Further Investigations on the Role of Diet-Induced Thermogenesis in the Regulation of Feed Intake in Chickens: Comparison of Age-Matched Broiler versus Layer Cockerels

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ABSTRACT The aim of this study was to investigate the role of diet-induced thermogenesis (DIT) in the regulation of feed intake in age-matched broiler and layer cockerels. In addition, the effect of genotype on endocrine functioning and key metabolites of the intermediary metabolism as well as on the expression of muscular uncoupling protein (avUCP) was explored. One-day-old male broiler (Ross) and layer (ISA Brown) chicks were reared under standard conditions on commercial broiler starter and finisher diets. From 22 d of age, twice per week, 3 broiler and 6 layer cockerels were placed in open circuit respiratory chambers. After adaptation, the animals were feed-deprived for 24 h, and heat production was measured by indirect calorimetry. During the subsequent 7-h refeeding period, feed intake and DIT were measured. Blood samples were taken after feed deprivation and refeeding. Muscle samples were taken after refeeding for determination of avUCP expression. A significantly higher heat production per metabolic BW (MBW) in the layer compared with the broiler cockerels, independent of nutritional state, suggests that the broilers used a greater proportion of the metabolizable energy intake for growth. The DIT per MBW and per gram of feed intake was higher for the layer than for the broiler cockerels. However, feed intake per MBW was also significantly higher in the layer cockerels. Thus, no feedback effect of DIT on feed intake per MBW was observed, and the model formulated for adult mammals relating feed intake to DIT could not be corroborated. The muscular expression of avUCP was not different between genotypes, which does not support the hypothesis of an involvement of avUCP in the higher DIT measured in layer cockerels. Circulating uric acid, glucose, triglyceride, and free fatty acid levels were significantly elevated in the layer compared with the broiler cockerels. As the diet was formulated according to broiler requirements, the higher metabolite levels of the layer cockerels might reflect a relative oversupply of dietary nutrients.

Key words: genotype, diet-induced thermogenesis, feed intake regulation, broiler chicken, layer chicken

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INTRODUCTION

Research with adult mammals has led to the propagation of the hierarchic oxidation/storage model that links diet-induced thermogenesis to feed intake (Stubbs et al., 1997). The heat production (**HP**) related to the oxidation of macronutrients has a negative feedback on feed intake, the magnitude of which depends on the macronutrient: proteins are preferentially combusted, followed by carbohydrates and finally fat, corresponding to their ability to induce satiety (Stubbs et al., 1997). However, in a previous experiment, broiler chickens were reared on isoenergetic diets with substitutions between protein and fat but with similar carbohydrate levels. Diet-induced thermogenesis (**DIT**) was calculated as the increase in HP above feed-deprived HP due to the ingestion of feed [kcal/metabolic BW (**MBW**) per g of feed intake]. No statistically verifiable differences in DIT were observed between broiler chickens reared on the low protein-high fat or the high protein-low fat diet in spite of differences in feed intake (Swennen et al., 2004). When genetically lean and fat broiler chickens (Leclercq et al., 1980) were raised on similar isoenergetic diets, again no significant effect of diet composition or genetic background on DIT could be observed (Swennen et al., 2006). This leads to the hypothesis that the macronutrient ratio in the diet (independent of energy supply) does not influence DIT and hence possibly has no effect on satiety in growing broiler chickens in contrast to observations in adult mammals. Next to the fact that the broilers used were juvenile, fast-growing animals in contrast to the (adult) mammals, a possible explanation could be that the impact of DIT on feed intake is less important as an appetite regulation mechanism in the broiler chicken, characterized by a voracious appetite (Dunnington and Siegel, 1996). Indeed, Denbow (1994) stated that mecha-

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nisms controlling feed intake in broilers have changed due to the genetic selection for growth. Therefore, it would be interesting to compare broiler chickens with poultry genotypes that have not been selected for growth, such as laying chickens, to investigate the mechanisms involved in feed intake regulation.

In mammals, uncoupling proteins are suggested to play a role in regulation of diet-induced thermogenesis and energy partitioning (Ricquier and Bouillaud, 2000). An uncoupling protein gene homologue was cloned and sequenced from skeletal muscle of the chicken, avian uncoupling protein (**avUCP**; Raimbault et al., 2001). This avUCP has been proposed to play a role in thermogenesis in birds (Collin et al., 2003a,b). When 2 experimental Rhode Island Red chicken genotypes, divergently selected for low or high residual feed intake were compared, avUCP was expressed more abundantly in the inefficient genotype (Raimbault et al., 2001). This genotype consumes 30 to 40% more feed for the same BW and egg production and shows a larger diet-induced thermogenesis than the more efficient genotype (Gabarrou et al., 1997), potentially establishing a link between avUCP expression and DIT.

Important objectives of the present study were to investigate the role of diet-induced thermogenesis in the control and regulation of voluntary feed intake in age-matched broiler and layer chickens. Furthermore, the effect of genetic background on endocrine functioning and key metabolites of the intermediary nutrient metabolism as well as on the muscular expression of avUCP of these divergent genotypes of chickens was studied.

MATERIALS AND METHODS

Experimental Design

Fifty 1-d-old male chicks of a broiler genotype (Ross) were obtained from a local hatchery (Avibel, Zoersel, Belgium). Fifty 1-d-old male chicks of a medium weight, brown egg layer type strain (ISA Brown) were purchased on the same day from another hatchery (VEPYMO, Poppel, Belgium). The chicks were divided over 2 floor pens (each genotype in a separate pen) in an environmentally controlled poultry house with wood shavings as litter. The temperature was set at 1-d-old at 35°C and was gradually decreased with 1°C every 1 to 2 or 4 to 5 d, reaching an end temperature of 19°C or 24°C for the broiler or the layer cockerels, respectively, at 30 d of age. The lighting schedule provided 23 h of light per d. Until 21 d of age, cockerels of both genotypes received a commercial broiler starter diet ad libitum (ME level = 2,890 kcal/kg). From 21 d of age onward, all chickens received a commercial broiler finisher diet ad libitum (ME level = 3,029 kcal/kg; for diet compositions, see Buyse et al., 2001). A number of chickens per genotype were weighed on a weekly basis (at hatch, wk 1, 2, 3, 4: n = 20; wk 5, 6: n = 10; wk 7: n = 6).

From 22 d of age, and repeated 2 times per week for 4 consecutive weeks, each time with different animals, 3 broiler cockerels and 6 (3 pairs) layer cockerels were taken from the floor pens and housed in 1 of 6 open circuit respiratory cells. The animals had feed and water ad libitum at their disposal. The same environmental conditions were used as for their floor-reared counterparts. During an adaptation period of 1 or 2 d, feed intake and BW of the chickens were closely monitored. Only the animals that were adapted properly—that gained weight and ate an appropriate amount of feed for their age—were used in the metabolic measurements. After this adaptation period, the animals were feed-deprived for 24 h though with drinking water available. Then they were given an unlimited, preweighed amount of feed during 7 consecutive hours for measuring diet-induced thermogenesis as well as feed intake. All measurements were performed on age-matched cockerels of both genotypes, and it is acknowledged that comparing BW-matched chickens might have been interesting as well. However, due to practical limitations, this comparison was not performed.

This research protocol was approved by the Ethical Commission for Experimental Use of Animals of the K.U. Leuven.

Heat Production Measurements

A detailed description of the respiratory unit is given elsewhere (Buyse et al., 1998). Briefly, it consisted of 3 separate light and temperature-controlled climatic chambers, each containing 2 respiratory cells, the gas analyzer unit, and the data acquisition system. The respiratory cells (550 × 300 × 500 mm) were made of stainless steel, and the inside temperature was measured by a platinum RTD (Pt-100, accuracy of 0.2°C; Farnell In One, Grace-Hollogne, Belgium). The paramagnetic O_2 analyzer (ADC 02-823A) and the infrared CO_2 analyzer (ADC D/8U/54/A; The Analytical Development Company, Hoddesdon, Herts, UK) were calibrated before every measurement by using gas mixtures with known (±0.001%) O_2 and CO_2 levels.

After the adaptation period, gas exchanges were measured continuously during the 24-h feed deprivation and the 7-h refeeding period. Oxygen and CO_2 levels in air samples coming out of each cell were measured for 90 s every 15 min. The CO_2 production and the O_2 consumption of the chickens were calculated based on 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): Heat production (kJ/h) = 16.18 O_2 (L/h) + 5.02 CO_2 (L/h). The third term for urinary N excretion was omitted because it typically induces an error of <1%. To assess DIT, the difference between the average value for HP during the last 8 h of the feed deprivation period and the HP at every measuring point during the refeeding period was calculated. The DIT was then estimated as the area under the HP-curve during the refeeding period and was expressed as a fraction of the feed intake during that interval.

Measurements and Sampling

The individual BW of the chickens was recorded before and after feed withdrawal and after the refeeding period, when feed intake was also determined. Blood samples were collected after feed deprivation and after refeeding from a wing vein using a heparinized syringe and were immediately put on crushed ice. After euthanasia, samples were taken of the gastrocnemius muscle, snap frozen in liquid nitrogen, and stored at –80°C for posterior determination of avUCP expression.

avUCP mRNA Expression

Total mRNA extraction from gastrocnemius muscle and reverse transcription were performed as described by Cassy et al. (2004). Expression of mRNA encoding for avUCP was analyzed by real-time PCR on 1/50 diluted reverse transcription products using specific primers (forward: CTACGACCTCATCAAGGACACA and reverse: GAAGGCAGCCACGAAGTGA). Efficiency of the real-time avUCP PCR was calculated from standard curves. The PCR products obtained were checked for their nucleotide sequence, and dissociation curves were determined to evaluate the quality of each run completed using an ABI prism apparatus and software (Applied Biosystems, Courtaboeuf, France). The level of 18S ribosomal RNA (18S rRNA), chosen as the reference gene, was determined using the Predeveloped TaqMan Ribosomal RNA control kit (Applied Biosystems, Courtaboeuf, France) according to the manufacturer's recommendations. Results were expressed as the ratio between expression of avUCP and that of 18S rRNA from each sample.

Plasma Metabolites and Hormones

Plasma glucose (IL Test kit, No. 182508-00), triglyceride (IL Test kit, No. 181610-60), and uric acid (IL Test kit, No. 181685-00) concentrations were measured spectrophotometrically with an automated apparatus (Monarch Chemistry System, Instrumentation Laboratories, Zaventem, Belgium). Plasma free fatty acid (**FFA**) concentrations were measured by the Wako NEFA C test kit (Wako Chemicals GmbH, Neuss, Germany), an enzymatic colorimetric test modified to use in the Monarch Chemistry System. Plasma 3,5,3′-triiodothyronine ($\mathbf{T_3}$) and thyroxine ($\mathbf{T_4}$) concentrations were measured using a specific radioimmunoassay as described by Darras et al. (1992). Intraassay coefficients were 4.1 and 4.7% for T_3 and T_4, respectively. Plasma leptin levels were measured using a multispecies leptin RIA kit (Linco Research Inc., St. Charles, MO) validated for chicken plasma (Dridi et al., 2000).

Statistical Analysis

All results were analyzed by ANOVA with genotype and age as classification variables (SAS for Windows, version 8e, SAS Institute Inc., Cary, NC). The level of $P < 0.05$ was considered significant. For the plasma metabolite and hormone concentrations, there were age effects for some of the parameters, but no significant interactions between genotype and age were found for any of the parameters. Therefore, to give a clear presentation of the results, these data were pooled over age and analyzed again with a 1-factor ANOVA.

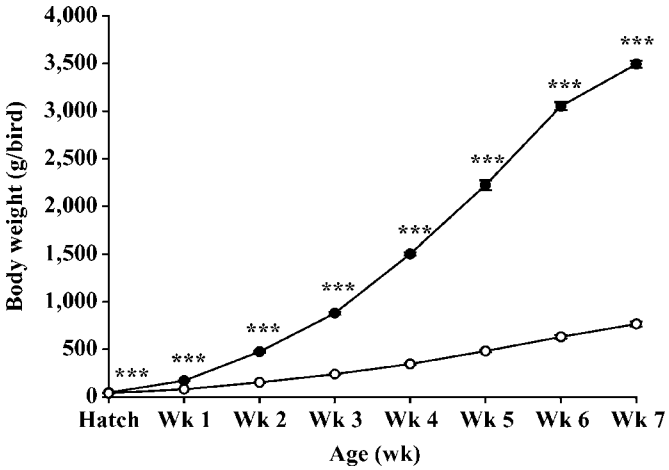

Figure 1. Weekly BW (g/bird) of broiler (●) and layer (○) cockerels reared on a commercial broiler starter and finisher diet. Values are means ± SEM (hatch and wk 1 until wk 5: n = 20 per genotype; wk 5 and 6: n = 10 per genotype; wk 7: n = 6 per genotype). ***Indicates a significant difference between the BW of the groups at the same age ($P < 0.0001$).

RESULTS

Body Weight and Feed Intake

The effect of genetic background on weekly BW is presented in Figure 1. Already at hatch, the broiler chickens had a significantly higher BW compared with the layer cockerels ($P < 0.0001$). Because of these pronounced differences in BW, results are presented per kilogram of MBW (kg of $BW^{0.75}$).

The layer cockerels had a significantly ($P < 0.01$) higher feed intake per MBW during the 7-h refeeding period in the respiratory cells compared with the broiler chickens. Feed intake per MBW of both genotypes decreased significantly ($P < 0.05$) from wk 4 until 6 of age and slightly increased again at 7 wk of age (Figure 2).

Heat Production and Diet-Induced Thermogenesis

During feed deprivation, the layer cockerels had a significantly elevated HP per MBW per 24 h compared with the broilers ($P < 0.05$; Figure 3A). In the subsequent 7-h refeeding period, the effect of genetic background on HP per MBW was sustained and even emphasized ($P < 0.0001$; results not shown). These HP parameters were not influenced by the age of the animals. When the HP per MBW was expressed per gram of feed ingested during the 7-h refeeding period, the difference between layer cockerels and broiler chickens was even more pronounced ($P <$

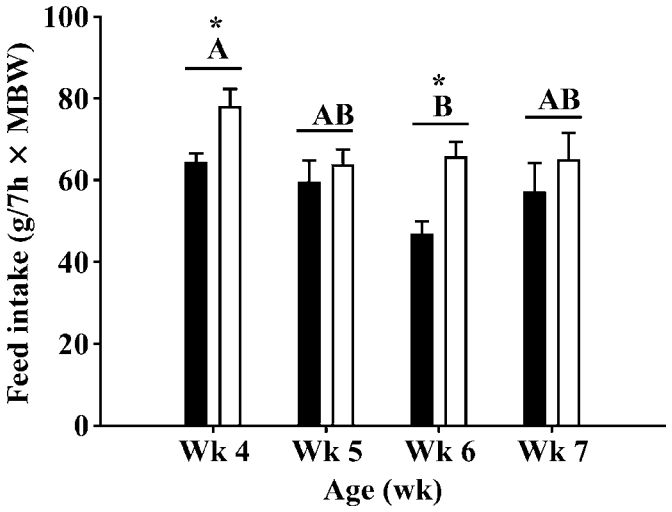

Figure 2. Feed intake by broiler (filled bars) and layer (open bars) cockerels during the 7-h refeeding period, expressed in grams per kilogram of metabolic BW (MBW) per 7 h. Values are means ± SEM (n = 6 per week; except for the broiler chickens at 7 wk of age: n = 5). *Indicates a significant difference between broiler and layer cockerels at the same age ($P < 0.05$). [A,B]Different letters indicate a significant overall age effect ($P < 0.05$)

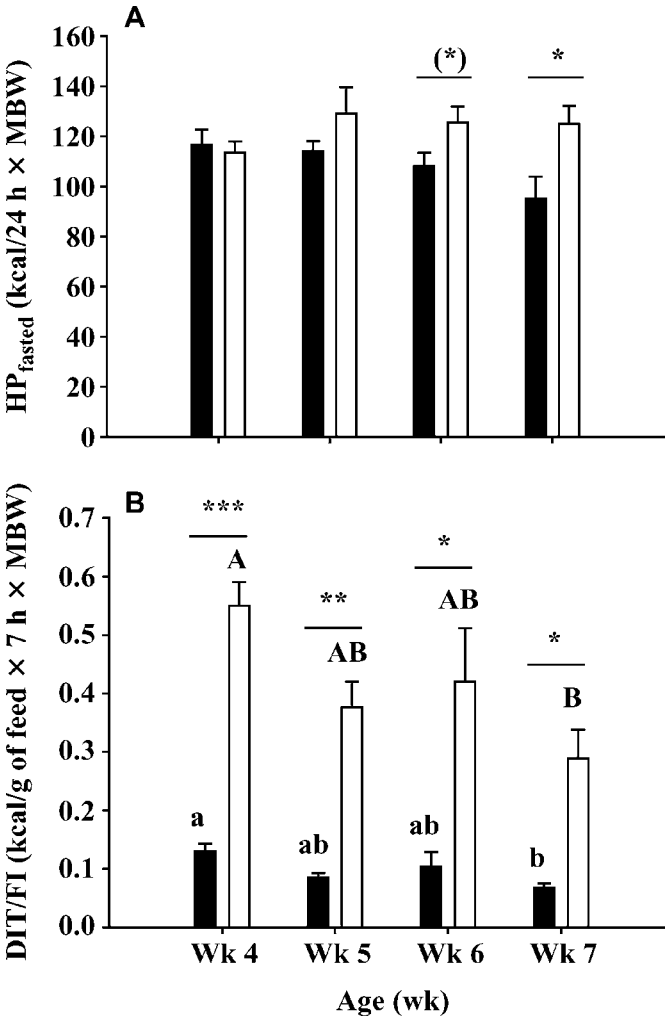

Figure 3. A) Heat production (HP) during the 24-h feed deprivation period, expressed in kilocalories per kilogram of metabolic BW (MBW) per 24 h; B) diet-induced thermogenesis, expressed as kcal/MBW per gram of feed intake per 7 h measured for broiler (filled bars) and layer (open bars) cockerels. All values are means ± SEM (n = 5 or 6 per genotype and per week). A significant difference between broilers and layer cockerels at the same age is indicated by (*)$0.1 < P < 0.05$, *$P < 0.05$, **$P < 0.001$, ***$P < 0.0001$. Different letters indicate a significant age effect for each genotype (a, b: broiler cockerels; A, B: layer cockerels; $P < 0.05$).

0.0001; results not shown). The HP per MBW and per feed intake was on average 193% higher during the 7-h refeeding period in the layer cockerels than in the broiler chickens. Values decreased significantly ($P < 0.001$) with age for both genotypes.

The diet-induced thermogenesis per MBW per 7 h and corrected for feed intake was significantly affected by genotype as well as by age. Again, values were significantly ($P < 0.0001$) higher for the layer cockerels compared with the broilers; on average, the difference between both genotypes was 323%. For both genotypes, the values decreased significantly ($P < 0.05$) with the age (Figure 3B). No interactions between genotype and age were observed for any of these parameters.

avUCP mRNA Expression

The data of avUCP mRNA expression, relative to the expression of the reference gene 18S, are given in Table 1. No effects were observed of age or genotype on avUCP mRNA expression. No correlations were found between uncoupling protein expression and HP parameters or plasma variables.

Plasma Metabolites and Hormones

Free fatty acid levels were significantly ($P < 0.0001$) higher in the plasma of the layer cockerels compared with the broiler chickens, independent of the nutritional state (24-h feed deprived or 7-h refed; Figure 4A). Plasma triglyceride levels were affected by the genotype of the animals in a similar way, irrespective of nutritional status (Figure 4B). The values were significantly higher in the plasma of the layer cockerels ($P < 0.05$ after feed deprivation and $P < 0.0001$ after refeeding). After 24 h of feed deprivation, plasma glucose levels were somewhat elevated in the plasma of the layer cockerels compared with the broilers, although not statistically verifiable at the 5% level ($P = 0.0515$). After the 7-h refeeding period, this effect of the genotype was more pronounced ($P < 0.05$;

Table 1. The effect of genotype on the ratio of the expression of avian uncoupling protein (avUCP) mRNA to the expression of 18S rRNA[1]

Age (wk)	Broiler cockerels	Layer cockerels
4	38 ± 7	74 ± 10
5	74 ± 13	48 ± 12
6	115 ± 37	107 ± 26
7	138 ± 51	104 ± 34

[1]Values are means ± SEM (n = 6, except at wk 7 for the broiler cockerels: n = 4).

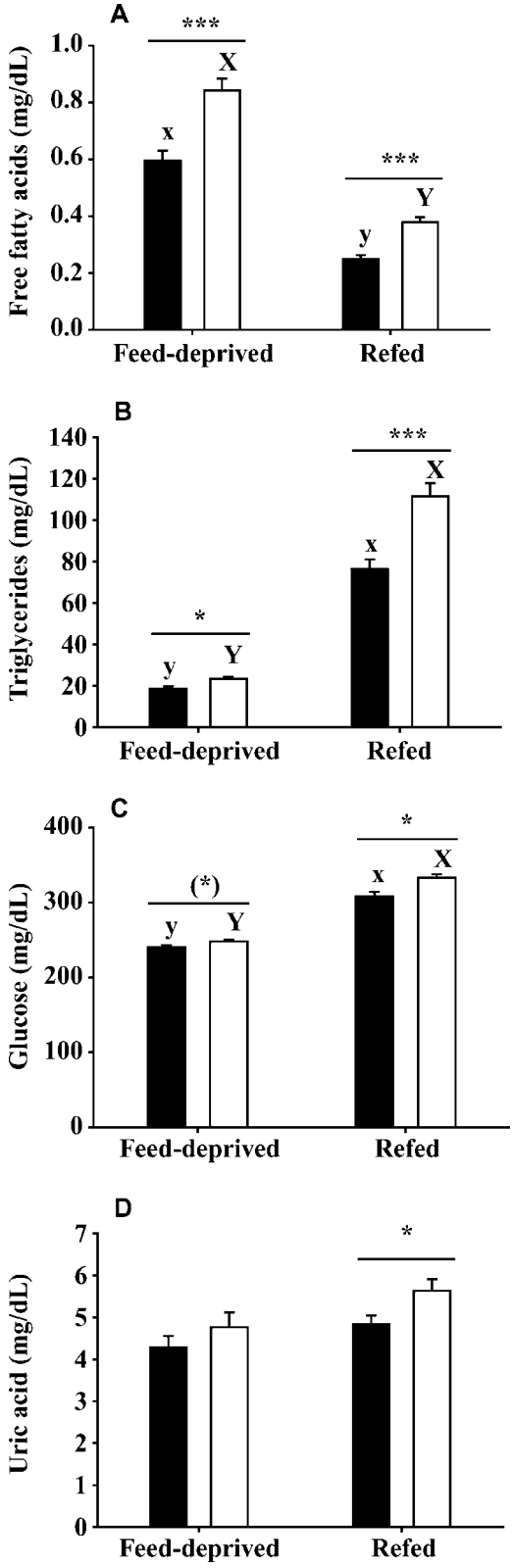

Figure 4. Plasma concentrations of free fatty acids (A; mg/dL), triglycerides (B; mg/dL), glucose (C; mg/dL) and uric acid (D; mg/dL) of broilers (filled bars) and layer (open bars) cockerels, per nutritional state (after 24 h of feed deprivation and after 7 h refeeding). Values are means ± SEM (n = 24). Significant differences between genotypes at the same nutritional state are indicated by (*)$0.1 < P < 0.05$; *$P < 0.05$; and ***$P < 0.0001$. Different letters indicate a significant effect of nutritional state per genotype (x, y: broiler cockerels; X, Y: layer cockerels; $P < 0.0001$).

Figure 4C). Uric acid concentrations in the plasma were also elevated in the plasma of the layer cockerels, but this difference was only significant ($P < 0.05$) after 7 h of refeeding (Figure 4D). Refeeding after a 24-h feed-deprivation period caused a significant ($P < 0.0001$) increase of circulating levels of triglycerides (Figure 4B) and glucose (Figure 4C) and a significant ($P < 0.0001$) decrease of the plasma FFA concentrations (Figure 4A), independent of genotype. Nutritional state had no statistically verifiable effect on plasma uric acid levels (Figure 4D).

The levels of T_3 in the plasma of the layer cockerels were augmented compared with the levels in the plasma of the broiler chickens, this genotypic difference being statistically verifiable only in the feed-deprived state ($P < 0.05$; Figure 5A). Independent of the nutritional state of the animals, T_4 concentrations were significantly ($P < 0.001$) lower in the plasma of the layer cockerels when compared with the broilers (Figure 5B). Refeeding after the 7-h feed deprivation caused a significant ($P < 0.0001$) increase and decrease of the circulating T_3 and T_4 levels, respectively. Plasma leptin levels were not influenced by genotype, by nutritional state, or by the age of the animals (Figure 5C).

DISCUSSION

Body Weight, Feed Intake, and Heat Production Parameters

As expected, the broiler chickens grew much faster than the layer cockerels, resulting in a nearly 5-fold higher BW at 7 wk of age. This faster BW gain was accompanied by a higher feed intake expressed in grams per chicken. Indeed, as demonstrated previously, broiler chickens have a higher appetite compared with layer chickens, which partly accounts for their faster growth rate (Considine, 1997; Bokkers and Koene, 2003). However, when feed intake was expressed in terms of MBW, the layer cockerels ate significantly more during the 7-h refeeding period. Thus, the difference in feed intake as such cannot be the only factor determining the divergence in BW between both genotypes. The ME absorbed from the ingested feed is used for maintenance on the one hand and for production, in casu growth, on the other hand. Variation in one of these parameters would affect the amount of ME available for the other component. The energy for maintenance can be partitioned into the basal metabolism, the energy expended on physical activity and the heat increment or diet-induced thermogenesis for maintenance. In the present study, the layer cockerels had a significantly higher daily HP per MBW during the feed deprivation period, suggesting an elevated basal metabolism compared with the broiler chickens, as also observed by King and Farmer (1961) and Muramatsu et al. (1987). The physical activity pattern of the animals was not monitored in the present experiment. Previous research has revealed, however, that broiler chickens spent less time and thus also less energy on feeding than layers do (Savory, 1974). Masic et al. (1974) revealed that broilers also spent more

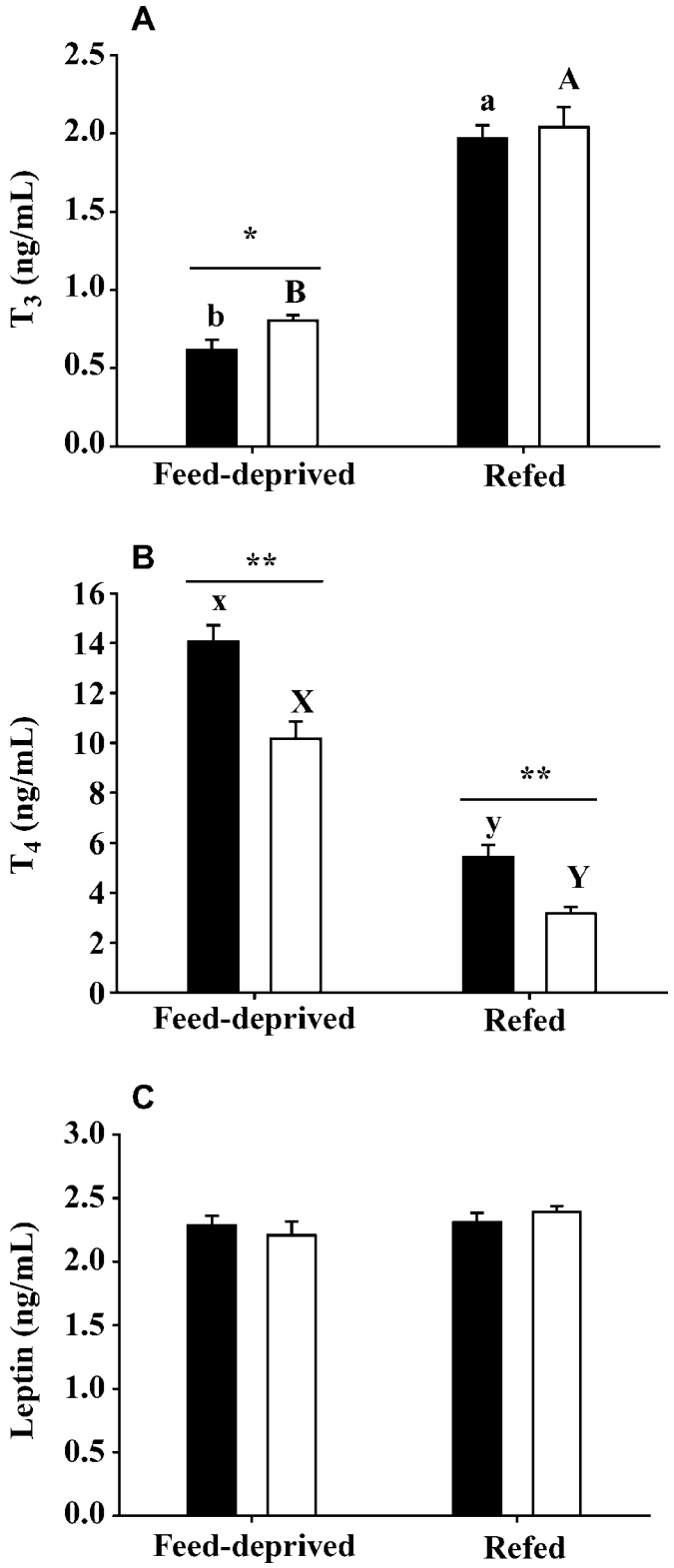

Figure 5. Plasma concentrations of 3,5,3′-triiodothyronine (T_3) (A; ng/mL), thyroxine (T_4) (B; ng/mL), and leptin (C; ng/mL) of broiler (filled bars) and layer (open bars) cockerels per nutritional state (after 24 h of feed deprivation and after 7 h of refeeding). Values are means ± SEM (n = 24). Significant differences between genotypes at the same nutritional state are indicated by $^*P < 0.05$, $^{**}P < 0.001$. Different letters indicate a significant effect of nutritional state per genotype (x, y: broiler cockerels; X, Y: layer cockerels; $P < 0.0001$).

time resting, suggesting that the total energy expenditure on activity by broiler chickens was considerably lower compared with that of animals of a layer genotype. In addition, Hocking et al. (1997) showed that laying-strain birds express more investigative or foraging behavior than broiler breeders. It can therefore be inferred that in the present experiment the broilers spent less of their ME on feeding behavior and physical activity. In addition, the DIT per gram feed intake was 323% higher in the layer cockerels. Taken together, the behavioral data found in literature combined with the lower basal metabolic rate and lower DIT found in this study confirm that broiler chickens are more efficient in retaining ME for productive purposes.

Previous research using isoenergetic diets with substitutions between protein and fat showed that the dietary macronutrient ratio did not affect DIT in a significant manner (Swennen et al., 2004, 2006). The results of these studies could not confirm the hierarchic oxidation/storage model as formulated by Stubbs et al. (1997) for adult mammals, nor could the model be refuted. The fact that the animals used in our previous experiments were juvenile, fast-growing broiler chickens might have an influence on energy and nutrient partitioning because a substantial amount of the ingested nutrients would be used for growth and a smaller fraction would be oxidized in comparison to adult birds. Furthermore, it is also possible that the macronutrient ratio in the diet (independent of energy supply) does not influence DIT and possibly also satiety in these broiler chickens in the same degree observed in mammals. It is consequently hypothesized that the mechanisms linking DIT to feed intake might be disturbed or at least less active in the broiler chicken. This could be a result of intensive selection for a high growth rate, which resulted in an increased appetite as reported by Denbow (1994), Dunnington and Siegel (1996), and others. Burkhart et al. (1983) proved that selection for growth influenced the hypothalamic satiety signals in a negative way. The same conclusion was reached by Bokkers and Koene (2003), who demonstrated that broilers did not show an upper set point for controlling eating behavior and probably eat to their maximal physical capacity. In contrast, the eating behavior of layer chickens is controlled equally by satiety and hunger mechanisms (Bokkers and Koene, 2003). Thus, it could be inferred that chickens of a layer strain, and thus not selected for growth, might show a stronger relation between DIT and feed intake. Indeed, the diet-induced thermogenesis per gram feed intake was significantly higher for the layer cockerels compared with the broiler chickens. However, in spite of the higher DIT found in the cockerels of the layer genotype, the feed intake per MBW (per 7 h) was also significantly higher in these animals compared with age-matched broilers. It is therefore tempting to conclude that in growing chickens, DIT might not regulate feed intake. The difference in physical capacity between both genotypes might have influenced the obtained data. In addition, it is possible that the diet composition affected DIT measurements. Because the cockerels of both geno-

types were reared on a commercial diet formulated according to the needs of broiler chickens, the layer cockerels possibly ingested a relative oversupply of nutrients, resulting in an increased HP after feed intake and consequently an increased DIT. However, because these parameters were not assessed in the present study, no firm conclusions can be drawn.

The DIT per MBW, per 7 h and per feed intake, decreased significantly with age for both genotypes. This was also observed in our previous study with genetically fat and lean genotypes of broiler chickens (Swennen et al., 2006). As a consequence of the smaller proportion of the ingested energy being combusted with age, a greater percentage is available for deposition in the body, most probably under the form of fat. Indeed, the abdominal fat pad is a late maturing tissue (Govaerts et al., 2000) because its weight (absolute and proportional) increases with age.

avUCP mRNA Expression

Expression of avUCP mRNA was not influenced by genotype or age in the present experiment. This uncoupling protein is possibly involved in HP (Raimbault et al., 2001; Collin et al., 2003a), and its expression is strongly regulated by thyroid status (Collin et al., 2003b). The avUCP is also suggested to be involved in the limitation of reactive oxygen species generation in mitochondria (Criscuolo et al., 2005). The present results do not support the hypothesis of an involvement of avUCP in the higher DIT measured in the layer strain, although Raimbault et al. (2001) showed a 30% higher avUCP mRNA expression in inefficient R+ cockerels characterized by a 84% higher DIT than efficient R– cockerels. However, our result is consistent with recent results from Cassy et al. (2005) showing similar avUCP mRNA expressions in broiler chickens from a single commercial broiler line but characterized by high (1.97) or low (1.70) feed conversion ratios. In the absence of any specific antibody recognizing the avUCP protein, it is difficult to draw conclusions about the avUCP protein expression, and its real involvement in HP and diet-induced thermogenesis is still debated (Mozo et al., 2005).

Plasma Metabolites and Hormones

The decrease in plasma levels of FFA and the increase of plasma concentrations of triglyceride and glucose after the refeeding period, compared with feed-deprived levels, are in agreement with previous findings (Buyse et al., 2000; Swennen et al., 2005). A more detailed discussion is provided by these authors.

The higher triglyceride concentration in the plasma of the layer cockerels might result from an augmented de novo lipogenesis on the one hand or from a decreased clearance of triglycerides to the adipose and other tissues on the other hand. Considering the genetic background and the age of the animals at experimentation, it is not likely that the layer cockerels had a higher de novo lipogenesis than the broilers. Indeed, unlike layer chickens, broilers are selected for an increased growth rate and an unfavorable indirect response to this selection is an increased fat deposition (Barbato, 1992; Havenstein et al., 2003). Thus, a decreased clearance is more likely. Indeed, March (1984) showed that the rate with which triglycerides left the circulation was generally faster in broilers than in White Leghorn chickens. In addition, Sato et al. (2006) found a tendency for a lower plasma triglyceride level in broiler compared with layer embryos. The authors suggested that broiler embryos used more lipids than layer embryos. A similar pattern was observed for circulating glucose levels. Mahagna and Nir (1996) found lower glucose concentrations in the plasma of young (up to 3 wk of age) broiler chickens compared with chicks of a layer strain. They hypothesized that the glucose uptake by the high-energy-demanding organs was larger in the broiler than in the layer chicks. The decreased circulating glucose levels in the broiler chickens compared with the layer cockerels found in the present study support this hypothesis.

Plasma FFA levels were significantly increased in the layer cockerels compared with the broiler chickens. Circulating FFA levels are the result of lipolysis on the one hand and cellular uptake of FFA by the cells for energy or lipogenesis (in liver) on the other hand. The elevated circulating FFA levels in the layer chickens were probably the consequence of a reduced uptake by peripheral tissues. An increase in lipolysis is not very likely; both genotypes were reared on commercial broiler diets, and consequently, dietary energy intake by the layer cockerels was probably more than sufficient for maintenance. Similarly, the layer cockerels were relatively overfed with dietary proteins, possibly resulting in an increased amino acid oxidation. This elevated amino acid catabolism is reflected by the increased uric acid levels in the plasma of the layer cockerels compared with the broilers after refeeding. The fact that no differences in circulating uric acid levels are found in feed-deprived state supports this hypothesis.

As discussed for the DIT measurements, it is possible that the higher metabolite concentrations in the plasma of the layer compared with the broiler cockerels reflect a relative oversupply of dietary nutrients in the former group due to the use of a commercial broiler diet for both genotypes. In this respect, it would have been interesting to study both genotypes when reared on a layer diet as well. Indeed, Zhao et al. (2004) showed that for certain parameters, the differences between layer and broiler chickens depended on the diet provided. They reared layer and broiler chickens on a commercial broiler diet and a commercial layer diet from 1 to 42 d of age. When reared on the same diet, the differences in growth potential between broiler and layer chickens were still significant, reflecting their genetic background. Similarly, muscle growth hormone receptor mRNA expression was not affected by diet exchange but maintained genotype-specific features. In contrast, pituitary growth hormone mRNA and hypothalamic somatostatin mRNA expres-

sion were inversed by the diet, suggesting that these genes are sensors to nutritional factors. However, due to practical limitations, it was not possible to compare the genotypes on both types of diet in the present study.

Plasma T_3 values significantly increased after the 7-h refeeding period compared with feed-deprived levels. Plasma T_4 levels showed an opposite pattern compared with T_3 values after refeeding, as observed previously (Geris et al., 1999; Buyse et al., 2000, 2002; Swennen et al., 2005, 2006). Feed-deprived plasma T_3 levels were significantly higher in the layer cockerels compared with the broiler chickens. Because the level of T_3 in the plasma is positively correlated with HP (Klandorf et al., 1981), this corroborates the higher feed-deprived HP in the former group. After refeeding, however, the difference in circulating T_3 levels between the genotypes disappeared, although the genotypic difference in refed HP was even more pronounced. It is possible that a larger proportion of the circulating T_3 in the layer cockerels is taken up by the cells for binding to a nuclear receptor, thus increasing oxidative phosphorylation and subsequently HP. Plasma T_4 levels were increased in the broiler chickens compared with the layer cockerels. The inversely related pattern of T_3 and T_4 due to genotype as well as nutritional state points 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; Decuypere et al., 2005). Thus, the driving forces for these effects are probably regulatory effects on deiodination (affecting T_3 formation as well as T_3 degradation).

In conclusion, broiler chickens were more efficient in retaining ME for productive purposes compared with age-matched layer cockerels. In spite of an elevated diet-induced thermogenesis relative to their MBW, the layer cockerels also had a higher feed intake during the 7-h refeeding period than the broilers. Thus, the model of Stubbs et al. (1997) as postulated for adult mammals could not be corroborated, or in other words, diet-induced thermogenesis did not have a feedback effect on feed intake in these genotypes. The avUCP mRNA expression was not influenced by genotype, and consequently, the hypothesis of an involvement of avUCP in the higher DIT measured in the layer compared with the broiler cockerels was not supported. The elevated levels of metabolites in the plasma of the layer compared with the broiler cockerels might reflect a relative oversupply of nutrients in the former group.

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