Research Note

The Influence of Dietary Sodium Chloride, Arginine:Lysine Ratio, and Methionine Source on Apparent Ileal Digestibility of Arginine and Lysine in Acutely Heat-Stressed Broilers

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ABSTRACT The present study was carried out to determine the ileal digestibility of Arg and Lys in acutely heat-stressed broilers using diets varying in Arg:Lys ratio, NaCl concentration, and Met Source. Male broilers were maintained at 22°C from 21 to 33 d of age and then at 32°C from 33 to 38 d of age. From 28 to 38 d of age, birds were fed a diet with an Arg:Lys ratio of 1.05 and 3 g of supplemental NaCl/kg of diet with or without L-arg free base to increase the Arg:Lys to 1.35, and with or without 3 g/kg of additional NaCl. Methionine was supplied as equimolar amounts of DL-Met or 2-hydroxy-4-(methylthio)-butanoic acid in a 2 × 2 × 2 design. At 38 d of age, digesta were collected from the terminal ileum, and amino acid analyses were conducted on feed and digesta samples and compared with acid-insoluble ash (dietary celite) to calculate the apparent ileal digestibilities of Lys and Arg. Increasing the NaCl concentration and the presence of HMB significantly decreased the digestibility of both Arg and Lys, whereas increasing the Arg:Lys ratio increased the digestibility of only Arg but did increase BW gain ($P = 0.08$). An interaction between dietary NaCl and Arg:Lys ratio as well as the 3-way interaction suggested that dietary NaCl could affect the apparent ileal digestibility of Arg and Lys at certain Arg:Lys ratios and the response may be influenced by the Met source.

(*Key words*: arginine, lysine, sodium chloride, ileal digestibility, amino acid)

2005 Poultry Science 84:294–297

INTRODUCTION

Recent studies have shown that high ambient temperatures affect the ideal amino acid balance for broilers (Brake et al., 1998; Chamruspollert et al., 2004) and that increasing the dietary Arg:Lys ratio improves broiler performance at high temperatures (Brake et al., 1998). In addition, interactions between dietary electrolytes and Arg:Lys ratio have been found (Brake et al., 1998; Balnave and Brake, 2001). In particular, Brake et al. (1998) observed that at a low dietary NaCl concentration (1.2 g/kg), the feed conversion ratio (FCR) and BW gain of heat-stressed broilers were significantly improved with increasing Arg:Lys ratios, but at a higher dietary NaCl concentration (2.4 g/kg), no such response occurred. Furthermore, the relative efficacy of the 2 most frequently used Met sources for heat-stressed broilers, DL-Met (DLM) and 2-hydroxy-4-(methylthio)-butanoic acid (HMB), has been shown to be related to the dietary Arg:Lys ratio (Balnave et al., 1999; Chen et al., 2003). An interaction between Arg and DLM has also been reported (Chamruspollert et al., 2004).

Differences in amino acid digestibilities will influence dietary amino acid specifications, and high environmental temperatures have been shown to influence amino acid digestibility (Wallis and Balnave, 1984; Balnave and Olivia, 1991). It has also been reported that the total uptake of Arg, but not Lys, from the intestines was significantly decreased during heat stress (Brake et al., 1998). The present study was carried out to determine the ileal digestibility of Lys and Arg in acutely heat-stressed broilers using diets varying in Arg:Lys ratio, NaCl concentration, and Met source supplementation.

MATERIALS AND METHODS

Experimental Birds

Cobb 500 male broilers obtained from a local hatchery at 1 d of age were used. The birds were reared in electrically heated battery brooders set at 30°C in a temperature-controlled room kept at 25°C; the brooder heat was removed at 15 d of age. At 21 d of age, the birds were

Received for publication May 26, 2004.
Accepted for publication October 25, 2004.
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Abbreviation key: DLM = DL-methionine; FCR = feed conversion ratio; HMB = 2-hydroxy-4(methylthio)-butanoic acid.

transferred to grower cages in temperature-controlled rooms maintained at 22°C. At 28 d of age, the birds were weighed and allocated based on BW to 48 grower cages of 5 birds each. From 28 to 33 d of age, the temperature was maintained at 22°C and was then increased to 32°C until 38 d of age when the experiment finished. At 38 d, the ileal digesta from all birds were collected for determination of amino acid digestibility (Siriwan et al., 1993). Birds were euthanized by an intracardial injection of pentobarbitone sodium. The ileum (from the caudal end of the jejunum to the ileocecal junction) was removed and divided into 2 parts. The digesta from the lower half of the ileum of all birds in each replicate were collected and pooled into containers by gently flushing with distilled water. The digesta were stored at −20°C before freeze-drying. The Lys, Arg, and acid-insoluble ash contents of diets and digesta were determined and the apparent ileal digestibility (%) of the 2 amino acids calculated using the following equation:

$$100\ [(AA/AIA)_d - (AA/AIA)_I]\ /\ (AA/AIA)_d$$

where $(AA/AIA)_d$ = ratio of amino acid and acid-insoluble ash in diet and $(AA/AIA)_i$ = ratio of amino acid and acid-insoluble ash in digesta.

Starter and Grower Diets

Birds were fed a commercial starter diet with the Met supplement being provided as DLM. Between 21 and 28 d of age, the birds were fed a pre-experimental grower mash diet in which the Met supplement was provided as equimolar amounts of DLM and HMB, assuming 88% efficacy for HMB (Table 1). Feed and water were available for ad libitum consumption and continuous fluorescent lighting was provided at all times. From 28 d of age, 8 experimental diets were fed with 2 replicates of each diet in each of 3 rooms. The wheat-sorghum diets, similar to those used in previous experiments (Balnave et al., 1999; Chen et al., 2003), contained celite as a source of acid-insoluble ash. The Lys, Met, and TSAA concentrations were similar to those of previous experiments where growth was normal when supplemental Met was added (Balnave et al., 1999). The experimental arrangement was a 2 × 2 × 2, consisting of 2 levels of NaCl (3 and 6 g/kg), 2 Arg:Lys ratios (1.05 and 1.35), and 2 Met sources (2.3 g of DLM/kg and 2.6 g of HMB/kg). The basal diet contained 3 g of supplemental NaCl/kg and an Arg:Lys ratio of 1.05 (Table 1). A second basal diet was prepared by the addition of 3 g of NaCl/kg. L-Arginine free base was then added to these 2 basal diets to increase the Arg:Lys ratio to 1.35. These diets were then supplemented with either 2.3 g of DLM/kg or 2.6 of HMB/kg. All supplements were added in lieu of Solka-Floc.

Statistics

Cages and pooled digesta were considered as replicates for statistical purposes. The feed intake and BW gain were measured between 33 and 38 d of age and the FCR was calculated. The data were analyzed by factorial ANOVA using the GLM procedure (SAS Institute, 1989) using a completely randomized block design with rooms as blocks. The data for rooms were pooled because no significant interactions of main effects with rooms were observed. All statements of statistical significance were based on $P < 0.05$ unless otherwise stated. Where significant effects were observed in the ANOVA, the treatment means were compared by least significant difference (Steel and Torrie, 1982).

TABLE 1. Composition of pre-experimental and basal experimental diets

Ingredient	Pre-experimental (g/kg)	Experimental (g/kg)
Wheat	325.90	315.30
Sorghum	289.30	300.00
Soybean meal	250.00	200.00
Soybean oil	54.10	55.80
Limestone	3.00	3.00
Fish meal	50.00	70.00
Dicalcium phosphate	20.00	20.00
Vitamin-mineral premix[1]	2.50	2.50
Sodium chloride	3.00	3.00
DL-Methionine[2]	0.75	—
L-Lysine HCl	—	0.80
Arginine free base	0.60	—
Alimet[3]	0.85	—
Choline chloride	—	0.60
Solka-Floc[4]	—	9.00
Celite	—	20.00
Total	1,000.00	1,000.00
Analyzed composition		
Crude protein	219.0	206.0
Lysine	11.6	11.6
Arginine	13.9	12.2
Methionine	5.4	3.5
TSAA	8.6	6.8
Calculated composition		
Calcium	9.4	10.2
Total phosphorus	8.5	8.6
Potassium	7.7	6.6
Sodium	1.8	1.9
Chloride	2.7	2.7
Arginine:Lysine	12.0	10.5
ME (kcal/kg)	3,105	3,065

[1]Contained (per kilogram of diet): vitamin A, 12,000 IU; vitamin D_3, 2,400 IU; vitamin E, 25 IU; vitamin K_3, 2 mg; thiamine, 2 mg; folic acid, 1.5 mg; riboflavin, 12.5 mg; pyridoxine, 6 mg; pantothenic acid, 15 mg; niacin, 60 mg; biotin, 0.15 mg; vitamin B_{12} 0.015 mg; Mn, 100 mg; Zn, 80 mg; Mg, 100 mg; Fe, 80 mg; Cu, 10 mg; Co, 0.1 mg; I, 0.5 mg; Mo, 0.1 mg; Se, 0.1 mg; antioxidant, 125 mg.

[2]Deguss Australia Pty. Ltd, Parramatta, NSW, Australia.

[3]Novus International Pty. Ltd, Sydney, NSW, Australia.

[4]NaCl (3 g/kg), Arg free base (3.4 g/kg), DL-Met (2.3 g/kg), and Alimet (2.6 g/kg) substituted as required for Solka-Floc.

RESULTS AND DISCUSSION

The primary aim of this study was to determine the Arg and Lys digestibility responses of acutely heat-stressed broilers to diets varying in NaCl concentration, Arg:Lys ratio, and dietary Met source. There were significant main effects of the dietary treatments on ileal Arg and Lys digestibilities (Table 2). The higher dietary NaCl concentration reduced both Arg ($P < 0.05$) and Lys ($P < 0.01$)

TABLE 2. The effect of NaCl, Arg:Lys ratio, and Met supplement on ileal Arg and Lys digestibilities at 38 d of age

Main effects			Lysine, %	Arginine, %	n[1]
NaCl (g/kg)					
3			88.9[A]	90.5[a]	24
6			86.8[B]	89.7[b]	24
SE[2]			0.34	0.26	
Arg:Lys ratio					
1.05			87.4	88.7[B]	24
1.35			88.2	91.6[A]	24
SE			0.34	0.26	
Supplement[3]					
HMB			86.0[B]	88.7[B]	24
DLM			89.6[A]	91.6[A]	24
SE			0.34	0.26	
Interactions					
NaCl (g/kg)	Arg:Lys ratio				
3	1.05		90.1[A]	90.3[B]	12
3	1.35		87.7[B]	90.8[B]	12
6	1.05		84.8[C]	87.1[C]	12
6	1.35		88.7[B]	92.3[A]	12
SE			0.48	0.37	
NaCl (g/kg)	Supplement				
3	DLM		90.6	92.2	12
3	HMB		87.2	89.0	12
6	DLM		88.6	91.0	12
6	HMB		84.9	88.4	12
SE			0.48	0.37	
Arg:Lys ratio	Supplement				
1.05	DLM		89.1	90.1	12
1.05	HMB		85.8	87.4	12
1.35	DLM		90.2	93.1	12
1.35	HMB		86.3	90.0	12
SE			0.48	0.37	
NaCl (g/kg)	Arg:Lys ratio	Supplement			
3	1.05	DLM	91.1[A]	91.7	6
3	1.35	DLM	90.2[A]	92.6	6
3	1.05	HMB	90.2[AB]	89.0	6
3	1.35	HMB	85.2[C]	89.0	6
6	1.05	DLM	87.2[B]	88.5	6
6	1.35	DLM	90.1[A]	93.5	6
6	1.05	HMB	82.4[D]	85.7	6
6	1.35	HMB	87.4[B]	91.0	6
SE			0.68	0.52	

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

[A–D]Means with no common superscripts differ significantly ($P < 0.01$).

[1]Number of replicate cages of 5 birds.

[2]Pooled SE for number of replicates shown.

[3]DLM = DL-methionine, HMB = 2-hydroxy-4(methylthio)-butanoic acid.

digestibilities compared with the lower dietary NaCl concentration. Inspection of the data confirmed that the magnitude of the effect was greater for Lys than for Arg. The effect of the Arg:Lys ratio differed for the 2 amino acids. At the Arg:Lys ratio of 1.35, Arg digestibility was ($P < 0.01$) improved compared with the 1.05 ratio, whereas Lys digestibility was not significantly different at the 2 ratios. The digestibilities of both amino acids were ($P < 0.01$) greater in the presence of DLM compared with HMB.

Both the responses to the higher Arg:Lys ratio and NaCl levels describe a movement toward an increased "effective" Arg:Lys ratio. Thus, the Arg:Lys response was consistent with improved broiler BW gain (298 vs. 318 g; $P = 0.08$) under heat-stress conditions and with previous studies (Brake et al., 1998; Balnave and Brake, 2001) where supplementing a diet with Arg to increase the dietary Arg:Lys ratio or feeding a diet with a higher Arg:Lys ratio improved the performance of heat-stressed broilers. There were no significant differences in feed intake or FCR (data not shown).

The only ($P < 0.01$) 2-way interactions were between the dietary NaCl concentration and the Arg:Lys ratio. At the lower concentration of NaCl (3 g/kg), the digestibility of Lys was significantly reduced at the 1.35 ratio compared with the 1.05 ratio, whereas the digestibility of Arg was not affected by the Arg:Lys ratio. At the higher concentration of NaCl (6 g/kg), the digestibilities of both Lys and Arg were significantly improved at the 1.35 Arg:Lys ratio compared with the 1.05 ratio. Increasing the dietary NaCl from 3 to 6 g/kg of diet significantly decreased the apparent ileal digestibility of both Arg and Lys at the Arg:Lys ratio of 1.05 (2-way interaction). However, at the Arg:Lys ratio of 1.35, the ileal digestibility was unaffected. This confirmed an earlier report that demonstrated the existence of an interaction of Arg:Lys

ratio and the dietary NaCl in chronically heat-stressed broilers (Brake et al., 1998). There was a 3-way interaction ($P < 0.01$) for digestibility of Lys but not Arg.

In the report of Chen et al. (2003), broilers exhibited incrementally increased BW gain with increasing Arg:Lys ratio (1.04 to 1.19 to 1.35) in the presence of HMB but not DLM at a dietary NaCl level of 2 g/kg during acute heat stress. On the contrary, in the chronic heat stress study (21 to 42 d of age) of Balnave et al. (1999), there was a significant positive correlation between BW gain and decreasing Arg:Lys ratio (1.34 to 1.20 to 1.03) at a dietary NaCl level of 3.0 g/kg in the presence of DLM but not HMB. Interestingly, as pointed out by Balnave and Brake (2002), purified crystalline amino acid diets used to evaluate the relative efficacy of DLM and HMB have been generally characterized by low Arg:Lys ratios and concentrations of NaCl above 5 g/kg. A possible explanation for the previous results may be provided by inspection of the ($P < 0.01$) 3-way interaction for Lys shown in Table 2. In the case of the dietary NaCl levels of 3 g/kg, the digestibility of Lys was significantly lower at an Arg:Lys ratio of 1:35 in the presence of HMB. We theorize that this lower digestibility of Lys helped maintain an appropriate Arg:Lys ratio required for maximum growth during acute heat stress.

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