Dietary energy, digestible lysine, and available phosphorus levels affect growth performance, carcass traits, and amino acid digestibility of broilers

Nishchal K. Sharma,[*] Mingan Choct,[*] Mehdi Toghyani,[*] Yan C. S. M. Laurenson,[*] C. K. Girish,[†] and Robert A. Swick[*,1]

[*]*School of Environmental and Rural Science, University of New England, Armidale, New South Wales 2351, Australia; and* [†]*Nutrition and Care, Animal Nutrition, Evonik (SEA) Pte Ltd, Singapore*

ABSTRACT A 3-factor, 3-level Box-Behnken design was used to investigate the interaction effect of dietary digestible lysine (dLys, 9.5, 10.5, 11.5 g/kg), apparent metabolizable energy (AMEn, 12.77, 13.19, 13.61 MJ/kg) and available P (avP, 3.0, 4.0, 5.0 g/kg) levels on performance and amino acid (AA) digestibility of Ross 308 male broilers (n = 1,050) from d 14 to 34. The design consisted of 15 treatments each replicated 5 times with 12 birds per replicate. On d 34, 3 birds were sampled from each pen to collect ileal digesta (pooled per pen) to analyze AA. Response surface was fitted by first-, second-, or third-degree polynomial regressions in JMP statistical software v. 12.0.1. Feed intake (FI), weight gain (WG) and feed conversion ratio (FCR) were affected by dLys (linear and quadratic, $P < 0.01$), AMEn (linear, $P < 0.01$) and AMEn × avP ($P < 0.01$). Increased dLys increased FI but increased AMEn decreased FI in the birds fed the low-avP diet. However, when the avP level in the diet was increased, FI decreased to 13 MJ/kg AMEn and remained constant thereafter. Increased dLys increased WG whereas an increase in AMEn decreased WG in the birds fed the low-avP diet but had no effect on WG in those fed the high-avP diet. Increased dLys decreased FCR whereas increased AMEn decreased FCR in the birds fed the low-avP diet but had no effect on FCR in those fed the high-avP diet. Increased dLys increased breast yield percentage (linear, $P < 0.01$ and quadratic, $P < 0.05$) whereas increased AMEn decreased breast yield percentage (linear, $P < 0.01$). Dietary levels of dLys or avP had positive, linear effects on apparent ileal digestibility (AID) of methionine ($P < 0.01$) and threonine ($P < 0.01$) but had no effect on other AA ($P > 0.05$). These results indicate that increasing dLys levels above current industry standard would improve broiler performance irrespective of AMEn or avP levels of the diet.

Key words: amino acid density, apparent metabolizable energy, broiler, performance, phosphorus

2018 Poultry Science 97:1189–1198
http://dx.doi.org/10.3382/ps/pex405

INTRODUCTION

Energy and amino acids (**AA**) are the 2 largest and most expensive components in broiler diets. Broilers generally perform better when fed high AA-density diets (Vieira and Angel, 2012), but the requirements for digestible lysine (**dLys**) vary greatly depending on the strains and genders (Kidd and Tillman, 2016). The response of growing broilers to dietary energy density is variable and may depend on several factors such as bird gender, breed, age, etc., including AA density of diets (Classen, 2016). There is no general current consensus on the interaction of energy and dietary AA density on broiler performance and thus it requires further investigation with modern genotypes.

Phosphorus (**P**) is the third most expensive dietary component after energy and AA (Woyengo and Nyachoti, 2011). Although P plays a vital role in skeletal development, energy metabolism, AA metabolism, and protein synthesis, the requirement for P has not been established with certainty. Evidence in the literature suggests that the P requirement for broilers is much lower than NRC recommendations and the values currently used by the industry (Li et al., 2016). It was hypothesized that the requirements of dietary dLys (based on the ideal ratio as suggested by Baker and Han, 1994), apparent metabolizable energy corrected for nitrogen retention (**AMEn**), and available P (**avP**) of broilers are not in the same proportion, and these nutrients may interact with each other to affect broiler performance. This experiment was conducted to investigate the interaction effect of dietary dLys, AMEn, and avP levels on growth performance and AA digestibility of broilers.

MATERIALS AND METHODS

Animal Ethics

All the experimental procedures were approved by the University of New England, Australia animal ethics committee (AEC16–031).

Received August 1, 2017.
Accepted November 23, 2017.
[1]Corresponding author: rswick@une.edu.au

Table 1. Box-Behnken design consisting of 3 factors each with 3 levels.

Factors (Independent variables)	Low (-1)	Levels used Medium (0), center point	High (1)
X_1: dLys (g/kg)	9.5	10.5	11.5
X_2: AMEn (MJ/kg)	12.77	13.19	13.61
X_3: avP (g/kg)	3.0	4.0	5.0

Table 2. Box-Behnken design for 3-factors each with 3-levels with a total of 15 treatments.

Treatments	Independent variables dLys (g/kg)	AMEn (MJ/kg)	avP (g/kg)
1	9.5	12.77	0.4
2	9.5	13.61	0.4
3	11.5	12.77	0.4
4	11.5	13.61	0.4
5	10.5	12.77	0.3
6	10.5	12.77	0.5
7	10.5	13.61	0.3
8	10.5	13.61	0.5
9	9.5	13.19	0.3
10	11.5	13.19	0.3
11	9.5	13.19	0.5
12	11.5	13.19	0.5
13 (center point)	10.5	13.19	0.4
14 (center point)	10.5	13.19	0.4
15 (center point)	10.5	13.19	0.4

Experimental Design

A 3-factor, 3-level Box-Behnken design (**BBD**) (De Leon et al., 2010) was used to investigate the effect of dietary dLys, AMEn, and avP levels on performance, carcass yield and amino acid digestibility of broilers. The independent variables (factors) used in this study were dLys (9.5, 10.5, 11.5 g/kg), AMEn (12.77, 13.19, 13.61 MJ/kg), and avP (3.0, 4.0, 5.0 g/kg) as shown in Table 1. The design consisted of 15 treatments (with 3 center points) each replicated 5 times with 12 birds per replicate. The values of center points (dLys 10.5 g/kg, AMEn 13.19 MJ/kg, and avP 4.0 g/kg) were chosen based on the nutrient requirement of birds (AMINOChick® software v. 2, Evonik Industries AG, Essen, Germany). The arrangement of dietary treatments is presented in Table 2. The evaluated responses (dependent variables) were growth performance (feed intake [**FI**], weight gain [**WG**] and feed conversion ratio [**FCR**]), toe ash percentage, breast yield, abdominal fat yield, and coefficient of apparent ileal amino acid digestibility of broilers. The adequacy of the model was evaluated by the coefficient of determination (adjusted R^2), model *P*-value, and lack of fit testing. The model that had non-significant lack of fit was chosen and the non-significant terms were excluded from the model, which resulted in recalculations of the equation for each response. When more than one model had non-significant lack of fit, the model that had the highest adjusted R^2 value was chosen. The response surface plots were constructed to examine the effect of changing levels of 2 selected factors on the desired response when the third factor was kept constant.

Table 3. Analyzed nutrient composition of ingredients (g/kg, as-fed basis)[1].

Items	Wheat	Soybean meal	Canola meal	Meat meal
CP	118	474	354	518
AMEn, MJ/kg	13.20	9.88	7.75	8.40
Total P	2.5	7.5	12.0	68.0
dLys	2.85	26.62	15.59	17.10
dM+C	4.19	10.93	12.06	6.30
dThr	3.01	15.59	11.14	8.99
dArg	4.85	31.97	18.87	28.75
dIso	3.80	19.09	10.89	8.50
dVal	4.51	19.85	14.09	13.23
dTrp	1.30	5.72	3.75	1.29
dLeu	7.02	32.09	20.09	19.31

[1]AMEn and digestible amino acids were analyzed using NIRS (AminoNIR, Evonik Industries AG, Essen, Germany).

Bird Management and Diets

A total of 1,050 day-old Ross 308 male broiler chicks were fed a common starter diet (dLys 12.0 g/kg, AMEn 12.77 MJ/kg, avP 4.5 g/kg) from 0 to 14 d and allocated to treatment diets from 14 to 34 d. The birds were allocated to 75 pens on the basis of body weight to ensure consistency in pen weights. Each pen consisted of 4 nipple drinkers, one tube feeder, and fresh wood shavings as bedding material. The diets contained wheat, soybean, canola, and meat meals as major ingredients. The ingredients were analyzed for nutrient content (Table 3) before feed formulation. Thirteen experimental diets were formulated to contain similar ideal amino acid ratios but with different levels of dLys (9.5, 10.5, 11.5 g/kg), AMEn (12.77, 13.19, 13.61 MJ/kg) and avP (3.0, 4.0, 5.0 g/kg). For each level of avP, Ca to avP ratio was kept constant at 2:1. The chemical composition and nutrient specifications of experimental diets are presented in Table 4 and the analyzed nutrient composition are presented in Table 5. Diets were mixed and pelleted at 65°C at the University of New England, Australia feed processing facility. All diets were fed in crumble form to 10 d and as 3 mm pellets thereafter until finishing the 34-d study period. Birds were housed in an environmentally controlled facility with unlimited access to feed and water under a light regime of 24 h light for the first 48 h after chicks' arrival followed by a 1 h darkness each day up to d 7 and gradually increased to 6 h darkness from d 10. An initial temperature of 33 ± 1°C was maintained for wk 1, gradually decreased to 22 ± 1°C by the end of wk 3 and maintained at the same temperature until the end of the feeding study. Initial and final feed and body weights were determined on d 14 and 34. The weight of dead or culled birds were recorded to adjust FCR calculations, which was done by dividing FI by WG of birds from 14 to 34 d.

Sample Collection and Chemical Analyses

On d 34, 3 birds were sampled from each pen and euthanized by cervical dislocation to collect ileal contents,

Table 4. Composition and nutrient specifications of experimental diets (g/kg, as-fed basis).

Ingredients	Starter Diet[2]	Experimental Diets[1] 1	2	3	4	5	6	7	8	9	10	11	12	13
Wheat	615.6	743.7	704.0	652.1	667.1	702.3	697.4	674.9	666.9	725.5	637.3	721.3	629.8	680.1
Soybean meal	244.5	161.3	167.1	242.5	194.9	212.7	187.5	230.0	200.5	177.7	259.7	151.7	232.9	202.9
Canola meal	40.0	40.0	40.0	40.0	40.0	40.0	40.0	18.0	24.8	40.0	35.9	40.0	40.0	40.0
Meat meal	44.2	16.1	16.5	14.8	16.2	0.0	30.6	0.0	31.7	0.0	0.0	31.5	30.2	15.7
Canola oil	35.9	13.6	47.1	24.0	50.0	18.8	18.4	50.0	50.0	30.8	40.5	30.1	40.6	35.3
Limestone	4.49	8.83	8.66	8.75	8.60	8.86	8.76	8.90	8.60	8.94	8.70	8.71	8.63	8.70
Dicalcium phosphate	–	–	–	–	–	–	–	0.54	–	0.40	–	–	–	–
Titanium dioxide	–	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Salt	3.22	2.16	2.15	2.13	2.14	2.37	1.93	2.42	1.95	2.38	2.36	1.94	1.91	2.14
Sodium bicarbonate	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
L-lysine HCl	3.02	2.47	2.42	2.70	4.19	2.55	2.73	2.53	2.71	2.35	2.59	2.54	2.76	2.62
D,L-methionine	2.78	1.45	1.56	2.46	2.92	1.97	2.07	2.18	2.25	1.46	2.48	1.55	2.56	2.07
L-threonine	1.52	0.91	0.93	1.25	1.93	1.00	1.10	1.06	1.16	0.86	1.22	0.97	1.32	1.06
Choline chloride	0.66	0.83	0.86	0.53	0.76	0.67	0.71	0.70	0.74	0.83	0.53	0.86	0.56	0.70
Mineral mixture	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Xylanase powder[3]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salinomycin[4]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin mixture[5]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Phytase[6]	–	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Calculated nutrients														
CP	231	191	190	218	205	202	206	199	204	188	215	192	220	203
AMEn, MJ/kg	12.77	12.77	13.61	12.77	13.61	12.77	12.77	13.61	13.61	13.19	13.19	13.19	13.19	13.19
Total P	7.3	6.6	6.6	6.9	6.6	5.8	7.7	5.6	7.5	5.6	5.9	7.5	7.8	6.7
avP	4.5	4.0	4.0	4.0	4.0	3.0	5.0	3.0	5.0	3.0	3.0	5.0	5.0	4.0
Ca	9.0	8.0	8.0	8.0	8.0	6.0	10.0	6.0	10.0	6.0	6.0	10.0	10.0	8.0
dLys	12.0	9.5	9.5	11.5	11.5	10.5	10.5	10.5	10.5	9.5	11.5	9.5	11.5	10.5
dM+C	8.9	7.2	7.2	8.7	8.7	8.0	8.0	8.0	8.0	7.2	8.7	7.2	8.7	8.0
dThr	8.0	6.4	6.4	7.7	7.7	7.0	7.0	7.0	7.0	6.4	7.7	6.4	7.7	7.0
dVal	9.0	7.2	7.2	8.7	8.7	8.0	8.0	8.0	8.0	7.2	8.7	7.2	8.7	8.0
dArg	12.8	10.2	10.2	12.3	12.3	11.2	11.2	11.2	11.2	10.2	12.3	10.2	12.3	11.2

[1]Diet 4 also contained added L-Arginine (1.41 g/kg), L-Isoleucine (0.41 g/kg) and L-Valine (0.69 g/kg) into the formulation to balance digestible amino acid contents.

[2]Starter diet contained added Zn bacitracin 0.33 g/kg into the formulation.

[3]Feedzyme XBC 1000 (Feedworks, Australia).

[4]Sacox 120 (coccidiostat) provided 60 mg/kg of salinomycin sodium.

[5]Vitamin-mineral concentrate supplied per kilogram of diet: retinol, 12,000 IU; cholecalciferol, 5,000 IU; tocopheryl acetate, 75 mg, menadione, 3 mg; thiamine, 3 mg; riboflavin, 8 mg; niacin, 55 mg; pantothenate, 13 mg; pyridoxine, 5 mg; folate, 2 mg; cyanocobalamine, 16 μg; biotin, 200 μg; cereal-based carrier, 149 mg; mineral oil, 2.5 mg; Cu (sulphate), 16 mg; Fe (sulphate), 40 mg; I (iodide), 1.25 mg; Se (selenate), 0.3 mg; Mn (sulphate and oxide), 120 mg; Zn (sulphate and oxide), 100 mg; cereal-based carrier, 128 mg; mineral oil, 3.75 mg.

[6]Axtra® PHY10000 TPT (Dupont Animal Nutrition) provided 500 FTU/kg of phytase with following nutrient matrix values- Ca (1.4 g/kg), av P (1.5 g/kg), Na (0.3 g/kg), amino acids (dLys 0.2 g/kg, dMet+ Cys 0.17 g/kg, dThr 0.17 g/kg, dArg 0.16 g/kg, dIle 0.16 g/kg, dTrp 0.05 g/kg, dVal 0.15 g/kg) and energy (ME 0.28 MJ/kg).

Table 5. Analyzed nutrient composition of experimental diets (g/kg, as-fed basis).

Items	Experimental diets 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
DM	906	906	900	908	905	905	904	908	905	907	907	907	904	910	905
CP	195	190	222	207	207	215	201	205	191	218	192	222	207	208	206
Total P	5.1	5.0	4.9	5.5	4.4	6.3	4.3	6.4	4.2	4.3	5.6	6.8	5.3	5.1	5.1
Met	4.1	4.1	5.3	5.6	4.8	4.9	4.9	5.0	4.1	5.4	4.2	5.5	4.8	4.9	4.7
Cys	3.5	3.4	3.7	3.4	3.6	3.6	3.5	3.4	3.5	3.8	3.5	3.7	3.6	3.6	3.4
Lys	10.5	10.0	12.1	11.9	11.2	11.7	11.1	11.2	10.2	12.1	10.4	12.2	11.2	11.0	11.1
Thr	7.3	7.1	8.5	8.4	7.9	8.3	7.7	7.9	7.2	8.6	7.4	8.6	7.9	7.9	7.7
Arg	11.3	11.0	13.3	12.9	12.0	12.8	11.7	12.2	11.1	12.8	11.3	13.3	12.1	12.1	11.9
Ile	7.4	7.2	8.6	8.0	8.1	8.2	8.0	7.9	7.6	8.7	7.3	8.5	7.9	8.0	8.0
Leu	13.2	12.8	15.1	13.5	14.2	14.6	14.0	14.0	13.3	15.1	13.1	15.0	14.0	14.1	14.0
Val	8.8	8.5	10.0	9.5	9.4	9.6	9.2	9.3	8.9	10.0	8.8	10.0	9.3	9.4	9.4
His	4.5	4.3	5.1	4.6	4.8	4.9	4.8	4.6	4.6	5.2	4.5	5.1	4.8	4.8	4.7
Phe	8.8	8.5	10.1	9.1	9.4	9.6	9.3	9.1	8.9	10.1	8.4	10.0	9.4	9.3	9.2
Gly	9.1	8.6	9.9	9.0	8.5	10.9	8.2	10.4	8.0	8.9	10.1	10.9	9.5	9.4	9.2
Ser	8.7	8.5	9.8	8.9	9.3	9.7	9.2	9.2	8.6	9.8	8.7	9.9	9.3	9.2	9.1
Pro	13.3	12.7	13.7	12.5	12.9	14.2	12.8	14.0	13.3	13.8	14.6	14.6	14.1	13.7	13.5
Ala	7.9	7.6	8.9	8.1	8.1	9.1	7.9	8.7	7.4	8.6	8.2	9.3	8.4	8.3	8.3
Asp	15.0	14.5	18.2	15.6	16.6	17.2	16.7	16.5	15.1	18.4	14.8	18.2	16.5	16.5	16.3
Glu	42.1	40.4	44.9	41.7	43.9	44.2	43.0	42.8	42.6	45.4	42.3	45.2	43.9	43.5	43.4

Table 6. Treatment means for live performance, toe ash and carcass yield of broilers.

Treatment	Independent variables			Experimental response (dependent variables)							
				d 14–34			d 34				
	dLys[1] (g/kg)	AMEn[2] (MJ/kg)	avP[3] (g/kg)	Weight gain (g/bird)	Feed intake (g/bird)	FCR (g/g)	Toe ash (%)	Breast weight, g (d 34)	Breast yield[4], % (d 34)	Abdominal fat weight, g (d 34)	Abdominal fat yield[5], % (d 34)
1	9.5	12.77	4.0	1718	2718	1.582	10.6	511	19.3	24.6	0.92
2	9.5	13.61	4.0	1602	2478	1.547	10.4	444	17.4	29.9	1.20
3	11.5	12.77	4.0	1999	2815	1.408	10.9	601	21.7	19.4	0.70
4	11.5	13.61	4.0	1911	2708	1.417	10.5	549	20.6	22.8	0.85
5	10.5	12.77	3.0	1893	2864	1.513	10.5	573	20.9	24.0	0.88
6	10.5	12.77	5.0	1875	2753	1.468	10.4	544	20.7	19.5	0.74
7	10.5	13.61	3.0	1796	2635	1.467	10.0	521	20.1	24.2	0.94
8	10.5	13.61	5.0	1885	2760	1.464	10.3	540	20.5	25.9	1.00
9	9.5	13.19	3.0	1702	2689	1.580	9.8	460	18.1	27.4	1.08
10	11.5	13.19	3.0	1909	2690	1.409	10.4	569	21.1	21.0	0.78
11	9.5	13.19	5.0	1670	2587	1.549	10.5	480	18.7	25.2	1.00
12	11.5	13.19	5.0	1949	2727	1.399	10.7	568	21.1	22.4	0.82
13	10.5	13.19	4.0	1880	2749	1.462	10.8	553	20.8	23.4	0.90
14	10.5	13.19	4.0	1801	2653	1.473	10.4	513	20.1	21.9	0.83
15	10.5	13.19	4.0	1851	2741	1.481	10.8	553	20.4	22.7	0.84
SEM				22.4	32.8	0.007	0.30	18.6	0.472	1.42	0.053

[1]Digestible lysine.
[2]Apparent metabolizable energy corrected for nitrogen.
[3]Available phosphorus.
[4,5]Percentage of live body weight.

middle toes, breast meat, and abdominal fat. The contents of the ileum (portion of the small intestine from Meckel's diverticulum to approximately 1 cm proximal to the ileo-cecal junction) from each bird were gently squeezed and pooled per pen, then frozen and stored at −20°C until processed. Digesta samples were freeze dried at −50°C for 7 d and finely ground with an electrical grinder to pass through a 0.5 mm sieve to ensure a homogenous mixture. The breast meat and abdominal fat were weighed and calculated as a percentage of live body weight. Toes were ashed at 580°C to constant weight to calculate percentage toe ash. The ingredients were analyzed for AMEn and digestible amino acids by near-infrared spectroscopy (**NIRS**; AminoNIR, Evonik Industries AG, Essen, Germany). The diets and digesta samples were analyzed for DM by placing duplicate samples in a drying oven at 105°C for 36 h to constant weight (method 930.15; AOAC, 1990). Nitrogen content of the ingredients and diets were determined on a 0.15 g sample with a combustion analyzer (Leco model FP-2000 N analyzer, Leco Corp., St. Joseph, MI) using ethylenediaminetetraacetic acid (**EDTA**) as a calibration standard. Crude protein was calculated by multiplying percentage N by 6.25. Minerals in the diets were analyzed using inductively coupled plasma optical emission spectrometer (ICP-OES, Model-725, Agilent Industries Inc., Santa Clara, CA) using perchloric acid and hydrogen peroxide for digestion of the samples (Anderson and Henderson, 1986). The diet and freeze-dried digesta samples were analyzed for AA content by AA analyzer according to AOAC (1990) by method 994.12 at Evonik's AMINOLab in Singapore. Titanium dioxide concentrations were determined in duplicate for diets and digesta samples by the colorimetric method described by Short et al. (1996).

Calculations and Statistical Analysis

The coefficient of apparent ileal digestibility of AA was calculated using the indigestible marker as follows:

$$\begin{aligned}&\text{Coefficient of apparent ileal digestibility}\\&= 1 - [\text{TiO}_2\text{diet}(\%)/\text{TiO}_2\text{digesta}(\%)]\\&\times [\text{AA digesta}(\%)/\text{AA diet}(\%)\}\end{aligned}$$

A 3-factor, 3-level BBD function in JMP statistical software v. 12.0.1 was used to generate the response surface plots. Response surface was fitted by first-, second-, or third-degree polynomial regressions. The experimental units were pen means and a 5% level of probability was considered to be significant.

RESULTS

Growth Performance and Toe Ash

The overall mortality during the entire study period was less than 3% and there was no diet-related mortality ($P > 0.05$, data not shown). The effects of dietary treatments on FI, WG, FCR, and toe ash are shown in Table 6. For demonstration, Table 8 shows model selection for FCR related to the inclusion level of dLys, AMEn and avP and the model selection for other parameters were conducted in a similar way as shown in Table 9. For FCR, models 1 and 2 had significant lack of fit ($P < 0.05$) although the independent variables

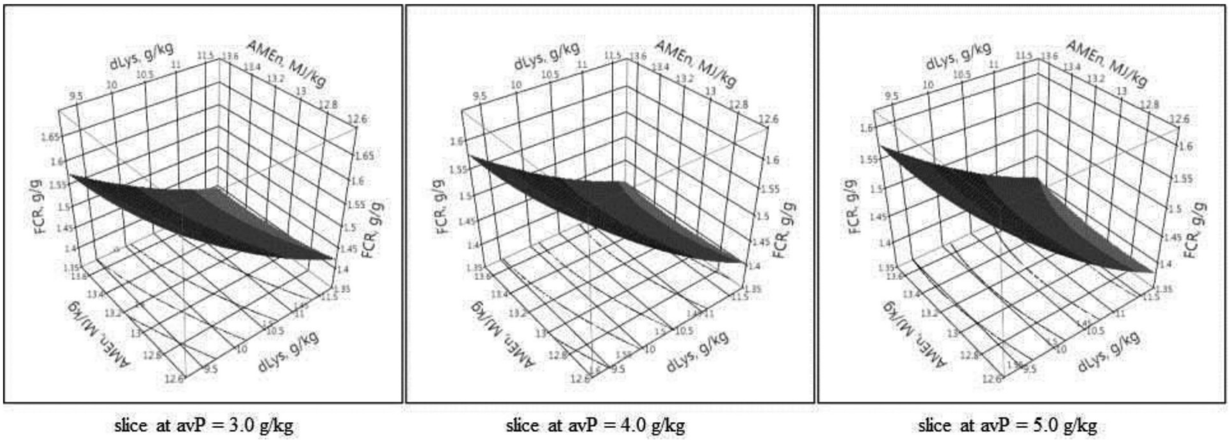

Figure 1. Response surface describing the relationship between FCR and dietary dLys, AMEn, and avP levels in broilers from d 14 to 34.

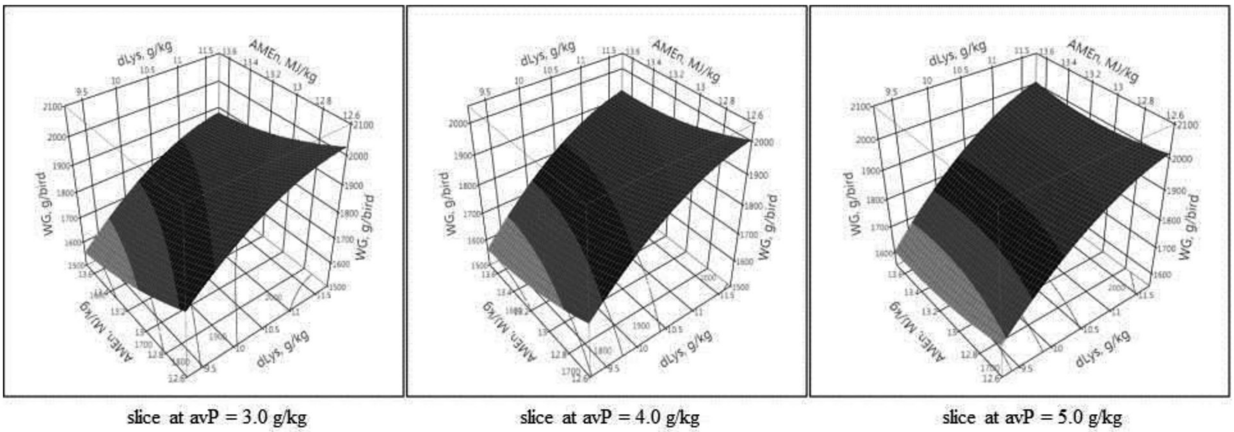

Figure 2. Response surface describing the relationship between weight gain and dietary dLys, AMEn, and avP levels in broilers from d 14 to 34.

were significant. Model 3 fitted well with significant linear, quadratic and interaction terms for estimating FCR. The response of FCR is described by the following equation (adj. $R^2 = 0.92$, $P < 0.001$),

$$Y = 1.4737 + (-0.078225 \times dLys) + (-0.008275 \times AMEn) + (-0.0097 \times avP) + (0.0105 \times AMEn \times avP) + (0.0101583 \times dLys^2)$$

The response surface for FCR is illustrated in Figure 1. Digestible lysine had linear and quadratic effect on FCR where increasing dLys decreased FCR irrespective of the levels of avP and AMEn in the diet. Digestible lysine tended to interact with AMEn ($P = 0.06$) to affect FCR. High dLys and AMEn tended to decrease FCR but this was not significant. There was an interaction between AMEn and avP levels in diet on FCR ($P < 0.01$). An increase in AMEn level decreased FCR in birds fed the low- and medium-avP diets but had no effect on FCR in those fed the high-avP diet.

Similarly, the response surface for WG is described by the following equation (adj. $R^2 = 0.80$, $P < 0.001$),

$$Y = 1830.9333 + (135.47782 \times dLys) + (-37.13206 \times AMEn) + (26.8 \times AMEn \times avP) + (-39.90861 \times dLys^2)$$

As shown in Figure 2, WG was affected by dLys (linear and quadratic, $P < 0.01$), AMEn (linear, $P < 0.01$) and AMEn × avP ($P < 0.05$). Increased dLys levels increased WG but increased AMEn levels decreased WG in the birds fed the low- and medium-avP diets but had no effect on WG in those fed the high-avP diet.

The response surface for FI is described as follows (adj. $R^2 = 0.53$, $P < 0.001$),

$$Y = 2698.1 + (57.37 \times dLys) + (-70.20 \times AMEn) + (59.05 \times AMEn \times avP) + (-48.97 \times dLys^2)$$

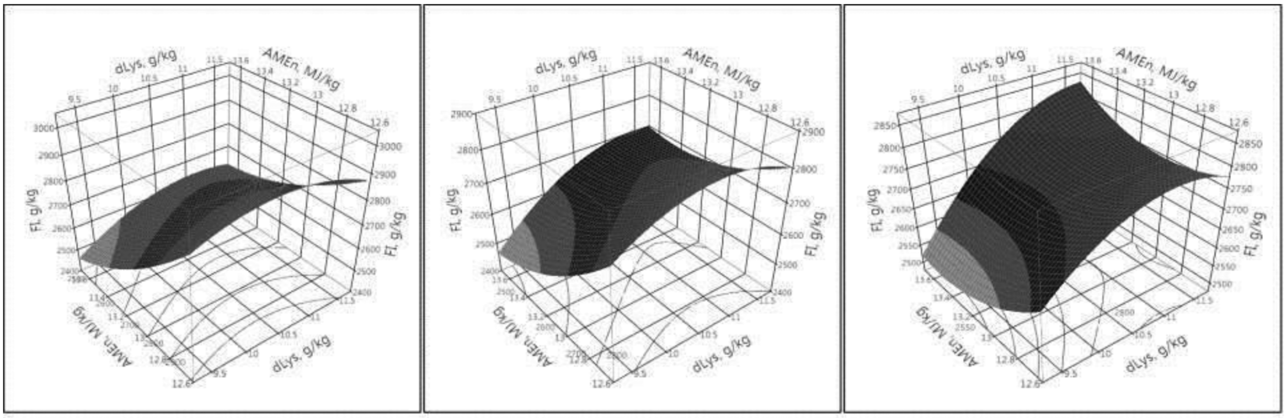

Figure 3. Response surface describing the relationship between feed intake and dietary dLys, AMEn, and avP levels in broilers from d 14 to 34.

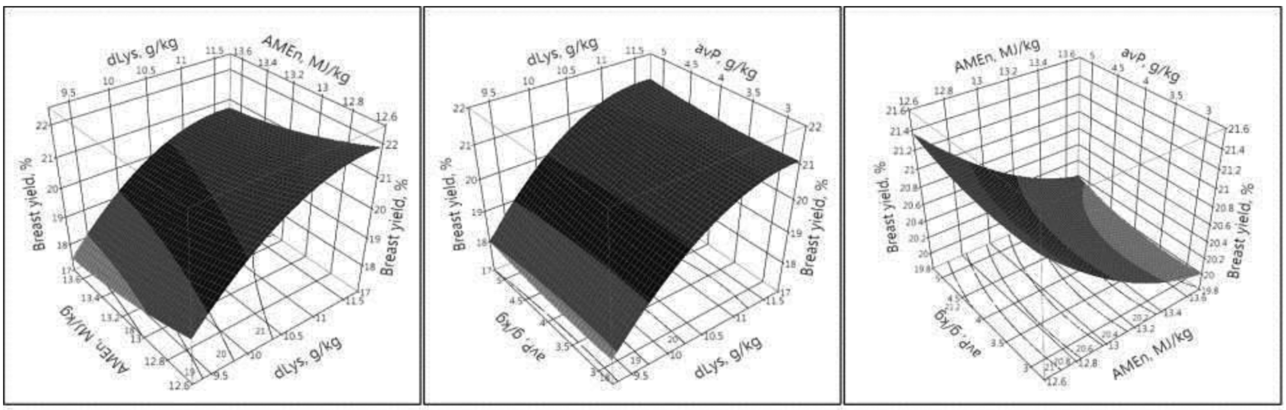

Figure 4. Response surface describing the relationship between breast yield and dietary dLys, AMEn, and avP levels in broilers at d 34.

As shown in Figure 3, FI was affected by dLys (linear and quadratic, $P < 0.01$), AMEn (linear, $P < 0.01$) and AMEn × avP ($P < 0.05$). Increased dLys levels increased FI but increased AMEn levels decreased FI in the birds fed the low-avP diet. When the avP level in the diet was increased to 5.0 g/kg, FI decreased up to a level of 13 MJ/kg AMEn but remained constant thereafter.

There was no significant difference in toe ash percent between any treatments and thus the response surface plots were not constructed for this parameter.

Carcass Traits

The effect of dietary treatments on breast and abdominal fat yields is presented in Table 6.

The response surface for breast yield is described by the following equation (adj. $R^2 = 0.50$, $P < 0.001$),

$$Y = 20.353333 + (1.305 \times dLys) + (-0.4275 \times AMEn) + (-0.581667 \times dLys^2)$$

As shown in Figure 4, increased dLys levels resulted in higher breast yield percentage (linear, $P < 0.01$ and quadratic, $P < 0.05$) but increased AMEn levels resulted in lower breast yield percentage (linear, $P < 0.01$).

The response surface for abdominal fat percentage is described by the following equation (adj. $R^2 = 0.50$, $P < 0.001$),

$$Y = 0.850 + (-0.125 \times dLys) + (0.095 \times AMEn) + (0.05 \times AMEn \times avP)$$

As shown in Figure 5, increased dLys levels resulted in lower abdominal fat percentage (linear, $P < 0.01$) but increased AMEn levels resulted in higher abdominal fat percentage at both low and high avP levels with a more distinct effect on high avP level (adj. $R^2 = 0.50$, $P < 0.001$).

Amino Acid Digestibility

The effect of dietary treatments on the coefficient of apparent ileal digestibility of amino acids (AID) of

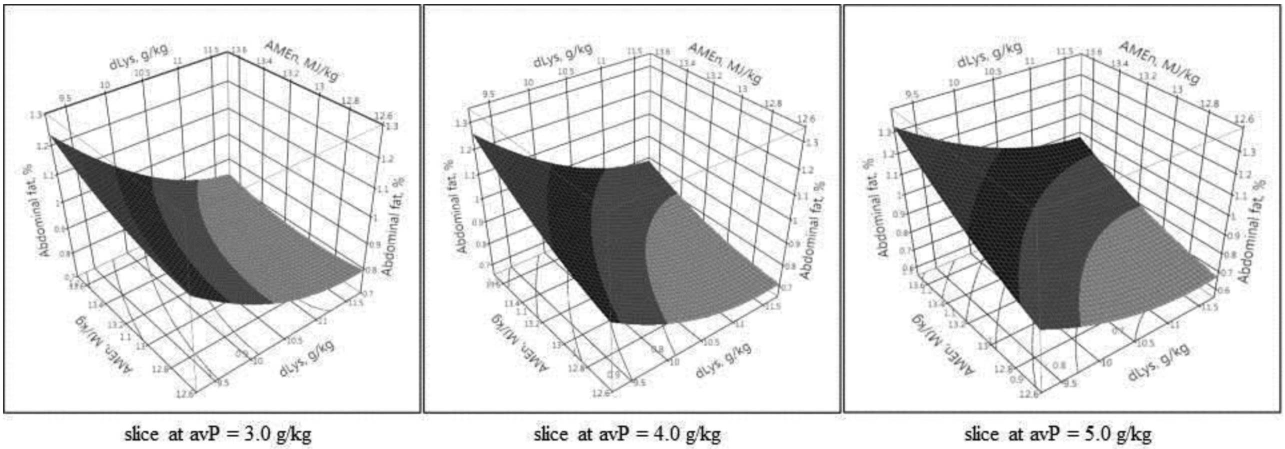

Figure 5. Response surface describing the relationship between abdominal fat yield and dietary dLys, AMEn, and avP levels in broilers at d 34.

Table 7. Treatment means for coefficient of apparent ileal digestibility of amino acids of broilers at d 34.

	Independent Variables			Experimental Response (Dependent Variables)											
Treatment	dLys[1] (g/kg)	AMEn[2] (kcal/kg)	avP[3] (g/kg)	Met	Cys	Lys	Thr	Arg	Ile	Leu	Val	His	Phe	Gly	Ser
1	9.5	12.77	4.0	0.897	0.755	0.861	0.786	0.860	0.824	0.831	0.811	0.832	0.854	0.773	0.806
2	9.5	13.61	4.0	0.906	0.785	0.860	0.796	0.875	0.834	0.843	0.822	0.845	0.866	0.798	0.819
3	11.5	12.77	4.0	0.922	0.729	0.857	0.780	0.860	0.822	0.826	0.805	0.828	0.849	0.756	0.797
4	11.5	13.61	4.0	0.930	0.780	0.886	0.830	0.887	0.850	0.847	0.841	0.851	0.869	0.794	0.826
5	10.5	12.77	3.0	0.911	0.771	0.864	0.794	0.873	0.828	0.835	0.816	0.840	0.857	0.791	0.816
6	10.5	12.77	5.0	0.916	0.772	0.869	0.813	0.873	0.841	0.845	0.829	0.847	0.865	0.784	0.823
7	10.5	13.61	3.0	0.912	0.745	0.867	0.791	0.863	0.830	0.834	0.815	0.836	0.855	0.783	0.809
8	10.5	13.61	5.0	0.915	0.773	0.862	0.802	0.859	0.833	0.836	0.819	0.834	0.855	0.767	0.814
9	9.5	13.19	3.0	0.889	0.742	0.844	0.763	0.863	0.813	0.822	0.799	0.825	0.850	0.767	0.793
10	11.5	13.19	3.0	0.917	0.752	0.865	0.795	0.869	0.829	0.833	0.815	0.839	0.855	0.789	0.810
11	9.5	13.19	5.0	0.908	0.791	0.866	0.805	0.865	0.839	0.843	0.826	0.846	0.861	0.786	0.820
12	11.5	13.19	5.0	0.938	0.801	0.896	0.842	0.888	0.870	0.872	0.857	0.871	0.888	0.810	0.849
13	10.5	13.19	4.0	0.910	0.742	0.861	0.786	0.856	0.822	0.830	0.807	0.831	0.852	0.760	0.801
14	10.5	13.19	4.0	0.915	0.765	0.867	0.803	0.865	0.836	0.838	0.821	0.839	0.859	0.781	0.815
15	10.5	13.19	4.0	0.917	0.777	0.876	0.812	0.875	0.846	0.851	0.834	0.851	0.868	0.796	0.827
SEM				0.008	0.013	0.011	0.013	0.008	0.012	0.012	0.012	0.010	0.010	0.013	0.012

[1]Digestible lysine.
[2]Apparent metabolizable energy corrected for nitrogen.
[3]Available phosphorus.

broilers is presented in Table 7. The response surface for coefficient of AID of methionine (Met) was described by the following equation (adj. $R^2 = 0.28$, $P < 0.001$),

$$Y = 0.889 + (0.0126 \times dLys) + (0.0060 \times avP)$$

As shown in Figure 6, increased level of dLys or avP increased coefficient of AID of Met (linear, $P < 0.001$) but increased AMEn levels had no such effect ($P > 0.05$). Similarly, the response surface for coefficient of AID of Threonine (Thr) was described by the following equation (adj. $R^2 = 0.19$, $P < 0.001$),

$$Y = 0.763 + (0.01296 \times dLys) + (0.01476 \times avP)$$

As shown in Figure 7, increased level of dLys or avP increased coefficient of AID of Thr (linear, $P < 0.01$) but increased AMEn had no such effect ($P > 0.05$). Dietary levels of dLys and avP had no effect on coefficient of AID of lysine, leucine, isoleucine, valine, arginine, histidine, phenylalanine, glycine and serine ($P > 0.05$).

DISCUSSION

It is common practice to use digestible AA (**dAA**) and ratios to dLys in formulating diets for broilers. The dLys level of the diet is critical as the minimums for other essential dAA are set based on it according to the ideal protein concept. With continuous improvement in genetics, it is important to determine the optimum level of dLys along with AMEn and avP level in diets. In this study, dLys level had the greatest influence on performance of birds. Increased levels of dLys resulted in improved FI, WG, FCR, breast yield and coefficient of AID of Met and Thr in broilers irrespective of AMEn (above 3050 kcal/kg) and/or avP (above

Table 8. Model selection for FCR of broilers from 14 to 34 d.

	Model 1		Model 2		Model 3	
Variables	Coefficient	*P*-value	Coefficient	*P*-value	Coefficient	*P*-value
First order						
dLys	−0.078225	<0.001	−0.078225	<0.001	−0.078225	<0.001
AMEn	−0.008275	<0.01	−0.008275	<0.01	−0.008275	<0.01
avP	−0.00970	<0.01	−0.00970	<0.01	−0.00970	<0.001
Second order						
dLys			0.0101583	<0.05	0.0101583	<0.05
AMEn			0.0069583	0.108	0.0069583	0.088
avP			−0.002692	0.531	−0.002692	0.506
Interactions						
dLys × AMEn					0.00755	0.055
AMEn × avP					0.0105	<0.01
dLys × avP					0.0027	0.487
Intercept	1.4814267	<0.001	1.47373	<0.001	1.4737333	<0.001
Adjacent R^2		0.903		0.910		0.920
P-value		<0.001		<0.001		<0.001
Lack of fit		<0.05		<0.05		0.414

Table 9. ANOVA, coefficient estimates and summary statistics of growth performance, carcass yield and apparent ileal amino acid digestibility in response to dLys, AMEn, and avP in broilers from 14 to 34 d.

	Body weight gain		Feed intake		Breast yield, %[1]		Abdominal fat, %[2]		Coefficient of AID methionine of		Coefficient of AID threonine of	
Variables	Coefficient	*P*-value	Coefficient	*P*-value	Coefficient	*P*-value	Coefficient	*P*-value	Coefficient	*P*-value	Coefficient	*P*-value
First order												
dLys	135.47782	<0.001	57.37	<0.001	1.305	<0.001	−0.125	<0.001	0.0126	<0.001	0.0129583	<0.01
AMEn	−37.13206	<0.001	−70.20	<0.001	−0.4275	<0.01	0.095	<0.001	0.0030	0.256	0.0052336	0.28
avP	–	–	−3.91	0.749	–	–	–	–	0.0060	<0.05	0.0147581	<0.01
Second order												
dLys	−39.90861	<0.01	−48.97	<0.01	−0.581667	<0.05	–	–				
AMEn	–	–	30.96	0.087	–	–	–	–				
avP	–	–	24.02	0.182	–	–	–	–				
Interactions												
dLys × AMEn	–	–	29.96	0.087			–	–				
AMEn × avP	26.8	<0.05	59.05	<0.001			0.05	<0.05				
dLys × avP	–	–	30.27	0.092			–	–				
Intercept	1830.9333	<0.001	2698.1	<0.001	20.353333	<0.001	0.850	<0.001	0.913	<0.001	0.8002035	<0.001
Adjacent R^2		0.800		0.529		0.500		0.503		0.280		0.18639
P-value		<0.001		<0.001		<0.001		<0.001		<0.001		<0.001
Lack of fit		0.061		0.111		0.862		0.708		0.879		0.4106

[1,2]Percentage of live body weight at d 34.

3.0 g/kg) levels in diets during 14 to 34 d of age. This is similar to the findings by Dozier et al. (2008), who reported no interaction between dietary AA density and AMEn to influence growth performance or meat yield, but main effects were observed. It has been reported that AA requirements increase proportionately faster than energy requirements and thus a higher AA to energy ratio is required in faster-growing broilers (Gous, 2010), which may explain the response observed in this study with increased levels of dLys. During the growing period of 14 to 35 d, broiler chickens have a high allometric growth for breast muscle compared to the whole body and thus the demand for Lys (or AA) is higher for breast muscle growth during this period (Vieira and Angel, 2012). Increased digestibility of Met and Thr with higher dLys level suggests that there may be a higher need for Met and Thr (higher than the level used in this study) along with Lys to maximize breast meat yield. This is because the maintenance requirement for AAs is increased as birds become heavier (Emmert and Baker, 1997) and Met+Cys requirements for maintenance are at least twice those for Lys (Edwards and Baker, 1999). Increased digestibility of methionine and threonine due to increased dLys level may simply be due to increased inclusion of these synthetic AA in the diets. Thus, any reduction in dLys levels in the diet during grower-finisher period may negatively impact performance and breast yield of broilers. The tendency for high dLys and high AMEn levels to decrease FCR observed in this study requires further investigation.

The lower levels (less than 3.0 g/kg) of avP in broiler diets have been shown to increase mortality, lower FI and WG (Kornegay et al., 1996; Fritts and Waldrup, 2006). Based on the current findings, it may be concluded that 3.0 g/kg avP (with 6.0 g/kg Ca) may be safely used in broiler diets at 14 to 34 d of age without

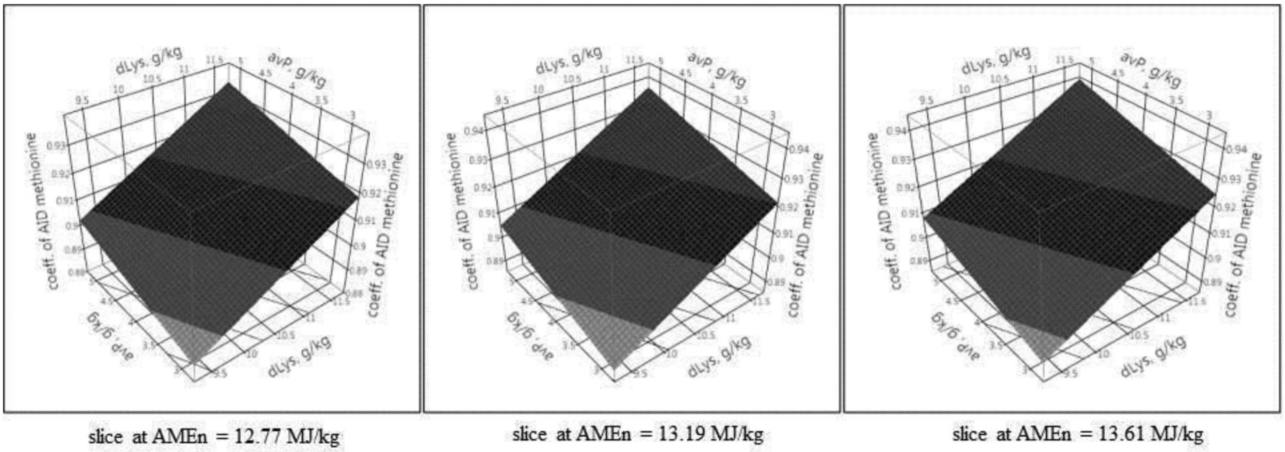

Figure 6. Response surface describing the relationship between coefficient of apparent ileal digestibility of methionine and dietary dLys, AMEn, and avP levels in broilers at d 34.

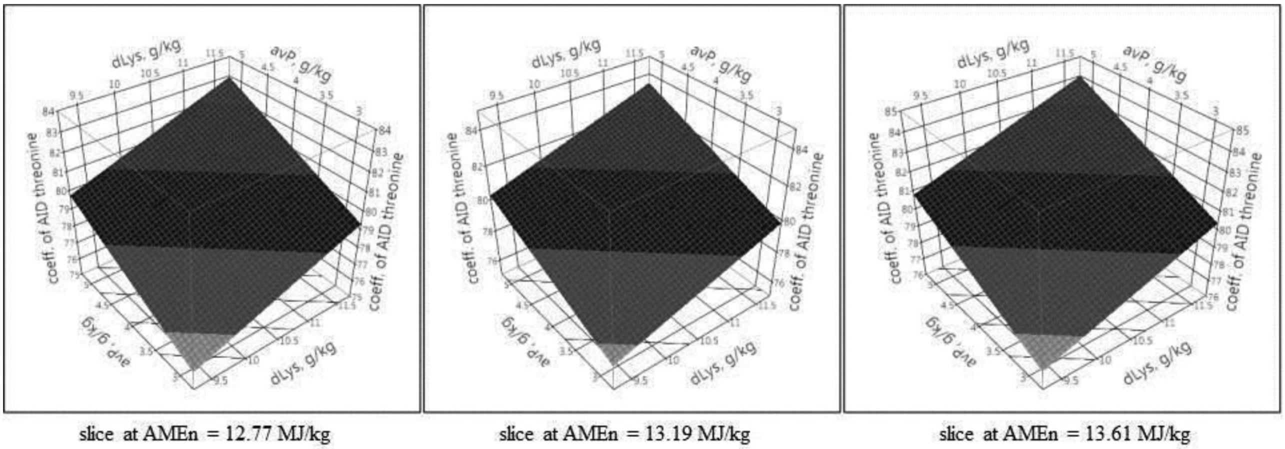

Figure 7. Response surface describing the relationship between coefficient of apparent ileal digestibility of threonine and dietary dLys, AMEn, and avP levels in broilers at d 34.

affecting FI, WG, FCR, and mortality. The results indicate that AMEn and avP levels interact to drive feed intake. With a high avP level (5.0 g/kg), increasing AMEn had very little effect on FI. With a low avP level (3.0 g/kg), increasing AMEn levels decreased FI. The effect of dietary AMEn on FI in broilers has been questioned with modern broiler genetics (Plumstead et al., 2007; Delezie et al., 2010; Classen, 2016). It has been suggested that factors other than AMEn are major drivers of FI including the first limiting nutrient, bird gender, age, breed, nutrient and physical density of feed, secondary metabolites in feed ingredients, environmental conditions, and disease (Classen, 2016). The current study showed that dietary avP level drives FI and interacts with AMEn in this regard. The lack of response of AMEn on WG at high avP level (5.0 g/kg) compared to sharp reduction in WG with increasing AMEn at low avP level (3.0 g/kg) in this study parallels that observed for FI. The decrease in WG and FCR with increasing dietary AMEn at low avP level may be due to the reduction in FI. The interaction between dietary ME and the quadratic effect of avP on final weight, slaughter weight, grower period growth, and total growth was also observed in a previous report (Venalainen et al., 2006) but the dietary ME levels used in that study were very low (11.0 and 12.0 MJ/kg) and perhaps not comparable with the present study. The decrease in breast yield and increase in abdominal fat with increased dietary AMEn in this study confirms that observed by Dozier et al. (2006) and may be largely due to an imbalance between energy and AA in the diet.

The current study indicates that increasing dLys levels in the grower feed above current industry standards may improve broiler performance without a concomitant increase in AMEn or avP levels. In addition, this study indicates that the influence of dietary AMEn on broiler performance depends on avP levels in the diet.

ACKNOWLEDGMENTS

The authors would like to thank Evonik (SEA) Pte. Ltd., Singapore, for providing the financial support and amino acid analysis for the current study.

REFERENCES

Anderson, D. L., and L. J. Henderson. 1986. Sealed chamber digestion for plant nutrient analysis. Agron. J. 78:937–938.

AOAC. 1990. Official Methods of Analysis. 15th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Baker, D. H., and Y. Han. 1994. Ideal amino acid profile for chicks during the first three weeks posthatching. Poult. Sci. 73:1441–1447.

Classen, H. L. 2016. Diet energy and feed intake in chickens. Anim. Feed. Sci. Technol. http://dx.doi.org/10.1016/j.anifeedsci.2016.03.004.

De Leon, A. C., M. T. Kidd, and A. Corzo. 2010. Box-Behnken design: alternative multivariate design in broiler nutrition research. World. Poult. Sci. J. 66:699–706.

Delezie, E., V. Bruggeman., Q. Swennen, E. Decuypere, and G. Huyghebaert. 2010. The impact of nutrient density in terms of energy and/or protein on live performance, metabolism and carcass composition of female and male broiler chickens of two commercial broiler strains. J. Anim. Physiol. Anim. Nutr. 94:509–518.

Dozier, W. A., M. T. Kidd, and A. Corzo. 2008. Dietary amino acid responses of broiler chickens. J. Appl. Poult. Res. 17:157–167.

Dozier, W. A., C. J. Price., M. T. Kidd, A. Corzo, J. Anderson, and S. L. Branton. 2006. Growth performance, meat yield, and economic responses of broilers fed diets varying in metabolizable energy from thirty to fifty-nine days of age. J. Appl. Poult. Res. 15:367–382.

Edwards, H. M., III, and D. H. Baker. 1999. Maintenance sulfur amino acid requirements of young chicks and efficiency of their use for accretion of whole-body sulfur amino acids and protein. Poult. Sci. 78:1418–1423.

Emmert, J. L., and D. H. Baker. 1997. Use of the ideal protein concept for precision formulation of amino acid levels in broiler diets. J. Appl. Poult. Res. 6:462–470.

Fritts, C. A., and P. W. Waldroup. 2006. Modified phosphorus program for broilers based on commercial feeding intervals to sustain live performance and reduce total and water-soluble phosphorus in litter. J. Appl. Poult. Res. 15:207–218.

Gous, R. M. 2010. Nutritional limitations on growth and development in poultry. Livest. Sci. 130:25–32.

Kidd, M. T., and P. B. Tillman. 2016. Key principles concerning dietary amino acid responses in broilers. Anim. Feed. Sci. Technol. 221:314–322.

Kornegay, E. T., D. M. Denbow, Z. Yi, and V. Ravindran. 1996. Response of broilers to graded levels of microbial phytase added to maize-soybean meal based diets containing three levels of non-phytate phosphorus. Br. J. Nutr. 75:839–852.

Li, X., D. Zhang., T. Y. Yang, and W. L. Bryden. 2016. Phosphorus bioavailability: a key aspect for conserving this critical animal feed resource with reference to broiler nutrition. Agricult. doi 10.3390/agriculture6020025.

Plumstead, P. W., H. Romero-Sanchez, N. D. Paton, J. W. Spears, and J. Brake. 2007. Effects of dietary metabolizable energy and protein on early growth responses of broilers to dietary lysine. Poult. Sci. 86:2639–2648.

Short, F. J., P. Gorton., J. Wiseman, and K. N. Boorman. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed. Sci. Technol. 59:215–221.

Vanalainen, E., J. Valaja., and T. Jalava. 2006. Effects of dietary metabolisable energy, calcium and phosphorus on bone mineralisation, leg weakness and performance of broiler chickens. Br. Poult. Sci. 47:301–310.

Vieira, S. L., and C. R. Angel. 2012. Optimizing broiler performance using different amino acid density diets: What are the limits? J. Appl. Poult. Res. 21:149–155.

Woyengo, T. A., and C. M. Nyachoti. 2011. Review: supplementation of phytase and carbohydrases to diets of poultry. Can. J. Anim. Sci. 91:177–192.