Research Notes

Effect of sequential feeding on nitrogen excretion, productivity, and meat quality of broiler chickens[1]

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ABSTRACT The aim of this trial was to investigate the effect of a 24-h cycle sequential feeding program on nitrogen excretion, incidence of foot pad lesions, productive performance, quality traits, and chemical composition of broiler chicken breast meat. In total, 1,320 one-day-old male Cobb 700 chicks were split into 2 groups of 6 replicates each. From 1 to 10 d of age, all of the chickens received the same prestarter diet (ME 3,058 kcal/kg; CP 226 g/kg). The control group (CON) received 1 of 3 diets for 24-h cycles: starter (ME 3,162 kcal/kg; CP 205 g/kg), grower (ME 3,224 kcal/kg; CP 192 g/kg), and finisher diets (ME 3,242 kcal/kg; CP 184 g/kg) from d 11 to 18, 19 to 38, and 39 to 44 of age, respectively. The sequential feeding group (SF) received the same diets as the CON birds for half of the day, and then low-protein and isoenergetic diets for the remaining half of the day. Birds submitted to the SF program showed better utilization of dietary nitrogen compared with the CON birds (45.0 vs. 46.1% of N excreted/N ingested, respectively; $P < 0.05$), and consequently the SF birds had lower nitrogen excretion compared with the CON birds (24.8 vs. 25.9 N g/kg of BW, respectively; $P < 0.01$). The SF birds exhibited a significantly lower incidence (7 vs. 13%) of foot pad lesions and consumed 70 g of feed/bird more than the CON birds. The SF birds also had a significantly higher feed conversion rate compared with that of the CON birds (1.84 vs. 1.78, respectively). The SF breast meat exhibited a significantly lower ultimate pH, a higher cook loss, and a lower lipid content compared with the values found for the CON group. The SF approach in poultry husbandry had positive repercussions on environmental and animal welfare aspects, but adversely affected feed efficiency, and altered some meat traits (mainly pH and cook loss).

Key words: sequential feeding, nitrogen excretion, meat quality, foot pad lesion, broiler chicken

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INTRODUCTION

The contribution of animal production to environmental pollution is a crucial issue worldwide, particularly in the European Union, and is mostly characterized by high concentrations of farmed animals and limited land availability to spread manure.

To limit water pollution, the European Union, through directive 91/676/CEE (European Commission, 1991), ruled that 170 kg of N was the maximum quantity that can be spread per year per hectare of land. In practice, the application of this directive may limit the number of animals that can be reared per unit of land. Moreover, the Integrated Pollution Prevention and Control (IPPC) directive 2008/1/EC (European Commission, 2008) requires that farmers adopt the best available techniques to reduce environmental pollution. These include equipment, facilities, and management, but also nutritional and feeding strategies.

In poultry production, the 2 main strategies focused on reducing N emission are the use of synthetic amino acids and enzyme supplementation (Nahm, 2007). A low-protein diet supplemented with essential amino acids reduced N excretion by 10 to 27% in broiler production and by 18 to 35% in chicks and layer production (Blair et al., 1999). Nitrogen output in broiler production can also be limited by the addition of dietary enzymes that improve protein digestibility by destroying antinutritional factors (Bedford and Morgan, 1996).

Sequential feeding (**SF**) is a strategy consisting of alternating diets with different nutritional contents over a cycle of a specified period of time. Sequential feeding has been studied and applied both in broiler and laying hens to improve nutrient utilization and welfare conditions. Umar Faruk et al. (2010) found that SF of whole

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wheat grain and protein mineral concentrate reduced the total feed intake and improved the feed efficiency of laying hens. By alternating low- and high-lysine diets during the day, Bizeray et al. (2002) reported better leg condition of meat-type chickens. However, the application of SF programs to control N excretion in poultry production, as well as to reduce the incidence of foot pad lesions, has not been studied.

The aim of this trial was to investigate the effect of a 24-h SF cycle on N excretion, incidence of foot pad dermatitis, and quality traits and chemical composition of broiler chicken-breast meat, as well as on productive performance.

MATERIALS AND METHODS

In total, 1,320 one-day-old male Cobb 700 chicks were split into 2 groups and randomly distributed in 12 pens (3 × 4 m, 110 chicks/pen) in an environmentally controlled poultry house. Birds received the same light regimen of 23L:1D. The pen floors were covered with wood shavings (4 kg/m^2). From 1 to 10 d of age all of the chickens received the same prestarter diet (ME 3,058 kcal/kg; CP 226 g/kg). The control group (**CON**) received starter (ME 3,162 kcal/kg; CP 205 g/kg), grower (ME 3,224 kcal/kg; CP 192 g/kg), and finisher diets (ME 3,242 kcal/kg; CP 184 g/kg) from 11 to 18, 19 to 38, and 39 to 44 d of age, respectively, for 24-h cycles. The SF group received the same diets as the CON birds for half of the day, and low-protein and isoenergetic diets for the remaining half of the day (Table 1). In detail, SF birds were fed control diets from 1200 to 2330 h and low-protein diets from 0030 (after 1 h of dark) to 1200 h. Feed and water were provided on an ad libitum basis. Each pen was equipped with a feeder designed with a specific edge to avoid feed spillage. At the beginning of each feeding phase, the feeds were put into 1 bin per pen, clearly labeled, weighed, sampled, and analyzed for N content. From the bin, the feed was manually taken and put into the feeder daily. At the end of the feeding phase, the remaining feed from the feeder and the bin was weighed. In the SF groups, 2 feeders and 2 bins were alternately used to supply the 2 different diets.

Feed intake and live BW were recorded at the end of each feeding phase. Mortality was calculated by recording the number, age, and weight of each dead bird. The feed conversion rate was calculated on a pen basis as total feed intake to total live BW, and corrected for mortality.

At the end of the trial, broilers were subjected to a total feed withdrawal for 12 h, which included a holding

Table 1. Composition and calculated analysis of diets for control (CON) and sequential fed (SF) chickens

	Prestarter	Starter		Grower		Finisher	
Item	CON and SF	CON	SF		SF	CON	SF
Bird age (d)	0–10	11–18		19–38		39–44	
Ingredients (g/kg)							
Corn	350	250	250	100	100	100	100
Wheat	201	273	327	446	503	475	523
Sorghum	—	50	50	100	100	100	100
Soybean extracted meal	210	194	131	210	163	186	148
Soybean whole seeds	150	100	100	50	50	50	50
Wheat shorts	0	30	49	0	0	0	0
Corn gluten meal	30	20	20	0	0	0	0
Vegetable oil	17	42	36	60	53	58	52
Dicalcium phosphate	13	10	10	7	7	5	5
Calcium carbonate	9	9	9	9	9	9	9
Sodium bicarbonate	1	1	1	1	1	1	1
Salt	3	2	2	2	2	2	2
Choline chloride	1	1	1	1	1	1	1
Methionine hydroxy-analog	2	1	1	1	1	1	1
DL-Methionine	1	1	1	2	1	2	1
Lysine sulfate	4	4	3	4	2	4	2
Threonine	0.6	0.5	0	0.7	0	0.8	0
Vitamin-mineral premix[1]	4	4	4	3	3	2	2
Hostazyme 500	0	0	0	0.5	0.5	0.5	0.5
Avyzyme	1	1	1	0	0	0	0
Calculated composition							
ME (kcal/kg)	3,058	3,162	3,155	3,224	3,219	3,242	3,237
DM (g/kg)	880	883	883	887	887	887	887
CP[2] (g/kg)	226	205	182	192	173	184	168
Lipid (g/kg)	63	79	73	84	78	82	77
Crude fiber (g/kg)	28	27	27	25	24	25	24
Ash (g/kg)	56	51	50	46	45	43	42
Energy:protein ratio	13.5	15.4	17.3	16.8	18.6	17.6	19.3

[1]Provided the following per kg of diet: vitamin A (retinyl acetate), 13,000 IU; vitamin D_3 (cholecalciferol), 4,000 IU; vitamin E (DL-α-tocopheryl acetate), 80 IU; vitamin K (menadione sodium bisulfite), 3 mg; riboflavin, 6.0 mg; pantothenic acid, 6.0 mg; niacin, 20 mg; pyridoxine, 2 mg; folic acid, 0.5 mg; biotin, 0.10 mg; thiamine, 2.5 mg; vitamin B_{12}, 20 μg; Mn, 100 mg; Zn, 85 mg; Fe, 30 mg; Cu, 10 mg; I, 1.5 mg; Se, 0.2 mg; ethoxyquin, 100 mg.

[2]Determined concentration.

time of 2 h at the processing plant. In total, 18 birds per group (3 birds/replicate) were gas stunned and killed by neck dislocation. Each bird was immediately frozen with liquid N, thoroughly ground, and analyzed for body N content in order to assess the N balance in accordance with the methodology proposed by the European Commission (2002). The value of excreted N is the difference between the nitrogen inputs (N content of 1-d-old chicks plus the N content from feed ingested) and the N retained in the body (computed by multiplying the BW of the birds at the slaughterhouse by the respective body concentration of N plus the amount of N contained in the birds that died during the rearing period). The N excretion was calculated as grams per kilogram of BW and as grams per bird. This indirect method of calculation for the estimation of N excretion was suggested by the European Commission (2002) because it is considered to be more precise than the direct method of calculation, which is based on the measurement of N in excreta. The latter method does not consider the N lost by ammonia emission.

The remaining birds were subsequently processed under commercial conditions using electrocution (120 V, 200 Hz) as a stunning system. For each replicate, all of the feet were systematically collected for macroscopic examination, and scored in 3 classes of foot pad dermatitis (**FPD**): 0 = no lesions, 1 = mild lesions, and 2 = severe lesions, according to the classification of Ekstrand et al. (1997). After chilling, 16 carcasses per group were randomly collected and used for subsequent meat quality analysis. Carcasses were deboned 24-h postmortem, and breast fillets (pectoralis major) were used to determine color profile, ultimate pH, drip and cook loss, and Allo-Kramer (**AK**) shear values after cooking. Moisture, protein, total lipid and ash were also determined. The CIE (1978) system for color profile of lightness (L*), redness (a*), and yellowness (b*) was performed by a reflectance colorimeter (Minolta Chroma Meter CR-300, Minolta Italia SpA, Milan, Italy) using illuminant source C. Breast meat color was evaluated by averaging 3 measurements taken on the medial surface of the fillet (bone side) in an area free of obvious color defects. Breast meat pH was measured 24-h postmortem (ultimate pH) using a modified iodoacetate method, initially described by Jeacocke (1977). Approximately 2.5 g of meat was removed from the cranial end of the pectoralis major, minced by hand, and homogenized in 25 mL of a 5 mM iodoacetate solution with 150 mM of potassium chloride for 30 s. The pH of the homogenate was determined using a pH meter (pH meter Crison Basic 20+, Crison Strumenti, SpA, Carpi, Italy) calibrated at pH 4.0 and 7.0. The drip loss was analyzed on 1 intact fillet from each of the 16 birds per group that were kept suspended in a sealed glass box for 48 h at 2 to 4°C, and calculated as percentage of weight loss during storage. The cook loss was measured by cooking intact muscles (pectoralis major) on aluminum trays in a convection oven (180°C) until the core of each sample reached 80°C. The samples were then equilibrated to room temperature and reweighed, after which the cook loss was determined as a percentage of weight loss. The shear values were determined using an Instron universal testing machine (ITW 3600 Instron, Glenview, IL) equipped with an AK shear cell. The cooked meat samples (approximately 2 × 4 × 1 cm) were cut parallel to the direction of the muscle fiber for each fillet (pectoralis major: cranial position), and sheared with the blades at a right angle to the fibers using a 250-kg load cell and crosshead speed of 500 mm/min (Papinaho and Fletcher, 1996). The AK shear values were reported as kilograms of shear per gram of sample. The percentage of moisture was determined in duplicate, according to the AOAC procedure (AOAC, 1990). Proteins were determined using the standard Kjeldahl copper catalyst method (AOAC, 1990). The total lipids were measured using the chloroform to methanol procedure described by Folch et al. (1957). Finally, the ash was determined using the procedure described by the AOAC (1990). The data were evaluated using a 1-way ANOVA, and percentage values were previously submitted to arcsine transformation (GLM; SAS Institute, 1988).

RESULTS AND DISCUSSION

The productive results are shown in Table 2. At the end of the trial (d 44), birds from both groups had reached remarkable live BW (3,913 for CON and 3,948 g for SF) that are higher than the performance goals of Cobb 700. The SF birds consumed 70 g of feed/bird more than the CON birds, and had a significantly higher feed conversion rate (1.84 vs. 1.78, respectively). Bouvarel et al. (2008) used a 48-h sequential feeding program with isoenergetic low- and high-protein content feeds (CP: 156 and 234 g/kg feed, respectively), and observed no difference in BW, and a significantly worse feed efficiency in birds submitted to the sequential feeding program.

The results for N balance are shown in Table 3. The amount of N intake was lower in SF birds compared with CON birds (55.2 vs. 56.2 g/kg of live BW, respectively; $P < 0.01$), whereas the N retained in the body was almost the same in both groups (30.4 and 30.3 g/kg of live BW, respectively). As a consequence, birds submitted to the SF program showed better utilization of dietary N compared with that of the CON birds (45.0 vs. 46.1% of N excreted/N ingested, respectively; $P < 0.05$), and lower N excretion compared with that of the CON birds (24.8 vs. 25.9 N g/kg of live BW, respectively; $P < 0.01$). In general, chickens fed sequential diets varying in energy content quickly develop a preference for the higher energy feed, and consequently adapt their intake. However, the variation of the protein content in sequential diets leads to lower effects on feed intake, occurring after a longer exposure (Bouvarel, 2009). On the basis of these observations, in our

Table 2. Production performance (means and SE) of broiler chickens fed control (CON) or sequential feeding (SF) diets from d 0 to 44 (slaughtering age)

Item	CON	SF	SE
Number of birds	660	660	—
Chick weight (g)	42.3	42.3	0.17
BW (g/bird)	3,913	3,948	22.19
Daily weight gain (g/bird per day)	88.0	88.8	1.41
Daily feed intake (g/bird per day)	153.3	154.7	3.48
Feed intake (kg/bird)	6.74	6.81	0.18
Feed conversion rate[1]	1.78[b]	1.84[a]	0.009
Mortality (%)	6.98	7.58	0.84

[a, b]Means within row with different superscripts differ significantly ($P < 0.01$).

[1]Corrected for mortality.

trial, a short sequential cycle (12 h) of low- and high-protein contents with isoenergetic diets was adopted to avoid any bird adaptation to feed intake. Although SF birds slightly increased their feed intake, the overall N excretion was reduced by almost 4%, confirming the efficacy of the feeding program for improving N utilization. Several attempts have been made to reduce N excretion in poultry, mainly by manipulating the CP and amino acid balance, and by using enzyme supplementation (Ferguson et al., 1998; Meluzzi et al., 2001; Ferket et al., 2002; Nahm, 2007). However, as animals are fed diets closer to their true N requirements, further reductions in dietary CP may result in a less pronounced reduction in N excretion.

The incidence of foot pad lesions is shown in Figure 1. In general, the number of birds affected by FPD was low in both groups; however, SF birds had a 46% reduction in incidence of birds with score 1 lesions. The footpad lesions, also named ammonia burns, are caused by a combination of factors, such as moisture (Youssef et al., 2011) and ammonia content, and other chemical factors of the litter (Berg, 2004). The incidence of FPD is an important aspect of poultry welfare, and has been used to characterize the health and welfare of broiler flocks (Berg, 1998; Meluzzi et al., 2008; Shepherd and Fairchild, 2010). Nagaraj et al. (2006, 2007) found that incidence and severity of pododermatitis in broiler chickens significantly increased with high-protein diets and related these outcomes to the higher level of N and NH_3-N in the litter. Youssef et al. (2011) attributed the higher severity of FPD of turkeys to higher moisture of the litter, and did not find any increase of FPD when birds were exposed for 8 h/d to wet litter enriched with NH_4Cl and uric acid.

Concerning the effect of the feeding program on carcass and cut-up yields, Widyaratne and Drew (2011) found an increased breast meat yield when administering high-protein diets to broiler chicks. In contrast, no differences emerged in our trial between the experimental groups (Table 4), which is in accordance with the findings of Bizeray et al. (2002).

The quality traits and chemical composition of breast meat are reported in Table 5. The SF breast meat exhibited a significantly lower ultimate pH in accordance with Bouvarel et al. (2008) who alternated high- and low-protein diets. This decrease in breast meat pH may be associated with higher muscle energy storage in the form of glycogen at death, which is negatively related to meat pH (Berri et al., 2005; Duclos et al. 2007). Yalcin et al. (2010), feeding broilers with low-protein diets, found reduced breast meat pH and attributed this outcome to the greater energy storage in muscle at death. Berri et al. (2008) observed that the final pH significantly decreased after reducing the dietary lysine from 1.03 to 0.83%. In the current experiment, the SF birds showed a slightly paler meat compared with that of the CON birds (L* 55.73 vs. 54.25, respectively), and a significantly higher cook loss (28 vs. 22%, respectively; $P < 0.01$). According to the characterization reported by Petracci et al. (2004), breast meat from SF birds may be considered as pale, soft, and exudative (PSE)-like meat because it shows a low ultimate pH, an L* value close to 56, and a high cook loss. In our study, SF breast meat showed higher a* values than those

Table 3. Nitrogen balance of broiler chickens fed control (CON) or sequential feeding (SF) diets (means and SE)

Item	CON	SF	SE
Number of replicates	6	6	—
Nitrogen intake (g/kg of live BW)	56.2[A]	55.2[B]	0.48
Nitrogen retained (g/kg of live BW)	30.3	30.4	0.29
Nitrogen excreted (g/kg of live BW)	25.9[A]	24.8[B]	0.22
Nitrogen excreted (g/bird)	105.6[a]	102.1[b]	1.05
Nitrogen excreted/N ingested (%)	46.1[A]	45.0[B]	0.33

[a,b]Means within row with different superscripts differ significantly ($P < 0.05$).

[A,B]Means within row with different superscripts differ significantly ($P < 0.01$).

Table 4. Slaughtering performance of broiler chickens fed control (CON) or sequential feeding (SF) diets

Item	CON	SF
Chilled carcass yield[1] (%)	72.9	72.5
Breast[2] (%)	21.6	21.3
Legs[2] (%)	27.3	27.5
Unseparated wings[2] (%)	12.1	12.2

[1]Ready to cook carcass/live BW.

[2]Calculated on live BW.

found in the CON meat (2.53 vs. 1.92, respectively; $P < 0.05$). In broilers, pale meat is often associated with high redness values (Qiao et al., 2001). In the current experiment, yellowness, AK shear values, and drip loss were unaffected by the dietary program.

The chemical composition of breast meat was similar in the 2 groups, with the exception of lipid content, which was significantly lower in SF birds compared with that in the CON birds (2.49 vs. 3.08 g/100 g, respectively; $P < 0.05$). In general, diets with a higher energy to protein ratio promote body energy retention as fat. Moreover, according to Si et al. (2001), a decrease in dietary CP causes a decrease in carcass protein and an increase in carcass fatness. However, it is questionable whether the higher energy to protein ratio of diets fed for only part of the day, as applied in this experiment, can modulate the lipogenesis in chickens slaughtered at a high live BW (4 kg). It can be argued that in birds undergoing an SF program, some modifications may occur in the energy metabolism; these modifications may be responsible for the increase in muscle glycogen storage, as shown by the lower meat pH, and also for the decrease in the energy substrates available for lipogenesis, as shown by the lower meat lipid content. However, this hypothesis needs to be confirmed by targeted investigations.

In conclusion, the SF program had positive repercussions on environmental and animal welfare by reducing N emission in poultry husbandry, and at the same time decreased the incidence of foot pad lesions. However, the SF program adversely affected feed efficiency and altered some meat traits, mainly pH and cook loss.

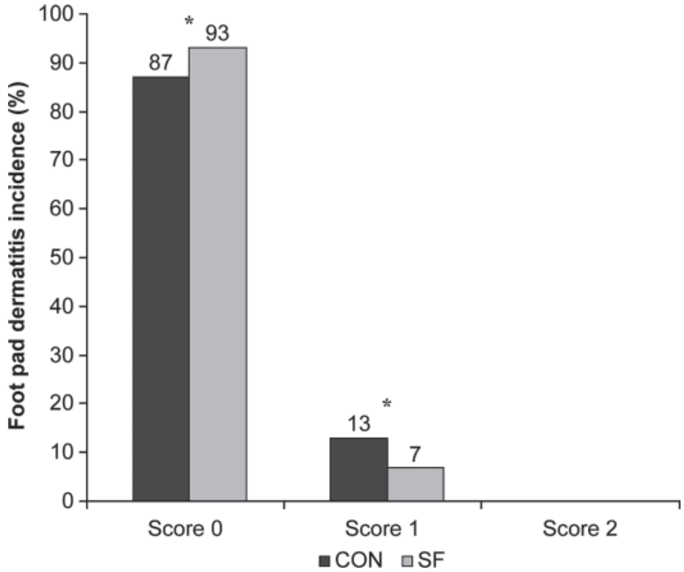

Figure 1. Incidence of foot pad lesions in broiler chickens fed control (CON) or sequential feeding (SF) diets. An asterisk indicates a difference at $P < 0.05$.

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Table 5. Quality traits and chemical composition of breast meat of chickens fed control (CON) or sequential feeding (SF) diets (means and SE)

Item	CON	SF	SE
Number of chickens	16	16	
Quality trait			
pH (24 h)	5.91[A]	5.76[B]	0.04
Color			
Lightness (L*)	54.25	55.73	0.53
Redness (a*)	1.92[b]	2.53[a]	0.14
Yellowness (b*)	4.85	4.48	0.31
Drip loss (%)	1.08	1.07	0.11
Cook loss (%)	22.0[B]	28.0[A]	1.37
Allo Kramer-shear (kg/g)	3.56	4.15	0.26
Chemical composition			
Moisture (%)	73.8	73.9	0.29
Protein (%)	22.6	22.3	0.19
Lipid (%)	3.08[a]	2.49[b]	0.20
Ash (%)	1.23	1.23	0.02

[a,b]Means within a row followed by differing superscript letters differ significantly ($P < 0.05$).

[A,B]Means within a row followed by differing superscript letters differ significantly ($P < 0.01$).

REFERENCES

AOAC. 1990. Official Methods of Analysis of the Association of Official Analytical Chemists, 15th Ed. AOAC International, Arlington, VA.

Bedford, M. R., and A. J. Morgan. 1996. The use of enzyme in poultry diets. World's Poult. Sci. J. 52:61–68.

Berg, C. 1998. Foot-pad dermatitis in broilers and turkeys—Prevalence, risk factors, and prevention. *Acta Universitatis Agriculturae Sueciae*, Veterinaria 36. PhD Thesis. Swedish University of Agricultural Sciences, Uppsala, Sweden.

Berg, C. 2004. Pododermatitis and hock burn in broiler chickens. Pages 37–49 in Measuring and Auditing Boiler Welfare. C. A. Weeks and A. Butterworth, ed. CABI publishing, Wallingford, UK.

Berri, C., J. Besnard, and C. Relandeau. 2008. Increasing dietary lysine increases final pH and decreases drip loss of broiler breast meat. Poult. Sci. 87:480–484.

Berri, C., W. Debut, W. Santè-Lhoutellier, C. Arnould, B. Boutten, N. Sellier, E. Baeza, N. Jehl, Y. Jego, M. J. Duclos, and E. Le Bihan-Duval. 2005. Variation in chicken breast meat quality: Implications of struggle and muscle glycogen content at death. Br. Poult. Sci. 46:572–579.

Bizeray, D., C. Leterrier, P. Constantin, M. Picard, and J. M. Faure. 2002. Sequential feeding can increase activity and improve gait score in meat-type chickens. Poult. Sci. 81:1798–1806.

Blair, R., J. P. Jacob, S. Ibrahim, and P. Wang. 1999. A quantitative assessment of reduced protein diets and supplements to improve nitrogen utilization. J. Appl. Poult. Res. 8:25–47.

Bouvarel, I. 2009. Variations d'ingestion chez le poulet de chair lors d'une alimentation séquentielle. Mémoire Thèse, AgroParisTech, 10 avril 2009. Institut des Sciences et Industries du Vivant et de l'Environnment, Paris, France.

Bouvarel, I., A. M. Chagneau, P. Lescoat, S. Tesseraud, and C. Leterrier. 2008. Forty-eight-hour cycle sequential feeding with diets varying in protein and energy content: Adaptation in broiler at different ages. Poult. Sci. 87:196–203.

CIE. 1978. International Commission on Illumination recommendations on uniform color spaces, color difference equations, and psychometric color terms. CIE Publication [No.15 (E-1.3.1) 1971/(TO-1.3)] Suppl. 15. Bureau Central de la CIE, Paris, France.

Duclos, M. J., C. Berri, and E. Le Bihan-Duval. 2007. Muscle growth and meat quality. J. Appl. Poult. Res. 16:107–112.

Ekstrand, C., B. Algers, and J. Svedberg. 1997. Rearing conditions and foot-pad dermatitis in Swedish broiler chickens. Prev. Vet. Med. 31:167–174.

European Commission, 1991. Council Directive 91/676/EEC of 12 December 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources. Off. J. L375:1–8.

European Commission. 2002. Nitrogen equivalents in livestock manure. Office for Official publications of European Communities, Luxembourg City, Luxembourg.

European Commission, 2008. Council Directive 2008/1/EC 15 January 2008 concerning integrated pollution prevention and control. Off. J. L24:8–29.

Ferguson, N. S., R. S. Gates, J. L. Taraba, A. H. Cantor, A. J. Pescatore, M. L. Straw, M. J. Ford, and D. J. Burnham. 1998. The effect of dietary protein and phosphorus on ammonia concentration and litter composition in broilers. Poult. Sci. 77:1085–1093.

Ferket, P. R., E. van Heugten, T. A. T. G. van Kempen, and R. Angel. 2002. Nutritional strategies to reduce environmental emission from nonruminants. J. Anim. Sci. 80:(E-Suppl. 2):E168–E182.

Folch, J., M. Lees, and G. H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497–509.

Jeacocke, R. E. 1977. Continuous measurement of the pH of beef muscle in intact beef carcasses. J. Food Technol. 12:375–386.

Meluzzi, A., C. Fabbri, E. Folegatti, and F. Sirri. 2008. Survey of chicken rearing conditions in Italy: Effects of litter quality and stocking density on productivity, foot dermatitis, and carcass injuries. Br. Poult. Sci. 49:257–264.

Meluzzi, A., F. Sirri, N. Tallarico, and A. Franchini. 2001. Nitrogen retention and performance of brown-laying hens fed on diets with different protein content and constant of amino acids and energy. Br. Poult. Sci. 42:213–217.

Nagaraj, M., F. Biguzzi, J. B. Hess, and S. F. Bilgili. 2006. Paw burns in broiler chickens are negatively affected by high-protein and all-vegetable diets. Poult. Sci. 85(Suppl. 1):169–170. (Abstr.)

Nagaraj, M., C. A. P. Wilson, J. B. Hess, and S. F. Bilgili. 2007. Effect of high-protein and all-vegetable diets on the incidence and severity of pododermatitis in broiler chickens. J. Appl. Poult. Res. 16:304–312.

Nahm, K. H. 2007. Feed formulations to reduce N excretion and ammonia emission from poultry manure. Bioresour. Technol. 98:2282–2300.

Papinaho, P. A., and D. L. Fletcher. 1996. The effect of stunning amperage and deboning time on early rigor development and breast meat quality of broilers. Poult. Sci. 75:672–676.

Petracci, M., M. Betti, M. Bianchi, and C. Cavani. 2004. Color variation and characterization of broiler breast meat during processing in Italy. Poult. Sci. 83:2086–2092.

Qiao, M., D. L. Fletcher, D. P. Smith, and J. K. Northcutt. 2001. The effect of broiler breast-meat color on pH, moisture, water holding capacity, and emulsification capacity. Poult. Sci. 80:676–680.

SAS Institute. 1988. SAS/STAT Guide for Personal Computers. Version 6.03 Edition. SAS Institute Inc., Cary, NC.

Shepherd, E. M., and B. D. Fairchild. 2010. Footpad dermatitis in poultry. Poult. Sci. 89:2043–2051.

Si, J., C. A. Fritts, D. J. Bournham, and P. W. Waldroup. 2001. Relationship of dietary lysine level to the concentration of all essential amino acids in broiler diets. Poult. Sci. 80:1472–1479.

Umar Faruk, M., I. Bouvarel, N. Même, N. Rideau, L. Roffidal, H. M. Tukur, D. Bastianelli, Y. Nys, and P. Lescoat. 2010. Sequential feeding using whole wheat and separate protein-mineral concentrate improved feed efficiency in laying hens. Poult. Sci. 89:785–796.

Widyaratne, G. P., and M. D. Drew. 2011. Effects of protein level and digestibility on the growth and carcass characteristics of broiler chickens. Poult. Sci. 90:595–603.

Yalçin, S., H. Özkul, S. Özkan, R. Gous, I. Yaşa, and E. Babacanoğlu. 2010. Effect of dietary regime on meat quality traits and carcass nutrient content of broilers from two commercial genotypes. Br. Poult. Sci. 51:621–628.

Youssef, I. M. I., A. Beineke, K. Ronh, and J. Kamphues. 2011. Effect of litter quality (moisture, ammonia, and uric acid) on development and severity of foot pad dermatitis in growing turkeys. Avian Dis. 55:51–58.