In vitro lipid metabolism, growth and metabolic hormone concentrations in hyperthyroid chickens*

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(*Received 20 February 1991 – Accepted 17 December 1991*)

Indian River male broiler chickens growing from 7 to 28 d of age were fed on diets containing energy:protein values varying from 43 to 106 MJ/kg protein and containing 0 or 1 mg triiodothyronine (T_3)/kg diet to study effects on growth, metabolic hormone concentrations and in vitro lipogenesis. In vitro lipid synthesis was determined in liver explants in the presence and absence of ouabain (Na^+, K^+-transporting ATPase (*EC* 3.6.1.37) inhibitor) to estimate the role of enzyme activity in explants synthesizing lipid. Growth and feed consumption increased ($P < 0.01$) when the energy:protein value decreased from 106 to 71 MJ/kg protein; however, both variables decreased as the value was further decreased from 53 to 43 MJ/kg protein. Triiodothyronine depressed ($P < 0.01$) growth, but not food intake. Large energy:protein diets (> 53 MJ/kg protein) and dietary T_3 lowered ($P < 0.01$) plasma growth hormone. Large energy:protein diets (> 53 MJ/kg protein) increased ($P < 0.01$) lipogenesis, plasma growth hormone (GH) and decreased plasma insulin-like growth factor 1 (IGF-1). Also, T_3 decreased plasma GH, IGF-1 in vitro lipogenesis. Ouabain inhibited a greater proportion of in vitro lipogenesis in those explants synthesizing fat at a high rate. Both dietary T_3 and in vitro ouabain decrease lipogenesis, but, when combined, the effects are not cumulative.

Lipogenesis: Dietary protein: ATPase: Thyroid hormones: Chickens

The effect of dietary protein on weight gain and body composition of broiler chickens is well known. Diets containing large energy:protein values (greater than 13 MJ/kg diet and more than 72 MJ/kg crude protein (nitrogen × 6·25)) promote high rates of in vitro lipogenesis (Rosebrough & Steele, 1985) as well as *de novo* carcass lipid synthesis by the liver (Donaldson, 1985). Diets with small energy:protein values (less than 13 MJ/kg diet and less than 56 MJ/kg crude protein) promote lean broiler carcasses (Donaldson *et al.* 1956; Thomas & Combs, 1967). Those diets containing large energy:protein values may direct nutrients from lean to fat synthesis. It is also reasonable to assume that an increase in nutrient flux to support *de novo* lipid synthesis may require an increase in energy-dependent transport processes. Maintenance of the cellular Na^+–K^+ gradient seems related to accelerated tissue metabolism. For example, Adeola *et al.* (1989) and Milligan & McBride (1985) have reported that one of these processes, Na^+, K^+-transporting ATPase (*EC* 3.6.1.37)-dependent respiration, can account for 20–30% of the total oxygen consumption of porcine muscle synthesizing protein.

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Although lipogenesis is another highly anabolic process, there are no findings that we are aware of that suggest a direct role for Na^+, K^+-transporting ATPase-dependent processes accompanying lipogenesis resulting from the feeding of diets containing large energy: protein values. Certain studies using rodents have shown that obese mice had lower Na^+, K^+-transporting ATPase activity than their lean littermates (Berdanier & Shubeck, 1981). Other work has shown that the number of ouabain-binding sites in liver could be taken as an estimate of Na^+, K^+-transporting ATPase units and that this estimate was lower in obese compared with lean littermates. Ismail-Beigi & Edelman (1970) reported that thyroid hormones stimulated O_2 consumption through an increase in Na^+, K^+-transporting ATPase. When thyroid hormones were given to either lean or obese rats, ouabain-binding sites were increased to a greater degree in the obese rat. Thus, it has been shown that a metabolic error resulting in severe obesity in rodents resides in that portion of mitochondrion involved in respiratory control (Berdanier & Shubeck, 1981; Kim & Berdanier, 1989). Furthermore, this error has also been related to thyroid hormones (York *et al.* 1978; Hillgartner & Romsos, 1985).

The purpose of the present study was to examine the thyroid hormone $\times$ Na^+, K^+-transporting ATPase axis in broiler chickens subjected to dietary regimens designed to promote different rates of lipogenesis. The hypothesis tested was that the regulation of lipogenesis during the feeding of diets containing large energy:protein values is similar to genetic obesity, e.g. reduced Na^+, K^+-transporting ATPase activity. Of particular interest was the determination of the effect of the known Na^+, K^+-transporting ATPase inhibitor ouabain (Glynn, 1964) on lipogenesis in the chicken made hyperthyroid by dietary additions of triiodothyronine (T_3). In addition, circulating T_3, thyroxine (T_4), growth hormone (GH) and insulin-like growth factor-1 (IGF-1) were monitored to determine the potency of dietary thyroid hormones as moderators of whole animal metabolism.

MATERIALS AND METHODS

Animals

Male, Indian River broiler chickens were grown under common conditions from 1 to 7 d of age. 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. Both feed and water were apportioned on an *ad lib.* basis. For purposes of statistical analyses, the observation was the pen mean.

Experimental treatments

At 7 d of age, chickens were randomly assigned to one of eight dietary treatments (106, 71, 53, 43 MJ/kg protein + 0 or 1 mg T_3/kg diet) for a 7–28 d growth trial. The eight dietary treatments formed a 4×2 factorial arrangement with a total of eight 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 in Table 1. In addition, a third treatment, 0 or 1 mM-ouabain, was superimposed on liver explants from all treatment groups to determine the effect of the two dietary treatments on in vitro intermediary metabolism.

In vitro metabolism

Optimizing conditions for in vitro metabolism have been described by Rosebrough & Steele (1987). Briefly, the methods are as follows: two 28-d-old chickens were randomly selected from each pen replicate treatment per treatment and killed by cervical dislocation at 09.00 hours. The livers were rapidly excised, weighed and placed in individual vessels containing 10 mM-HEPES (*N*-2-hydroxyethyl piperazine-*N'*-2-ethane sulphonic acid) and 155 mM-

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

Dietary energy:protein (MJ/kg protein)...	106	43
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
Crude protein (nitrogen × 6·25) (g/kg)	120	300
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 selenium/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, niacin 100, thiamine 10, vitamin B_6 10, menadione sodium bisulphite 1·5, cyanocobalamin 0·1, folic acid 2 and ethoxyquin 150.

sodium chloride (pH 7·5). Pieces of fresh livers were sliced with a tissue chopper (MacIlwain Tissue Chopper; Mickle Laboratory Engineering Company, Gomshall, Surrey, UK) at a setting corresponding to a thickness of 0·3 mm to give explants weighing from 35 to 75 mg. The explants were placed in 75 mm Petri dishes containing the chilled buffer and randomly allocated to in vitro treatments. Duplicate explants were incubated at 37° for 2 h in Hanks' balanced salts (Hanks & Wallace, 1949) supplemented with 10 mM-HEPES and 20 mM-sodium[2-^{14}C]acetate (New England Nuclear, Boston, Massachusetts 02118) (20 disintegrations/min per nmol) + 0 or 1 mM-ouabain. All incubations were conducted in a volume of 3 ml at 37° for 2 h under an O_2–CO_2 (95:5, v/v) atmosphere. Slices were removed from incubation flasks and extracted for 24 h in 15 ml chloroform–methanol (2:1, v/v) in 20 ml glass scintillation vials. Slices were then discarded and 2 ml 117 mM-potassium chloride added to the chloroform–methanol extract (Folch *et al.* 1957). After vigorous shaking, phase separation was allowed and the upper phase discarded. The lower phase was washed with 5 ml methanol–117 mM-NaCl (3:2, v/v), shaken and again allowed to separate. The upper phase was discarded and the lower phase evaporated to dryness, dispersed in 10 ml scintillation cocktail, and counted by liquid-scintillation spectroscopy.

The remainder of each liver was homogenized in 100 mM-HEPES, pH 7·5 (Research Organics Inc., Cleveland, Ohio 44125)–3·3 mM-mercaptoethanol (1:10, w/v) and centrifuged (Beckman J2-21; Beckman Instruments, Inc., Palo Alto, CA 94304) at 50000 ***g*** for 60 min. The supernatant fractions were kept at 0° until analysed for malic enzyme (*EC* 1.1.1.40; ME), isocitrate dehydrogenase ($NADP^+$) (*EC* 1.1.1.42; ICD) and glutamic–oxaloacetic transaminase (*EC* 2.6.1.1; GOT).

ME activity was determined by a modification of the method of Hsu & Lardy (1969). The reaction contained 50 mM-HEPES (pH 7·5), 1 mM-NADP, 5 mM-manganese chloride and

the substrate, 2·2 mM-L-malate (disodium salt). A 50 μl portion of the 50000 *g* supernatant fraction was pre-incubated for 15 min in the presence of the first three ingredients. The reaction was initiated by adding L-malate and following the rate of reduction of NADP at 340 nm at 25°. The reaction proceeded linearly for at least 60 min providing that the reaction contained less than 100 μg supernatant-fraction protein.

ICD activity was determined by a modification of the method of Cleland *et al.* (1969). The reaction contained 50 mM-HEPES (pH 7·5), 1 mM-NADP, 5 mM-$MnCl_2$ and the substrate, 4·4 mM-DL-isocitrate. A 25 μl portion of the 50000 *g* supernatant fraction was pre-incubated for 15 min in the presence of the first three ingredients. The reaction was initiated by adding DL-isocitrate and following the rate of reduction of NADP at 340 nm at 25°. The reaction proceeded linearly for at least 60 min providing that the reaction contained less than 50 μg supernatant-fraction protein.

GOT activity was determined by a modification of the method of Martin & Herbein (1976). The reaction contained 50 mM-HEPES, 200 mM-L-aspartate, 0·2 mM-NADH, 1000 units malic dehydrogenase (*EC* 1.1.1.37)/l and the substrate, 15 mM-2-oxoglutarate. A 25 μl portion of the 50000 *g* supernatant fraction was pre-incubated for 15 min in the presence of the first four ingredients. The reaction was initiated by adding 2-oxoglutarate and following the rate of oxidation of NADH at 340 nm at 25°. The reaction proceeded linearly for at least 30 min providing that the reaction contained less than 50 μg supernatant-fraction protein.

Hormone and metabolite assays

Both T_3 and T_4 concentrations were estimated with commercial, solid-phase kits (Immuchem Corp, Carson, CA). These assays were validated for avian samples by dispersing standards in charcoal-stripped chicken serum and by noting recovery of added T_3 and T_4 (98%). All hormone assays were conducted as single batches to eliminate inter-assay variation. The intra-assay coefficients of variation averaged 1·6 and 1·8% for T_3 and T_4 respectively.

Plasma GH was estimated with a homologous chicken GH radioimmunoassay that has been extensively described (Vasilatos-Younken, 1986). Plasma IGF-1 was estimated with a heterologous radioimmunoassay as previously described (Dawe *et al.* 1988). I^{125}-labelled IGF-1 was purchased from Amersham Corp and purified human sequence IGF-1 for standard was supplied by Bachem, Torrance, CA. Primary antisera (rabbit anti-human IGF-1 was kindly provided by Dr Geoff Francis, CSIRO, Adelaide, Australia. Plasma glucose, triacylglycerols (Sigma Chemical Co., St Louis, MO) and non-esterified free fatty acids (NEFA; NEFA-C, Wako Chemical Co., Dallas) were determined with commercially available kits.

Statistical analyses

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 the energy:protein (106, 71, 53 and 43) were used as the independent variables. In addition, the energy:protein value was split into linear and quadratic components for tests of statistical significance. All interactions involving the blocking factor were pooled with the residual. The condensed analyses of variance tables presented with the data only describe significance of pooled linear and quadratic effects. Data for enzyme activities were subjected to natural log transformations to decrease the mean square residual in the analysis of variance. This transformation was tried with all other data, but did not alter *F* ratios. Data for in vitro lipogenesis were analysed as a split design with two error strata. The main treatment effects of diet and T_3 were tested against the between-plot error term,

Table 2. *The effects of dietary energy:protein value and triiodothyronine (T_3) on chicken growth**

(Mean values for eight pen means (six chickens per pen) per dietary treatment)

Dietary energy:protein (MJ/kg protein)...	106		71		53		43	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
28 d body weight (g)								
Control	453	17·2	953	26·7	992	13·9	871	20·3
+1 mg T_3/kg diet	304	11·0	807	14·0	796	35·0	650	14·0
Food efficiency (food intake/wt gain)								
Control	2·34	0·23	1·72	0·06	1·67	0·03	1·75	0·07
+1 mg T_3/kg diet	4·35	0·25	1·69	0·05	1·83	0·08	2·35	0·11
Food intake 7–28 d (g)								
Control	750	69·1	1404	14·0	1443	27·1	1297	65·0
+1 mg T_3/kg diet	748	32·2	1145	36·9	1203	46·0	1298	49·0
Diet variables	28 d body-wt		Food efficiency		Food intake			
Energy:protein (E:P)	$P < 0·01$		$P < 0·01$		$P < 0·01$			
Dietary T_3 (T)	$P < 0·01$		$P < 0·01$		$P < 0·01$			
E:P × T	$P = 0·23$		$P < 0·01$		$P = 0·02$			

* For details of diets, see Table 1 and p. 668.

while ouabain and its associated interactions were tested against the within-plot error term. The general linear models procedure (GLM) was used for the analyses of transformed data, least squares means, and mean square components (Remington & Schork, 1972).

RESULTS

Growth and feed efficiency

There were significant differences ($P < 0·01$) among treatment means for 28 d body-weights that could be attributed both to the energy:protein values of the diets as well to dietary T_3 (Table 2). In addition, the main treatment effect of the energy:protein value (an increase in body-weight with a decrease in the ratio from 106 to 53 MJ/kg protein) could be further delineated into both significant linear and quadratic components. There were significant ($P < 0·01$) linear and quadratic effects of the energy:protein value on feed efficiency (an improvement in efficiency as the ratio decreased from 106 to 53 MJ/kg protein) as well as an apparent significant ($P < 0·01$) T_3 × energy:protein interaction. A further examination of data revealed that this finding of significance could be attributed to simple effects of T_3 when fed in conjunction with either the large (106 MJ/kg protein) or the small (43 MJ/kg protein) energy:protein diets. There were also significant ($P < 0·01$) linear and quadratic effects of the energy:protein value on food intake efficiency (a decrease in intake as the value decreased from 106 to 53 MJ/kg protein. In contrast to effects on body-weight, T_3 had no effect on food intake.

Plasma metabolites

Neither energy:protein value nor dietary T_3 changed plasma glucose concentrations (Table 3). Plasma-free fatty acids were also unaffected by the energy:protein value of the diets; however, there was a significant ($P < 0·01$) effect of dietary T_3 when data were pooled across the four energy:protein values. The overall mean for dietary T_3 was 225 μmol/l compared with 144 μmol/l for the unsupplemented groups. In particular, the addition of

Table 3. *The effects of dietary energy:protein and triiodothyronine (T_3) on plasma metabolites in chickens**

(Mean values for eight pen means (two observations per pen) per dietary treatment)

Dietary energy:protein (MJ/kg protein)...	106		71		53		43	
	Mean	SE	Mean	SE	Mean	SE	Mean	se
Plasma glucose, mg/100 ml								
Control	216	9·0	225	4·7	228	6·4	223	4·7
+1 mg T_3/kg diet	227	6·6	200	5·9	232	5·9	211	10·3
Free fatty acids (μmol/l)								
Control	127	13·4	151	15·2	141	10·7	156	19·1
+1 mg T_3/kg diet	175	26·1	297	41·6	223	33·9	205	42·9
Triacylglycerols (mg/l)								
Control	1310	110	1080	118	980	64	860	101
+1 mg T_3/kg diet	1680	124	1350	99	1250	144	1330	84
Uric acid (mg/l)								
Control	27	4	41	4	50	7	92	14
+1 mg T_3/kg diet	21	3	30	2	35	3	74	17
Diet variables	Glucose		Free fatty acids		Triacylglycerols		Uric acid	
Energy:protein (E:P)	$P = 0·11$		$P = 0·12$		$P < 0·01$		$P < 0·01$	
Dietary T_3 (T)	$P = 0·29$		$P < 0·01$		$P < 0·01$		$P = 0·01$	
E:P × T	$P = 0·08$		$P = 0·32$		$P = 0·79$		$P = 0·90$	

* For details of diets, see Table 1 and p. 668.

T_3 increased NEFA in that group of chickens fed on the diet containing 71 MJ/kg protein. A decrease in the energy:protein value decreased ($P < 0·01$) plasma triacylglycerols. In contrast, the overall mean triacylglycerol concentration for those chickens fed on T_3 was significantly greater than the pooled value for chickens fed on the respective control levels of crude protein (1400 *v.* 1060 mg/l). Decreasing the energy:protein value increased ($P < 0·01$) plasma uric acid concentrations. In contrast, pooling data for T_3 treatments across protein levels revealed a significant ($P = 0·01$) decrease due to the inclusion of T_3 in the diets (41·7 *v.* 52·5 mg/l).

Plasma hormones

Plasma T_3 was slightly influenced ($P < 0·05$) by dietary energy:protein and was markedly increased ($P < 0·01$) by dietary T_3 (Table 4). Overall mean plasma T_3 was significantly greater for chickens fed on T_3 (5·9 *v* 2·4 ng/ml). The apparent significant ($P < 0·01$) effect of the energy:protein value on plasma T_4 could be traced to a significant ($P < 0·01$) energy:protein × T_3 interaction. On further examination of possible causes of this interaction, it was shown that plasma T_4 concentrations in groups fed on T_3 were similar across all protein levels, contrasting with differences in T_4 among the unsupplemented groups. The overall mean for plasma T_4 was less for chickens fed on T_3 (2·2 *v* 10·8 ng/ml).

There was an inverse, significant ($P < 0·01$) effect of the energy:protein value on plasma IGF-1 where decreasing the value from 106 to 71 MJ/kg protein increased plasma IGF-1. There was no effect of dietary T_3 on plasma IGF-1. Plasma GH declined as the energy:protein value decreased from 106 to 71 MJ/kg protein, remained unchanged as the value further decreased from 71 to 53 MJ/kg protein and increased as the value finally decreased from 53 to 43 MJ/kg protein. This pattern of change was noted whether or not T_3 was provided in the diets. It should be noted that dietary T_3 did result in an overall decrease ($P < 0·01$) in plasma GH (11·6 *v.* 43·5 ng/ml).

Table 4. *The effects of dietary energy:protein and triiodothyronine (T_3) on plasma hormone concentrations in chickens**

(Mean values for eight pen means (two observations per pen) per dietary treatment)

Dietary energy:protein (MJ/kg protein)...	106		71		53		43	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
T_3 (ng/ml)								
Control	2·4	0·3	2·8	0·2	2·3	0·1	2·1	0·2
+1 mg T_3/kg diet	8·2	1·0	4·8	0·9	6·7	1·3	4·2	0·6
T_4 (ng/ml)								
Control	5·1	0·5	10·9	0·9	15·3	1·4	11·7	0·9
+1 mg T_3/kg diet	1·8	0·1	2·1	0·1	2·7	0·2	2·4	0·2
IGF-1 (ng/ml)								
Control	12·5	0·5	18·8	0·6	19·3	0·5	17·3	0·6
+1 mg T_3/kg diet	12·5	0·9	18·3	1·7	17·3	0·5	16·3	1·4
GH (ng/ml)								
Control	78·1	11·2	20·8	4·1	24·8	6·6	50·3	8·9
+1 mg T_3/kg diet	22·3	2·3	6·2	0·9	7·1	1·2	10·7	1·2
Diet variables	T_3		T_4		IGF-1		GH	
Energy:protein (E:P)	$P = 0·05$		$P < 0·01$		$P < 0·01$		$P < 0·01$	
Dietary T_3 (T)	$P < 0·01$		$P < 0·01$		$P = 0·21$		$P < 0·01$	
E:P × T	$P = 0·05$		$P < 0·01$		$P = 0·78$		$P = 0·01$	

T_3, triiodothyronine; T_4, thyroxine; IGF-1, insulin-like growth factor 1; GH, growth hormone.
* For details of diets, see Table 1 and p. 668.

Table 5. *The effects of dietary energy:protein, triiodothyronine (T_3) and ouabain on in vitro hepatic lipogenesis in chickens*†*

(Mean values for eight pen means (two observations per pen) per dietary treatment are expressed as nmol substrate incorporated per 100 mg liver)

Dietary energy:protein (MJ/kg protein)...		106		71		53		43	
T_3/kg diet	Ouabain	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	0	2845	227	2318	230	1705	270	632	68
0	1 mM	1457	198	1618	144	1415	202	671	80
1	0	2182	278	1201	185	1075	294	347	71
1	1 mM	1390	146	1051	141	865	197	390	81
Diet variables									
Energy:protein (E:P)		$P = 0·025$							
Dietary T_3 (T)		$P = 0·046$							
P × T		$P = 0·35$							
Ouabain (O)		$P < 0·01$							
E:P × O		$P < 0·01$							
T × O		$P = 0·09$							

* For details of diets, see Table 1 and p. 668.
† For details of procedures, see pp. 668–670.

The effects of the energy:protein value and dietary T_3 as well as in vitro ouabain are presented in Table 5. There was an overall decrease in lipogenesis corresponding to a decrease in the energy:protein value ($P = 0·01$). Pooling data across crude protein levels showed that dietary T_3 decreased lipogenesis by 30% (10440 *v.* 15780 ng/g liver; $P < 0·01$).

Table 6. *The effects of dietary energy:protein and triiodothyronine (T_3) on hepatic enzyme activities in chickens (one unit is that amount of enzyme resulting in the production of 1 μmol oxidized or reduced NAD(P)/min at 25°)*†*

(Mean values for eight pen means (two observations per pen) per dietary treatment are expressed as units/kg body weight)

Dietary energy:protein (MJ/kg protein)...	106		71		53		43	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
GOT:								
Control	774	84	1062	70	1446	13·1	2586	547
+1 mg T_3/kg diet	678	83	977	106	1456	13·2	2365	278
ICD (NADP):								
Control	221	35	349	33	563	3·3	858	136
+1 mg T_3/kg diet	173	22	384	50	631	9·9	849	111
ME:								
Control	159	22	180	24	109	18	35	8
+1 mg T_3/kg diet	141	20	121	10	75	18	32	9
Diet variables	GOT		NADP(ICD)		ME			
Energy:protein (E:P)	$P < 0·01$		$P < 0·01$		$P < 0·01$			
Dietary T_3 (T)	$P = 0·75$		$P = 0·84$		$P = 0·03$			
E:P × T	$P = 0·93$		$P = 0·90$		$P = 0·47$			

* For details of diets, see Table 1 and p. 668.

† GOT, glutamic-oxaloacetic aminotransferase (EC 2.6.1.1); ICD (NADP), isocitrate dehydrogenase (NADP) (EC 1.1.1.42); ME, malic enzyme (EC 1.1.1.40.).

A similar effect for in vitro ouabain was also noted (11010 *v.* 15210 ng/g liver; $P < 0·01$). A significant ouabain × T_3 interaction was not observed. A significant ($P < 0·01$) energy:protein × ouabain interaction may imply that effects of ouabain in vitro are specific to the high rates of lipogenesis that accompany the feeding of diet containing the largest energy:protein value (106 MJ/kg of protein).

The effects of the energy:protein value and dietary T_3 on the activities of certain hepatic enzymes are presented in Table 6. Both GOT and ICD activities increased significantly ($P < 0·01$) with a decrease in the energy:protein value. 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 contrast, ME activity decreased significantly ($P < 0·01$) with an increase in the energy:protein value. Dietary T_3 did result in an overall significant decrease ($P < 0·05$) in ME activity (92 *v.* 120·7 units/kg body-weight).

DISCUSSION

The objectives of the present study were to determine the effects of dietary energy:protein values and T_3 on in vitro lipid metabolism, growth and metabolic hormone concentrations. 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 values. Although, depressed IGF-1 values in humans can be returned to normal with T_4 replacement, it was somewhat surprising that dietary T_3 lacked effect on plasma IGF-1 concentrations in the present study.

The findings 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/kg diet (71 and 53 MJ/kg protein respectively) to approach typical broiler production diets, there will be few differences in the concentrations of the plasma hormones implicated in growth regulation. Likewise, dietary thyroid hormones may not alter growth or food utilization if this optimal dietary energy range is utilized. A further implication is that neither GH nor IGF-1 reflect either growth or protein nutrition. Our findings 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 as the dietary crude protein was increased from 120 to 180 g/kg diet (energy:protein decreased from 106 MJ/kg protein to 71 MJ/kg protein).

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. Yeh & Leveille (1969) found an inverse relationship between the level of the 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. The enzyme activities in the present study also suggest that ICD may function in both lipid and protein metabolism by providing a residual capacity for the production of reducing equivalents during a period of decreased ME activity. Intracellular competition may exist between acetyl-CoA carboxylase (*EC* 6.4.1.2) and the aconitase (*EC* 4.2.1.3)–ICD pathway for limited cytoplasmic citrate. Thus, the requirement for 2-oxoglutarate (a product of the reaction catalysed by ICD) as a reactant for transamination occurring during increased dietary protein intakes, would depress citrate levels to a point that activation of acetyl-CoA carboxylase would not occur.

Weaving together the three elements in the present study (dietary energy:protein, dietary T_3 and in vitro inhibition of Na^+, K^+-transporting ATPase activity) did not produce a satisfactory explanation of a diet × thyroid axis that involved an increase in T_3-dependent lipogenesis. It was subsequently determined that both dietary T_3 and in vitro ouabain decreased lipogenesis to an equal degree. It was originally proposed that feeding T_3 would increase that proportion of lipogenesis sensitive to inhibition of Na^+–K^+ transport. It was reasonable to assume that transport systems also exist for lipogenic substrates and that these systems also require significant O_2 consumption to support Na^+, K^+-transporting ATPase activity. A recent series of reports (Spratt *et al.* 1990*a*, *b*) examined tissue respiration rates in poultry and found that ouabain-sensitive respiration was much lower in poultry than in other livestock species.

Both in vitro ouabain and dietary T_3 constrain lipogenesis in chickens fed on a diet containing 106 MJ/kg crude protein. It is unknown at the present time if these effects are specific to lipogenesis in general or to the feeding of a diet containing a large energy:protein value. Moreover, it could not be shown that dietary T_3 treatments altered the portion of *de novo* lipogenesis susceptible to inhibition by ouabain. Thus,it can be implied that the role of dietary T_3 in the regulation of lipid metabolism in broilers does not involve changes in Na^+, K^+-transporting ATPase.

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Printed in Great Britain