Increasing Dietary Lysine Increases Final pH and Decreases Drip Loss of Broiler Breast Meat

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ABSTRACT Responses to increased dietary Lys concentrations were evaluated on 1,584 Ross 308 male broilers between 21 and 42 d of age housed according to 2 bird densities. The experimental design was composed of 8 factorial treatments: 2 bird densities (22 or 44 broilers/1.7 m^2 pen) × 4 true digestible (TD) Lys levels (0.83, 0.93, 1.03, and 1.13%). There were 6 repetitions per treatment. Birds were weighed individually at d 21 and 42. Feed consumption was recorded per pen. Body weight gain and feed conversion were calculated over the experimental period. Forty-eight broilers per treatment were dissected at 42 d of age. Final pH and drip loss during storage were measured on the pectoralis major. Density adversely affected feed intake (169 ± 1 and 160 ± 1 g/d with 22 and 44 birds per pen, respectively, $P < 0.05$), growth rate (97.4 ± 0.5 and 91.0 ± 0.7 g/d, $P < 0.05$), and feed conversion (1.730 ± 0.008 and 1.760 ± 0.006, $P < 0.05$). Except for feed intake, there was no interaction between the effects of bird density and dietary Lys. An increase in dietary TD Lys from 0.83 to 0.93% resulted in an increased growth rate (from 91.8 ± 1.6 to 95.5 ± 0.8 g/d, $P < 0.05$), improved feed conversion (from 1.783 ± 0.008 to 1.742 ± 0.009, $P < 0.05$), and increased breast meat yield (22.0 ± 0.1% to 22.7 ± 0.2%, $P < 0.01$). Performance and body composition traits were not significantly improved for concentrations of TD Lys higher than 0.93%. However, final breast pH increased from 0.83 up to 1.03% TD Lys in the diet (6.02 ± 0.01 vs. 5.91 ± 0.01, $P < 0.05$), and drip loss correlatively decreased (0.85 ± 0.03% vs. 1.10 ± 0.06, $P < 0.05$). This result opens new way of research for the definition of an amino acid requirement and on metabolic pathways involved in variations of breast muscle pH.

Key words: broiler growth, lysine, density, pH, breast meat

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INTRODUCTION

Many studies have been published dealing with the response of broiler chickens to variable dietary Lys concentrations. Most of them aimed at defining dietary Lys requirement regarding feed efficiency, growth performance, and carcass composition (Leclercq, 1998) but barely considered Lys requirements in relation to meat properties. However, because a predominant proportion of poultry go on further processing (Mead, 2004), poultry companies are more interested in food technology as it relates to meat processing and product development. Only 1 study reported that increasing dietary Lys decreased light reflectance of breast fillets and increased green muscle disease incidence in tenders (Corzo et al., 2002). As widely described, increasing dietary Lys generally results in improved feed intake, feed conversion, and BW gain (Holsheimer and Veerkamp, 1992; Sterling et al., 2006). It can also alter carcass yield and composition by both increasing meat yield and reducing carcass fatness (Moran and Bilgili, 1990; Grisoni et al., 1991; Leclercq, 1998; Sterling et al., 2006). Recent advances highlighted that breast meat quality, in particular its processing ability, can be modulated by growth or body composition (Berri et al., 2001, 2007; Le Bihan-Duval et al., 2001; Duclos et al., 2007). Indeed, broilers with greater BW, breast yield, or lower abdominal fatness would exhibit breast with higher final pH, darker color, and greater water-holding capacity. The objectives of the present study were to (1) measure the effect of Lys concentration in the diet on growth performance, body composition, and some breast meat quality traits of fast-growing male broilers and (2) test the effect of the number of chickens per square meter (bird stocking density) on the Lys response, because most of the current experimental determinations were obtained in research stations using a bird stocking density lower than in practical conditions.

MATERIALS AND METHODS

A total of 1,730 one-day-old male chicks Ross (308) were received at the experimental unit of the Institut

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Table 1. Composition (%) and characteristics of the diets

Item	Starter (0 to 7 d)	Grower (8 to 20 d)	Experimental finisher (21 to 42 d) 0.83%[1]	0.93%[1]	1.03%[1]	1.13%[1]
Ingredients						
Maize	40.63	24.23	37.52	37.52	37.52	37.52
Wheat	10.00	30.00	18.00	18.00	18.00	18.00
Soybean meal, 48%	30.00	25.00	21.00	21.00	21.00	21.00
Full-fat soybean	12.00	12.00	8.58	8.58	8.58	8.58
Maize starch	—	—	0.38	0.255	0.129	0.004
Rapeseed (whole seed)	—	—	5.00	5.00	5.00	5.00
Soya oil	3.00	4.50	4.00	4.00	4.00	4.00
$CaCO_3$	1.00	1.00	0.60	0.60	0.60	0.60
Dicalcium phosphate	2.00	1.80	1.70	1.70	1.70	1.70
L-Thr	—	0.07	0.13	0.13	0.13	0.13
L-Lys HCl	0.17	0.17	0.00	0.125	0.251	0.376
DL-Met	0.25	0.26	0.32	0.32	0.32	0.32
Trace elements,[2] additives	0.95	0.97	0.97	0.97	0.97	0.97
Bentonite	—	—	1.80	1.80	1.80	1.80
ME,[3] kcal/kg	3,028	3,120	3,162	3,162	3,162	3,162
Calculated composition, %						
CP	22.6	21.1	18.5	18.7	18.8	19.1
Lys	1.33	1.23	0.94	1.05	1.14	1.25
Met	0.54	0.54	0.56	0.56	0.55	0.56
Met + Cys	0.89	0.88	0.87	0.87	0.86	0.88
Thr	0.84	0.83	0.80	0.81	0.80	0.81
Trp	0.27	0.26	0.23	0.22	0.23	0.23
Ca	1.12	1.07	0.89	0.89	0.89	0.89
Available P	0.44	0.42	0.40	0.40	0.40	0.40

[1]Percentage of true digestible Lys in the experimental finisher diets.

[2]Supplied the following per kilogram of diet: NaCl, 4 g; vitamin A (retinyl acetate), 10,000 IU; vitamin D_3, 2,000 IU; vitamin E (DL-α-tocopheryl acetate), 30 mg; vitamin K, 2 mg; thiamin, 1.5 mg; riboflavin, 4 mg; pyridoxine, 2.5 mg; vitamin B_{12}, 0.015 mg; niacin, 30 mg; folic acid, 0.4 mg; biotin, 0.2 mg; calcium pantothenate, 10 mg; cholin, 500 mg; manganese, 85 mg; zinc, 60 mg; iron, 50 mg; copper, 25 mg; iodine, 1 mg; selenium, 0.25 mg; cobalt, 0.6 mg; butylated hydroxytoluene, 125 mg; enzyme, 0.2 g (Zyble, only in grower and finisher, Lohmann Animal Health, Ploufragan, France); coccidiostat (Clinacox, 0.5 g, Janssen Animal Health, Beerse, Belgium).

[3]Calculated value.

National de la Recherche Agronomique Avian Research Center (Nouzilly, France). Chicks were randomly distributed in 24 pens (71 chicks per pen) of a room containing 48 pens with an effective surface (that did not include the space taken up by the feeder) of 1.7 m^2. Rearing temperature was 31°C at 1 d, reduced by 1°C every other day to achieve 26°C at 11 d, then 25°C at 16 d, 24°C at 20 d, 23°C at 24 d, 22°C at 28 d, and 21°C after 32 d of age. The lighting program was constant at 20 lx for 24 h of light until 3 d, 3 to 5 lx for 14L:10D until 14 d, 3 to 5 lx for 18L:6D from 14 to 21 d, and 3 lx that increased from 18L:6D to 24 h of light from 21 to 42 d. At d 7, chicks were individually tagged. At d 17, chicks from each pen were allocated by tag number in 2 groups of 22 and 44 chicks to obtain housing densities of 13 and 26 birds/m^2, respectively. The groups of 44 chickens were placed in the empty pen, and the groups of 22 chickens stayed in their pen of origin. The extra chickens were euthanized. During the experiment, birds were watered and fed ad libitum (Table 1). Starter feed was provided as crumbles (small from 0 to 2 d and large from 3 to 7 d). Then, chicks received a grower diet from 8 to 21 d in 2.5-mm pellets. At d 21, the chickens were individually weighed after a night of starvation, and the neutrality of the design was checked. The following day, grower feed was offered; grower feed refusals were weighed, and the growing diet was replaced by an experimental finisher feed that was, after L-Lys HCl supplementation, pelleted 2.5 mm. The experimental basal feed was supplemented with 3 levels of Lys base (0.1, 0.2, and 0.3% using, respectively, 0.125, 0.251, and 0.376% L-Lys HCl in addition to the basal content [0.95% total Lys including 0.83% true digestible (**TD**) Lys]. The TD Lys of the diets was calculated according to Sauvant et al. (2004). The introduction of L-Lys HCl was made at the expenses of maize (*Zea mays*) starch (Table 1).

At d 42, the chickens were weighed individually after 6 h of starvation. Eight chickens by pen, representing the average and the variability of the pen, were selected to be slaughtered the next day. After 8 h of feed withdrawal, the 384 selected broilers were slaughtered at the Institut National de la Recherche Agronomique Avian Research Center experimental processing plant. The chickens were weighed, stunned in a water bath (125 Hz AC, 80 mA/bird, 5 s) and then killed by ventral neck cutting. After partial evisceration (only gut was removed), whole carcasses were air-chilled (airflow of 7 m^3/s) and stored at 2°C until the next day. Carcasses were weighed and deboned 1 d after slaughter. Abdominal fat, left thigh, and left pectoral muscles (major and minor) were removed and weighed. All variables were expressed in absolute weight and as percentages of the carcass weight.

Table 2. Effect of stock density and true digestible Lys level on mortality, average daily BW (DBW), weight gain (WG), feed intake (FI), feed conversion (FC), carcass, and breast meat traits[1]

	Low				High				Level of significance			
True digestible Lys	0.83%	0.93%	1.03%	1.13%	0.83%	0.93%	1.03%	1.13%	Density (D)	Lys (L)	D × L	SEM
Mortality, %	0.76	1.52	2.27	1.55	8.75	5.33	3.79	3.43	0.002	NS	NS	1.56
Growth traits												
BW at 21 d, g	725	731	733	724	713	717	722	716	0.02	NS	NS	6.6
BW at 42 d, g	2,747	2,782	2,784	2,789	2,546	2,679	2,639	2,646	<0.001	0.02	NS	26
Daily weight gain 21 to 42 d, g/d	96.3	97.6	97.6	98.3	87.3	93.4	91.3	91.9	<0.001	0.02	NS	1.15
FI 21 to 41 d, g/d	171[a]	168[a]	168[a]	168[a]	156[c]	165[ab]	159[c]	161[bc]	<0.001	NS	0.009	1.52
FC 21 to 42 d	1.77	1.72	1.72	1.71	1.79	1.76	1.75	1.75	<0.001	<0.001	NS	0.01
Carcass traits[2,3]												
Carcass yield, %	77.4	77.8	78.0	78.1	77.9	78.0	78.0	78.2	NS	NS	NS	0.19
Abdominal fat yield, %	3.39	3.11	2.87	2.89	3.39	3.40	3.31	3.28	0.003	0.04	NS	0.13
Breast muscle weight, g	475	500	506	518	450	481	476	476	0.001	0.001	NS	7.9
Breast muscle yield,[3] %	22.1	22.8	23.0	23.2	21.9	22.6	22.6	22.6	0.03	0.001	NS	0.21
Thigh yield,[3] %	30.8	30.9	30.8	30.4	31.2	31.3	31.4	31.1	0.001	NS	NS	0.18
Breast meat traits												
Final pH	5.93	5.97	6.00	6.04	5.89	5.98	6.04	6.00	NS	<0.001	NS	0.017
Drip loss,[4] %	1.01	0.86	0.81	0.82	1.18	1.00	0.88	0.91	0.02	<0.001	NS	0.060

[a–c]Means within a row with no common superscripts are significantly different ($P \leq 0.05$).

[1]Data presented as means (n = 6 pens per treatment).

[2]Carcass yield is expressed as a percentage of BW.

[3]Abdominal fat, breast, and thigh yields are expressed as percentages of carcass weight.

[4]Drip loss is expressed as a percentage of pectoralis major weight at 24 h postmortem.

At 24 h postmortem, the final pH of the breast pectoralis major was measured with a portable pH meter (model 506, Crison Instruments SA, Alella, Barcelona, Spain) by inserting a glass electrode directly in the thickest part of the pectoralis major. The water-holding capacity of meat has been estimated by measuring drip loss of the raw meat after storage: the pectoralis major muscle was weighed 24 h postmortem and immediately placed in a plastic bag, hung from a hook, and stored at 2°C for 4 d. After hanging, the sample was wiped with absorbent paper and weighed again. The difference in weight corresponded to the drip loss and was expressed as the percentage of the initial muscle weight.

There were 8 treatments representing a factorial 2 (animal stock densities) × 4 (TD Lys contents of the feed). Those 8 treatments were repeated 6 times according to a plan in 6 randomized blocks. A block was established by 8 neighboring pens. All data were subjected to a 2-way ANOVA (bird density × feed) with supplementary evaluation of the block effect. The averages were distinguished by the multiple mean comparison test of Newmann and Keuls.

RESULTS AND DISCUSSION

Mortality was higher ($P = 0.002$) in the high-density groups, and there was no significant effect of dietary Lys on this trait (Table 2). However, there was a trend for mortality to decrease with increasing dietary Lys under high-density conditions. But because most of mortality events occurred early during the experimental period (between 25 and 27 d), this observation may not be relevant and not related to the dietary Lys level. A significant negative effect of density was observed on BW at 21 d of age. This effect might be put in relation with mortality observations and suggests that birds reared with a high density might undergo greater stress or discomfort than birds reared at low density. Even though most studies did not report increased mortality with increased density (Feddes et al., 2002; McLean et al., 2002; Dawkins et al., 2004), it has been suggested that broilers reared with a space lower than 0.05 m^2/bird (i.e., more than 20 birds/m^2) would be more stressed than birds housed under lower density (Cravener et al., 1992).

There were both a significant density and a significant Lys effect on the final BW (42 d) and weight gain without significant interaction on average values. Between 21 and 42 d of age, feed intake, feed conversion, and growth were significantly lower at the higher stocking density. These observations are in agreement with several studies in which feed intake and growth rate were adversely affected by increasing the density (Cravener et al., 1992; Feddes et al., 2002; McLean et al., 2002; Dawkins et al., 2004, 2005). The lowest TD Lys level (0.83%) significantly reduced BW gain compared with higher Lys levels ($P < 0.05$). Between 21 and 42 d, a significant effect of the density was observed on feed intake. There was also a significant interaction between density and Lys level. Feed intake was significantly lower in high-density birds, except for birds fed with 0.93% TD Lys. Dietary Lys level had no effect on feed intake in the low-density groups, whereas under high density, there was an effect with broilers fed with 0.83 and 1.03% TD Lys showing the lowest intake. Feed conversion was significantly improved under both density situations with the first level of Lys supplementation (Table 2). Some studies reported that the positive effect on broiler BW gain of increasing dietary Lys is obtained through the improvement of both feed intake and feed conversion (Tesseraud et al., 1999; Fatufe et al., 2004; Sterling et al., 2006), whereas other reported that increasing Lys only improved feed conversion without affecting feed intake (Corzo et al., 2002, 2003, 2006). In the present study, there was no clear effect of dietary Lys on feed intake. However, supplemental TD Lys from 0.83 to 0.93% clearly improved feed conversion and BW gain. From a strictly growth and feed conversion approach, 0.93% Lys in the feed resulted in optimal performance regardless of bird density under our experimental conditions.

Regarding body composition traits, there was no significant interaction between bird density and Lys level in the diet (Table 2). High bird density compared with low density increased carcass fatness, decreased breast weight and yield, and concomitantly increased the proportion of thigh. This is in agreement with previous studies in which several carcass traits were adversely affected by increasing the density (Bilgili and Hess, 1995; Martrenchar et al., 1997; Dozier et al., 2006). The main effect of dietary Lys was an increase in breast meat weight and yield. Under our experimental conditions, only the differences in breast yield between broilers fed with 0.83% and those fed with higher levels of TD Lys were significant. The positive effect of increasing dietary Lys on breast yield has been described in many studies (Hickling et al., 1990; Moran and Bilgili, 1990; Garcia et al., 2006; Sterling et al., 2006). According to Tesseraud et al. (1996, 1999), the pectoralis major is more sensitive to Lys deficiency than muscles from the thigh. As already suggested in rats (Garlick et al., 1989), the higher response to Lys of the fast-twitch glycolytic pectoralis major could be partly attributed to the type and composition of muscle fiber. Increasing Lys in the diet also reduced carcass fatness. As already described by Leclercq (1998), the level of TD Lys for minimum abdominal fat appears higher (1.03%) than that for maximal weight gain and breast yield. Finally, increasing Lys level did not compensate for the negative effects of high stocking density conditions on feed efficiency, growth performance, and body composition.

Regarding breast meat properties, there was no significant interactions between bird density and Lys level in the diet (Table 2). The final pH steadily and significantly increased from 5.91 at 0.83% TD Lys to 6.02 at above 1.03% TD Lys in the diet. The muscle drip loss exhibited relatively symmetrical variations. From a meat quality standpoint, and at the difference of growth parameters, the optimal level of Lys in the diet was clearly above 0.93%.

Although a higher stocking density reduced growth and body composition parameters, there were very few significant interactions between this factor and level of dietary Lys. Additional regression analyses were performed (data not shown) to test the linearity of the dietary Lys response and if it was different under low or high stocking density. Results indicated no significant differences between the 2 groups of density, suggesting similar responses to dietary Lys in both conditions. In the present study, broken-line or Spline regression was not performed to determine the exact requirement in Lys, but our data suggest it can be estimated to be between 0.93 and 1.03% when feed efficiency, growth, and body composition performances are considered and may be at least 1.03% when breast meat properties are taken into account. Ojano-Dirain and Waldroup (2002) found that 1.02% TD Lys was necessary to maximize breast meat yield in 21- to 42-d-old broilers submitted to a moderate heat stress. Labadan et al. (2001) determined that the Lys requirement for maximal breast meat growth at similar age was 0.92% TD Lys. Therefore, our results are in good agreement with literature data. One of the most important observations in this study is the significant improvement in meat quality (yield and pH of breast meat) beyond the feed conversion and growth response. It is well established that as the final pH increases, the water-holding capacity and potentially the processing ability of broiler breast meat are improved (Barbut, 1997; Le Bihan-Duval et al., 2001; Zhang and Barbut, 2005). Because of the great development of further-processed product (Mandava and Hoogenkamp, 1999), technological properties have become major criteria for poultry processors to improve processing yields and profits. Recent advances highlighted that final pH of the breast meat is negatively related to the muscle glycogen stores at the time of death (Berri et al., 2005; Duclos et al., 2007). In addition, the glycogen content and therefore final pH of breast meat depends on muscle growth and more generally on body composition. Experimental selection for increased breast meat yield and reduced carcass fatness was associated with breast meat exhibiting lower muscle glycogen reserves, ultimately greater pH, darker color, and lower drip loss (Le Bihan-Duval et al., 1999; Berri et al., 2001). Moreover, chicken breast muscle glycogen levels decrease as muscle fiber diameter and more generally muscle weight and yield increase (Berri et al., 2007). These changes resulted in breast meat with greater pH and consequently darker color and improved water-holding capacity. The evolution of final meat pH with dietary Lys reported in the present study appears consistent with previous findings that suggest a cumulative effect of increasing breast meat and reducing carcass fatness on muscle metabolism, decreasing glycogen storage, and thereby reducing the amplitude of acidification postmortem. This result opens new ways of research for the definition of an amino acid requirement but also for the metabolic reasons that might explain these variations of muscle breast pH in relation with protein and carbohydrate metabolism in fast-growing broilers.

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