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Short-term nutritional strategies before slaughter are effective in modulating the final pH and color of broiler breast meat

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ABSTRACT The poultry meat industry is faced with various quality issues related to variations in the ultimate pH of breast meat. The aim of this study was to evaluate the possibility to control breast ultimate pH by distributing finishing diets varying in amino acid (AA) and energy content for a short period before slaughter. Experimental diets were distributed to PM3 broilers on the last 3 d before slaughter (36 d of age). They consisted of a control (C) diet (3,150 kcal/kg; 200 g/kg of CP; 10.0 g/kg of true digestible Lys) with adequate amounts of AA other than Lys, 6 diets isocaloric to the control diet including 3 Lys-deficient (8.0 g/kg) diets with an adequate (Lys-/AA), low (Lys-/AA-), or high (Lys-/AA+) amount of other essential AA calculated in relation to Lys, and 3 Lys-rich (12.0 g/kg) diets with an adequate (Lys+/AA), low (Lys+/AA-), or high (Lys+/AA+) amount of other essential AA calculated in relation to Lys, and 2 diets isoproteic

to C with a high (3,300 kcal/kg, E+) or low (3,000 kcal/kg, E-) energy content. Broiler feed consumption and growth performance were slightly affected by AA and energy content during the finishing period. Feed intake (33–36 d) was lower with the Lys+/AA+ and E+, and FCR between 24 and 36 d was higher with the Lys-/AA- and E- than with the C diet. Body weight at d 36 was lower in Lys-/AA-, Lys+/AA+, and E+ than in C, whereas the breast meat yield and abdominal fatness were not affected by diet. Lower pH values were observed in broilers fed Lys-deficient diets containing a high amount of other AA (Lys-/AA+) than in broilers fed diets containing low (AA-) or adequate (AA) amounts of other AA. This study shows that it is possible to alter the pH of breast meat by changing AA profile over a short period before slaughter, with limited impact on broiler growth and carcass composition.

Key words: nutrition, amino acid, energy, meat quality, broiler

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INTRODUCTION

Optimization of nutritional strategies in poultry production requires integration of classical parameters, such as BW and feed efficiency, and also meat yield and processing ability, that strongly affect competitiveness in the poultry meat industry. Currently, several quality issues are a concern for the industry related to meat aspects, texture, and processing ability (Zhang and Barbut, 2005; Petracci et al., 2013). In chickens, processing ability is largely influenced by the ultimate pH (pHu) of the meat (Sheard et al., 2012), which itself depends on muscle glycogen content (Le Bihan-Duval et al., 2008). Recent reports have suggested the possibility of modifying these characteristics by varying

protein or energy intake in broilers (Berri et al., 2008; Bouvarel et al., 2008; Yalçın et al., 2010; Jlali et al., 2012; Zhao et al., 2012). However, most of these studies have considered long-term feeding strategies that also impair broiler growth performance and body composition. One study on sequential feeding suggests, however, that a change in dietary protein to energy ratio can affect the breast muscle glycogen content from day to day (Mameri et al., 2010), probably with a limited impact on bird growth.

The aim of the study presented here was to evaluate the possibility to improve the quality of breast meat without impairing broiler growth performance and body composition by distributing over a very short period (3 d) before slaughter diets varying in amino acid (AA) and energy content. Our hypothesis was that changes in dietary AA, energy content, or both can affect the intermediate metabolism of birds, thus orienting the use of nutrients to protein synthesis or energy storage as body fat or muscle glycogen. The experimental design

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included a control diet (3,150 kcal/kg, 200 g/kg of CP, 10.0 g/kg of true digestible Lys), 6 diets isocaloric to the control diet and varying in both the digestible Lys content (8 or 12.0 g/kg) and the amounts of other essential AA calculated in relation to Lys (low, adequate, and high), and 2 diets isoproteic to the control diet but varying in energy content (3,000 or 3,300 kcal/kg). The effects of treatments were evaluated on growth, feed intake and efficiency, carcass yield, and breast meat quality traits such as pHu and color parameters.

MATERIALS AND METHODS

Birds, Experimental Design, and Diets

All procedures involving animals were performed in accordance with the European Union Guidelines for animal care and under authorization 37–112 delivered to C. Berri by the French Ministry of Agriculture.

A total of 1,140 one-day-old male Ross (PM3) chicks were purchased from a commercial hatchery (Grelier, Volnay, France) and reared at the experimental poultry unit (PEAT) of the Institut National de la Recher-

che Agronomique (INRA, Nouzilly, France). They were randomly distributed in 45 pens of 3 m². Pens each contained 32 birds at a density of 10.7 birds per m². The ambient temperature program applied was 32°C from d 1 to 3, 30°C from d 4 to 6, 28°C from d 7 to 13, 27°C from d 14 to 20, 24°C from d 21 to 27, 22°C from d 28 to 34, and 20°C from d 35 until the end of the experiment at d 36. Birds were exposed to the following lighting schedule: 23 h of light d 1 to 3, 18 h of light d 4 to 36. Chickens were individually wing tagged at d 7. During the experiment, feed and water were administered ad libitum. Starter feed was provided as short pellets from d 1 to 13 (Table 1). Chickens were then fed with a grower diet from d 14 to 23 and with a finisher diet from d 24 to 32, both as 2.5-mm pellets (Table 1). From d 33 to 36, chickens received the same finisher diet (control diet) or 1 of the 8 experimental diets described in Table 2.

The experiment consisted of 9 dietary treatments replicated in 5 randomized blocks. There were 160 birds by treatment. The dietary treatments were based on maize, soybean meal, wheat, soybean oil, maize gluten meal, and extruded soybean seed (Table 2). They consisted of

Table 1. Composition (%) and characteristics of diets fed to chickens from d 1 to 32

| Item | Starter (0 to 13 d) | Grower (14 to 23 d) | Finisher ¹ (24 to 32 d) |
|---|------------------------|------------------------|---------------------------------------|
| Ingredient | | | |
| Maize | 29.70 | 33.50 | 39.90 |
| Soybean meal | 37.86 | 30.73 | 30.44 |
| Wheat | 25.00 | 25.00 | 20.00 |
| Soybean oil | 3.70 | 4.50 | 5.20 |
| Maize gluten meal | — | 2.48 | 1.08 |
| Dicalcium phosphate | 1.74 | 1.65 | 1.68 |
| Calcium carbonate | 0.65 | 0.77 | 0.61 |
| Mineral-vitamin premix ² | 0.50 | 0.50 | 0.50 |
| Sodium chloride | 0.30 | 0.30 | 0.30 |
| DL-Met | 0.30 | 0.26 | 0.18 |
| Lys-HCl | 0.14 | 0.20 | 0.10 |
| Thr | 0.06 | 0.06 | 0.003 |
| AME _n , ³ kcal/kg | 2,950 | 3,078 | 3,150 |
| Calculated composition, ⁴ % | | | |
| CP | 22.8 | 21.3 | 20.2 |
| Fat | 6.2 | 7.1 | 7.9 |
| Lys | 1.20 | 1.10 | 1.00 |
| Met | 0.59 | 0.55 | 0.46 |
| Met + Cys | 0.88 | 0.84 | 0.73 |
| Trp | 0.24 | 0.21 | 0.21 |
| Thr | 0.79 | 0.73 | 0.65 |
| Leu | 1.64 | 1.67 | 1.55 |
| Ile | 0.89 | 0.82 | 0.78 |
| Val | 0.94 | 0.88 | 0.84 |
| Arg | 1.38 | 1.22 | 1.18 |
| Calcium | 1.00 | 1.00 | 0.81 |
| Available phosphorus | 0.42 | 0.40 | 0.38 |
| Measured composition, % | | | |
| Protein | 23.38 | 21.31 | 20.44 |
| Lipid | 6.43 | 6.73 | 7.56 |

¹Also distributed as control diet between the experimental period (d 33 to 36).

²Supplied per kilogram of diet: Co, 0.6 mg; Cu, 20 mg; Fe, 58 mg; I, 2 mg; Mn, 80 mg; Se, 0.2 mg; Zn, 90 mg; retinyl acetate, 15,000 IU; cholecalciferol, 4,300 IU; DL- α -tocopheryl acetate, 100 mg; thiamine mononitrate, 5 mg; riboflavin, 8 mg; calcium pantothenate, 25 mg; cyanocobalamin, 0.02 mg; menadione, 5 mg; pyridoxine hydrochloride, 7 mg; folic acid, 3 mg; biotin, 0.3 mg; niacin, 100 mg; choline chloride, 550 mg; antioxidant (butylhydroxyanisole, propyl gallate, ethoxyquin), 50 mg.

³Calculated value.

⁴Amino acids are given as true digestible values calculated according to Sauvante et al. (2004).

Table 2. Composition (%) and characteristics of experimental finisher diets¹ fed to chickens from d 33 to 36

| Item | Lys-/AA- | Lys-/AA | Lys-/AA+ | Lys+/AA- | Lys+/AA | Lys+/AA+ | E- | E+ |
|---|----------|---------|----------|----------|---------|----------|-------|--------|
| Ingredient | | | | | | | | |
| Maize | 56.80 | 52.60 | 47.10 | 35.50 | 31.31 | 24.43 | 43.00 | 39.055 |
| Soybean meal | 16.86 | 20.50 | 25.30 | 33.35 | 35.50 | 36.33 | 30.22 | 22.12 |
| Wheat | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 | 20.00 |
| Soybean oil | 2.79 | 3.45 | 4.31 | 5.67 | 6.00 | 6.00 | 2.60 | 6.00 |
| Maize gluten meal | — | — | — | 1.97 | 3.67 | 5.00 | 0.83 | 4.32 |
| Extruded soybean seed | — | — | — | — | — | 4.89 | — | 5.00 |
| Dicalcium phosphate | 1.57 | 1.56 | 1.549 | 1.50 | 1.50 | 1.467 | 1.51 | 1.53 |
| Calcium carbonate | 0.81 | 0.79 | 0.78 | 0.73 | 0.715 | 0.69 | 0.75 | 0.76 |
| Mineral-vitamin premix ² | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Sodium chloride | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 | 0.30 |
| DL-Met | 0.10 | 0.125 | 0.152 | 0.21 | 0.25 | 0.267 | 0.183 | 0.157 |
| Lys-HCl | 0.25 | 0.15 | 0.008 | 0.262 | 0.19 | 0.045 | 0.104 | 0.212 |
| Thr | 0.01 | 0.013 | 0.001 | — | 0.027 | 0.03 | — | 0.015 |
| Trp | 0.01 | 0.012 | — | 0.008 | 0.015 | 0.023 | — | 0.021 |
| Val | — | — | — | — | 0.023 | 0.028 | — | — |
| Arg | — | — | — | — | — | — | — | 0.01 |
| AME _n , ³ kcal/kg | 3,150 | 3,150 | 3,150 | 3,150 | 3,150 | 3,150 | 3,000 | 3,300 |
| Calculated composition, ⁴ % | | | | | | | | |
| CP | 14.9 | 16.1 | 17.7 | 21.9 | 23.5 | 25.6 | 20.2 | 20.0 |
| Fat | 6.0 | 6.6 | 7.3 | 8.3 | 8.5 | 9.1 | 5.5 | 9.5 |
| Lys | 0.80 | 0.80 | 0.80 | 1.20 | 1.20 | 1.20 | 1.00 | 1.00 |
| Met | 0.31 | 0.35 | 0.39 | 0.50 | 0.57 | 0.32 | 0.46 | 0.45 |
| Met + Cys | 0.53 | 0.58 | 0.64 | 0.79 | 0.88 | 0.96 | 0.73 | 0.73 |
| Trp | 0.15 | 0.17 | 0.18 | 0.23 | 0.25 | 0.28 | 0.21 | 0.21 |
| Thr | 0.47 | 0.52 | 0.57 | 0.70 | 0.78 | 0.86 | 0.65 | 0.65 |
| Leu | 1.18 | 1.26 | 1.36 | 1.68 | 1.87 | 2.06 | 1.55 | 1.68 |
| Ile | 0.54 | 0.60 | 0.68 | 0.85 | 0.92 | 1.02 | 0.78 | 0.76 |
| Val | 0.61 | 0.67 | 0.74 | 0.91 | 1.00 | 1.10 | 0.84 | 0.83 |
| Arg | 0.81 | 0.90 | 1.03 | 1.28 | 1.36 | 1.50 | 1.18 | 1.11 |
| Calcium | 0.84 | 0.83 | 0.84 | 0.82 | 0.82 | 0.82 | 0.83 | 0.84 |
| Available phosphorus | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 |
| AA/Lys ratio, ⁵ % | 90 | 100 | 110 | 90 | 100 | 110 | 100 | 100 |
| Measured composition, % | | | | | | | | |
| Protein | 15.27 | 15.89 | 18.00 | 22.30 | 22.82 | 24.71 | 20.37 | 20.19 |
| Lipid | 5.84 | 6.77 | 6.64 | 7.67 | 8.43 | 8.76 | 5.23 | 8.90 |

¹Lys-/AA = Lys-deficient (8.0 g/kg) diet with an adequate amount of other essential amino acids (AA) calculated in relation to Lys; Lys-/AA- = Lys-deficient (8.0 g/kg) diet with a low amount of other essential AA calculated in relation to Lys; Lys-/AA+ = Lys-deficient (8.0 g/kg) diet with a high amount of other essential AA calculated in relation to Lys; Lys+/AA = Lys-rich (12.0 g/kg) diet with an adequate amount of other essential AA calculated in relation to Lys; Lys+/AA- = Lys-rich (12.0 g/kg) diet with a low amount of other essential AA calculated in relation to Lys; Lys+/AA+ = Lys-rich (12.0 g/kg) diet with a high amount of other essential AA calculated in relation to Lys; E- = low energy content (3,000 kcal/kg) diet; E+ = high energy content (3,300 kcal/kg) diet.

²Supplied per kilogram of diet: Co, 0.6 mg; Cu, 20 mg; Fe, 58 mg; I, 2 mg; Mn, 80 mg; Se, 0.2 mg; Zn, 90 mg; retinyl acetate, 4,300 IU; cholecalciferol, 4,300 IU; DL- α -tocopheryl acetate, 100 mg; thiamine mononitrate, 5 mg; riboflavin, 8 mg; calcium pantothenate, 25 mg; cyanocobalamin, 0.02 mg; menadione, 5 mg; pyridoxine hydrochloride, 7 mg; folic acid, 3 mg; biotin, 0.3 mg; niacin, 100 mg; choline chloride, 550 mg; antioxidant (butylhydroxyanisole, propyl gallate, ethoxyquin), 50 mg.

³Calculated value.

⁴Amino acids are given as true digestible values calculated according to Sauvant et al. (2004).

⁵Ratio between essential amino acids (other than Lys) and Lys, 100% corresponding to the control diet and to the ideal protein content described in Mack et al. (1999).

a control (**C**) diet (3,150 kcal/kg; 200 g/kg of CP; 10.0 g/kg of true digestible Lys) with adequate amounts of AA other than Lys (optimum profile), 6 diets isocaloric to the control diet including 3 Lys-deficient (8.0 g/kg) diets with an adequate (**Lys-/AA**), low (**Lys-/AA-**), or high (**Lys-/AA+**) amount of other essential AA calculated in relation to Lys, and 3 Lys-rich (12.0 g/kg) diets with an adequate (**Lys+/AA**), low (**Lys+/AA-**), or high (**Lys+/AA+**) amount of other essential AA calculated in relation to Lys, and 2 diets isoproteic to C with a high (3,300 kcal/kg, **E+**) or a low (3,000 kcal/kg, **E-**) energy content.

Measurements

Diet composition and pellet hardness were analyzed. Protein and lipid content were measured in each diet according to the Kjeldahl (AOAC Method 976.05; AOAC, 1990) and Folch et al. (1957) methods, respectively. Hardness was measured in 100 pellets of each diet using an Instron 5543 machine (Instron, Guyancourt Cedex, France) as described in Lecuelle et al. (2011). Hardness was expressed as the maximum load necessary to break the outer surface area of the pellet: $H = ML / (\pi \times l \times R)$, where **H** is hardness (MPa), **ML** is the maximum load (N), **l** is the length of the pellet (mm), and **R** is the radius of the pellet (mm).

Growth performance was recorded during the whole rearing period. At d 1, birds from the same pen were weighed together, and thereafter weighed individually at d 10, 24, and 36 after 6 h of fasting. We chose not to weigh broilers at d 33 (when finishing experimental diets started to be distributed) to avoid too many manipulations close to slaughter. Feed intake was recorded per pen at d 14, 24, 33, and 36. Average daily gain (**ADG**) and feed conversion ratio (**FCR**) were calculated between d 1 and 13, d 14 and 23, and d 24 and 36.

Slaughter Procedure and Carcass Measurements

At d 36, 8 chickens per pen (representing the average and the variability of the pen) were selected to be slaughtered the next day. The 360 chickens were slaughtered after 8 h of feed withdrawal at the experimental processing plant of the experimental poultry unit (PEAT, INRA, Nouzilly, France). The chickens were weighed, stunned in a water bath (120 Hz AC, 80 mA/bird, 5 s), and then killed by ventral neck cutting. After feather pecking and partial evisceration (only the gut was removed), whole carcasses were air-chilled (airflow of 7 m³/s) and stored at 2°C until the next day. Carcasses were weighed and deboned 1 d after slaughter. Abdominal fat and left pectoral muscles (pectoralis major and minor) were removed and weighed. At 24 h postmortem, the pHu of the breast pectoralis major muscle was measured with a portable pH meter (model 506, Crison Instruments SA, Alella, Barcelona, Spain)

by inserting a glass electrode directly in the thickest part of the pectoralis major. Breast color also was measured on the cranial, ventral side of the pectoralis major muscle by using a Miniscan spectrophotometer (Hunterlab, Reston, VA). Color was measured by the CIELAB trichromatic system as lightness (**L***), redness (**a***), and yellowness (**b***) values.

Statistical Analyses

All data were analyzed using SAS (SAS Institute Inc., Cary, NC). The type I error accepted was 5%. The effects of diet in relation to growth, body composition, and meat quality were analyzed by 1-way ANOVA (GLM procedure). The effects of AA and energy content were analyzed separately using the same control diet as comparison for each analysis. Orthogonal contrast analyses were performed to assess the respective effects of dietary Lys and AA (other than Lys) amounts and to compare each of these treatments against the control and each other. Pairwise comparisons of means for each significant effect were performed by Scheffe test using the LSMEANS statement of the GLM procedure. Values are expressed as least squares means and SEM. Pearson correlation coefficients were analyzed with the procedure CORR of SAS to assess the relationship between traits. Linear or nonlinear regression models were fitted between the pellet hardness, protein and lipid content, and feed intake to assess the relationship between these variables.

RESULTS

Growth Traits

Growth performance was similar for all groups until d 24 (data not shown). Body weight was not measured at d 33, but feed intake was similar between treatments during the d 24 to 32 period (Table 3). Between d 33 and 36, broiler feed intake was lower ($P < 0.001$) in Lys+/AA+ than in C and all Lys-deficient (Lys-) diet birds. The AA content significantly affected broiler growth performance during the finishing period. The FCR between d 24 and 36 were lower ($P < 0.001$) in broilers fed C and the 3 high Lys content (Lys+) diets than in those fed the Lys-/AA- diet. The ADG during the d 24 to 36 period was lower ($P < 0.001$) in Lys-/AA-, Lys+/AA, and Lys+/AA+ than in C birds, with the other diets yielding intermediate values. The ADG and the BW at d 36 were lower ($P < 0.001$) in Lys-/AA- and Lys+/AA+ than in C birds. Orthogonal contrast analyses showed that increasing dietary Lys content (from 8.0 to 12.0 g/kg) decreased feed intake between d 33 and 36 ($P < 0.001$) and FCR between d 24 and 36 ($P < 0.001$). When compared with C, Lys- diets increased FCR ($P < 0.001$) and decreased ADG between d 24 and 36 ($P < 0.001$) and final BW ($P = 0.003$). When compared with C, Lys+ diets decreased feed intake between d 33 and 36, with

negative effects ($P < 0.001$) on FCR between d 24 and 36, ADG between d 24 and d 36, and BW at d 36. The amount of AA relative to Lys also affected feed intake and growth performances. Feed intake between d 33 and 36 was lower in AA+ compared with AA ($P = 0.003$) and AA- ($P = 0.05$). When compared with AA, AA- showed higher FCR ($P = 0.004$) and lower ADG ($P < 0.001$) between d 24 and 36, and lower BW at d 36 ($P = 0.006$). The AA+ showed also lower feed intake between d 33 and 36 ($P = 0.003$), ADG between d 24 and 36 ($P = 0.02$), and final BW ($P = 0.03$) than AA. When compared with C, AA- diets increased FCR ($P = 0.003$) and decreased ADG between d 24 and 36, and decreased BW at d 36 ($P < 0.001$). When compared with C, AA+ diets decreased feed intake between d 33 and 36 ($P = 0.01$) and increased FCR between d 24 and 36 ($P = 0.03$), negatively affecting final BW ($P < 0.001$).

Feed consumption and growth performance varied according to dietary energy content (Table 3). Feed intake between d 33 and 36 was lower ($P < 0.001$) with the E+ than with the C and E- diets whereas FCR between d 24 and 36 was higher ($P = 0.03$) with the E- than with the C diet. The ADG during this period

was lower ($P < 0.001$) with E- and E+ compared with C. Over the whole period, feed intake was unchanged by dietary energy content but FCR was higher ($P = 0.02$) with the E- and E+ compared with the C diet. Overall ADG and BW were the highest with the control diet, and significantly different ($P < 0.006$) from the E+ diet.

Diet Composition and Hardness, and Relationship with Feed Intake

Protein and lipid content and pellet hardness of finishing diets are shown in Tables 2 and 4, respectively. The lipid and protein content of the experimental diets were consistent with the calculated values (Table 2). Pellet hardness differed significantly ($P < 0.001$) among diets varying in AA content, the highest value being observed in the Lys-/AA- and the lowest in the Lys+/AA+ diets that had the lowest and highest lipid and protein content, respectively. The pellets of the E+ diet were also softer ($P < 0.001$) than those of the C and E- diets. There was a negative exponential correlation between pellet hardness and the lipid content of the diet [$y = 621.6e^{-0.27x}$, where x is diet lipid

Table 3. Effects of variations in amino acid (AA) and energy content on broiler feed intake, feed conversion ratio (FCR), average daily gain (ADG), and BW at slaughter¹

| Item ² | Feed intake, g/d 33 to 36 d | FCR 24 to 36 d | ADG, g/d 24 to 36 d | BW 36 d, g |
|--------------------------------|--------------------------------|--------------------|------------------------|---------------------|
| Diet | | | | |
| Control | 216.0 ^a | 1.64 ^b | 117.0 ^a | 2,763 ^a |
| Lys-/AA- | 216.1 ^a | 1.77 ^a | 106.2 ^c | 2,636 ^b |
| Lys-/AA | 223.8 ^a | 1.70 ^{ab} | 112.8 ^{ab} | 2,696 ^{ab} |
| Lys-/AA+ | 214.8 ^a | 1.70 ^{ab} | 112.1 ^{abc} | 2,710 ^{ab} |
| Lys+/AA- | 206.1 ^{ab} | 1.66 ^b | 112.4 ^{ab} | 2,695 ^{ab} |
| Lys+/AA | 205.3 ^{ab} | 1.67 ^b | 110.6 ^b | 2,687 ^{ab} |
| Lys+/AA+ | 190.1 ^b | 1.67 ^b | 108.4 ^b | 2,646 ^b |
| SEM | 4.2 | 0.02 | 1.4 | 18 |
| Level of significance | <0.001 | <0.001 | <0.001 | <0.001 |
| Contrast estimate ³ | | | | |
| Lys- vs. C | NS | +0.08 (<0.001) | -6.6 (<0.001) | -83 (<0.001) |
| Lys+ vs. C | -15.5 (0.003) | -0.03 (<0.001) | -6.5 (<0.001) | -88 (<0.001) |
| Lys+ vs. Lys- | -17.7 (<0.001) | -0.05 (<0.001) | NS | NS |
| AA- vs. C | NS | NS | -7.7 (<0.001) | -98 (<0.001) |
| AA+ vs. C | -13.5 (0.01) | +0.04 (0.03) | -6.8 (<0.001) | -85 (<0.001) |
| AA+ vs. AA- | -8.7 (0.05) | NS | NS | NS |
| AA- vs. AA | NS | +0.03 (0.004) | -2.4 (<0.001) | -26 (0.006) |
| AA+ vs. AA | -12.1 (0.003) | NS | -1.5 (0.02) | -14 (0.03) |
| Control | 216.0 ^a | 1.64 ^b | 117.0 ^a | 2,763 ^a |
| E- | 222.1 ^a | 1.72 ^a | 111.0 ^b | 2,701 ^{ab} |
| E+ | 199.9 ^b | 1.70 ^{ab} | 108.1 ^b | 2,651 ^b |
| SEM | 2.8 | 0.02 | 1.6 | 20 |
| Level of significance | <0.001 | 0.03 | <0.001 | 0.006 |

^{a-c}Least squares means within a column with no common superscripts are significantly different ($P \leq 0.05$).

¹Data presented as least squares means (n = 5 pens by treatment).

²Control (C) = diet containing 10.0 g/kg of true digestible Lys and an adequate amount of other essential AA calculated in relation to Lys; Lys-/AA = Lys-deficient (8.0 g/kg) diet with an adequate amount of other essential AA calculated in relation to Lys; Lys-/AA- = Lys-deficient (8.0 g/kg) diet with a low amount of other essential AA calculated in relation to Lys; Lys-/AA+ = Lys-deficient (8.0 g/kg) diet with a high amount of other essential AA calculated in relation to Lys; Lys+/AA = Lys-rich (12.0 g/kg) diet with an adequate amount of other essential AA calculated in relation to Lys; Lys+/AA- = Lys-rich (12.0 g/kg) diet with a low amount of other essential AA calculated in relation to Lys; Lys+/AA+ = Lys-rich (12.0 g/kg) diet with a high amount of other essential AA calculated in relation to Lys; E- = low energy content (3,000 kcal/kg) diet; E+ = high energy content (3,300 kcal/kg) diet.

³Contrast estimates between treatments are only presented if significant (level of significance in parentheses). A vs. B means A - B. C = control diet; Lys- = Lys-deficient (8.0 g/kg) diets; Lys+ = Lys-rich (12.0 g/kg) diets; AA = diets with an adequate amount of other essential AA calculated in relation to Lys; AA+ = diets with a high amount of other essential AA calculated in relation to Lys; AA- = diets with a low amount of other essential AA calculated in relation to Lys.

Table 4. Effects of variations in amino acid (AA) and energy content on pellet hardness of experimental finisher diets

| Item ¹ | Pellet hardness, MPa |
|-----------------------|----------------------|
| Diet | |
| Control | 96.7 ^c |
| Lys-/AA- | 194.0 ^a |
| Lys-/AA | 136.7 ^b |
| Lys-/AA+ | 103.2 ^{bc} |
| Lys+/AA- | 68.1 ^{cd} |
| Lys+/AA | 72.6 ^{cd} |
| Lys+/AA+ | 51.6 ^d |
| SEM | 8.4 |
| Level of significance | <0.001 |
| Control | 96.7 ^a |
| E- | 96.4 ^a |
| E+ | 53.8 ^b |
| SEM | 6.0 |
| Level of significance | <0.001 |

^{a-d}Least squares means within a column with no common superscript are significantly different ($P \leq 0.05$), sample size = 100.

¹Control = diet containing 10.0 g/kg of true digestible Lys and an adequate amount of other essential AA calculated in relation to Lys; Lys-/AA = Lys-deficient (8.0 g/kg) diet with an adequate amount of other essential AA calculated in relation to Lys; Lys-/AA- = Lys-deficient (8.0 g/kg) diet with a low amount of other essential AA calculated in relation to Lys; Lys-/AA+ = Lys-deficient (8.0 g/kg) diet with a high amount of other essential AA calculated in relation to Lys; Lys+/AA = Lys-rich (12.0 g/kg) diet with an adequate amount of other essential AA calculated in relation to Lys; Lys+/AA- = Lys-rich (12.0 g/kg) diet with a low amount of other essential AA calculated in relation to Lys; Lys+/AA+ = Lys-rich (12.0 g/kg) diet with a high amount of other essential AA calculated in relation to Lys; E- = low energy content (3,000 kcal/kg) diet; E+: high energy content (3,300 kcal/kg) diet.

content (%) and y is pellet hardness (MPa); $r = -0.79$, $P < 0.01$, $n = 9$] for all the finisher diets. The negative correlation between pellet hardness and dietary lipid content was even greater ($y = 1,883e^{-0.4x}$, $r = -0.94$, $P < 0.01$, $n = 6$) when only diets differing in AA content were considered. A strong negative correlation was also found between diet protein content and pellet hardness [$y = 1 \times 10^5 x^{-2.343}$, where x is diet protein content (%) and y is pellet hardness (MPa); $r = -0.97$, $P < 0.001$, $n = 6$] in these conditions.

There were linear negative correlations between bird feed intake and the protein and lipid content of the diet ($r = -0.84$ and $r = -0.75$, $P < 0.01$, respectively, $n = 9$) during the d 33 to 36 period. A strong linear negative relationship ($r = -0.95$, $P < 0.01$, $n = 6$) was also revealed for broiler feed intake and the hardness differential between the control and experimental diets that were softer than the control diet (Figure 1). This relationship was not found for diets harder than the control diet.

Carcass and Breast Meat Traits

Body composition (i.e., breast meat yield and abdominal fat percentage) was not different between diets (Table 5). However, increasing dietary Lys from 8.0 to

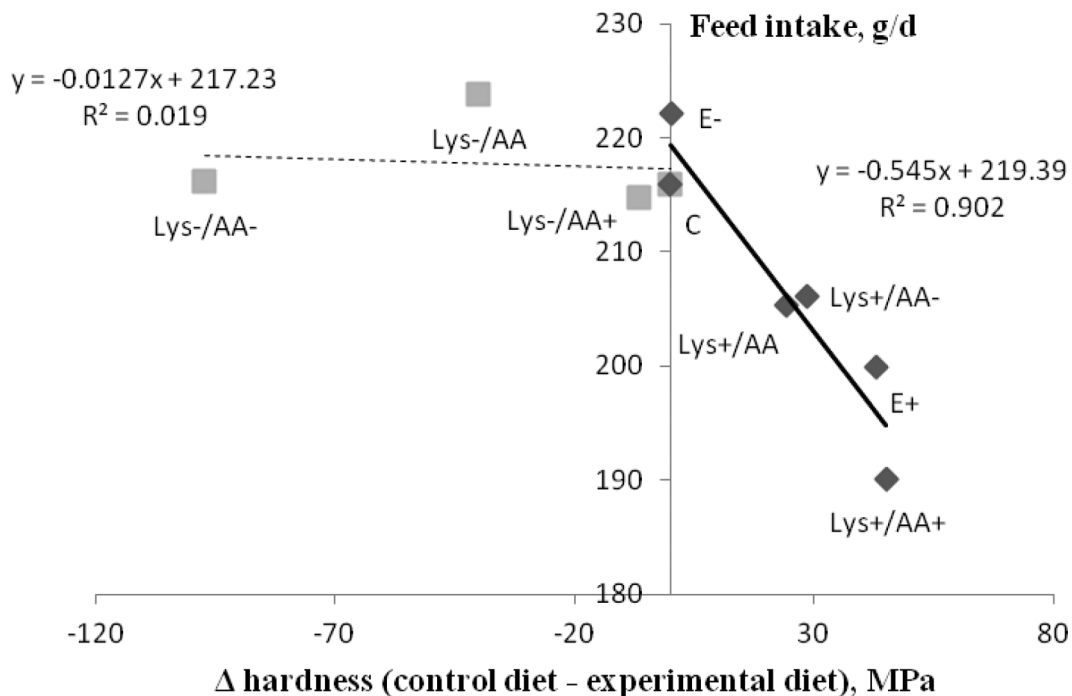


Figure 1. Effects of hardness differential [Δ hardness (control diet – experimental diet), in MPa] between control and experimental diets on chicken feed intake (in g/d) between d 33 and 36. Linear regression lines were calculated separately for harder diets (diets are represented by ■, and regression lines by a dotted line) and softer diets (diets are represented by ◆, and regression lines by a solid line) compared with the control diet. C = diet containing 10.0 g/kg of true digestible Lys and an adequate amount of other essential amino acids (AA) calculated in relation to Lys; Lys-/AA = Lys-deficient (8.0 g/kg) diet with an adequate amount of other essential AA calculated in relation to Lys; Lys-/AA- = Lys-deficient (8.0 g/kg) diet with a low amount of other essential AA calculated in relation to Lys; Lys-/AA+ = Lys-deficient (8.0 g/kg) diet with a high amount of other essential AA calculated in relation to Lys; Lys+/AA = Lys-rich (12.0 g/kg) diet with an adequate amount of other essential AA calculated in relation to Lys; Lys+/AA- = Lys-rich (12.0 g/kg) diet with a low amount of other essential AA calculated in relation to Lys; Lys+/AA+ = Lys-rich (12.0 g/kg) diet with a high amount of other essential AA calculated in relation to Lys; E- = low energy content (3,000 kcal/kg) diet; E+: high energy content (3,300 kcal/kg) diet.

12.0 g/kg decreased ($P = 0.05$) the abdominal fat percentage relative to BW.

The AA content of the finisher diet affected breast pHu and color. The pHu was lower ($P < 0.001$) in broilers fed the Lys-/AA+ diet than in those fed the Lys-/AA-, Lys-/AA, Lys+/AA-, and Lys+/AA diets. The pHu was negatively correlated with the L* and b* values ($r = -0.67$ and -0.35 , $P < 0.0001$, respectively) but not with a*. In consequence, the breast meat from broilers fed the Lys-/AA+ diet showed higher L* and b* values ($P < 0.001$ for both) than that from broilers fed the Lys-/AA-, Lys-/AA, and Lys+/AA- diets. Increasing dietary Lys from 8.0 to 12.0 g/kg increased ($P = 0.007$) the pHu and decreased ($P = 0.006$) the a* of breast meat. The a* of breast meat was also lower ($P = 0.03$) in AA- than in AA. An excess amount of other essential AA relative to Lys (AA+) led to lower pHu and higher L* and b* of breast meat ($P < 0.001$) compared with AA and AA- diets. Variations in pHu according to the amount of digestible Lys and other AA reported in Figure 2 emphasize that the highest pH values (>5.87) were observed in broilers fed diets containing high Lys content and low or adequate amounts of other essential AA calculated in relation to Lys. In contrast, the lowest pH values (<5.81) were observed in broilers fed Lys-deficient diets containing a high amount of other essential AA relative to Lys. Moreover, significant positive and negative linear relationships were observed between pHu and Lys content of the diet ($y = 0.11x + 5.75$; $r = 0.95$) and between pHu and amounts of other AA relative to Lys ($y = -0.004x + 6.25$; $r = -0.93$), respectively. The pHu and color of breast meat were not affected by dietary energy content (Table 5).

DISCUSSION

Our hypothesis was that changes in dietary AA or energy content in the diet distributed a few days before slaughter could affect breast muscle meat characteristics, with a limited impact on broiler growth performance and body composition. Indeed, the pHu and color parameters of breast meat were significantly affected by changes in AA content but not by changes in energy content of the diet distributed to broilers 3 d before slaughter. In chickens, the pHu of breast meat is mainly determined by the amount of glycogen stored in muscle at death (also referred as glycolytic potential; Le Bihan-Duval et al., 2008), muscle with the highest glycogen content producing the meat with the lowest final pH value. Variations in pH caused by changes in dietary AA content is therefore probably a consequence of changes in glycogen content of broiler breast muscle, although this parameter was not measured in the present study. As widely reported in the literature, Lys supplementation above the recommendations promotes protein synthesis and increases breast muscle yield in chickens (Tesseraud et al., 1996a, 1999; Berri et al., 2008). This may limit the use of other AA for energy

purposes, including storage as muscle glycogen, especially when the amounts of other essential AA are low. This is consistent with our findings indicating that the highest pH values were obtained when the Lys ratio to other AA was the highest (Figure 2). On the other hand, protein synthesis is limited in cases of Lys deficiency (Tesseraud et al., 1996b, 2001) and the proportion of nutrients used for energy storage may increase, especially when chickens are subjected to an excess intake of AA relative to Lys, leading to the decreased pH observed in such conditions (Figure 2).

Changes in pHu caused by variations in dietary AA profiles were fairly moderate (0.14 pH unit separating treatments with the highest and the lowest average pH value); however, they significantly affected breast meat lightness, which is strongly correlated with water holding capacity of meat and therefore processing yield (Qiao et al., 2002; Petracci et al., 2004; Zhuang and Savage, 2010). Given that broiler breast meat is mainly used for portioning or further processing, these findings are of particular importance for the poultry meat industry.

Lysine supplementation at contents above the requirements for maximum growth results in specific and significant effects on body composition (Leclercq, 1998). The breast meat yield is increased and the abdominal fat percentage is reduced (Hickling et al., 1990; Moran and Bilgili, 1990; Grisoni et al., 1991; Berri et al., 2008). In the present study, increasing dietary Lys from 8.0 to 12.0 g/kg 3 d before slaughter had no effect on breast meat yield and had only a limited impact on abdominal fat percentage, which was lower. However, increasing dietary Lys before slaughter decreased feed intake and therefore affected broiler performance, especially when a high amount of Lys was combined with a high amount of other essential AA. Increasing dietary energy content from 3,150 to 3,300 kcal/kg negatively affected feed intake and final BW, whereas decreasing it from 3,150 to 3,000 kcal/kg principally increased FCR without significant impact on BW at slaughter. Our results indicated that decreased feed consumption may be related to feed pellet softness, which, as previously shown, increases with the amount of dietary lipids and proteins (Chagneau et al., 2006): the more softness increased between the control and the experimental diets, the greater the decrease in feed consumption. By contrast, increasing pellet hardness over that of the control diet barely altered broiler feed intake, suggesting greater acceptance of hard feed pellets by broilers. Changing feed composition may also impair feed efficiency and growth, especially when all AA including Lys are deficient. It is worthy to mention possible diluted effects on ADG and FCR values, which were calculated over the whole finishing (d 24 to 36) instead of the experimental (d 33 to 36) period. The consequences on feed consumption, feed efficiency, and breast muscle characteristics must therefore be considered to optimize food transition and to determine the best strategies based on production and quality objectives.

Table 5. Effects of variations in amino acid (AA) and energy content on body composition and breast meat quality

| Item ¹ | Carcass trait | | | Breast meat trait | | |
|--------------------------------|-----------------------|------------------------------|--------------------|--------------------|---------------------|---------------------|
| | Breast meat yield (%) | Abdominal fat percentage (%) | Ultimate pH | Lightness (L*) | Redness (a*) | Yellowness (b*) |
| Diet | | | | | | |
| Control | 19.5 | 2.32 | 5.85 ^{ab} | 49.6 ^{ab} | -0.57 ^{ab} | 9.90 ^{ab} |
| Lys-/AA- | 19.6 | 2.42 | 5.87 ^a | 48.9 ^b | -0.63 ^{ab} | 9.76 ^b |
| Lys-/AA | 19.1 | 2.24 | 5.86 ^a | 48.3 ^b | -0.19 ^a | 9.72 ^b |
| Lys-/AA+ | 19.3 | 2.27 | 5.76 ^b | 51.2 ^a | -0.46 ^{ab} | 10.55 ^a |
| Lys+/AA- | 19.4 | 2.27 | 5.90 ^a | 49.3 ^b | -0.75 ^b | 9.46 ^b |
| Lys+/AA | 19.6 | 2.17 | 5.90 ^a | 49.5 ^{ab} | -0.58 ^{ab} | 9.69 ^b |
| Lys+/AA+ | 19.5 | 2.19 | 5.84 ^{ab} | 50.1 ^{ab} | -0.62 ^{ab} | 10.03 ^{ab} |
| SEM | 0.2 | 0.06 | 0.02 | 0.45 | 0.11 | 0.18 |
| Level of significance | NS | 0.09 | <0.001 | <0.001 | 0.01 | 0.002 |
| Contrast analysis ² | | | | | | |
| Lys- vs. C | NS | NS | NS | NS | NS | NS |
| Lys+ vs. C | NS | NS | NS | NS | NS | NS |
| Lys+ vs. Lys- | NS | -0.10 (0.05) | +0.05 (0.007) | NS | -0.24 (0.006) | NS |
| AA- vs. C | NS | NS | NS | NS | NS | NS |
| AA+ vs. C | NS | NS | NS | NS | NS | NS |
| AA+ vs. AA- | NS | NS | -0.08 (<0.001) | +1.5 (<0.001) | NS | +0.66 (<0.001) |
| AA- vs. AA | NS | NS | NS | NS | -0.28 (0.03) | NS |
| AA+ vs. AA | NS | NS | -0.08 (<0.001) | +1.6 (<0.001) | NS | +0.59 (<0.001) |
| Control | 19.5 | 2.32 | 5.85 | 49.64 | -0.57 | 9.90 |
| E- | 19.6 | 2.25 | 5.85 | 49.42 | -0.28 | 10.19 |
| E+ | 19.3 | 2.38 | 5.88 | 49.39 | -0.48 | 9.78 |
| SEM | 0.2 | 0.07 | 0.02 | 0.42 | 0.13 | 0.21 |
| Level of significance | NS | NS | NS | NS | NS | NS |

^{a,b}Least squares means within a column with no common superscript are significantly different ($P \leq 0.05$), sample size = 40.

¹Control = diet containing 10.0 g/kg of true digestible Lys and an adequate amount of other essential AA calculated in relation to Lys; Lys-/AA- = Lys-deficient (8.0 g/kg) diet with a low amount of other essential AA calculated in relation to Lys; Lys-/AA+ = Lys-deficient (8.0 g/kg) diet with an adequate amount of other essential AA calculated in relation to Lys; Lys+/AA- = Lys-rich (12.0 g/kg) diet with a low amount of other essential AA calculated in relation to Lys; Lys+/AA+ = Lys-rich (12.0 g/kg) diet with a high amount of other essential AA calculated in relation to Lys; E- = low energy content (3,000 kcal/kg) diet; E+ = high energy content (3,300 kcal/kg) diet.

²Contrast estimates between treatments are only presented if significant (level of significance in parentheses). A vs. B means A - B. C = control diet; Lys- = Lys-deficient (8.0 g/kg) diets; Lys+ = Lys-rich (12.0 g/kg) diets; AA- = diets with an adequate amount of other essential AA calculated in relation to Lys; AA+ = diets with a high amount of other essential AA calculated in relation to Lys; AA- = diets with a low amount of other essential AA calculated in relation to Lys.

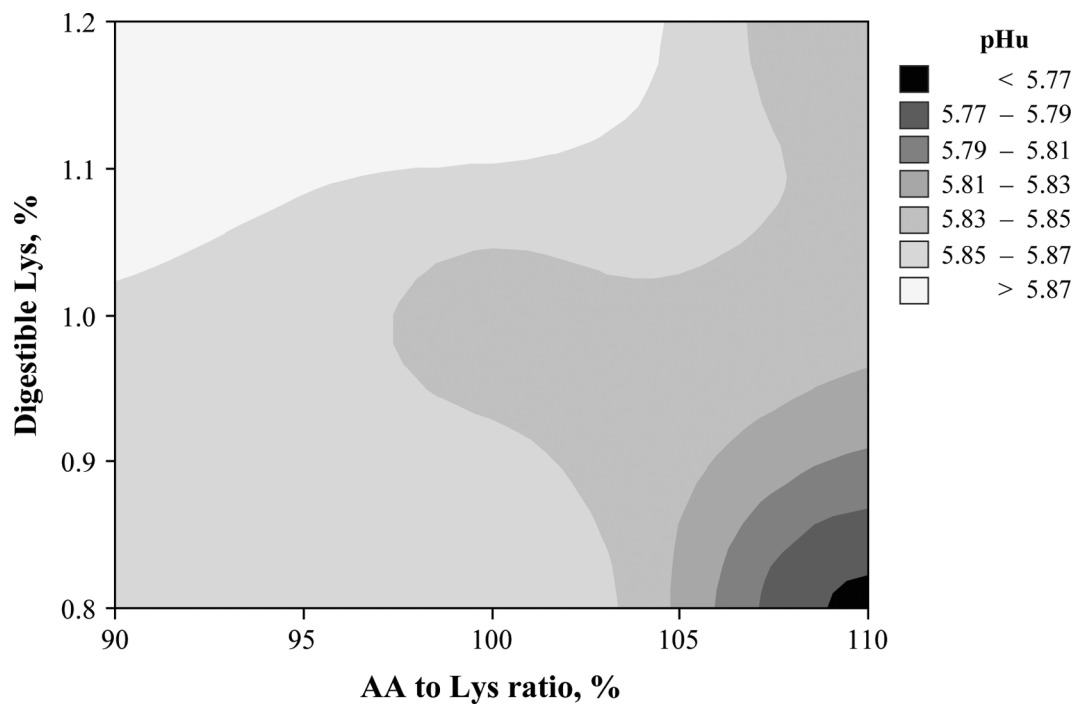


Figure 2. Graphical representation of the respective effects of digestible Lys content (%) and the other essential amino acids (AA; amino acid to Lys ratio, 100% corresponding to the control diet ratio) on the ultimate pH (pHu) of chicken breast meat.

Taken together, our findings show that changing AA content in the diet 3 d before slaughter can affect the final pH and color of broiler breast meat. This may slightly impair broiler growth performance (variations less than 4 and 3% for FCR and BW, respectively) and carcass composition (less than 1% for breast meat yield) because of changes in pellet hardness that affected feed consumption. On the hypothesis that raising the pH improves the processing ability of the meat, it is possible to achieve this by increasing the dietary supply of Lys while decreasing other AA over a short period before slaughter. This opens up new scope for improving and homogenizing the quality of poultry meat through broiler nutrition, although improving feed acceptability and modeling the metabolic response thresholds of the birds to changes in AA content is essential before considering the introduction of this new concept in feeding strategies for poultry species.

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