Effects of Substitution Between Fat and Protein on Feed Intake and Its Regulatory Mechanisms in Broiler Chickens: Energy and Protein Metabolism and Diet-Induced Thermogenesis

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ABSTRACT The objective of this study was to investigate the effect of dietary macronutrient ratio on energy, protein, and lipid metabolism and on the involvement of diet-induced thermogenesis in feed intake regulation of broiler chickens. Male broilers were reared from 1 to 7 wk on isoenergetic diets with substitutions between fat and protein and similar carbohydrate content [low protein (LP): 126 vs. 242 g of protein/kg; low fat (LF): 43 vs. 106 g of fat/kg]. Every week from 21 d onward, 3 chickens per group were placed in open-circuit respiratory cells to measure energy and protein metabolism in fasting, short-term refeeding (5 h) and ad libitum conditions. As LP chickens had a significantly lower BW from 2 wk onward, all parameters were expressed per kilograms of metabolic BW. Feed intake, gross energy intake, and apparent metabolizable energy intake were significantly higher in LP than LF birds. The excessive energy relative to protein intake resulted in significantly increased heat production and energy retention as fat. The latter effect and a significantly increased respiratory quotient indicated higher de novo lipogenesis in the LP chickens. Furthermore, the efficiency of protein retention was significantly better in LP broilers. Neither diet-induced thermogenesis nor feed intake during a 5-h refeeding period was affected by diet composition. Our results indicate that isoenergetic substitution of fat for protein has a strong effect on growth and on energy and protein balance in broilers. The theory linking diet-induced thermogenesis to feed intake could not be corroborated or countered, and further research is warranted.

(*Key words*: broiler chicken, macronutrient, energy and protein metabolism, diet-induced thermogenesis)

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INTRODUCTION

Energy deposition is the net result of energy intake and expenditure and is controlled by multiple regulatory mechanisms. Next to genetic factors, exogenous factors such as environmental conditions and nutritional factors (e.g., diet quantity and composition) interact strongly with the control and regulation of the energy flow. With respect to the dietary macronutrient content, mammals exhibit a hierarchy in which recently ingested nutrients are combusted. First proteins are combusted, followed by carbohydrates, then the oxidation of fat, which corresponds to their ability to induce satiety but is reciprocal to their relative storage capacity (hierarchical oxidation/storage model; Stubbs et al., 1997). It is well documented that dietary composition also has a major impact on body composition of chickens (Buyse et al., 1992; MacLeod, 1990, 1992; Nieto et al., 1997; Collin et al., 2003). In general, diets with a high ME content or a high energy to protein ratio promote energy retention as fat. Excess dietary CP results in a leaner bird but reduces feed efficiency, whereas a less-than-optimal protein content increases fat accretion (Buyse et al., 1992). The relationship between ME intake and changes in energy expenditure is still unclear in poultry. Broiler chickens fed high fat diets with a very high energy to protein ratio had a lower heat production (HP) despite higher ME intake (MacLeod 1990, 1992). In contrast, Buyse et al. (1992) found that broilers reared on a 15% protein diet increased their feed intake in an attempt to meet their protein and amino requirements but also consumed an excessive amount of energy in this process. The result of this excess energy intake was increased fat deposition and higher HP compared with broilers fed an isoenergetic diet with 20% CP.

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Abbreviation Key: AMEI = apparent metabolizable energy intake; DIT = diet-induced thermogenesis; EB = energy balance; EE = excreted energy; GE = gross energy; GEI = gross energy intake; HP = heat production; LF = low fat; LP = low protein; MBW = metabolic body weight; RQ = respiratory quotient

Increased HP and fat accretion were also observed by Kita et al. (1993) and Nieto et al. (1997) when increasing the energy to protein ratio of broiler diets, whereas Collin et al. (2003) noted an increased fat deposition but no effect on total HP. Given these discrepancies between a limited number of studies, more research is warranted to explain the effect of isoenergetic diets with different macronutrient contents on the components of the energy balance (EB), including diet-induced thermogenesis (DIT).

The regulation of voluntary feed intake is a very complex and multifactorial mechanism, with several levels of control. Several models have been proposed in an attempt to understand the mechanisms that match energy and nutrient balance with feed intake and energy expenditure to maintain body homeostasis in mammals (Flatt, 2000; Stubbs and O'Reilly, 2000). Included in all of these models are central controllers located in the brain and peripheral control systems (e.g., sensory, gustatory, metabolic) that monitor a variety of events (Stubbs, 1999). However, far less is known about the regulation of these processes in avian species and, in particular, domestic poultry. Because some control systems (e.g., gustatory, sensory) seem to differ between avian species and mammals (Denbow, 1994), extrapolation of findings on mammals to avian species should be done cautiously. An important question is whether the energy or nutrient metabolism is involved in the regulation of feed intake.

In this experiment, fat and protein were substituted in 2 isoenergetic diets to determine the role of these macronutrients in regulating energy, protein and lipid balance, and voluntary feed intake in broiler chickens. An important objective of the study was to explore the role of DIT in the control and regulation of voluntary feed intake in chickens, as postulated in the hierarchical oxidation/storage model for mammalian species. Information on the satiety power of macronutrient oxidation in avian species is scarce. The effect of this isoenergetic substitution on endocrine functioning and key metabolites of intermediary protein, fat, and carbohydrate metabolism was also established and is discussed separately (Swennen, unpublished data).

MATERIALS AND METHODS

Experimental Design

One-day-old male Cobb broiler chickens were purchased from a local hatchery.[2] The chicks were divided over 2 floor pens in an environmentally controlled poultry house with wood shavings as litter. For 1-d-old birds, the temperature was set at 35°C and was decreased by 1°C every 2 d until a final temperature of 22°C was reached. The lighting schedule provided 23L:1D. Until 7 d of age, a commercial starter diet (Buyse et al., 2001) was provided ad libitum.

[2]Avibel, Zoersel, Belgium.

TABLE 1. Experimental diets

	Diet	
Item	Low protein	Low fat
Ingredient (g/kg)		
Peas (<200 g CP/kg)	31.369	31.369
Wheat	24.229	24.229
Maize starch	16.800	16.800
Soya oil	9.507	1.961
Soybean meal	5.124	5.124
Monocalcium phosphate	2.365	2.365
Premix[1]	1.400	1.400
Chalk	1.307	1.307
L-Lysine	0.770	0.770
DL-Methionine	0.622	0.622
L-Threonine	0.393	0.393
Salt	0.337	0.337
L-Tryptophan	0.121	0.121
Soy protein	—	13.2
Celite	6.104	—
Energy and nutrient content		
Gross energy content (kcal/kg)	3,975	3,998
Protein (g/kg)	126.1	242.2
N-free extract (g/kg)	513.9	504.3
Fat (g/kg)	106.1	43.4
Ash (g/kg)	120.7	66.1
Fiber (g/kg)	23.0	22.4
Moisture (g/kg)	110.2	121.6
Starch (g/kg)	447.5	442.0
Sugars (g/kg)	21.7	21.7
Gross energy to protein ratio (kcal/g protein)	31.52	16.51

[1]Premix supplied the following amounts of vitamins and minerals per kilogram of diet: vitamin A, 12,000 IU; vitamin D_3, 3,000 IU; vitamin E, 50 IU; vitamin K_3, 2.5 mg; vitamin B_1, 2.2 mg; vitamin B_2, 7.5 mg; vitamin B_3, 13 mg; vitamin B_6, 5.5 mg; vitamin B_{12}, 0.035 mg; vitamin PP, 38 mg; folic acid, 1 mg; biotin, 0.2 mg; choline-Cl, 650 mg; Fe, 45 mg; Cu, 25 mg; Mn, 60 mg; Co, 1 mg; Zn, 70 mg; I, 2 mg; Se, 0.4 mg; ethoxyquin, 35 mg; butylated hydroxytoluene, 25 mg.

From 7 d of age, each group received one of the isoenergetic diets (Table 1). The diets contained the same ingredients, although some in different quantities. The levels of soybean oil, soybean protein, and celite were manipulated to create changes in protein and fat content and to keep gross energy (GE) content more or less similar for both diets, as was the carbohydrate level. The low protein (LP) diet contained 3,974 kcal of GE/kg, 126 g of protein/kg, 106 g of fat/kg, and 514 g of N-free extract/kg. The low fat (LF) diet contained 3,998 kcal of GE/kg, 242 g of protein/kg, 43 g of fat/kg, and 504 g of N-free extract/kg.

At 21 d of age, 12 chicks from each group were taken from the floor pens and housed in wire cages for adaptation to restraint housing conditions, and they were provided assigned diets and water ad libitum. For the next 4 wk and repeated weekly with other chickens, 3 LP and 3 LF chickens were each placed in 1 of the 6 respiratory cells for measuring the energy and protein metabolism, as indicated in Figure 1. Birds in the respiratory cells were provided continuous lighting and the same temperature schedule as used in the floor pens. After the adaptation period, the birds were fasted for 24 h, and then they were allowed to eat for 5 h to measure DIT. For the rest of the experimental period, they were fed ad libitum to calculate a total energy balance (EB). This research was approved

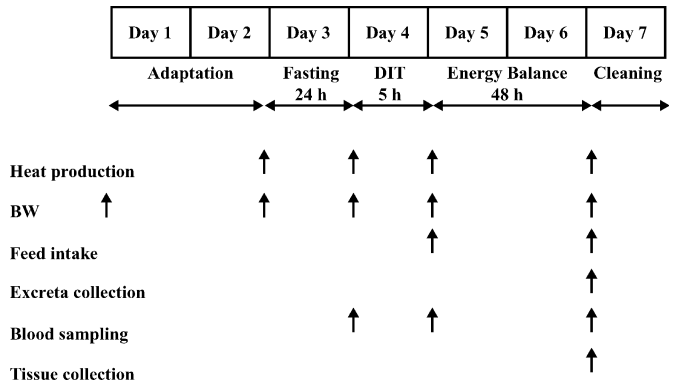

FIGURE 1. Experimental protocol. From 21 d of age and repeated for 4 consecutive weeks with other chickens, 3 chickens on a low protein (LP) diet and 3 chickens on a low fat (LF) diet were placed in 1 of the 6 respiratory cells for measuring the energy and protein metabolism. Arrows indicate the time of data collection. DIT = diet-induced thermogenesis.

by the Ethical Commission for Experimental Use of Animals of the Katholieke Universiteit Leuven.

HP Measurements

A respiration unit consisted of 6 respiratory cells, placed 2 by 2 in 3 separate, light- and temperature-controlled climatic chambers, a gas analyzer unit, and a data acquisition system as described by Buyse et al. (1998). The respiratory cells (550 × 300 × 500 mm) were made of stainless steel with little insulation, and the temperature inside was measured by a resistance temperature detector (Pt-100,[3] accuracy of 0.2°C). The paramagnetic O_2 analyzer (ADC 02-823A) and the infrared CO_2 analyzer (ADC D/8U/54/A) were calibrated before each measurement by using gas mixtures with known exact O_2 and CO_2 contents.

After the period of adaptation to the respiratory cells, gas exchanges (CO_2 and O_2) were measured continuously during fasting and the short (DIT) and long (EB) refeeding periods. Briefly, O_2- and CO_2 concentrations from air samples coming out of each cell were measured for 60 s every 15 min. The CO_2 production and the O_2 consumption by the chicken were calculated from the differences between the gas concentrations of the outside fresh air (measured for 180 s) and the cell air. Heat production was calculated from these data according to the short formula of Romijn and Lokhorst (1961):

$$HP\ (kJ/h) = 16.18\ O_2\ (l/h) + 5.02\ CO_2\ (l/h).$$

The third term for urinary N excretion was omitted as it typically induced an error of < 1%.

[3]Farnell In One, Grace-Hollogne, Belgium.
[4]Parr Instrument Company, Moline, Illinois.
[5]SAS for Windows, Version 8e, 1988, SAS Institute Inc., Cary, NC.

Excreta and Tissue Sampling

Individual BW were measured at the start of the experimental period and after the fasting DIT and EB periods (Figure 1). Feed intake per cell was measured after the DIT and EB periods. Excreta were collected quantitatively after EB measurements, weighed, packed in plastic foil, and stored frozen (–20°C) until analyzed.

Energy and Protein Balance

After being thawed and homogenized, duplicate excreta and feed samples were dried in a vacuum oven, and their GE content was determined using an isoperibol calorimeter.[4] Nondried samples of feed and excreta were analyzed, again in duplicate, by the Kjeldahl method to determine their nitrogen content (Association of Official Analytical Chemists, 1984). Apparent metabolizable energy intake (AMEI) was calculated as the difference between the gross energy intake (GEI) and energy excreted (EE) in excreta. Apparent metabolizability was calculated as the ratio between AMEI and GEI. Energy retention was determined by subtracting HP from AMEI. 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 with the caloric value of protein, 5.66 kcal/g (23.7 kJ/g) (Zaniecka, 1967). Energy retention as fat was then determined as the difference between total energy retention and energy retention as protein. The amount of fat deposited was obtained by dividing energy retention as fat by its energy content of 9.37 kcal/g (39.2 kJ/g) (Zaniecka, 1967). Results were calculated per kilogram of metabolic BW (MBW; $kg^{0.75}$). To assess DIT, the difference between the average value for HP during the last 5 h of fasting and HP at every measuring point during the short refeeding period was calculated. Diet-induced thermogenesis was then considered as the area under the HP-curve during the refeeding period and was expressed as fraction of AMEI (%) during the same interval.

Statistical Analysis

All results were analyzed by ANOVA with age and diet composition as classification variables.[5] There were age effects for a few parameters, but no interactions between diet and age were found for any of the parameters. Therefore, to give a clear presentation of the results, the data were pooled over ages and analyzed again with a one-factor ANOVA.

RESULTS

BW and Feed Intake

The effect of dietary composition on BW is presented in Figure 2. An effect ($P < 0.0001$) of diet on the BW of chickens was observed from 2 until 7 wk of age, with the

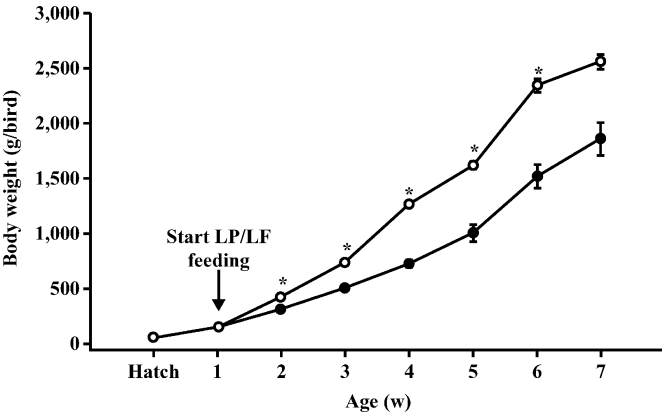

FIGURE 2. Weekly BW of broiler chickens (g/bird) fed the low protein (LP) diet (•) or the low fat (LF) diet (○). Values are means ± SEM (hatch and week 1: n = 70; week 2 to 4: n = 30; week 5: n = 15; week 6: n = 9; week 7: n = 3). *Significant effect of the diets at the same age ($P < 0.0001$).

LF birds having a higher BW compared with their LP counterparts. Because of the pronounced diet-induced differences in BW, results will be presented per kilograms of MBW ($kg^{0.75}$).

Feed intake per MBW was 29% higher ($P < 0.001$) in the birds of the LP group (Figure 3). There was also a significant effect of the diet on the intake of each macronutrient calculated per MBW. On a daily basis, the LP birds consumed 8.8 g or 241% more fat ($P < 0.0001$) and 13.8 g or 33% more carbohydrates ($P < 0.01$), whereas they ingested 6.5 g or 32% less protein ($P < 0.001$) compared with the LF birds.

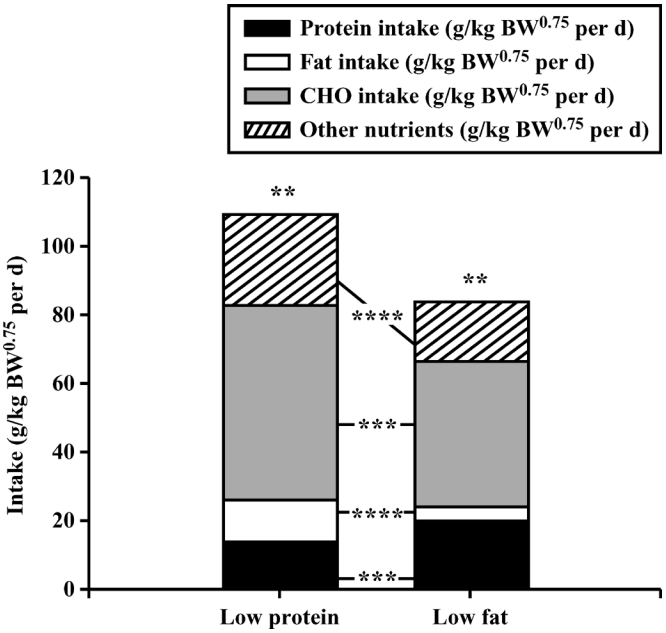

FIGURE 3. Feed intake, partitioned in macronutrients and other nutrients per 24 h for the experimental diets (n = 12 per group). Values are means and are expressed per metabolic BW ($kg^{0.75}$). Significant effects of the diets are indicated by $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$. $BW^{0.75}$, metabolic BW; CHO, carbohydrate.

Energy and Protein Balance

The GE intake per MBW (Table 2) by the LP group was 29% higher ($P = 0.0096$) compared with the LF group, whereas there was no effect of the diet on EE per MBW. The AMEI per MBW (Figure 4) by the LP birds was 45% higher ($P < 0.001$). Apparent metabolizability was also significantly ($P = 0.0006$) affected by diet composition, resulting in a 13% higher metabolizability for the LP diet (Table 2). A higher ($P < 0.05$) HP per MBW was observed for the LP birds (Figure 4). However, there was no effect of diet composition on HP per MBW relative to the feed ingested or relative to the amount of protein retained (Table 2). Total energy retention as well as the efficiency of energy retention of the LP chickens was higher than that of the LF chickens ($P < 0.01$). The fraction of the energy that was retained as protein was not different between groups, whereas the fraction retained as fat was 156% higher ($P < 0.001$) in the LP group (Figure 4). The ratio between both fractions was 63% higher ($P = 0.0013$) for the LF chickens compared with their LP counterparts (Table 2).

The N intake and excretion were higher ($P < 0.001$ and $P < 0.001$, respectively) for the LF chickens. Protein retention was not influenced by the diet, whereas efficiency of protein retention was higher ($P < 0.05$) and, therefore, better for the LP birds (Table 2). Respiratory quotients (RQ) for both groups measured during 3 phases of the experimental period are presented in Figure 5. During fasting and the short refeeding period, RQ values were not different between diets. RQ values measured throughout the ad libitum period were influenced ($P < 0.0001$) by the diet composition, which was 7% higher for the LP than the LF chickens.

DIT

There was no significant diet effect on feed intake, GEI, or AMEI per MBW during the short refeeding period, although the values for these parameters were somewhat elevated for chickens of the LP group compared with the LF group (Figure 6). DIT calculated as a percentage of AMEI was also similar for both groups with a slightly higher value for the LF birds.

DISCUSSION

Feed and Macronutrient Intakes and Growth Rate

The results of the present study showed a marked difference in growth rate between broiler chickens reared on the LP diet and those receiving the LF diet. The LP birds, which were provided with a diet of a much higher energy to protein ratio but similar GE content, exhibited much slower rate of growth than their LF counterparts, as previously observed (Bartov and Bornstein, 1976; Jones and Smith, 1986; Buyse et al., 1992; Collin et al., 2003). As the LP birds ingested more feed per MBW on a daily

TABLE 2. Effect of dietary macronutrient content on parameters of energy and protein metabolism of 4- to 7-wk-old broiler chickens (means ± SEM)[1]

Parameter	Diet			
	Low protein (LP; n = 12)	Low fat (LF; n = 12)	Change in low protein vs. low fat[4] (%)	*P*-value
Gross energy intake[2]	414.51 ± 22.78	321.70 ± 23.45	+29	0.0096
Energy excreted[2]	98.61 ± 8.30	104.40 ± 10.13	–6	NS
N intake[3]	2.197 ± 0.121	3.242 ± 0.236	–32	0.0007
N excretion[3]	0.945 ± 0.081	1.705 ± 0.150	–44	0.0002
N retention[3]	1.252 ± 0.055	1.537 ± 0.166	–19	NS
Protein retention[3]	7.826 ± 0.347	9.605 ± 1.037	–19	NS
Fat retention[3]	13.04 ± 1.70	5.088 ± 0.752	+156	0.0003
RE[6] as protein:RE as fat	0.504 ± 0.134	1.359 ± 0.189	–63	0.0013
Apparent metabolizability	0.766 ± 0.016	0.679 ± 0.015	+13	0.0006
Efficiency of energy retention	0.468 ± 0.041	0.304 ± 0.027	+54	0.0032
Efficiency of protein retention	0.580 ± 0.026	0.470 ± 0.041	+23	0.0318
HP[5]/g protein retained (kcal/g protein retained, kg $BW^{0.75}$ per d)	18.86 ± 1.64	16.31 ± 4.75	+16	NS
HP/g feed (kcal/kg $BW^{0.75(7)}$ per g feed per d)	1.36 ± 0.11	1.39 ± 0.056	–2	NS

[1]Data obtained during 48 h periods of measurements in respiratory cells.
[2]kcal/kg $BW^{0.75}$ per d.
[3]g/kg $BW^{0.75}$ per d.
[4]Calculated as (LP-LF)*100/LF.
[5]HP, heat production.
[6]RE, retained energy.
[7]Metabolic body weight.

basis, feed intake per se was not a causal factor for the observed growth difference. However, regardless of their relative higher feed intake, protein intake by the LP group was 32% lower than that of their LF counterparts, which was the most likely cause for their reduced growth rate. A marginally reduced dietary CP level can induce hyperphagia (Rosebrough and Steele, 1985; Carew and Alster, 1997), whereas a more pronounced reduction might result in hypophagia (Buyse et al. 1992; Rosebrough et al. 1996). However, in spite of a 50% reduction of the protein level in the LP compared with the LF diet, the absolute feed intake (g/bird) of the LP chickens was not different, indicating that the possibilities to display compensatory feed intake by chickens to meet their protein requirements are not unlimited. This plateau in feed intake capacity is likely to be the consequence of limitations to the filling of the gastrointestinal tract as well as negative feedback of peripheral neural and neuroendocrine signals on the central

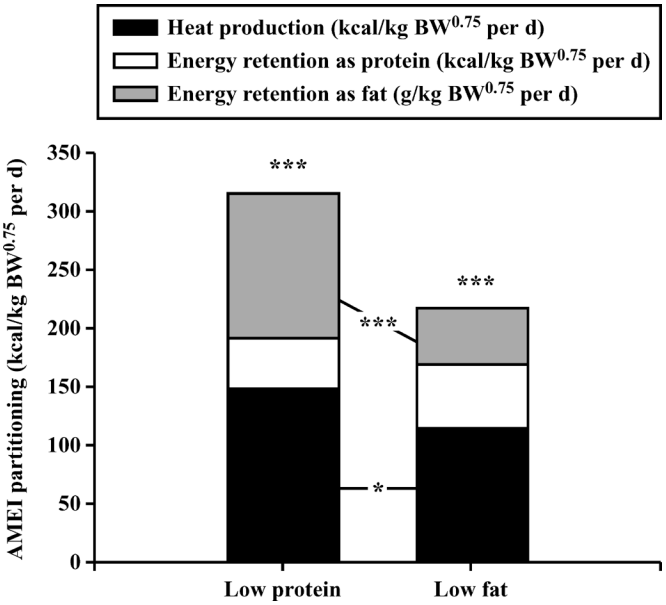

FIGURE 4. Apparent metabolizable energy intake (AMEI) partitioned in its components: heat production, energy retention as fat, and energy retention as protein (n = 12 per group). Values are means and are expressed per metabolic BW ($BW^{0.75}$) and per 24 h. Significant effects of the diets are indicated by *$P < 0.05$; ***$P < 0.001$.

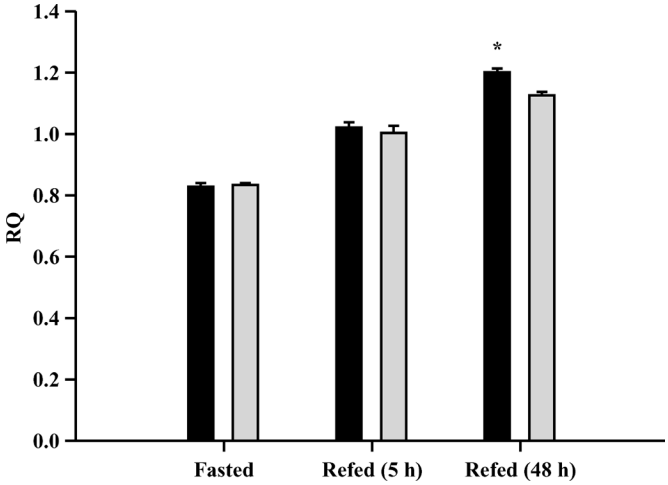

FIGURE 5. Respiratory quotients (RQ) of chickens reared on the low protein (filled bars) and the low fat diet (open bars) measured during 3 phases of the experimental period (n = 9 during fasting, n = 12 during the refeeding periods). Values are means ± SEM. A significant effect of the diets is indicated with an asterisk ($P < 0.0001$).

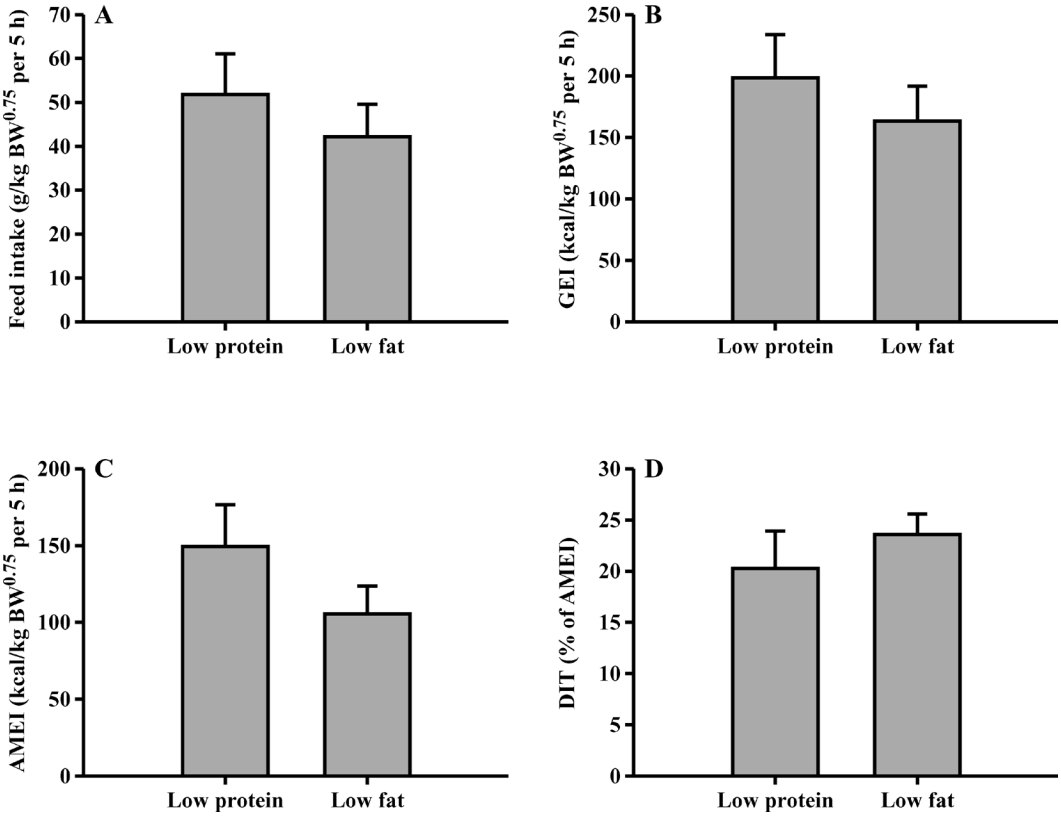

FIGURE 6. A) Feed intake (low protein: n = 9; low fat: n = 12), B) gross energy intake (GEI) (low protein: n = 9; low fat: n = 8), C) apparent metabolizable energy intake (AMEI) (low protein: n = 9; low fat: n = 8) are expressed per metabolic BW ($BW^{0.75}$) and per 5 h (refeeding period). D) Diet-induced thermogenesis (DIT) (n = 8 for both groups) was measured during 5 h and is expressed as a percentage of AMEI. All values are means ± SEM.

control of voluntary feed intake. In this context, the influences of dietary composition on organ weights and of relevant plasma metabolite and hormone concentrations have been determined and will be discussed in an accompanying paper (Swennen, unpublished results).

Energy and Protein Metabolism

Because feed intake per MBW was significantly higher by LP chickens, and both diets contained about the same amount of GE, it follows that LP chickens had a significantly higher GE uptake per MBW. As AMEI per MBW is calculated as the difference between GEI and EE (and EE was not influenced by dietary composition), LP chickens were characterized by a significantly higher AMEI per MBW than their LF counterparts. Consequently, LP chickens had excessive ingestions of energy relative to their protein intakes. There are 3 ways to counteract this overconsumption of energy: increased HP, increased fat retention, or a combination of both. Our experimental data are in favor of the latter possibility. Energy retention was significantly higher in the LP chickens, which was mainly caused by a significant increase of energy retention as fat, as was also reported by Collin et al. (2003). In accordance with other studies (Buyse et al. 1992; Kita et al. 1993, Nieto et al. 1997), chickens reared on the LP diet had a significantly higher total HP. Because feed intake and HP per MBW were similarly influenced by dietary composition, there was no significant effect on HP per gram of feed ingested or on HP per gram of protein retained.

Is it possible for a broiler to increase its HP to a large extent? The ratio of body surface to BW is negatively correlated with the size of chickens. Therefore, the smaller LP chickens have a larger ratio and a proportionally greater surface to dissipate the produced heat, allowing a higher HP, even expressed per MBW (Figure 4). However, the increase of HP is not unlimited. When the upper limit of the metabolic rate is reached, excess energy intake above this metabolic ceiling will not be converted into HP but will lead to storage of fat.

A fundamental question is why energy excretion by the LP broilers does not increase in view of their excessive energy consumption. The absence of an effect of diet on the EE might be caused by the extracaloric effect of the higher fat content of the LP diet. Indeed, supplementation of a diet with a higher fat content has a positive effect on energy use as well as on metabolizable energy intake (Mateos and Sell, 1980). This effect is caused by the better metabolizability of fat compared with proteins and by the longer transition time of high fat diets through the digestive tract. Because diets used in this experiment were isoenergetically formulated by substitution of protein and fat, the LP diet had a higher fat content compared with

the LF diet. On the other hand, the LF birds ingested significantly more proteins that were only partially digested or more absorbed amino acids might have been oxidized, leading to a higher urinary N excretion, which is voided with feces by avian species. This resulted in a higher N content in the excreta of the LF chickens and thus a similar EE for both groups. In addition, lower protein digestion can contribute to lower metabolizability of the LF diet.

Intake and excretion of N per MBW were significantly increased to the same extent for the LF chickens. Because N retention is calculated as the difference between intake and excretion, it follows that protein retention was similar for both groups. However, efficiency of protein retention was significantly influenced by the diet with a significantly higher and therefore better efficiency of protein retention for LP chickens, suggesting a more efficient protein metabolism compared with the LF counterparts. Better protein retention with LP diets has also been reported by Jackson et al. (1982).

During fasting, RQ values were about 0.8, evolving toward basal conditions (RQ = 0.7) (Mitchell and Haines, 1927). There was no effect of the diet on RQ during this phase, suggesting that these values were independent of the body composition of the birds and of the diet ingested before fasting. Throughout DIT measurements, RQ values averaged about one independent of the diet, which indicated that the birds of both groups were combusting carbohydrates. The RQ values measured during EB were greater than one, which was indicative of a high de novo fatty acid synthesis (Ferrannini, 1988). As RQ values of the LP chickens were significantly higher compared with the LF birds during this period, fatty acid synthesis would be higher in LP chickens. Thus, these values corroborated the significantly higher values for energy retention as fat and fat retention in the LP group. MacLeod (1997) formulated the hypothesis that the negative correlation between RQ values and N excretion is caused by a decrease in the oxidation of amino acids. In the present experiment, the LP chickens had a lower N excretion and a higher RQ, which supported this hypothesis. Because these birds had a more efficient protein metabolism, it is likely that their amino acid catabolism was lower compared with that of the LF group.

DIT

Short-term feed intake and, hence, GEI per MBW were not influenced by the dietary composition. Because feces were not collected during this short refeeding period, AMEI was calculated on basis of the apparent metabolizability of the diets as calculated during the ad libitum period. This result implies some incorrectness, because the first feed ingested after a period of fasting is somewhat less well metabolized (Buyse et al., 1993). Because this effect stimulates further uptake of feed, this is a way to compensate for losses sustained during fasting. The AMEI per MBW was slightly higher for the LP birds compared with their LF counterparts, although not significant. The DIT measured in this experiment were 20 and 23% of the AMEI for the LP and LF groups, respectively. This result is in contrast to the findings of Geraert et al. (1988), who found that the heat increment of feeding is <20% AMEI. In all other studies, DIT has been calculated by taking the difference between the average HP in the fed and fasted states (e.g., Geraert et al., 1988; Gabarrou et al., 1997). However, in our experiment, DIT was determined as the area between the average HP during the last 5 h of fasting and the curve of HP during the short refeeding period, taking every measuring point during this period into account. As we believe that our method of calculation is more accurate, it is possible that heat increment of feeding was underestimated in earlier research.

Stubbs and O'Reilly (2000) formulated a hypothesis that links DIT to feed intake; HP caused by the oxidation of macronutrients would have a negative feedback on feed intake and the magnitude of this feedback depends on the macronutrient: the hierarchic oxidation/storage model. Our results show that DIT, expressed as a percentage of AMEI, was 13% higher and feed intake was 24% lower for the LF birds. However, these effects of diet composition on DIT and feed intake during the 5-h refeeding period were not significant. Thus, the hypothesis of Stubbs and O'Reilly (2000) could not be proven nor refuted by our experimental data. An important difference to note is that the above hypothesis was formulated after research with adult mammals, whereas in the current experiment, growing broiler chickens were used. Therefore, a substantial amount of the ingested macronutrients would have been used for growth and the fraction that would have been oxidized and, hence, the DIT would be smaller when compared with fully-grown birds.

In conclusion, our findings indicate that isoenergetic substitution of fat energy for protein energy has a strong impact on growth as well as on energy and protein balance in broiler chickens. The overconsumption of energy compared with protein energy leads to a combination of increased fat accretion and increased HP. The theory linking DIT to feed intake as developed for adult mammals could not be corroborated nor countered in growing chickens, and further research is warranted on this aspect.

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