PRODUCTION, MODELING, AND EDUCATION

Productive performance and histological features of intestinal mucosa of broiler chickens fed different dietary protein levels

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ABSTRACT To evaluate the effect of decreasing dietary protein on growth performance, carcass traits, and intestinal mucosal morphometry, 180 female Hubbard strain broiler chickens were divided into 3 groups and fed 3 isoenergetic diets ad libitum from 14 d of age until slaughter age (49 d). The treatments varied according to 3 protein levels: high-protein diet (HiP, 22.5% CP, DM basis), medium-protein diet (MedP, 20.5% CP), and low-protein diet (LowP, 18.5%). Diets were obtained by replacing wheat middlings with soybean meal and were formulated to meet or exceed broiler amino acid requirements of the NRC. Morphometric indices of duodenum, jejunum, and ileum were measured at the end of the feeding period and included villus height, crypt depth, villus-to-crypt ratio, and apparent villus surface area. The dietary protein level had a significant effect on final BW of birds, whereas ADG, ADFI, and feed efficiency remained unaffected by dietary treatment. The muscle (breast and drumstick) yields were significantly higher in birds fed the HiP diet compared with those of the MedP and LowP diets. Meat quality traits were not affected by the protein level. The villus surface area of all intestinal segments did not change among groups. Instead, reducing the dietary protein level to 20.5% resulted in a higher villus height and villus height to crypt depth ratio in the duodenum and ileum. On the basis of our findings, even if the high-protein diet promoted meat yield, a medium-protein diet could positively support broiler growth performance, as confirmed by favorable morphometric features of the intestine.

Key words: broiler, protein level, growth, carcass trait, gut morphology

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INTRODUCTION

Dietary protein is a crucial regulator of poultry growth and reproductive performance, but also of the development of the gastrointestinal tract. A major concern for the modern poultry industry is to reduce feed cost for optimal economic return because feed represents the main component of total production cost, and CP is one of the major cost components of poultry diets (Kamran et al., 2004). A significant number of trials have been conducted to assess effects of low-protein, amino acid-supplemented diets on the growth and carcass characteristics of broiler chickens. However, conflicting results from these studies prevent a clear conclusion on the effects of these diets in practical broiler production. Some trials conducted with low-CP diets fed to broilers have generally led to poor performance when comparison is made with broilers receiving adequate amino acids (Berres et al., 2010), whereas other trials have reported no effect on performance when low-protein diets are fed (Widyaratne and Drew, 2011). Feeding a low-CP diet has also been reported to improve the performance of birds in hot climates (Thim et al., 1997) and decrease the N in excreta, thus reducing the N loss to the environment (Sterling et al., 2005; Kamran et al., 2010). The only consistent result reported in the abovementioned studies was the increased accumulation of abdominal fat in broilers fed low-protein diets (Kidd et al., 1996). The changes in carcass yield caused by changing the dietary protein level may be of the order of 4%, which is advantageous from an economic perspective (Nawaz et al., 2006). The efficiency of utilization of dietary protein in poultry depends partly on the features of the gastrointestinal tract (Swatson et al., 2002). The small intestine, especially crypts and villi

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of the absorptive epithelium, plays a significant role in the final phase of nutrient digestion and assimilation (Wang and Peng, 2008). Intestinal development can be assessed through measurements of the crypt, a region in which new intestinal cells are formed, as well as villus height and surface area, to determine the area available for digestion and absorption (Swatson et al., 2002; Franco et al., 2006). Reports of anatomical changes of the gut, depending on the type of diet (Incharoen et al., 2010), and alterations of villus shape in different species have been published. However, there is a lack of report linking the effects of dietary nutrients and especially that of protein to the development of the gastrointestinal tract of poultry. According to Yamauchi (2002), the morphological changes of the intestinal villi in broilers are dependent on the presence of digested nutrients in the small intestinal lumen. Maneewan and Yamauchi (2003) reported that semipurified protein-free diets were the slowest in promoting histological recovery after feed withdrawal, suggesting that protein is the most important factor in recovery after feed withdrawal. Recently, Buwjoom et al. (2010), assessing the histology of intestinal villi and epithelial cells in broilers using long-term feeding of low-CP diets, found that the ileal villi did not show specific alterations and that the chronic feeding of a low-CP diet induced a histological alteration. Moreover, this report suggests that long villi, large cell area, and many cells undergoing mitosis might be observed even in hyponutritional conditions to obtain deficient nutrients, not just in the hypernutritional conditions. This suggests that the hypotrophied histological alterations can indicate that a diet is not nutritionally well-balanced. Thus, the aim of the present study was to determine the effect of different dietary CP contents, with constant ME, on broiler performance and to confirm the hypothesis that intestinal morphology is dependent on protein level.

MATERIALS AND METHODS

Birds, Housing, and Diets

The trial was performed observing the animal welfare Legislative Decree 116/92, Council Directive 98/58/EC, received in Italy by Legislative Decree 146/2001, Council Directive 2007/43/CE, received in Italy by Legislative Decree 181/2010 and Legislative Decree 267/2003. In total, 180 female Hubbard-strain broiler chickens were randomly allotted to 18 floor pens on arrival at the research facility. Each pen was equipped with a pan feeder, a manual drinker, and wood shavings. On d 14, birds were individually weighed and divided among pens and randomly assigned to 1 of 3 dietary treatment groups. Each diet (treatment) was replicated 6 times, with each replicate comprising 1 pen of 10 birds. From d 14 to slaughtering age (49 d), birds were fed 3 diets containing different levels of CP, which were obtained by replacing wheat middlings with soybean meal and were formulated to meet or exceed broiler amino acid requirements (NRC, 1994): a low-protein diet (LowP, 18.5%), a medium-protein diet (MedP, 20.5% CP), and a high-protein diet (HiP, 22.5% CP), as reported in Table 1. The ME of the diets was estimated using the Carpenter and Clegg equation (Leeson and Summers, 2001): ME (MJ/kg) = 53 + 38 × [CP (%) + 2.25 × ether extract (%) + 1.1 × starch (%) + sugar (%)]. The experimental diets were based on wheat middlings obtained from durum wheat (*Triticum durum* Desf. cv. Appulo). The wheat middlings were previously sieved to separate the fibrous components in order to obtain a product with an average crude fiber content of <3% (Laudadio and Tufarelli, 2011). Feed (pelleted form) and water were provided ad libitum throughout the entire experiment; feed intake was measured and ADG as well as feed conversion ratio were calculated.

On d 49 of the trial, 2 broilers from each pen (12 birds/dietary treatment) were selected based on the average BW within each pen. Following a 12-h fast, individual broilers were weighed; electrically stunned in a water bath to deliver a constant current; killed by cervical dislocation; and then immediately bled, plucked, and eviscerated. The abdominal fat, breast (pectoralis major), and drumstick (peroneous longus) muscles were removed and weighed. Some muscles from the breast and drumstick were stored at −80°C for an assessment of fat content and others were stored individually in plastic bags at 4°C for meat quality analysis.

Chemical Analysis

Samples of the diet were ground in a hammer mill with a 1-mm screen and analyzed in triplicate for DM (method 945.15), ash (967.05), CP (Kjeldahl N × 6.25, 990.03), and ether extract (945.16) according to AOAC (2000). Neutral detergent fiber (without sodium sulphite), acid detergent fiber, and acid detergent lignin contents were analyzed according to Mertens (2002), AOAC (2000; method 973.187), and Van Soest et al. (1991), respectively, using the sequential procedure and the filter bag system (Ankom Technology, New York, NY). Meat samples were analyzed for moisture and ash by the oven method, protein by the Kjeldahl method (AOAC, 2000), and total lipids were extracted according to the method of Folch et al. (1957). Values for meat samples are expressed as a percentage on a fresh-matter basis.

Tissue Sampling and Morphometric Indices of Duodenum, Jejunum, and Ileum

At the end of the feeding trial (d 49), 2 birds per pen (12 birds/diet) were killed by cervical dislocation. Intestinal segment samples (approximately 2 cm in length) of duodenum, jejunum, and ileum were excised and flushed with 0.9% saline to remove the contents. Gut segments were fixed in 10% neutral-buffered formalin for histology. The segments of intestine collected were the loop of the duodenum, midpoint between the

Table 1. Ingredients and chemical composition of experimental diets

Item	HiP[1]	MedP[1]	LowP[1]
Ingredients, g/kg			
Wheat middlings	721.2	838.0	895.6
Soybean meal (48% CP)	205.0	90.0	30.0
Soybean oil	25.0	17.0	15.0
Dicalcium phosphate	12.0	12.0	12.0
Calcium carbonate	11.0	11.0	12.0
Monocalcium phosphate	10.0	10.0	10.0
L-Lysine HCl	4.0	8.1	10.0
L-Threonine	1.8	3.2	4.5
DL-Methionine	2.5	3.2	3.4
Sodium chloride	2.5	2.5	2.5
Sodium bicarbonate	2.0	2.0	2.0
Vitamin-mineral premix[2]	2.0	2.0	2.0
Enzyme[3]	0.5	0.5	0.5
Choline chloride	0.5	0.5	0.5
Chemical composition, %			
ME, MJ/kg	13.43	13.43	13.43
CP	22.52	20.51	18.51
Neutral detergent fiber	5.03	4.95	4.89
Acid detergent fiber	1.41	1.32	1.27
Acid detergent lignin	0.392	0.37	0.35
Crude fat	4.69	4.52	4.32
Ash	6.42	6.33	6.29
Calcium	1.14	1.13	1.14
Available phosphorus	0.59	0.59	0.59
Sodium	0.20	0.21	0.21
Lysine	1.28	1.27	1.27
Methionine	0.57	0.60	0.60
Methionine + cysteine	0.99	0.96	0.93
Threonine	0.87	0.85	0.88

[1]HiP = high-protein diet (22.5%); MedP = medium-protein diet (20.5%); and LowP = low-protein diet (18.5%).

[2]Supplied per kilogram of diet: vitamin A, 12,000 IU; vitamin E, 10 mg; vitamin D, 2,200 IU; niacin, 35.0 mg; D-pantothenic acid, 12 mg; riboflavin, 3.63 mg; pyridoxine, 3.5 mg; thiamine, 2.4 mg; folic acid, 1.4 mg; biotin, 0.15 mg; vitamin B, 0.03 mg; Mn, 60 mg; Zn, 40 mg; Fe, 1,280 mg; Cu, 8 mg; I, 0.3 mg; and Se, 0.2 mg.

[3]Provided per kilogram of product: endo-1,4-β-glucanase, 800,000 U; endo-1,3(4)-β-glucanase, 1,800,000 U; and endo-1,4-β-xylanase, 2,600,000 U.

bile duct entry and Meckel's diverticulum (jejunum), and midway between Meckel's diverticulum and the ileo-cecal junction (ileum). Samples were dehydrated, cleared, and paraffin embedded. Intestinal segments from 12 birds per dietary treatment were sectioned at a 6-μm thickness, placed on glass slides, and processed in Masson's trichrome stain for examination by light microscopy according to Culling et al. (1985). The morphometric indices evaluated were villus height from the tip of the villus to the crypt, crypt depth from the base of the villi to the submucosa, and the villus height to crypt depth ratio (Zhang et al., 2009). The apparent villus surface area was calculated by the following formula: [(villus width at one-third + villus width at two-thirds of the height of the villus) $\times 2^{-1} \times$ villus height], according to Iji et al. (2001). Morphometric investigations were performed on 20 intact villi and 30 crypts chosen from each intestinal segment of broiler chickens.

Statistical Analyses

Data collected for growth performance, carcass traits, and histological examination were statistically analyzed by one-way ANOVA of SAS (SAS Institute Inc., 2004). Three treatments with 6 replications were used, and each pen was an experimental unit. Duncan's multiple range test was applied to compare the significance of differences between the means (Steel and Torrie, 1980). Statistical significance was considered at $P \leq 0.05$.

RESULTS

Productive Performance

The effects of dietary treatments on growth performance of broiler chickens are presented in Table 2. The average final BW tended to increase with increasing dietary protein level and was significantly higher ($P = 0.045$) in the MedP group than in the HiP and LowP groups, respectively. The ADG did not change with the decrease in dietary CP. Feed intake was unaffected by protein level, and moreover, there were no differences in feed conversion ratio among groups throughout the trial. The mortality rate did not differ significantly among the dietary treatments.

The carcass traits, expressed as percentages of BW at slaughter, as well as meat quality are reported in Table 3. The different levels of protein in the diet had no significant effect on dressing percentage and abdominal fat pad of chicks. Conversely, the yield of breast and drumstick muscles of broilers was modified by dietary protein level, being significantly higher ($P = 0.041$ and $P = 0.032$, respectively) in broilers fed HiP diet compared with the other treatments. Meat quality parame-

Table 2. Effect of dietary protein level on growth performance of broiler chickens

Item[1]	HiP[2]	MedP[2]	LowP[2]	SEM	*P*-value
BW, g	2,592[b]	2,663[a]	2,552[b]	31.5	0.045
ADG, g/d	52.9	54.5	55.9	0.61	0.082
ADFI, g/d	89.1	90.4	88.8	0.69	0.098
FCR, g/g	1.67	1.65	1.59	0.02	0.067
Mortality, %	1.7	1.3	1.8	—	0.498

[a,b]Means within a row with no common superscript differ significantly ($P < 0.05$).

[1]BW = body weight at 49 d of age; FCR = feed conversion ratio.

[2]HiP = high-protein diet (22.5%); MedP = medium-protein diet (20.5%); and LowP = low-protein diet (18.5%).

ters, in terms of moisture, protein, fat, and ash content, were similar between groups.

Gut Morphometric Indices

The data for the morphology measurements of the small intestine of broilers are reported in Table 4. The dietary CP level influenced the morphology of the mucosa in the small intestine. In the duodenum, the villus height was higher ($P = 0.006$) in the MedP group than in the other dietary groups. At this intestinal region, crypts were deeper in broilers fed a LowP diet compared with those of MedP and HiP groups (372 vs. 273 and 298 μm, respectively). As a consequence, the villus height to crypt depth ratio varied significantly ($P = 0.002$) between groups. The same situation was observed for the ileum in terms of villus height, crypt depth, and the ratio between them. In contrast, the histological parameters examined in the jejunum were not affected by dietary protein level. When CP varied, villus surface area was not significantly different ($P > 0.05$) between groups.

DISCUSSION

At the end of the feeding period (d 49), broilers reached an overall live BW of more than 2.5 kg, particularly the broilers fed the medium-protein level. Our level of CP (20.5%) in the diet could be considered optimal to obtain the same or better productive performance in broilers fed conventional feed formulations, as the feed efficiency values among groups were similar. Therefore, the performance of birds fed the diets with 20.5% CP was considered valid as a standard diet (HiP) of comparison for the factors evaluated in the trial. It is well-documented that varying the protein level in diets for broilers can influence performance (Kerr and Kidd, 1999). Swatson et al. (2002) reported that poorer performance observed for birds fed diets of low energy-to-protein ratios suggests that, when surplus protein is fed, the energy content should also be increased to ensure that sufficient energy is available for the efficient utilization of the dietary protein. The results of the present study are in accordance with the recent findings of Berres et al. (2010), who observed that broilers fed low-CP diets with synthetic amino acids had the same cumulative BW gain, feed intake, feed conversion, and carcass and abdominal fat yields compared with birds fed diets with a higher protein level. In fact, in our study, the best final BW was achieved in broilers fed 20.5% CP, if compared with birds fed 22.5% CP (the most common protein level used by broiler producers). Our results are in line with the previous findings of Widyaratne and Drew (2011), who reported

Table 3. Effect of dietary protein level on some carcass traits and meat-quality parameters of broiler chickens

Item	HiP[1]	MedP[1]	LowP[1]	SEM	*P*-value
Carcass trait[2]					
Eviscerated carcass	71.0	70.1	69.5	0.29	0.059
Breast	19.6[a]	18.5[b]	18.1[b]	0.23	0.041
Drumstick	15.6[a]	14.4[ab]	13.5[b]	0.21	0.032
Abdominal fat	1.6	1.8	2.2	0.07	0.063
Meat parameter					
Breast, %					
Moisture	74.1	73.9	74.2	0.31	0.177
Protein	23.7	24.1	23.8	0.58	0.075
Fat	1.52	1.41	1.35	0.09	0.095
Ash	0.63	0.59	0.62	0.13	0.312
Drumstick, %					
Moisture	75.9	76.4	76.3	0.45	0.255
Protein	20.4	20.1	19.9	0.33	0.096
Fat	3.11	2.95	3.19	0.24	0.062
Ash	0.55	0.55	0.53	0.11	0.144

[a,b]Means within a row with no common superscript differ significantly ($P < 0.05$).

[1]HiP = high-protein diet (22.5%); MedP = medium-protein diet (20.5%); and LowP = low-protein diet (18.5%).

[2]Percentages of BW at slaughter.

Table 4. Effect of dietary protein level on intestinal mucosal morphometry of broiler chickens[1]

Item	HiP[2]	MedP[2]	LowP[2]	Pooled SEM	*P*-value
Duodenum					
Villus height, µm	1,763[b]	1,802[a]	1,623[c]	42.2	0.006
Crypt depth, µm	298[b]	273[b]	372[a]	8.7	0.039
Villus surface area, mm^2	0.16	0.16	0.14	0.01	0.215
Villus height to crypt depth	5.91[a]	6.61[a]	4.36[c]	0.43	0.002
Jejunum					
Villus height, µm	1,178	1,188	1,201	31.3	0.065
Crypt depth, µm	187	191	194	7.5	0.052
Villus surface area, mm^2	0.19	0.22	0.21	0.02	0.163
Villus height to crypt depth	6.30	6.22	6.19	0.39	0.058
Ileum					
Villus height, µm	355[b]	561[a]	341[b]	19.6	0.005
Crypt depth, µm	187[b]	217[ab]	231[a]	5.2	0.036
Villus surface area, mm^2	0.13	0.11	0.12	0.02	0.189
Villus height to crypt depth	1.90[ab]	2.58[a]	1.48[b]	0.24	0.012

[a–c]Means within a row with no common superscript differ significantly ($P < 0.05$).

[1]Each value represents the mean of 12 birds per treatment.

[2]HiP = high-protein diet (22.5%); MedP = medium-protein diet (20.5%); and LowP = low-protein diet (18.5%).

that low-protein diets can support growth performance of broiler chickens similar to that of high-protein diets when highly digestible feed ingredients are used.

Dietary treatments did not affect most carcass traits. However, broilers fed the low-CP diet had lower breast and drumstick yields and abdominal fat percentage than did birds fed diets with high and medium CP levels. Our findings are in agreement with those of Bu-wjoom et al. (2010), who found that dietary protein did not affect carcass yield in chicks. The results of the present trial support the findings of Corzo et al. (2005) and Widyaratne and Drew (2011), who reported that the maximum breast meat yield requires a high-protein diet and, moreover, is not affected by ingredient digestibility. In agreement with our study, Widyaratne and Drew (2011) showed that dietary protein levels did not influence the abdominal fat percentage in broilers. However, previous studies have reported that birds fed low CP have increased feed intake with a concurrent increase in the deposition of abdominal fat (Rosebrough and McMurtry, 1993). These changes in fat deposition most likely resulted from energy excess.

In our study, dietary protein level affected the structure of the small intestine in broilers. Intestinal morphology is the main indicator of gut health. The functional status of the small intestine is characterized in part by villus height and crypt depth. Previous studies have been conducted to evaluate the effect of protein level on the intestinal morphology in other species, such as pigs (Gu and Li, 2004) and rabbits (Iyeghe-Erakpotobor et al., 2005). However, relatively few studies have been carried out in broilers. Available data showed that diets differing in the level of CP did not increase the villus height and crypt depth or villus surface area (Bu-wjoom et al., 2010). In our study, a significant increase in villus height and decrease in crypt depth and in the ratio of the two was observed in the small intestinal mucosa of chickens fed the high- and medium-CP diets compared with that of broilers given the lower-CP diet. The improved villus height and villus height to crypt depth ratio for various gut segments of birds fed the pellet diet were in agreement with adequate growth performance and increased metabolizability of nutrients. In fact, lengthening of villi may increase total luminal villus absorptive area and subsequently result in satisfactory digestive enzyme action and higher transport of nutrients at the villus surface (Tufarelli et al., 2010). Moreover, the higher villus height to crypt depth ratio in the broilers fed the medium-protein-level diet resulted in a decreased turnover of the intestinal mucosa. A slower turnover rate of the intestinal epithelium results in a lower maintenance requirement, which can finally lead to a higher growth rate or growth efficiency of the animal (Van Nevel et al., 2005). Thus, the changes in intestinal morphology may influence nutrient metabolizability and performance. In addition, a deeper crypt may indicate faster tissue turnover to permit renewal of the villus, which suggests that the host's intestinal response mechanism is trying to compensate for normal sloughing or atrophy of villi due to inflammation from pathogens and their toxins (Gao et al., 2008). An increase in villus height in the duodenum and ileum has been previously reported in broilers fed a diet with a low CP level compared with a diet containing a standard protein level (≥22%, Sterling et al., 2005), and has been explained by the development of the intestinal villi, thus enhancing the efficiency of digestion and absorption. Because long villi are correlated with improved gut health (Baurhoo et al., 2007), in our study, the diet containing 20.5% CP should offer a competitive advantage over the other diets (with high or low CP levels, 22.5 or 18.5%, respectively), resulting in improved intestinal health status of the broilers, and it supported growth performance without any negative effect on meat quality.

In conclusion, decreasing the dietary protein concentration in the diet by up to 20.5% significantly improved broiler BW and supported feed consumption and efficiency. Moreover, the villus height and villus height to crypt depth ratio were also greater as the level of protein decreased. Therefore, the present study suggests that a level of 20.5% protein concentration in the diet is optimal for maximizing growth performance and absorptive capacity in broiler chickens. In addition, it is widely accepted that the choice of dietary protein level is an economic decision to be made for each poultry company to increase the cost-effective benefits.

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