Spot 14β mRNA expression was increased 1.5 h after chicks were switched from the basal to the low protein diet and was decreased when chicks were switched from the basal to the high protein diet (Hidalgo et al., 2005). The mRNA expression of spot 14α was influenced by dietary protein intake in the same manner, however, the response did not mirror the expression of spot 14β until 6 h after the dietary protein changes.

The mechanism by which dietary protein intake rapidly modifies the mRNA expression of hepatic spot 14 in chicks is not understood, but the dietary intake of

specific amino acids as well as the plasma concentrations of glucagon and/or 3,5,3'-triiodothyronine (T_3) may play a role. Several reports indicate that dietary sulfur amino acid intake can modify lipid metabolism (Rukaj and Serougne, 1983, Yagasaki et al., 1986, Sugiyama et al., 1986 and Seougne et al., 1988) and decrease the activity of malic enzyme in rats (Ayala et al., 1991 and Ide et al., 1992). In chicks, abdominal fat mass relative to total body weight declined as sulfur amino acids were added in greater amounts to a low protein diet (Medonca and Jensen, 1989 and Pesti et al., 1996). Additionally, Takahashi and Akiba (1996) reported that the activity of malic enzyme decreased in chickens fed diets supplemented with sulfur amino acids. Finally, Yeh and Leveille (1969) and Rosebrough et al. (1986a) reported that the addition of lysine to a low protein diet depressed fatty acid synthesis in chicks.

Plasma glucagon concentrations are elevated within 1 h of feeding chicks a high protein diet (Chendrimada, 2003). In addition, work with mammalian species indicates that arginine may be involved with the secretion of glucagon (Henningsson and Lundquist, 1998) while the concentration of histidine may be involved in regulating preproglucagon mRNA levels (Paul et al., 1998).

Chicks fed a protein deficient diet for several days have elevated plasma T_3 concentrations (Alster and Carew, 1984, Keagy et al., 1987, Buyse et al., 1992 and Rosebrough et al., 1996). The elevated plasma T_3 concentration in chicks fed a low protein diet was independent of the carbohydrate and fat content of the diet and the deficiencies of specific amino acids seemed to play a significant role in the elevation of the plasma T_3 concentration (Carew and Alster, 1997, Carew et al., 1997 and Carew et al., 2003).

The current study was conducted to determine the role of dietary essential and nonessential amino acids in regulating spot 14α and β mRNA expression, as well as to determine if glucagon and T_3 could play a role in the rapid alteration of hepatic spot 14 mRNA expression caused by changes in dietary protein intake.

MATERIALS AND METHODS

Experiment 1-3

Total RNA isolated from four previous experiments (Chendrimada, 2003) was used to determine the effect of essential and nonessential amino acids as well as nonprotein nitrogen sources on the mRNA expression of chicken spot 14α and β. In all three experiments, the experimental diets were provided ad libitum for 24 h. There were six replicate pens of two birds for each dietary treatment. At the end of the 24 h experimental period, food consumption was determined and liver samples were taken for RNA extraction and subsequent Northern analysis.

In experiment 1 the dietary treatments consisted of a basal protein diet (22 g/100 g diet), a high protein diet (40 g/100 g diet) and the basal diet supplemented with either 9.16 g/100 g diet essential amino acids or 8.32 g/100 g diet nonessential amino acids. The composition of the essential and nonessential amino acid supplements was formulated so that when each was added to the basal diet the total dietary concentration of the essential or nonessential amino acids equaled the concentration found in the high protein diet. All of the diets were isocaloric.

The goal of experiment 2 was to determine if the results obtained in experiment 1 could be obtained by utilizing just one essential or nonessential amino acid. The dietary treatments consisted of the basal protein diet, the high protein diet, and the basal diet supplemented with either 9.5 g/100 g diet glutamic acid or 5 g/100 g diet glycine. The amount of total nitrogen present in the basal supplemented diets was equivalent to the nitrogen present in the nonessential supplemented diet used in experiment 1. All of the diets were isocaloric.

The results from experiment 2 suggested that possibly just the addition of dietary nitrogen might influence spot 14 mRNA expression in chicks. Therefore, in experiment 3 nonprotein nitrogen sources were used to supplement the basal diet. The dietary treatments consisted of the basal protein diet, the high protein diet, and the basal diet supplemented with either ammonium phosphate (4.25 g/100 g diet), ammonium bicarbonate (5.08 g/100 g diet) or a combination of both ammonium phosphate (2.13 g/100 g diet) and ammonium bicarbonate (2.54 g/100 g diet). All of the supplemented diets were isocaloric. The amount of total nitrogen present in the basal supplemented diets was equivalent to the nitrogen present in the nonessential supplemented diet used in experiment 1.

Experiment 4.

The goal of this experiment was to determine if glucagon altered hepatic spot 14 mRNA expression in chicks. This experiment utilized stored total RNA extracted from a previous experiment (Chendrimada, 2003). In this previous experiment, chicks (4 replicate pens with 2 chicks each per treatment) were injected (brachial vein) with either

glucagon (240 µg glucagon/kg body weight) or saline. After the injection, the chicks continued to have free access to the basal protein diet. At the end of the 3 h experimental period, liver samples were collected and pooled by pen for RNA extraction.

Experiment 5.

In this experiment, the goal was to determine if T_3 altered hepatic spot 14 mRNA expression. Day old Cobb by Cobb broiler chicks hatched at the University of Georgia Poultry Research Farm Hatchery were raised in thermostatically controlled, electrically heated battery brooder cages with wire floors. The cages were lighted for 24 h/d. Chicks had free access to water and a practical chick starter diet. Seven days after hatching, the chicks were sorted and those with extreme weights discarded. The remaining chicks were assigned to experimental groups so as to achieve similar weight distributions among the pens, and the chicks were fed the basal protein diet used in the previous experiments. After 5 d of having free access to the basal protein diet, the 36 pens each containing two chicks, were randomly split into two experimental groups. The birds in half of the pens were injected (brachial vein) with T_3 (20 mg/kg body weight, Sigma Chemical Company, St. Louis, MO) while the birds in the other pens were injected with an equivalent volume of saline. After injection, the birds continued to have free access to the experimental basal diet for 1, 2 or 3 h. At the end of each of the three experimental periods, the birds from 6 of the pens from each experimental group were killed by cervical dislocation. Liver samples were collected and pooled by pen for RNA extraction. The Institutional Animal and Care and Use Committee of the University of Georgia approved all animal procedures.

RNA extraction and Northern blot analysis.

The procedures utilized for total RNA extraction and the Northern blot analysis were the same as those described previously (Hidalgo et al., 2005).

Statistical analysis.

Data from each experiment were subjected to ANOVA according to the General Linear Model procedure. Tukey's multiple-comparison procedure (Neter et al., 1990) was used to detect significant differences among diets. All statistical procedures were done with the Minitab Statistical software package (Release 13, State College, PA), and differences were considered significant when *P*-values were < 0.05.

RESULTS

Experiment 1.

Food consumption data for this experiment has been published previously (Chendrimada, 2003). There were no significant differences in food consumption except for the chicks fed the high protein diet and those fed the basal diet supplemented with nonessential amino acids. The chicks fed the high protein diet consumed less food than the chicks consuming the basal diet supplemented with nonessential amino acids.

Hepatic spot 14α and β mRNA expression was significantly lower in chicks fed the basal diet supplemented with the essential amino acids when compared to chicks fed

the basal diet (**Fig.2**). However, the depression in spot 14α and β mRNA expression caused by the essential amino acid supplement was not as robust as that caused by feeding the high protein diet. In contrast, the depression in spot 14α and β mRNA expression caused by the nonessential supplement was statistically equivalent to that caused by feeding the high protein diet (**Fig. 3**).

Experiment 2.

As reported previously, (Chendrimada, 2003) food consumption was 52, 50, 32 and 49 g/chick for the basal, basal plus glutamic acid, basal plus glycine and high protein diets, respectively. The only significant difference in food consumption was for the chicks fed the glycine-supplemented diet, and they consumed less food than the chicks fed the other three diets.

Spot 14α mRNA expression was significantly lower in chicks consuming the basal diets supplemented with glutamic acid or glycine (**Fig. 4A**). The glycine supplement was more effective in depressing spot 14α mRNA expression than the glutamic acid supplement (Fig. 3A). Spot 14β mRNA expression was the same for chicks consuming the basal diet and the basal diet supplemented with glutamic acid (**Fig 4B**). Chicks consuming the glycine-supplemented basal diet and the high protein diet had equivalent spot 14β mRNA expression **(Fig. 4B).**

Experiment 3.

Food consumption was 58, 50, 23, 39 and 39 g/chick for the basal, basal plus ammonium bicarbonate, basal plus ammonium phosphate, basal plus ammonium bicarbonate and ammonium phosphate and high protein diets, respectively (Chendrimada, 2003). Chicks fed the ammonium phosphate supplemented diets had significantly lower food consumption than birds fed the other diets. In addition, the chicks fed the basal and ammonium bicarbonate supplemented diets had significantly higher food intake than the birds fed the high protein diet and the basal diet supplemented with a combination of ammonium phosphate and ammonium bicarbonate diet. There was no significant difference in food consumption in chicks fed the basal and the ammonium bicarbonate supplemented basal diet.

Spot 14α and β mRNA expression was significantly lower in the chicks consuming any of the basal diets supplemented with the nonprotein nitrogen sources when compared to the chicks fed the basal diet (**Fig. 5**).

Experiment 4.

Three hours post-injection, food consumption was equivalent for the chicks injected with either saline or glucagon (Chendrimada, 2003). Glucagon significantly depressed spot 14α mRNA expression, but had no effect on spot 14β mRNA expression (**Fig. 6**).

Experiment 5.

Food consumption did not differ between the saline and T_3 injected birds for any experimental duration. For the 1-h experimental period, food consumption was (mean ± SEM) 4.0 ± 1.00 and 4.6 ± 0.73 g/chick, while for the 2-h experimental period, food consumption was 5.8 ± 1.33 and 6.0 ± 2.25 g/chick, whereas for the 3-h experimental period, food consumption was 7.2 ± 0.44 and 6.0 ± 0.44 g/chick for the saline and T_3 injected chicks, respectively. T_3 significantly enhanced hepatic spot 14α and β mRNA expression at all three experimental duration's **(Fig. 7).**

DISCUSSION

Previously our laboratory reported that when chicks are fed diets with increased dietary protein concentrations, hepatic spot 14 mRNA expression decreased (Hidalgo et al., 2005). The results from the current research strongly suggest that the decrease in spot 14 mRNA expression is not related to specific amino acids. Adding a mixture of essential or nonessential amino acids to the basal diet were both effective in reducing spot 14 mRNA expression. Furthermore, even the addition of one essential, one nonessential amino acid or nonprotein nitrogen sources reduced hepatic spot 14 mRNA expression. Although the nitrogen from the nonprotein nitrogen sources could be used to synthesize amino acids, its effect on spot 14 mRNA expression raises the interesting possibility that simply the total nitrogen content of the diet may regulate spot 14 expression.

The spot 14 mRNA expression results obtained with some of the amino acid and nonprotein nitrogen sources were complicated by the significant decrease in food consumption caused by these supplements. Spot 14α and β mRNA expression are both

decreased in chicks by fasting (Wang et al., 2004). Although the chicks fed the glycine-supplemented or the ammonium phosphate-supplemented diets still consumed some of this diet, the depression in food intake was severe, and it is likely that it contributed to the very low spot 14α and β mRNA expression seen in these chicks.

In rats, spot 14 appears to function as a transcription factor that regulates the expression of lipogenic enzymes such as malic enzyme (Kinlaw et al., 1995, and Brown et al., 1997). It is interesting to note that the expression of spot 14 in the current experiments mirrored the expression of malic enzyme mRNA reported previously (Chendrimada, 2003) for these experiments. Therefore, the current results further strengthen the potential role of spot 14 as an important metabolic mediator for the well-documented (Tanaka et al., 1983b, Rosebrough and Steele, 1985 a, b, c, 1990, Rosebrough et al., 1986b, 1987, 1988, 1996 ,1999, 2002 and Adams and Davis, 2001) inhibition of malic enzyme and *de novo* fatty acid synthesis in birds by dietary protein. The results also suggest that further research should be conducted to determine the role that dietary supplements of nitrogen could have on *de novo* fatty acid synthesis and subsequent body fat content in broiler chicks. Nonprotein nitrogen sources are relatively cheap compared to dietary protein sources and the level of supplementation could be small if the compound contained a relatively high nitrogen content relative to that delivered by protein.

Chendrimada (2003) reported that the plasma glucagon concentration of chicks was significantly elevated at 1 and 3 h, but not 2 h, after these chicks were fed a high protein diet when compared to chicks fed the basal protein diet. In addition, chicks injected with glucagon had a significant depression in hepatic malic enzyme mRNA

expression when measured 3 h after the injection. In the current research that utilized RNA stored form this previous experiment, the injection of glucagon significantly depressed hepatic spot 14α mRNA expression, but had no effect on spot 14β mRNA expression. Besides clearly indicating that the two spot 14 genes in chicks exhibit differential regulation, this result established that a change in the plasma glucagon concentration is not the metabolic signal that directly stimulated the decrease in spot 14β mRNA expression seen previously at 1.5 h after chicks initiated consumption of a high protein diet (Hidalgo et al., 2005). Although spot 14α mRNA expression was very rapidly stimulated by glucagon, the early response (1.5 h) to an increase in dietary protein intake is mediated by spot 14β and not spot 14α (Hidalgo et al., 2005).

Another potential mediator of the response of spot 14β mRNA expression to dietary protein intake might be T_3. The results from the current research clearly indicate that T_3 can very rapidly (1 h) induce spot 14α and β mRNA expression. Further research is needed to determine if feeding a high protein diet quickly depress plasma T_3 concentrations. If there is a depression in the plasma T_3 concentration, then this finding could be correlated with the lower spot 14α and β mRNA expression observed when feeding a high protein diet. Interestingly, it is already known that feeding a low protein diet for several days results in an elevated plasma concentration of T_3 in chicks (Alster and Carew, 1984, Keagy et al., 1987, Buyse et al., 1992 and Rosebrough et al 1996, Carew and Alster, 1997, Carew et al., 1997 and Carew et al., 2003).

In summary, spot 14α and β are differentially regulated in the chick. Spot 14α mRNA expression is quickly decreased in chicks injected with glucagon, however, spot 14β mRNA expression is unaffected. Chicks injected with T_3, have enhanced mRNA

expression of both spot 14α and β, and this increase in expression happens vary quickly. The mRNA expression of both spot 14α and β are depressed by dietary supplements of individual amino acids or nonprotein nitrogen sources. Controlling the expression of spot 14 through dietary supplements of nonprotein nitrogen may be a way to reduce *de novo* fatty acid synthesis in chicks and reduce body fat content.

LITERATURE CITED

Adams, K. A. and A. J. Davis. 2001. Dietary protein concentration regulates the mRNA expression of chicken hepatic malic enzyme. J. Nutr. 131:2269-2274.

Alster, F. A. and L. B. Carew, 1984. Energy balance and thyroid function in protein deficient chicks. Nut. Rep. Intern. 30:1231-1240.

Ayala, A., E. Gordillo, , M. F. Lobato and A. Machado. 1991. Involvement of diminution of glutathione, produced by deficiency of methionine in the diet, in the elevation of malic enzyme level in rat liver. Biochim. Biophys. Acta. 1084:48-52.

Brown, S. B., M. Maloney and W. Kinlaw. 1997. Spot 14 protein functions at the pretranslational level in the regulation of hepatic metabolism by thyroid hormone and glucose. J. Biol. Chem. 272:2163-2166.

Buyse, J., M. A. Decuypere, E. Berghsmn, L. Kun and F. Vandesande. 1992. Effect of dietary protein content on episodic growth hormone secretion and on heat production of male broiler chickens. Brit. Poult. Sci. 33:1101-1109.

Carew, L. B. and F. A. Alster. 1997. Dietary carbohydrate and fat do not alter the thyroid response to protein deficiency in chicks. Proc. Soc. Exp. Biol. Med. 215: 82-86.

Carew, L. B., K. G. Evarts and F. A. Alster. 1997. Growth and plasma thyroid hormone concentrations of chicks fed diets deficient in essential amino acids. Poult. Sci. 76:1398-1404.

Carew, L. B., J. P. McMurtry and F. A. Alster. 2003. Effect of methionine deficiencies on plasma levels of thyroid hormones, insulin-like growth factors-I and –II, liver and body weights, and feed intake in growing chickens. Poul. Sci. 82:1932-1938.

Chendrimada, T. P. 2003. Molecular cloning of chicken hepatic histidase and protein regulation of the mRNA expression of chicken hepatic histidase and malic enzyme. Ph.D. Thesis, University of Georgia, Athens, GA. pp. 1-116.

Goodridge, A.G. and E. G. Ball. 1966. Lipogenesis in the pigeon: In vitro studies. Am. J. Physiol. 211:803-808.

Goodridge, A. G. and E. G. Ball. 1967. Lipogenesis in the pigeon: In vivo studies. Am. J. Physiol. 213:245-249.

Goodridge, A. G. 1968a. Citrate-cleavage enzyme, 'malic' enzyme and certain dehydrogenases in embryonic and growing chicks. Biochem. J. 108:663-666.

Henningsson, R. and I. Lundquist. 1998. Arginine-induced insulin release is decreased and glucagon increased in parallel with islet NO production. Am. J. Physiol. 275: E500-E506.

Hidalgo M., K. A. Adams and A. J. Davis. 2005. Increasing dietary protein levels alter chicken hepatic Spot 14 α and β mRNA expression at different rates. Thesis dissertation PhD. University of Georgia.

Ide, T., M. Murata and Y. Sunada 1992. Soybean protein-dependent changes in triacylglycerol synthesis and concentration of diacylglycerol in the liver microsomes of fasted-refed rats. Ann. Nutr. Metab. 36:87-96.

Kinlaw, W. B., J. L. Church, J. Harmon and C. N. Mariash. 1995. Direct evidence for a role of the "spot 14" protein in the regulation of lipid synthesis. J. Biol. Chem. 270(28):6615-6618.

Keagy, E. M., L. B. Carew, F. A. Alster and R. S. Tyzbir. 1987. Thyroid function, energy balance, body composition and organ growth in protein-deficient chicks. J. Nutr. 117:1532-1540.

Leveille, G. A., E. K. O'Hea and K. Chakrabarty. 1968. In vivo lipogenesis in the domestic chicken. P.S.E.M.B. 128:398-401.

Mendonca, C. X. and L. S. Jensen. 1989. Influence of protein concentration on the sulfur amino acid requeriment of broiler chickens. Brit. Poult. Sci. 30:889-898.

Neter, J., W. Wasserman and M. H. Kutner. 1990. Applied Linear Statistical Models, 3rd ed., p. 580. Irwin, Boston, MA.

Pesti, G. M., B. Leclercq, A.M. Chagneau, T. Cochard. 1996. Effects of the naked neck (NA) gene on the sulfur-containing amino acid requirement of broilers. Poult. Sci. 75:375-380.

Paul, G. L., A. Waegner, H. R. Gaskins and N. F. Shay. 1998. Histidine availability alters glucagon gene expression in murine alphaTC6 cells. J. Nutr. 128:973-976.

Rosebrough, R. W. and N. C. Steele. 1985a. Energy and protein relations in the broiler chicken. 2. Effect of varied protein and constant carbohydrate levels on body composition and lipid metabolism. Growth 49:479-489.

Rosebrough, R. W. and N. C. Steele. 1985b. Energy and protein relationships in the broiler. 1. Effect of protein levels and feeding regimens on growth, body composition, and in vitro lipogenesis of broiler chicks. Poult. Sci. 64:119-126.

Rosebrough, R. W. and N. C. Steele. 1985c. Protein and energy relations in the broiler. 3. Growth and in vitro metabolism in male and female chickens used as parent stock. Growth 49:63-77.

Rosebrough, R. W., Mitchell and N. C. Steele. 1986a. Lysine status and in vivo lipid metabolism in chicks. Poul. Sci. 65 (Sup 1), 115.

Rosebrough, R. W., N. C. Steele, J. P. McMurtry, M. P. Richards, A. D. Mitchell and C. C. Calvert. 1986b. Energy and protein relations in the broiler chicken. 4. Role of sex, line and substrate on in vitro lipogenesis. Growth 50:461-471.

Rosebrough, R. W., J. P. McMurtry and N. C. Steele. 1987. Protein and energy relations in the broiler. 5. Lipogenesis, glucose production and metabolic hormone levels as functions of age and dietary protein levels. Growth 51:309-320.

Rosebrough, R. W., J. P. McMurtry, A. D. Mitchell and N. C. Steele. 1988. Chicken hepatic metabolism in vitro. Protein and energy relations in the broiler chicken-VI. Effect of dietary protein and energy restrictions on in vitro carbohydrate and lipid metabolism and metabolic hormone profiles. Comp. Biochem. Physiol. 90B:311-316.

Rosebrough, R. W., A. D. Mitchell and J. P. McMurtry. 1996. Dietary crude protein changes rapidly alter metabolism and plasma insulin–like growth factor I concentration in broiler chickens. J. Nutr. 126:2888-2898.

Rosebrough, R. W., J. P. McMurtry and R. Vasilatos-Younken. 1999. Dietary fat and protein interactions in the broiler. Poult. Sci. 78:992-998.

Rosebrough, R. W., S. M. Poch , B. A. Rusell and M. P. Richards. 2002. Dietary protein regulates in vitro lipogenesis and lipogenic gene expression in broilers. Comp. Biochem. Physiol. 132:423-31.

Rukaj, A. and C. Serougne 1983. Effect of excess dietary cystine on the biodynamics of cholesterol in the rat. Biochim. Biophys. Acta. 753:1-5.

Serougne, C. and A. Rukaj 1988. Induction of long-lasting hypercholesterolemia in the rat fed cystine-enriched diet. Lipids 23:930-936.

Sugiyama, K. and M. Mizuno. 1986. Effect of individual amino acids on plasma cholesterol level in rats fed a high cholesterol diet. J. Nutr. Sci. Vitaminol. (Tokyo) 32:623-633.

Takahashi, K. and Y. Akiba. 1996. Influence of dietary protein, sulfur amino acid content and their ratio on activities of malic enzyme, fatty acid synthetase and hormone sensitive lipase in male broilers. Anim. Sci. Technol. 67:305-309.

Tanaka, K., S. Ohtani and K. Shigeno. 1983a. Effect of increasing dietary energy on hepatic lipogenesis in growing chicks. I. Increasing energy by carbohydrate supplementation. Poult. Sci. 62:445-451.

Tanaka, K., S. Ohtani and K. Shigeno. 1983b. Effect of increasing dietary energy on hepatic lipogenesis in growing chicks. II increasing energy by fat or protein supplementation. Poult. Sci. 62:452-458.

Wang, X., W. Carre, H. Zhou, S. Lamont and L. Cogburn. 2004. Duplicated Spot genes in the chicken: Characterization and identification of polymorphisms associated with abdominal fat traits. Gene 332:79-88.

Yagasaki, K., M. Machida and R. Funabiki. 1986. Effects of dietary methionine, cystine, and glycine on endogenous hypercholesterolemia in hepatoma-bearing rats. J. Nutr. Sci. Vitaminol. (Tokyo) 32:643-51.

Yeh, Y.Y. and G. Leveille. 1969. Effect of dietary protein on hepatic lipogenesis in the growing chick. J. Nutr. 98:356-366.

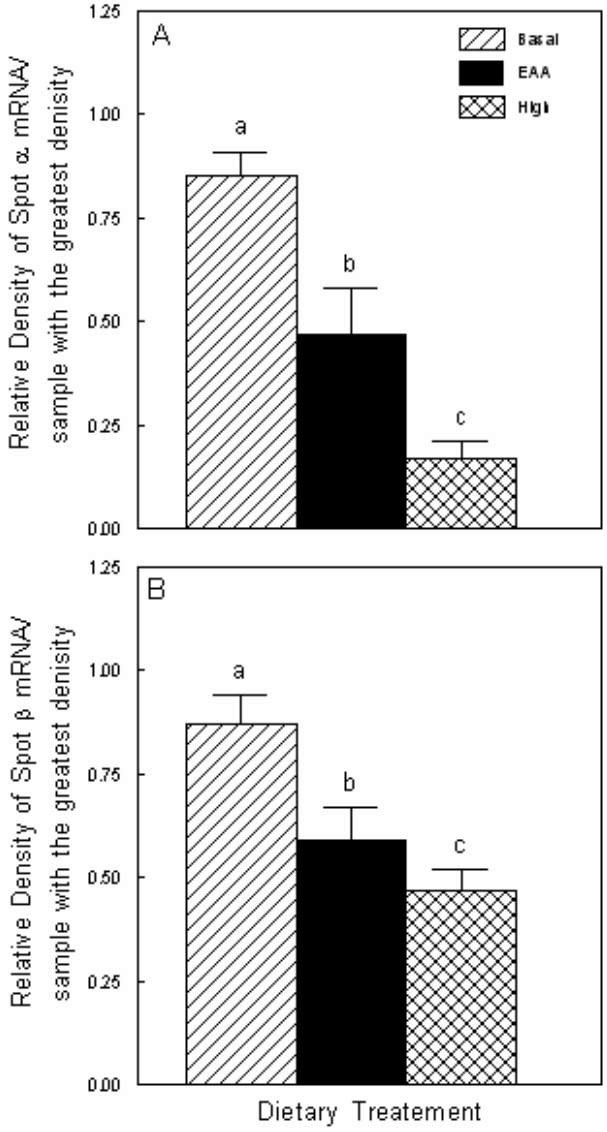

Figure 6 The relative density of hepatic spot 14α (A) and spot 14β (B) mRNA of chicks fed three dietary treatments (experiment 1). Values are means ± SEM, n = 6 replicate pens. Means with different letters differ, $P < 0.05$. Abbreviation: EAA = essential amino acids.

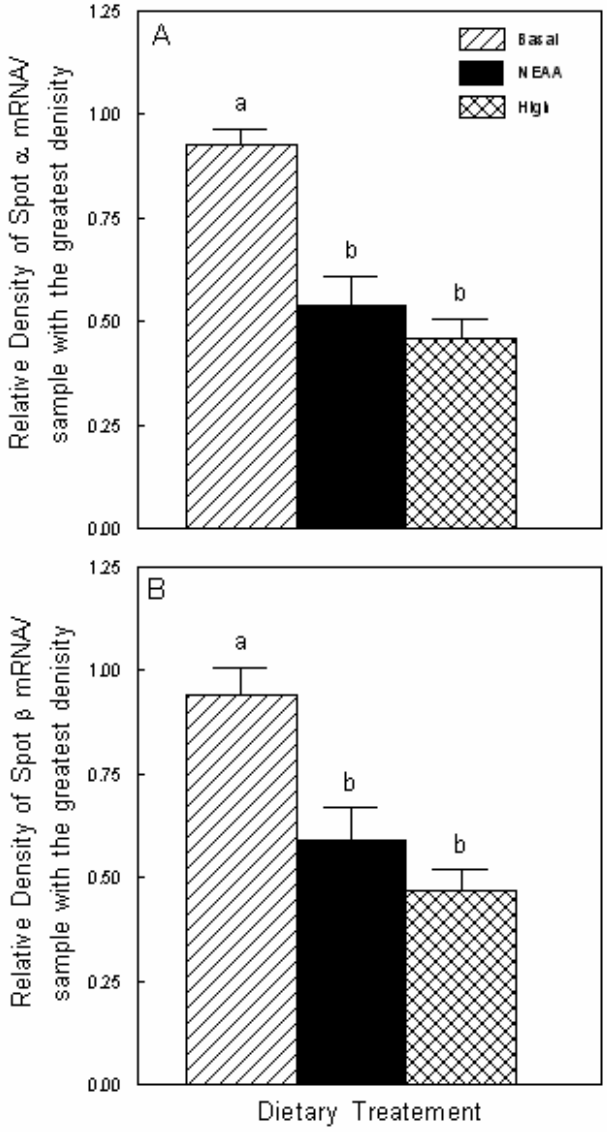

Figure 7 The relative density of hepatic spot 14α (A) and spot 14β (B) mRNA of chicks fed the three dietary treatments (experiment 1). Values are means ± SEM, n = 6 replicate pens. Means with different letters differ, $P < 0.05$. Abbreviation: NEAA = nonessential amino acids.

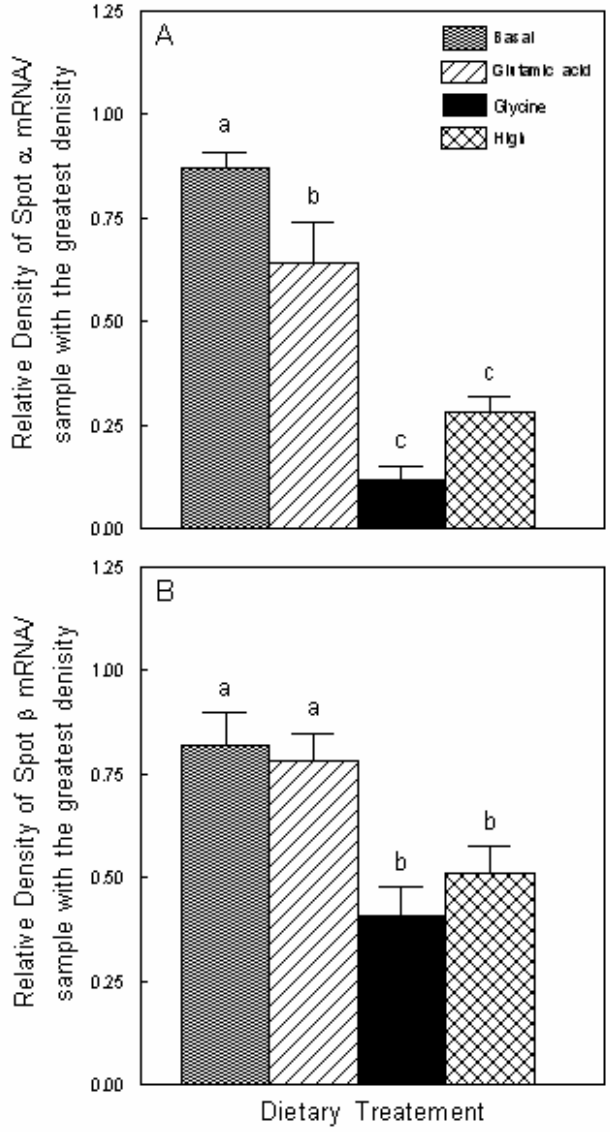

Figure 8 The relative density of hepatic spot 14α (A) and spot 14β (B) mRNA of chicks fed the four dietary treatments (experiment 2). Values are means ± SEM, n = 6 replicate pens. Means with different letters differ, $P < 0.05$.

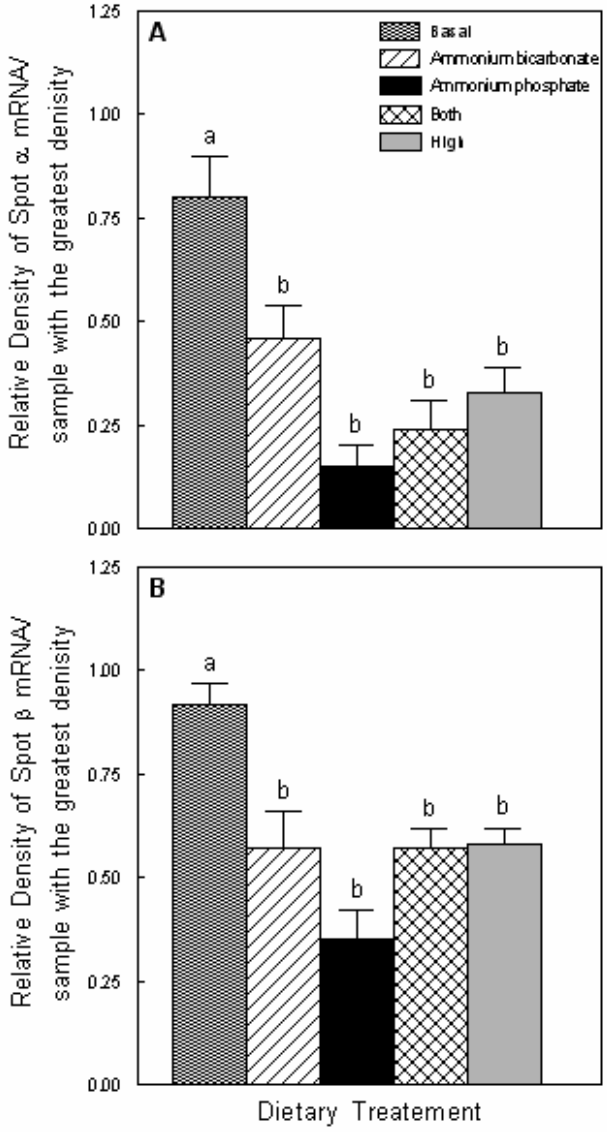

Figure 9 The relative density of hepatic spot 14α (A) and spot 14β (B) mRNA of chicks fed the five dietary treatments (experiment 3). Values are means ± SEM, n = 6 replicate pens. Means with different letters differ, $P < 0.05$.

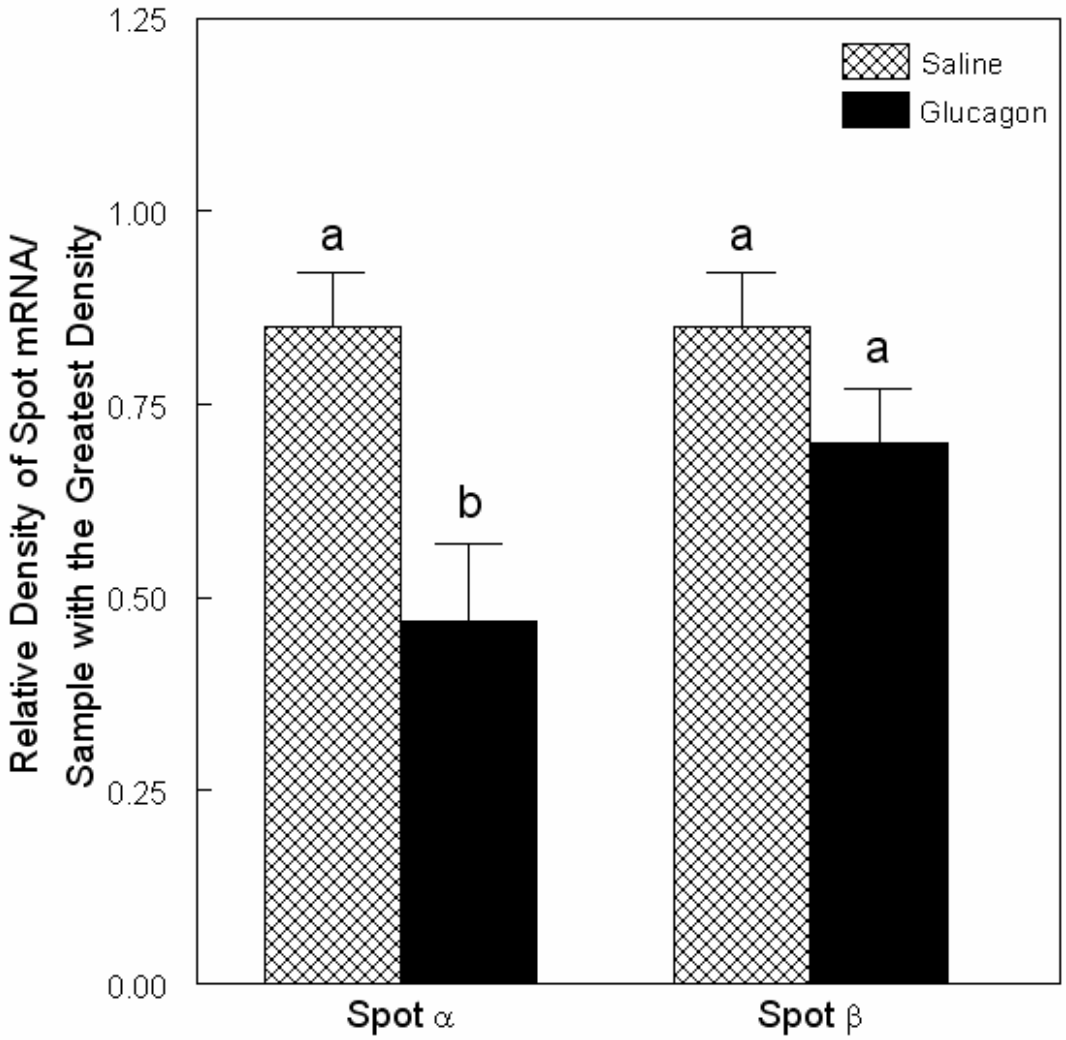

Figure 10 The relative density of hepatic spot 14α and β mRNA of chicks 3 h after a brachial vein injection of either saline or glucagon (240 μg/kg body weight, experiment 4). Values are means ± SEM, n = 4 replicate pens. Means with different letters differ, $P < 0.05$. Note that the relative densities of spot 14β and β mRNA are specific to one another and that all statistical comparisons are for either spot 14β or spot 14β.

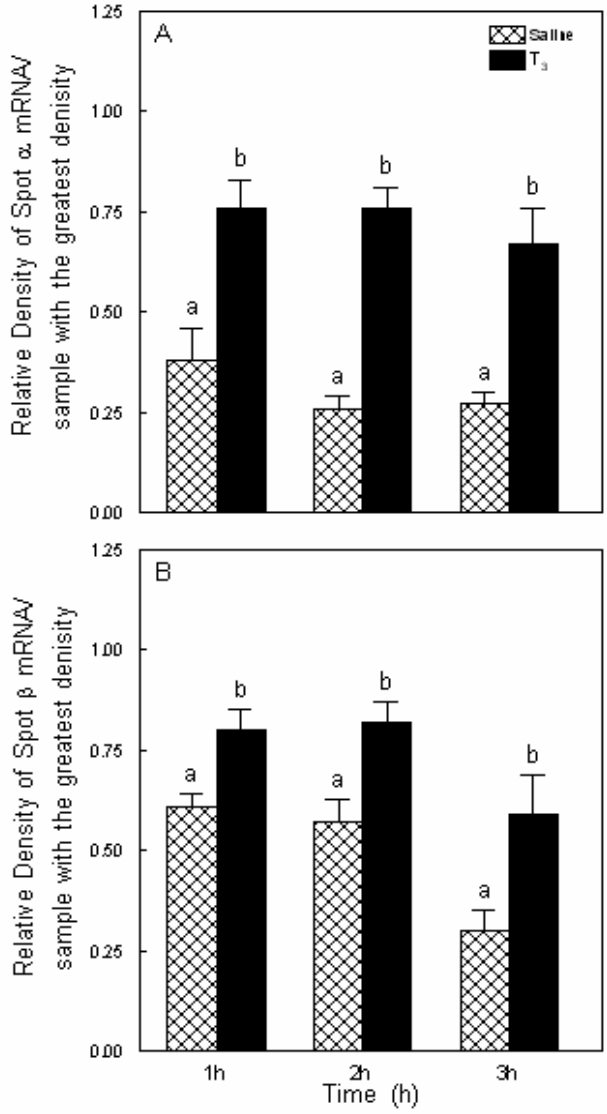

Figure 11. The relative density of hepatic spot 14α (A) and spot 14β (B) mRNA of chicks after 1, 2 or 3 h after a brachial vein injection of either saline or T_3 (20 mg/kg body weight, experiment 5). Values are means ± SEM, n = 6 replicate pens. Means with different letters differ, $P < 0.05$. Note that the relative densities of spot 14α and β mRNA are specific for each time point and that all statistical comparisons are within a given time period.

CHAPTER 6

GENERAL SUMMARY

Body fat accumulation is a major concern in the poultry industry. For broilers, excessive adipose tissue deposition decreases both feed efficiency and the yield of lean meat after processing. In addition, health conscious consumers prefer a lean product. For laying hens, the accumulation of body fat during the laying cycle is associated with decreased egg production and with interfering with normal follicular development and maturation. Although body fat can be derived from fatty acids consumed in the diet, in chickens which are not fed a lipid rich diet most of the fat is derived from *de novo* fatty acid synthesis. Therefore, knowledge about the molecular mechanisms that control *de novo* fatty acid synthesis and applied nutritional research that limits fat production in chickens is of critical value to the poultry industry.

Jones and Taylor (2001) reported that abdominal fat pad weights relative to total body weight were significantly increased in broilers fed diets containing whole wheat versus ground wheat. In the current research, feeding whole pearl millet was also associated with an increased body fat content as well. It is not clear why birds fed low levels of whole grains have increased fat pad weights. It could be assumed that more energy would be required to digest whole grain leaving less for carcass fat deposition, or there is some evidence suggesting that feeding whole grains may enhance digestion and nutrient absorption (Hetland et al., 2002 and Svihus et al. 2001). If nutrient absorption is increased when feeding whole grains and more energy is available to the bird, it remains unclear why this energy is not utilized more for lean growth than fat, especially given that the broilers in the previous research and the current research did not weigh more than the control birds. None the less, more research is needed to determine if feeding whole

grains does indeed increase body fat deposition and if increased fat accumulation can be prevented by decreasing the total energy content of diets containing whole grains.

Feeding increasing concentrations of dietary protein inhibits fat synthesis in birds (Rosebrough et al., 1985a, 1985b, 1986a, 1988, 1990, 1996, 1999 and 2002; Skinner et al., 1991; Kouba et al., 1992; Eits et al., 2002; Sklan and Plavnik, 2002 and Wylie et al., 2003). In the current research, this knowledge was used to prevent increased abdominal fat accumulation in broilers fed diets high in fat. By formulating diets so that the metabolizable energy to crude protein ratio remains constant, broilers can be fed diets containing fat concentrations up to 8.5 percent, without having an increased amount of abdominal fat present at the end of a 38 day production cycle.

The ability for increased dietary protein concentrations to decrease *de novo* fatty acid synthesis in birds is correlated with a decrease in malic enzyme activity that is preceded by a decrease in malic enzyme mRNA expression (Adams and Davis, 2001). More importantly, the changes in malic enzyme mRNA expression occur with in 3 h of a change in the dietary protein level (Adams and Davis, 2001). The current research established that the changes in malic enzyme mRNA expression are proceeded by changes in the mRNA expression of the transcription factor spot 14.

In chickens a gene duplication event has resulted in the presence of two forms of spot 14 (spot 14α and β). Both forms were detected by Northern analysis in livers obtained from broilers. Interestingly, although the hepatic mRNA expression of both forms of spot 14 in broilers decreased as the level of dietary protein ingested increased, the rate of this response differed between the two forms of spot 14. When broilers were fed one of three isocaloric diets containing 13, 22 or 40 % protein, the hepatic mRNA

expression of spot 14β was significantly different between the broilers fed the three diets within 1.5 h. A similar pattern of significance in hepatic spot 14α mRNA expression, however, was not seen until 6 h. Therefore, it appears only spot 14β contributes to the quick (3 h) change in malic enzyme transcription in response to a change in dietary protein intake.

The current research also indicates that dietary protein's influence on spot 14 mRNA expression may be mediated by glucagon and/or thyroid hormone. Increasing dietary protein consumption by chicks is associated with a rapid (1 h) elevation in the plasma concentration of glucagon (Chendrimada, 2003). Injections of glucagon into broiler chicks significantly decreased the expression of spot 14α , but did not alter spot 14β mRNA expression. Thus, the very early (1.5 h) depression in spot 14β mRNA observed when dietary protein intake is increased, must be the result of another mediator and not a direct effect of an elevated glucagon concentration.

This other mediator could be thyroid hormone. Injecting broiler chicks with T_3 rapidly (1 h) increased the mRNA expression of both spot 14α and β. The plasma T_3 concentration in broilers fed different levels of dietary protein has not been investigated for short periods of time after the change in dietary protein intake. The plasma concentration of T_3 in chicks fed a protein or amino acid deficient diet for several days, however, is significantly elevated (Alster and Carew, 1984, Keagy at al., 1987, Buyse et al., 1992 and Rosebrough et al. 1996). Therefore, future research that investigates whether a change in dietary protein intake quickly (1 h) alters plasma T_3 concentrations in broilers is warranted.

Interestingly, as with malic enzyme expression (Chendrimada, 2003), the expression of spot 14 α and β does not require changes in dietary protein per se, but rather just a change in dietary nitrogen intake. In fact even feeding broilers nonprotein nitrogen sources will successfully decrease spot 14α and β mRNA expression. Feeding these nonprotein nitrogen sources may be a way to economically reduce fatty acid synthesis and thus body fat content in broilers.

In conclusion, it is well established that increasing dietary protein concentrations will reduce *de novo* fatty acid synthesis in birds and subsequently body fat content. As researchers continue to delineate the biochemical mechanisms by which protein causes this effect, practical dietary manipulations may be identified to control the body fat content of broilers and the identification of critical biochemical mediators in the process may lead to their use as genetic markers in the selection of broilers for leanness.