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Dietary protein regulates in vitro lipogenesis and lipogenic gene expression in broilers[☆]

R.W. Rosebrough*, S.M. Poch, B.A. Russell, M.P. Richards

*Growth Biology Laboratory, Animal and Natural Resources Institute,
United States Department of Agriculture-Agricultural Research Service, Beltsville Agricultural Research Center, Beltsville,
MD 20705, USA*

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Abstract

The purpose of this experiment was to determine the possible relationship between certain indices of lipid metabolism and specific gene expression in chickens fed graded levels of dietary crude protein. Male, broiler chickens growing from 7 to 28 days of age were fed diets containing 12, 21 or 30% protein ad libitum. In addition, another group of birds was fed on a regimen consisting of a daily change in the dietary protein level (12 or 30%). This latter group was further subdivided such that one-half of the birds received each level of protein on alternating days. Birds were sampled from 28 to 30 days of age. Measurements taken included in vitro lipogenesis, malic enzyme activity the expression of the genes for malic enzyme, fatty acid synthase and acetyl coenzyme carboxylase. In vitro lipogenesis and malic enzyme activity were inversely related to dietary protein levels (12–30%) and to acute changes from 12 to 30%. In contrast, expression of malic enzyme, fatty acid synthase and acetyl CoA carboxylase genes were constant over a dietary protein range of 12–21%, but decreased by feeding a 30% protein diet (acute or chronic feeding). Results of the present study demonstrate a continued role for protein in the regulation of broiler metabolism. It should be pointed out, however, that metabolic regulation at the gene level only occurs when feeding very high levels of dietary protein. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Gene expression; Diets; Metabolism; Chickens; Lipogenesis; Enzyme activities

1. Introduction

Over a period of several years, we have studied the effects of dietary protein levels on various indices of lipid metabolism. Briefly, increased dietary protein decreases de novo lipogenesis and the activities of several associated enzymes (Rosebrough et al., 1989). In contrast, increased dietary protein increases plasma thyroxine (T_4) and insu-

lin-like growth factor I (IGF-I) while decreasing plasma triiodothyronine (T_3) and growth hormone (GH; Rosebrough et al., 1990). Likewise, acute changes in dietary protein, whether changing from a high to a low protein diet or vice versa, will cause a rapid increase or decrease, respectively, in de novo lipogenesis (Rosebrough et al., 1996).

Similar results concerning a relationship between dietary protein levels and body composition have been described by many groups. Donaldson (1985) reported a negative relationship dietary protein levels and body fat of chickens. Summers et al. (1992) suggested that the relationship between body fat and dietary protein could be explained based on the quantity of essential amino acids present in diets. Leeson et al. (1996) found

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*Corresponding author. Tel.: +1-301-504-8866; fax +1-301-504-8623.

E-mail address: rosebro@anri.barc.usda.gov (R.W. Rosebrough).

that both energy and protein had a comparable effect on growth rate although there was a linear decrease in carcass weight and breast meat yield as dietary protein decreased. Likewise, MacLeod (1997) reported differences in energy expenditure could be explained almost entirely by differences in quantities, and therefore costs, of protein and fat accretion.

Carbohydrates, fats and protein participate along with the endocrine system in changes in gene expression elicited by nutrients (Clark and Abraham, 1992). It may be possible to regulate gene expression anywhere from transcription to the actual enzyme protein. It would seem more efficient for regulation to occur at steps down stream from the actual transcription process. It should be emphasized that nutritional factors could regulate enzyme activity by any combination of factors impacting translation and post-translational events (Hesketh et al., 1998).

The purposes of this experiment were to further study the metabolic effects of chronic or acute dietary protein treatments and to determine if changes the levels of mRNA for certain lipogenic enzymes related to changes in metabolic rates noted with various levels of dietary crude protein. We chose to analyze malic enzyme activity and gene expression because of its central role in providing reducing equivalents to support de novo lipogenesis. In our experience, measurements of in vitro lipogenesis approximate the rate limiting activities of both acetyl CoA carboxylase and fatty acid synthase (Rosebrough and Steele, 1988; Rosebrough and Russell, 1993).

2. Materials and methods

2.1. Animals—general

All chickens were held under a quarantine that was certified by the station veterinarian. Chickens were observed daily for healthiness. One authorized animal caretaker was assigned to maintain the chickens over the course of the experiments. In addition, the research protocols were approved by the Beltsville Agricultural Research Animal Care Committee. Birds were allowed to consume both feed and water on an ad libitum basis. Birds were kept in electrically heated battery-brooders (four birds/pen replicate) in an environmentally controlled room (22 °C). A 12-h light (06:00 h to 18:00 h), 12-h dark (18:00 h to 06:00 h) cycle

Table 1
Composition of the experimental diets (g/kg diet)

Ingredient	Dietary crude protein (%)		
	12	21	30
Isolated soy protein ^a	20	180	280
Soybean meal	100	0	0
Corn meal	600	575	500
Glucose	180	180	120
Corn oil	10	0	40
Sand	25	0	0
Dicalcium phosphate	40	40	40
Limestone	10	10	10
L-Methionine ^b	5	5	5
Selenium premix ^c	1	1	1
Mineral premix ^d	1	1	1
Vitamin premix ^e	5	5	5
Iodized salt	3	3	3
Metabolizable energy (MJ/kg)	12.9	12.2	12.8
Calculated carbohydrate (%)	75	67	61

^a Soybean protein grade II (900 g/kg crude protein, 21726); Nutritional Biochemicals, P.O. Box 22400, Cleveland, OH 44122.

^b L-Methionine (18915), US Biochemicals.

^c Provided 0.2 mg Se/kg of diet.

^d Provided (mg/kg of diet): manganese 100, iron 100, copper 10, cobalt 1, iodine 1, zinc 100 and calcium 89.

^e Provided (mg/kg of diet): retinol 3.6, cholecalciferol 0.075, biotin 1, vitamin E (d- α -tocopherol) 10, riboflavin 10, pantothenic acid 20, choline 2 g, niacin 100, thiamine 10, vitamin B₆ 10, menadione sodium bisulfite 1.5, cyanocobalamin 0.1, folic acid 2 and ethoxyquin 150.

^f Calculated from the derived fat and protein energy contents of each of the dietary ingredients and subtracting that figure from the total energy value of the diet.

was maintained. The birds were killed by decapitation at 09:00 h to minimize possible diurnal variation.

2.2. Animals—experimental

Male broiler chickens growing from 7 to 28 days of age were fed diets containing 12, 21 or 30% protein (Table 1). The calculated carbohydrate content of each of the diets was determined by using published values for the protein and fat contents of each of the dietary ingredients, multiplying by 16.7 J for protein and 37.6 J for fat and subtracting those figures from the total metabolizable energy of the ingredient. It was assumed that this remainder was the carbohydrate contribution to the energetic value of that ingredient. In addition, another group of birds was fed on a regimen consisting of a daily change in the dietary protein level (12 or 30%). The latter treatment was con-

tinued for 10 cycles. Additionally, This group was also subdivided such that one-half of the birds received each level of protein on an alternating day. This subdivision was necessary to account for possible daily variation in responses. Birds were selected on days 28 to 30, weighed, bled by cardiac puncture and sacrificed. There were six pen replicates for each dietary treatment.

2.3. *In vitro* techniques—tissue preparation

Livers were rapidly excised into phosphate-buffered saline, rinsed to removed debris and held at 2 °C for later use for *in vitro* lipogenic studies. Portions of each liver were also snap frozen in liquid N₂ and stored at –80 °C for subsequent extraction of total RNA for later message expression studies.

2.4. *In vitro* techniques—in vitro metabolism—lipogenesis

Livers were excised, washed in 155 mM NaCl to remove blood and debris and then sliced (MacIlwain Tissue Chopper; 0.4–0.5 mm). Quadruplicate explants were incubated at 37 °C for 2 h in Hanks' balanced salts containing (Hanks and Wallace, 1949), 10 mM-HEPES and 10 mM-sodium[2-¹⁴C]acetate (166 MBq/mol). All incubations were conducted in 3 ml volumes at 37 °C for 2 h under a 95% O₂–5% CO₂ atmosphere (Rosebrough and Steele, 1985a,b, 1988). At the end of the stated incubation periods, the explants were placed in 10 ml of 2:1 chloroform/methanol for 18 h according to Folch et al. (1957). The extracts were evaporated to dryness and dispersed in scintillation fluid. Radioactivity in the extracts

was measured by liquid scintillation spectroscopy. *In vitro* lipogenesis was expressed as μmol of acetate incorporated into lipids per g of tissue.

2.5. *In vitro* metabolism—enzyme assay

Liver tissues were homogenized (1:10, w/v) in 100 mM HEPES (pH 7.5) 3.3 mM β-mercaptoethanol and centrifuged at 12 000×g for 30 min (Rosebrough and Steele, 1985a,b). The supernatant fractions were kept at –80 °C until analyzed for the activity of malate: NADP⁺ oxidoreductase-[decarboxylating] (malic enzyme, EC 1.1.1.40). Malic enzyme activity was determined by a modification of the method of Hsu and Lardy (1969). Reactions contained 50 mM HEPES (pH 7.5), 1 mM NADP, 10 mM MgCl₂ and the substrate, 2.2 mM-L-malate (disodium salt) in a total volume of 1 ml. Portions (50 μl) of the 12 000×g supernatants (diluted 1:10) were preincubated in the presence of the first three ingredients. Reactions were initiated by adding the substrate and following the rate of reduction per minute of NADP at 340 nm at 30 °C.

2.6. Lipogenic enzyme gene expression

Reverse transcription polymerase chain reaction (RT-PCR): Total RNA was isolated using the Tri-Reagent procedure (Life Technologies, Rockville, MD) and measured spectrophotometrically. RT reactions (50 μl) consisted of 5 μg total RNA, 100 units MMLV reverse transcriptase (RNase H minus, point mutation), 40 units RNasin, 1.0 mM of each dNTP, and 6 pmol random hexamer primers (Promega, Madison, WI). Hot-started PCR was performed in separate 27.5 μl reactions containing:

Table 2
Effects of diets containing either 12, 21 or 30% crude protein on the growth of broiler chickens from 7 to 28-days^{a,b}

Variable	Dietary crude protein (%)			
	12	21	30	Rotation
Body weight (g)	721 ± 25.9 ^a	1477 ± 31.2 ^{b,c}	1559 ± 27.3 ^c	1392 ± 25.9 ^b
Food eaten (g)	1741 ± 75.6 ^a	1973 ± 45.1 ^{b,c}	1894 ± 45.8 ^{a,b}	2113 ± 53.8 ^c
Carbohydrate intake ^d (g)	1300 ± 56.7	1323 ± 30.2	1255 ± 27.9	1358 ± 38.3
g gain/g food	0.33 ± 0.02 ^a	0.68 ± 0.02 ^b	0.75 ± 0.02 ^c	0.60 ± 0.03 ^b
g gain/g protein	2.77 ± 0.14 ^{a,b}	3.22 ± 0.09 ^c	2.50 ± 0.08 ^a	3.01 ± 0.13 ^b

^a Birds were fed diets containing 12, 21 or 30% on an ad libitum basis or on a rotation consisting of a daily change in dietary protein (12–30%).

^b Values are means ± S.E.M., n = 6.

^c Calculated from the recorded intakes of both the 12 and 30%.

^d Derived from multiplying the calculated carbohydrate percentage from Table 1 by the total feed intake.

PCR buffer, 0.5 (fatty acid synthase, acetyl CoA-carboxylase) or 1.25 (malic enzyme) units of Platinum Taq DNA polymerase (Life Technologies), 0.2 mM of each dNTP, 10 pmol each of each gene specific primer including a set for β -actin (see below), the internal standard. Each PCR was run as a duplex with primer sets added for a particular lipogenic enzyme and for β -actin. The final concentration of magnesium in the reaction was either 1.4 mM (fatty acid synthase, acetyl CoA-carboxylase) or 1.8 mM (malic enzyme). PCR thermal cycling parameters were as follows: 1 cycle 94 °C for 2 min, followed by 30 cycles, 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 1 min with a final extension at 72 °C for 8 min. RT-PCR produced dsDNA amplicons of 423, 431, 447 and 300 bp for fatty acid synthase, malic enzyme, acetyl-CoA carboxylase, and β -actin, respectively.

Gene-specific primers: The following gene-specific primer sets were used:

Gene	Sense primer	Antisense primer
Fatty acid synthase	5'-AAGGAGATTC-	5'-GGAGTCAAAC-
	CAGCATCGTGCAGC	AGTTATCCATGGCC
	5'-ATGAAGAGGGG-	5'-CGTTCTCCATC-
Malic enzyme	CTACGAGGT	TGTCACCAC
	5'-CACTTCGAGGC-	5'-GGAGCAAATCC-
Acetyl-CoA carboxylase	GAAAACTC	ATGACCACT
β -Actin	5'-TGCCTGACATC-	5'-TGCCAGGGTAC-
	AAGGAGAAG	ATTGTGGTA

Capillary electrophoresis (CE): Aliquots (2 μ l) of RT-PCR samples were diluted 1:100 with deionized water prior to analysis by CE. A P/ACE MDQ (Beckman Coulter, Inc., Fullerton, CA) configured for reversed polarity and equipped with an argon ion laser-induced fluorescence (LIF) detector was used to separate and detect the dsDNA amplicons. A μ SIL-DNA capillary (J and W Scientific, Folsom, CA) with a 75- μ m I.D., 0.075 μ m film thickness, and length of 32 cm was used at 25 °C. The dsDNA separation buffer was from Sigma (St. Louis, MO). Enhance™ dye (1 mg/ml stock in methanol) was added to the separation buffer to produce a final concentration of 0.5 μ g/ml. Diluted RT-PCR samples were loaded by electrokinetic injection at 3.5 kV for 5–10 s. Separations were performed at a field strength of 300 V/cm (8.1 kV) for 4.5 min.

Data analysis: P/ACE MDQ software (Beckman Coulter, Inc.) was used to calculate integrated peak areas. A lipogenic enzyme/ β -actin peak area ratio was then calculated and used to compare

tissue samples with respect to relative lipogenic enzyme gene expression activities. Ratio values are expressed as mean \pm S.E.M.

2.7. Statistical methods

Significance of differences was determined with Dunn's Multiple Comparison procedure using the pooled standard error derived from an analysis of variance (Remington and Schork, 1970).

3. Results

Table 2 summarizes the effect of the dietary treatments on growth of the chickens in the present study. The 12% protein diet decreased ($P < 0.01$) body weight, feed intake efficiency of feed utilization compared with the other three dietary treatments. The rotational protein-feeding regimen resulted in feed and protein efficiencies that were similar to feeding the 21% protein diet. In contrast, feeding the 30% protein diet resulted in the poorest ($P < 0.01$) utilization of dietary protein. There were no significant differences in calculated carbohydrate intakes among the treatments. The latter observation will be used as a justification in assuming metabolic differences were due to differences in protein intake per se and not to complications in carbohydrate intake.

Fig. 1 depicts a rather precipitous decrease ($P < 0.01$) in vitro lipogenesis accompanying an increase in dietary protein (12 < 21 < 30%). The decrease was particularly noticeable ($P < 0.01$), as protein was increased from 21 to 30%. The daily change in protein also affected in vitro lipogenesis as the birds were switched from 12 to 30% protein ($P < 0.05$). In contrast, it should be noted that the rate noted in birds fed the 12% diet as a part of a rotational regimen was similar to that noted in birds fed the 21% protein diet on an ad libitum basis.

Fig. 2 demonstrates that malic enzyme activity also paralleled the decrease in lipogenesis noted as dietary protein increased from 12% to 30% (12 < 21 < 30%). Again, a rather large decrease ($P < 0.01$) in activity was noted as dietary protein was increased from 21 to 30%. In contrast to its effect on lipogenesis, a daily change from 12 to 30% protein had no effect on malic enzyme activity. Moreover, activities were similar to that activity note in birds fed the 21% protein on an ad libitum basis.

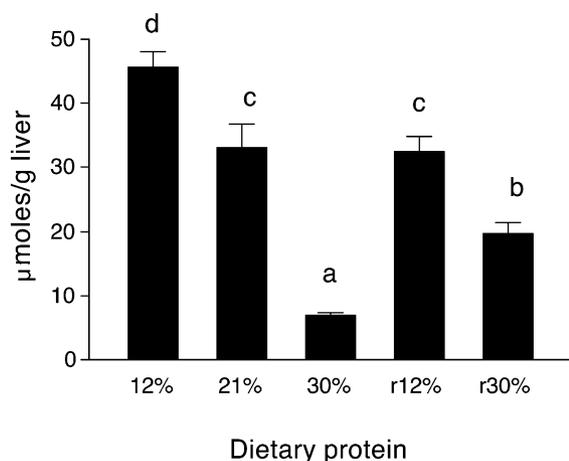


Fig. 1. In vitro lipogenesis in chickens fed diets containing 12, 21 or 30% and a rotational scheme consisting of a daily change in dietary protein (12 or 30%). Chickens were fed on these regimens from 7 to 28 days of age. Birds were selected and killed on days 28, 29 and 30. In vitro lipogenesis was determined by culturing liver explants for 2 h in the presence of 10 mmol/l-[2-¹⁴C]sodium acetate and by noting incorporation of acetate into hepatic lipids. Values for in vitro lipogenesis (IVL) are expressed as μmol of substrate incorporated/g of liver. Data are means for $n=6$ chickens at each time point. Bars represent S.E.M. Different superscripts indicate a significant difference ($P<0.05$) between values.

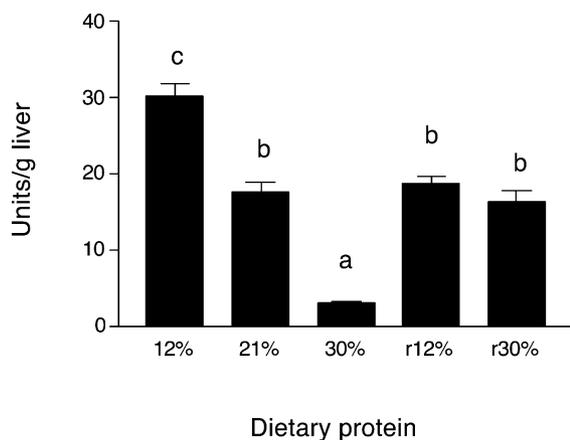


Fig. 2. Malic enzyme activity in chickens fed diets containing 12, 21 or 30% and a rotational scheme consisting of a daily change in dietary protein (12 or 30%). Chickens were fed on these regimens from 7 to 28 days of age. Malic enzyme (ME, EC 1.1.1.40) was determined in a $10\,000\times$ g liver supernatant fraction and expressed as μmol of reduced NADP formed per minute under standard assay conditions. Data are means for $n=6$ chickens at each time point. Bars represent S.E.M. Different superscripts indicate a significant difference ($P<0.05$) between values.

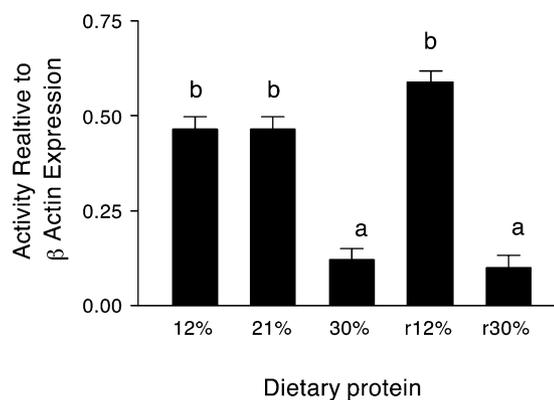


Fig. 3. Malic enzyme gene expression in chickens fed diets containing 12, 21 or 30% and a rotational scheme consisting of a daily change in dietary protein (12 or 30%). Gene expression is noted as the ratio of malic enzyme gene expression: β -actin gene expression. Data are means for $n=6$ chickens at each time point. Bars represent S.E.M. Different superscripts indicate a significant difference ($P<0.05$) between values.

Figs. 3–5 illustrate the role of dietary protein feeding regimens on the expression of certain genes whose products are implicated in supporting or regulating lipogenesis. Malic enzyme gene expression (gene expression relative to β -actin gene expression) was very nearly identical in birds fed diets containing either 12 or 21% protein, but was less ($P<0.01$) in birds fed the 30% protein diet (Fig. 3). The daily switch in dietary protein

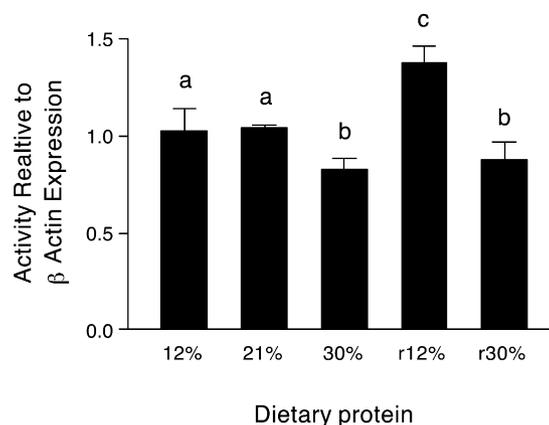


Fig. 4. Acetyl CoA carboxylase gene expression in chickens fed diets containing 12, 21 or 30% and a rotational scheme consisting of a daily change in dietary protein (12 or 30%). Gene expression is noted as the ratio of acetyl CoA carboxylase gene expression: β -actin gene expression. Data are means for $n=6$ chickens at each time point. Bars represent S.E.M. Different superscripts indicate a significant difference ($P<0.05$) between values.

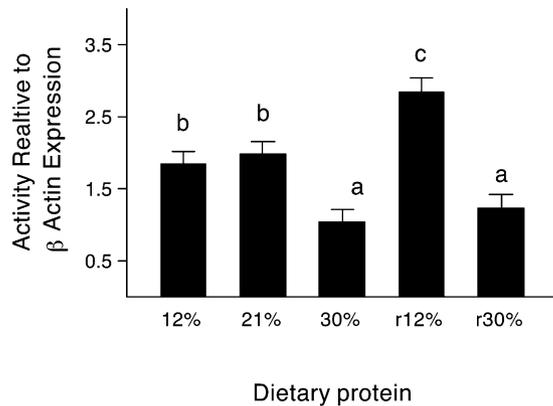


Fig. 5. Fatty acid synthase gene expression in chickens fed diets containing 12, 21 or 30% and a rotational scheme consisting of a daily change in dietary protein (12 or 30%). Gene expression is noted as the ratio of fatty acid synthase gene expression: β -actin gene expression. Data are means for $n=6$ chickens at each time point. Bars represent S.E.M. Different superscripts indicate a significant difference ($P<0.05$) between values.

from 12 to 30% resulted in a rapid decrease in gene expression. Acetyl CoA carboxylase gene expression was identical for birds fed 12 and 21% protein diets, but was decreased ($P<0.01$) by feeding the 30% protein diet (Fig. 4). In contrast, the decrease, although significant ($P<0.05$), was not as pronounced as the decrease in malic enzyme gene expression. The daily switch in dietary protein from 12 to 30% again, resulted in a rapid decrease ($P<0.01$) in gene expression. Fatty acid synthase gene expression followed the same pattern as previously noted for malic enzyme expression (Fig. 5). It should be noted that expression rates for acetyl CoA carboxylase and fatty acid synthase were greatest in chickens fed the 12% protein diet as a part of a daily change in dietary protein.

4. Discussion

We have noticed certain general observations concerning the effects of dietary protein on avian metabolism that were confirmed in the present study. Likewise, the present experiment demonstrated that increasing dietary protein would also decrease in vitro lipogenesis as well as malic enzyme activity. Few groups have offered any biochemical explanation for the decrease in lipogenesis coincident to the feeding of high-protein

diets. Yeh and Leveille (1969) found an inverse relationship between the level of the dietary protein and the subsequent rate of in vitro lipogenesis. They speculated that an increase in the dietary protein level decreased the flow of substrates through glycolysis and increased the production of glucose from substrates that were formerly in the pathways leading to fat synthesis. In addition, Tanaka et al. (1983) wrote that the supply of reducing equivalents (NADPH) regulates de novo lipogenesis in chickens. Based on a high correlation between malic enzyme activity and de novo lipogenesis, Yeh and Leveille (1969) originally proposed that availability of NADPH regulates lipid metabolism in chickens fed on high-protein diets. Furthermore, work from our group has rather convincingly shown that dietary protein per se influences avian lipid metabolism (Rosebrough and Steele, 1985a,b, 1986; Rosebrough et al., 1986, 1988, 1989, 1990). In these studies, we formulated diets containing similar amounts of carbohydrate and varied amounts of protein to prove that dietary carbohydrate availability was not the sole determinant in regulating lipogenesis in birds. In this respect, the present experiment supports our previous findings concerning the role of dietary protein per se in regulating avian lipid metabolism.

Goodridge et al. (1986) reported that feeding increased and starvation decreased malic enzyme and fatty acid synthase. This group concluded that steps in gene transcription regulated both enzymes during dietary perturbations. It was interesting to note that increased transcription and mRNA stability were responsible for the increase in malic enzyme seen in fasted-refed birds. It was proposed that some kind of inhibitor to malic acid degradation be synthesized during this regimen. In contrast, this same regimen only increased transcription of the fatty acid synthase gene. Goodridge (1987) reported a positive correlation between the rate of synthesis of long chain fatty acids and fatty acid synthase activity. Moreover, in the same work, Goodridge (1987) reported that feeding caused a rapid increase in fatty acid synthase gene transcription prior to any increase in enzyme activity. A much later work by Goodridge et al. (1996) further described the relationship between fasting–refeeding and stimulation of the transcription of the avian gene for malic enzyme. Ma et al. (1990) reaffirmed that feeding

fasted chickens would stimulate malic enzyme gene transcription and that this increase could occur in as little as 1.5 h following refeeding. We (Rosebrough and Steele, 1985a,b) hypothesized and described the similarities between lipogenic responses noted after fasting–refeeding and a switch from a high to a low protein diet. A more recent study (Rosebrough, 2000) indicated that changing from a high to a low protein diet or from a fasted to a fed state rapidly increased lipogenesis in a fashion similar to the meal-feeding response seen in rodents. The latter study also indicates that this increase is also greater when chickens are repeatedly fasted and refed or fed alternating high–low protein diets. It should be noted that similarities between high–low protein feeding and fasting–refeeding were noted in either chronic or acute bouts. Ma et al. (1990) reported that feeding fasted chickens stimulated malic enzyme gene transcription which would seem to support our finding that switching dietary protein levels changed the expression of the genes for both malic enzyme and fatty acid synthase.

Semenkovich et al. (1993) reported that glucose availability could control fatty acid synthase mRNA levels without having any effect on transcription initiation. This group seemed to indicate that mRNA stability could be affected by glucose availability.

This observation could very easily explain changes in malic enzyme activity and the lack of noted changes in the expression of the genes for malic enzyme, fatty acid synthase or acetyl CoA carboxylase in rodents.

The above report may not necessarily explain divergence between genes transcription and enzyme activity in the present study, as carbohydrate intakes among the dietary treatments were nearly equal. The disconnection between gene expression and enzyme activity has been noted in studies on glycogen synthase (Nur et al., 1995). This study revealed that starvation did not change glycogen synthase mRNA but did decrease enzyme activity and that fraction of mRNA associated with polyribosomes. Some of the early work by Goodridge et al. (1986) alluded to the possibility that both increased transcription and mRNA stability were responsible for the increase in malic enzyme mRNA in fasted–refed ducklings.

Bordas and Merat (1981) first described selection experiments based on feed intake. Tixier et

al. (1988) and El-Kazzi et al. (1995) later reported that selection for feed intake altered fat content of skin and breast muscle as well as changing the size of the abdominal fat. Differences between the selected lines were attributed to differences in fatty acid metabolism. A more recent study (Lagarigue et al., 2000) showed that mRNA levels of genes coding for certain lipogenic enzymes (acetyl-CoA carboxylase and fatty acid synthase) were not greater in a line of chickens selected for high feed intake in spite of consistently larger abdominal fat pads. Previous suggestions had been made that lipogenic enzyme activities were regulated at transcriptional steps (Goldman et al., 1985; Goodridge et al., 1986, 1996; Goodridge, 1987) and, by inference, lipogenesis. It is interesting to note that Carlson and Kim (1974) reported that reversible phosphorylation of acetyl CoA carboxylase dramatically changed fatty acid synthesis. We have shown that incubation of liver explants in the presence of either isoproterenol or cAMP decreased *in vitro* lipogenesis 80%, suggesting that rapid changes in lipogenic rates were under the control of phosphorylation-dephosphorylation steps. It is our hypothesis that *in vitro* lipogenesis is an *in situ* measurement of, at the very least, acetyl CoA carboxylase activity. A more liberal interpretation would be an *in situ* measurement of the acetyl CoA carboxylase–fatty acid synthase couple.

Hesketh et al. (1998) reported that although nutrition could alter the amount of enzyme protein by transcriptional, post-transcriptional or translational events, increasing evidence indicated that the latter two events were likely candidates for nutritional events. Indeed, the results of the present study are supportive of the above work. We have shown decreases in malic enzyme activity and *in vitro* lipogenesis without concomitant decreases in measurements of gene expression.

In summary, we have shown that feeding chickens diets containing crude protein levels from 12 to 21% will dramatically decrease *in vitro* lipogenesis without changing the expression of genes coding for enzymes involved in lipogenesis. In contrast, an increase in crude protein to 30% will further decrease lipogenesis in addition to decreasing the expression of genes coding for lipogenic enzymes. Rapid changes in dietary protein will change lipogenesis and expression of lipogenic

enzyme genes. The data from these experiments suggest the possibility that a combination of mRNA stability and post-transcriptional events interact to regulate lipogenesis in the chicken.

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