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Protein and energy relationships in the broiler chicken

11. Effects of protein quantity and quality on metabolism*

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Male broiler chickens growing from 7 to 35 d were fed on a diet containing 150 g crude protein (N × 6·25)/kg diet supplemented with lysine to equal that in diets containing 166, 183 and 200 g crude protein/kg diet (Expt 1). A second group of male broiler chickens growing over the same period were fed on a diet containing 120 g crude protein/kg supplemented with lysine, arginine, tryptophan, threonine and isoleucine equal to that in diets containing 144, 172 and 200 g crude protein/kg diet (Expt 2). Growth was improved by lysine supplementation but not to the level attained by feeding 200 g crude protein/kg (Expt 1). Lysine, arginine, tryptophan, threonine and isoleucine supplementation of a low-protein diet also improved growth, but growth again fell short of that attained by feeding a diet containing 200 g crude protein/kg. Plasma insulin-like growth factor-1 and thyroxine concentrations increased and triiodothyronine decreased as the crude protein level increased from 150 to 200 g/kg diet. Supplemental lysine did not affect plasma levels of these hormones. Although dietary crude protein levels noticeably changed rates of *in vitro* lipogenesis, changing either the level of a single limiting amino acid or the levels of several limiting amino acids did not change lipogenesis.

Lipogenesis: Protein quality: Enzyme activity: Broiler chickens

Both the quantity (crude protein (N × 6·25) concentration) and quality (amino acid composition relative to the required balance) of the dietary protein affect the body composition of chickens (Khalil *et al.* 1968). For example, diets with small energy:protein values promote lean broiler carcasses (Donaldson *et al.* 1956; Thomas & Combs, 1967) while diets containing large energy:protein values promote high rates of *in vitro* lipogenesis (Rosebrough & Steel, 1985) and *de novo* carcass lipid synthesis by the liver (Donaldson, 1985). Recently, the effects of different energy:protein values have been studied and different possible interpretations of results are possible (Rosebrough & Steele, 1985). For example, compared with a diet containing a large energy:protein value, feeding a very small energy:protein diet will result in a very lean carcass when crude protein is expressed relative to dry matter. On the other hand, if carcass protein is expressed as g protein amassed over time, the diet containing a larger energy:protein value resulted in more total carcass protein. It is possible that diets containing very small energy:protein values 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 & Boorman, 1976).

Dietary protein quality reflects a balance of amino acids required for maximum growth and lean tissue synthesis and the limiting amino acid levels in a protein source (Fisher *et*

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al. 1959). Dietary protein level can be described by the lysine content of the diet relative to the crude protein level. For example, Yeh & Leveille (1969) proposed that there should be 60 g lysine/kg protein in the diet of the chicken. Although the relationship of a limiting amino acid to crude protein may regulate lipogenesis, it is not known if the effect is due to the presence of a limiting amino acid at the ribosomal level or to a shift in cellular metabolism caused by a need to process excess amino acid-C. In the process of converting gluconeogenic amino acid-C into glucose-C, reducing equivalents (NADPH) normally required for the addition of acetyl residues during *de novo* lipogenesis are utilized and may be unavailable for lipogenesis.

The purposes of the experiments described in the present report were: (1) to examine lipid metabolism in chickens fed on additional amounts of the limiting amino acid lysine as either lysine hydrochloride or soya-bean meal, (2) to continue the previously described investigation in chickens fed on a lower level of crude protein, but supplemented with the purported first five limiting amino acids (lysine, arginine, tryptophan, threonine and isoleucine). The null hypothesis tested was that amino acid supplementation of low-protein diets would influence metabolism similarly to a diet containing a greater amount of crude protein. These regimens allow the testing of hypotheses relating to either the first limiting amino acid in lipid metabolism or natural *v.* synthetic amino acids as improvers of protein quality. In the first regimen a maize–soya-bean-meal basal diet was formulated to be adequate for all essential amino acids except lysine. Lysine hydrochloride was then added to meet requirements. In the second regimen an essential amino acid-deficient basal diet was formulated, and then blended with either a diet containing 200 g protein/kg or a low-protein diet supplemented with amino acids to be equal to the diet containing 200 g protein/kg.

MATERIALS AND METHODS

Animals and diets

Expt 1. Ross male broiler chicks (7-d-old; *n* 336) were assigned to one of seven dietary treatments (Table 1). Each treatment consisted of eight pen replicates of six chickens per pen. The dietary treatments consisted of three stock diets: 150 g crude protein and 8 g lysine/kg (diet A); 150 g crude protein and 12 g lysine/kg (diet G) and 200 g crude protein and 12 g lysine/kg (diet D). Diets B and C were prepared by blending diets A and D, and diets E and F were prepared by blending diets A and G. Diet A was formulated as adequate in all required amino acids except lysine (National Research Council, 1984). Diet G was formulated by adding lysine hydrochloride to the first diet so that this diet was now adequate for all required amino acids, but contained a low level of crude protein. Diet D met all amino acid needs for the 7- to 28-d-old chickens and served as a control diet.

Exp 2. Ross male broiler chicks (7-d-old; *n* 336) were assigned to one of seven dietary treatments (Table 1). Each treatment consisted of eight pen replicates of six chickens per pen. These treatments were again obtained by blending three diets (H, M and D). Diet H was formulated to be deficient in the following amino acids, lysine, arginine, tryptophan, leucine and isoleucine. Diet M was a modification of diet H such that the amino acids mentioned were added to reach the following levels (g/kg diet): lysine 12, tryptophan 2·1, threonine 5·7, isoleucine 7·8, arginine 14·2. Diet D was the same as in Expt 1 and served again, as the control diet.

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.

Table 1. *Expt 1. Composition of the diets*

Diet...	A	B	C	D	E	F	G
Ingredient (g/kg)							
Maize meal	800	766	733	700	800	800	800
Soya-bean meal	0	33	66	100	0	0	0
Soya-bean oil	20	22	23	25	20	20	20
Soya-bean protein*	85	90	95	100	85	85	85
L-Lysine hydrochloride†	0	0	0	0	2	4	6
L-Methionine†	5	5	5	5	5	5	5
Dicalcium phosphate	40	40	40	40	40	40	40
Limestone	10	10	10	10	10	10	10
Se premix‡	1	1	1	1	1	1	1
Mineral premix§	1	1	1	1	1	1	1
Vitamin premix‖	5	5	5	5	5	5	5
Iodized salt	3	3	3	3	3	3	3
Sand	30	23	17	10	28	26	24
Composition (g/kg diet)							
Crude protein (N × 6·25)	150	170	183	200	150	150	150
Methionine + cystine	9·5	9·9	10·4	10·8	9·5	9·5	9·5
Lysine	8·2	9·4	10·6	11·9	9·4	10·7	12·0
Tryptophan	1·5	1·7	1·9	2·2	1·5	1·5	1·5
Threonine	6·1	6·8	7·5	8·2	6·1	6·1	6·1
Isoleucine	7·1	8·1	9·1	10·1	7·1	7·1	7·1
Arginine	10·1	11·5	12·9	14·3	10·1	10·1	10·1

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

† 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, Ca 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, thiamin 10, pyridoxine 10, menadione sodium bisulphite 1·5, cyanocobalamin 0·1, folic acid 2, ethoxyquin 150.

Metabolic studies

In vitro *lipogenesis*

Chickens were randomly selected from each pen at 35 d and killed by cervical dislocation at 09·00 hours. The livers were rapidly excised, weighed and placed in individual vessels containing 10 mM-N-2-hydroxyethyl piperazine-N′-ethane sulphonic acid (HEPES) and 155 mM-NaCl (pH 7·5). A portion of the liver was sliced with a MacIlwain tissue chopper (50–75 mg/explant) and duplicate explants were incubated for 2 h at 37° in 25 ml Erlenmeyer flasks containing 3 ml Hanks' balanced salts (Hanks & Wallace, 1949) supplemented with 10 mM-HEPES (pH 7·4) and 10 g bovine serum albumen/l. Under the assay conditions described previously, reactions are linear from 1 to 4 h with substrate concentrations from 5 to 40 mM (Rosebrough & Steele, 1987). Explants were incubated in the presence of 10 mM-sodium [2-^{14}C]acetate (18 disintegrations/min per nmol). Following a 2 h incubation period, the liver explants were extracted for 24 h in 15 ml chloroform–methanol (2:1, v/v) and then partitioned into aqueous and lipid phases with 3 ml 155 mM-KCl (Folch *et al.* 1957). The bottom phase was evaporated to dryness, dispersed in liquid-scintillation cocktail and counted by liquid-scintillation spectroscopy.

Table 2. *Expt 2. Composition of the diets*

Diet...	H	I	J	D	K	L	M
Ingredient (g/kg)							
Maize meal	800	766	733	700	800	800	800
Soya-bean meal	40	60	80	100	40	40	40
Soya-bean oil	30	28	27	25	20	20	30
Soya-bean protein*	25	50	75	100	45	45	25
L-Lysine hydrochloride†	0	0	0	0	2·5	5	7·5
L-Arginine†	0	0	0	0	2·5	5	7·5
L-Methionine†	5	5	5	5	5	5	5
L-Tryptophan†	0	0	0	0	0·3	0·6	1
L-Threonine†	0	0	0	0	0·3	0·6	1
L-Isoleucine†	0	0	0	0	0·9	1·8	2·8
Dicalcium phosphate	40	40	40	40	40	40	40
Limestone	10	10	10	10	10	10	10
Se premix‡	1	1	1	1	1	1	1
Mineral premix§	1	1	1	1	1	1	1
Vitamin premix‖	5	5	5	5	5	5	5
Iodized salt	3	3	3	3	3	3	3
Sand	40	30	20	10	28	26	24
Composition (g/kg diet)							
Crude protein (N × 6·25)	120	144	172	200	123	129	134
Methionine + cystine	8·3	8·8	9·4	10·8	8·4	8·4	8·4
Lysine	5·9	7·9	9·9	11·9	7·9	9·8	12·0
Tryptophan	1·2	1·5	1·9	2·2	1·5	1·8	2·1
Threonine	4·8	5·9	7·5	8·2	5·0	5·4	5·7
Isoleucine	5·2	6·8	9·1	10·1	6·0	6·9	7·8
Arginine	7·3	9·6	12·9	14·3	9·6	11·9	14·2

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

† 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, Ca 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, thiamin 10, pyridoxine 10, menadione sodium bisulphite 1·5, cyanocobalamin 0·1, folic acid 2, ethoxyquin 150.

Enzyme assays

Remaining liver tissue was homogenized in 100 mM-HEPES (pH 7·5)–3·3 mM-β-mercaptoethanol (1:10, w/v) 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) (*EC* 1.1.1.40; MDH-NADP), isocitrate:NADP+ oxidoreductase (decarboxylating) (*EC* 1.1.1.42; ICD-NADP) and glutamic-oxaloacetic aminotransferase (*EC* 2.6.1.1; GOT).

MDH-NADP 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-$MnCl_2$ 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 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 μg supernatant fraction protein.

ICD-NADP 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 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 μg supernatant fraction protein.

GOT 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 malate:NAD+ oxidoreductase (*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 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 μg supernatant fraction protein. Enzyme activities are expressed as μmol product formed/min under the assay conditions (Rosebrough & Steele, 1985*a*).

Hormone assays

Both triiodothyronine (T_3) and thyroxine (T_4) concentrations were estimated with a double-antibody procedure that has been outlined by May (1978). Plasma insulin-like growth factor-1 (IGF-1) was estimated with a heterologous radioimmunoassay as previously described (Ballard *et al.* 1990). I^{125}-labelled IGF-1 was purchased from Amersham Corp. and recombinant chickens IGF-1 for the standard was supplied by Gropep Ltd, Adelaide, SA, Australia. Primary antisera (rabbit anti-human IGF-1) was kindly provided by Dr Geoff Francis, CSIRO, Adelaide, SA, Australia. All hormone assays were conducted as single batches to remove inter-assay variation.

Statistical analyses

Both experiments were replicated twice with each treatment replicated a total of eight times. Data were analysed as a randomized-block design with each replicate being considered as a block. All interactions involving the blocking factor were pooled with the residual. Before analyses, data for *in vitro* lipogenesis were ranked according to the magnitude of values. The rank value was then used in an analysis of variance to decide statistical significance. This transformation was necessary because of a lack of homogeneity of error variances. Although standard errors are presented in the respective tables, mean separation was accomplished by using the transformed value. The general linear models procedure (GLM) of the analysis of variance was used to test the overall null hypothesis of inequality of means. The Newman-Keuls range statistic was used to decide significance of pair-wise comparisons (Remington & Schork, 1970).

RESULTS

Body weights, feed efficiencies and *in vitro* lipogenesis values for Expt 1 are presented in Table 3. Chickens fed on diet A (150 g crude protein and 8 g lysine/kg) were equal in body weight to chickens fed on diets B (166 g crude protein/kg) or E (150 g crude protein and 9·4 g lysine/kg diet). These chickens were lighter ($P < 0·05$) than chickens fed on diets G (150 g crude protein and 12 g lysine/kg) or D (200 g crude protein/kg) which were the heaviest chickens in the present experiment). Feed conversion efficiency was poorest in chickens fed on diet A and best in the group fed on diet D. All levels of lysine supplementation (diets E, F and G) resulted in feed conversion efficiencies which were poorer than that of diet D.

The greatest rate of lipogenesis ($P < 0·05$) was noted in chickens fed on diet A. There was a significant ($P < 0·05$) linear decrease in lipogenesis as the dietary crude protein level increased (diets B, C and D). In contrast, additions of lysine hydrochloride to diet A to

Table 3. *Expt 1. The effects of dietary crude protein (nitrogen × 6·25) and lysine supplementation* on chicken body weight and* in vitro *lipogenesis at 35 d of age*†

(Mean values with their standard errors for eight pen means per dietary treatment)

Diets	Wt (g)		Feed efficiency (gain/feed)		Lipogenesis (μmol/g liver)	
	Mean	SE	Mean	SE	Mean	SE
A	896[a]	15·0	0·43[a]	0·013	43·2[cd]	2·44
B	960[a]	63·6	0·49[b]	0·026	36·6[bc]	2·74
C	1156[bc]	44·2	0·50[b]	0·020	30·1[ab]	1·67
D	1244[c]	49·4	0·59[c]	0·017	23·7[a]	1·67
E	913[a]	9·2	0·50[b]	0·025	48·3[de]	3·03
F	1116[b]	11·2	0·51[b]	0·028	53·5[ef]	3·23
G	1136[b]	14·6	0·52[b]	0·013	59·5[f]	3·20

[a–f] Mean values with unlike superscript letters were significantly different ($P < 0·05$).

* Chickens (7-d-old; average of 135 g) were assigned to one of the following dietary treatments (crude protein–lysine; g/kg diet): A 150–8; B 170–9·4; C 183–10·6; D 200–11·9; E 150–9·4; F 150–10·7; G 150–12·0; for details of composition, see Table 1. Chickens were fed for a 28 d experimental period and then selected from each treatment to determine the effects of these diets on intermediary metabolism.

† *In vitro* lipogenesis was determined by culturing liver explants for 2 h in the presence of 10 mM sodium [2-^{14}C]acetate and by noting incorporation of acetate into hepatic lipids. Values are expressed as μmol substrate incorporated per g liver.

Table 4. *Expt 1. The effects of dietary crude protein (nitrogen × 6·25) level and lysine supplementation* on metabolic hormone levels in chickens*

(Mean values with their standard errors for eight pen means per dietary treatment, expressed as ng/ml plasma)

Diets	IGF-1		T_4		T_3		T_4:T_3	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
A	16·8[a]	0·60	6·8[a]	0·69	2·1[c]	0·12	2·9[a]	0·34
B	16·8[a]	0·92	8·2[a]	0·67	1·7[ab]	0·14	4·8[ab]	0·77
C	19·0[a]	1·04	9·0[ab]	0·76	1·6[ab]	0·08	5·6[b]	0·79
D	21·7[b]	0·89	10·9[b]	0·69	1·4[a]	0·06	7·8[cd]	0·85
E	18·6[a]	1·10	8·2[a]	1·11	1·8[bc]	0·10	4·6[ab]	0·68
F	17·4[a]	0·92	10·8[b]	1·57	1·4[a]	0·14	7·7[d]	1·33
G	17·5[a]	0·67	7·0[a]	0·58	1·7[ab]	0·13	4·1[ab]	0·54

[a–d] Mean values with unlike superscript letters were significantly different ($P < 0·05$).

IGF-1, insulin-like growth factor-1; T_3, triiodothyronine; T_4, thyroxine.

* Chickens (7-d-old; average of 135 g) were assigned to one of the following dietary treatments (crude protein–lysine; g/kg diet): A 150, 8; B 170, 9·4; C 183, 10·6; D 200, 11·9; E 150, 9·4; F 150, 10·7; G 150, 12; for details of composition, see Table 1. Chickens were fed for a 28 d experimental period and then selected from each treatment to determine the effects of these diets on intermediary metabolism.

equal those at the higher levels of crude protein were accompanied by an increase ($P < 0·05$) in lipogenesis.

Plasma IGF-1 was greatest ($P < 0·05$) in chickens fed on diet D (Table 4). There were no significant differences between the other treatment groups. Plasma T_4 was also greater ($P < 0·05$) in chickens fed on diet D when compared with values obtained from chickens fed

Table 5. *Expt 2. The effects of dietary crude protein (nitrogen × 6·25) level and amino acid supplementation* on chicken body weight and* in vitro *lipogenesis† at 35 d of age*

(Mean values with their standard errors for eight pen means per dietary treatment)

Diet	Weight (g)		Feed efficiency (gain/feed)		Lipogenesis (μmol/g liver)	
	Mean	SE	Mean	SE	Mean	SE
H	668[a]	14·1	0·27[a]	0·016	28·5[d]	2·11
I	859[b]	20·2	0·37[b]	0·013	24·7[cd]	2·03
J	930[cd]	11·2	0·43[cb]	0·033	14·6[ab]	1·75
D	1083[e]	21·4	0·46[c]	0·022	9·4[a]	2·19
K	894[bc]	25·7	0·41[cb]	0·019	17·9[b]	3·84
L	916[cd]	21·8	0·46[c]	0·010	19·6[bc]	3·59
M	952[d]	15·7	0·46[c]	0·027	19·6[bc]	3·59

[a–e] Mean values with unlike superscript letters were significantly different ($P < 0·05$).

* Chickens (7-d-old; average of 135 g) were assigned to one of the following dietary treatments: D, H–J, deficient in lysine, arginine, tryptophan, threonine and isoleucine but with increasing levels of crude protein (200, 120, 144, 172 g/kg respectively; K–M, supplemented with increasing amounts of lysine, arginine, tryptophan, threonine and isoleucine, so that the levels in diet M were equal to those in diet D, but with low crude protein levels (123, 129, 134 g/kg respectively); for details of composition, see Table 2. Chickens were then fed for a 28 d experimental period and then selected from each treatment to determine the effects of dietary treatments on intermediary metabolism.

† *In vitro* lipogenesis was determined by culturing liver explants for 2 h in the presence of 10 mM sodium [2-^{14}C]acetate and by noting incorporation of acetate into hepatic lipids. Values are expressed as μmol substrate incorporated per g liver.

on the diets containing either 166 crude protein/kg diet (diet B) or the two higher levels of lysine supplementation (diets F and G). Plasma T_3 was lowest in chickens fed on diet D and greatest in chickens fed on diet A. Lysine supplementation to give either 10·7 or 12 g lysine/kg diet (diets F and G respectively) also decreased plasma T_3. Likewise, increasing the dietary crude protein level decreased plasma T_3. Plasma T_4:T_3 increased as dietary crude protein increased; however, analysing this trend was not as simple when lysine was added to basal diet. For example, the addition of lysine hydrochloride to give 10·7 g/kg diet (diet F) significantly increased ($P < 0·05$) the ratio (from 2·9 to 7·7); however, a further addition of lysine hydrochloride to give 12 g/kg diet (diet G) decreased the ratio (from 7·7 to 4·1) compared with diet A.

The effects of feeding diets containing either increasing levels of crude protein or low-protein diets supplemented with lysine, arginine, tryptophan, threonine and isoleucine on growth, feed efficiency and *in vitro* lipogenesis are shown in Table 5. There was, again, a significant ($P < 0·05$) linear effect of crude protein on body weight. The addition of lysine, arginine, tryptophan, threonine and isoleucine also resulted in a linear increase in growth. Feed conversion efficiency was also improved by amino acid supplementation, although the two higher levels of supplementation gave the same efficiency value. As in Expt 1, there was a linear improvement in efficiency as the crude protein level increased.

As in Expt 1, increasing dietary crude protein described ($P < 0·05$) *in vitro* lipogenesis. Compared with diet D, amino acid supplementation resulted in higher rates of lipogenesis; however, these same diets did result in rates lower than the unsupplemented low-protein basal diet (diet H).

Table 6 shows the effects of diet upon plasma hormone levels. There were no significant differences in plasma T_4 values between chickens consuming diet H (120 g crude

Table 6. *Expt 2. The effects of dietary crude protein (nitrogen × 6·25) level and amino acid supplementation* on metabolic hormone levels in chickens*

(Mean values with their standard errors for eight pen means per dietary treatment, expressed as ng/ml plasma)

	IGF-1		T_4		T_3		T_4:T_3	
Diets	Mean	SE	Mean	SE	Mean	SE	Mean	SE
H	$18·6^{a}$	0·40	$9·1^{a}$	0·83	$1·9^{d}$	0·10	$5·0^{a}$	0·39
I	$20·3^{ab}$	0·72	$10·9^{abc}$	0·92	$1·6^{c}$	0·13	$7·2^{b}$	0·99
J	$19·5^{a}$	0·68	$12·4^{bc}$	0·58	$1·3^{ab}$	0·07	$9·4^{c}$	0·44
D	$21·5^{b}$	0·62	$12·9^{c}$	0·66	$1·2^{a}$	0·08	$11·8^{d}$	1·27
K	$18·5^{a}$	0·74	$10·7^{ab}$	0·49	$1·4^{abc}$	0·07	$8·1^{bc}$	0·62
L	$20·1^{ab}$	0·93	$9·3^{a}$	0·59	$1·4^{abc}$	0·09	$6·9^{ab}$	0·55
M	$19·9^{ab}$	0·79	$12·0^{bc}$	0·94	$1·5^{bc}$	0·09	$7·9^{bc}$	0·79

$^{a-d}$ Mean values with unlike superscript letters were significantly different ($P < 0·05$).

IGF-1, insulin-like growth factor 1; T_3, triiodothyronine; T_4, thyroxine.

* Chickens (7-d-old; average of 135 g) were assigned to one of the following dietary treatments: D, H–J, deficient in lysine, arginine, tryptophan, threonine and isoleucine but with increasing levels of crude protein (200, 120, 144, 172 g/kg respectively); K–M, supplemented with increasing amounts of lysine, arginine, tryptophan, threonine and isoleucine, so that the levels in diet M were equal to those in diet D, but with low crude protein levels (123, 129, 134 g/kg respectively); for details of composition, see Table 2. Chickens were then fed for a 28 d experimental period and then selected from each treatment to determine the effects of dietary treatments on intermediary metabolism.

Table 7. *Expt 2. The effects of dietary crude protein (nitrogen × 6·25) level and amino acid supplementation* on hepatic enzyme activities† in chickens*

(Mean values with their standard errors for eight pen means per dietary treatment, expressed as units/g liver)

	MDH-NADP		GOT		ICD-NADP	
Diets	Mean	SE	Mean	SE	Mean	SE
H	$17·0^{bc}$	0·74	$50·2^{ab}$	5·96	$23·4^{ab}$	0·74
I	$15·4^{b}$	1·01	$49·5^{ab}$	2·84	$21·9^{a}$	1·75
J	$13·1^{b}$	1·16	$55·0^{ab}$	4·98	$26·0^{ab}$	2·09
D	$8·6^{a}$	1·32	$68·2^{c}$	3·46	$31·5^{c}$	1·32
K	$23·6^{e}$	1·95	$57·0^{b}$	5·24	$28·4^{bc}$	2·76
L	$19·4^{cd}$	2·71	$48·7^{ab}$	3·44	$22·8^{a}$	1·83
M	$21·0^{de}$	1·83	$46·6^{a}$	2·39	$23·7^{ab}$	1·83

$^{a-e}$ Mean values with unlike superscript letters were significantly different ($P < 0·05$).

MDH-NADP, malate:NADP+ oxidoreductase (decarboxylating) (*EC* 1.1.1.40); GOT, glutamic-oxaloacetic aminotransferase (*EC* 2.6.1.1); ICD-NADP, isocitrate:NADP+ oxidoreductase (decarboxylating) (*EC* 1.1.1.42).

* Chickens (7-d-old; average of 135 g) were assigned to one of the following dietary treatments: D, H–J, deficient in lysine, arginine, tryptophan, threonine and isoleucine but with increasing levels of crude protein (200, 120, 144, 172 g/kg respectively; K–M, supplemented with increasing amounts of lysine, arginine, tryptophan, threonine and isoleucine, so that the levels in diet M were equal to those in diet D, but with low crude protein levels (123, 129, 134 g/kg respectively); for details of composition, see Table 2. Chickens were then fed for a 28 d experimental period and then selected from each treatment to determine the effects of dietary treatments on intermediary metabolism.

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

protein/kg) and diets I (144 g crude protein/kg), K and L (first two increments of amino acid supplementation). Both diet D (200 g crude protein/kg) and diet M (highest rate of amino acid supplementation) increased T_4 compared with diet H. All dietary treatments decreased T_3 relative to diet H. Increments of lysine, arginine, lysine, tryptophan, threonine and isoleucine (diets K and L respectively) gave similar plasma IGF-1 values, as did increases in crude protein (diets I and J respectively). Compared with diet H, the greatest IGF-1 values were noted in chickens fed on diet D (200 g crude protein/kg diet).

The activities of certain hepatic enzymes are presented in Table 7. The activities of GOT and ICD-NADP were greater in the group fed on diet D than in any of the other treatment groups. Although amino acids were added to the basal diet (H) to equal those in diet D, the activities of these two enzymes were not decreased compared with those obtained with diet H. MDH-NADP activity was decreased by increasing the dietary crude protein level from 120 to 200 g/kg diet (diets H > I > J > D). Adding methionine, lysine, tryptophan, threonine and isoleucine to diet H to equal those in the control diet (D) did not change MDH-NADP activity.

DISCUSSION

A logical progression in the study of the role of protein *per se* in the regulation of metabolism involves further experiments concerning protein quality (amino acid composition). The first experiment in the present study addresses lysine as a limiting factor in protein quality. The second experiment involves supplementation of a low-protein diet with several amino acids to obtain both the proper balance and quantity. The results of the present study concerning metabolism in chickens fed on diets containing a marginal crude protein level both complement and conflict with previous work (Rosebrough *et al.* 1990) concerning the feeding of lysine-adequate and -inadequate diets. In that study a marginal level of crude protein (adequate in all amino acids with exception of lysine) was fed to growing chickens. It was shown that a diet containing a marginal level of crude protein (150 g/kg) could be supplemented with lysine to give a growth rate similar to that attained with a diet containing a higher level of crude protein (200 g/kg). In contrast, changes in intermediary metabolism such as oxidation, lipogenesis and liver glucose production, however, were not equal under these conditions. In the present study growth was not completely restored by feeding this level of lysine supplementation. It should be pointed out, however, that different age birds were used in the study of Rosebrough *et al.* (1990; 28-d-old *v.* 35-d-old chickens in the present study).

The present study also expands upon our previous efforts by showing that graded amounts of lysine or protein will improve both growth and feed efficiency, although it should be noted that lysine supplementation to equal that in the control diet did not result in equal growth. Likewise, a very low level of crude protein (120 g/kg diet) was used to test the effect of the supplementation of several amino acids. In this case chicken growth performance was also improved compared with the basal, unsupplemented diet. Again, growth did not equal that in the group fed on the control diet.

Although growth could be improved by incremental increases in either lysine (Expt 1) or a balanced mix of limiting amino acids (Expt 2), plasma IGF-1 differed only when control values (200 g crude protein) were compared with basal, unsupplemented diets (150 g crude protein in Expt 1 and 120 g crude protein in Expt 2). The usefulness of assaying plasma IGF-1 concentrations as an indicator of the metabolic status of animals has met with varying degrees of success. For example, in comparing plasma IGF-1 concentrations among animal species, lower concentrations were noted in the chicken (Leung *et al.* 1986) than in the growing rat (Prewitt *et al.* 1982), although trends associated with growth are similar in both species. Growth retardation in chickens may be due also to reduced systemic IGF-1 as well

as increased catabolic hormones. Buyse *et al.* (1986) used exogenous corticosterone to reduce growth and increase body fat of chickens and found a decrease in plasma IGF-1 concentration. Huybrechts *et al.* (1985) surveyed plasma IGF-1 concentrations in growing meat-and egg-type chickens and found an age-dependent decrease in plasma IGF-1 in the egg-type chicken but not in the meat-type chicken. The latter observation is not surprising because the meat-type chicken has been intensively selected for rapid growth.

Several recent studies provide evidence for nutrient control of plasma IGF-1. Refeeding a diet containing non-essential amino acids increased plasma IGF-1 in rats to a lesser extent than refeeding a diet containing essential amino acids (Isley *et al.* 1984; Clemons *et al.* 1985*a*, *b*). Prewitt *et al.* (1982) also noted a linear increase in plasma IGF-1 with increasing protein intake coupled to a moderate energy restriction (75% of *ad lib.* intake). A more recent study (Lauterio & Scanes, 1987) compared plasma IGF-1 concentrations in chickens as a function of dietary protein and showed a decrease in plasma IGF-1 when chicks were switched from a diet containing 200 g crude protein/kg to one containing 50 g crude protein/kg. Correspondingly, the opposite feeding regimen increased plasma IGF-1. The change in plasma IGF-1 in this study was attributed to changes in protein nutritional status. An examination of feed intakes in the latter study cast some doubt on this hypothesis because feed intake was not controlled. Thus, protein intake *per se* could not be separated from the energy intake of the chickens in this study. In a later study (Rosebrough *et al.* 1988) growing chickens were used to test the relationship between protein and energy intakes and various indices of growth; in this study a feed restriction regimen was practised so that fixed quantities of both protein and energy were fed. Under the condition of a restricted energy intake (70% of *ad lib.* intake) and two levels of crude protein intake, plasma IGF-1 was greater in those chickens given the greater amount of protein. Plasma IGF-1 concentrations matched both growth and relative breast muscle size, suggesting that this hormone regulated lean tissue development. It is tempting to speculate that the increase in muscularity (increase in relative breast muscle size) in chickens given a higher daily protein allotment may relate to IGF-1. It was concluded from the Rosebrough *et al.* (1988) study that plasma IGF-1 concentration was a useful indicator of dietary protein adequacy given that energy intake was also considered.

Analyses of data also showed that thyroid hormone values may be of limited usefulness in determining the protein nutritional status of chickens. In the present study large differences among dietary treatments were required because of the large variation in values. 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. The findings of Yang *et al.* (1987) suggested that: (1) growth could be separated from thyroid function and (2) growth retardation was related to the quantity of the dietary energy rather than its composition.

Few studies have presented any biochemical logic for the decrease in lipogenesis accompanying the feeding of high-protein diets. Bartov (1979) stated that excess dietary protein also forced the chicken to use energy to excrete excess N as uric acid. Thus, less energy would be available for lipogenesis (Buttery & Boorman, 1976). Yeh & Leveille (1969) found an inverse relationship between the level of the dietary protein and the ensuing rate of *in vivo* lipogenesis. They thought that 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. Reducing equivalents produced through the normal operation of the Krebs Cycle would be used for glucose production because of a higher state of reduction compared with glucose precursors (Yeh & Leveille, 1969). 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 & Steele, 1986*b*). 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. Intracellular competition may exist between acetyl-CoA carboxylase (*EC* 6.4.1.2) and the aconitase (*EC* 4.2.1.3)–isocitrate dehydrogenase (*EC* 1.1.1.42) pathway for limited cytoplasmic citrate. The requirement for 2-oxoglutarate (a product of the reaction catalysed by ICD-NADP as a co-reactant for transamination, occurring during increased dietary protein intake, depresses citrate levels to that activation of acetyl-CoA carboxylase would not occur. Clark *et al.* (1979) reported that avian acetyl-CoA carboxylase was particularly sensitive to citrate levels. The latter report seems to offer some support for the role of high-protein diets as regulators of lipogenesis via citrate availability.

The results of the present study support those of Tanaka *et al.* (1983) that show that in the face of a constant energy intake an increase in protein energy will result in a decrease in lipogenic enzyme activities. Although MDH-NADP may provide the necessary NADPH for lipogenesis, the enzyme may not strictly regulate lipogenesis according to the findings in the present study. For example, the lowest noted activity (attained by feeding a diet containing 200 g crude protein/kg diet) would result in the formation of nearly five times the NADPH required for the *de novo* synthesis of the fatty acids noted for this group.

In summary, the present study using growing chickens was designed to test the relationship between either crude protein or protein quality and various indices of growth and intermediary metabolism. Plasma IGF-1 concentrations paralleled growth and may either regulate lean tissue development or reflect changes in lean tissue synthetic rates. It is likely that plasma IGF-1 concentrations can be used as an indication of dietary protein adequacy given dietary treatment effects are large and that energy intake is considered with both protein quantity and quality. In contrast, an apparent improvement in the protein quality of a low-protein diet did not depress lipogenesis when compared with feeding an unimproved diet.

The hypothesis of the present study was that an improvement in protein nutriture, attained by adding either a limiting amino acid to an otherwise adequate diet or by adding several limiting amino acids to a marginal level of crude protein, would repartition energy away from lipogenesis. This hypothesis was not substantiated by the present findings. Therefore, the regulation of lipogenesis in the broiler is more complex than merely meeting some amino acid need for lean tissue synthesis, and thereby, directing energy away from fat synthesis.

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