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Impact of Dietary Isoleucine Status on Heavy-Broiler Production

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Abstract: Two experiments were conducted to evaluate the importance of Ile as a limiting amino acid in diets fed to broilers up to heavy market weights (35-54 d). The first experiment compared a control diet formulated to meet all critical limiting amino acids vs. a diet that also met all limiting amino acid needs except for Ile (0.71 vs. 0.58% standardized ileal digestibility). Results from Experiment 1 showed poorer BW gain, feed conversion and feed cost/BW gain in birds fed the Ile-marginal diet when compared to the control. Experiment 2 evaluated the supplementation of three graded levels of Ile (0.58, 0.62 and 0.66% standardized ileal digestibility) to the Ile-reduced diet used in Experiment 1. Results for Experiment 2 showed that BW gain and feed conversion improved when L-Ile was supplemented to the lowest dietary Ile level fed. Supplementation with equal amounts of Arg did not alleviate the dietary Ile limitation, thus validating the essentiality and marginality of Ile in practical corn-soybean meal diets when at least 2% of meat-and-bone meal is present in diet formulation.

Key words: Isoleucine, lysine, broiler, standardized ileal digestibility

Introduction

Nutritionists must maintain adequate levels of the less limiting amino acids (i.e. Ile, Val, Trp, Arg, Gly) as supplemental critical amino acids enter the diet because soybean meal and meat meals inclusion levels are reduced. It has been predicted that practical corn and soybean meal diets that contain meat meals typically have the nutrient Ile as 4th limiting (Kidd and Hackenhaar, 2005; Corzo, 2007) and thus, the inclusion of dietary L-Thr requires advanced knowledge of the Ile minimum recommendations. Wang *et al.* (1997) conducted a series of amino acid addition and deletion studies and reported that Ile was the first limiting of the branched chain amino acids in meat-and-bone meal, as well as the first limiting of those amino acids not available in commercial feed-grade form. Recent data regarding Ile needs of heavy broilers, particularly beyond 30 d of age, has mostly addressed the needs of Ile requirement estimates and not the potential growth performance reduction of minimal dietary Ile in a practical diet (Corzo *et al*., 2004; Hale *et al*., 2004; Kidd *et al*., 2004). Therefore, two experiments were conducted to examine the impact of failure to meet dietary Ile recommendations in heavy broilers fed practical diets.

Materials and Methods

Treatments: Two experiments were conducted to evaluate the dietary Ile needs of the Ross × Ross 708 broiler males at a stage in life where breast tissue accretion is being maximized, thus theoretically susceptible to dietary amino acid inadequacies. Birds used in the two experiments were fed a common starter diet in crumbled form (0-14 d) and subsequent feeds were provided as whole pellets (14-35 d common to all birds; 35-54 d experimental phase). All phases were formulated to meet or exceed NRC (1994) nutrient recommendations. During the experimental feeding phase of both experiments (35-54 d), formulation was accomplished with the use of digestible amino acid values based from broiler standardized ileal digestibility (SID) assays of feed ingredients (Lemme *et al*., 2004). Composite samples of feed ingredients (prior to formulating) and dietary treatments were obtained and analyzed for total protein bound and supplemental crystalline L-Ile (Llames and Fontaine, 1994) to assure that calculated and analyzed total amino acid values were in agreement. The first experiment was conducted to examine the impact of disregarding the dietary Ile nutrient minimum in a practical formula that was potentially limiting in this amino acid, during the feeding period of 35 to 54 d of age. Experimental diets consisted of a control diet (0.71% SID Ile) formulated to meet or exceed nutrient recommendations (NRC, 1994), and a diet theoretically marginal in dietary Ile (0.58% SID). Experiment 2 evaluated the addition of three graded levels of dietary Ile in L-Ile form to the Ile-marginal diet used in Experiment 1 and monitor any recovery in performance (0.58, 0.62, 0.66% SID Ile). A similar amount (weight basis) of Arg was supplemented to that same diet (+0.08% L-Arg) in L-Arg form to observe if any possible Ile response was specific for Ile, non-essential N, or a limitation of the essential amino acid arginine. L-Ile and L-Arg were supplemented at the expense of an inert filler (sand). During Experiments 1 and 2, each treatment was represented with replicate 8 pens, using a floor pen as a replicate experimental unit.

Bird husbandry: Ross × Ross 708 broiler chicks were obtained from a commercial hatchery, and randomly distributed into floor pens (0.9 × 1.2 m) in a solid-sided house equipped with forced air furnaces. Each pen was composed of 12 broilers per pen at the start of experimentation, for a total of 192 and 384 broilers used in Experiments 1 and 2, respectively. Built-up litter was used in all pens, and the pens were equipped with a nipple drinker line (3 nipples/pen) and a hanging pan feeder (22.5 kg capacity). Feed and water were offered for *ad-libitum* consumption. The lighting program consisted of 23 h of light and 1 h of dark. Tunnel ventilation was accomplished by negative air pressure from two 1.22 m fans and evaporative cooling pads. Chicks were vaccinated for Marek's disease (via *in ovo* administration at d 18) and Newcastle disease and infectious bronchitis (via coarse spray at hatch). Stocking densities of 15 birds/pen (0.07 m^2/bird) and 12 birds/pen (0.09 m^2/bird) prior to- and during the experimental phase were used, respectively. All animal procedures were approved by Mississippi State University Institutional Animal Care and Use Committee.

Measurements: Parameters measured in both experiments were BW gain, feed conversion and mortality. Body weight gain was determined by measuring the mean bird weight of all pens at the initiation and termination of the experimental phase and calculating the difference. Mortality was recorded daily. Feed conversion was corrected for mortality and represents g of feed consumed by all birds in a pen divided by g of BW gain per pen plus the BW of the birds that died. In Experiment 1 monetary income was also calculated. This was computed by determining the feed cost using consumption of feed during the study and feed ingredient prices (Feed Ingredient Weekly, 2006) at the time diets were formulated, divided by the BW gain of the birds. These calculations were created in Microsoft Excel[2] using mathematical calculations, using a similar model to that used by Kidd *et al.* (2005). In Experiment 2, all birds were individually weighed, wing-banded, and placed into transportation coops for 12 h. Subsequently, birds were transported to the Mississippi State University processing plant, were birds were manually hanged, and mechanically stunned, bled, scalded, feather picked, eviscerated, their necks removed, and washed. Hot carcass weight and abdominal fat weights were then recorded. Carcasses were then chilled for 4 h and the breast muscles (boneless-skinless) weighed and recorded.

Statistics: Each experiment was conducted as a randomized complete block design. Pen was used as the experimental unit for analysis. Percentage data for mortality were transformed to arcsine √% for analysis. All data were analyzed by the GLM procedure of SAS (2006). Due to the elevated experimental variability observed when experimenting with heavy-broilers, one-way analysis of variance was considered significant when the global P value was P = 0.10. Mean separation was performed using Tukey's multiple range test at P = 0.05. In Experiment 2, broilers fed the Ile-marginal diet and the two Ile-supplemented diets were tested for linear trends using the GLM procedure of SAS (2006).

Table 1: Experimental diet composition, % as-is

Ingredient	+Control	Ile-marginal
Corn	65.87	74.36
Soybean meal	26.27	18.38
Poultry fat	3.257	1.864
Meat & bone meal	2.0	2.0
Dicalcium phosphate	1.165	1.222
Limestone	0.518	0.635
Salt	0.408	0.411
Vitamin/mineral premix[1]	0.25	0.25
DL-Methionine	0.153	0.226
L-Lysine	0.055	0.311
Filler[2]	-	0.16
L-Threonine	-	0.114
Sacox[3]	0.05	0.05
Choline chloride	-	0.02
Calculated composition[4]		
AME (kcal/kg)	3,200	3,200
CP (%)	19.83	16.96
Ca (%)	0.84	0.84
Av P (%)	0.42	0.42
Choline (ppm)	1,513	1,500
Lys (% SID)	0.95	0.95
TSAA (% SID)	0.71	0.71
Thr (% SID)	0.64	0.64
Val (% SID)	0.80	0.80
Arg (% SID)	1.17	1.06
Ile (% SID)	0.71	0.58

[1]The vitamin and mineral premix contained in the diet: vitamin A, 8,820 IU/kg; vitamin D, 3,528 IU/kg; vitamin E, 13,230 IU/kg; menadione, 1,166 mg/kg; B_{12}, 11 mg/kg; folic acid, 441 mg/kg; choline, 835 mg/kg; d-pantothenic acid, 14,112 mg/kg; riboflavin, 4,410 mg/kg; niacin, 70,560 mg/kg; thiamin, 2.205 mg/kg; d-biotin, 66 mg/kg; pyridoxine, 1,600 mg/kg; manganese, 68 g/kg; zinc, 68 g/kg; iron, 48 g/kg; copper, 6 g/kg; iodine, 0.6 g/kg; selenium, 0.24 g/kg.

[2]Filler represented inert space (sand) in the diet to which L-Ile or L-Arg were added at the expense of the filler to derive the different experimental diets.

[3]Dietary inclusion of 60 g salinomycin sodium per 907.2 kg of feed.

[4]Calculated and analyzed (in parenthesis) total Ile values are as follows (%), Experiment 1: + Control 0.76 (0.71); Ile deficient 0.62 (0.56). Experiment 2: 0.62 (0.59), 0.66 (0.66), 0.70 (0.67). The +Arg treatment in Experiment 2: 1.04 (1.03) Arg.

Results and Discussion

Growth performance of the birds immediately prior to the initiation of the experimental phase (35 d) for Experiment 1 was satisfactory across all pens (BW mean: 2.08 kg;

Table 2: Live performance and feed cost/BWG of broilers fed diets varying with adequate vs. deficient dietary Ile (Experiment 1)

Treatment	BWG (g)	FCR[1]	Mortality (%)	Feed cost/BWG[2]
+ Control (0.71% SID)	1,880 [a]	2.08 [b]	1.3	0.255 [b]
Ile-marginal (0.58% SID)	1,792 [b]	2.21 [a]	2.5	0.268 [a]
SEM	33.2	0.035	1.51	0.004
P value	0.08	0.03	0.56	0.05

[a-b]Means within a column not sharing a common superscript differ (P = 0.05).
[1]Values represent feed conversion ratio after being adjusted for mortality.
[2]Values represent = [feed cost ($/kg) / BW gain (kg)].

Table 3: Live performance and carcass traits of broilers fed various dietary levels of Ile (Experiment 2)

	Live performance			Yield (% of live BW)		
Treatment	BWG (g)	FCR (g/g)[1]	Mortality (%)	Carcass	Abdominal fat	Breast
0.58% SID Ile	1,791[ab]	2.21[ab]	2.5[ab]	71.4	2.16	22.8
0.62% SID Ile	1,846[ab]	2.14[bc]	2.5[ab]	71.0	2.13	23.1
0.66% SID Ile	1,881[a]	2.07[c]	1.3[b]	71.1	2.00	22.7
0.58% SID Ile + 0.08% Arg	1,777[b]	2.24[a]	3.8[a]	71.4	2.28	23.1
SEM	32.3	0.031	0.76	0.53	0.118	0.29
P value	0.10	0.003	0.09	0.89	0.46	0.55
Linear Ile[2]	0.14	0.006	0.56	0.50	0.35	0.71

[a-c]Means within a column not sharing a common superscript differ (P = 0.05).
[1]Values represent feed conversion ratio after being adjusted for mortality.
[2]Values represent linear trend analysis for Ile supplementation (discarding the +0.08% Arg supplementation treatment).

SD=0.065). Analysis of the experimental feed ingredients resulted in a close relationship between calculated vs. analyzed total dietary Ile (Table 1; footnote 4). The expected range in dietary Ile between the treatments in Experiment 1, and the progressive increase of dietary Ile accomplished via L-Ile supplementation was validated after analysis of the experimental diets. Furthermore, Arg supplementation was also corroborated after analysis of the respective experimental treatment (Table 1; footnote 4).

Experiment 1 showed a depression in BW gain in broilers that were fed the Ile-marginal diet when compared to the control birds (Table 2). This effect was also observed for feed conversion, where Ile-deficient diets resulted in broilers with 13 points higher when compared to broilers fed the control diet. Though no effect was observed for mortality of the birds during the experimental phase of this study, the calculated feed cost/kg of BW gain exhibited negative impact on profitability when broilers were fed the Ile-marginal diet (Table 2). Birds fed the Ile-marginal diet yielded a lower monetary income when compared to the control fed birds. Although the Ile-marginal diet had a lower manufacturing least-cost value ($133.41/ton) than that of the control ($134.86), the concomitant reduction in BW gain was responsible for the effect observed in this study. Kidd *et al.* (2005) had previously documented the effect that amino acid density may have on monetary income, and results from this study confirms the need to satisfy all critical amino acids in the diet if deleterious consequences in growth and economic feasibility are to be avoided due to amino acid marginality or deficiency.

In Experiment 2, the supplementation of dietary Ile via L-Ile addition to the Ile-marginal diet used in Experiment 1 resulted in an improvement of feed conversion ($P < 0.003$) and a partial linear response ($P = 0.13$) in BW gain (Table 3). A linear improvement ($P = 0.006$) in feed conversion was observed as Ile was supplemented to the Ile-marginal diet (Table 3). The addition of 0.08% of L-Arg to the Ile-marginal diet showed no recovery in BW gain or feed conversion when compared to the Ile-marginal diet. This indicates that the response noted when Ile was supplemented to the Ile-marginal diet was Ile specific and not non-essential N or Arg related. Furthermore, the addition of 0.08% of L-Arg, even though is similar in mass weight to the addition of 0.08% L-Ile, corresponds to 3 times the amount of N supplementation due to the existence of the guanidyl group present in the structure of Arg, which gives this amino acid a higher CP equivalency value. Finally, it also suggests that the decrease in performance between the treatments in Experiment 1 was not due to the difference in CP between the treatments, but exclusive to an Ile-related deficiency. No effects were observed for yields of carcass or breast meat, or abdominal fat percentage in Experiment 2.

In conclusion, the limitation of Ile as the fourth limiting amino acid in practical broiler diets containing a minimum inclusion level of 2% of meat-and-bone meal has been corroborated, and concurs with previous models that projected Ile as the likely candidate to be the 4th limiting amino acid under these dietary circumstances (Kidd and Hackenhaar, 2005; Corzo, 2007).

The importance of maintaining adequate amounts of subsequent limiting amino acids after Thr has been shown and emphasized, and in this case of dietary Ile, particularly when using L-Thr in formulas that reduce dietary nutrient excesses and costs.

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Abbreviations: Standardized ileal digestibility, SID.

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