

Interactions between diet composition and genotype of broiler chickens on feed intake regulation.

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Abstract

Day-old male broiler chickens of fat line (FL) and lean line (LL) (Leclercq *et al.*, 1980) were obtained from INRA (Nouzilly, France). From two weeks of age onwards, both lines were divided into 2 groups, each receiving 1 of 2 isoenergetic diets with substitutions between fat and protein and a constant gross energy and carbohydrate level (low protein diet (LP): 126 vs 242 g protein/kg and low fat diet (LF): 106 vs 43 g fat/kg). There were no effects of genetic background on the carbohydrate (e.g. glucose oxidation rate as measured by stable isotope breath test) or protein metabolism of the broilers. The line differences in carcass fat content are therefore likely to be due to differences in fat metabolism. The theory linking diet-induced thermogenesis to feed intake could not be corroborated nor refuted.

Introduction

Research in mammals has shown a hierarchy in the extent to which recently ingested nutrients are combusted. First proteins are oxidized, followed by carbohydrates and finally fat, which corresponds to their ability to induce satiety but is reciprocal to their relative storage capacity (Stubbs *et al.*, 1997). Causal relationships were observed between diet composition, diet-induced thermogenesis, nutrient balance and the satiety power of the different macronutrients. However, for avian species, information is scarce.

The use of stable isotope tracers for studying a wide variety of metabolic processes and pathways *in vivo* has remarkable potentials. Stable isotope breath tests offer a safe and non-invasive 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. Recently, the methodology to perform stable isotope breath tests in chickens has been developed (Buyse *et al.*, 2004).

Models for genetic obesity are numerous for mammals, but are rare for birds. Genetically fat and lean lines of broiler chickens have been developed by Leclercq *et al.* (1980) in order to investigate the mechanisms controlling fattening in chickens. These lines differ in carcass lipid content, but exhibit a similar live body weight, feed consumption and energy expenditure. Another difference between both lines is related to the plasma glucose-insulin balance. In this experiment, fat and protein were substituted in two isoenergetic diets to determine the role of these macronutrients in regulating voluntary feed intake in two genetically diverse 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 and to determine the glucose oxidation of these broiler chickens, known to differ in glucose-insulin balance.

Materials and Methods

Day-old male broiler chickens of fat line (FL) and lean line (LL) (Leclercq *et al.*, 1980) were obtained from INRA (Nouzilly, France). The animals were divided over floor pens and raised under standard conditions. Until 14 days of age, the chickens received a commercial starter diet. At 14 days of age, each line was divided into 2 groups (4 groups in total), each receiving

one of two isoenergetic diets. These diets contained the same ingredients, though in varying quantity to create differences in fat and protein level, while maintaining a similar gross energy and carbohydrate level. The low protein (LP) diet contained 16.63 MJ gross energy/kg, 126 g protein/kg, 106 g fat/kg and 514 g N-free extract/kg. The low fat (LF) diet contained 16.73 MJ gross energy/kg, 242 g protein/kg, 43 g fat/kg and 504 g N-free extract/kg.

Starting at 21 days of age, for 5 consecutive weeks and repeated two times per week with different animals, 3 chickens of each group were placed in one of the 6 open-circuit respiratory cells for measuring the energy metabolism. After an adaptation period (48 h), the chickens were fasted for 24h. Then they were given a weighed amount of their specific diet for 7h. Gas exchanges (CO_2 and O_2) were measured continuously during fasting and refeeding. Heat production (HP) was calculated from these data according to the short formula of Romijn and Lokhorst (1961): $\text{HP (kJ/h)} = 16.18 \text{ O}_2 \text{ (l/h)} + 5.02 \text{ CO}_2 \text{ (l/h)}$.

The postprandial carbohydrate oxidation rate was measured by using $\text{U-}^{13}\text{C}_6$ -glucose. First, a 10 ml air sample was taken at the outlet side of the respiratory cell to determine the background $^{13}\text{CO}_2$ that is normally produced by the animal. Samples were taken with a syringe and delivered into a 10 ml vacutainer tube. The animals were then intubated with 2 mg $\text{U-}^{13}\text{C}_6$ -glucose per kg body weight, dissolved in water. After intubation, air samples were collected for 7h at 30 min intervals. The enrichment of $^{13}\text{CO}_2$ in air was measured using isotope ratio mass spectrometry. The end results of the analyses are expressed in percent dose recovery (PDR) and cumulative PDR (CPDR), as described by Buyse *et al.* (2004) (Figure 1).

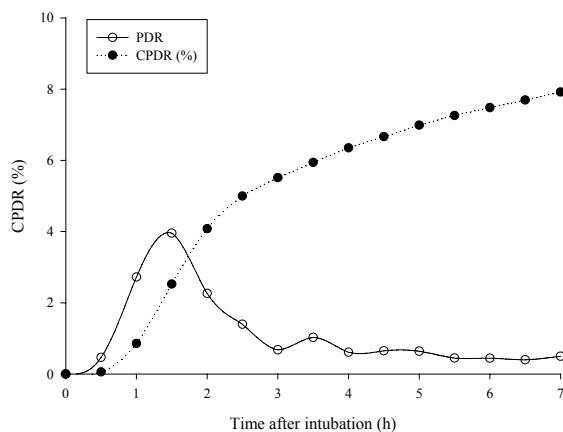


Figure 1. Percentage dose recovery (% per h) and cumulative percentage dose recovery (% dose) in function of time after intubation (h).

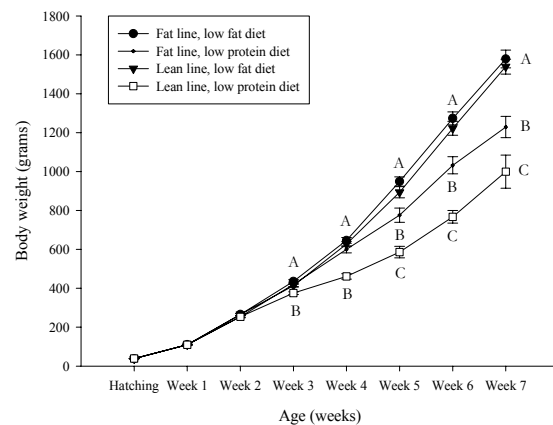


Figure 2. Weekly body weight of broilers of fat and lean line, fed a low fat or a low protein diet (mean \pm SEM; n=20) A significant difference between line*diet combinations at the same age is indicated by letters

The obtained CPDR curves were fitted according to t 1825): $O_t = A * \exp(-B * \exp(-Ct))$ where O_t is the oxidation of $\text{U-}^{13}\text{C}_6$ -glucose (% dose) at time t (min). A is the asymptote of the curve (% dose). B and C are constants (min^{-1}) that are indicative for the rate of increase and decrease in oxidation during the ascending and descending phase of the $\text{U-}^{13}\text{C}_6$ -glucose oxidation curve, respectively.

After fasting and after diet-induced thermogenesis (DIT) measurements, the body weight of the animals was recorded and blood samples were taken. After DIT, feed intake was determined and after euthanasia, the weight of the abdominal fat pad was recorded.

Plasma glucose, triglyceride, free fatty acid and uric acid concentrations were measured spectrophotometrically with an automated apparatus (Monarch Chemistry System, Instrumentation Laboratories, Belgium).

All data were analyzed by analysis of variance with diet composition and line as classification variables (SAS Institute Inc., Cary, N.C., 1998).

Results and Discussion

From week 3 until 5, the lean line-LP animals had a significantly lower BW than the other 3 groups. From week 5 until 7, these animals had a significantly lower BW than the fat line-LP chickens, which had a significantly lower BW compared to the LF fed groups (Figure 2).

Proportional abdominal fat pad weight was significantly affected by diet as well as by line, with an interaction between both factors. The LP diet induced a significant increase in abdominal fat pad, but this augmentation was more pronounced for the FL chickens. No differences were found in the plasma triglyceride and free fatty acid concentrations during fasting. After 7 hours of refeeding, triglyceride levels were augmented, with a more pronounced increase in the plasma of the chickens that were fed the LP diet ($P < 0.001$). Free fatty acid levels in the plasma decreased after 7 hours of refeeding, with a less pronounced decrease in the chickens on the LP diet ($P < 0.0001$). These differences are probably attributable to the higher fat level in the LP diet compared to the LF diet. No significant effect of the genetic background on plasma triglycerides and free fatty acids was observed.

During fasting and after refeeding, the LP chickens had a lower uric acid level in their plasma ($P < 0.001$ and $P < 0.0001$, respectively). Since the uric acid concentration in the plasma is a measure for protein catabolism, this indicates that the LP animals had a significantly lower protein oxidation compared to their LF counterparts. There was no effect of line on this parameter suggesting that the protein oxidation of fat line and lean line is not different.

Plasma glucose levels were not influenced by diet composition during fasting and refeeding. There was an effect of line during fasting, with lower glucose concentrations ($P < 0.0001$) in the plasma of the fat line broilers, but not after refeeding as observed before (e.g. Leclercq *et al.*, 1984). Furthermore, there were no significant effects of diet or line on PDR and CPDR, nor on the coefficients of the Gompertz curve. These results indicate that there was no difference in carbohydrate metabolism between both lines or induced by the diet composition. In conclusion, our results show no significant effect of the genetic background of the broilers on their carbohydrate and protein metabolism. Considering the difference in the carcass composition (fat content) of chickens 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, possible differences might be found in fat oxidation or/and in *de novo* lipogenesis. The latter possibility is currently being investigated. Diet-induced thermogenesis per metabolic body weight ($\text{kg BW}^{0.75}$) per 24 h, expressed per gram of feed intake was 4 % increased for chickens of both lines when reared on the LF diet, but this difference was not significant. This confirms the hierarchical oxidation model in mammals. However, the expected feedback of the augmented DIT on the feed intake of the animals was not observed. Thus, the model of Stubbs *et al.* (1997) that links the diet-induced thermogenesis to feed intake, as postulated for adult mammals could not be corroborated nor countered for growing broiler chickens, and further research is warranted.

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