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Carry-over effects of dietary crude protein and triiodothyronine (T_3) in broiler chickens*

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Indian River male broiler chickens growing from 7 to 30 d of age were fed on diets containing crude protein levels ranging from 120 to 300 g/kg plus 0 or 1 mg triiodothyronine (T_3)/kg diet. The purpose of this study was to examine the effects of these treatments on lipogenesis after a common diet was fed (180 g crude protein/kg diet from 30 to 56 d of age). Dietary treatment groups were sampled at 30 and 56 d. *In vitro* lipogenesis was determined by incubating liver explants for 2 h at 37° in Hanks' salts containing 25 mM-HEPES and 10 mM-[2-^{14}C]acetate and then measuring acetate incorporation into total lipid. Growth and feed consumption from 7 to 30 d increased ($P < 0{\cdot}01$) as dietary protein increased from 120 to 210 g/kg diet. Both measurements decreased as crude protein increased from 210 to 300 g/kg diet. T_3 decreased ($P < 0{\cdot}01$) growth and feed intake during this period. Low-protein (< 180 g/kg) diets increased ($P < 0{\cdot}05$) and T_3 decreased lipogenesis in 30-d-old chickens. Although birds given T_3 from 7 to 30 d grew at the greatest rate from 30 to 56 d of age, the final body weight was still less than controls. *In vitro* lipogenesis at 56 d of age was not affected by either of the two dietary treatments. In contrast, the relative size of the abdominal fat pad (g/kg body weight) at 56 d was decreased by feeding T_3 from 7 to 30 d. Any changes in metabolism elicited by either dietary protein levels or hormone treatments may be specific to the particular dosing interval and are not sustained when a common diet is fed during a repletion period.

Dietary protein: Triiodothyronine: Lipogenesis: Chicken

Diets containing high energy:protein ratios promote *de novo* lipogenesis resulting in obese broiler chickens (Donaldson, 1985; Rosebrough & Steele, 1985). On the other hand, diets with low energy:protein ratios promote lean broiler carcasses (Donaldson *et al.* 1956; Thomas & Combs, 1967). Feeding a very low energy:protein diet (43 MJ/kg crude protein) results in a very lean carcass (crude protein as a percentage of DM) when compared with results attained by feeding a higher energy:protein diet (65 MJ/kg crude protein; Rosebrough & Steele, 1985). Bartov (1979) has proposed that the excretion of excess amino acid N from diets containing high levels of crude protein would require energy. This requirement for energy would come at the expense of that used for fat synthesis.

Later work has suggested an interaction between thyroid status and dietary crude protein on lipid metabolism. Dietary triiodothyronine (T_3) depressed lipogenesis in chickens fed on a diet containing 106 MJ/kg crude protein. Artificial changes in thyroid metabolism gave conflicting results in poultry (Leung *et al.* 1984*a*, *b*, 1985). For example, a daily injection of thyrotropin-releasing hormone increased both plasma growth hormone and thyroid hormone levels (Cogburn *et al.* 1989). These increases were accompanied by an increase in

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body weight, an effect not seen when plasma thyroid hormones were altered by diets (Decuypere *et al.* 1987). Other sets of data suggest that dietary T_3 decreases body fat (Cogburn *et al.* 1989) although body weight is also decreased (Harvey, 1983). Chemical hypothyroidism, caused by either propylthiouracil (PTU) or methimazole, also decreases growth (Chiasson *et al.* 1979).

The purposes of the studies in the present report were (1) to examine further the interaction of dietary thyroid hormones and crude protein levels on metabolism of broilers and (2) to determine carry-over effects of these treatments after birds were switched to a common dietary treatment.

MATERIALS AND METHODS

Animals and diets

At 7 d of age, male, Indian River broiler chickens were assigned to one of six dietary treatments (120, 210 or 300 g protein containing either 0 or 1 mg T_3/kg diet) for a 7–30 d growth trial. These dietary treatments formed a factorial arrangement with a total of four pen replicates (six birds per pen) for each dietary treatment. The chickens were housed in battery-brooders in an environmentally controlled room maintained at 23° with a 12 h light–dark cycle (06.00–18.00 hours light). Treatments were randomly assigned to pens in each battery. One chicken from each pen was randomly selected and killed at 30 d by cervical dislocation to determine effects of dietary treatments on intermediary metabolism. Chickens from the above treatment groups were also given a common dietary treatment (180 g protein/kg diet) and grown from 30 to 56 d. These birds were also killed to determine any carry-over effects of starter protein or thyroid status. The experimental diets are described in Table 1. The intermediate level of crude protein was obtained by mixing equal portions of the low- and high-protein diets.

In vitro *metabolism: lipogenesis*

Livers were excised, washed in 155 mM-NaCl to remove blood and debris and then sliced (MacIlwain Tissue Chopper; 0·3 mm). Quadruplicate explants were incubated at 37° for 2 h in Hanks' balanced salts (Hanks & Wallace, 1949; Rosebrough & Steele, 1987) supplemented with 10 mM-HEPES, 10 g/l bovine serum albumin and 10 mM-sodium[2-^{14}C]acetate (18 d.p.m./nmol). All incubations were conducted in 3 ml volumes at 37° for 2 h under an O_2–CO_2 (95:5, v/v) atmosphere. At the end of the stated incubation periods the explants were placed in 10 ml chloroform–methanol (2:1, v/v) for 18 h and washed according to the method of 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 *metabolism: enzyme assays*

Remaining liver tissues were homogenized (1:10, w/v) in 100 mM-HEPES (pH 7·5)–3·3 mM-β-mercaptoethanol and centrifuged at 12000 *g* for 30 min (Rosebrough *et al.* 1988). The supernatant fractions were kept at 0° until analysed for the activities of malate:$NADP^+$ oxidoreductase-(decarboxylating) (MDH(NADP), *EC* 1.1.1.40), isocitrate:$NADP^+$ oxidoreductase-(decarboxylating) (ICD(NADP), *EC* 1.1.1.42) and aspartate amino-transferase (AAT, *EC* 2.6.1.1). The activity of MDH(NADP) was monitored because of the enzyme's role in providing reducing equivalents (NADPH) for the synthesis of fatty acids. ICD(NADP) may function as both a residual source for the provision of NADPH and as a source of a coreactant for transamination. AAT functions to remove excess amine groups formed as a result of the feeding of high-protein diets.

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

	Dietary crude protein (N × 6·25) (g/kg diet)	
	120	300
Ingredients		
Isolated soyabean protein*	—	100
Soyabean 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
Fat (MJ/kg)	1·8	2·2
Protein (MJ/kg)	2·1	5·4
Carbohydrate (MJ/kg)	8·9	5·2
Lysine (g/kg)	6·0	17·3
Sulphur amino acids (g/kg)	10·3	10·3

* Soyabean protein grade II (900 g crude protein/kg, 21 726); Nutritional Biochemicals, Cleveland, Ohio 44122, USA.

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

‡ Provided 0·2 mg Se/kg diet.

§ Provided (mg/kg diet): Mn 100, Fe 100, Cu 10, Co 1, I 1, Zn 100 and Ca 89.

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

MDH(NADP) activity was determined by a modification of the method of Hsu & Lardy (1969). Reactions contained 50 mM-HEPES (pH 7·5), 1 mM-NADP, 5 mM-$MnCl_2$ and the substrate, 2·2 M-L-malate (disodium salt), in a total volume of 1 ml. Portions (50 μl) of the 12000 *g* supernatant fractions (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 of NADP at 340 nm at 30°.

ICD(NADP) activity was determined by a modification of the method of Cleland *et al.* (1969). Reactions 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. Portions (50 μl) of the 12000 *g* supernatant fractions (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 of NADP at 340 nm at 30°.

AAT activity was determined by a modification of the method of Martin & Herbein (1976). Reactions contained 50 mM-HEPES, 200 mM-L-aspartate, 0·2 mM-NADH, 1000 units/l malate: NAD^+ oxidoreductase (*EC* 1.1.1.37) and the substrate, 15 mM-2-oxoglutarate, in a total volume of 1 ml. Portions (25 μl) of the 12000 *g* supernatant fractions (diluted 1:10) were pre-incubated in the presence of the first four ingredients. Reactions were initiated by adding the substrate and following the rate of oxidation of

NADH at 340 nm at 30°. Enzyme activities are expressed as μmol product formed/min under the assay conditions (Rosebrough & Steele, 1985).

It was decided not to sample any chickens at 30 d of age because abdominal fat pads (AFP) were not present in chickens fed on diets containing either 210 or 300 g crude protein and 1 mg T_3/kg diet. At the time of slaughter at 56 d, the AFP were quickly removed and frozen at −80°. Lipoprotein lipase (LPL: *EC* 3.1.1.34) was determined by modifications of the methods of Nilsson-Ehle & Schotz (1976), Quig *et al.* (1983) and Griffin *et al.* (1987). Preliminary experiments revealed that LPL was stable for at least 120 d in AFP stored at −80°. Samples of the AFP were homogenized (1:10, w/v) in 100 mM-HEPES (pH 7·5)–3·3 mM-β-mercaptoethanol–100 μg/ml heparin and centrifuged at 12000 *g* for 30 min. The supernatant fractions were kept at 0° until analysed. The assay involves the enzymic hydrolysis of 2 mM-tri-[1-^{14}C]oleoylglycerol in a glycerol emulsion containing L-α phosphatidyl choline, 16% fasted chicken serum and 50 mM-Tris (pH 8·0) in a total volume of 200 μl (100 μl supernatant fraction and 100 μl emulsion). The reactions were initially found to proceed linearly for 120 min at 37°, but were routinely stopped after 30 min. The reactions were stopped with 3 ml methanol–chloroform–heptane (1·41:1·25:1, by vol.) followed by 1·00 ml 100 mM-potassium-borate buffer (pH 10·5) and then mixing for 30 s. The mixtures were centrifuged for 15 min at 600 *g* to form an aqueous phase. These phases were transferred into vials and counted by liquid scintillation spectroscopy. Enzyme activities are expressed as μmol oleic acid liberated/kg body weight per h under the above assay conditions.

Hormone and metabolite assays

Both T_3 and thyroxine (T_4) concentrations were estimated with a solid-phase single-antibody procedure that is commercially available (CN Biomedicals, Irvine, CA, USA). These assays were validated for avian samples (Rosebrough *et al.* 1988) by dispersing standards in charcoal-stripped chicken serums and by noting recovery of added T_3 and T_4 (98%). Plasma insulin-like growth factor-I (IGF-I) was estimated with a radio-immunoassay as previously described (McMurtry *et al.* 1994). All assays were conducted as single batches to remove inter-assay variation.

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 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. 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. The general linear models (GLM) procedure was used for the analyses of transformed data, least squares means, and mean square components (Remington & Schork, 1970).

RESULTS

Growth and efficiency of feed utilization

There were significant differences ($P < 0·05$) among treatment means for body weights at 30 d of age that could be ascribed to both crude protein content of the diets as well as dietary T_3 (Table 2). The body weights of chickens were increased by increasing crude protein from 120 to 210 g/kg diet. A further increase in crude protein from 210 to 300 g/kg

Table 2. *Effects of dietary crude protein and triiodothyronine (T_3) on growth of broiler chickens**

(Mean values for four observations per dietary treatment)

	30 d variables		30–56 d variables		
7–30 d diet	Body wt (g)	F/G†	Body wt (g)	Wt gain (g)	F/G†
120-Control	624[b]	2·25[c]	2168[b]	1544[e]	1·88[a]
120-1 mg T_3/kg	548[a]	2·69[d]	2008[a]	1460[d]	1·86[a]
210-Control	1610[d]	1·66[a]	2770[e]	1160[a]	2·12[b]
210-1 mg T_3/kg	1293[c]	1·85[b]	2531[d]	1238[b]	2·17[b]
300-Control	1541[d]	1·95[b]	2889[e]	1348[c]	2·09[b]
300-1 mg T_3/kg	1227[c]	2·27[c]	2554[c]	1327[c]	2·17[b]
Pooled SEM	12·8	0·028	29·1	16·3	0·027

[a,b,c,d,e] Mean values within a column not sharing a common superscript letter were significantly different ($P < 0·05$).

* Male, Indian River broiler chickens (7 d old, average weight 145 g) were fed on diets containing 120, 210, and 300 g crude protein/kg diet + 0 or 1 mg T_3/kg diet for a 21 d growth period. Chickens were then selected from each treatment to determine the effects of dietary treatments on intermediary metabolism. For details of diets, see Table 1 and p. 574.

† F/G = feed intake/body-weight gain.

resulted in a slight, insignificant decrease in body weight. Regardless of crude protein content, dietary T_3 decreased body weight and increased the amount of feed required per unit of body-weight gain in birds growing from 7 to 30 d.

Significant effects of dietary treatments from 7 to 30 d persisted at 56 d even though birds had been fed on a common diet from day 30. Birds fed on the lowest level of crude protein from 7 to 30 d of age were smaller than the other protein treatment groups at 56 d. In contrast, these birds grew at a greater rate from 30 to 56 d than did these other groups. Pooling data across all protein treatments from 7 to 30 d revealed a persistent negative effect of T_3 at 56 d even though the common diet fed from 30 to 56 d did not contain T_3.

The results for body composition are presented per kg body weight (Table 3). This conversion allowed comparisons to be made among chickens of vastly different body weights. The relative lean tissue mass was increased by increasing crude protein from 120 to 210 g/kg diet but not from 210 to 300 g/kg diet. In contrast, both total body lipid and AFP weight were decreased by increasing dietary protein. The effect of dietary T_3 was additive to the effect of crude protein on both total body fat and AFP weight. In birds fed on the two higher levels of crude protein and T_3, the AFP was not detectable. The effects of 7 to 30 d dietary treatments on 56 d body composition were inconsistent. Although total body lipid did not vary among the treatments, the AFP at 56 d of age was greatest in those birds fed on the diet containing 120 g crude protein and 0 mg T_3/kg diet from 7 to 30 d. The smallest AFP at 56 d of age were noted in those birds fed on diets containing either 210 or 300 g crude protein and 1 mg T_3/kg diet or 300 g crude protein and 0 mg T_3/kg diet from 7 to 30 d of age.

Enzyme activities

The effects of the energy:protein ratio and dietary T_3 on hepatic enzyme activities in 30-d-old chickens are presented in Table 4. Both AAT and ICD activities increased significantly ($P < 0·05$) with an increase in dietary crude protein content. In contrast, MDH activities decreased significantly ($P < 0·05$) with an increase in crude protein content (120 *v.* 300 g crude protein/kg diet; Table 4). Dietary T_3 did not change enzyme activities.

Table 3. *Effects of different levels of dietary crude protein and triiodothyronine (T_3) on body composition of broiler chickens**

(Mean values for four observations per dietary treatment)

	30 d variables			56 d variables				
7–30 d diet	AFP (g/kg BW)	Lipid (g/kg BW)	Protein (g/kg BW)	AFP (g/kg BW)	Lipid (g/kg BW)	Lipid†	Protein (g/kg BW)	Protein†
120-Control	14·6^{d}	152·3^{d}	157·4^{a}	14·6^{c}	117·1^{a}	152·8^{a}	172·2^{a}	266·3^{b}
120-1 mg T_3/kg	2·4^{b}	96·5^{c}	165·5^{a}	7·8^{b}	114·5^{a}	176·5^{ab}	174·5^{a}	256·6^{b}
210-Control	7·6^{c}	99·3^{c}	187·4^{b}	7·9^{b}	124·2^{a}	183·0^{ab}	176·2^{a}	189·7^{a}
210-1 mg T_3/kg	ND	48·7^{a}	204·9^{d}	4·3^{ab}	112·0^{a}	191·6^{ab}	182·5^{a}	167·3^{a}
300-Control	2·6^{b}	76·4^{b}	187·1^{b}	4·4^{ab}	118·3^{a}	217·5^{c}	175·9^{a}	222·2^{ab}
300-1 mg T_3/kg	ND	41·8^{a}	195·7^{cd}	3·2^{a}	121·5^{a}	201·2^{bc}	181·0^{a}	228·6^{ab}
Pooled SEM	1·05	5·00	2·18	1·02	6·18	12·81	2·08	21·11

AFP, abdominal fat pad; ND, not detectable; BW, body weight.

^{a,b,c,d} Mean values within a column not sharing a common superscript letter were significantly different ($P < 0·05$).

* For details of diets and procedures, see Table 1 and p. 574.

Table 4. *Hepatic enzyme activities (units*/g liver) in broiler chickens fed on diets containing different levels of crude protein, with or without triiodothyronine (T_3)†*

(Mean values for four observations per dietary treatment)

	30 d activities			56 d activities		
7–30 d diet	MDH	ICD	AAT	MDH	ICD	AAT
120-Control	22·1^{c}	14·6^{a}	45·8^{a}	10·3^{ab}	19·3^{a}	83·8^{a}
120-1 mg T_3/kg	20·1^{c}	18·5^{ab}	51·4^{ab}	14·7^{b}	15·8^{a}	77·8^{a}
210-Control	9·5^{b}	21·0^{ab}	66·2^{bc}	9·4^{a}	14·8^{a}	72·4^{a}
210-1 mg T_3/kg	6·2^{ab}	27·9^{b}	78·1^{c}	11·3^{ab}	16·6^{a}	74·5^{a}
300-Control	2·6^{a}	27·7^{b}	76·1^{c}	12·8^{ab}	16·3^{a}	70·4^{a}
300-1 mg T_3/kg	2·1^{a}	54·1^{c}	101·9^{d}	13·8^{ab}	16·5^{a}	84·9^{a}
SEM	1·41	2·75	4·69	1·06	1·09	5·27

MDH, malate:NADP^{+} oxidoreductase-(decarboxylating) (*EC* 1.1.1.40); AAT, aspartate aminotransferase (*EC* 2.6.1.1); ICD, isocitrate:NADP^{+} oxidoreductase-(decarboxylating) (*EC* 1.1.1.42).

^{a,b,c} Mean values within a column not sharing a common superscript letter 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 30°.

† For details of diets and procedures, see Table 1 and pp. 574–576.

Significant diet × T_3 interactions were not observed for the activities of either of these enzymes. There were no consistent trends in enzyme activities in 56-d-old chickens that could be attributed to prior dietary treatments.

The effects of dietary crude protein and T_3 on lipogenesis are presented in Table 5. There was an overall decrease in lipogenesis that corresponded to an increase in dietary crude protein content ($P < 0·01$). Pooling data across crude protein levels showed that dietary T_3 decreased lipogenesis 33% (20·1 *v.* 31·5 μmol/g liver; $P < 0·01$). The AFP could not be measured in birds fed on either of the two higher levels of crude protein in conjunction with T_3. Therefore, LPL was not determined in 30-d-old chickens. There were no significant

Table 5. *Effects of different levels of dietary crude protein and triiodothyronine (T_3) on* in vitro *hepatic lipogenesis (IVL) and abdominal fat pad lipoprotein lipase (LPL; EC 3.1.1.34) activity in broiler chickens**

(Mean values with their standard errors for four observations per dietary treatment)

	30 d activities IVL (μmol/g liver)		56 d activities IVL (μmol/g liver)		56 d activities LPL (μmol/pad per h)	
7–30 d diet	Mean	SEM	Mean	SEM	Mean	SEM
120-Control	65·6^{e}	3·41	29·0^{a}	1·83	336·2^{e}	13·9
120-1 mg T_3/kg	48·6^{d}	1·87	30·8^{a}	2·57	164·0^{c}	9·8
210-Control	21·7^{c}	1·51	36·5^{a}	1·05	217·4^{d}	5·9
210-1 mg T_3/kg	9·3^{b}	0·32	32·8^{a}	1·96	118·6^{b}	9·9
300-Control	7·3^{ab}	0·25	27·5^{a}	1·94	120·9^{b}	12·7
300-1 mg T_3/kg	4·6^{a}	0·26	34·9^{a}	2·15	69·8^{a}	12·7

^{a,b,c,d} Mean values within a column not sharing a common superscript letter were significantly different ($P < 0·05$).

* For details of diets see Table 1 and p. 574. IVL was determined by culturing liver explants for 2 h in the presence of 10 mM-[2-^{14}C]sodium acetate and by noting incorporation of acetate into hepatic lipids. Values are expressed as μmol substrate incorporated per g liver. LPL was determined by incubating portions of a 12000 *g* supernatant fraction derived from an abdominal fat pad homogenate in the presence of 1 mM-[1-^{14}C]triolein and by measuring radioactivity in fatty acids. Values are expressed as μmol oleic acid released per abdominal fat pad per h.

Table 6. *Plasma hormone concentrations of broiler chickens fed on diets containing different levels of crude protein and triiodothyronine (T_3)**

(Mean values for four observations per dietary treatment)

	30 d concentrations (ng/ml)			56 d concentrations (ng/ml)		
7–30 d diet	T_3	T_4	IGF-I	T_3	T_4	IGF-I
120-Control	4·0^{b}	5·4^{b}	31·6^{a}	3·5^{a}	7·9^{a}	54·6^{a}
120-1 mg T_3/kg	9·1^{d}	2·2^{a}	32·4^{ab}	3·9^{a}	8·6^{a}	56·3^{a}
210-Control	2·9^{a}	12·1^{c}	47·9^{d}	4·1^{a}	8·5^{a}	52·0^{a}
210-1 mg T_3/kg	8·1^{c}	3·3^{ab}	38·8^{c}	4·0^{a}	8·5^{a}	52·9^{a}
300-Control	2·7^{a}	11·9^{c}	47·9^{d}	4·4^{a}	8·3^{a}	53·7^{a}
300-1 mg T_3/kg	8·4^{cd}	2·9^{a}	36·5^{bc}	3·6^{a}	8·1^{a}	51·6^{a}
SEM	0·23	0·72	1·13	0·35	0·96	1·87

T_4, thyroxine; IGF-I, insulin-like growth factor-I.

^{a,b,c,d} Mean values within a column not sharing a common superscript letter were significantly different ($P < 0·05$).

* For details of diets and procedures, see Table 1 and pp. 574–576.

effects of 7–30 d dietary treatments on lipogenesis in 56-d-old chickens. In contrast, because of the persistent effects of both dietary protein and T_3 on AFP size, LPL per AFP reflected prior dietary treatments ($P < 0·05$).

Plasma hormones

An increase in dietary crude protein decreased ($P < 0·05$) plasma T_3 and increased ($P < 0·05$) plasma T_4 in 30-d-old chickens (Table 6). The addition of T_3 to the diets attenuated

these effects of dietary protein, resulting in uniformly high values for plasma T_3 and low values for plasma T_4. Chickens fed on the diets containing either 210 or 300 g crude protein/kg diet and no added T_3 had greater ($P < 0.05$) levels of IGF-I than did chickens fed on the diet containing 120 g crude protein and no added T_3. There were no significant carry-over effects of either crude protein or T_3 at 56 d of age.

DISCUSSION

Few studies have presented any biochemical logic for the decrease in lipogenesis accompanying the feeding of high-protein diets. Yeh & Leveille (1969) found an inverse relationship between the level of the dietary protein and the ensuing rate of *in vitro* lipogenesis. Possibly an increase in the dietary protein level decreased the flow of substrates through glycolysis and increased the production of glucose from substrates formerly in the pathways leading to fat synthesis. It has also been hypothesized that under conditions of feeding high-protein diets, transamination of amino acids would deplete 2-oxoglutarate and force citrate from lipid synthetic pathways to 2-oxoglutarate production to support transamination (Rosebrough *et al.* 1988). In addition, malate transport from the mitochondrion requires 2-oxoglutarate influx. Thus, malate availability for the reaction catalysed by MDH(NADP) may depend on citrate utilization and the production of 2-oxoglutarate. The enzyme activities in the present study also suggest that ICD(NADP) may function in both lipid and protein metabolism by providing a residual capacity for the production of reducing equivalents during a period of decreased MDH(NADP) activity and by providing 2-oxoglutarate (a product of the reaction catalysed by ICD(NADP) as a coreactant for transamination.

Although total LPL at 56 d appeared to be influenced by 7–30 d treatments, activity per g tissue did not vary among treatments. In this respect, Griffin *et al.* (1987) provide similar findings in studying differences between broiler and egg-laying chickens. In both strains the pattern of its increase relative to body weight was similar to that of abdominal fat. Differences in the LPL activity of abdominal fat between strains were attributed to differences in adipocyte number. Moreover, differences were reduced if values were expressed relative to tissue weight. A later study of Guo *et al.* (1988) indicated that although total LPL was significantly correlated to fat-pad weight, activity per unit of AFP was unrelated to total fat-pad weight. In both of these studies the authors state that LPL is of little value in predicting fat content of birds.

Dietary protein levels from 120 to 210 g/kg diet profoundly affected IGF-I levels in the 30-d-old chicken. Although depressed IGF-I values in humans can be returned to normal with T_4 replacement (Valcavi *et al.* 1987), dietary T_3 did not uniformly change plasma IGF-I concentrations in the present study. There was no carry-over effect of 30 d dietary treatments on subsequent IGF-I values at 56 d.

In summary, it was shown that both dietary crude protein and T_3 influenced certain indices of intermediary metabolism and both growth and body composition. The effects on intermediary metabolism were temporary and did not persist when a common diet was fed for a subsequent period of time. Further research must focus on the first few days following the switch from test diets to the common diet.

REFERENCES

Bartov, I. (1979). Nutritional factors affecting quantity and quality of carcass fat in chickens. *Federation Proceedings* **38**, 2627–2639.

Chiasson, R. B., Sharp, P. J., Klandorf, H., Scanes, C. G. & Harvey, S. (1979). The effect of rapeseed meal and methimazole on levels of plasma hormones in growing broiler cockerels. *Poultry Science* **58**, 1575–1583.

Cleland, W. W., Thompson, V. M. & Barden, R. E. (1969). Isocitrate dehydrogenase (TPN specific) from pig heart. In *Methods in Enzymology*, vol. 13, pp. 30–33 [J. M. Lowenstein, editor]. New York: Academic Press.

Cogburn, L. A., Liou, S. S., Alfonso, C. P., McGuiness, M. C. & McMurtry, J. P. (1989). Dietary thyrotropin-releasing hormone stimulates growth rate and increases the insulin:glucagon molar ratio of broiler chickens. *Proceedings of the Society for Experimental Biology and Medicine* **192**, 127–134.

Decuypere, E., Buyse, J., Scanes, C. G., Huybrechts, L. & Kuhn, E. R. (1987). Effects of hyper- or hypothyroid status on growth, adiposity and levels of growth hormone, somatomedin C and thyroid metabolism in broiler chickens. *Reproduction Nutrition Développement* **27** B, 555–565.

Donaldson, W. E. (1985). Lipogenesis and body fat in chicks: effects of calorie:protein ratios and dietary fat. *Poultry Science* **64**, 1199–1204.

Donaldson, W. E., Combs, G. F. & Romoser, G. L. (1956). Studies on energy levels in poultry rations. 1. The effect of calorie-protein of the ration on growth, nutrient utilization and body composition of chicks. *Poultry Science* **35**, 1100–1204.

Folch, J., Lees, M. & Sloan-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* **226**, 497–509.

Griffin, H. D., Butterwirth, S. C. & Goddard, C. (1987). Contribution of lipoprotein lipase to differences in fatness between broiler and layer-strain chickens. *British Poultry Science* **28**, 197–206.

Guo, K., Griffin, H. D. & Butterwirth, S. C. (1988). Biochemical indicators of fatness in meat-type chickens: lack of correlation between lipoprotein lipase activity in post-heparin plasma and body fat. *British Poultry Science* **29**, 343–350.

Hanks, J. H. & Wallace, R. E. (1949). Relation of oxygen and temperature in the preservation of tissues by refrigeration. *Proceedings of the Society for Experimental Biology and Medicine* **71**, 196–200.

Harvey, S. (1983). Thyroid hormones inhibit growth hormone secretion in domestic fowl. (*Gallus domesticus*). *Journal of Endocrinology* **98**, 129–135.

Hsu, R. Y. & Lardy, H. A. (1969). Malic enzyme. In *Methods in Enzymology*, vol. 13, pp. 230–235 [J. M. Lowenstein, editor]. New York: Academic Press.

Leung, F. C., Taylor, J. E. & Vanderstine, A. (1984*a*). Effects of dietary thyroid hormones on growth, serum T_3, T_4 and growth hormone in sex-linked dwarf chickens. *Proceedings of the Society for Experimental Biology and Medicine* **177**, 77–81.

Leung, F. C., Taylor, J. E. & Vanderstine, A. (1984*b*). Thyrotropin-releasing hormone stimulates body weight gain and increases thyroid hormones and growth hormone in plasma of cockerels. *Endocrinology* **115**, 736–740.

Leung, F. C., Taylor, J. E. & Vanderstine, A. (1985). Effects of dietary thyroid hormones on growth, plasma T_3, T_4 and growth hormone in normal and hypothyroid chickens. *General and Comparative Endocrinology* **59**, 91–99.

McMurtry, J. P., Francis, G. L., Upton, F. Z., Rosselot, G. & Brocht, D. M. (1994). Developmental changes in chicken and turkey insulin like growth factor I (IGF-I) studied with a homologous radioimmunoassay for chicken IGF-I. *Journal of Endocrinology* **142**, 225–234.

Martin, R. J. & Herbein, J. H. (1976). A comparison of the enzyme levels and in vitro utilization of various substrates for lipogenesis in pair-fed lean and obese pigs. *Proceedings of the Society for Experimental Biology and Medicine* **151**, 231–235.

Nilsson-Ehle, P. & Schotz, M. (1976). A stable, radioactive substrate emulsion for assay of lipoprotein lipase. *Journal of Lipid Research* **17**, 536–541.

Quig, D. W., Layman, D. K., Bechtel, P. J. & Heckler, L. R. (1983). The influence of starvation and refeeding on the lipoprotein lipase activity of skeletal muscle and adipose tissue of lean and obese Zucker rats. *Journal of Nutrition* **113**, 1150–1156.

Remington, R. D. & Schork, M. A. (1970). *Statistics with Applications to the Biological and Health Sciences*. Engelwood Cliffs, NJ: Prentice-Hall.

Rosebrough, R. W., McMurtry, J. P., Mitchell, A. D. & Steele, N. C. (1988). Protein and energy restrictions in the broiler chicken. 6. Effect of dietary protein and energy restrictions on carbohydrate and lipid metabolism and metabolic hormone profiles. *Comparative Biochemistry and Physiology* **90**, 311–316.

Rosebrough, R. W. & Steele, N. C. (1985). Energy and protein relations in the broiler. 1. Effect of protein levels and feeding regimes on growth, body composition and *in vitro* lipogenesis in broiler chickens. *Poultry Science* **64**, 119–126.

Rosebrough, R. W. & Steele, N. C. (1987). Methods to assess glucose and lipid metabolism in avian liver explants. *Comparative Biochemistry and Physiology* **88** A, 1041–1049.

Thomas, O. P. & Combs, G. F. (1967). Relationship between serum protein level and body composition in the chick. *Journal of Nutrition* **91**, 468–472.

Valcavi, R., Dieguez, C., Preece, M. & Taylor, A. (1987). Effect of thyroxine replacement therapy on plasma insulin-like growth factor I levels and growth hormone responses to growth hormone releasing factor in hypothyroid patients. *Clinical Endocrinology* **27**, 85–90.

Yeh, Y. Y. & Leveille, G. A. (1969). Effect of dietary protein on hepatic lipogenesis in the growing chick. *Journal of Nutrition* **98**, 356–366.

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