# Protein and energy relationships in the broiler chicken 12. Dietary protein and triiodothyronine ($T_3$) effects on the response of broilers to isoproterenol and cyclic adenosine monophosphate *in vitro**

BY R. W. ROSEBROUGH AND A. D. MITCHELL

*Nonruminant Animal Nutrition Laboratory, Livestock and Poultry Science Institute, United States Department of Agriculture–Agricultural Research Service, Beltsville Agricultural Research Center, Beltsville, MD 20705, USA*

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**Indian River male broiler chickens (7-d-old) were fed on diets containing 120, 210 or 300 g crude protein/kg + 0 or 1 mg triiodothyronine ($T_3$)/kg diet (Expt 1) and 120, 150, 180 or 210 g crude protein/kg + 0 or 1 mg $T_3$/kg diet (Expt 2) to determine the effects of crude protein level and $T_3$ on growth and metabolism. Body composition of chickens was determined by a combination of dissection of muscle and abdominal fat pads, and chemical extraction (Expt 1). *In vitro* lipogenesis (IVL) was determined in both experiments by incubating liver explants for 2 h at 37° in the presence of $10^{-4}$ M-dibutyryl cyclic AMP (cAMP) or $10^{-5}$ M isoproterenol (ISO) and $10^{-2}$ M-[2-$^{14}$C]acetate. Acetate incorporation into total lipid was an indication of IVL. Activity ratios for each of these additions relative to control (−cAMP−ISO) were calculated to ascertain basal *v.* inhibited rates of IVL. The relative muscle mass was increased by increasing crude protein from 120 to 210 g/kg diet but not from 210 to 300 g/kg diet. Dietary $T_3$ decreased total body lipid regardless of the dietary crude-protein level. Increasing dietary crude protein decreased ($P < 0{\cdot}05$) basal IVL (−cAMP−ISO) but not IVL (+cAMP). Dietary $T_3$ decreased basal IVL in birds fed on the diets containing 120 and 210 g crude protein/kg but had little effect on the two inhibited states of lipogenesis (+cAMP or +ISO). The component of lipogenesis sensitive to *in vitro* inhibition is also the component under dietary control.**

**Dietary protein: Triiodothyronine: Lipogenesis: Chicken**

Changes in the thyroid metabolism of poultry have been shown to give conflicting results. For example, a daily injection of thyrotropin-releasing hormone was shown to increase both plasma growth hormone and thyroid hormone levels (Cogburn *et al.* 1989). These increases were accompanied by an increase in growth, an effect not seen when plasma thyroid hormones were altered by diets (Decuypere *et al.* 1987). A recent study by our group shows that dietary thyroxine ($T_4$) is detrimental to growth (Rosebrough *et al.* 1992), a finding that contradicts work showing either no effect (Scanes *et al.* 1986) or a positive effect of $T_4$ (May, 1980). Other sets of data suggest that dietary triiodothyronine ($T_3$) decreases body fat (Cogburn *et al.* 1989) although body weight is also decreased (Harvey, 1983). In a similar fashion, chemical hypothyroidism, caused by either propylthiouracil or methimazole, also decreases growth (Chiasson *et al.* 1979). These two sets of observations

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show that both an artificial increase in plasma $T_4$ and a decrease in $T_3$ decrease body weight. These findings show that simple manipulations in circulating thyroid hormones do not increase growth.

The primary purpose of the present studies was to examine the interaction of dietary thyroid hormones and crude-protein levels on the effects of either a $\beta$-adrenergic agonist (isoproterenol) or cyclic AMP (cAMP) on *in vitro* lipogenesis. We have previously found that catecholamine metabolism is altered in the hyperthyroid chicken (Rosebrough, 1994) and have surmised that the relationship of metabolic measurements in the presence of a $\beta$-adrenergic agonist and cAMP would indicate coupling of the hormone to its second messenger system (Rosebrough & Jones, 1992). Peptide hormones such as catecholamines, or their synthetic analogues such as isoproterenol, interact with cell membrane receptors to change intracellular concentrations of cAMP which change metabolic processes.

A secondary purpose was to attempt to measure accurately crude-protein effects on metabolism. Prior work from our laboratory indicated a linear decrease in lipogenesis as dietary crude protein was increased from 120 to 300 g/kg (Rosebrough *et al.* 1988). This was accomplished by first measuring responses (growth, body composition, *in vitro* lipogenesis and hepatic enzyme activities) at three levels of crude protein (120, 210 and 300 g crude protein/kg diet). Diets were then formulated and fed based upon the level of crude protein giving the maximum inhibition of lipid metabolism.

## MATERIALS AND METHODS

### *Animals and diets*

*Expt 1.* Male, Indian River broiler chickens were grown under common conditions from 1 to 7 d of age. At 7 d of age, chickens were randomly assigned to one of six dietary treatments (120, 210 or 300 g crude protein/kg diet and 0 or 1 mg $T_3$/kg diet) for a 7 to 28 d growth trial. The six dietary treatments formed a three by two factorial arrangement with a total of four pen replicates (six birds per pen) for each dietary treatment. The different levels of dietary crude protein were obtained by mixing the two basal diets described in Table 1. Two chickens from each pen were randomly selected and killed by cervical dislocation to determine effects of dietary treatments on body composition and intermediary metabolism. One chicken from each pen was randomly selected and killed at 28 d by cervical dislocation to determine effects of dietary treatments on intermediary metabolism. Another bird was selected at 32 d for analysis of body composition.

*Expt 2.* Male, Indian River broiler chickens were grown under common conditions from 1 to 7 d of age. At 7 d of age, chickens were randomly assigned to one of eight dietary treatments (120, 150, 180 or 210 g crude protein/kg diet and 0 or 1 mg $T_3$/kg diet) for a 7 to 28 d growth trial. The eight dietary treatments formed a four by two factorial arrangement with a total of four pen replicates (six birds per pen) for each dietary treatment. The different levels of dietary crude protein were obtained by mixing the two basal diets described in Table 1. One chicken from each pen was randomly selected and killed at 28 d by cervical dislocation to determine effects of dietary treatments on intermediary metabolism.

### *Body composition*

Chickens from Expt 1 were killed by $CO_2$ asphyxiation to determine effects of dietary treatments on body composition. Determination of body composition was first accomplished by a combination of dissection and physical separation and weighing of the dissected parts. The abdominal fat pad was dissected from the viscera and weighed. Total muscle was calculated by adding together the weights of the wings, thighs, calves

Table 1. *Composition of the basal diets (g/kg diet)*

| | Dietary crude protein (N × 6·25) (g/kg diet) | |
|---|---|---|
| | 120 | 300 |
| Ingredient | | |
| Isolated soya-bean protein* | — | 100 |
| Soya-bean meal | 112 | 400 |
| Maize meal | 767 | 400 |
| Maize oil | 17 | 40 |
| Sand | 15 | — |
| Dicalcium phosphate | 40 | 40 |
| Limestone | 10 | 10 |
| L-Methionine† | — | 5 |
| Selenium premix‡ | 1 | 1 |
| Mineral premix§ | 1 | 1 |
| Vitamin premix‖ | 5 | 5 |
| Cellulose | 30 | — |
| Calculated composition | | |
| Metabolizable energy (MJ/kg) | 12·8 | 12·8 |
| Lysine (g/kg) | 6·0 | 17·3 |
| Sulphur amino acids (g/kg) | 10·3 | 10·3 |

* Soya-bean protein grade II (900 g crude protein/kg; 21726) Nutritional Biochemicals, PO Box 22400, Cleveland, Ohio 44122, USA.

† L-Methionine (18915), US Biochemicals, PO Box 22400, Cleveland, Ohio 44122, USA.

‡ Provided 0·2 mg Se/kg diet.

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

‖ Provided (mg/kg diet): retinol 3·6, cholecalciferol 0·075, biotin 1, vitamin E 10, riboflavin 10, pantothenic acid 20, choline 2 g, nicotinic acid 100, thiamin 10, pyridoxine 10, menadione sodium bisulphite 1·5, cyanocobalamin 0·1, pteroylmonoglutamic acid 2 and ethoxyquin 150.

(drumsticks) and breast. These muscles, as well as the remainder of the carcass (feathers removed), were homogenized, freeze-dried and extracted with chloroform–methanol (2:1, v/v) (Folch *et al.* 1957) to remove fat which was then measured gravimetrically. The viscera were then processed in a similar fashion as muscle to determine fat. Values for dissected and chemically extracted fat were then added together to give total body lipid. All values are reported per kg body weight.

### In vitro *metabolism: lipogenesis*

Livers of birds from both experiments were excised and washed initially with 155 mM-NaCl to remove blood and debris and then placed in chilled 10 mM-$Na_2HPO_4$–135 mM-NaCl (PBS; pH 7·4). The livers were then sliced (MacIlwain Tissue Chopper, Mickle Laboratory Engineering Company, Gomshall, Surrey) at a setting corresponding to a thickness of 0·3 mm to give explants weighing 35–75 mg. The explants were placed in 75 mm Petri dishes containing chilled PBS and then randomly allocated to *in vitro* treatments. Quadruplicate explants were incubated at 37° for 2 h in Hanks' balanced salts (Hanks & Wallace, 1949) supplemented with 10 mM-HEPES, 10 g/l bovine serum albumin (Bovuminar, Armour Pharmaceutical Company, Kankakee, IL, USA) and 10 mM-sodium[2-$^{14}$C]acetate (0·25 mBq/nmol). In addition, incubations contained $10^{-4}$ M-cAMP, $10^{-5}$ M-isoproterenol or no additions other than acetate. All incubations were conducted in 3 ml volumes at 37° for 2 h under an $O_2$–$CO_2$ (95:5, v/v) atmosphere (obtained by gassing vessels for 30 s). The

given concentration of isoproterenol was chosen because previous work from our laboratory indicated that this dosage caused a maximum inhibition of *in vitro* lipogenesis (Rosebrough & Steele, 1987). At the end of the stated incubation periods the explants were placed in 15 ml chloroform–methanol (2:1, v/v) for 18 h. The extract was partitioned into chloroform and aqueous phases with 3 ml 8·8 g/l KCl (Folch *et al.* 1957). The chloroform phase was evaporated to dryness and lipids were then dispersed in scintillation fluid and radioactivity measured by liquid scintillation spectroscopy.

### In vitro *metabolism: enzyme assays*

Remaining liver tissue was homogenized (1:10, w/v) in 50 mM-HEPES (pH 7·5)–3·3 mM-$\beta$-mercaptoethanol and centrifuged at 50000 ***g*** for 60 min (Rosebrough *et al.* 1988). The supernatant fractions were kept at 0° until analysed for the activities of malate:$NADP^+$ oxidoreductase (decarboxylating) (malic enzyme; *EC* 1.1.1.40), isocitrate:$NADP^+$ oxidoreductase (decarboxylating) (ICD(NADP); *EC* 1.1.1.42), fatty acid synthase (FAS; *EC* 2.3.1.85), tyrosine amino transferase (TAT; *EC* 2.6.1.5) and glutamic–oxaloacetic aminotransferase (GOT; *EC* 2.6.1.1).

Malic enzyme activity was determined by a modification of the method of Hsu & Lardy (1969). The reaction medium contained 50 mM-HEPES (pH 7·5), 1 mM-NADP, 5 mM-$MnCl_2$ and the substrate, 2·2 mM-L-malate (disodium salt) in a total volume of 1 ml. A 50 $\mu$l portion of the 50000 ***g*** supernatant fraction (diluted 1:10) was preincubated for 5 min in the presence of the first three ingredients. The reaction was initiated by adding the substrate and following the rate of reduction of NADP at 340 nm at 25°. The reaction was found to proceed linearly for at least 60 min providing that the reaction contained no more than 100 $\mu$g supernatant-fraction protein.

ICD (NADP) activity was determined by a modification of the method of Cleland *et al.* (1969). The reaction medium contained 50 mM-HEPES (pH 7·5), 1 mM-NADP, 5 mM-$MnCl_2$ and the substrate, 4·4 mM-DL-isocitrate in a total volume of 1 ml. A 50 $\mu$l portion of the 50000 ***g*** supernatant fraction (diluted 1:10) was preincubated in the presence of the first three ingredients. The reaction was initiated by adding the substrate and following the rate of reduction of NADP at 340 nm at 25°. The reaction was found to proceed linearly for at least 60 min providing that the reaction contained no more than 50 $\mu$g supernatant-fraction protein.

FAS was determined by a modification of the method of Mersmann *et al.* (1973). The reaction medium contained 50 mM-phosphate (pH 7·5), 3 mg/ml defatted bovine serum albumin, 0·15 mM-acetyl coenzyme A, 0·15 mM-NADPH and the substrate 0·3 mM-malonyl coenzyme A in a total volume of 1 ml. A 25 $\mu$l portion of the 50000 ***g*** supernatant fraction was preincubated in the presence of the first four ingredients. The reaction was initiated by adding the substrate and following the rate of oxidation of NADPH at 340 nm at 25°. The reaction was found to proceed linearly for 5 to 10 min providing that the reaction contained no more than 2·0 mg supernatant-fraction protein.

TAT activity was determined by the method of Granner & Tomkins (1970). The reaction contained 0·125 M-$K_2HPO_4$ (pH 7·4), 6 mM-L-tyrosine, 10 mM-pyridoxal phosphate (PLP) and 50 mM-2-oxoglutarate in a total volume of 1 ml. A 200 $\mu$l portion of the 50000 ***g*** supernatant fraction was preincubated at 37° in the presence of the first three ingredients. The reaction was then initiated by adding 2-oxoglutarate and allowing the reaction to proceed for 15 to 30 min. The reaction was stopped with 200 $\mu$l 10 M-NaOH and the absorption of the subsequent product, $\rho$-hydroxybenzaldehyde, was monitored at 331 nm.

GOT activity was determined by a modification of the method of Martin & Herbein (1976). The reaction medium contained 50 mM-HEPES, 200 mM-L-aspartate, 0·2 mM-NADH, 1000 units malate: $NAD^+$ oxidoreductase (*EC* 1.1.1.37)/l and the substrate,

15 mM-2-oxoglutarate, in a total volume of 1 ml. A 25 $\mu$l portion of the 50000 *g* supernatant fraction (diluted 1:10) was preincubated for 15 min in the presence of the first four ingredients. The reaction was initiated by adding the substrate and following the rate of oxidation of NADH at 340 nm at 25°. The reaction was found to proceed linearly for at least 30 min providing that the reaction contained no more than 50 $\mu$g supernatant-fraction protein. Enzyme activities are expressed as $\mu$mol product formed/min under the assay conditions (Rosebrough & Steele, 1985).

### *Statistical analyses*

Pen observations were considered as individual replicates. In the case of growth data this observation was derived for body-weight changes and feed consumption for any entire pen of birds. In the case of both body composition and metabolic data this observation was derived from one bird per pen. All data, except for those for *in vitro* lipogenesis, were analysed according to the model: Y = hormone (control or $T_3$), diet (level of crude protein) and hormone × diet. The actual values for crude-protein levels were used as the independent variables. In addition, crude-protein content was split into linear and quadratic components for tests of statistical significance. All interactions involving the blocking factor were pooled with the residual. Data for lipogenesis were subjected to natural log transformations because of a lack of homogeneity of error variances and because of profound differences in treatment means. Then, data for *in vitro* lipogenesis were analysed as a split-plot design with two error strata. The main treatment effects of diet and $T_3$ were tested against the between-plot error term while cAMP and isoproterenol, and their associated interactions, were tested against the within-plot error term. Pairwise comparisons of means were not conducted because of the previously mentioned lack of homogeneity of variances. The general linear models procedure (GLM) was used for the analyses of transformed data, least squares means, and mean square components (Remington & Schork, 1970).

## RESULTS

### *Growth and feed efficiency*

There were significant differences ($P < 0{\cdot}05$) among treatment means for body weights that could be attributed both to the crude-protein content of the diets and to dietary $T_3$ (Table 2; Expt 1). The body weights of chickens were decreased by increasing crude protein from 210 to 300 g/kg diet. The main treatment effect of crude-protein content (an increase in body weight with an increase in content from 120 to 210 g/kg diet) could be further delineated into both significant linear and quadratic components (Table 3; Expt 2). There were no significant interactions between crude protein and $T_3$ on either growth or food conversion efficiency in either experiment.

### *Body composition*

The data for body composition are presented per kg body weight (Table 4). This conversion allowed comparisons to be made among chickens of vastly different body weights. The relative muscle mass was increased by increasing crude protein from 120 to 210 g/kg diet but not from 210 to 300 g/kg diet. Likewise, breast muscle as a percentage of total muscle was not increased by increasing crude protein from 210 to 300 g/kg diet. The effect of $T_3$ was inconsistent. For example, relative muscle mass was increased by $T_3$ in birds consuming the diet containing 210 g crude protein/kg. Breast muscle as a percentage of total muscle was increased by $T_3$ when chickens were given either of the two lower levels of crude protein.

Table 2. *Expt 1. Feed intake, feed efficiency, and body weight (BW) of broiler chickens fed for 21 d on diets containing different levels of crude protein (N × 6·25), with or without triiodothyronine ($T_3$)**

(Mean values for four pen means per dietary treatment)

| Dietary crude protein (g/kg diet) | Weight (g) | | Feed efficiency (g gain/g feed) | | Feed intake (g/bird) | | Average $T_3$ intake (μg/d per kg BW) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| 120 | | | | | | | | |
| Control | 619[b] | 48·4 | 0·47[ab] | 0·048 | 1039[b] | 37·3 | | |
| +1 mg $T_3$/kg diet | 443[a] | 16·4 | 0·39[a] | 0·018 | 806[a] | 13·2 | 133 | 2·1 |
| 210 | | | | | | | | |
| Control | 1031[d] | 52·6 | 0·73[c] | 0·031 | 1241[c] | 76·0 | | |
| +1 mg $T_3$/kg diet | 701[bc] | 36·6 | 0·54[b] | 0·026 | 1051[b] | 32·3 | 122 | 3·7 |
| 300 | | | | | | | | |
| Control | 942[d] | 34·9 | 0·73[c] | 0·055 | 1139[bc] | 72·2 | | |
| +1 mg $T_3$/kg diet | 795[c] | 66·1 | 0·56[b] | 0·045 | 1189[bc] | 50·9 | 125 | 5·3 |

[a,b,c,d] Values within a column with unlike superscript letters were significantly different ($P < 0·05$).
* For details of diets and procedures, see Table 1 and p. 74.

Table 3. *Expt 2. Feed intake, feed efficiency, and body weight (BW) of broiler chickens fed for 21 d on diets containing different levels of crude protein (N × 6·25), with or without triiodothyronine ($T_3$)**

(Mean values for four pen means per dietary treatment)

| Dietary crude protein (g/kg diet) | Weight (g) | | Feed efficiency (g gain/g feed) | | Feed intake (g/bird) | | Average $T_3$ intake (μg/d per kg BW) | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| 120 | | | | | | | | |
| Control | 645[b] | 16·7 | 0·46[ab] | 0·022 | 1124[b] | 69·6 | | |
| +1 mg $T_3$/kg diet | 491[a] | 23·3 | 0·44[a] | 0·020 | 812[a] | 43·8 | 128 | 6·9 |
| 150 | | | | | | | | |
| Control | 954[c] | 42·2 | 0·57[d] | 0·014 | 1457[c] | 52·7 | | |
| +1 mg $T_3$/kg diet | 676[b] | 13·8 | 0·51[bc] | 0·036 | 1085[b] | 50·0 | 132 | 6·0 |
| 180 | | | | | | | | |
| Control | 1007[c] | 20·1 | 0·63[e] | 0·013 | 1399[c] | 11·4 | | |
| +1 mg $T_3$/kg diet | 719[b] | 16·6 | 0·55[c] | 0·015 | 1081[b] | 18·8 | 125 | 2·1 |
| 210 | | | | | | | | |
| Control | 1004[c] | 47·6 | 0·59[de] | 0·039 | 1480[c] | 50·2 | | |
| +1 mg $T_3$/kg diet | 763[b] | 13·6 | 0·56[cd] | 0·026 | 1143[b] | 47·2 | 112 | 4·6 |

[a,b,c,d,e] Values within a column with unlike superscript letters were significantly different ($P < 0·05$).
* For details of diets and procedures, see Table 1 and p. 74.

### *Enzyme activities*

The effects of the energy:protein ratio and dietary $T_3$ on the activities of certain hepatic enzymes are presented in Table 5 (Expt 1) and Table 6 (Expt 2). Both GOT and ICD (NADP) activities increased significantly ($P < 0·05$) with an increase in dietary crude-protein content. TAT activities were equal in birds fed on diets containing either 120 or

Table 4. *Expt 1. Body composition of broiler chickens fed for 21 d on diets containing different levels of crude protein (N × 6·25), with or without triiodothyronine ($T_3$)**

(Mean values for four pen means per dietary treatment)

| Dietary crude protein (g/kg diet) | Muscle† (g/kg BW) | | Breast muscle (% muscle) | | Body lipid (g/kg BW) | | Abdominal fat pad (g/kg BW) | | Muscle lipid (g/kg BW) | | Viscera lipid (g/kg BW) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| 120 | | | | | | | | | | | | |
| Control | 225[a] | 3·4 | 31·8[b] | 0·59 | 188[c] | 12·4 | 28[c] | 6·6 | 23[d] | 0·9 | 27[c] | 2·5 |
| +1 mg $T_3$/kg diet | 238[a] | 1·4 | 29·6[a] | 0·50 | 94[b] | 8·7 | 3[a] | 0·9 | 14[c] | 0·9 | 9[a] | 1·3 |
| 210 | | | | | | | | | | | | |
| Control | 278[b] | 7·4 | 35·6[c] | 0·87 | 94[b] | 9·2 | 9[b] | 3·2 | 11[b] | 0·4 | 17[b] | 0·8 |
| +1 mg $T_3$/kg diet | 299[c] | 6·1 | 38·1[d] | 0·97 | 37[a] | 3·4[a] | ND[a] | | 6[a] | 0·6 | 9[a] | 1·4 |
| 300 | | | | | | | | | | | | |
| Control | 302[c] | 8·5 | 35·9[c] | 0·86 | 83[b] | 6·9 | 7[b] | 1·1 | 10[b] | 0·8 | 18[b] | 1·4 |
| +1 mg $T_3$/kg diet | 291[bc] | 7·6 | 37·4[cd] | 0·61 | 35[a] | 5·8 | ND[a] | | 6[a] | 0·6 | 7[a] | 0·6 |

BW, body weight; ND, not detectable.

[a, b, c, d] Values within a column with unlike superscript letters were significantly different ($P < 0·05$).

* For details of diets and procedures, see Table 1 and pp. 74–75.

† Calculated by adding the weights of the wings, thighs, calves (drumsticks) and breast and dividing by total body weight.

Table 5. *Expt 1. Hepatic enzyme activities (units*/g liver) in broiler chickens fed for 21 d on diets containing different levels of crude protein (N × 6·25), with or without triiodothyronine ($T_3$)†*

(Mean values for four pen means per dietary treatment)

| Dietary crude protein (g/kg diet) | Malic enzyme | | ICD (NADP) | | FAS | | TAT | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| 120 | | | | | | | | |
| Control | 18·5[c] | 1·40 | 9·2[a] | 0·93 | 2·9[de] | 0·28 | 0·19[a] | 0·021 |
| +1 mg $T_3$/kg diet | 19·3[c] | 1·88 | 13·7[a] | 2·09 | 3·2[e] | 0·28 | 0·21[a] | 0·013 |
| 210 | | | | | | | | |
| Control | 12·6[b] | 1·54 | 20·7[b] | 2·12 | 2·5[cd] | 0·20 | 0·25[a] | 0·039 |
| +1 mg $T_3$/kg diet | 14·6[b] | 0·72 | 20·5[b] | 1·09 | 2·2[bc] | 0·20 | 0·16[a] | 0·008 |
| 300 | | | | | | | | |
| Control | 5·4[a] | 0·94 | 26·2[c] | 1·32 | 1·7[ab] | 0·22 | 0·63[b] | 0·089 |
| +1 mg $T_3$/kg diet | 2·5[a] | 0·48 | 36·3[d] | 3·21 | 1·4[a] | 0·08 | 0·69[b] | 0·062 |

Malic enzyme, malate:$NADP^+$ oxidoreductase (decarboxylating) (*EC* 1.1.1.40); ICD(NADP), isocitrate:$NADP^+$ oxidoreductase (decarboxylating) (*EC* 1.1.1.42); FAS, fatty acid synthase (*EC* 2.3.1.85); TAT, tyrosine amino transferase (*EC* 2.6.1.5).

[a, b, c, d, e] Values within a column with unlike superscript letters were significantly different ($P < 0·05$).

* One unit is that amount of enzyme resulting in the production of 1 μmol oxidized or reduced NAD(P)/min at 25°.

† For details of diets and procedures, see Table 1 and pp. 74–76.

Table 6. *Expt 2. Hepatic enzyme activities (units*/g liver) in broiler chickens fed for 21 d on diets containing different levels of crude protein (N × 6·25), with or without triiodothyronine ($T_3$)*†

(Mean values for four pen means per dietary treatment)

| Dietary crude protein (g/kg diet) | Malic enzyme | | ICD (NADP) | | FAS | | GOT | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| 120 | | | | | | | | |
| Control | 14·4$^{c}$ | 1·93 | 9·9$^{a}$ | 1·11 | 2·0$^{b}$ | 0·29 | 14·9$^{a}$ | 1·47 |
| +1 mg $T_3$/kg diet | 13·8$^{bc}$ | 1·37 | 9·0$^{a}$ | 0·56 | 1·6$^{ab}$ | 0·26 | 14·4$^{a}$ | 0·56 |
| 150 | | | | | | | | |
| Control | 12·8$^{bc}$ | 1·68 | 11·7$^{a}$ | 1·56 | 1·9$^{b}$ | 0·11 | 15·3$^{a}$ | 0·66 |
| +1 mg $T_3$/kg diet | 13·6$^{bc}$ | 2·44 | 12·9$^{a}$ | 1·49 | 1·7$^{ab}$ | 0·32 | 15·4$^{a}$ | 1·47 |
| 180 | | | | | | | | |
| Control | 8·8$^{ab}$ | 2·18 | 21·1$^{b}$ | 2·20 | 1·9$^{b}$ | 0·10 | 21·4$^{b}$ | 1·19 |
| +1 mg $T_3$/kg diet | 8·2$^{a}$ | 1·95 | 22·3$^{b}$ | 2·70 | 1·4$^{ab}$ | 0·38 | 19·9$^{b}$ | 1·51 |
| 210 | | | | | | | | |
| Control | 8·7$^{ab}$ | 1·61 | 21·4$^{b}$ | 1·44 | 1·2$^{a}$ | 0·20 | 23·7$^{c}$ | 1·32 |
| +1 mg $T_3$/kg diet | 9·4$^{ab}$ | 1·28 | 21·4$^{b}$ | 3·42 | 1·2$^{a}$ | 0·09 | 19·1$^{b}$ | 1·35 |

Malic enzyme, malate:$NADP^+$ oxidoreductase (decarboxylating) (*EC* 1.1.1.40); ICD(NADP), isocitrate:$NADP^+$ oxidoreductase (decarboxylating) (*EC* 1.1.1.42); FAS, fatty acid synthase (*EC* 2.3.1.85); GOT, glutamic–oxaloacetic aminotransferase (*EC* 2.6.1.1).

$^{a,b,c}$ Values within a column with unlike superscript letters were significantly different ($P < 0·05$).

* One unit is that amount of enzyme resulting in the production of 1 μmol oxidized or reduced NAD(P)/min at 25°.

† For details of diets and procedures, see Table 1 and pp. 74–77.

180 g crude protein/kg; however, these activities were less than TAT in birds fed on the diet containing 300 g crude protein/kg. In contrast, FAS and malic enzyme activities decreased significantly ($P < 0·05$) with an increase in crude-protein content (120 *v.* 300 g crude protein/kg diet, Table 5 and 120 *v.* 210 g crude protein/kg, Table 6). In contrast, dietary $T_3$ did not change enzyme activities. Significant diet × $T_3$ interactions were not observed for the activities of either of these enzymes.

## In vitro *lipogenesis*

The effects of dietary protein and $T_3$ on lipogenesis are presented in Table 7 (Expt 1) and Table 8 (Expt 2). Pooling data in Expt 1 across incubation conditions revealed an overall decrease in lipogenesis of 89% (25·5 *v.* 2·8 μmol/g liver; $P < 0·05$) that accompanied an increase in the protein level from 12 to 30%. Pooling data across protein levels and incubations showed that dietary $T_3$ decreased lipogenesis 25% (14·9 *v.* 11·2 μmol/g liver; $P < 0·05$). A significant protein level × $T_3$ interaction was also noted in Expt 1. In this context it should be noted that a significant dietary protein × $T_3$ interaction implies that effects of the hormone are specific to the high rate of lipogenesis that accompanied the feeding of the lowest levels of crude protein (120 g/kg diet). The effect of dietary $T_3$ was not noted when incubations were conducted in the presence of $10^{-5}$ M-isoproterenol although the crude-protein effect was still noticeable. The effects of both dietary crude protein and $T_3$ were not noted when incubations were conducted in the presence of $10^{-4}$ M-cAMP.

Table 8 summarizes Expt 2, an experiment that attempted to define the level of dietary crude protein between 120 and 210 g/kg that resulted in the greatest change in lipogenesis. The greatest decrease was noted when crude protein was increased from 180 to 210 g/kg

Table 7. *Expt 1.* In vitro *lipogenesis (μmol substrate incorporated/g liver) in cultured liver explants from broiler chickens fed for 21 d on diets containing different levels of crude protein (N × 6·25), with or without triiodothyronine ($T_3$)**

(Mean values for four pen means per dietary treatment)

| Dietary crude protein (g/kg diet) | *In vitro* additions: Control Mean | SE | $10^{-5}$ M-Isoproterenol Mean | SE | $10^{-4}$ M-cAMP Mean | SE |
|---|---|---|---|---|---|---|
| 120 | | | | | | |
| Control | 30·8 | 2·51 | 10·9 | 2·13 | 4·5 | 0·52 |
| +1 mg $T_3$/kg diet | 20·3 | 2·40 | 9·5 | 2·17 | 1·2 | 0·66 |
| 210 | | | | | | |
| Control | 10·9 | 2·33 | 4·3 | 1·09 | 2·1 | 0·69 |
| +1 mg $T_3$/kg diet | 10·8 | 1·61 | 3·9 | 0·85 | 1·3 | 0·49 |
| 300 | | | | | | |
| Control | 3·2 | 0·69 | 2·1 | 0·42 | 1·3 | 0·36 |
| +1 mg $T_3$/kg diet | 2·5 | 0·83 | 0·8 | 0·17 | 0·4 | 0·09 |

* For details of diets and procedures, see Table 1 and pp. 74–76.

Table 8. *Expt 2.* In vitro *lipogenesis (μmol substrate incorporated/g liver) in cultured liver explants from broiler chickens fed for 21 d on diets containing different levels of crude protein (N × 6·25), with or without triiodothyronine ($T_3$)**

(Mean values for four pen means per dietary treatment)

| Dietary crude protein (g/kg diet) | *In vitro* additions: Control Mean | SE | $10^{-5}$ M-Isoproterenol Mean | SE | $10^{-4}$ M-cAMP Mean | SE |
|---|---|---|---|---|---|---|
| 120 | | | | | | |
| Control | 26·9 | 3·34 | 8·7 | 3·53 | 2·8 | 0·44 |
| +1 mg $T_3$/kg diet | 15·8 | 4·53 | 4·8 | 1·56 | 2·7 | 0·83 |
| 150 | | | | | | |
| Control | 21·4 | 3·45 | 10·0 | 0·87 | 2·0 | 1·18 |
| +1 mg $T_3$/kg diet | 12·8 | 3·47 | 5·1 | 1·57 | 1·9 | 0·81 |
| 180 | | | | | | |
| Control | 18·9 | 4·70 | 6·4 | 1·75 | 2·1 | 0·86 |
| +1 mg $T_3$/kg diet | 8·2 | 1·48 | 3·6 | 0·99 | 1·1 | 0·54 |
| 210 | | | | | | |
| Control | 7·5 | 3·61 | 7·4 | 1·31 | 1·6 | 0·43 |
| +1 mg $T_3$/kg diet | 3·7 | 0·91 | 3·8 | 0·74 | 0·6 | 0·24 |

* For details of diets and procedures, see Table 1 and pp. 74–76.

diet (13·6 *v.* 5·6 μmol/g). The decreases noted as crude protein was increased from 120 to 150 and from 150 to 180 g/kg diet were similar (20% decrease in lipogenesis). Dietary $T_3$ decreased lipogenesis at all levels of crude protein. In contrast, lipogenesis in the presence of $10^{-5}$ M-isoproterenol was not decreased when crude protein was increased from 120 to 150 g/kg diet. Instead, this measurement required an increase of dietary protein from 150

to 180 g/kg diet to show a decrease in lipogenesis. Again, the effects of both dietary crude protein and $T_3$ were not noted when incubations were conducted in the presence of $10^{-4}$ M-cAMP.

## DISCUSSION

The objectives of the present study were to study further the interactions of dietary crude-protein level and $T_3$ on *in vitro* lipid metabolism and growth of chickens. The results given in the present report seem to indicate that crude-protein levels greater than 210 g/kg diet have no more effect on body composition than diets containing 210 g/kg. Further work is needed to define that level of crude protein between 120 and 210 g/kg diet that results in both a depression of *de novo* lipogenesis and a decrease in carcass lipid. There are many reports concerning the relationship between dietary energy and protein and subsequent effects on intermediary metabolism; few studies attempt to explain the basis of changes in the lean:fat ratio in the animal carcass and the energy:protein relationship in the diet. For example, diets with small energy:protein ratios promote lean broiler carcasses (Donaldson *et al.* 1956; Thomas & Combs, 1967) while diets containing large energy:protein ratios promote high rates of *in vitro* lipogenesis (Rosebrough & Steele, 1985) and *in vivo* lipid synthesis by the liver (Donaldson, 1985). Yeh & Leveille (1969) found an inverse relationship between the level of dietary protein and the subsequent rate of *in vitro* lipogenesis and 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. It is also possible that diets containing very small energy:protein ratios promote lean broilers by restricting energy consumption (Bartov, 1979). Excretion of surplus amino acid N may also require metabolic energy that would not be available for fat synthesis (Buttery & Borman, 1976).

In contrast to mammalian data, the thyroid–growth hormone relationship may be one of the most controversial areas in avian growth and development. This relationship can be altered either by changing dietary protein levels (Lauterio & Scanes, 1987) or by providing dietary thyroid hormones (Harvey, 1983; Scanes *et al.* 1986). In the former case plasma growth hormone increased and in the latter case plasma growth hormone decreased. Neither of these studies examined permutations of the relationship and effects on metabolism. In two later studies the relationship was altered by either pulsatile administration of chicken growth hormone (increased plasma growth hormone; Rosebrough *et al.* 1991) or by feeding $T_3$ (decreased plasma growth hormone; Rosebrough *et al.* 1992). In both cases *de novo* lipogenesis was depressed although the mechanisms remain obscure.

The results of the present study indicate that $T_3$ depresses lipogenesis, but has little effect on the activities of certain lipogenic enzymes. In this respect our results provide a contrast to other work. For example, Clarke & Hembree (1990) injected $T_3$ into rats (20–150 $\mu$g/kg body weight per d) and found an increase in malic enzyme activity. Previous articles describing metabolism in rodents also showed that $T_3$ increased lipogenesis (Roncari & Murthy, 1975) and lipogenic enzyme activities (Mariash *et al.* 1980). $T_3$ administration has been reported to increase rat liver malic enzyme mRNA abundance, possibly by altering transcriptional events, nuclear processing or mRNA turnover (Magnuson & Nikodem, 1983). $T_3$ stimulates transcription by forming a nuclear hormone–receptor complex that is the enhancer of transcription (Dozin *et al.* 1986; Perez-Castillo *et al.* 1987; Jump *et al.* 1988). It follows that a relative increase in mRNA abundance results in an increase in enzyme protein synthesis and an increase in activity (Clarke & Hembree, 1990).

Although results in the present study were obtained by feeding $T_3$, the daily dose given to birds in the present study was similar to the dose given to the rats in the above study

(110–130 $\mu$g/kg body weight per d). There is no reason to believe that the mode of delivery should be a consideration in evaluating effects, however. It is also important to realize that although malic enzyme may provide the necessary NADPH for lipogenesis, the enzyme may not strictly regulate lipogenesis according to the results of the present study. A more plausible explanation is that malic enzyme reflects NADPH utilization and does not *per se* regulate fatty acid synthesis. For example, the lowest noted activity in Expt 1 (birds fed on the diet containing 300 g crude protein/kg; Table 4) would result in the formation of NADPH far in excess of that required for the *de novo* synthesis of the fatty acids noted for this group.

The present study further supports the hypothesis that catecholamines can be used to inhibit *in vitro* lipogenesis in avian explants in a fashion that has been described for hepatocytes (Capuzzi *et al.* 1975; Cramb *et al.* 1982; Campbell & Scanes, 1985). Control of lipogenesis in chicken liver may be more complex than a simple mechanism involving cAMP and a cAMP-dependent protein kinase-catalysed phosphorylation of a rate-limiting enzyme (acetyl coenzyme A carboxylase; *EC* 6.4.1.2). For example, Clarke *et al.* (1979) proposed that the level of cellular citrate in chicken liver is particularly important in the regulation of the conversion of the protomeric to the polymeric form of acetyl coenzyme A carboxylase. The latter form is the more active of the two forms. Therefore, regulation of glycolytic flux and the turnover of citrate may also influence *de novo* lipogenesis. At first glance the data of Campbell & Scanes (1985) indicate that hepatocytes are preferable to explants because of their greater degree of sensitivity to catecholamines; however, certain differences in methodologies (as well as intact tissue *v.* isolated cells) make comparisons among studies difficult. The possibility also exists that either spare $\beta$-adrenergic receptors are unmasked or that further binding ability is gained during hepatocyte isolation techniques (Nakamura *et al.* 1983).

In summary, the present experiments demonstrated that feeding a diet containing a crude-protein level of 210 g/kg resulted in the greatest relative muscle mass. Even though *in vitro* lipogenesis was less in chicken fed on a diet containing 300 g crude protein/kg, body lipid was not further decreased.

It was also shown that *in vitro* cAMP, isoproterenol and dietary $T_3$ depress lipogenesis. Both the *in vitro* treatments were most noteworthy when low-protein diets were fed. It cannot be determined, at present, whether this effect is characteristic of low-protein diets *per se* or high rates of lipogenesis. The ratio of lipogenesis in the presence of isoproterenol to that in the presence of cAMP may be useful in studying catecholamine effects under various dietary treatments.

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