Further studies on 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 28 d of age were fed on diets containing either 120 or 210 g crude protein and 0 or 1 mg triiodothyronine (T_3)/kg diet to study *in vitro* lipogenesis (IVL). In addition, a carry-over period (180 g crude protein/kg diet from 28 to 40 d of age) was used to test the persistence of prior treatment effects. The higher protein level increased, but T_3 decreased ($P < 0.01$) growth and feed consumption at 28 d of age. The lower protein level increased ($P < 0.05$) and T_3 decreased IVL in 28-d-old chickens. These effects were only sustained for 6 d following the switch to a common diet at 28 d. IVL at 40 d of age was not affected by either crude protein or T_3 fed during the 7–28 d period. The higher protein level increased plasma insulin-like growth factor-1 during the period from 7 to 28 d; however, this effect lasted for only 6 d following the switch to a common diet. Plasma growth hormone (GH) at 28 d of age was inversely related to dietary protein level. Changing to a common level of crude protein did not change plasma GH values at 12 d, indicating that the nutritional state of the young chicken may affect GH at a later period of life. Metabolic changes noted in this study were rapid and maintained for a short period of time following the feeding of a common diet.

Lipogenesis: Dietary protein: Triiodothyronine

Although it is well known that the thyroid gland is involved in controlling avian growth, artificial changes in thyroid hormone levels do not always change growth in a predictable manner (Leung *et al.* 1984*a*, *b*, 1985). For example, a daily injection of thyrotropin-releasing hormone increased both plasma growth hormone (GH) and thyroid hormone levels and body weight (Cogburn *et al.* 1989). On the other hand, when plasma thyroid hormones were altered by diets, growth was not affected (Decuypere *et al.* 1987). Other sets of data suggest that dietary triiodothyronine (T_3) decreases body fat (Cogburn *et al.* 1989) as well as body weight (Harvey, 1983). Chemical hypothyroidism, caused by either propylthiouracil or methimazole, also decreased growth (Chiasson *et al.* 1979).

Later work suggested an interaction between thyroid status and dietary crude protein on lipid metabolism. Although diets containing large energy : protein ratios (120 g crude protein and 12·6 MJ metabolizable energy (ME)/kg) promote *de novo* lipogenesis and result in obese broiler chickens (Donaldson, 1985; Rosebrough & Steele, 1985), dietary T_3 will attenuate some of these effects (Rosebrough *et al.* 1992). In fact, feeding T_3 in conjunction with a low-protein diet (120 g crude protein/kg) resulted in lipogenic rates similar to those attained by feeding a diet containing a higher level of crude protein (210 g crude protein and 12·6 MJ ME/kg) (Rosebrough *et al.* 1992). The original hypothesis was that the high rate of lipogenesis caused by feeding low-protein diets was similar to genetic obesity (reduced Na^+,K^+ ATPase (*EC* 3.6.1.3 activity) and that feeding T_3 would alter that proportion of lipogenesis associated with Na^+/K^+ transport. It was subsequently determined that both dietary T_3 and *in vitro* ouabain

Abbreviations: AAT, aspartate aminotransferase; GH, growth hormone; ICD(NADP), isocitrate : $NADP^+$ oxidoreductase-(decarboxylating); IGF-I, insulin-like growth factor-1; IVL, *in vitro* lipogenesis; MDH(NADP), malate : $NADP^+$ oxidoreductase-(decarboxylating); ME, metabolizable energy; T_3, triiodothyronine; T_4, thyroxine.

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decreased lipogenesis to an equal degree (Rosebrough *et al.* 1992). Furthermore, inhibition of Na^+,K^+-ATPase with ouabain did not alter lipogenesis in either the euthyroid or hyperthyroid chicken.

The purposes of the studies in the present report were (1) to further examine the interaction of dietary thyroid hormones and crude protein levels on metabolism of broilers at different ages and (2) to determine carry-over effects of these treatments after birds were switched to a common dietary treatment. In addition, circulating T_3, thyroxine (T_4), and insulin-like growth factor-1 (IGF-1) levels were monitored to determine the potency of dietary thyroid hormones as moderators of whole animal metabolism.

Materials and methods

Animals and diets

At 7 d of age, male, Indian River broiler chickens were assigned to one of four dietary treatments (120 or 210 g crude protein and either 0 or 1 mg T_3/kg diet) for a 7–28 d growth trial. These dietary treatments formed a factorial arrangement with 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 systematically assigned to pens in each battery to balance positional effects across all treatment. Chickens were killed at 7 d, before the initiation of the experiment, to establish basal values and then at 14, 21 and 28 d to determine effects of dietary treatments on intermediary metabolism. Chickens from the treatment groups were also given a common dietary treatment (180 g crude protein/kg without T_3) and grown from 28 to 40 d. These birds were then killed at 30, 33, 35, 37 or 40 d to determine any carry-over effects of prior dietary protein or thyroid status. The diet fed during the 28–40 d interval was formulated by blending the two diets given during the earlier interval. The experimental diets are described in Table 1.

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

	Dietary crude protein (N × 6·25) (g/kg diet)		
	120	180	210
Ingredient			
Isolated soyabean protein*	–	33	50
Soyabean meal	112	208	256
Maize meal	767	645	584
Maize oil	17	25	29
Sand	15	10	7·5
Dicalcium phosphate	40	40	40
Limestone	10	10	10
L-Methionine†	–	1·7	2·5
Selenium premix†	1	1	1
Mineral premix§	1	1	1
Vitamin premix‖	5	5	5
Cellulose	30	10	15
Calculated composition			
Metabolizable energy (MJ/kg)	12·6	12·6	12·6
Lysine (g/kg)	6·0	11·8	11·7
Sulfur amino acids (g/kg)	10·3	10·8	10·3

* Soyabean protein grade II (900 g crude protein/kg, 21726); Nutritional Biochemicals, Cleveland, OH 44122, USA.
† L-Methionine (18915), Nutritional Biochemicals.
† 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, thiamin 10, pyridoxine 10, menadione sodium bisulfite 1·5, cyanocobalamin 0·1, pteroylmonoglutamic acid 2 and ethoxyquin 150.

In vitro *metabolism: lipogenesis*

Livers were excised, washed in 155 mM-NaCl to remove blood and debris and then sliced (MacIlwain Tissue Chopper, Mickel Laboratory Engineering Company, Gomshall, Surrey, UK; 0·3 mm). Quadruplicate explants were incubated at 37° for 2 h in Hanks' balanced salts (Hanks & Wallace, 1949; Rosebrough & Steele, 1987) containing 10 mM-HEPES and 10 mM-sodium[2-^{14}C]acetate (166 MBq/mol). All incubations were conducted in 3 ml volumes at 37° for 2 h under an atmosphere of O_2–CO_2 (95 : 5, v/v). 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* lipogenesis (IVL) was expressed as μmol acetate incorporated into lipids per g tissue.

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 12 000 ***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) (*EC* 1.1.1.40; MDH(NADP)), isocitrate: $NADP^+$ oxidoreductase-(decarboxylating) (*EC* 1.1.1.42; ICD(NADP)) and asparate aminotransferase (*EC* 2.6.1.1; AAT). 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 aids in the removal of excess amine groups formed by feeding high-protein diets.

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, 10 mM-$MgCl_2$ 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*** 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, 10 mM-$MgCl_2$ and the substrate, 4·4 mM-DL-isocitrate in a total volume of 1 ml. Portions (50 μl) of the 12 000 ***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-asparate, 0·2 mM-NADH, 1000 U/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 12 000 ***g*** supernatant fractions (diluted 1 : 10) were preincubated 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).

Plasma hormone assays

Both T_3 and T_4 concentrations were estimated with a solid-phase single antibody procedure that is commercially available (ICN Biochemicals, Irvine, CA, USA). These assays were validated for avian samples (Rosebrough *et al.* 1988) by dispersing standards in charcoal-stripped chicken sera and by noting recovery of added T_3 and T_4 (98 %). Plasma was estimated by a radioimmunoassay as previously described (McMurtry *et al.* 1994). All assays were conducted as single batches to remove inter-assay variation. Plasma GH concentrations were by a homologous chicken GH radioimmunoassay (Vasilatos-Younken, 1986) using pituitary-derived chicken GH (cGH, Lot RVY03) as a standard and for iodination, and a rabbit anti-cGH serum as primary antibody. The anti-cGH antibody was raised against recombinant cGH (Lucky Biotech Corp., Emeryville, CA, USA), and detects both the pituitary-derived and recombinant cGH preparations with equal affinity as determined by competitive displacement curves.

Statistics

Data for the period from 7 to 28 d were analysed by ANOVA as a 2 × 2 × 4 factorial arrangement. Terms in the model were dietary crude protein levels, T_3 supplementation and age. Measurements were taken at four ages (7, 14, 21 and 28 d). Data for the 28–40 d period were analysed as a 2 × 2 × 6 factorial arrangement. Terms in the model were the 7–28 d crude protein levels, T_3 supplementation and time (d) following the change to a common diet. All models have been described by Remington & Schork (1970).

Results

In vitro *lipogenesis*

By day 14, it was observed that IVL was decreased both by T_3 and by a higher level of crude protein (Table 2, $P < 0.001$ and $P < 0.001$ respectively). Feeding T_3 to birds fed on the lower level of crude protein resulted in IVL rates at day 21 which were similar to feeding the higher level of protein without T_3. At 28 d, all of the dietary treatment groups were different from each other. Table 3 summarizes the adaptation in IVL when chickens were switched from their 7–28 d dietary treatments to a common diet containing

Table 2. Effect of age, dietary crude protein level and triiodothyronine (T_3) administration on *in vitro* lipogenesis (IVL) and enzyme activities in broiler chickens*

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

	Value at 7 d†			Age						Statistical significance of effect of: $P <$		
				14 d		21 d		28 d				
Measurement	Mean	SE	Dieary treatment (7–28 d)	Mean	SE	Mean	SE	Mean	SE	Protein	T_3	Age
IVL‡	44·9	4·5	120	47·8	1·9	51·7	2·0	51·9	1·1	0·001	0·001	0·12
			120 + T_3	45·6	2·0	32·1	2·3	27·4	3·1			
			210	43·8	3·3	31·8	2·2	17·2	1·3			
			210 + T_3	17·0	2·4	8·4	0·5	9·1	0·7			
MDH§	18·9	1·0	120	25·7	1·0	30·2	1·2	25·4	1·8	0·001	0·001	0·001
			120 + T_3	27·1	0·9	26·7	1·5	22·8	0·3			
			210	20·8	2·1	16·8	1·4	12·8	1·8			
			210 + T_3	14·4	0·8	10·5	1·0	11·2	1·7			
ICD§	23·4	0·9	120	19·5	1·3	13·8	1·0	14·2	1·6	0·001	0·001	0·001
			120 + T_3	21·4	1·0	18·5	0·8	17·5	1·3			
			210	25·7	1·5	23·7	1·8	18·3	2·0			
			210 + T_3	24·8	1·4	23·1	1·4	26·1	2·2			
AAT§	54·3	2·0	120	50·5	4·4	51·8	3·1	49·4	3·3	0·001	0·10	0·65
			120 + T_3	57·1	1·0	54·1	2·7	59·6	6·0			
			210	67·2	2·9	60·3	2·4	62·3	6·0			
			210 + T_3	70·9	6·7	69·7	1·4	75·7	4·2			

MDH, malate : $NADP^+$ oxidoreductase-(decarboxylating); ICD, isocitrate : $NADP^+$ oxidoreductase-(decarboxylating); AAT, aspartate aminotransferase.
* For details of diets and procedures, see Table 1 and pp. 90–91.
† Values obtained at 7 d, before the initiation of dietary treatments.
‡ Values are expressed as µmol substrate incorporated per g liver.
§ Values represent µmol oxidized or reduced NAD(P) produced.

Table 3. Persistence of the effects of dietary protein and triiodothyronine (T_3) administration on *in vitro* lipogenesis (IVL) and enzyme activities in broiler chickens*

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

Measurement	Dietary treatment (7–28 d)	Time following change to a diet containing 180 g crude protein/kg										Statistical significance of effect of: $P <$		
		2 d		5 d		7 d		9 d		12 d				
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Protein	T_3	Age
IVL†	120	55·6	1·4	47·7	1·4	37·8	1·9	37·8	1·6	34·6	2·8	0·001	0·03	0·001
	120 + T_3	44·9	1·9	47·2	2·3	39·6	3·2	36·9	2·5	32·2	2·1			
	210	22·0	3·9	33·7	1·8	32·8	3·7	34·3	2·2	35·1	1·4			
	210 + T_3	13·7	2·8	21·7	0·7	31·8	3·3	31·7	1·2	36·9	1·2			
MDH‡	120	22·5	0·6	20·8	2·4	18·5	1·2	16·5	0·7	13·4	1·5	0·001	0·001	0·02
	120 + T_3	20·5	0·5	16·2	1·9	15·9	1·5	14·1	0·9	14·7	1·4			
	210	14·6	0·9	17·0	1·7	16·6	1·4	15·7	2·3	17·2	2·8			
	210 + T_3	11·2	1·4	11·4	1·9	17·4	0·6	19·9	0·9	20·8	0·6			
ICD‡	120	14·2	0·5	16·8	0·7	23·7	0·9	21·8	0·8	24·2	2·7	0·001	0·27	0·001
	120 + T_3	15·2	1·1	17·5	2·0	21·6	1·9	22·1	1·6	28·4	1·6			
	210	32·9	1·3	30·2	2·0	27·4	1·6	18·3	1·5	18·1	3·3			
	210 + T_3	33·8	0·7	28·1	1·8	27·1	1·0	22·2	4·8	16·7	2·4			
AAT‡	120	48·8	4·3	54·1	2·9	59·2	2·4	61·2	3·8	57·8	3·9	0·001	0·97	0·001
	120 + T_3	46·0	3·0	52·9	5·4	59·3	3·8	69·4	8·2	70·1	5·4			
	210	83·2	3·4	78·3	4·7	65·1	5·9	59·0	4·6	46·4	7·3			
	210 + T_3	86·8	2·6	68·1	5·2	61·5	7·2	58·1	10·9	46·5	4·9			

MDH, malate : $NADP^+$ oxidoreductase-(decarboxylating); ICD, isocitrate : $NADP^+$ oxidoreductase-(decarboxylating); AAT, aspartate aminotransferase.
* For details of diets and procedures, see Table 1 and pp. 90–91.
† Values are expressed as μmol substrate incorporated per g liver.
‡ Values represent μmol oxidized or reduced NAD(P) produced.

180 g crude protein/kg. There were persistent effects of both dietary crude protein and T_3 ($P < 0·001$ and $P < 0·001$ respectively) lasting until 5 d following the switch to the common diet.

Enzyme activities

Table 2 also summarizes the effects of dietary crude protein, T_3 and age on the activities of certain hepatic enzymes in the broiler chicken. By 14 d of age, there were significant effects ($P < 0·05$) of dietary protein, but not of T_3, on MDH(NADP) activity. MDH(NADP) activity remained lower in birds fed on the higher level of protein for the remainder of the 7–28 d period. The opposite trends were noted for ICD(NADP) and AAT activities.

Table 3 summarizes the adaptation in enzyme activities when chickens were switched from their 7–28 d dietary treatments to a common diet. There were persistent effects of both dietary crude protein and T_3 ($P < 0·001$ and $P < 0·001$ respectively) on MDH(NADP) activities until 7 d following the switch to the common dietary treatment. Prior supplemental T_3 status did not affect the rate of adaptation to the common diet, although a significant age × T_3 interaction may confound interpretation of the adaptation in MDH(NADP) activities. The adaptation in ICD(NADP) and AAT activities following the change to a common diet was opposite to that for MDH(NADP), as activities in birds fed on 120 g crude protein/kg diet increased and activities decreased in birds fed on 210 g crude protein/kg diet.

Plasma hormones

Table 4 summarizes the effects of both dietary protein and supplemental T_3 on certain plasma hormones in birds growing from 7 to 28 d of age. There was a significant effect of dietary T_3 ($P < 0·001$), but not of either dietary protein or age ($P < 0·12$ and $P < 0·76$ respectively), on plasma T_3 levels. In contrast, there were significant effects of dietary crude protein, supplemental T_3 and age on plasma T_4 concentrations ($P < 0·001$, $P < 0·001$ and $P < 0·001$ respectively). Briefly, an increase in dietary crude protein increased the plasma T_4 level. In contrast, feeding T_3 decreased plasma T_4. In general, dietary treatment effects on plasma T_4 were noted as early as 14 d of age. Plasma IGF-1 values were directly related to both the level of the dietary crude protein and the age of the bird ($P < 0·001$ and $P < 0·001$ respectively), but not to T_3 supplementation ($P < 0·71$). The effects of both dietary protein and age on IGF-1 were similar to those for T_4. Neither dietary protein ($P < 0·17$) nor age ($P < 0·51$) affected plasma GH in birds growing from 7 to 28 d. Plasma GH level was decreased ($P < 0·03$) by dietary T_3.

Table 5 summarizes hormone concentrations in chickens following the change to a common level of dietary protein. Plasma T_3 concentrations fell rapidly over time ($P < 0·001$) due mainly to switching birds from diets containing T_3 to a common diet that did not contain T_3. It should be noted that plasma T_3 concentrations were equal in all treatment groups 5 d after the change to the common diet. The apparent significant ($P < 0·001$) effect of prior dietary protein status on plasma T_3 levels was probably due to the values noted at day 0 (the time of the switch to the common diet).

Table 4. Effect of age, dietary crude protein level and triiodothyronine (T_3) administration on plasma hormone levels in broiler chickens*
(Mean values with their standard errors for four observations per dietary treatment)

Measurement	Value at 7 d†		Dieary treatment (7–28 d)	Age						Statistical significance of effect of: $P<$		
				14 d		21 d		28 d				
	Mean	SE		Mean	SE	Mean	SE	Mean	SE	Protein	T_3	Age
T_3 (nmol/l)	7·1	0·2	120	7·6	0·5	5·9	0·3	6·9	0·5	0·12	0·001	0·76
			120 + T_3	11·6	0·8	11·1	1·5	12·9	2·0			
			210	5·4	0·3	5·4	0·2	4·3	0·4			
			210 + T_3	9·2	1·1	12·2	0·9	10·4	0·9			
T_4 (nmol/l)	8·1	1·6	120	4·4	0·5	7·4	0·7	9·3	1·4	0·001	0·001	0·001
			120 + T_3	2·7	0·1	2·1	0·2	3·1	0·2			
			210	6·5	1·2	10·6	1·2	14·9	1·8			
			210 + T_3	2·4	0·2	2·7	0·1	3·9	0·2			
IGF-1 (nmol/l)	7·7	0·4	120	7·4	0·2	8·8	0·3	9·1	0·6	0·001	0·71	0·001
			120 + T_3	7·3	0·2	8·3	0·3	7·6	0·2			
			210	10·1	0·3	11·0	1·1	11·7	0·6			
			210 + T_3	9·6	0·4	10·8	0·1	12·9	0·1			
GH (nmol/l)	0·71	0·18	120	1·20	0·65	1·43	0·73	0·85	0·22	0·17	0·03	0·51
			120 + T_3	0·26	0·06	0·31	0·04	0·27	0·02			
			210	0·83	0·32	1·01	0·32	0·40	0·08			
			210 + T_3	0·13	0·08	0·09	0·17	0·17	0·16			

T_4, thyroxine; IGF-1, insulin-like growth factor-1; GH, growth hormone.
* For details of diets and procedures, see Table 1 and pp. 90–91.
† Values obtained at 7 d, before the initiation of dietary treatments.

Table 5. Persistence of the effects of dietary protein and triiodothyronine (T_3) administration on plasma hormone levels in broiler chickens*
(Mean values with their standard errors for four observations per dietary treatment)

Measurement	Dietary treatment (7–28 d)	Time following change to a diet containing 180 g crude protein/kg										Statistical significance of effect of: $P<$		
		2 d		5 d		7 d		9 d		12 d				
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Protein	T_3	Age
T_3 (nmol/l)	120	6·3	0·2	4·6	0·5	6·8	0·5	5·5	0·2	6·0	0·3	0·001	0·07	0·001
	120 + T_3	3·2	0·0	4·5	0·2	6·9	0·6	4·8	0·2	5·5	0·4			
	210	3·7	0·2	3·9	0·2	6·1	0·3	4·3	0·4	4·1	0·4			
	210 + T_3	2·7	0·2	3·7	0·1	6·1	0·3	4·7	0·0	5·1	0·6			
T_4 (nmol/l)	120	9·6	1·6	11·7	2·2	10·6	1·8	11·8	1·2	10·4	1·2	0·001	0·001	0·001
	120 + T_3	4·3	0·6	6·6	0·4	9·2	1·4	6·9	0·2	13·9	1·6			
	210	15·6	1·7	13·3	2·3	22·5	5·4	17·3	0·7	26·6	3·0			
	210 + T_3	4·3	0·1	6·9	0·4	8·9	0·2	10·7	1·8	11·2	0·3			
IGF-1 (nmol/l)	120	11·1	0·9	12·1	0·2	11·3	0·2	9·4	0·6	9·9	0·5	0·001	0·001	0·001
	120 + T_3	10·0	0·5	10·6	0·5	11·2	0·6	10·0	0·5	9·3	0·4			
	210	13·1	0·2	13·6	0·3	10·6	1·1	10·7	0·5	10·2	0·6			
	210 + T_3	12·8	0·4	12·8	0·2	10·0	0·1	10·7	0·5	0·5	0·6			
GH (nmol/l)	120	1·11	0·15	1·03	0·19	0·77	0·19	0·80	0·25	0·80	0·18	0·001	0·83	0·06
	120 + T_3	0·79	0·21	0·72	0·30	1·07	0·28	1·23	0·26	0·76	0·22			
	210	0·69	0·18	0·45	0·06	0·35	0·06	0·41	0·18	0·48	0·09			
	210 + T_3	0·27	0·17	0·63	0·17	0·70	0·12	0·67	0·07	0·27	0·04			

T_4, thyroxine; IGF-1, insulin-like growth factor-1; GH, growth hormone.
* For details of diets and procedures, see Table 1 and pp. 90–91.

Following the change to a common diet, plasma T_4 increased in both dietary protein groups previously fed with T_3, taking 7 d to reach a stable concentration ($P < 0·001$). Likewise, plasma T_4 also increased when birds were changed from the diet containing 120 g crude protein–T_3 to the diet containing 180 g crude protein/kg, although the adaptation process did not start until 5 d after the change. Following the change from a diet containing 210 to one containing 180 g crude protein/kg, plasma IGF-1 remained at 28 d levels for 5 d and then declined ($P < 0·06$) to a constant level 7 d after the change. Dietary T_3 during the 7–28 d period significantly affected the adaptation process as values remained depressed for 5 d following the change to the common diets. Dietary protein levels during the 7–28 d period affected ($P < 0·001$) plasma GH values. Even after 12 d of consuming the common diet, values for birds

consuming the diet containing 120 g crude protein/kg for 7–28 d were greater than were values for birds consuming the higher level of crude protein for the same interval.

Discussion

This report has two components. The first objective was to describe the effect of age on metabolism in chickens fed on two different levels of crude protein that were supplemented with T_3. The initial objective involved determining the effects of dietary energy : protein ratios and T_3 on *in vitro* lipid metabolism, growth and metabolic hormone concentrations after 21 d of treatment. The second objective involved changing the birds to a diet containing a common level of protein without supplemental T_3 to determine carry-over effects of both crude protein and T_3.

A previous study showed that feeding diets containing a range of crude protein levels from 120 to 210 g/kg profoundly affected IGF-1 levels in the 30-d-old chicken (Rosebrough *et al.* 1996). In this same study, treatment effects did not persist when these birds were fed on a diet containing 180 g crude protein/kg from 30 to 56 d. Likewise, IVL at 56 d of age was not affected by diets offered between 7 and 28 d of age although 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.

Plasma IGF-1 concentration was monitored in the present study to determine if the hormone was altered by feeding diets containing different levels of crude protein or supplemental T_3. An initial study from our laboratory showed that low levels of crude protein reduced plasma IGF-1 compared with higher levels of crude protein (Rosebrough *et al.* 1988). We can consistently show a highly significant relationship between plasma IGF-1 and chicken growth over the range of dietary crude protein levels from 120 to 180 g crude protein/kg diet. In contrast, we noted an almost total lack of relationship when higher levels of crude protein were given. Perhaps the latter observation relates to the amino acid needs of the bird. Earlier work demonstrated that protein quality (amino acid balance of the diet) could affect plasma IGF-1 concentration in rats (Isley *et al.* 1984; Clemons *et al.* 1985*a*,*b*). A recent study provides further evidence for nutrient control of IGF-1 (Tomas *et al.* 1991).

GH gene expression may be selectively regulated by IGF-1. For example, Yamashita *et al.* (1986) found that stimulation of GH secretion by GH-releasing hormone could be prevented by simultaneously treating cells with IGF-1. Furthermore, this group also reported that IGF-1 suppresses both basal and GH-releasing hormone-dependent GH secretion in pituitary adenoma cells. A recent study provides evidence for nutrient control of IGF-1 and ultimately GH levels (Tomas *et al.* 1991).

Plasma GH measurements are necessary because the efficacy of dietary thyroid hormone treatments can be verified by monitoring changes in plasma GH (Harvey, 1983; Cogburn *et al.* 1989). Plasma IGF-1 was measured to determine if the putative GH–IGF-1 axis was altered by feeding either a thyroid hormone or diets containing different energy : protein ratios. Although depressed IGF-1 values in human subjects can be returned to normal with T_4 replacement (Valcavi *et al.* 1987), it was somewhat surprising that dietary T_3 did not uniformly change plasma IGF-1 concentrations in broiler chickens used in previous studies (Rosebrough *et al.* 1992, 1996).

Analysis of data also revealed that thyroid hormone values may be of limited usefulness in determining the dietary protein status of broiler chickens. In the present study, a large difference in dietary crude protein level was used because of the large amount of variation in values noted in previous studies (Rosebrough *et al.* 1988, 1992). Even though large variations are normal and make hypothesis testing difficult, there are many reports in the literature concerning diet and thyroid hormone levels. Yang *et al.* (1987) indicated that, although a reduction in carbohydrate energy decreased body weight, there were no changes in either T_3 or T_4. This study seemed to imply that in rats growth could be separated from thyroid function. Generally speaking, plasma thyroid hormones will respond to changes in diet that elicit large changes in growth. In contrast, using thyroid hormones as indicators of changes in nutritional status during a production environment may be difficult because of subtle differences in diets.

The data in the present study also point out the caution that must be used in analysing effects of dietary crude protein on metabolism. Specifically, responses may not always follow linear trends as crude protein increases. For example, if dietary crude protein levels in experimental diets are kept between 180 and 230 g crude protein/kg to simulate typical broiler production diets, there will be few differences in the concentrations of the plasma hormones implicated in growth regulation. Dietary thyroid hormones also have little effect on growth or the efficiency of food utilization when used in conjunction with these levels of dietary crude protein. Furthermore, observations derived from birds fed on optimum levels of crude protein seem to indicate that neither GH nor IGF-1 reflects either growth or protein nutriture (Rosebrough *et al.* 1988, 1992). Our data show that, when wide fluctuations are made in the dietary energy : crude protein level, metabolic hormone concentrations can reflect changes in growth. This theory is especially noticeable after examining changes in growth and hormone concentrations in birds fed on diets containing either 120 or 210 g crude protein/kg.

In summary, it was shown that both dietary crude protein and T_3 affected intermediary metabolism, 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. A majority of the metabolic processes adapted to the change in 6 d or less. Thus, it is apparent that changes in body composition, brought about by early planes of nutrition can persist even though differences in intermediary metabolism do not.

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