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Effects of dietary macronutrient content on energy metabolism and uncoupling protein mRNA expression in broiler chickens

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The objective of the present study was to investigate the effects of dietary macronutrient ratio on energy metabolism and on skeletal muscle mRNA expression of avian uncoupling protein (UCP), thought to be implicated in thermogenesis in birds. Broiler chickens from 2 to 6 weeks of age received one of three isoenergetic diets containing different macronutrient ratios (low-lipid (LL) 30 *v.* 77 g lipid/kg; low-protein (LP) 125 *v.* 197 g crude protein (N × 6·25)/kg; low-carbohydrate (LC) 440 *v.* 520 g carbohydrate/kg). LP chickens were characterised by significantly lower body weights and food intakes compared with LL and LC chickens (−47 and −38 % respectively) but similar heat production/kg metabolic body weight, as measured by indirect calorimetry, in the three groups. However, heat production/g food ingested was higher in animals receiving the LP diet (+41 %, $P<0·05$). These chickens also deposited 57 % less energy as protein ($P<0·05$) and 33 % more as fat. No significant differences in energy and N balances were detected between LL and LC chickens. The diets with the higher fat contents (i.e. the LP and LC diets) induced slightly but significantly higher relative expressions of avian UCP mRNA in *gastrocnemius* muscle, measured by reverse transcription–polymerase chain reaction, than the LL diet (88 and 90 *v.* 78 % glyceraldehyde-3-phosphate dehydrogenase respectively, $P<0·05$). Our present results are consistent with the recent view that UCP homologues could be involved in the regulation of lipid utilisation as fuel substrate and provide evidence that the macronutrient content of the diet regulates energy metabolism and especially protein and fat deposition.

Macronutrients: Energy metabolism: Uncoupling proteins: Broiler chickens

Energy deposition is the result of energy intake on the one hand and energy expenditure on the other and is controlled by a variety of mechanisms. As well as genetic factors, exogenous factors such as environmental conditions and of particular interest nutritional factors (diet quantity and composition) interact strongly with the control and regulation of the energy flow. With regard to the dietary macronutrient ratio, protein has the highest oxidation rate followed by carbohydrate and then fat, which corresponds to their ability to induce satiety, but is reciprocal to their storage capacity in mammals (Stubbs *et al.* 1997). In chickens, dietary composition also has a major impact on energy metabolism and body composition (e.g. MacLeod 1990; Buyse *et al.* 1992; Nieto *et al.* 1997). Indeed, diets with a high metabolisable energy content or high energy: protein ratio induce energy deposition as fat. In contrast, increasing the crude protein (CP, N × 6·25) content of the diet above normal requirements will result in leaner birds but with a poor food efficiency, whereas reducing it will promote fat accretion (Buyse *et al.* 1992). However, the impact of macronutrients on adaptive thermogenesis in poultry is still unclear. Broiler chickens reared on diets with a very high energy:protein ratio obtained by the addition of fat showed a reduction in heat production (HP), notwithstanding their elevated energy intake (MacLeod, 1990, 1992). Broilers reared on 150 g protein/kg diet (compared with an isoenergetic diet, 200 g CP/kg) consumed relatively more food per g body-weight gain in an attempt to meet their protein requirements, but consumed in this way more energy ('luxus' energy consumption). This 'luxus' energy consumption is dealt with in two ways: increased energy retention as fat and elevated HP (Buyse *et al.* 1992). Kita *et al.* (1993) also observed an increase in HP in male White Leghorn chicks with a low CP intake compared with those having a normal and high CP intake. In view of these discrepancies between the limited number of studies available, more research is needed to clarify the effect of isoenergetic diets containing different

Abbreviations: av, avian; CP, crude protein (N × 6·25); HP, heat production; LC, low carbohydrate; LL, low lipid; LP, low protein; T_3, 3,5,3′-triiodothyronine; T_4, thyroxine; UCP, uncoupling protein.

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amounts of macronutrients on all components of energy metabolism, including thermogenesis.

The physiological mechanisms that regulate and link appetite, energy balance and energy expenditure are also poorly understood in poultry. An avian (av) homologue of the mammalian uncoupling proteins (UCP) was recently shown to be over-expressed in ducklings and to a lesser extend in chickens subjected to low temperatures (Collin *et al.* 2003), in cockerels from the line R+with a high diet-induced thermogenesis and in glucagon-treated ducklings (Raimbault *et al.* 2001). These results support the role of this protein in thermogenesis in birds. In mammals, UCP are also suggested to play a role in the regulation of diet-induced thermogenesis and energy partitioning (Ricquier & Bouillaud, 2000) and in lipid utilisation as fuel (Dulloo & Samec, 2001).

Therefore, the aim of the present experiment, conducted with three (almost) isoenergetic diets with different macronutrient (fat, protein and carbohydrate) ratios, was to identify mechanisms involved in the macronutrient-dependent regulation of the components of energy balance, and some relevant plasma hormone and metabolite levels as well as the expression level of avUCP in rapidly growing broiler chickens.

Materials and methods

Experimental design

Male broiler chicks (1-d-old) from a commercial meat-type strain (Cobb) were purchased from a local hatchery (Avibel, Zoersel, Belgium) and were divided over three floor pens in an environmentally controlled poultry house. Temperature was set at 1-d-old at 34°C during the first week, and was gradually lowered by 2°C per 1 or 2 d until a temperature of 20°C was reached at the age of 28 d. The lighting schedule provided 23 h light per d, and wood-shavings were used as litter. Until 7 d of age, a commercial starter diet (for diet composition see Buyse *et al.* 2001) was provided *ad libitum*.

From the second week of age, chicks in each pen received one of the three (almost) isoenergetic diets (Table 1). The diets contained the same ingredients, though some in different quantities, and 80 % of the diet content was similar for each diet. The levels of soyabean-protein hydrolysate, starch, soyabean oil and inert celite were manipulated to create pair-wise changes in macronutrient levels and to keep metabolisable energy content similar for all diets. The low-protein (LP) diet contained (per kg): metabolisable energy 11·69 MJ, protein 125 g, fat 77 g, non-fibre carbohydrate 530 g. The low-lipid (LL) diet contained (per kg): metabolisable energy 11·78 MJ, protein 196 g, fat 30 g, non-fibre carbohydrate 511 g. The low-carbohydrate (LC) diet contained (per kg): metabolisable energy 11·78 MJ, protein 197 g, fat 77 g, non-fibre carbohydrate 440 g.

At 14 d of age, a sufficient number of chicks from each diet group were taken from the floor pens and housed in wire-mesh cages for adaptation to restrained-housing conditions. At the ages indicated in Table 2, chickens were placed in pairs (days 15 and 19) or singly in one of the six respiration cells (two cells per diet) to measure energy metabolism. The same temperature and lighting schedule was applied as for their floor-reared

Table 1. Experimental diets

	Diet		
	Low-protein	Low-lipid	Low-carbohydrate
Ingredients (g/kg)			
Maize	596·9	596·9	596·9
Soyabean meal	145·8	145·8	145·8
Soyabean oil	55·0	8·0	55·0
Soyabean-protein hydrolysate	0·0	88·0	88·0
Starch	112·0	112·0	0·0
Dicalcium phosphate	23·3	21·8	21·6
Calcium carbonate	16·0	16·0	16·0
DL-Methionine	4·3	4·3	4·3
Lysine	6·9	6·9	6·9
L-Threonine	2·6	2·6	2·6
L-Tryptophan	0·8	0·8	0·8
Sodium chloride	2·8	2·8	2·8
Premix	4·0	4·0	4·0
Celite	29·6	17·0	55·3
Energy and nutrient contents			
Apparent metabolisable energy (MJ/kg)*	11·69	11·78	11·78
Protein (g/kg)	124·6	196·3	197·7
Non-fibre carbohydrate (g/kg)	530·3	511·2	440·1
Fat (g/kg)	76·9	30·0	77·1
Ash (g/kg)	62·6	60·6	60·7
Fibre (g/kg)	19·5	19·2	19·0
Ca (g/kg)	11·8	11·4	11·4
Non-phytate P (g/kg)	4·5	4·5	4·5
DM (g/kg)	858·7	868·9	845·9

* Calculated from gross energy intake and energy excreted.

Table 2. Schedule of measurements of heat production (HP) in the respiratory cells and of tissue sampling

Age at entry into respiratory cells (d)	Animals (*n*)	Training days (*n*)	Age at HP measurement (d)	Age at tissue sampling (d)
15	2 × 6	2·0	17–19	19
19	2 × 6	2·5	22–24	24
24	6	2·5	27–29	29
29	6	2·0	31–33	33
33	6	2·5	36–38	38
38	6	2·5	41–43	43

counterparts, and the chickens still received their specific diet *ad libitum*.

Heat production measurements

The respiration unit consisted of six equal-sized respiration cells, placed two by two in three separate, light- and temperature-controlled climatic chambers; the gas analyser unit and the data acquisition system were described in detail by Buyse *et al.* (1998). The respiratory cells (550 × 300 × 500 mm) were made of stainless steel and the temperature inside was measured by a Pt resistance temperature detector (Pt-100, accuracy of 0·2°C; Farnell In One, Grace-Hollogne, Belgium). Light was provided by a fluorescent lamp (60 W). Humidity was not controlled. The paramagnetic O_2 analyser (ADC 02-823A) and the infrared CO_2 analyser (ADC D/8U/54/A; The Analytical Development Company, Hoddesdon, Herts., UK) were calibrated before each measurement by using gas mixtures with precisely known O_2 and CO_2 contents.

After at least 2 d of adaptation to the respiratory cells, gas exchanges (CO_2 and O_2) were measured continuously over 48 h. Briefly, O_2 and CO_2 concentrations from air samples coming out of each cell were measured by the gas analysers every 15 min and the O_2 consumption and CO_2 production by the chicken were deduced from the difference between outside fresh air and cell air. HP was calculated from O_2 consumption and CO_2 production according to the formula of Romijn & Lokhorst (1961): HP (kJ/h) = 16·18 O_2 (litres/h) + 5·02 CO_2 (litres/h). The third term for urinary N secretion was omitted as it typically induces an error of <1 %.

Excreta and tissue sampling

On sampling days, the body weight of each chick was measured and food intake per respiratory cell was recorded. Excreta were collected quantitatively, weighed, packed in plastic foil and stored frozen until analysed. Blood was withdrawn from a wing vein by using a heparinised syringe and collected in ice-cold tubes. After killing, *gastrocnemius* muscles from both legs were immediately excised, frozen in liquid N_2 and stored at -80°C for further study of avUCP expression. Liver and abdominal fat pads were excised and weighed.

Energy and protein balance

After thawing and homogenisation, two excreta samples and several food samples were analysed by the Kjeldahl method to determine their N content. Other food and excreta samples were dried in a vacuum oven and their energy content was measured using an isoperibol calorimeter (Parr Instrument Company, Moline, IL, USA).

Apparent metabolisable energy intake was calculated as the difference between gross energy intake and energy excretion in excreta. Apparent metabolisability was calculated as the apparent metabolisable energy intake:gross energy intake ratio. Energy retention was calculated subtracting HP from apparent metabolisable energy intake. Body protein deposition was calculated as the difference between protein intake (N intake × 6·25) and amount of protein excreted (N excretion × 6·25). Energy retained as protein was calculated by multiplying protein retention by its energy value of 23·7 kJ/g (Znaniecka, 1967). Energy retention as fat was then calculated as the difference between total energy retention and energy retained as protein. The amount of fat deposited was obtained by dividing energy retention as fat by its energy content (39·2 kJ/g; Znaniecka, 1967). Results were calculated per kg metabolic body weight (body weight (kg) raised to the power 0·75) and expressed on a 24 h basis.

Avian uncoupling protein mRNA expression

avUCP mRNA expression was determined in *gastrocnemius* muscle by reverse transcription–polymerase chain reaction. The reaction was repeated four times for each sample, and the amount of amplified avUCP DNA from 1 μg total RNA was each time point calculated relative to the amount of glyceraldehyde-3-phosphate dehydrogenase DNA amplified from 1 μg of the same total RNA during the same reverse transcription–polymerase chain reaction. Complementary DNA was synthesised by the extension of avUCP antisense primer for avUCP (5′-TCTCCACAGCCCACGCACTC-3′) or glyceraldehyde-3-phosphate dehydrogenase, (5′-TTGCTGGGGTCACGCTCCTG-3′). The reverse transcription reaction was carried out in a DNA thermal cycler (MJ-PTC-200; Biozym, Landgraaf, The Netherlands) at 37°C for 45 min followed by 5 min at 95°C. The samples were placed immediately on ice and used for polymerase chain reaction amplification. This reaction was performed using the same antisense primers and the sense primers (5′-GCTTCGCCTCCATCCGCATC-3′ for avUCP and 5′-TTGGCCGTATTGGCCGCCTG-3′ for glyceraldehyde-3-phosphate dehydrogenase, 20 μM; Life Technologies NV, Merelbeke, Belgium). The antisense and sense primers for the avUCP fragment were chosen from the sequence of Raimbault *et al.* (2001). The reaction was started with 5 min at 95°C followed by twenty-five cycles of 30 s, at 94°C for 40 s at 59°C and for 20 s at 72°C. The final extension lasted 10 min at 72°C. Negative

control reverse transcription–polymerase chain reaction with diethyl pyrocarbonate-treated water was included in all experiments. Polymerase chain reaction products (15 μl; 266 bp for avUCP and 220 bp for glyceraldehyde-3-phosphate dehydrogenase) was electrophoresed on an agarose (2 %) gel (Life Technologies NV) containing ethidium bromide. After electrophoresis, the gel was scanned using a densitometer (Image Master VDS, Pharmacia Biotech, Uppsala, Sweden). The linearity of the determination was checked by reverse transcription-polymerase chain reaction made on diluted RNA samples (0–50 ng/μl) derived from an avUCP DNA probe (Fig. 1). These RNA samples were obtained by *in vitro* transcription as described by Stofflet *et al.* (1988).

Blood variable concentrations

Plasma glucose, uric acid and triacylglycerol concentrations were determined by using commercially available kits from Instrumentation Laboratory (Lexington, KY, USA). Non-esterified fatty acid concentrations were measured in plasma using kits from Wako Chemicals (Neuss, Germany). All samples were run in the same assay to avoid inter-assay variability.

Thyroid hormone concentrations and deiodase activities

Plasma 3,5,3′-triiodothyronine (T_3) and thyroxine (T_4) concentrations were measured by radioimmunoassay as described by Darras *et al.* (1992). Intra-assay CV were 4·5 and 5·4 % for T_3 and T_4, respectively. Antisera and T_3 and T_4 standards were purchased from Byk-Belga (Brussels, Belgium). The same radioimmunoassay was used for the determination of the concentration of both thyroid hormones in liver and kidney after extraction. A detailed description of the extraction protocol is given elsewhere (Reyns *et al.* 2002). Outer-ring deiodinating type I and inner-ring deiodinating type III activities were measured in microsomal fractions in liver and kidney (Darras *et al.* 1992).

Statistical analysis

Because some animals did not adapt well to the respiration chambers (i.e. weight loss) during the training period, data were analysed for twelve, sixteen and fifteen animals receiving the LP, LL and LC diets respectively. Results for energy balance and avUCP mRNA expression were analysed by ANOVA using a Fisher probability least significant difference test (Statview, 2000; SAS Institute, Cary, NC, USA).

Results

Body weight, energy intake and food conversion ratio

Offering isoenergetic diets with different macronutrient ratios markedly altered zootechnical variables (Table 3). Results indicate a significantly ($P=0·0056$) lower voluntary food intake in animals receiving the LP diet as compared with the LL and the LC diets (−37 and −40 % respectively). The apparent metabolisable energy intake was not different between groups when calculated per kg metabolic body weight. Apparent metabolisability was also not different between the diets and averaged 0·754, 0·724 and 0·729 for the LP, LL and LC diets respectively. The lower food intake resulted in a significantly ($P=0·0181$) lower mean body weight in the LP group compared with the LL and LC diets (−44 and −51 % respectively). Broilers reared on the LP diet were much less efficient in converting food into body-weight gain than their LL and LC counterparts (+54 and +79 % respectively).

Heat production and nitrogen and energy balance

All groups had similar values for HP/kg metabolic body weight. However, HP relative to the food ingested was significantly higher in animals receiving the LP diet as compared with the LL and LC birds (+41 and +42 % respectively, $P=0·0046$). No significant differences were observed in energy retention/kg metabolic body weight between the three diets. However, animals on the LP diet deposited on average 57 % less energy as protein ($P<0·05$) and 33 % more energy as fat than broilers receiving the normal-protein diets. In accordance with the higher value for fat deposition in birds on the LP diet, proportional liver and abdominal fat pad weights were the highest in this group (on average +28 and +107 % respectively). The RQ was close to unity in all groups

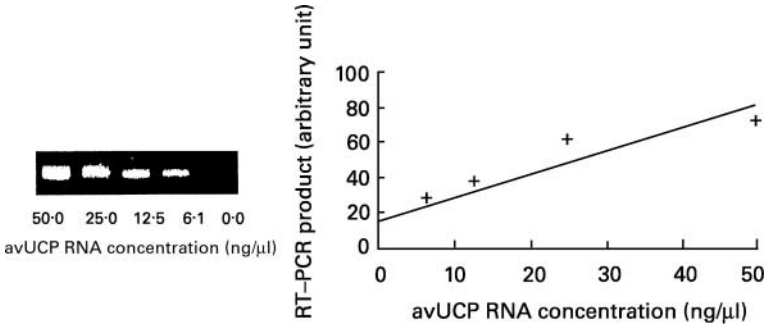

Fig. 1. Results of reverse transcription–polymerase chain reaction (RT–PCR) made on RNA samples derived from avian (av) uncoupling protein (UCP) DNA probe by *in vitro* transcription. For details of procedures, see p. 263. R^2 0·82.

Table 3. Effect of nutrient composition of the diet on energy metabolism in 2- to 6-week-old chickens*†
(Mean values with their standard errors)

Diet...	Low-protein (*n* 12)		Low-lipid (*n* 16)		Low-carbohydrate (*n* 15)		
Variable	Mean	SE	Mean	SE	Mean	SE	Statistical significance of effect: *P*
BW (kg)	0·485[b]	0·068	0·893[a]	0·111	1·019[a]	0·159	0·0181
Food intake (g/d)	64[b]	8	101[a]	7	106[a]	10	0·0056
Food conversion ratio (g/g)	2·82[a]	0·43	1·84[b]	0·11	1·57[b]	0·07	0·0020
Apparent metabolisable energy intake (kJ/kg $BW^{0·75}$ per d)	1303	71	1363	85	1304	51	0·7859
HP (kJ/kg $BW^{0·75}$ per d)	456	53	510	45	504	35	0·6706
HP/g food (kJ/$BW^{0·75}$ per g food per d)	3·64[a]	0·27	2·59[b]	0·18	2·56[b]	0·25	0·0046
RQ	1·061[a]	0·036	1·002[ab]	0·030	0·956[b]	0·032	0·1071
RE (kJ/kg $BW^{0·75}$ per d)	846	87	854	83	801	66	0·8729
RE as protein (kJ/kg $BW^{0·75}$ per d)	123[b]	8	296[a]	26	273[a]	16	<0·0001
RE as fat (kJ/kg $BW^{0·75}$ per d)	723	82	558	64	528	55	0·1189
RE as protein:RE as lipid (kJ/kJ)	0·21[b]	0·05	0·58[a]	0·07	0·56[a]	0·04	0·0001
Protein retention (g/kg $BW^{0·75}$ per d)	5·2[b]	0·3	12·5[a]	1·1	11·5[a]	0·7	<0·0001
Fat retention (g/kg $BW^{0·75}$ per d)	18·4	2·1	14·2	1·6	13·5	1·4	0·1192
Liver weight (g/kg BW)	40·1[a]	3·2	33·4[b]	1·4	29·2[b]	1·5	0·0022
Abdominal fat weight (g/kg BW)	20·1[a]	2·3	9·3[b]	0·7	9·5[b]	0·5	<0·0001
HP/g protein retained (kJ/g protein retention per d)	89[a]	11	44[b]	5	46[b]	5	<0·0001

BW, body weight; HP, heat production; RE, retained energy.
[a,b]Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, see Tables 1 and 2 and p. 262.
† Results were obtained during 2 d periods of measurements in respiratory cells.

but had the highest value in animals receiving the LP diet, being 1·061, as compared with the LL (1·002) and the LC (0·956) diets. Finally, the HP expressed per g protein retained in LP chickens was twice the value of that of the LL and LC groups ($P<0·0001$).

Avian uncoupling protein mRNA expression

The LP and LC diets, which had the highest fat content, induced slightly but significantly higher relative expressions of avUCP mRNA than the LL diet (88 and 90 *v.* 78 % relative to glyceraldehyde-3-phosphate dehydrogenase mRNA expression respectively, $P<0·05$; Fig. 2). Fig. 2 also shows that avUCP mRNA expression was not significantly different between the LP and the LC chickens.

Metabolite and endocrine variables

Offering the LP diet resulted in slightly higher plasma glucose concentrations as compared with the LL and LC diets ($P<0·10$) and in 43 and 51 % higher plasma triacylglycerol concentrations respectively ($P=0·0004$; Table 4). Plasma uric acid concentrations were lower in LP than in LL and LC birds ($P<0·05$), whereas no significant differences in non-esterified fatty acid levels between the diets could be discerned.

Plasma T_4 concentrations were significantly ($P<0·05$) lower in LP chickens than in LL and LC chickens (Table 5). The lower plasma T_4 concentrations in LP birds were associated with lower liver T_4 concentrations in these birds. Plasma T_3 concentrations were not significantly different between the dietary treatments nor were there any differences in hepatic T_3 concentrations.

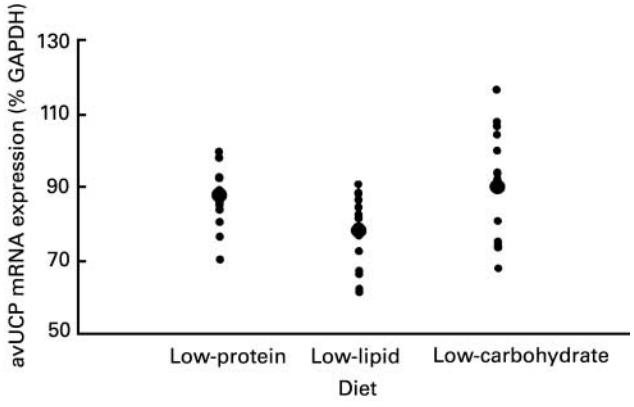

Fig. 2. Effect of the nutrient composition of the diet on the expression of avian (av) uncoupling protein (UCP) mRNA relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). For details of diets and procedures, see Tables 1 and 2 and p. 262. •, Individual values; ●, mean values for low-protein (*n* 12), low-lipid (*n* 16) and low-carbohydrate (*n* 15) diets. Mean value of the low-lipid diet was significantly different from those of the low-protein and low-carbohydrate diets: *$P<0·05$.

Table 4. Effect of nutrient composition of the diet on plasma variables in 2- to 6-week old chickens*
(Mean values with their standard errors)

Diet...	Low-protein (*n* 12)		Low-lipid (*n* 16)		Low-carbohydrate (*n* 15)		
Plasma variable	Mean	SE	Mean	SE	Mean	SE	Statistical significance of effect: *P*
Glucose (g/l)	2·85[a]	0·14	2·69[ab]	5·26	2·58[b]	5·06	0·0935
Triacylglycerol (mg/l)	636[a]	68	452[b]	29	372[b]	32	0·0004
Non-esterified fatty acids (mM)	0·55	0·08	0·49	0·04	0·64	0·06	0·1815
Uric acid (mM)	5·48[b]	0·67	7·23[ab]	0·59	7·70[a]	0·84	0·1141

[a,b]Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, see Tables 1 and 2 and p. 262.

Chickens on the LC diet had higher hepatic outer-ring deiodinating type I activity than LL and LP birds ($P<0·05$), but similar renal outer-ring deiodinating type I activity. In contrast, renal inner-ring deiodinating type III activity was the highest in birds on the LL diet ($P<0·05$), whereas no differences could be found between groups concerning the hepatic inner-ring deiodinating type III activity.

Discussion

The present study revealed marked differences in energy balance between chickens receiving the LP diet and those reared on the LL or LC diets. Indeed, the LP chickens exhibited a much lower growth rate and an altered repartitioning in body composition (lower lean:fat ratio) compared with the other birds fed normal CP levels, as has already been observed by others (e.g. Lin *et al.* 1980; Jones & Smith, 1986; Buyse *et al.* 1992). As food intake and growth of the LP chickens were so different from that of the LL and LC groups, their whole energy metabolism might be disturbed, and their metabolic state might have been close to maintenance energy level. Therefore, the effects of lower growth and low-protein content of the diet *per se* are difficult to distinguish in the present study, without a proper pair-fed group. Another constraint of the present experiment was the relatively close macronutrient composition of the LL and LC diets, which did not allow changes in energy balance between chickens fed with these diets to be identified. However, the experimental diets were chosen to be not too different from commercial broiler diets.

Broiler chickens fed on a diet with a CP content below normal requirements will adapt their ingestive behaviour, though differentially according to the CP level. In general, when the CP level is only marginally reduced, normo- or hyperphagia (g/bird) might occur (Rosebrough & Steele, 1985; Carew & Alster, 1997), probably in an attempt to meet protein requirements for sustaining their growth potential. On the other hand, too low a CP content will result in hypophagia (Buyse *et al.* 1992; Rosebrough *et al.* 1996). However, in all cases there will be an 'involuntary' over-consumption of energy, relative to CP intake. This 'luxus' energy consumption has to be dealt with by two ways: augmented fat deposition or increased adaptive thermogenesis, or more likely both. In the present study, LP chickens did indeed deposit more fat, but did not show an increase in total HP/kg metabolic body weight. This is not necessarily in conflict with results from other studies (Buyse *et al.* 1992; Kita *et al.* 1993) reporting

Table 5. Effect of dietary macronutrient ratio on plasma and liver concentrations of 3,5,3′-triiodothyronine (T_3) and thyroxine (T_4) and *in vitro* renal and hepatic outer-ring deiodinase type I (ORD-I) and inner-ring deiodinase type III (IRD-III) activities*
(Mean values with standard errors)

Diet...	Low-protein (*n* 12)		Low-lipid (*n* 16)		Low-carbohydrate (*n* 15)		
	Mean	SE	Mean	SE	Mean	SE	Statistical significance of effect: *P*
Plasma thyroid hormone concentrations (pmol/ml)							
T_4	3·73[b]	0·57	6·14[a]	0·55	6·32[a]	0·78	0·0177
T_3	2·89	0·22	2·85	0·19	2·63	0·17	0·5862
Hepatic thyroid hormone concentrations (pmol/g tissue)							
T_4	8·1	2·5	14·2	2·3	15·1	3·0	0·1733
T_3	8·4	1·0	8·4	0·8	7·8	0·9	0·8607
ORD-I activity (pmol reverse T_3 deiodinated/mg protein per min)							
Liver	103[b]	7	107[b]	4	126[a]	4	0·0043
Kidney	122	8	108	6	108	6	0·2751
IRD-III activity (fmol T_3 deiodinated/mg protein per min)							
Liver	0·19	0·09	0·13	0·02	0·26	0·08	0·3704
Kidney	1·12[b]	0·11	1·51[a]	0·15	1·07[b]	0·13	0·0464

[a,b]Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, see Tables 1 and 2 and p. 262.

a significant increase in total HP by chickens fed on LP diets. As the heat increment of feeding is $<20\%$ metabolisable energy intake (Geraert *et al.* 1988), the observed 1·4- to 1·5-fold increase in HP/g food ingested in the present study was probably not sufficient to raise total HP significantly. It seems, therefore, that there is a link between the magnitude of the over-consumption of energy (relative to the CP intake) and the diet-induced thermogenic response. This relationship may also be dependent on how the energy from protein is substituted by energy from fat or carbohydrate or both, as carbohydrates elicit a more pronounced heat increment than fat, at least in mammalian models (Stubbs *et al.* 1997). Although a direct comparison between the present study and the one reported previously (Buyse *et al.* 1992) should be done cautiously, it seems that when the heat increment of feeding is stimulated to such an extent that total energy expenditure is elevated as well, this coincides with high plasma T_3 concentrations. The lower plasma uric acid concentrations in LP birds as compared with the LL and LC birds suggests a lower amino acid degradation, resulting directly from the low amino acid supply, but a sparing mechanism of protein could also be involved.

The energy retained as fat was 33 % higher in the LP group as compared with the 'normal-protein content' groups, and this was associated with significantly higher proportional liver and abdominal fat pad weights. As a consequence, fat retention per animal and per d was not different between groups, in spite of the much lower body weight and food intake of LP chickens (results not shown). The significantly higher plasma triacylglycerol concentrations in LP birds are likely to be the result of a stimulated hepatic lipogenesis, as suggested by *in vitro* studies, as well as after measuring lipogenic enzyme activities (Yeh & Leveille, 1969; Tanaka *et al.* 1983; Donaldson, 1985; Rosebrough & Steele, 1985; Rosebrough *et al.* 1999). Further evidence of a high fatty acid synthesis of LP chickens is found in their RQ value being >1 (Ferrannini, 1988). Finally, as HP is due mainly to the metabolic rate of lean tissues rather than adipose tissues and protein retention was much depressed in the LP group, HP/g protein retained in this group was twice that of the LL and LC groups.

There were no significant differences in plasma T_3 levels between the dietary treatments, which is in contrast with most studies reporting an increase in plasma T_3 levels of chickens fed on an LP diet (Buyse *et al.* 1992; Rosebrough *et al.* 1996, 1999; Carew & Alster, 1997). These latter authors (except Buyse *et al.* 1992) did not measure total energy expenditure, which might have been significantly elevated, and hence associated with augmented plasma T_3 levels (see earlier). In accordance with all other studies (Rosebrough & Steele, 1985; Rosebrough *et al.* 1996, 1999; Carew & Alster, 1997), the LP chickens were characterised by significantly lower plasma T_4 level concentrations. This is likely to be the consequence of a reduced thyroidal T_4 output, and not by an enhanced hepatic T_4 uptake, as the T_4 concentrations in this tissue were also significantly lower in LP birds. Hepatic T_3 and T_4 concentrations were in parallel with their respective plasma levels, which points to the establishment of a rapid hormone exchange between plasma and liver until equilibrium is reached. Furthermore, deiodinase activity regulation seems to be tissue-specific, as LC chickens were characterised by the highest outer-ring deiodinating type I activity in liver but lowest activity in kidney. On the other hand, the absence of differences in plasma T_3 levels does not fit with the alterations in deiodinase enzyme activities in both tissues. This inconsistency is difficult to explain at present. The intrahepatic use of T_3 for metabolising the varying levels of lipid, protein and carbohydrate may differ between the diet groups.

In order to explain the molecular mechanisms of the higher HP/g food ingested in LP birds, mRNA muscular expression of the avUCP recently described by Raimbault *et al.* (2001) was measured. This UCP homologue is a potential mitochondrial indicator of the energy inefficiency of ATP synthesis and energy metabolism, and its mammalian homologues are known to be regulated by endocrine factors such as thyroid hormones (Masaki *et al.* 2000). The avUCP mRNA expression was found to be higher in LP birds than in LL birds, which is congruous with their higher HP/g food ingested compared with LL chickens. The difference in avUCP expression was found to be quite small (even if significant), probably because the three diets used in the present experiment were rather close to each other in terms of macronutrient content. This relationship supports a role for avUCP in adaptive thermogenesis, as suggested by the mRNA avUCP over-expression observed in R+cockerels (Raimbault *et al.* 2001), which manifest an 85 % higher diet-induced thermogenesis than R− birds (Gabarrou *et al.* 1997). The very low food intake observed in LP chickens could have an effect on avUCP mRNA expression in this group, as it was shown in rats that a 50 % food restriction strongly inhibits the mRNA expression of muscle UCP3 (Boss *et al.* 1998). Nevertheless, our present results do not show any decrease in avUCP mRNA in LP chickens with low voluntary food intake as compared with LL and LC chickens, consistent with the lack of effect of a 43 % food restriction in UCP2 and -3 mRNA expression in rat adipose tissue (Scarpace *et al.* 1998). This result could be due to the changes in energy metabolism in LP chickens, which exhibit lower voluntary food intakes when fed with suboptimal levels of protein.

In our present study, however, LL chickens had a slightly lower avUCP expression than LC chickens, but a similar HP/g food consumption. Our results, therefore, are also in accordance with the reconsiderations of Dulloo & Samec (2001) that the skeletal UCP homologues are more likely to be involved in the utilisation of lipids as fuel ('lipid handling' hypothesis) than in diet-induced thermogenesis; the avUCP mRNA over-expression in chickens fed the diets with the higher-fat contents (LP and LC) is in accordance with the findings of Matsuda *et al.* (1997) and Tsuboyama-Kasaoka *et al.* (1999), who described higher UCP3 expression in muscle of rats receiving an LL diet compared with their counterparts fed a normal-fat diet. We realise that the difference in expression between LP and LC *v.* LL groups is rather small (but significant because of a high number of animals) as compared with other studies, and the biological significance of the

observed effect in this experiment must be interpreted cautiously. Further investigations at the protein and functional level are necessary to assess the potential role of skeletal muscle avUCP gene expression in adaptive thermogenesis, lipid utilisation or even as free radical scavengers in chickens, as postulated in mammals (for a review in mammals, see Dulloo & Samec, 2001).

In conclusion, the present study confirms that the macronutrient content, and especially the protein content, of the diet regulates protein and fat deposition in chickens. Our results support the recent view that UCP homologues could be involved in the regulation of lipid utilisation as fuel substrate in skeletal muscles.

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References

Boss O, Samec S & Kühne F, *et al.* (1998) Uncoupling protein-3 expression in rodent skeletal muscle is modulated by food intake but not by changes in environmental temperature. *J Biol Chem* **273**, 5–8.

Buyse J, Decuypere E, Berghman L, Kühn ER & Vandesande F (1992) The effect of dietary protein content on episodic growth hormone secretion and on heat production of male broilers. *Br Poult Sci* **33**, 1101–1109.

Buyse J, Janssens GP & Decuypere E (2001) The effects of dietary L-carnitine supplementation on the performance, organ weights and circulating hormone and metabolite concentrations of broiler chickens reared under a normal or low temperature schedule. *Br Poult Sci* **42**, 230–241.

Buyse J, Michels H & Vloeberghs J, *et al.* (1998) Energy and protein metabolism between 3 and 6 weeks of age of male broiler chickens selected for growth rate for improved food efficiency. *Br Poult Sci* **39**, 264–272.

Carew LB & Alster FA (1997) Dietary carbohydrate and fat do not alter the thyroid response to protein deficiency in chicks. *Proc Soc Exp Biol Med* **215**, 82–86.

Collin A, Buyse J, van As P, *et al.* (2003) Cold-induced enhancement of avian uncoupling protein expression, heat production and triiodothyronine concentrations in broiler chicks. *Gen Comp Endocrinol* **130**, 70–77.

Darras VM, Visser TJ, Berghman LR & Kuhn ER (1992) Ontogeny of type I and III deiodinase activities in embryonic and posthatch chicks: relationship with changes in plasma triiodothyronine and growth hormone levels. *Comp Biochem Physiol* **103**A, 131–136.

Donaldson WE (1985) Lipogenesis and body fat in chicks: Effect of calorie:protein ratio and dietary fat. *Poult Sci* **64**, 191–204.

Dulloo AG & Samec S (2001) Uncoupling proteins: their roles in adaptive thermogenesis and substrate metabolism reconsidered. *Br J Nutr* **86**, 123–139.

Ferrannini E (1988) The theoretical bases of indirect calorimetry: a review. *Metabolism* **37**, 287–301.

Gabarrou J-F, Geraert P-A, Picard M & Bordas A (1997) Diet-induced thermogenesis in cockerels is modulated by genetic selection for high or low residual feed intake. *J Nutr* **127**, 2371–2376.

Geraert PA, MacLeod MG & Leclercq B (1988) Energy metabolism in genetically fat and lean chickens: diet- and cold-induced thermogenesis. *J Nutr* **118**, 1232–1239.

Jones R & Smith WK (1986) Effect of dietary protein concentration on the growth and carcass composition of male broilers from hatch to maturity. *Br Poult Sci* **27**, 502–503.

Kita K, Muramatsu T & Okumura J (1993) Effect of dietary protein and energy intakes on whole-body protein turnover and its contribution to heat production in chicks. *Br J Nutr* **69**, 681–688.

Lin CY, Friars GW & Moran ET (1980) Genetic and environmental aspects of obesity in broilers. *Worlds Poult Sci J* **36**, 103–111.

MacLeod MG (1990) Energy and nitrogen intake, expenditure and retention at 20° in growing fowl given diets with a wide range of energy and protein contents. *Br J Nutr* **64**, 625–637.

MacLeod MG (1992) Energy and nitrogen intake, expenditure and retention at 32° in growing fowl given diets with a wide range of energy and protein contents. *Br J Nutr* **67**, 195–206.

Masaki T, Yoshimatsu H & Sakata T (2000) Expression of rat uncoupling protein family mRNA levels by chronic treatment with thyroid hormone. *Int J Obes Relat Metab Disord* **24**, S162–S164.

Matsuda J, Hosoda K, Itoh H, *et al.* (1997) Cloning of rat uncoupling protein-3 and uncoupling protein-2 cDNAs: their gene expression in rats fed high-fat diet. *FEBS Lett* **418**, 200–204.

Nieto R, Aguilera JF, Fernandez-Figares I, Prieto C (1997) Effect of a low protein diet on the energy metabolism of growing chickens. *Arch Tierernahr* **50**, 105–109.

Raimbault S, Dridi S & Denjean F, *et al.* (2001) An uncoupling protein homologue putatively involved in facultative thermogenesis in birds. *Biochem J* **353**, 441–444.

Reyns GE, Janssens KA, Buyse J, Kühn ER & Darras VM (2002) Changes in thyroid hormone levels in chicken liver during fasting and refeeding. *Comp Biochem Physiol* B **132**, 239–245.

Ricquier D & Bouillaud F (2000) The uncoupling protein homologues: UCP1, UCP2, UCP3, StUCP and AtUCP. *Biochem J* **345**, 161–179.

Romijn C & Lokhorst W (1961) Some aspects of energy metabolism in birds. In *Proceedings of the IInd Symposium on Energy Metabolism. European Association for Animal Production*, pp. 49–59 [E Brouwer and EJH van Es, editors]. Lunteren: European Association for Animal Production.

Rosebrough RW, McMurtry JP & Vasilatos-Younken R (1999) Dietary fat and protein interactions in broilers. *Poult Sci* **78**, 992–998.

Rosebrough RW, Mitchell AD & McMurtry JP (1996) Dietary crude protein changes rapidly alter metabolism and plasma insulin-like growth factor-I concentrations in broiler chickens. *J Nutr* **126**, 2888–2898.

Rosebrough RW & Steele NC (1985) Energy and protein relationships in the broiler. 1. Effect of protein levels and feeding regimens on growth, body composition, and *in vitro* lipogenesis of broiler chicks. *Poult Sci* **64**, 119–126.

Scarpace PJ, Nicolson M & Matheny M (1998) UCP2, UCP3 and leptin gene expression: modulation by food restriction and leptin. *J Endocrinol* **159**, 349–357.

Stofflet ES, Koebel DD, Sakar G & Sommer SS (1988) Genomic amplification with transcript sequenceing. *Science* **239**, 491–494.
Stubbs RJ, Prentice AM & James WPT (1997) Carbohydrates and energy balance. *Ann NY Acad Sci* **819**, 44–69.
Tanaka K, Ohtani S & Shigeno K (1983) Effect of increasing dietary energy on hepatic lipogenesis in growing chickens. II. Increasing energy by fat or protein supplementation. *Poult Sci* **62**, 452–458.
Tsuboyama-Kasaoka N, Takahashi M, Kim H & Ezaki O (1999) Up-regulation of liver uncoupling protein-2 mRNA by either fish oil feeding or fibrate administration in mice. *Biochem Biophys Res Commun* **257**, 879–885.
Yeh YY & Leveille GA (1969) Effect of dietary protein on hepatic lipogenesis in the growing chick. *J Nutr* **98**, 356–366.
Znaniecka G (1967) Calorific value of protein and fat in the chicken's body. In *Proceedings of the IVth European Association for Animal Production Symposium on the Energy Metabolism of Farm Animals*, pp. 407–408 [KL Blaxter, J Kielanowski and G Thorbek, editors]. Newcastle-upon-Tyne: Oriel Press.