

Evaluation of Lysine, Methionine and Threonine Needs of Broilers Three to Six Week of Age under Moderate Temperature Stress

C. P. Ojano-Dirain and P. W. Waldroup*

Poultry Science Department, University of Arkansas, Fayetteville, AR 72701, USA

Email: Waldroup@uark.edu

Abstract: Information on amino acid requirements of broilers raised under warm environments is very limited. Two studies of identical design were conducted to evaluate Lys, Met and Thr needs of 3 to 6 week-old male broilers held in battery pens at 26.7EC. A basal diet was supplemented with amino acids in a 4 x 3 x 2 factorial arrangement with Lys levels of 1.03, 1.08, 1.12, and 1.16%, Met levels of 0.38, 0.44, and 0.50%, and Thr levels of 0.78 and 0.87%. Feed consumption, body weight (BW) gain and carcass yield were determined. Lys levels had no significant effect on 21 to 42 d BW gain, feed conversion ratio (FCR), or dressing percentage (DP). Increasing Lys levels from 1.03 to 1.12% significantly ($P < 0.05$) improved breast yield (BY) and reduced abdominal fat. Increasing Met to 0.44% resulted in significant improvements in BW gain, FCR, DP, BY, and a numerical ($P=0.08$) reduction in AF. Threonine levels used in this study had no significant effect on any parameter. There was a significant interaction of Lys and Thr on breast yield; however it followed no consistent trend. These results suggest that under moderate heat stress, the present levels of Lys and Met suggested by NRC may be inadequate for maximum live performance or breast meat yield; however, suggested levels of Thr appear to be adequate for this age period.

Key words: Amino acids, broilers, temperature, heat stress

Introduction

Methionine, lysine and threonine are considered to be the first, second and third most limiting amino acids in broilers fed practical corn-soybean meal diets, respectively. Adequate dietary levels of these amino acids are needed to support optimum growth and carcass yield of fast-growing commercial broilers. Many poultry nutritionists use the levels recommended by the National Research Council (NRC, 1994) as a guideline in establishing their own amino acid requirements, regardless of location and environmental conditions. A limitation of using the NRC recommendations in warm weather feeding is that these levels are derived primarily from studies conducted in thermoneutral environments and do not indicate possible differences in amino acid needs due to environmental temperatures. Since very little information is available on the amino acid needs of broilers raised in warm environments, nutritionists confronted with warm weather feeding generally add margins of safety to the NRC levels. Although the NRC also suggests increasing the amino acid levels in warm environments to compensate for the expected reduction in feed intake, the NRC noted that this adjustment should be exercised with caution since some studies have indicated that minimizing excesses of dietary crude protein (Austic, 1985) and amino acids (Waldroup *et al.*, 1976) with improved amino acid balance may be more beneficial for broilers raised in warm environments.

In recent years, a number of studies have demonstrated that elevated environmental temperatures influence the amino acid needs of broilers, either as a function of reduced amino acid digestibility (Wallis and Balnave, 1984; Zuprizal *et al.*, 1993; Hai *et al.*, 2000) or as a result of decreased feed intake (e.g., Deaton *et al.*, 1978; Howlider and Rose, 1987). However, a review of the literature shows that very few studies have been done to determine the amino acid needs of growing-finishing broilers raised under warm environments. In addition, previous studies on protein/amino acid nutrition of heat stressed broilers have shown conflicting results.

Waldroup *et al.* (1976) showed that minimizing dietary amino acid excesses improved growth of broilers housed in hot temperatures. Sinurat and Balnave (1985) also suggested that one way of improving body weight and feed consumption at high temperature is to induce a slight deficiency in lysine or provide a lower Lys:ME ratio. A series of studies conducted by Cheng *et al.* (1997) showed no advantage of increasing dietary amino acid

levels of 3 to 7 week-old broilers when raised above 26.7EC thus, they recommend feeding 90 to 100% of NRC suggested amino acid levels.

Conversely, other researchers have reported that heat-stressed birds respond positively to increased amino acid consumption (e.g., Fuller and Mora, 1973; Dale and Fuller, 1980). The Maryland broiler amino acid requirements also indicate that broilers raised at 80 and 90EF need higher levels of amino acids than birds raised at 70EF (Thomas *et al.*, 1992). Currently, no amino acid recommendations have been established for 3- to 6-week-old broilers raised under warm environments.

Since the greatest portion of the total feed intake is consumed during the period 3- to 6- week posthatching, meeting the bird's needs for amino acids at this growth period is necessary for economical production. This study was conducted to evaluate the lysine, methionine, and threonine requirements of broilers raised from 3 to 6 weeks of age under constant moderate temperature stress (26.7EC).

Materials and Methods

Birds and Housing: Two consecutive trials of identical design were conducted. All animals used in the experiments were provided proper care and management, and without unnecessary discomfort. One-day-old male broiler chicks of a commercial strain (Cobb 500) were obtained from a local hatchery and randomly distributed among compartments in electrically heated battery brooders with raised wire floors. For the first 3 weeks, the birds were fed a common starter diet that met the nutrient requirements suggested by NRC (1994). At 21 d, the birds were weighed and placed on grower experimental diets. Six birds were randomly assigned to each of 96 compartments of wire-floored unheated grower batteries maintained in a room at 26.7 EC. The experimental diets and tap water were provided for ad libitum consumption with 24-h fluorescent lighting.

Diets and Treatments: A corn-soybean meal basal diet formulated according to NRC (1994) nutrient analysis and amino acid compositional values of yellow corn, soybean meal, and corn gluten meal and fortified with complete vitamin and trace mineral mixes was used in the study (Table 1). It was supplemented with sodium bicarbonate to provide a minimum electrolyte balance [(Na + K)-Cl] of 200 meq/kg. Sodium and chloride levels were 0.16 and 0.15%, respectively.

Dirain and Waldroup: Amino Acids in Heat Stress

Table 1: Composition and nutrient content of basal diet for broilers 3 to 6 week-old under constant moderate heat stress (26.7EC)

Ingredient	Content (g/kg)	
Yellow corn	621.83	
Soybean meal (SBM)	255.57	
Corn gluten meal	30.90	
Animal fat	49.18	
Limestone	16.73	
Monocalcium phosphate	11.41	
L-Arginine	0.28	
L-Isoleucine	0.05	
Salt	1.75	
Choline Chloride, 60% ^A	2.00	
Vitamin premix ^B	2.00	
Mineral premix ^C	1.00	
Sodium bicarbonate	2.29	
L-Lysine HCl (98%)	1.91	
DL-Methionine (98%)	1.21	
L-Threonine	1.01	
Antioxidant-Mold inhibitor	0.88	
Total	1000.00	
Nutrient	Calculated ^D	Analyzed
ME	3200	...
Crude protein	19.35	20.67
Calcium	0.90	...
Phosphorus, nonphytate	0.35	...
Methionine	0.32	0.38
TSAA	0.66	0.74
Lysine	0.95	1.03
Threonine	0.72	0.78
Tryptophan	0.24	0.18
Isoleucine	0.80	0.82
Histidine	0.51	0.56
Valine	0.90	0.88
Leucine	1.89	1.87
Arginine	1.21	1.33
Glycine + Serine	1.74	1.85
Phenylalanine + Tyrosine	1.72	1.64

^A Provides per kg of diet: choline 1040 mg. ^B Provides per kg of diet: vitamin A (from vitamin A acetate) 7714 IU; cholecalciferol 2204 IU; vitamin E (from dl-alpha tocopheryl acetate) 16.53 IU; vitamin B12 0.013 mg; riboflavin 6.6 mg; niacin 39 mg; pantothenic acid 10 mg; menadione (from menadione dimethylpyrimidinol) 1.5 mg; folic acid 0.9 mg; thiamin (from thiamine mononitrate) 1.54 mg; pyridoxine (from pyridoxine hydrochloride) 2.76 mg; d-biotin 0.066 mg; ethoxyquin 125 mg; selenium 0.1 mg). ^C Provides per kg of diet: Mn (from MnSO₄ · H₂O) 100 mg; Zn (from ZnSO₄ · 7H₂O) 100 mg; Fe (from FeSO₄ · 7H₂O) 50 mg; Cu (from CuSO₄ · 5H₂O) 10 mg; I (from Ca (IO₃)₂ · H₂O) 1 mg. ^DBased on NRC (1994) adjusted to analyzed nutrient content of corn and SBM used in the study.

The basal diet was formulated to meet a minimum of 110% of all amino acids (NRC, 1994) other than Lys, Met, TSAA and Thr (Table 1). It was calculated to contain 0.95% Lys, 0.32% Met, 0.65% TSAA and 0.72% Thr. However, amino acid analysis by a commercial laboratory indicated that it contained 1.03% Lys, 0.38% Met and 0.78% Thr (Table 1). Hence, our dietary treatments were adjusted accordingly and the lysine, methionine and threonine levels in our dietary treatments were the actual analyzed values.

From a large lot of this basal diet, experimental diets were mixed by supplementing aliquots of the basal diet with different amounts of Lysine HCl, DL-Methionine and L-Threonine in a 4 x 3 x 2 factorial arrangement with Lys levels of 1.03, 1.08, 1.12 and 1.16%, Met levels of 0.38, 0.44 and 0.50%, and Thr levels of 0.78 and 0.87%. Each of the 24 dietary treatments was fed to four pens of six male chicks from 21 to 42 d in each of two successive trials.

The basal diet was analyzed for moisture, crude protein and total amino acids (Llames and Fontaine, 1994). Supplemental amino acid assays were conducted on all test diets (Fontaine and Eudaimon, 2000). The levels of lysine, methionine and threonine reported in this study were adjusted based on total amino acid assay of our basal diet and the calculated level of added lysine, methionine and threonine. In general, analyzed values of added amino acid in our experimental diets were in close agreement to our calculated levels (Table 2).

Measurements: Birds were group weighed by pen at 21 and 42 d and feed consumption during the period from 21 to 42 d was determined. Mortality was checked twice daily and weights of dead birds were used to adjust feed conversion ratio (FCR). At the conclusion of the study, all remaining birds were processed after a 12-h fast to determine dressing percentage, parts yield (percentage breast yield, leg quarter, wing) and percentage abdominal fat.

Statistical Analysis: Pen means served as the experimental unit for all measurements. The results of the experiments were subjected to the analysis of variance (ANOVA) by the General Linear Models procedure of SAS (SAS Institute, 1999-2000). Significant differences among or between treatment means were separated by repeated t-test using probabilities generated by the LS means option. Body weight at 21 d was used as a covariate for body weight gain analysis. All percentage data were subjected to arc sine transformation prior to analysis; data presented as natural numbers. Mortality was analyzed by Chi-square. The level of significance was based on a probability of P < 0.05.

Results and Discussion

The results of this study are presented in Tables 3 and 4, and Fig. 1. Data from our experiments using typical corn-soybean meal diets demonstrated that dietary levels of lysine (Lys) influenced growth, but not carcass characteristics of broilers grown under moderate temperature stress from 3 to 6 week of age. In contrast, methionine (Met) levels affected both growth and carcass yield, whereas threonine (Thr) supplementation had no significant effect on any parameter. Mortality was low and was not significantly different (P > 0.05) between treatments (Table 3).

Lysine Requirement: Lys is considered as the second limiting amino acid in practical broiler diets after Met and/or TSAA. Lys has no precursor role in the body and its utilization is solely for protein accretion. Dietary Lys levels evaluated in this study had no significant effect (P > 0.05) on 42 d body weight, 21 to 42 d body weight gain, total feed intake, or feed conversion ratio (Table 3). Broilers fed diets with 1.16% Lys had numerically higher body weight gain (1.415 kg) but was not significantly different (P = 0.08) from the weight gain of birds fed the lower lysine levels. Sinurat and Balnave (1985) and Han and Baker (1993) suggested that lysine requirement of male broilers is not modified by high temperature. McNaughton *et al.* (1978) conducted two experiments to evaluate the lysine requirement of 2- to 4-week-old broilers maintained at 15.6 EC or 29.4 EC. They found that 1.10% and 1.0% Lys was needed to maximize body weight gain and feed utilization of 4-week-old birds raised at 15.6 EC and 29.4 EC, respectively. Using a mathematical model approach, Hurwitz *et al.* (1980) predicted that amino acid requirements of 6-week old broilers should increase at temperatures up to 27 EC and decline between temperatures of 27 to 34 EC. Under the conditions of our experiments, Lys levels from 1.03 to 1.16% had no significant effect on growth performance of male broilers.

In two different studies, Han and Baker (1993; 1994) found that the Lys requirement to maximize feed efficiency is higher than to maximize weight gain. In our study we found that under moderate heat stress the lowest level of Lys tested supported maximum weight gain and feed efficiency of 3 to 6 week-old broilers. March and Biely (1972) conducted two experiments to

Dirain and Waldroup: Amino Acids in Heat Stress

Table 2. Amount of supplemental amino acids in test diets^A

Diet	Lysine		Methionine		Threonine	
	Calculated	Analyzed	Calculated	Added %	Calculated	Analyzed
1	0	0.018	0	0.004	0	0.006
2	0	0.016	0	0.005	0.101	0.093
3	0	0.014	0.060	0.063	0	0.006
4	0	0.017	0.060	0.054	0.101	0.095
5	0	0.010	0.121	0.128	0	0.006
6	0	0.012	0.121	0.114	0.101	0.094
7	0.063	0.057	0	0.004	0	0.006
8	0.063	0.058	0	0.002	0.101	0.093
9	0.063	0.057	0.060	0.070	0	0.008
10	0.063	0.066	0.060	0.060	0.101	0.093
11	0.063	0.055	0.121	0.101	0	0.005
12	0.063	0.060	0.121	0.118	0.101	0.093
13	0.127	0.108	0	0.005	0	0.006
14	0.127	0.096	0	0.003	0.101	0.094
15	0.127	0.109	0.060	0.062	0	0.005
16	0.127	0.100	0.060	0.070	0.101	0.092
17	0.127	0.106	0.121	0.106	0	0.005
18	0.127	0.126	0.121	0.132	0.101	0.096
19	0.191	0.140	0	0.003	0	0.005
20	0.191	0.146	0	0.004	0.101	0.090
21	0.191	0.146	0.060	0.055	0	0.005
22	0.191	0.168	0.060	0.060	0.101	0.092
23	0.191	0.197	0.121	0.123	0	0.005
24	0.191	0.180	0.121	0.117	0.101	0.096

^AAnalyzed by the method of Fontaine and Eudaimon, 2000.

Table 3: Live performance of broilers raised in constant moderate heat stress (26.7EC) from 3 to 6 weeks of age and fed different levels of Lys, Met and Thr^A

		Live performance parameters				
Treatment ^B		42 d Body weight	21 to 42 d BW gain ^C	21 to 42 d FCR ^D	Mortality	
Amino acid	Levels (%)	kg/bird	kg/bird	kg:kg	%	
Lysine	1.03	2.166	1.386	1.951	1.500	
	1.08	2.161	1.381	1.994	1.400	
	1.12	2.168	1.389	1.969	1.000	
	1.16	2.194	1.415	1.955	2.900	
Methionine	0.38	2.126 ^b	1.347 ^b	2.011 ^a	1.100	
	0.44	2.196 ^a	1.417 ^a	1.949 ^b	1.900	
	0.50	2.194 ^a	1.415 ^a	1.943 ^b	2.200	
Threonine	0.78	2.163	1.384	1.965	1.200	
	0.87	2.182	1.402	1.971	2.200	
Source of Variation		ANOVA P-Values				
Lys		0.347	0.082	0.126	0.396	
Met		< 0.0001	< 0.0001	< 0.0001	0.580	
Thr		0.197	0.192	0.680	0.230	
Lys x Met		0.571	0.458	0.839	0.378	
Lys x Thr		0.808	0.828	0.363	0.549	
Met x Thr		0.893	0.846	0.123	0.221	
Lys x Met x Thr		0.310	0.466	0.151	0.849	
21 d Body Weight		< 0.0001	< 0.0001	0.648		
CV		4.415	6.866	4.603	2.670	
Pooled SEM		0.035	0.035	0.034	2.000	

^A Means of two trials with four replicate battery pens of 6 males per diet for each trial. ^B Means within column for each amino acid with no common superscript differs significantly at $P < 0.05$. ^C Mean 21d wt of 780g. Body weight at 21d used as covariate. ^D FCR = feed conversion ratio, total feed intake/ weight gain.

evaluate the effects of differences in energy input from both diet and from environmental temperature (18.3 and 31.1 EC) on the response of chicks to diets containing different levels of Lys. They presented evidence that increasing energy input by increasing either environmental temperature or metabolic heat increased the efficiency of feed utilization. In addition, Hurwitz *et al.* (1980) reported that at 27 EC, the requirement for maintenance energy is minimum. This may be a reason why we did not observe

different Lys needs to attain maximal weight gain and feed efficiency of 3 to 6 week-old broilers grown at constant temperature of 26.7 EC.

Table 4 shows the carcass composition of birds fed different levels of Lys. Lysine did not influence ($P > 0.05$) dressing percentage, leg quarter yield or wing yield. However, increasing the dietary Lys levels from 1.03 to 1.12% significantly improved breast meat yield and reduced abdominal fat ($P < 0.05$). Breast meat yield (*Pectoralis*

Dirain and Waldroup: Amino Acids in Heat Stress

Table 4: Carcass characteristics of broilers raised in constant moderate heat stress (26.7EC) from 3 to 6 weeks of age and fed different levels of Lys, Met and Thr^A

Treatment ^A	Carcass composition (%)					
Amino acid	Levels (%)	Dressed yield	Breast meat	Leg quarter	Wing	Abdominal fat
Lysine	1.03	69.73	22.99 ^b	33.68	11.50	0.832 ^a
	1.08	69.66	23.14 ^b	33.65	11.52	0.814 ^a
	1.12	69.58	23.44 ^a	33.54	11.53	0.709 ^b
	1.16	69.50	23.55 ^a	33.43	11.52	0.663 ^b
Methionine	0.38	69.37 ^b	22.49 ^c	33.71 ^a	11.56	0.798
	0.44	69.72 ^a	23.45 ^b	33.59 ^{ab}	11.52	0.746
	0.50	69.76 ^a	23.94 ^a	33.43 ^b	11.47	0.719
Threonine	0.78	69.61	23.26	33.55	11.50	0.764
	0.87	69.63	23.30	33.60	11.53	0.745
Source of Variation		ANOVA P-Values				
Lys		0.337	0.0001	0.177	0.841	< 0.0001
Met		0.001	< 0.0001	0.033	0.082	0.082
Thr		0.809	0.759	0.593	0.353	0.683
Lys x Met		0.926	0.381	0.090	0.894	0.388
Lys x Thr		0.725	0.003	0.214	0.128	0.914
Met x Thr		0.259	0.507	0.646	0.571	0.796
Lys x Met x Thr		0.767	0.453	0.425	0.917	0.757
CV		1.669	3.747	2.446	2.348	42.018
Pooled SEM		0.223	0.245	0.215	0.079	0.067

^A Means of two trials with four replicate battery pens of 6 males per diet for each trial.

^B Means within column for each amino acid with no common superscript differs significantly at P < 0.05.

major and *Pectoralis minor*), expressed as percentage of the chilled carcass weight, increased linearly (P < 0.0001) in response to increased dietary Lys levels. However, no significant response to breast meat yield (P > 0.05) was observed when dietary Lys was increased beyond 1.12%. These results support the findings of Hickling *et al.* (1990) and Moran and Bilgili (1990) who reported that supplementing the diet with Lys levels above the requirement for maximal growth performance resulted in increased breast meat yield. In contrast, Summers *et al.* (1988) showed that edible meat yield was not improved with higher levels of supplemental Lys and Met. A study by Mendes *et al.* (1997), also found no differences in breast meat yield of broilers fed 1.0 to 1.2% Lys when birds were grown under hot, cyclic (25 to 33.3 EC) or neutral environment (21.1 EC).

The increase in carcass fat of broilers raised under hot ambient temperature is another concern since the fat content of meat products has become increasingly important to consumer perceptions of the healthfulness of meat. Previous studies have shown that birds grown under hot environment tend to have higher abdominal fat (Howlinder and Rose, 1987; Ain Baziz *et al.*, 1996; Mendes *et al.*, 1997). Hussein and Al-Batshan (1999) and Mendes *et al.* (1997), found that increasing dietary lysine in diets of heat-stressed 3 to 6 week-old broilers significantly reduced abdominal fat. Similarly, we found that increased Lys supplementation resulted in significant (P < 0.0001) linear reduction in abdominal fat content, expressed as percentage of the chilled carcass weight. Birds fed 1.03% and 1.08 Lys had greater percentage of abdominal fat than those fed 1.12 or 1.16% Lys. Again, no significant differences were observed between the two higher Lys levels (P > 0.05).

Our results and those of other authors suggest that the current NRC (1994) recommendation of 1.0% Lys for 3- to 6- week-old broilers is adequate to support comparable growth performance but a higher level (1.12%) is needed to obtain optimum breast meat yield and reduced abdominal fat if birds are grown from 3 to 6 weeks of age under moderate temperature stress.

Methionine Requirement: Met, the first limiting amino acid in broiler diets, has numerous functions in the body. It serves as an integral portion of body protein, is a precursor for cystine, and an important source of dietary sulfur. S-adenosyl methionine is a potent donor of methyl groups, which contributes to the

synthesis of many important substances including epinephrine, choline and creatine (Bender, 1975).

As shown in Tables 3 and 4, the higher levels of Met resulted in significant improvements (P < 0.05) in 42 d body weight, 21 to 42 d body weight gain, feed conversion ratio, dressing percentage, breast meat yield, and a numerical (P = 0.08) reduction in abdominal fat.

Broilers fed diets with higher levels of dietary Met had significantly (P < 0.0001) higher body weight and body weight gain than broilers fed the NRC Met level of 0.38% (Table 3). However, a further increase in Met level to 0.50% did not result in significantly higher body weight and body weight gain from those fed 0.44% Met (P > 0.05). The benefit of increasing Met level from NRC recommendation to 0.44% was a 5.2% (70 g per bird) increase in 21 to 42 d body weight gain while weight gain of birds fed 0.44 and 0.50% Met were the same. The higher levels of Met added to the diets also resulted in significantly improved feed conversion ratio (P < 0.0001). Again, there was no significant difference between birds fed 0.44% Met and 0.50% Met (Table 3).

In our evaluation on the effects of Met levels on carcass composition of 3- to 6-week-old broilers under moderate heat stress (Table 4), we observed significant improvements (P < 0.05) in dressing percentage and breast meat yield between the broilers fed NRC Met level and those fed higher levels. Birds fed diets with 0.44% Met had significantly higher dressing percentage (P < 0.001) than did birds fed diets with 0.38% Met; dressing percentage did not differ between those fed 0.44% and 0.50% Met.

Breast meat yield increased linearly (P < 0.0001) with increasing Met level. Feeding birds diets with 0.44% Met resulted in 0.96% increase in breast meat yield compared to those fed NRC level. A further increase in Met supplementation to 0.50% only resulted in another 0.49% improvement in breast meat yield. Methionine levels also significantly influenced leg quarter yield (P < 0.05) with the optimum yields at 0.44% Met. The tendency of leg quarter yield to decrease with increasing Met may not be a treatment effect. It can be attributed to the fact that parts yield are expressed as percentage of the whole carcass. Therefore, leg quarter decreased as breast meat yield increased. Although there was a trend for abdominal fat, expressed as percentage of the chilled carcass weight, to decrease linearly with increasing dietary Met supplementation, it was not significantly different between the three Met levels evaluated herein (P = 0.08).

Dirain and Waldroup: Amino Acids in Heat Stress

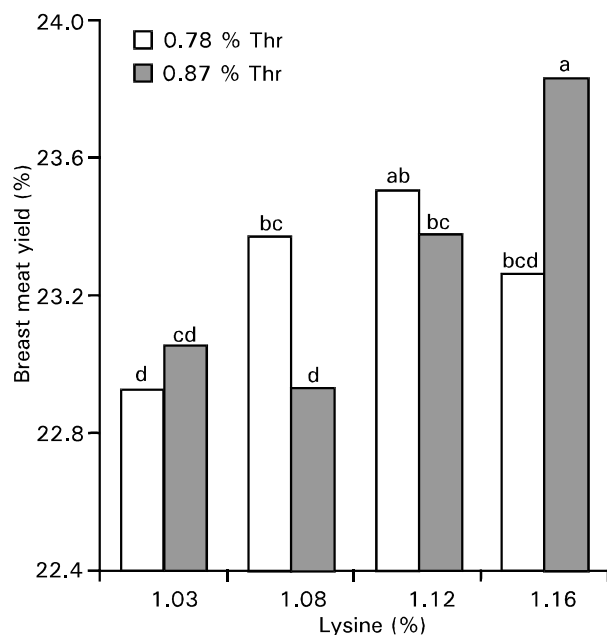


Fig. 1: Lysine and threonine interactions on breast meat yield of 42-d old male broilers raised in constant moderate heat stress (26.7EC) from 3 to 6 weeks of age^A

^A Means of two trials with four replicate battery pens of 6 males per diet for each trial.

^{a-d} Bars with no common superscript for each threonine level differ significantly ($P < 0.05$).

The Met level found adequate for optimum growth performance and meat yield of 3 to 6 week-old male broilers raised under moderate temperature stress was higher than the NRC recommended level of 0.38% and values observed by previous studies. According to Cheng *et al.* (1997), heat-stressed broilers from 3 to 6 weeks of age should not be fed diets with crude protein (CP) greater than 20 and 100% NRC amino acid levels since such practices may lead to more detrimental effects. Moreover, they reported that supplementing 16 and 18% CP diets with Met, Lys, Thr, Trp, and Arg to provide 100 to 110% NRC levels did not improve weight gain of heat-stressed broilers compared to those fed 90% NRC level. Balnave and Oliva (1990) examined the responses of finishing broilers at high temperature to dietary Met source and supplementation levels. These authors concluded that the methionine requirement for 3 to 6 week-old male broilers decreased from 1.21 g/Mcal ME at 21 EC to 1.09 g/Mcal ME at diurnally cycling temperatures of 25 to 35 EC and 0.92g/Mcal ME at constant high temperature (30EC).

Threonine Requirement: In practical broiler diets, Thr is considered as the third limiting amino acid after Met and Lys. Thr serves as a component of body protein, plays an important role in feather synthesis as a component of feather protein and precursor of glycine and serine, is involved in immune responses, needed in gastrointestinal mucin production, and it has been shown to improve livability of heat-stressed broilers (Lemme, 2001). Dietary threonine levels had no significant effect ($P > 0.05$) on growth or carcass parameters evaluated in this study (Table 3 and 4). As the lowest level examined was in excess of the NRC (1994) recommendation we cannot determine if Thr levels recommended by NRC are adequate or extra Thr beneficial under moderate heat stress. In the last decade, several studies have been conducted to evaluate the Thr requirement of growing broilers. The majority of these studies (e.g., Thomas *et al.*, 1986; Weibel *et al.*, 1996; Kidd *et al.*, 1997; Kidd and Kerr, 1997) also indicate that NRC Thr level

for the 3- to 6- week growth period is adequate or more than adequate. In a review of the literature, Lemme (2001) reported that depending on the performance criteria chosen, the optimum dietary Thr levels for 20- to 42-d-old broilers to achieve 95% of the asymptotic response for body weight gain, feed conversion ratio and breast meat yield were 0.66, 0.68 and 0.70%, respectively, compared to the 0.74% suggested by NRC (1994) for 21 to 42 d-old broilers.

Interactions: The only significant interaction that we observed in our study was that of Lys and Thr on breast meat yield ($P < 0.05$). However, it followed no consistent trend, with the higher level of Thr significantly improving breast meat yield at some levels of Lys and significantly decreasing breast meat yield at other levels of Lys (Fig. 1). Kidd *et al.* (1997) also observed an interaction between Lys and Thr on breast meat yield and found no consistency either. In contrast, Kerr *et al.* (1999) reported that Lys and Thr interacted on breast meat weight so that 100% of the NRC Thr level was required to maximize breast meat in broilers fed the 105% Lys diet, and 107.5% Thr was necessary to maximize breast meat in broilers fed the 120% Lys diets.

The present study demonstrates that under conditions of moderate temperature stress, supplementing the diet of broilers for the period from 3 to 6 weeks of age with 0.44% Met resulted in significant improvements in body weight gain, feed conversion ratio, dressing percentage and breast yield. Feeding heat-stressed broilers diets with Lys level from 1.03 to 1.16% did not influence growth parameters; however, a higher level (1.12%) was needed to increase breast meat yield and reduce abdominal fat. Growth and carcass yield were not affected by Thr concentrations of 0.78 and 0.87%. An interaction between Lys and Thr level on breast meat yield was observed but showed no consistent trend.

Conclusions: Broilers grown under constant moderate temperature stress (26.7EC) from 3 to 6 weeks of age fed a corn-soybean meal diet with 3250 kcal/kg ME need 0.44% methionine to achieve optimum body weight gain, feed conversion ratio, dressed yield and breast meat yield. A further increase in dietary Met level to 0.50% did not result in significant improvements in growth and carcass parameters.

Body weight gain and feed conversion ratio of male broilers under moderate temperature stress and fed diets with lysine level from 1.03 to 1.16% was not significantly different. However, a higher level (1.12%) was necessary to increase breast meat yield and reduce abdominal fat.

Feeding diets with threonine levels of 0.78 and 0.87% had no significant effects on growth or carcass quality of 3 to 6-week-old male broilers kept constantly at 26.7 °C. Breast meat yield of heat-stressed broilers was significantly influenced by an interaction of Lys and Thr but was without consistent trend. The higher level of Thr improved breast meat yield at some levels of Lys and decreased breast meat yield at other levels of Lys.

Acknowledgement

The authors would like to thank Ajinomoto Heartland, Inc., Chicago, IL 60631 for conducting the amino acid assay of the diets used in the experiments.

References

- Ain Baziz, H., P.A. Geraert, J.C.F. Padilha and S. Guillaumin, 1996. Chronic heat exposure enhances fat deposition and modifies fat partition in broiler carcasses. *Poult. Sci.*, 75:505-513.
- Austic, R.E., 1985. Feeding poultry in hot climates. Pages 123-136 in: *Stress Physiology in Livestock*. M.K. Yousef, ed. CRC Press, Boca Raton, FL.
- Balnave, D. and A. Oliva, 1990. Responses of finishing broilers at high temperatures to dietary methionine source and supplementation levels. *Aust. J. Agric. Res.*, 41:557-564.
- Bender, D.A., 1975. Amino acid metabolism. John Wiley and Sons, Ltd., Great Britain.

Dirain and Waldroup: Amino Acids in Heat Stress

- Cheng, T.K., M.L. Hamre and C. N. Coon, 1997. Responses of broilers to dietary protein levels and amino acid supplementation to low protein diets at various environmental temperatures. *J. Appl. Poult. Res.*, 6:18-33.
- Dale, N.M. and H.L. Fuller, 1980. Effect of diet composition on feed intake and growth of chicks under heat stress. II. Constant vs. cycling temperatures. *Poult. Sci.*, 59:1434-1441.
- Deaton, J.W., F.N. Reece and J.L. McNaughton, 1978. The effect of temperature during the growing period on broiler performance. *Poult. Sci.*, 57: 1070-1074.
- Fontaine, J. and M. Eudaimon, 2000. Liquid chromatographic determination of lysine, methionine, and threonine in pure amino acids (food grade) and premixes: Collaborative study. *J. AOAC Int.*, 83:771-783.
- Fuller, H.L. and G. Mora, 1973. Effect of diet composition on heat increment, feed intake, and growth of chicks subjected to heat stress. *Poult. Sci.*, 52:2029 (Abs).
- Hai, L., D. Rong and Z.Y. Zhang, 2000. The effect of thermal environment on the digestion of broilers. *J. Anim. Physiol. Anim. Nutr.*, 83: 57-64.
- Han, Y. and D.H. Baker, 1993. Effects of sex, heat stress, body weight and genetic strains on the dietary lysine requirements of broiler chicks. *Poult. Sci.*, 72:791-798.
- Han, Y. and D.H. Baker, 1994. Digestible lysine requirement of male and female broiler chicks during the period three to six weeks posthatching. *Poult. Sci.*, 73:1739-1745.
- Hickling, D., M. Guenter and M.E. Jackson, 1990. The effect of dietary methionine and lysine on broiler performance and breast meat yield. *Can. J. Anim. Sci.*, 70: 673-678.
- Howlader, M.A.R and S.P. Rose, 1987. Temperature and growth of broilers. *World's Poult. Sci. J.*, 43:228-237.
- Hurwitz, S., M. Weisberg, U. Eisner, I. Bartov, G. Riesenfeld, M. Sharvit, A. Niv and S. Bornstein, 1980. The energy requirements and performance of growing chickens and turkeys as affected by environmental temperature. *Poult. Sci.*, 59:2290-2299.
- Hussein, E.O.S. and H.A. Al-Batshan, 1999. Performance and carcass composition of broilers under heat stress: The effects of dietary lysine. *Asian-Australasian J. Anim. Sci.*, 12:1923-1931.
- Kidd, M.T. and B.J. Kerr, 1997. Threonine responses in commercial broilers at 30 to 42 days. *J. Appl. Poultry Res.*, 6:362-367.
- Kidd, M.T., B.J. Kerr, and N.B. Anthony, 1997. Dietary interactions between lysine and threonine in broilers. *Poult. Sci.*, 7:608-614.
- Kerr, B.J., M.T. Kidd, G.W. McWard and C.L. Quarles, 1999. Interactive effects of lysine and threonine on live performance and breast yield in male broilers. *J. Appl. Poult. Res.*, 8:391-399.
- Llames, C. R. and J. Fontaine, 1994. Determination of amino acids in feeds: Collaborative study. *J. AOAC Int.*, 77:1362-1402.
- Lemme, A., 2001. Responses of broilers to dietary threonine: A survey of the international literature. *Amino News* 02 (01):1-6, Degussa Corporation.
- March, B.E. and J. Biely, 1972. The effect of energy supplied from the diet and from the environmental heat on the response of chicks to different levels of dietary lysine. *Poult. Sci.*, 51:665-668.
- McNaughton, J.L., J.D. May, F.N. Reece and J.W. Deaton, 1978. Lysine requirements of broilers as influenced by environmental temperatures. *Poult. Sci.*, 57:57-65.
- Mendes, A.A., S.E. Watkins, J.A. England, E.A. Saleh, A.L. Waldroup and P.W. Waldroup, 1997. Influence of dietary lysine levels and arginine:lysine ratios on performance of broilers exposed to heat or cold stress during the period of three to six weeks of age. *Poult. Sci.*, 76:472-481.
- Moran, E.T and S.F. Bilgili, 1990. Processing losses, carcass quality and meat yields of broiler chickens receiving diets marginally deficient to adequate lysine prior to marketing. *Poult. Sci.*, 69:702-710.
- National Research Council, 1994. Nutrient Requirements of Poultry. 9th rev. ed., Natl. Acad. Press, Washington, D.C.
- SAS Institute Inc., 1999-2000. SAS/STAT[®] User's Guide. Version 8. SAS Institute Cary, N.C.
- Sinurat, A.P., and D. Balnave, 1985. Effect of dietary amino acids and metabolizable energy on the performance of broilers kept at high temperatures. *Br. Poult. Sci.*, 26:117-128.
- Summers, J.D., S. Leeson, and D. Spratt, 1988. Yield and composition of edible meat from male broilers as influenced by dietary protein level and amino acid supplementation. *Can. J. Anim. Sci.*, 68:241-248.
- Thomas, O.P., A.I. Zuckerman, M. Farran and C.B. Tamplin, 1986. Updated amino acid requirements. Pages 79-85 in: *Proc. Maryland Nutr. Conf.*, Baltimore, MD.
- Thomas, O.P., M. Farran and C. B. Tamplin, 1992. Broiler nutrition update. Pages 45-53 in: *Proc. Maryland Nutr. Conf.*, College Park, MD.
- Waldroup, P.W., R.J. Mitchell, J.R. Payne and K.R. Hazen, 1976. Performance of chicks fed diets formulated to minimize excess levels of essential amino acids. *Poult. Sci.*, 55:243-253.
- Wallis, I. R. and D. Balnave, 1984. The influence of environmental temperature, age and sex on the digestibility of amino acids in growing broilers. *Br. Poult. Sci.*, 25:401-407.
- Webel, D.M., S.R. Fernandez, C.M. Parsons and D.H. Baker, 1996. Digestible threonine requirement of broiler chickens during the period three to six and six to eight weeks posthatching. *Poult. Sci.*, 75:1253-1257.
- Zuprizal, M. Larbier, A. M. Chagneau and P. A. Geraert, 1993. Influence of ambient temperature on true digestibility of protein and amino acids of rapeseed and soybean meals in broilers. *Poult. Sci.*, 72:289-295.