# Growth performance and accretion of selected amino acids in response to three levels of dietary lysine fed to fast- and slow-growing broilers

D. H. Tran,<sup>\*</sup> J. Th. Schonewille,<sup>†</sup> C. Pukkung,<sup>\*</sup> and S. Khempaka<sup>\*,1</sup>

\*School of Animal Technology and Innovation, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima 30000, Thailand; and <sup>†</sup>Department of Population Health Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

ABSTRACT Literature data indicate that feed intake is sensitive to the dietary Lys content particularly in fastgrowing birds. From a conceptual and a practical viewpoint, an interaction between genotype (i.e., fastgrowing vs. slow-growing birds) and dietary Lys content is of interest, but it needs confirmation owing to a dearth of studies addressing this issue. A study was conducted with 266 Cobb 500 birds and 266 Thai native crossbreed birds serving as models for fast-growing broilers (FGB) and slow-growing broilers (SGB), respectively. Within genotype, chicks were randomly high allocated to diets containing either a  $(\mathbf{H}-\mathbf{LYS} = 1.36\%)$ , medium (1.17%), or low Lys (1.01%)content. Growth performance and the accretion of protein and selected amino acids were determined in birds from 1 to 21 d of age. Treatments were arranged in a factorial design with 6 replications/treatment. Low Lys vs. H-LYS caused a 42.1% lower feed intake in FGB (P < 0.001), but not in SGB (P = 0.596). The feed

conversion ratio (FCR (g feed/g BW gain)) was lowest in FGB (P < 0.001) and increased with decreasing dietary Lys contents (P < 0.001). The Lys induced increase in FCR, however, was more pronounced in SGB (P = 0.025). The absolute protein gain (g/bird) was influenced by the Lys content of feed and decreased by  $\sim 54\%$  and  $\sim 23\%$  in FGB and SGB, respectively (P < 0.001). The efficiency (% of intake) of protein accretion was found to be greater in FGB ( $P \le 0.001$ ) and decreased with decreasing dietary Lys ( $P \leq 0.001$ ). The efficiency of Lys accretion was found to be negatively affected by the dietary Lys content in FGB (P < 0.001) but not SGB ( $P_{\text{genotype}} \times \text{dietary Lys} = 0.008$ ). It can be concluded that a dietary Lys content of 1.01% does not safeguard both growth performance and body protein accretion efficiency in both FGB and SGB. The suboptimal growth performance in FGB, but not SGB, is partially counteracted by a Lys-induced reduction in feed intake.

Key words: dietary lysine, genotype, feed intake, growth performance, amino acid accretion

## INTRODUCTION

Worldwide consumption of poultry meat is projected to increase by 12.5% from 2019 to 2028 (Shahbandeh, 2019). In view of its potential competition with human food resources, environmental reasons and economics, efficient production of poultry meat is required, and thus, the use of a fast-growing, modern genotype broiler is warranted. On the other hand, a public debate on the use of fast-growing broilers (**FGB**) is currently ongoing

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and in several countries (i.e., China, Italy, Japan, Botswana, Vietnam, and Thailand among others), a tendency exists toward the production of poultry meat with the use of slow-growing, native, broiler breeds (Wattanachant et al., 2004, 2005; Chen et al., 2008; Rikimaru and Takahashi, 2010; Kgwatalala et al., 2013; Lan Phuong et al., 2015; Zotte et al., 2019a,b).

For obvious reasons, feed intake is a principal determinant of growth performance. Next to environmental factors such as temperature, RH, and stocking density (Feddes et al., 2002; Lin et al., 2006), feed intake in poultry is also influenced by dietary factors such as water supply, palatability, energy density, and fiber content of the feed (Kondra et al., 1974; Alenier and Combs, 1981; Ross et al., 1981; Ferket and Gernat, 2006; Maliwan et al., 2018). There are however also indications that the dietary Lys content affects feed intake in

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<sup>&</sup>lt;sup>1</sup>Corresponding author: khampaka@sut.ac.th

poultry. Indeed, Tesseraud et al. (1992) reported a  $\sim 57\%$  decrease in feed intake in Shaver-France broilers when the diet contained 0.65% instead of 1.01% Lys. Urdaneta-Rincon and Leeson (2004) also reported that feed intake in Ross 308 birds responded to a decrease in the dietary Lys content, that is,  $\sim 21\%$  lower feed intake when the diet contained 0.86% vs. 1.34% Lys. These results are in line with those reported by Fatufe et al. (2004) who found a  $\sim 35\%$  decrease in feed intake in male Ross broilers when the dietary Lys content decreased from 1.28 to 0.88%. In the same study (Fatufe et al., 2004), however, the feed intake of slowgrowing male chickens of a layer genotype (Lohmann White) did not respond to a decrease in the dietary Lys content. It thus appears that the inhibitory effect of a low Lys diet on feed intake depends on the genotype of the birds in question. Unfortunately, the study reported by Fatufe et al. (2004) appears the only one showing an interaction between the genotype of the chickens and the Lys content of their feed.

Both from a conceptual and a practical viewpoint, a genotype  $\times$  dietary Lys interaction is of interest, but the results published by Fatufe et al. (2004) need to be confirmed. Moreover, it is currently not known whether the interaction between genotype and dietary Lys on feed intake can be extrapolated to other genotypes such as slow-growing broilers (SGB) vs. FGB. This lack of knowledge prompted us to conduct the present study, and it was hypothesized that a low vs. a high dietary Lys content decreases feed intake in the fastgrowing, modern genotype broilers but not in slowgrowing, native crossbreed broilers. Obviously, a decrease in feed intake will also lower the amount of protein that can be deposited in the body and as such it may affect the efficiency of protein/amino acid accretion. It can be hypothesized that the efficiency of protein/amino acid accretion is favorably affected in FGB when feed intake, and thus protein intake is sensitive to a decrease in the dietary Lys content. We therefore measured, next to growth performance, also the accretion of protein and selected amino acids in the 2 genotypes of broilers.

## MATERIALS AND METHODS

## Ethical Considerations

The current experiment was approved by the Animal Ethics Committee of Suranaree University of Technology (**SUT**) (approval number: SUT3-303-58-36-06) and based on the Ethics of Animal Experimentation of the National Research Council of Thailand.

## Animals, Housing, and Experimental Design

Two genotypes of chicken were used in the present study, that is, Cobb 500 birds that served as a model for FGB, while a Thai native crossbreed was selected to serve as a model for a SGB. The 2 genotypes differ substantially in their growth capacity, thereby potentially enhancing the interpretation of the data. From each genotype, 266 birds were used. Fourteen hatchlings from each genotype were killed on arrival to determine the initial body composition of the birds. The FGB birds were obtained from a commercial hatchery (Pak Thong Chai hatchery, Nakhon Ratchasima, Thailand). The SGB chickens are a cross between ♂ Thai native chicken (Leung Hang Khao) and SUT synthetic breeder lines through a crossbreeding program and they were obtained from a hatchery belonging to SUT, Nakhon Ratchasima, Thailand. In an attempt to reduce potential effects on feed intake and BW gain owing to differences in time point of hatching and first access of feed after hatch (Lamot et al., 2014), we only used birds (both FGB and SGB) that hatched within a 24-hour time window. The SGB were unsexed because the gender of these birds cannot be established 1 d after hatch. We therefore also used unsexed FGB in the current experiment. At day 7, all birds were inoculated with Newcastle disease and infectious bronchitis vaccines (FATRO S.p.A., Bologna, Italy). At day 14, the birds were vaccinated against infectious bursal disease vaccine (FATRO S.p.A., Bologna, Italy).

The diets were fed in a mash form. Each pen  $(1.0 \times 1.5 \text{ m}^2)$  was equipped with a tray feeder and 1 drinker during the first 10 d of age. From day 11 onwards, a nipple-type drinker line (6 nipples) and round-bottomed hanging feeders were used to supply feed. Both feed and water were available for ad libitum consumption throughout the experiment. All birds were raised in an open-sided, naturally ventilated barn, with a 23-hour photoperiod using a fluorescent bulb as a light source, and they were housed on a concrete floor covered by rice husks disinfected with a disinfectant solution (glutaraldehyde). Brooding heat was provided throughout this phase by using an infrared heat lamp bulb (175 W) above the birds (1 for each pen). A brooding temperature of 35°C was provided for the first week after hatch, and it was reduced by 3°C per week.

The experiment had a  $2 \times 3$  factorial design with an experimental period of 21 d. There were 6 replicates per treatment with 14 birds per replicate. On arrival, all hatchlings were weighed, and the birds were, within genotype, stratified by BW to attain similar BW, between pens. Then, the pens were, within genotype, randomly allocated to the 3 experimental diets, that is, a high Lys (H-LYS) (1.36%), medium Lys (M-LYS) (1.17%), or a low Lys (**L-LYS**) (1.01%) content. With the exception of the Lys content, all experimental diets were formulated to meet or exceed the nutrient requirements as recommended by the Cobb broiler management guide (2015). Corn starch and L-glutamic acid were used to replace the variable Lys contents so as to keep the diets isonitrogenous and isocaloric (Tables 1 and 2).

## Data and Sample Collection

Feed was sampled directly after mixing of the various feed ingredients (Table 1). Duplicate samples ( $\sim 500$  g) were stored at  $-20^{\circ}$ C pending analysis. The birds were

Table 1. The ingredient composition of the experimental diets, that is, diets with a high- (H-LYS), medium- (M-LYS), or a low lysine (L-LYS) content.<sup>1</sup>

	Ex	perimental die	ets
Item	H-LYS	M-LYS	L-LYS
Ingredients			
Corn	56.81	56.81	56.81
Soybean meal	25.01	25.01	25.01
Corn DDGS	8.00	8.00	8.00
Rice bran oil	1.24	1.24	1.24
Calcium carbonate	1.79	1.79	1.79
Monocalcium phosphate	1.54	1.54	1.54
NaCl	0.48	0.48	0.48
$\operatorname{Premix}^2$	0.50	0.50	0.50
Cornstarch	1.91	1.76	1.61
L-Glutamic acid, purity 99%	0.61	1.01	1.41
L-Lys HCl, purity 78%	0.50	0.25	0.00
DL-Met, purity 99%	0.48	0.48	0.48
L-Thr, purity 98.5%	0.41	0.41	0.41
L-Arg, purity 99%	0.33	0.33	0.33
L-Ile, purity 99%	0.18	0.18	0.18
L-Val, purity 99%	0.16	0.16	0.16
L-Trp, purity 98.5%	0.05	0.05	0.05

Unless otherwise indicated, values are expressed as % as fed.

Abbreviation: DDGS, dried distiller's grains with soluble.

<sup>1</sup>Total lysine content for H-LYS, M-Lys, and L-LYS are 1.36%, 1.17%, and 1.01%, respectively.

<sup>2</sup>Premix contained the following nutrients (units are expressed per kg of diet): vitamin A, 15,000 IU; vitamin D<sub>3</sub>, 3,000 IU; vitamin E, 25 IU; vitamin K<sub>3</sub>, 5 mg; vitamin B<sub>1</sub>, 2 mg; vitamin B<sub>2</sub>, 7 mg; vitamin B<sub>6</sub>, 4 mg; vitamin B<sub>12</sub>, 25 mg; pantothenic acid, 11.04 mg; nicotinic acid, 35 mg; folic acid, 1 mg; biotin, 15  $\mu$ g; choline chloride, 250 mg; Cu, 1.6 mg; Mn, 60 mg; Zn, 45 mg; Fe, 80 mg; I, 0.4 mg; Se, 0.15 mg.

weighed on arrival and 21 d thereafter (Table 2). The remaining feed were collected at day 21 of the experimental period. On arrival, 14 hatchlings from each genotype were killed with the use of chloroform (99.8%; RCI LABSCAN, Bangkok, Thailand), and the carcasses were subsequently stored at  $-20^{\circ}$ C for further processing. After 21 d, 4 birds (2 males and 2 females) were selected on the basis of their BW (birds with BW closest to the pen mean) from each pen and housed for 24 h in metabolism cages so as to facilitate the fasting of the birds (i.e., reduction of gut fill). During fasting, the animals had unrestricted access to water. After the 24-hour fasting period, the animals were killed as already described. Then, the carcasses were stored at  $-20^{\circ}$ C until processing. The carcasses of both the hatchlings and the 21-dayold birds were processed as described by Edwards and Baker (1999). Briefly, the frozen carcasses were chopped with the use of a cutter (Crown Machinery, Taoyuan, Taiwan). Then, the parts from the 21-day-old birds that originated from the same pen were combined and subsequently ground (Xingtai Leibin commercial Co., Ltd., Hebei, China) for 3 times. A 6-mm die was used for the first 2 grindings, while a 3-mm die was used for the third grinding. After grinding, a subsample  $(\sim 300 \text{ g})$  was stored in plastic bags at  $-20^{\circ}$ C. Thereafter, the subsamples were freeze-dried (Gamma 2-16 LSC; Christ, UK) and subsequently ground again with the use of a blender (Panasonic, Osaka, Japan) to pass a 1-mm sieve. Thereafter, the samples were stored at  $-20^{\circ}$ C until analysis.

### Chemical Analysis

The DM content of the experimental diets was determined by drying at 135°C for 3 h (AOAC, 1990; ID 930.15). The ash content was determined by combustion at 550°C for 3 h (Thiex and Novotny, 2012). The nitrogen content was determined using the Dumas combustion technique (AOAC, 2006; ID 990.03) by means of a nitrogen analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) using L-aspartic acid (Sigma-Aldrich, St Louis, MO) as a calibration standard. Nitrogen was converted into CP by multiplying nitrogen with 6.25. Ether extract in feed and carcasses was determined with the use of a fully automated Soxhlet system (Foss, Soxtec 8000, Hilleroed, Denmark) as described by Association of Official Analytical Chemists (AOAC, [2006]) procedures ID 2003.05 and ID 991.36, respectively. The crude fiber content was determined as described by the Association of Official Analytical Chemists (AOAC, [1990]) procedure ID 962.09.

Amino acids in feedstuffs, diets, and the carcasses were analyzed as per the Association of Official Analytical Chemists (AOAC, [2000], procedure ID994.12). Briefly, performic acid (mixed phenol crystals,  $H_2O_2$ ) 30%, and formic acid solution 88%) was used to oxidize cystine and methionine to cysteic acid and methionine sulfone, respectively. Then, the samples were hydrolyzed for 30 min by means of HCl (6 M) phenol solution under nitrogen gas (Multiwave 3000; Anton Paar GmbH, London, UK) at a temperature of 150°C. Norleucine (Sigma-Aldrich, St Louis, MO) was used as an internal standard. Inherent to the oxidation with performic acid and hydrolysis with 6 MHCl, tyrosine and tryptophan could not be determined. Separation of amino acids was achieved with the use of an amino acid analyzer (Biochrom 30+, Cambridge, UK) using appropriate sodium buffers and ninhydrin reagent (Biochrom 30+, Cambridge, UK). Proline extinction was measured at 440 nm, and for all other amino acids, extinction was measured at 570 nm (Fountoulakis and Lahm, 1998).

## Statistical Analysis

Pen was considered as a statistical unit. All data were subjected to ANOVA using the GLM procedure in SPSS 18.0, using the model (SPSS Inc. 2010):

$$Y_{ij} = \mu + LYS_i + GENOTYPE_j + (LYS \times GENOTYPE)_{ii} + e_{ij}$$

where  $Y_{ij} = a$  response variable (e.g., growth performance, nutrient deposition);  $\mu = overall mean$ ; LYS<sub>i</sub> = level of dietary Lys (i = High, Medium or Low);  $F_j = GENOTYPE$ (j = FGB or SGB); (LYS × GENOTYPE)<sub>ij</sub> = interaction term between level of dietary Lys and genotype of bird; and  $e_{ij} = residual error$ . Both, LYS and GENOTYPE were set as fixed factors in the statistical model. Tukey's test was used to identify rations with different effects on the variable involved. Throughout, the level of statistical significance was preset at  $P \leq 0.05$ .

**Table 2.** The chemical composition of the experimental diets, that is, diets with a high- (H-LYS), medium- (M-LYS) or a low lysine (L-LYS) content.

	E	xperimental die	ts
Item	H-LYS	M-LYS	L-LYS
Analyzed composition			
DM	89.7	89.8	89.9
CP	21.2	20.9	20.7
Ether extract	4.1	4.2	4.2
Crude ash	5.3	5.3	5.3
Crude fiber	2.9	2.9	2.9
Total Lys	1.36	1.17	1.01
Total Met	0.85	0.79	0.83
Total Met $+$ Cys	1.19	1.14	1.16
Total Thr	1.12	1.09	1.09
Total Arg	1.60	1.50	1.53
Total Ile	1.00	0.98	0.98
Total Val	1.17	1.22	1.16
Calculated nutrient contents			
ME (kcal/kg)	2,984	2,982	2,980
CP	21.3	21.3	21.3
Ca	1.02	1.02	1.02
Available P	0.46	0.46	0.46
Linoleic acid	2.14	2.14	2.14
Digestible Lys <sup>1</sup>	1.27	1.07	0.87
Digestible Met	0.76	0.76	0.76
Digestible Met $+$ Cys	1.04	1.04	1.04
Digestible Thr	0.93	0.93	0.93
Digestible Arg	1.45	1.45	1.45
Digestible Ile	0.93	0.93	0.93
Digestible Val	1.04	1.04	1.04
Digestible Leu	1.62	1.62	1.62
Digestible His	0.58	0.58	0.58
Digestible Phe	0.83	0.83	0.83
Digestible Trp	0.23	0.23	0.23
$DEB, mEq/kg^2$	193	193	193

Unless otherwise indicated, values are expressed as % as fed.

<sup>1</sup>Digestible amino acid values of the diets were calculated using the digestibility coefficients reported by Ajinomoto Heartland LLC (2009) for the individual feedstuffs (i.e., corn, soybean meal, and corn DDGS), while digestibility coefficients of synthetic amino acids were assumed to be 100%.

 $^{2}$ DEB = dietary electrolyte balance, using the following conversion factors; 434.98, 255.74 and 282.06 for Na, K, and Cl, respectively (Hooge, 1995).

#### RESULTS

## Growth Performance

Initial BW was neither affected by genotype  $\times$  dietary treatments (P = 0.959) nor by dietary treatment (P = 0.788), but initial BW in SGB was found to be 10.1% greater (P < 0.001) compared with the FGB hatchlings (Table 3). Both, the final BW and BW gain were influenced by the interaction between the level of dietary Lys and the genotype of the birds (P < 0.001). In SGB, the feeding of L-LYS vs. H-LYS caused a 15.2% lower final BW (P < 0.001), but the final BW was found to be 47.5% lower (P < 0.001) in FGB when the birds were fed L-LYS instead of H-LYS. Gain of BW in the 2 genotypes was likewise affected. Feed intake was affected by an interaction between genotype and the level of dietary Lys (P < 0.001). Feed intake was 42.1% lower in FGB when the birds were fed L-LYS instead of H-LYS, but the level of dietary Lys did not affect feed intake in SGB (P < 0.001). Values on the feed conversion ratio (FCR) (g feed/g BW gain) were lowest in FGB (P < 0.001) and increased with decreasing dietary Lys contents (P < 0.001). The increase in FCR value, however, was more pronounced in SGB (P = 0.025), that is, 28% vs. 16% in FGB. Mortality was found to be 0.4% in both genotypes and was appeared to be unrelated with a specific dietary treatment.

### **Body Composition**

Upon ANOVA, the water content of the carcasses was found to be significantly different between the 2 genotypes of birds, that is, 71.3 and 72.3% in FGB and SGB, respectively (P = 0.012) (Table 4). Both, the protein and fat content of the carcasses were affected by genotype × level of dietary Lys ( $P \le 0.050$ ). The protein contents of carcasses decreased with a decreasing dietary Lys content, but the decrease was more pronounced in FGB, that is, 8.4 and 3.8% in FGB and SGB, respectively (P < 0.001). In contrast to the protein content, the fat content of the carcasses was inversely related to the dietary Lys content (P < 0.001), and the increase in fat content was more pronounced in SGB compared with FGB (P < 0.001).

The absolute gains (g/bird) of water, protein, fat, and energy retention (Table 4) were all affected by the interaction between the genotype of the birds and the dietary Lys (P < 0.001). The absolute gains in water, protein, and fat decreased by ~50, ~54, and ~61% respectively in FGB (P < 0.001) when L-LYS was fed instead of H-LYS. The effects of Lys on the absolute gain of water and protein were, however, less pronounced in SGB compared with FGB (P < 0.001), while the absolute fat gain did not respond to dietary Lys in SGB (P < 0.001). Likewise, energy retention decreased by ~47% in FGB in response to the decrease in the dietary Lys content (P < 0.001) but not in SGB (P = 0.094).

# Profile of Accreted Amino Acids (g/100 g Retained Protein)

The profile of accreted amino acids was not affected  $(P \ge 0.107)$  by genotype × dietary Lys content (Table 5). The relative accretions of Met + Cys, Thr, Phe, and His were similar  $(P \ge 0.146)$  between the 2 genotypes, while the relative accretions of Arg, Ile, Val, and Leu tended  $(0.057 \le P \le 0.094)$  to be lower in SGB than in FGB, that is, 8.0, 7.7, 5.7, and 2.5%, respectively. In contrast, the relative accretion of Lys was statistically different between the 2 genotypes and was found to be 6.0% lower (P = 0.001) in SGB vs. FGB.

The Lys accretion was also affected (P = 0.025) by the level of dietary Lys (Table 5) and values were found to be 6.3% lower when M-LYS instead of H-LYS was fed. Likewise, the lowest values on Thr, Ile, Val, and Phe accretion were also found when the diet contained a medium level of dietary Lys ( $P \le 0.037$ ). The relative accretion of Arg was greatest (P = 0.048) when L-LYS was fed, that is, 15.2% greater compared with M-LYS. The greatest values on Met + Cys and Leu accretion were also found when the animals consumed L-LYS vs. M-LYS diets but values were borderline statistically

**Table 3.** Growth performance of broilers from 1 to 21 d after hatch, in response to 3 dietary lysine levels and genotype of the broilers<sup>1</sup>, that is, fast-growing (FGB) or slow-growing (SGB) chickens.

$Genotype^2(G)$	FGB			SGB				<i>P</i> -value		
Lysine level <sup><math>^{3}</math></sup> (L)	High	Medium	Low	High	Medium	Low	SEM	L	G	$L \ge G$
BW										
Initial	$41.8^{b}$	$41.7^{\mathrm{b}}$	$41.3^{\mathrm{b}}$	$45.9^{\mathrm{a}}$	$45.8^{\mathrm{a}}$	$45.7^{\mathrm{a}}$	0.400	0.788	< 0.001	0.959
Final	$836.0^{\mathrm{a}}$	$789.2^{\mathrm{b}}$	$438.7^{\circ}$	$320.0^{\mathrm{d}}$	$313.5^{\mathrm{d,e}}$	$271.3^{\mathrm{e}}$	39.780	< 0.001	< 0.001	< 0.001
Gain	$794.2^{\mathrm{a}}$	$747.5^{\rm b}$	$397.4^{\circ}$	$274.2^{\rm d}$	$267.7^{\mathrm{d,e}}$	$225.7^{\mathrm{e}}$	40.080	< 0.001	< 0.001	< 0.001
Feed intake	$1,033.5^{\rm a}$	$1,019.2^{\rm a}$	$597.9^{ m b}$	$418.4^{\rm c}$	$430.4^{\circ}$	$442.0^{\circ}$	46.500	< 0.001	< 0.001	< 0.001
FCR, g/g	$1.30^{d}$	$1.37^{\rm c,d}$	$1.51^{\rm b,c}$	$1.53^{b,c}$	$1.62^{\mathrm{b}}$	$1.96^{\mathrm{a}}$	0.039	< 0.001	< 0.001	0.025

<sup>a,-e</sup>Means within each row with different superscripts are significantly different ( $P \le 0.05$ ).

Unless otherwise indicated, values are expressed as % as fed. Abbreviation: FCR, feed conversion ratio, calculated as g feed/g BW gain.  $^{1}n = 6$  replicates (pen) per treatment with 14 birds per replicate.

 ${}^{2}FGB = Cobb 500; SGB = Thai native crossbreed chickens.$ 

<sup>3</sup>High lysine level = 1.36%; Medium lysine level = 1.17%; Low lysine level = 1.01%.

significant, that is, P = 0.086 and P = 0.052, respectively. Relative His accretion was not influenced (P = 0.104) by the level of dietary Lys.

# Efficiency of Protein and Amino Acid Accretion (% of Intake)

The efficiency of protein accretion (Table 6) was not influenced by an interaction between genotype and the level of dietary Lys (P = 0.414). In case the birds were either fed with H-LYS or M-LYS, the efficiency of protein accretion (% of intake) was similar with respective values of 61.9 and 59.3%. However, when the birds were fed L-LYS instead of H-LYS, the efficiency of protein accretion dropped by ~13 percentage units (P < 0.001). In FGB, the efficiency of protein accretion was 59.7% of intake and the corresponding value in SGB was 6.5 percentage units lower (P < 0.001).

In contrast to total protein, the efficiency of Lys accretion was affected by genotype × dietary Lys (P = 0.008). Lys accretion was inversely affected by the dietary Lys content in FGB (P < 0.001) but not in SGB. In SGB, the efficiency of Lys accretion was similar between the dietary treatments (P = 0.224). Next to Lys, the efficiency of both Leu and His accretion was also affected by genotype × dietary Lys  $(P \le 0.037)$ . In both genotypes, Leu and His accretion decreased

with a decreasing dietary Lys content, but the decline in the efficiency of both Leu and His accretion was greater in SGB when the birds were fed M-LYS instead of L-LYS (P < 0.001). A tendency ( $P \le 0.094$ ) toward an interaction between genotype of the birds and the level of dietary Lys was found for the relative accretions of Met + Cys, Thr, and Phe. In all 3 cases, the accretions of Met + Cvs, Thr, and Phe in FGB birds decreased only when M-LYS instead of H-LYS was fed  $(P \le 0.004)$ . In contrast, the accretions of Met + Cys, Thr, and Phe in SGB were lowered when the birds ingested L-LYS vs. M-LYS ( $P \leq 0.001$ ). The accretions of Arg, Ile, and Val were not affected by genotype  $\times$  dietary Lys (P > 0.102). The relative accretion of Arg was not influenced by the intake of Lys (P = 0.107), but efficiency of Arg accretion was different (P = 0.001) between genotypes, that is, 46.2 and 37.7% in FGB and SGB, respectively. The relative accretions of Ile and Val were likewise influenced (P < 0.001), and the following values were found: in FGB, 37.8 and 37.7% accretion of Ile and Val, respectively, and in SGB, 31.1 and 31.9% accretion of Ile and Val, respectively. Furthermore, the relative accretions of both Ile and Val decreased with decreasing Lys content of the diet (P < 0.001), and values (% of intake) dropped from 41.2% (Ile) or 40.2% (Val) when H-LYS was fed to 30.9% (Ile) or 31.0% (Val) when L-LYS was fed.

**Table 4.** Chemical composition of whole carcass and weight gain from 1 to 21 d after hatch, in response to 3 levels of dietary lysine and genotype of broilers<sup>1</sup>, that is, fast-growing (FGB) or slow-growing (SGB) chickens.

$Genotype^2(G)$	FGB				SGB			P-value		
Lysine $evel^3(L)$	High	Medium	Low	High	Medium	Low	SEM	L	G	$L \ge G$
Carcass										
Water, % fresh	71.5	71.4	71.0	71.8	72.8	72.3	0.20	0.510	0.012	0.364
Protein, % DM	$63.0^{ m b,c}$	$61.5^{ m c}$	$57.7^{\rm d}$	$66.0^{ m a,b}$	$68.4^{\mathrm{a}}$	$63.5^{ m b,c}$	0.64	< 0.001	< 0.001	0.050
Fat, % DM	$24.2^{\mathrm{b,c}}$	$25.7^{\mathrm{a,b}}$	$27.5^{\mathrm{a}}$	$18.7^{\mathrm{d,e}}$	$16.0^{\mathrm{e}}$	$21.7^{\mathrm{c,d}}$	0.73	< 0.001	< 0.001	0.017
Weight gain, g/bird										
Water	$568.0^{\mathrm{a}}$	$533.4^{\mathrm{b}}$	$282.1^{\circ}$	$196.8^{\mathrm{d}}$	$194.8^{d}$	$163.3^{\mathrm{e}}$	28.48	< 0.001	< 0.001	< 0.001
Protein	$142.3^{\rm a}$	$131.2^{\rm b}$	$65.1^{\circ}$	$52.0^{d}$	$51.2^{d}$	$40.2^{\mathrm{e}}$	7.02	< 0.001	< 0.001	< 0.001
Fat	$55.1^{a}$	$55.6^{\mathrm{a}}$	$32.5^{\mathrm{b}}$	$13.4^{\rm c}$	$10.1^{\rm c}$	$12.8^{\circ}$	3.41	< 0.001	< 0.001	< 0.001
Energy (kcal/bird)	$1311^{\rm a}$	$1146^{\mathrm{b}}$	$618^{\rm c}$	$387^{\rm d}$	$351^{d}$	$325^{d}$	68.68	< 0.001	< 0.001	< 0.001

<sup>a,-e</sup>Means within each row with different superscripts are significantly different ( $P \leq 0.05$ ).

 $^{1}n = 6$  replicates (pen) per treatment with 14 birds per replicate.

 $^{2}$ FGB = Cobb 500; SGB = Thai native crossbreed chickens.

<sup>3</sup>High lysine level = 1.36%; Medium lysine level = 1.17%; Low lysine level = 1.01%.

**Table 5.** Main effects of 3 dietary lysine levels and genotype of broilers, that is, fast-growing (FGB) or slowgrowing (SGB) chickens on total body accretion of selected amino acids (g/16 g N) from 1 to 21 d after hatch<sup>1</sup>.

Item	$Genotype^2(G)$		Leve	l of dietary lysin	$e^{3}(L)$		P-value	
	FGB	SGB	High	Medium	Low	SEM	G	L
Lys	5.6	5.2	$5.6^{\mathrm{a}}$	$5.2^{\mathrm{b}}$	$5.5^{\mathrm{a,b}}$	0.06	0.001	0.025
Met + Cys	3.4	3.4	3.4	3.3	3.5	0.04	0.352	0.086
Thr	3.4	3.3	$3.4^{\mathrm{a}}$	$3.1^{ m b}$	$3.5^{\mathrm{a}}$	0.04	0.259	0.002
Arg	5.8	5.3	$5.4^{\mathrm{b}}$	$5.2^{\mathrm{b}}$	$6.1^{\hat{a}}$	0.15	0.074	0.048
Ile	3.1	2.8	$3.2^{\mathrm{a}}$	$2.5^{ m b}$	$3.1^{\mathrm{a}}$	0.08	0.057	0.001
Val	3.5	3.3	$3.5^{\mathrm{a}}$	$3.1^{ m b}$	$3.5^{\mathrm{a}}$	0.06	0.086	0.001
Leu	5.3	5.2	5.3	5.1	5.4	0.05	0.094	0.052
Phe	3.5	3.4	$3.5^{\mathrm{a}}$	$3.3^{ m b}$	$3.5^{\mathrm{a}}$	0.04	0.180	0.037
His	3.3	3.4	3.4	3.5	3.3	0.03	0.146	0.104

<sup>a,b</sup>Means within each row with different superscripts are significantly different ( $P \le 0.05$ ).

<sup>1</sup>*P* genotype × level of dietary lysine was  $\geq 0.107$  (n = 6 replicates (pen) per treatment with 14 birds per replicate). <sup>2</sup>FGB = Cobb 500; SGB = Thai native crossbreed chickens.

<sup>3</sup>High lysine level = 1.36%; Medium lysine level = 1.17%; Low lysine level = 1.01%.

#### DISCUSSION

## Dietary Lys Content, Genotype, and Feed Intake

The current data show clearly that the feed intake of FGB, but not SGB, was sensitive to the Lys content of the diet thereby confirming our hypothesis. The magnitude of the Lys-induced effect on feed intake in the present study was similar to that reported by Tesseraud et al. (1992), that is, 42.1 and 57%, respectively. Urdaneta-Rincon and Leeson (2004) and Fatufe et al. (2004) reported somewhat lower values on the inhibitory effect of a L-LYS diet on feed intake in Ross broilers, that is, 21 and 35%, respectively. In the present study, Lys contents ranged from 1.01 to 1.36%, while the dietary Lys contents of the diets used by Urdaneta-Rincon and Leeson (2004) ranged from 0.86 to 1.34 and 0.88 to 1.28%, respectively. Thus, the difference in the magnitude of response in feed intake

between the present study and the values reported by Urdaneta-Rincon and Leeson (2004) and Fatufe et al. (2004) are at first sight not related the Lys content of the L-LYS diets in the present study. It must be noted, however, that the ingredient compositions of the experimental diets differed between the present study and those already mentioned in this section, thereby implicating that the range in total Lys contents of the various experimental diets may not properly reflect the differences in digestible Lys contents of the diets used.

The inhibitory effect of low Lys diets on feed intake in FGB vs. SGB is not easy to explain, but it might be related to a difference in protein turnover between FGB and SGB (Maeda et al., 1990). In FGB, the fractional degradation rate of muscle protein is relatively lower than in SGB, while the fractional synthesis rate of muscle protein is similar between FGB and SGB (Hayashi et al., 1985; Tomas et al., 1991; Tesseraud et al., 2000). It can thus be speculated that FGB chickens have lower plasma Lys concentrations than

**Table 6.** Total body accretion of protein and selected amino acids (% of intake) from 1 to 21 d after hatch, in response to 3 dietary lysine levels and genotype of broilers<sup>1</sup>, that is, fast-growing (FGB) or slow-growing (SGB) chickens.

$Genotype^2(G)$	FGB			SGB				P-value		
Lysine $\operatorname{level}^3(L)$	High	Medium	Low	High	Medium	Low	SEM	L	G	$L \times G$
Protein	$65.0^{\mathrm{a}}$	$61.7^{\mathrm{a,b}}$	$52.5^{\circ}$	$58.7^{\mathrm{b,c}}$	$56.9^{ m b,c}$	$44.2^{\mathrm{d}}$	1.21	< 0.001	< 0.001	0.414
Lys	$56.2^{\mathrm{b}}$	$57.1^{a,b}$	$63.0^{\mathrm{a}}$	$48.0^{\circ}$	$51.1^{ m b,c}$	$47.6^{\circ}$	1.08	0.103	< 0.001	0.008
Met + Cys	$42.3^{\mathrm{a}}$	$37.0^{\mathrm{b}}$	$34.8^{\mathrm{b}}$	$36.8^{ m b}$	$35.5^{ m b}$	$27.8^{\circ}$	0.83	< 0.001	< 0.001	0.051
Thr	$46.6^{\mathrm{a}}$	$39.3^{ m b}$	$38.3^{ m b}$	$41.0^{\mathrm{b}}$	$37.0^{ m b}$	$30.2^{\circ}$	0.95	< 0.001	< 0.001	0.094
Arg	$48.4^{\mathrm{a}}$	$46.3^{\mathrm{a}}$	$43.8^{\mathrm{a,b}}$	$41.9^{\mathrm{a,b}}$	$37.0^{ m a,b}$	$34.1^{\mathrm{b}}$	1.36	0.107	0.001	0.824
Ile	$44.0^{\mathrm{a}}$	$33.4^{\mathrm{b,c,d}}$	$36.0^{\mathrm{a,b,c}}$	$38.3^{ m a,b}$	$29.3^{ m c,d}$	$25.7^{\mathrm{d}}$	1.25	< 0.001	< 0.001	0.293
Val	$42.7^{\mathrm{a}}$	$34.7^{b}$	$35.6^{\mathrm{b}}$	$37.7^{\mathrm{a,b}}$	$31.8^{\mathrm{b,c}}$	$26.3^{\circ}$	1.00	< 0.001	< 0.001	0.102
Leu	$41.7^{\rm a}$	$37.3^{ m b}$	$34.5^{\mathrm{b}}$	$36.7^{\mathrm{b}}$	$34.8^{\mathrm{b}}$	$27.1^{\circ}$	0.79	< 0.001	< 0.001	0.027
Phe	$54.0^{\mathrm{a}}$	$45.9^{\mathrm{b}}$	$43.6^{\mathrm{b}}$	$47.3^{\mathrm{b}}$	$43.5^{\mathrm{b}}$	$33.7^{\rm c}$	1.15	< 0.001	< 0.001	0.054
His	$72.1^{\mathrm{a}}$	$66.1^{\mathrm{a,b}}$	$54.5^{\rm c}$	$63.8^{\mathrm{b}}$	$65.0^{\mathrm{b}}$	$47.2^{\rm d}$	1.47	< 0.001	< 0.001	0.037

<sup>a,-d</sup>Means within each row with different superscripts are significantly different ( $P \leq 0.05$ ).

 $^{1}n = 6$  replicates (pen) per treatment with 14 birds per replicate.

 ${}^{2}FGB = Cobb 500; SGB = Thai native crossbreed chickens.$ 

<sup>3</sup>High lysine level = 1.36%; Medium lysine level = 1.17%; Low lysine level = 1.01%.

SGB. In case L-LYS diets are fed, plasma Lys concentrations might drop below, a currently unknown, threshold thereby causing, at least to a certain extent, inactivation of the hepatic vagus nerve which ultimately affects feed intake (Alam et al., 2014). Alternatively, there are indications that the feeding of Lvs deficient diets affects the production of thyroid hormones (Pastro et al., 1969; Carew et al., 1997) which may negatively influence feed intake. May (1979), however, reported that the feeding of Lys-deficient diets (50% lower than requirement) did not affect levels of circulating thyroid hormones, while Carew et al. (2005) reported a reduced feed intake when a Lys-deficient diet was fed, but in their study (Carew et al., 2005) plasma T3 levels were increased compared with the control (i.e., Lysadequate diet, 1.1%). Clearly, the relationship between Lys deficiency, thyroid hormones, and feed intake is not yet settled. Needless to say that the aforementioned notions are highly speculative, and future studies are required to elucidate the mechanism by which L-LYS diets depress feed intake in broilers.

# Growth Performance and Accretion of Body Protein and Fat

Except for L-LYS, the observed BW gains and FCR values in FGB and SGB were in line with those reported by Garcia et al. (2006), Siqueira et al. (2013), Maliwan et al. (2018, 2019). It thus appears that H-LYS and M-LYS provided enough Lys to safeguard the growth performance in both FGB and SGB. In case L-LYS was fed, feed intake dropped almost proportionally to BW gain in FGB but not SGB, thereby explaining the greater increase in FCR of the SGB vs. FGB birds. For obvious reasons, the absolute gains of water, protein, and fat were in line with total BW gain, but the current data indicate that the Lys content of the diet also affected the absolute (g/d) gains of protein and fat. In both genotypes, the protein content of the carcass was inversely related to the Lys content of the diet. It can thus be argued that L-LYS limited net protein synthesis. This notion is corroborated by Tesseraud et al. (1992, 1996) who reported that feeding of Lys deficient diets decrease net protein synthesis owing to a greater fractional breakdown of body protein. The latter reasoning implies greater plasma Lys levels in case Lys-deficient vs. Lysadequate diets are fed but apparently not to such an extent that it counteracts the Lys-induced effect on feed intake in FGB. Unfortunately, plasma Lys concentrations were not measured in the present study, and this lack of information hinders further substantiation on this issue. Next to the protein content of the carcass, the fat content of the carcass also, points to a decreased net protein synthesis. Indeed, it is generally accepted that the consumption of a diet containing a low Lysto-energy ratio enhances the accretion of body fat owing to relative excess of dietary energy (Leenstra, 1986; Leeson et al., 1996; Rosa et al., 2007; Abudabos and Aljumaah, 2012).

# Profile of Accreted Amino Acids

Overall, the profile of accreted amino acids was found to be almost similar between the 2 genotypes of birds, but Lys accretion was found to be greater in FGB. Perhaps, the greater Lys accretion in FGB is related to the intensive genetic selection of modern genotype broilers. The process of genetic selection led to greater proportions of breast meat (i.e., 12% of BW in 1957 compared with 23% in 2014 [Aftab, 2019]) and beast meat has the highest Lys contents within the broiler's carcass, that is, ~8% (Kerr et al., 1999). Unfortunately, the current data do not provide further clues to substantiate this notion.

Across the 2 genotypes, the lowest accretion values for all selected amino acids were found when the birds were fed M-LYS. The lower amino acid accretions after M-LYS, but not L-LYS, feeding are most likely not caused by a systemic error in the experimental design because within breed, birds were randomly allocated to their dietary treatments and the ingredient matrix of the experimental diets were almost identical, that is, ~97% similarity in ingredient composition (Table 1). Moreover, carcasses from the 3 dietary treatments were evenly distributed across the various amino acid assays. On the other hand, it seems unlikely that the lower amino acid accretion is specifically caused by M-LYS. For the lack of a better explanation, we therefore qualify these observations as an unfortunate coincidence.

# *Efficiency (% of Intake) of Protein and Amino Acid Accretion*

In view of the aforementioned notions related to M-LYS feeding, only the observations on H-LYS and L-LYS are considered in the current section. Overall, the efficiency (% of intake) of protein/amino acid accretion was greater in FGB. This observation can be explained, at least partly, by a difference in protein/amino acid absorption. Schmidt et al. (2009) reported that jejunal and ileal sections are relatively longer in modern genotype broilers compared with heritage lines thereby, at least potentially, enhancing the efficiency of protein absorption. Moreover, when the mean daily feed intake is expressed as % of mean BW, feed intake in FGB vs. SGB was found to be lower, that is, 11.2 and 11.9% for H-LYS and L-LYS, respectively, in FGB with corresponding values of 10.9 and 13.3% for SGB. It is well established that a lower level of feed intake causes a lower passage rate of feed particles, thereby enhancing protein absorption (Sibbald, 1979; Noy and Sklan, 1995). Next to a potential difference in protein absorption, the difference in the efficiency of protein/amino acid accretion between FGB and SGB may also be explained by a difference in amino acid oxidation between the 2 genotypes (Wang and Nesheim, 1972; MacLeod et al., 1988; Geraert et al., 1990). Unfortunately, the current experiment does not provide specific clues that warrants further speculation on this issue.

With the exception of Lys, the efficiency of protein/ amino acid accretion dropped with decreasing content of dietary Lys in both FGB and SGB. It was already mentioned that L-LYS most likely limited net protein synthesis, thereby preventing the incorporation of other amino acids in body protein (Sklan and Noy, 2004). The current finding on the efficiency of Lys accretion in FGB, but not SGB, is noteworthy and in line with previous findings reported by Fatufe et al. (2004) and Siqueira et al. (2013). Perhaps, modern genotype broilers, such as Cobb 500, are genetically primed to preserve Lys for the synthesis of body proteins rather than its use to fuel either oxidation (Edwards et al., 1999; Fatufe et al., 2004) or de novo fat synthesis (Ahmed et al., 2019).

In conclusion, a dietary Lys content of 1.01% does not safeguard both growth performance and efficient accretion of body protein in both FGB and SGB. The suboptimal growth performance in FGB, but not SGB, is partially counteracted by a Lys-induced reduction in feed intake. The current data support the use of FGB to produce poultry meat owing to the efficient conversion of feed protein into animal protein, thereby implicating a more favorable ecological footprint compared with SGB.

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## DISCLOSURES

The authors declare no conflicts of interest.

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