

Diet-Induced Thermogenesis and Glucose Oxidation in Broiler Chickens: Influence of Genotype and Diet Composition

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ABSTRACT The main objectives of this study were to explore the role of diet-induced thermogenesis in the regulation of voluntary feed intake and to determine the glucose oxidation of broiler chicken strains, known to differ in glucose-insulin balance. From 2 to 7 wk of age, male broiler chickens of a fat and a lean line were reared on 1 of 2 isoenergetic diets with constant gross energy and carbohydrate levels but with substitutions between fat and protein. The low protein (LP/HF) diet contained 126 g of protein/kg and 106 g of fat/kg, whereas the low fat (LF/HP) diet contained 242 g of protein/kg and 43 g of fat/kg. There was no significant effect of the genetic background of the broilers on the glucose oxidation rate (as measured by stable isotope breath test) or protein

oxidation (as measured by plasma uric acid levels). Considering the difference in carcass composition (fat content) of both lines, this leads to the hypothesis that the lines differ predominantly in fat metabolism. Although there was no line effect on plasma triglyceride and free fatty acid concentrations, it was hypothesized that there might be differences in fat oxidation or de novo lipogenesis, or both, between the genotypes. Diet-induced thermogenesis per metabolic body weight (kg of BW^{0.75}) per 24 h, expressed per gram of feed intake, was not significantly influenced by genetic background or by diet composition. Therefore, a model linking feed intake to diet-induced thermogenesis, as postulated for adult mammals, could not be corroborated for growing broiler chickens.

Key words: broiler chicken, genotype, macronutrient, diet-induced thermogenesis, glucose oxidation

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INTRODUCTION

The regulation of voluntary feed intake is a very complex and multifactorial mechanism, with numerous 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). One of the hypotheses linking feed intake to diet-induced thermogenesis (DIT) in adult mammals was formulated in the hierarchic oxidation/storage model by Stubbs and O'Reilly (2000). Heat production caused by the oxidation of macronutrients would have a negative feedback on feed intake, which depends on the macronutrient in question. Proteins are preferentially combusted, followed by carbohydrates and then the oxidation of fat, which corresponds to their abil-

ity to induce satiety but is reciprocal to their relative storage capacity (Stubbs et al., 1997; Stubbs and O'Reilly, 2000). In a previous experiment (Swennen et al., 2004) broiler chickens were reared on isoenergetic diets with substitutions between protein and fat but with similar carbohydrate levels. The authors reported that feed intake was 24% lower and DIT, expressed as a percentage of apparent metabolizable energy intake, was 13% higher for birds fed a low fat/high protein (LF/HP) diet vs. a low protein/high fat (LP/HF) diet. However, because these changes were not statistically significant, the hypothesis of Stubbs and O'Reilly (2000) could not be proven. Thus, further research on the relationship between DIT and feed intake in poultry is necessary.

To study macronutrient-specific oxidation, stable isotope breath tests offer a safe and noninvasive method to study several aspects of physiological functioning. A substrate labeled with ¹³C is ingested, followed by serial measurements of the ¹³C:¹²C ratio in the exhaled CO₂ (Amarri et al., 1998). In human research, this technique is commonly used, but its application in farm animals has only just been initiated. Recently, the methodology to perform stable isotope breath tests using U-¹³C₆-glucose

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with chickens has been developed (Buyse et al., 2004), opening a window of opportunity in research on nutrient oxidation in avian species.

In rodent studies, it has been shown that subjects of different genetic background react differently to dietary compositions in terms of appetite, energy expenditure, adiposity, etc. Pronounced differences in the response to dietary manipulations have also been reported for humans according to body composition (Rolls, 1995; Stubbs et al., 1997). However, in contrast to the numerous models for genetic obesity for mammals, such models are sparsely used in avian species. Genetically fat and lean lines of broiler chickens have been developed by Leclercq et al. (1980) to investigate the mechanisms controlling fattening in chickens. These lines differ in carcass lipid content, especially abdominal fat content, but exhibit a similar live BW, feed consumption, and energy expenditure. Another difference between the lines lies in the plasma glucose-insulin balance. Chickens of the fat line have lower plasma levels of glucose and insulin under fasted and fed conditions compared with animals of the lean line. In addition, during oral glucose tolerance tests, the fat line broilers show a better glucose tolerance and higher insulin concentrations in the plasma (Leclercq et al., 1988b; Saadoun et al., 1988).

In the present experiment, fat and protein were substituted in 2 isoenergetic diets to determine the role of these macronutrients in regulating voluntary feed intake in 2 genetically divergent lines of broiler chickens. Important objectives of the study were to explore the role of diet-induced thermogenesis in the regulation of voluntary feed intake on the one hand and, on the other hand, to determine the postprandial glucose oxidation rates of these particular broiler chickens differing in glucose-insulin balance. Furthermore, the effects of the isoenergetic diets on endocrine functioning and key metabolites of the intermediary nutrient metabolism of these broiler lines were examined and related to the feed intake-associated parameters.

MATERIALS AND METHODS

Experimental Design

One hundred forty 1-d-old male broiler chickens each of genetically fat and lean lines (Leclercq et al., 1980) were obtained from the Institut National de la Recherche Agronomique (INRA), Nouzilly, France. The animals were divided between 2 floor pens, each line in a separate pen, in an environmentally controlled poultry house with wood shavings as litter. The temperature was set at 35°C when birds were 1 d of age and was gradually decreased by 1°C every 1 or 2 d to reach a final temperature of 23°C. The lighting schedule provided 23 h of light per day. Until 14 d of age, birds received a commercial starter diet ad libitum (for diet composition, see Buyse et al., 2001).

From 14 d of age, chickens of each line were divided into 2 groups, each receiving 1 of 2 isoenergetic diets

Table 1. Experimental diets

| Ingredient (g/kg) | Diet | |
|--|--------------------------|--------------------------|
| | Low protein/ high fat | Low fat/ high protein |
| Peas (<i>Pisum sativum</i> ; 18% CP) | 31.369 | 31.369 |
| Wheat | 24.229 | 24.229 |
| Maize starch | 16.800 | 16.800 |
| Soy oil | 9.507 | 1.961 |
| Soybean meal (44% CP) | 5.124 | 5.124 |
| Monocalcium phosphate | 2.365 | 2.365 |
| Premix ¹ | 1.400 | 1.400 |
| CaCO ₃ | 1.307 | 1.307 |
| L-Lysine | 0.770 | 0.770 |
| DL-Methionine | 0.622 | 0.622 |
| L-Threonine | 0.393 | 0.393 |
| NaCl (noniodized) | 0.337 | 0.337 |
| L-Tryptophan | 0.121 | 0.121 |
| Soybean protein ² (83.2% CP) | — | 13.2 |
| Celite ³ | 6.104 | — |
| Analyzed energy and nutrient content ⁴ | | |
| Gross energy content (kcal/kg) | 3,974 | 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 of protein) | 1.52 | 16.51 |

¹Premix supplied the following amount of vitamins and minerals per kilogram of diet: vitamin A, 12,000 IU; vitamin D₃, 3,000 IU; vitamin E, 50 IU; vitamin K₃, 2.5 mg; vitamin B₁, 2.2 mg; vitamin B₂, 7.5 mg; vitamin B₃, 13 mg; vitamin B₆, 5.5 mg; vitamin B₁₂, 0.035 mg; vitamin niacin, 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.

²Nurish 1500, Du Pont Protein Technologies, St. Louis, MO.

³Brenntag, Deerlijk, Belgium.

⁴The values represent the means of 2 determinations.

(Table 1), resulting in a total of 4 groups of 70 chickens each. The isoenergetic diets contained many of the same ingredients, although contents of soybean oil, soybean protein, and celite were varied to make the LP/HF and LF/HP diets (Table 1). The LP/HF diet contained 3,974 kcal of gross energy/kg, 126 g of protein/kg, 106 g of fat/kg and 514 g of N-free extract/kg, whereas the LF/HP diet contained 3,998 kcal of gross energy/kg, 242 g of protein/kg, 43 g of fat/kg, and 504 g of N-free extract/kg. The diets were analyzed in duplicate according to the methods of the Association of Official Analytical Chemists (1984). The gross energy content of the diets was calculated with the formula of Schiemann et al. (1971). At 21 d of age, 3 chickens from each group were taken from the floor pens and housed in wired cages for adaptation to restraint housing conditions. The animals were provided with their specific diet as well as water for ad libitum consumption. For the next 5 wk, with 2 trials per wk and each trial with different animals, 3 chickens of each group were placed in 1 of the 6 open circuit respiratory cells for measuring their metabolism. The same envi-

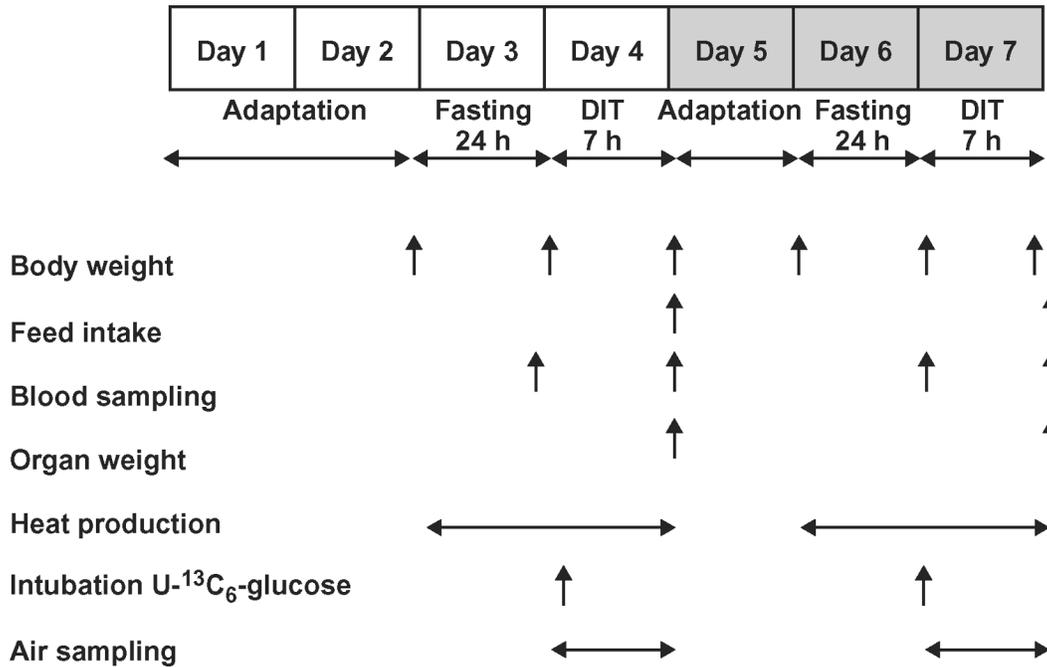


Figure 1. Experimental protocol. From 21 d of age, the experiment was carried out for 5 consecutive weeks, 2 trials per week. For the first trial of 4 d (d 1 to 4), 3 chickens from each group [Fat line – low protein/high fat (LP/HF) diet; Fat line – low fat/high protein (LF/HP) diet; Lean line – LP/HF diet; Lean line – LF/HP diet] were each placed in 1 of the 6 respiratory cells for measuring the energy metabolism and the postprandial glucose oxidation. For the second trial of 3 d (d 5 to 7), different animals, again 3 per group, were placed in the cells. Arrows indicate the time or period of data collection. DIT = diet-induced thermogenesis.

ronmental conditions were used as for their floor-reared counterparts. The weekly measurements are represented in Figure 1. After the adaptation period, the chickens were feed deprived for 24 h though water remained at their disposal. The chickens were then given a pre-weighed amount of their specific diet for 7 consecutive hours to measure DIT. Feed intake was measured after this 7-h period. Due to technical problems, DIT measurements were not available for wk 5 of the study.

This research was approved by the Ethical Commission for Experimental Use of Animals of the Katholieke Universiteit Leuven.

Heat Production Measurements

The respiratory unit consisted of 3 separate, light- and temperature-controlled climatic chambers each containing 2 respiratory cells, a gas analyser unit, and a data acquisition system, as described in detail by Buyse et al. (1998). The respiratory cells (550 × 300 × 500 mm) were made of stainless steel, with little insulation, and the inside temperature was measured by a resistance temperature detector (Pt-100, Farnell In One, Grace-Hollogne, Belgium), accuracy of 0.2°C). The paramagnetic O₂ analyser (ADC 02-823A, The Analytical Development Company, Hoddesdon, Herts, UK) and the infrared CO₂ analyser (ADC D/8U/54/A, The Analytical Development Company) were calibrated before every measurement by using gas mixtures with known levels (±0.001%) of O₂ and CO₂.

After adaptation to the respiratory cells, gas exchanges were measured continuously during the feed deprivation

and subsequent refeeding period. The levels of O₂ and CO₂ in the outlet air were measured for 120 s every 20 min. The CO₂ production and the O₂ consumption of the animal were calculated from the differences between the gas concentrations of the fresh outside air (measured for 120 s every 20 min) and the outlet air of each cell. Heat production was calculated from these data according to the formula of Romijn and Lokhorst (1961): heat production (kJ/h) = 16.18 O₂ (l/h) + 5.02 CO₂ (l/h). The term for urinary N excretion was omitted as it typically induces an error of ≤1% (Romijn and Lokhorst, 1961). To assess DIT, the difference between the average value for heat production during the last 8 h of feed deprivation and the heat production at every measuring point during the refeeding period was calculated. The DIT was then calculated as the area under the heat production curve during the refeeding period and was expressed as a fraction of the feed intake during that interval.

Glucose Oxidation Studies

The postprandial carbohydrate oxidation rate was measured using U-¹³C₆-glucose (D-[U-¹³C₆]-Glucose, 99% atom ¹³C; Euriso-top, Bat 547 91191 Gif-Sur-Yvette, France). Before the cell was opened for intubating the animals, a 10-mL air sample was taken at the outlet side of the respiratory cell to determine the background ¹³CO₂ that is normally produced by the animal. Each air sample was taken with a 10-mL syringe, and the sample was delivered into a 10-mL Vacutainer tube (Labco limited, Buckinghamshire, UK). Then, the animals were weighed

and given a single oral dose of 2 mg per kilogram of body weight of U-¹³C₆-glucose, which was dissolved in water in a concentration of 2 mg/mL. During the 7-h refeeding period, air samples were taken every 30 min to measure glucose oxidation rate. The enrichment of ¹³CO₂ was measured using isotope ratio mass spectrometry, as described by Buyse et al. (2004).

Curve Fitting

The obtained cumulative percentage dose recovery curves showed a typical sigmoid pattern and were therefore fitted according to the Gompertz equation (Gompertz, 1825): $O_t = A * \exp(-B * \exp(-Ct))$ with O_t the oxidation of U-¹³C₆-glucose (% dose) at time t (min). A is the asymptote of the curve (% dose). B and C are constants (min^{-1}) and are indicative for the rate of increase and decrease in oxidation during the ascending and descending phase of the U-¹³C₆-glucose oxidation curve, respectively. The point of inflection (POI) is calculated as B/C and is the time (min) at which the oxidation is maximal (M). Finally, this maximal oxidation (% dose) at POI is calculated as A/e , where A is the asymptote of the curve, and e is Euler's number ($e = 2.71828183$). (Buyse et al., 2004).

Tissue Sampling

At the start of the experimental period, after feed deprivation and after DIT measurements, individual body weights of the animals were recorded, and after DIT, feed intake was determined. Blood samples were collected after feed deprivation and after DIT measurements from a wing vein with a heparinized syringe and were put on ice immediately. Thereafter, the animals were euthanized, and livers and abdominal fat pads were removed and weighed (Figure 1).

Plasma Metabolites and Hormones

Plasma glucose, triglycerides, and uric acid concentrations were measured spectrophotometrically with an automated apparatus (Monarch Chemistry System, Instrumentation Laboratories, B-1930, Zaventem, Belgium). Plasma free fatty acid concentrations were measured by the WAKO NEFA C (Wako Chemicals GmbH, Neuss, Germany) test kit, an enzymatic colorimetric test, modified for use in the Monarch Chemistry System. Plasma 3,5,3'-triiodothyronine (T₃) and thyroxine (T₄) concentrations were measured using a specific radioimmunoassay as described by Darras et al. (1992). Intraassay variation coefficients were 3.9 and 5.7% for T₃ and T₄, respectively.

Statistical Analyses

It is acknowledged that the age of the animals increased with the duration of the trial. Therefore, we have chosen to use the term duration of the trial consequently throughout the manuscript when this parameter contributed to

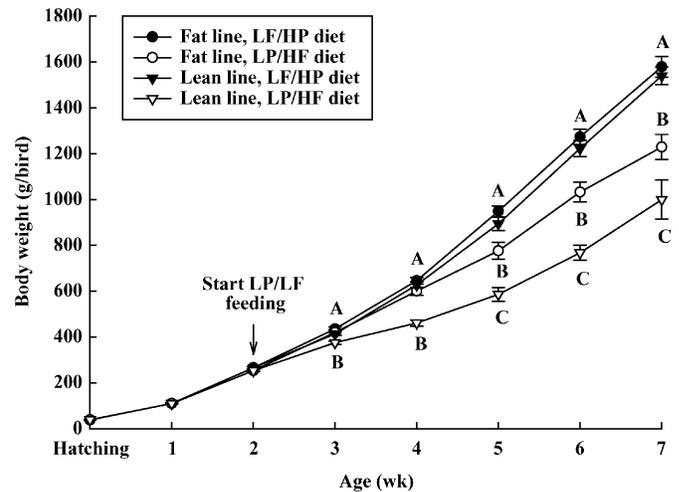


Figure 2. Weekly body weight of broiler chickens (g/bird) of genetically fat and lean lines fed the low protein/high fat (LP/HF) or the low fat/high protein (LF/HP) diets. Values are means \pm SEM (hatch and wk 1 and 2: $n = 30$ per line; wk 3 and 4: $n = 30$ per line and diet; wk 5 and 6: $n = 20$ per line and diet; wk 7: $n = 10$ for the lean line-LP/HF chickens, $n = 20$ for the other 3 groups). ^{A-C}Different letters indicate a significant difference between the BW of the groups at the same age ($P \leq 0.05$).

the model. All results were analyzed by ANOVA with diet composition, line, and duration of the trial as classification variables (SAS, 1998), and interactions between variables were studied. When duration of the trial had a significant input in the model, a 2-factor ANOVA with line and diet composition as classification variables was performed separately for each week. A Tukey posthoc analysis was used to identify which group means differed at $P \leq 0.05$.

RESULTS

Body Weight and Feed Intake

The effect of diet composition and genetic background on weekly BW is presented in Figure 2. At 3 and 4 wk of age, a significant ($P \leq 0.001$ and $P \leq 0.01$, respectively) effect of line and diet on BW was observed, the chickens of the lean line fed the LP/HF diet having a lower BW compared with the other 3 groups. From the age of 5 wk, the lean line chickens on the LP/HF diet had a significantly lower BW compared with the fat line chickens on the LP/HF diet, which in turn had a significantly ($P \leq 0.0001$) lower BW compared with the fat line and the lean line on the LF/HP diet. The LP/HF diet induced a significant reduction in BW, but this effect was more pronounced in the lean line chickens, resulting in a significant ($P \leq 0.05$) interaction between line and diet. Because of the pronounced differences in BW, results are presented per kilogram of metabolic body weight [MBW; BW (in kilograms) raised to the power 0.75].

Feed intake per MBW (after 24 h of feed deprivation) decreased significantly ($P \leq 0.0001$) with the duration of

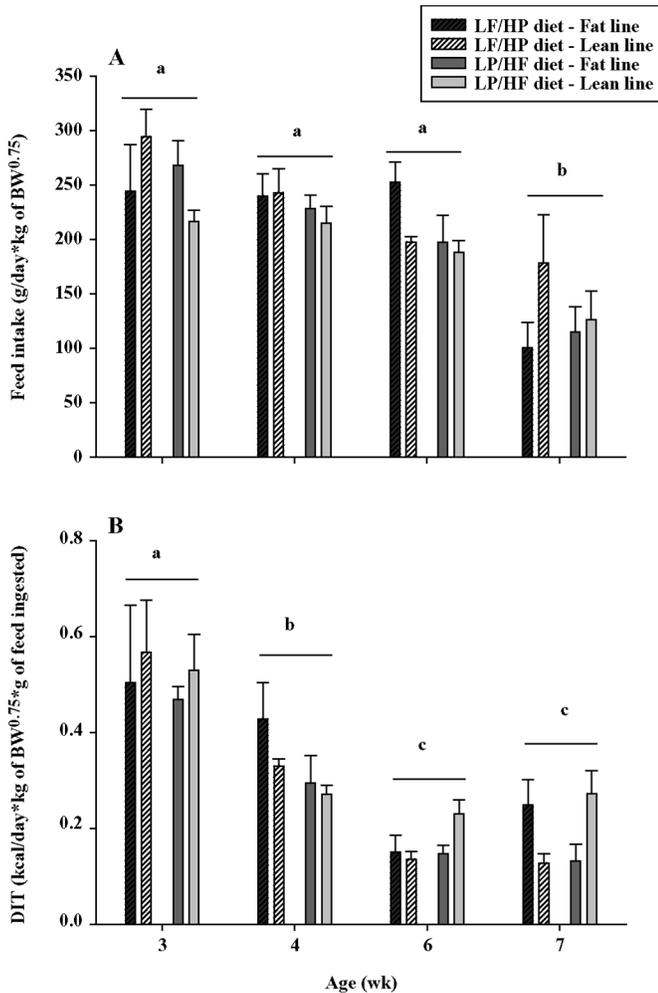


Figure 3. A) Feed intake ($n = 3$ per group and per week), expressed in grams per metabolic BW and per day and B) diet-induced thermogenesis (DIT; $n = 3$ per group and per week, except for the fat line-low protein group at wk 6: $n = 2$), expressed as kilocalories per metabolic BW ($BW^{0.75}$), per gram feed ingested and per day of broiler chickens of genetically fat and lean lines fed the low protein/high fat (LP/HF) or the low fat/high protein (LF/HP) diets. All values are means \pm SEM. ^{A-C}Different letters indicate a significant effect of duration of the trial on overall means ($P \leq 0.02$).

the trial and was not affected by genetic background or by diet composition (Figure 3A).

Diet-Induced Thermogenesis

Diet-induced thermogenesis per gram of feed ingested, per day, and per MBW decreased significantly from 3 to 6 wk of age (Figure 3B). Values were similar for all treatment groups, and no effects of diet composition or genetic background were observed.

Glucose Oxidation

The estimated parameters A, B, and C of the Gompertz curve and the calculated POI and M values are summarized in Table 2. There were no effects of line of the broilers or diet composition on these parameters. However, there was an effect of the duration of the trial on

all parameters except POI. Values for the asymptote (A) of the Gompertz curve as well as for the maximal oxidation at the POI (M) decreased, whereas values for constants B and C increased with the duration of the trial.

Organ Weights

The proportional liver weights significantly decreased with the duration of the trial (Figure 4A) and were increased in the groups fed the LP/HF diet ($P = 0.0129$).

Proportional abdominal fat pad weights were significantly influenced by duration of the trial ($P = 0.0084$), diet composition ($P \leq 0.0001$), and genetic background of the animals ($P \leq 0.0001$) (Figure 4B). Animals reared on the LP/HF diet were characterized by a significantly (diet effect: $P \leq 0.0001$) higher abdominal fat content compared with that of their LF/HP counterparts. In addition, this diet-induced difference was more pronounced for the fat line chickens, resulting in a significant ($P \leq 0.0001$) interaction between diet and line. Indeed, the fat line chickens on the LP/HF diet had a significantly increased proportional abdominal fat pad weight compared with the other groups from wk 4 until the end of the trial. The percentage of abdominal fat clearly increased with the duration of the trial in this group and to a lesser extent in the lean line chickens on the LP/HF diet, whereas it remained constant in the LF/HP-fed animals, resulting in a duration \times diet ($P = 0.0008$) as well as a duration \times line ($P = 0.0443$) interaction.

Plasma Parameters

The outcome of the statistical analyses of plasma metabolite and hormone concentrations obtained after 24 h of feed deprivation and after 7 h of refeeding is summarized in Table 3. Only main effects are shown; there were no significant interactions between these classification variables for any of the plasma parameters.

Plasma glucose concentrations after feed deprivation were significantly ($P \leq 0.0001$) influenced by the genetic background of the chickens; the chickens of the lean line had a higher plasma glucose concentration compared with the fat line animals (Figure 5). After the 7-h refeeding period, glucose concentrations in the plasma were increased compared with feed-deprived levels, but no effects were observed of line, diet, or duration of the trial.

Regardless of the duration of the trial, uric acid concentrations in the plasma of animals of both lines fed the LF/HP diet were significantly higher as compared with that of the chickens on the LP/HF diet after 24-h feed deprivation as well as after 7 h of refeeding ($P = 0.0004$ and $P \leq 0.0001$, respectively; Figure 6A). A decrease in the uric acid levels with duration of the trial was observed in both feeding conditions ($P = 0.0104$ after feed deprivation and $P \leq 0.0001$ after refeeding). Refeeding caused an increase in the plasma uric acid levels. There was no effect of the genetic background of the animals on plasma uric acid levels, irrespective of nutritional state.

Table 2. Estimates of the parameters of the Gompertz curve fitted to postprandial glucose oxidation patterns in relation to duration of the trial, dietary macronutrient¹

| | Duration of trial ² | Low fat diet | | Low protein diet | |
|------------------------|--------------------------------|---------------|---------------|------------------|---------------|
| | | Fat line | Lean line | Fat line | Lean line |
| A (% dose) | 0.003 | | | | |
| Wk 3 | | 12.7 ± 1.3 | 15.8 ± 2.5 | 17.0 ± 1.7 | 16.4 ± 2.1 |
| Wk 4 | | 13.8 ± 1.8 | 10.3 ± 1.6 | 18.5 ± 2.7 | 12.9 ± 2.0 |
| Wk 5 | | 19.9 ± 3.1 | 13.1 ± 4.7 | 16.6 ± 4.1 | 10.1 ± 2.1 |
| Wk 6 | | 9.9 ± 1.0 | 13.0 ± 3.6 | 9.6 ± 0.4 | 9.6 ± 2.8 |
| Wk 7 | | 11.8 ± 1.4 | 9.2 ± 1.3 | 8.0 ± 0.5 | 10.2 ± 0.9 |
| B (min ⁻¹) | ≤0.0001 | | | | |
| Wk 3 | | 1.1 ± 0.04 | 0.8 ± 0.1 | 0.83 ± 0.09 | 0.87 ± 0.08 |
| Wk 4 | | 1.1 ± 0.2 | 1.3 ± 0.02 | 1.08 ± 0.19 | 0.86 ± 0.03 |
| Wk 5 | | 0.9 ± 0.1 | 1.1 ± 0.2 | 1.03 ± 0.08 | 1.03 ± 0.16 |
| Wk 6 | | 1.0 ± 0.2 | 1.2 ± 0.2 | 1.08 ± 0.33 | 1.22 ± 0.07 |
| Wk 7 | | 1.6 ± 0.1 | 1.4 ± 0.1 | 1.42 ± 0.06 | 1.56 ± 0.1 |
| C (min ⁻¹) | 0.0081 | | | | |
| Wk 3 | | 0.010 ± 0.003 | 0.008 ± 0.001 | 0.006 ± 0.001 | 0.007 ± 0.001 |
| Wk 4 | | 0.010 ± 0.001 | 0.011 ± 0.001 | 0.007 ± 0.001 | 0.008 ± 0.001 |
| Wk 5 | | 0.008 ± 0.001 | 0.009 ± 0.002 | 0.008 ± 0.002 | 0.014 ± 0.002 |
| Wk 6 | | 0.009 ± 0.001 | 0.010 ± 0.001 | 0.010 ± 0.001 | 0.012 ± 0.002 |
| Wk 7 | | 0.013 ± 0.002 | 0.010 ± 0.002 | 0.013 ± 0.002 | 0.010 ± 0.001 |
| POI (min) | NS | | | | |
| Wk 3 | | 129.3 ± 36.6 | 109.1 ± 30.2 | 132.6 ± 12.5 | 127.6 ± 2.6 |
| Wk 4 | | 105.0 ± 12.4 | 111.6 ± 11.9 | 158.4 ± 21.1 | 102.6 ± 6.6 |
| Wk 5 | | 107.5 ± 9.1 | 123.6 ± 25.3 | 152.4 ± 35.1 | 72.7 ± 6.4 |
| Wk 6 | | 111.4 ± 26.1 | 110.9 ± 17.0 | 107.6 ± 19.9 | 109.0 ± 23.3 |
| Wk 7 | | 126.1 ± 27.3 | 136.7 ± 15.9 | 119.8 ± 25.0 | 158.2 ± 26.9 |
| M (% dose) | 0.003 | | | | |
| Wk 3 | | 4.7 ± 0.5 | 5.8 ± 0.9 | 6.3 ± 0.6 | 6.0 ± 0.8 |
| Wk 4 | | 5.1 ± 0.7 | 3.8 ± 0.6 | 6.8 ± 1.0 | 4.7 ± 0.8 |
| Wk 5 | | 7.3 ± 1.2 | 4.8 ± 1.7 | 6.1 ± 1.5 | 3.7 ± 0.8 |
| Wk 6 | | 3.6 ± 0.4 | 4.8 ± 1.3 | 3.5 ± 0.2 | 3.5 ± 1.0 |
| Wk 7 | | 4.3 ± 0.5 | 3.4 ± 0.5 | 2.9 ± 0.2 | 3.8 ± 0.3 |

¹Ratio and genetic background of broiler chickens from a fat and a lean line. Values are means ± SEM (n = 3 for all groups at all ages). Gompertz curve $O_t = A * \exp(-B * \exp(-Ct))$ with O_t : oxidation of U-¹³C-glucose (% dose) at time t (min); A: asymptote of the curve (% dose); B and C: constants (min⁻¹); point of inflection (POI): time (min) at which the oxidation is maximal (B/C); M: maximal oxidation (% dose) at point of inflection; POI (=A/e).

²P-value of 1-factor ANOVA with duration of the trial as classification variable.

After 24 h of feed deprivation, the animals of both lines kept on the LP/HF diet had significantly increased plasma triglyceride levels compared with the LF/HP-fed chickens (diet effect: $P = 0.0041$; Figure 6B). Refeeding caused an increase in the triglyceride levels and accentuated the effect of the diet ($P \leq 0.0001$). For all groups, a significant decrease of plasma triglyceride concentrations with the duration of the trial was observed in feed deprived ($P = 0.0007$) as well as refeed conditions ($P \leq 0.0001$).

Plasma nonesterified free fatty acid (NEFA) levels of feed-deprived chickens were not influenced by line, diet, or duration of the trial (Figure 6C). After the 7-h refeeding period, the NEFA concentrations in the plasma were decreased, which seemed to be most pronounced for the chickens fed the LF/HP diet, whereas plasma NEFA levels of refeed LP/HF chickens remained similar to before feeding. This resulted in significantly (diet effect: $P \leq 0.0001$) higher NEFA levels in the plasma of the LP/HF chickens compared with that of LF/HP chickens after refeeding.

Plasma T₃ concentrations after feed deprivation significantly ($P = 0.0002$) decreased with the duration of the trial (Figure 7A). The chickens on the LP/HF diet had

higher plasma T₃ levels compared with their LF/HP-fed counterparts (diet effect: $P \leq 0.0001$), and this difference was more pronounced in the lean line chickens (line × diet interaction: $P = 0.0748$). As a consequence, the animals of the lean line showed an increased T₃ level in the plasma after fasting compared with the fat line chickens, and this difference approached significance ($P = 0.0688$). Refeeding the chickens caused the plasma T₃ concentrations to increase significantly ($P \leq 0.0001$), independent of line, duration of the trial, or diet composition. After the 7-h refeeding period, the LP/HF chickens were characterized by significantly (diet effect: $P = 0.026$) higher plasma T₃ levels. After feed deprivation ($P \leq 0.0002$) as well as after refeeding ($P = 0.002$), T₃ concentrations decreased with the duration of the trial.

There was no overall effect of the line on plasma T₄ concentrations of the feed-deprived animals (Figure 7B). Chickens of both lines fed the LF/HP diet had higher fasting plasma T₄ concentrations compared with their LP/HF-fed counterparts, resulting in an overall effect of the diet ($P \leq 0.0001$). Plasma T₄ levels decreased significantly ($P \leq 0.0001$) after refeeding, independent of genetic background, diet composition, or duration. After 7 h of refeed-

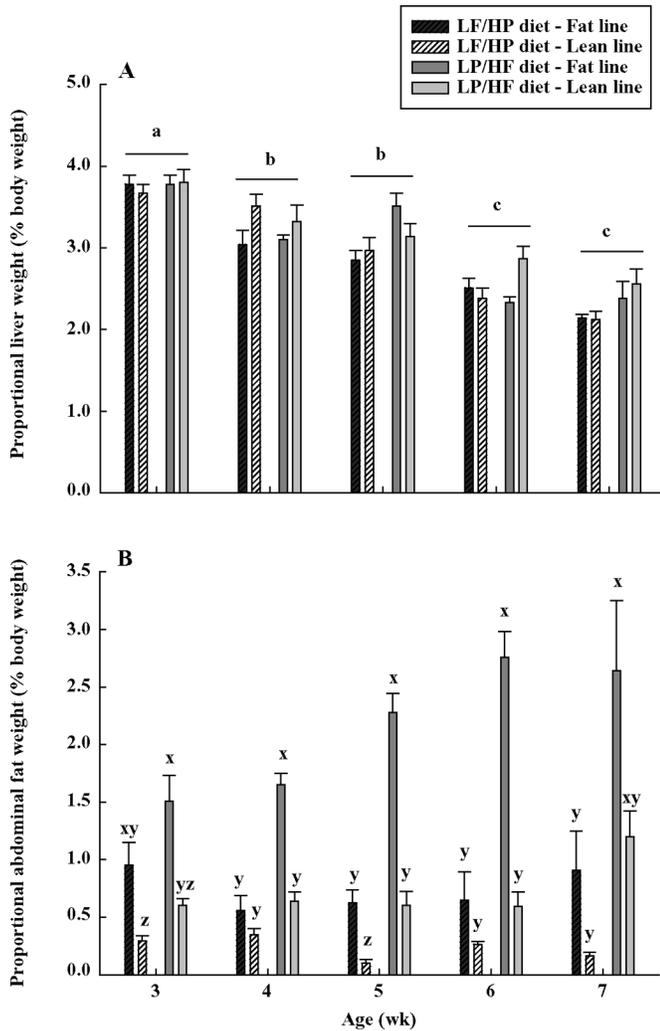


Figure 4. Proportional liver (A) and abdominal fat pad (B) weights, expressed as percentage of body weight ($n = 6$ per group in wk 3, 4, and 5; $n = 3$ in wk 6 and 7) of broiler chickens of genetically fat and lean lines fed the low protein/high fat (LP/HF) or the low fat/high protein (LF/HP) diets. Values are means \pm SEM. The letters a, b, and c indicate a significant effect of the duration of the trial ($P \leq 0.0001$). The letters x, y, and z indicate significant differences between the groups at the same age ($P \leq 0.05$).

ing, the genetic background of the chickens had a significant ($P \leq 0.0001$) effect on T_4 concentrations, with higher overall means in the plasma of the fat line chickens. Plasma T_4 levels showed a significant ($P \leq 0.0001$) increase with duration of the trial during feed deprivation as well as during refeeding.

DISCUSSION

Body Weight and Feed Intake

The growth delay due to the LP/HF diet was more pronounced in the animals of the lean line, as reflected by a line \times diet interaction. This indicates that the animals of the lean line might be more sensitive to the suboptimal protein content of the diet, when BW is taken as a criterion, as previously observed (Touchburn et al., 1981;

Leclercq, 1983; Alleman et al., 2000). In both diets, standard essential amino acid requirements for broiler chickens (National Research Council, 1994) were met. However, Alleman et al. (2000) reported that the lean line chickens have a higher requirement for essential amino acids than the fat line. It cannot be excluded that the amino acid composition of the diets was sufficient for the fat line but not for the lean line broilers, which could have influenced the results obtained. On the other hand, it is also acknowledged that the LP/HF diet might not provide enough nitrogen for nonessential amino acid synthesis.

Feed intake was recorded only during the 7-h refeeding period after 24 h of feed withdrawal. No effects were found of diet composition or genetic background. The absence of differences in feed intake between the lean and fat line chickens corroborates previous statements that hyperphagia is not the cause for the higher fat deposition by the fat line broilers (Touchburn et al., 1981; Leclercq and Saadoun, 1982).

Diet-Induced Thermogenesis

Diet-induced thermogenesis, expressed per gram of feed ingested, per MBW, and per day, was similar in both lines, which is in accordance with the data of Geraert et al. (1988). Furthermore, DIT per gram feed intake was not influenced by the diet composition. Geraert et al. (1990) came to the same conclusion using diets containing 131, 166, 192, 218, and 251 g of protein/kg and an approximately constant metabolisable energy level. In humans, it has been repeatedly shown that the protein content of a diet is one of the major determinants of diet-induced thermogenesis, next to the energy and the alcohol level. Protein plays a key role in body weight regulation through satiety-related DIT (for review see Westerterp, 2004). Stubbs and O'Reilly (2000) formulated the hierarchic oxidation/storage model for adult mammals: the heat production caused by the oxidation of macronutrients has a negative feedback effect on feed intake, and the magnitude of this feedback effect depends on the macronutrient. Because the diet composition did not have an effect on the DIT corrected for the feed intake, this model could not be confirmed by the present study. In a previous experiment (Swennen et al., 2004), broilers were reared on diets with an isoenergetic substitution between protein and fat and with similar carbohydrate and gross energy levels. The researchers reported that feed intake was 24% lower, and DIT, expressed as a percentage of apparent metabolizable energy intake, was 13% higher for birds fed a LF/HP diet vs. a LP/HF diet. However, these changes were not statistically significant. Thus, it is possible that the lack of influence of the macronutrient ratio in the diet in both experiments is due to the age of the animals, as suggested by Swennen et al. (2004). Indeed, the hierarchic oxidation theory was formulated for adult mammals, whereas in the 2 experiments conducted, growing broiler chickens were used. Because a substantial proportion of the ingested macronutrients are used for

Table 3. Results of ANOVA according to diet composition (low protein and low fat diet), genetic background of the animals (fat line and lean line), and duration of the trial on plasma concentrations of metabolites and hormones of 24-h feed-deprived and 7-h refed chickens¹

| | Model | Age | Diet | Line |
|--------------------|---------|---------|---------|---------|
| 24-h feed-deprived | | | | |
| Glucose | ≤0.0001 | 0.0025 | NS | ≤0.0001 |
| Uric acid | 0.0024 | 0.0104 | 0.0004 | NS |
| Triglycerides | 0.0374 | 0.0007 | 0.0041 | NS |
| NEFA | NS | NS | NS | NS |
| T ₃ | ≤0.0001 | 0.0002 | ≤0.0001 | NS |
| T ₄ | ≤0.0001 | ≤0.0001 | ≤0.0001 | NS |
| 7-h refed | | | | |
| Glucose | NS | NS | NS | NS |
| Uric acid | ≤0.0001 | ≤0.0001 | ≤0.0001 | NS |
| Triglycerides | ≤0.0001 | ≤0.0001 | ≤0.0001 | NS |
| NEFA | 0.0004 | NS | ≤0.0001 | NS |
| T ₃ | 0.0534 | 0.002 | 0.026 | NS |
| T ₄ | ≤0.0001 | ≤0.0001 | ≤0.0001 | ≤0.0001 |

¹Only main effects are shown as there were no significant interactions between these classification variables for any of the plasma parameters.

growth, the fraction that would be oxidized postprandially, and therefore contributes to the DIT, will be smaller than in adult birds.

The DIT per gram of feed intake decreased significantly with the duration of the trial. As proportionally less of the ingested energy is combusted, a greater percentage is available for storage, most probably in the form of fat. Indeed, the abdominal fat pad is a late-maturing tissue (Govaerts et al., 2000); its weight increases with age.

Other factors that might have an influence on the energy metabolism are activity and body composition of the broilers. Skinner-Noble et al. (2005) showed that the effective caloric value of a diet is dependent on behavioral patterns, and it is known that bird activity has an effect

on heat production and also on DIT. Unfortunately, no reports on the behavior of these selected broiler lines have been published so far. Also in the present study, the birds' activity was not evaluated, and thus an effect of activity on the DIT measurements cannot be excluded. In addition, the body composition of the chickens might play a role in heat production and in DIT. Indeed, the contribution of fat free mass to metabolic rate is much larger than that of the fat mass (Lührmann et al., 2001). It has been shown that the animals of the lean line have more body protein (Leclercq et al., 1994), which could have an effect on heat production. However, because neither bird activity nor body composition was determined in the present study, the contribution of both factors to DIT cannot be established.

Glucose Oxidation

Because of the well-known difference between these specific lean and fat lines for their glucose-insulin balance (Touchburn et al., 1981; Simon and Leclercq, 1982), it was suggested in the present study to investigate for the first time postprandial glucose oxidation in these animals. However, no effect of the genetic background on the PDR, cumulative percentage dose recovery, or Gompertz parameters was found, suggesting that lean and fat growing chickens had similar postprandial glucose oxidations. There also was no effect of the diet composition on glucose oxidation rate, which might be due to the fact that diets were formulated with a constant carbohydrate content. Furthermore, no diet effects were seen on the plasma glucose levels, indicating that the diet did not have an effect on the carbohydrate metabolism, which confirmed the findings of earlier studies (Malheiros et al., 2003; Swennen et al., 2005).

Changes in glucose oxidation rate related to the duration of the trial as found in this study are different from those reported by Buyse et al. (2004) as a function of age. However, the composition of the diets (commercial broiler grower diet; see Buyse et al., 2001 for composition)

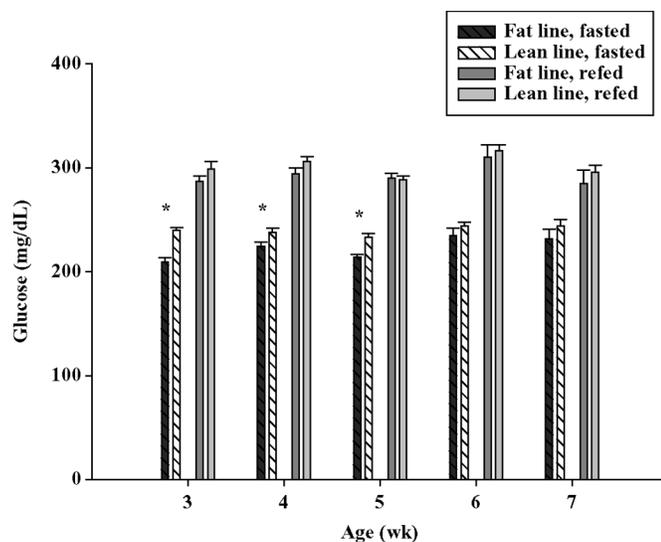


Figure 5. Plasma concentrations of glucose (milligrams/deciliter) per line, per nutritional state (after 24 h fasting and after 7 h refeeding) and per week ($n = 3$) of broiler chickens of genetically fat and lean lines fed the low protein/high fat (LP/HF) or the low fat/high protein (LF/HP) diets. Values are means \pm SEM. A significant effect of the line at the same treatment duration and the same nutritional state is indicated by $*P \leq 0.05$.

as well as the genetic background of the chickens (Cobb broiler chickens) used in the study of Buyse et al. (2004) were different from those used in the present study, which might account for the discrepancy between the studies.

Organ Weights and Plasma Parameters

There was no effect of the diet on the plasma glucose levels regardless of nutritional state, which might be due to the similar carbohydrate level in both experimental diets. After 24 h of feed deprivation, the broilers of the

fat line had significantly lower plasma glucose concentrations compared with the animals of the lean line. After refeeding, this effect of the genetic background disappeared, corroborating the results of Simon and Leclercq (1982) and Saadoun et al. (1988). In addition, Touchburn et al. (1981) found reduced glucose levels in the plasma of the fat line chickens compared with the lean line animals when they were reared on isocaloric diets with 16, 20, or 24% protein, in feed-deprived as well as fed state. The fat line animals were considered to have a slightly increased fasting insulin level (Touchburn et al., 1981). Dupont et al. (1999) showed a significantly higher activation of the early steps of the insulin pathway in the liver of the fat line chickens. Because insulin stimulates lipogenesis in the liver of chickens (Joshi and Wakil, 1978; Joshi and Aranda, 1979), this could contribute to the higher fatness of the fat line chickens.

The chickens of both lines reared on the LP/HF diet showed significantly lower plasma levels of uric acid compared with the animals kept on the LF/HP feed, which confirmed previous findings (Geraert et al., 1990; Collin et al., 2003; Malheiros et al., 2003; Swennen et al., 2005). Because uric acid is a measure for protein catabolism in birds, these observations corroborate the previous finding (Swennen et al., 2005) that when reared on a LP/HF diet, chickens reduce their amino acid oxidation rate as a protein sparing mechanism, besides their enhanced capacity to retain dietary protein. There was no effect of the line on uric acid levels in the plasma, suggesting that the lines do not differ in protein oxidation. This confirmed the results of earlier studies with labeled amino acids using 5-wk-old chickens that were fasted or fed a standard diet. Geraert (1987) reported that no significant differences in the oxidation of amino acids were found between fat and lean line broilers.

The chickens of both lines fed the LP/HF diet showed significantly increased proportional liver weights compared with their LF/HP-reared counterparts, which can probably be ascribed to the 1.9-fold higher energy to protein ratio in the LP/HF compared with the LF/HP diet (31.5 vs. 16.5 kcal of GE/g of CP). This was not unexpected because high energy to protein ratio in the diet enhances de novo lipogenesis (Yeh and Leveille, 1969; Rosebrough and Steele, 1985). An additional indication of an enhanced diet-induced lipogenic activity in the liver is the significantly increased triglyceride levels in the plasma of the LP/HF chickens after feed withdrawal as well as after refeeding. These results confirmed the findings of Swennen et al. (2005) in an experiment with broiler chickens reared on similar isocaloric diets with a protein-fat substitution. Leclercq et al. (1984) found increased plasma triglyceride levels in fat line compared with lean line chickens reared on a fatty diet, whereas no line effect was observed in our study. However, this discrepancy between the studies can be ascribed to differences in diet composition (corn vs. wheat and corn oil vs. soy oil) and hence genotype \times diet interactions.

As a consequence of the enhanced lipogenesis, animals fed the LP/HF diet had a significantly increased propor-

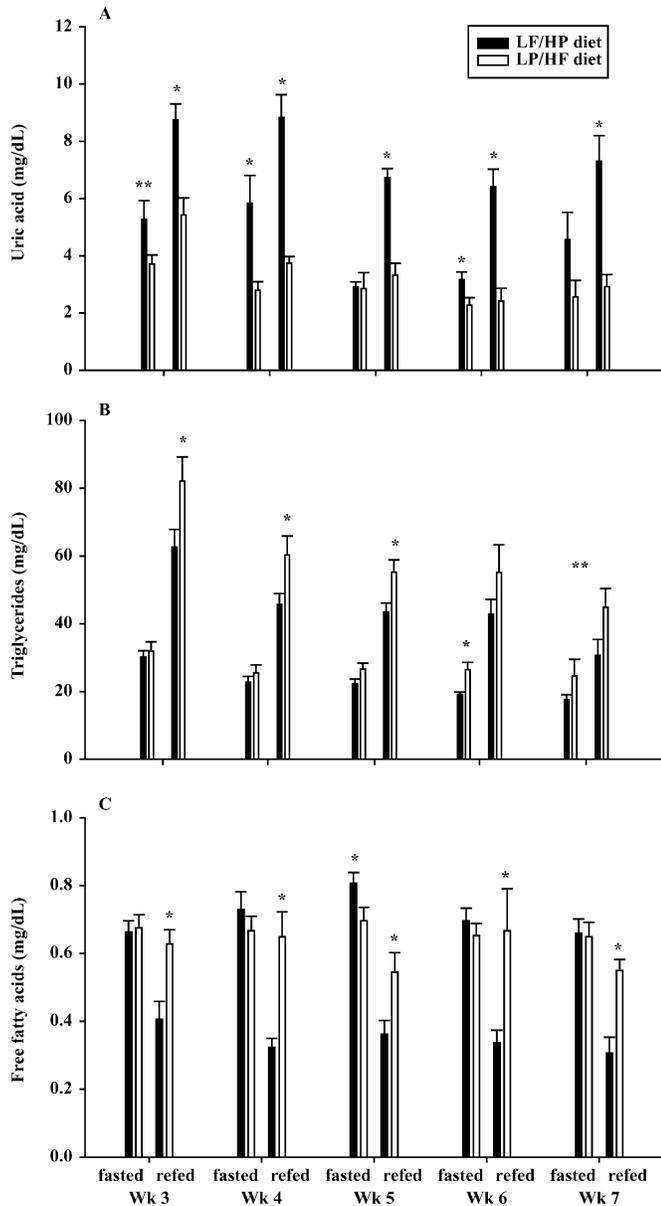


Figure 6. Plasma concentrations of uric acid (A; milligrams/deciliter), triglycerides (B; milligrams/deciliter) and free fatty acids (C; milligrams/deciliter) per diet (low fat diet or low protein diet), per nutritional state (24 h feed-deprived or 7 h refeed) and per week ($n = 3$) of broiler chickens of genetically fat and lean lines fed the low protein/high fat (LP/HF) or the low fat/high protein (LF/HP) diets. Values are means \pm SEM. A significant effect of the diets at the same nutritional state and the same treatment duration is indicated by ** ($0.05 \leq P \leq 0.1$), * ($P \leq 0.05$).

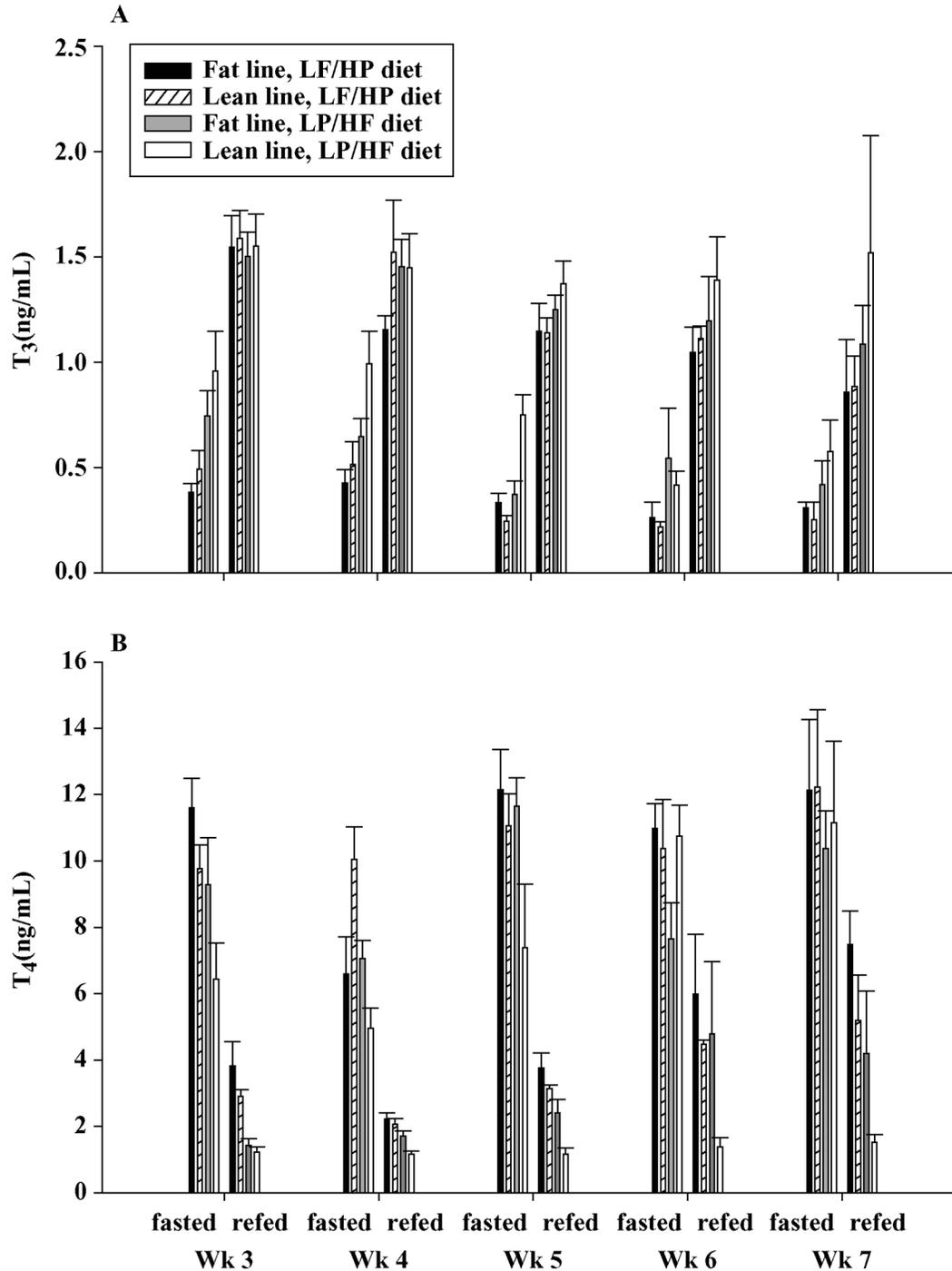


Figure 7. Plasma concentrations of 3,5,3'-triiodothyronine (T_3 ; A; ng/ml) and thyroxine (T_4 ; B; ng/ml) per line, per dietary treatment, per nutritional state (after fasting, after 5 h and after 48 h refeeding) and per week ($n = 3$) of broiler chickens of genetically fat and lean lines fed the low protein/high fat (LP/HF) or the low fat/high protein (LF/HP) diets. Values are means \pm SEM.

tional abdominal fat weight compared with the LF/HP-fed animals of the same line, and this difference was more pronounced in the fat line chickens, resulting in a line \times diet interaction. It has been shown before that chickens of the fat line had a significantly higher abdominal fat content than the lean line animals as a consequence of the selection strategy (Saadoun and Leclercq, 1983; Leclercq et al., 1989; Geraert et al., 1990). In addition,

decreasing the protein level in the diet resulted in an augmented abdominal fat deposition relative to the BW in both lines (Leclercq, 1983; Geraert et al., 1990). Plasma free fatty acid levels were not influenced by the genetic background of the broilers independent of the nutritional state, as also observed by Leclercq et al. (1984) in an experiment with commercial broiler diets. Significantly higher levels were found in the fed LP/HF animals, con-

firming the results of Swennen et al. (2005). This might have been due to a decreased uptake of free fatty acids by peripheral tissues in the LP/HF chickens.

The plasma T_3 concentration after feed deprivation was decreased ($P = 0.07$) in the chickens of the fat line. After 7 h refeeding, this tendency was still present but less pronounced. Previous studies have revealed clear differences in plasma T_3 levels between the lean line and the fat line broilers; the latter genotype was characterized by lower T_3 levels (Leclercq et al., 1988a; Saadoun et al., 1988; Buyse et al., 1999). Although not statistically significant, the findings of the present study point into the same direction. The genotype differences in fed plasma T_4 levels, with the animals of the fat line having significantly ($P \leq 0.0001$) higher T_4 concentrations than the lean line chickens, are also in agreement with previous reports (Saadoun et al., 1988). Refeeding the chickens after a 24-h period of feed deprivation caused plasma T_3 levels to increase and T_4 levels to decrease significantly, corroborating earlier findings (Buyse et al., 2002). In addition, T_3 levels decreased and T_4 levels increased significantly with the duration of the trial, irrespective of the nutritional state, as observed in earlier studies (Decuypere and Kühn, 1988; Buyse et al., 1991, 1999). The inversely related patterns of T_3 and T_4 throughout genotype, diet, and fasted-refed variables point to the T_3 -driven feedback on thyroid functioning (through thyrotropin-releasing hormone/thyroid-stimulating hormone) and hence T_4 production (Decuypere and Kühn, 1988; Kühn et al., 1993). Therefore the regulatory effects on deiodination (affecting T_3 formation as well as T_3 degradation) are probably the driving forces for the observed differences.

In conclusion, no effect of diet composition on DIT corrected for feed intake was found in the present study. Therefore the model of Stubbs et al. (1997) linking feed intake to diet-induced thermogenesis—as postulated for adult mammals—could not be corroborated for growing broiler chickens. Furthermore, the genetic background of these broilers had no significant influence on the parameters of the carbohydrate and protein metabolism that were measured. Considering the difference in the body fat contents between the lines, this leads to the conclusion that these chickens differ mainly in fat metabolism. Although no line effects were found on plasma triglyceride and free fatty acid concentrations, possible differences might be found in fat oxidation or in de novo lipogenesis, or both. Indeed, it has already been shown that the expression of some genes involved in lipid synthesis and secretion are differentially expressed in the liver of these specific lines of chickens (Daval et al., 2000).

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