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Decreasing Diet Density: Direct Fed Microbials and L-Threonine[1,2]

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Abstract: Alterations in nutritional strategies are becoming more prevalent as broiler integrators are faced with high feed costs compared to historical averages. If broiler diets are devoid of antimicrobials and contain lower than average nutrient content, could the addition of a direct fed microbial ingredient aid in performance recovery? And, although the dietary addition of L-Thr decreases diet cost, will inclusion of up to a pound per ton result in a loss of yield? Two floor pen experiments were conducted to assess the previous questions. In experiment 1, broilers were fed diets varying in amino acid and energy density with and without Primalac from d 1-48. Feeding reduced density diets decreased ($p<0.05$) some live performance measurements and increased ($p<0.05$) mortality, but interactions or Primalac main effects did not occur ($p<0.05$). In experiment 2, broiler performance and N excretion were evaluated from 25-43 d of age after birds were fed variations in dietary L-Thr. The dietary inclusion of L-Thr did not decrease bird performance, but reduced ($p = 0.13$) percent N excreted. Although topics addressed in these experiments should be further studied, these results provide possible strategies for integrators to reduce feed costs.

Key words: Diet density, primalac, broiler, L-threonine

INTRODUCTION

Since the early 2000's feed grade L-threonine has emerged from an opportunity ingredient to a commercially used micro-ingredient in commercial broiler diets. Key factors that have circumvented this include a cyclic spread in the corn to soybean meal ratio, an L-threonine price between $1.00 and $1.50 USD/pound and a better understanding of how much L-threonine to use without allowing CP to fall to insufficient levels. Although some nutritionist have not adopted the ideal protein concept in diet formulation, most agree that threonine as a digestible nutrient is best expressed relative to 64-67% of the digestible lysine nutrient.

Research assessing threonine needs in broilers is decreasing and has been partially replaced by experimentation measuring the impact of L-threonine on broiler performance by identifying and quantifying the fourth limiting amino acid. As L-threonine enters least-cost diet formulation it reduces diet cost and amino acid nutrient excesses; however, care must be taken by the nutritionist during formulation as its inclusion can render some amino acids (e.g., isoleucine, valine, or arginine) marginally deficient. Most notably, marginal deficiencies of the former less limiting amino acids will decrease live productive efficiency and breast meat yield, especially in late-maturing, high-yield broilers. Two independent experiments were conducted to evaluate broilers fed diets with reduced levels of CP that would require incorporation of dietary L-threonine. The objective of Experiment 1 was to evaluate live performance and carcass characteristics of broilers up to 48 d of age fed diets reduced in CP and containing L-threonine with and without dietary addition of a direct fed microbial. The objective of experiment 2 was to evaluate live performance, carcass characteristics, and nitrogen excretion in broilers from 25 to 43 d of age fed balanced diets containing 0, 0.5, or 1.0 pound of L-threonine/2000 pounds of feed.

MATERIALS AND METHODS

Experiment 1: Live production measurements, percentage abdominal fat and white meat yield were measured after nutritional treatments were administered from 1-48 d of age. Six hundred feather sexed broilers (Ross 708 cross; 300/sex) were obtained from a commercial hatchery and transported to the Mississippi State University research farm. At the commercial hatchery chicks underwent normal processing and vaccinations, excluding Gumboro. Chickens were sorted in boxes (6 per sex), wing tagged, weighed, and individual boxes were distributed across 48 floor pens of a 96 pen house (576 total chicks). Chicks were placed in every other floor pen so that an empty pen was adjacent to each pen of birds. Each pen measured 12 ft^2 and contained built-up litter, a pan feeder with a 35 lb capacity tube connected to the pan and one nipple drinker line that provided three nipples/pen. House heating was accomplished by forced air furnaces and

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Table 1: Experiment 1 diets (%) and calculated composition[1]

		Grower, d 16-29			Withdrawal, d 30-43			Final withdrawal, d 44-48		
Ingredients	Starter, d 1-15	1	2	3	1	2	3	1	2	3
Corn	55.24	59.99	61.89	68.48	64.85	67.72	70.35	66.90	69.04	71.49
Soybean meal	31.89	28.07	26.36	20.57	23.43	21.55	19.24	21.39	19.64	17.44
Poultry meal	5.00	3.50	3.50	3.50	3.00	3.00	3.00	3.00	3.00	3.00
Poultry fat	4.67	4.92	4.61	3.50	5.49	4.32	3.88	5.62	5.01	4.61
Dical. P	1.23	1.38	1.39	1.44	1.20	1.22	1.24	1.17	1.18	1.20
Limestone	0.95	0.98	0.98	0.99	0.89	0.90	0.90	0.85	0.85	0.89
Salt	0.47	0.49	0.49	0.49	0.54	0.54	0.54	0.54	0.54	0.54
Premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Met	0.24	0.18	0.20	0.25	0.19	0.20	0.22	0.15	0.16	0.18
L-Lys HCl	0.19	0.01	0.07	0.26	0.04	0.17	0.24	0.01	0.17	0.24
L-Thr	0.01		0.025	0.033		0.025	0.03		0.025	0.06
Filler[3]	0.11	0.101	0.101	0.101	0.051	0.051	0.051	0.091	0.091	0.091
Choline Cl	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Biocox 60	0.05	0.05	0.05	0.05						
Monteban-45					0.06	0.06	0.06			
3-Nitro-20		0.025	0.025	0.025						
Calculated composition[4]										
CP (%)	22.54	20.16	19.61	17.71	18.14	17.66	16.90	17.32	16.87	16.16
ME, kcal/lb	1,406	1,430	1,430	1,430	1,467	1,450	1,450	1,481	1,474	1,474
Ca (%)	0.92	0.90	0.90	0.90	0.80	0.80	0.80	0.77	0.77	0.77
Avail. P (%)	0.46	0.45	0.45	0.45	0.40	0.40	0.40	0.39	0.39	0.39
Na (%)	0.22	0.23	0.23	0.23	0.24	0.24	0.24	0.24	0.24	0.24
DEB, mEq/kg[5]	201	194	183	149	172	158	144	165	149	136
TSAA (%)	0.97	0.85	0.85	0.85	0.80	0.80	0.80	0.74	0.74	0.74
d TSAA (%)	0.86	0.76	0.76	0.76	0.71	0.72	0.72	0.66	0.66	0.66
Lys (%)	1.34	1.06	1.06	1.06	0.95	1.00	1.00	0.87	0.95	0.95
d Lys (%)	1.20	0.94	0.94	0.96	0.84	0.90	0.90	0.77	0.85	0.85
Thr (%)	0.88	0.78	0.78	0.71	0.70	0.70	0.67	0.67	0.67	0.67
d Thr (%)	0.76	0.68	0.68	0.61	0.60	0.61	0.58	0.58	0.58	0.58
Ile (%)	0.91	0.82	0.79	0.69	0.73	0.69	0.65	0.69	0.66	0.62
d Ile (%)	0.83	0.75	0.72	0.63	0.66	0.64	0.60	0.63	0.60	0.57
Val (%)	1.03	0.93	0.90	0.80	0.84	0.81	0.77	0.80	0.77	0.73
d Val (%)	0.92	0.84	0.81	0.72	0.75	0.73	0.69	0.72	0.69	0.66
Arg (%)	1.46	1.30	1.25	1.09	1.15	1.10	1.03	1.09	1.04	0.98
d Arg (%)	1.35	1.21	1.16	1.01	1.06	1.02	0.95	1.01	0.96	0.90

[1]The design represents a 2 (Primalac) x 3 (diet densities) factorial array. The starter diet represents with or without Primalac only (inclusion of 2 pounds per ton in place of the filler). Subsequent diets contain Primalac at an inclusion of 1 pound per ton and diet series 1 represents control; 2 represents adjusted control; and 3 represents adjusted and reduced control;
[2]Premix provided the following per kg of diet: Vitamin A (Vitamin A acetate) 7,718 IU; cholecalciferol 2,200 IU; Vitamin E (source unspecified) 10 IU; menadione, 0.9 mg; B_{12}, 11µg; choline, 379 mg; riboflavin, 5.0 mg; niacin, 33 mg; D-biotin, 0.06 mg; pyridoxine, 0.9mg; ethoxyquin, 28 mg; manganese, 55 mg; zinc, 50 mg; iron, 28 mg; copper,7 mg; iodine, 1 mg; selenium, 0.2 mg;
[3]Filler represents play sand to which Primalac or the drug program (BMD-50 in the grower and Stafac-20 in the final WD) was added in place of; [4]d = digestible; [5]DEB = Dietary Electrolyte Balance

cooling was accomplished by negative pressure ventilation using two 48 inch fans pulling air through evaporative cooling pads.

The experimental design consisted of six treatments (3 diet density regimes with and without Primalac; 8 replications/trt). Primalac was administered at a level of 2 lb/ton in the starter diet (1-15 d) and at a level of 1 lb/ton in subsequent diets (16-29, 30-43 and 44-48 d) (Table 1). In the latter three diets, nutrient density (amino acids and energy) were fed from adequacy (Bushong, 2006) to the extent to which some nutrients would be marginal. Hence, only two treatments (with and without Primalac) were administered in the starter (1-15 d) period.

Pen BW was obtained at d 1, 15 and 48. Feed consumption was measured from the 1-15 and 1-48 d periods. If mortality occurred, dead birds were removed from pens, weighed, and recorded twice daily. At 48 d of age all male broilers (6/pen) were processed in a pilot processing facility. After carcasses chilled for four h, breast meat was obtained through cone deboning.

Experiment 2: Live production performance, percentage white meat and abdominal fat and N excretion were measured in male broilers from 25-43 d of age. Straight run broilers (Cobb 500 fast-feather cross) were obtained from a commercial hatchery after standard vaccinations (excluding Gumboro), transported to the Mississippi

State University research farm, placed in floor pens and reared on common feeds known to support good growth (Johnson, 2007). At d 24, birds were phenotypically sexed, with only males being placed, and males were placed in 24 floor pens (6 birds/pen) of 48 pen blocks. This blocking arrangement was done so that birds were placed in every other pen to avoid possible contamination during excreta collection. Each pen consisted of one pan feeder with a 35 lb tube reservoir, one nipple drinker line (3 nipples per pen), and built up litter. The floor space in each pen was 12 ft^2. Although the allotted space was over twice that used in practice, it aided in the collection of excreta. Heating during brooding was provided with forced air furnaces and evaporative cooling was used during ventilation.

Birds were fed one of three diets (Table 2) resulting in eight replications per treatment. Treatments were derived by mixing and steam-pelleting two diets: one devoid of L-Thr and one least cost formulated to contain one pound of L-Thr. The additional treatment was derived by blending equal portions of the former diets post pelleting.

Table 2: Experiment 2 diets (%) fed from 25-43 d of age and calculated composition[1]

Ingredients	No L-Thr per ton	L-Thr/ton (1.0 pound)
Corn	62.15	66.13
Soybean meal	26.15	22.43
Poultry meal	5.00	5.00
Poultry fat	3.61	3.06
Dicalcium P	1.20	1.23
Limestone	0.90	0.91
NaCl	0.46	0.46
Promix[2]	0.25	0.25
L-Lys SO_4	0.04	0.23
DL-Met	0.18	0.21
L-Thr	0.00	0.05
Sacox 60	0.05	0.05
Calculated composition[3]		
CP (%)	19.88	18.62
ME, kcal/Lb	1,430	1,430
Ca (%)	0.88	0.88
Avail. P (%)	0.44	0.44
Na (%)	0.22	0.22
DEB, mEq/kg[4]	188	172
d TSAA (%)	0.75	0.75
d Lys (%)	1.00	1.00
d Thr (%)	0.65	0.65
d Ile (%)	0.71	0.66
d Val (%)	0.80	0.75
d Arg (%)	1.20	1.10

[1]Equal amounts of these diets were blended to obtain the diet containing 0.5 pounds of L-Thr per 2,000 pounds of feed.
[2]Premix provided the following per kg of diet: Vitamin A (Vitamin A acetate) 7,718 IU; cholecalciferol 2,200 IU; Vitamin E (source unspecified) 10 IU; menadione, 0.9 mg; B_{12}, 11µg; choline, 379mg; riboflavin, 5.0mg; niacin, 33mg; D-biotin, 0.06mg; pyridoxine, 0.9mg; ethoxyquin, 28mg; manganese, 55mg; zinc, 50mg; iron, 28mg; copper, 7mg; iodine, 1mg; selenium, 0.2mg.
[3]d = digestible, [4]DEB = Dietary Electrolyte Balance

At d 32, birds were removed from pens and polyethylene sheets were placed on litter and stapled to pen framing. Feed weight was recorded and birds were reallocated to pens on polyethylene sheets. After 24 h, birds were removed, feed weight was recorded and sheets were removed to collect excreta. Excreta was collected under a well illuminated area so that feathers, feed, and litter was not collected with excreta. Excreta was analyzed for N in duplicate using a combustion analysis. Percent N excretion was calculated [(N excreted/N intake) x 100] with the % N excretion data being reported.

The BW of birds in each pen was obtained at d 25 and 43. Feed consumption was measured for the 25-43 d period. All birds were processed at d 43 mimicking commercial conditions, except for a lengthened carcass chilling time (4 h). All breast meat was removed via cone deboning.

Statistical analysis: Pen was the experimental unit in both experiments. All data were analyzed by ANOVA using the General Linear Models procedure of SAS (2006). Repeated *t* test was used to separate ($p \leq 0.05$) main effects and interaction means in experiment 1 and treatment effects in experiment 2. In experiment 1, contrast analysis was performed in the reduced diet density regimes with and without Primalac. Analysis of covariance was used for initial (d 25) BW in experiment 2.

RESULTS AND DISCUSSION

Both experiments followed a general theme of allowing L-Thr to enter formulation resulting in a decrease in the less limiting amino acids (i.e., Val, Ile, Arg and Trp). In addition, ME was reduced in Experiment 1 from the 30 to 48 d period. Diet samples were collected post steam pelleting, analyzed for amino acids and CP and were in agreement with calculated levels.

Two important poultry traits that processing plants try to optimize are white meat yield and foot pad ‘paw’ quality. Both of these poultry products are tied to diet density. Diets reduced in amino acids will impair breast meat yield accretion, but will reduce N excretion and the potential for litter ammonia production. Hence, rejected and downgraded paws can be tied to ammonia burns.

Interactions between diet density and Primalac did not occur (Table 3 and 4). Overall BW gain (d 1-48) was suppressed in birds fed reduced diet density (diet 2) as compared to the control. Feed conversion (d 1-48) was poorer in birds fed reduced density diets (diets 2 and 3). However, the most reduced diet regime (diet 3) resulted in higher mortality over birds fed diets 1 (control) and 2. Few processing differences occurred; however, abdominal fat yields relative to BW and carcass weight were higher ($p = 0.09$ and 0.08, respectively) in the reduced diet density diets. The effects of feeding suboptimal amino acid levels on productive efficiency of broilers is documented (Fancher and Jensen, 1989). Dietary balance of amino acids can help mitigate the

Table 3: Nutrient density by primalac dietary effects on live performance at 48 d (kg weights)

Treatment[1]		1-15 d			1-48 d		
Diet	Primalac	BW gain	FCR[2]	Mortality (%)	BW gain	FCR[2]	Mortality
1					2.927[a]	1.75[b]	0.52[b]
2					2.762[b]	1.80[a]	0.60[b]
3					2.814[ab]	1.82[a]	3.65[a]
	+	0.349	1.21	0.72	2.805	1.79	2.13
	-	0.353	1.22	0.69	2.863	1.80	1.04
1	+				2.891	1.75	1.04
1	-				2.963	1.76	0.00
2	+				2.709	1.79	1.19
2	-				2.815	1.81	0.00
3	+				2.816	1.82	4.17
3	-				2.813	1.83	3.13
SEM		0.0051	0.018	0.610	0.0606	0.019	1.269
Source of variation							
Diet					0.029	0.002	0.028
Primalac		0.547	0.609	0.972	0.244	0.490	0.304
Diet x Primalac					0.665	0.904	0.998

[1]The design represents a 2 (Primalac) x 3 (diet densities) factorial array. The starter diet represents with or without Primalac only (inclusion of 2 pounds per ton in place of the filler). Subsequent diets contain Primalac at an inclusion of 1 pound per ton and diet series 1 represents control; 2 represents adjusted control; and 3 represents adjusted and reduced control.
[2]FCR = Feed Conversion

Table 4: Nutrient density by primalac dietary effects on processing 48 d (6 male birds per pen)

Treatment[1]		Yields relative to process BW (%)			Yields relative to hot carcass weight (%)	
Diet	Primalac	Carcass	Total breast	Fat	Total breast	Fat
1		71.34	19.94	2.12	27.96	2.96
2		72.26	20.24	2.34	28.13	3.24
3		72.24	19.71	2.48	27.26	3.43
	+	71.75	20.04	2.28	27.99	3.17
	-	72.14	19.88	2.35	27.58	3.25
1	+	71.08	19.44	2.03	27.37	2.83
1	-	71.60	20.44	2.21	28.55	3.09
2	+	72.01	20.69	2.38	28.95	3.31
2	-	72.50	19.79	2.30	27.32	3.17
3	+	72.16	20.00	2.43	27.66	3.36
3	-	72.33	19.41	2.53	26.86	3.49
SEM		1.521	0.578	0.159	0.957	0.199
Source of variation						
Diet		0.796	0.657	0.093	0.628	0.077
Primalac		0.755	0.734	0.623	0.599	0.613
Diet x Primalac		0.992	0.233	0.726	0.341	0.599

[1]The design represents a 2 (Primalac) x 3 (diet densities) factorial array. The starter diet represents with or without Primalac only (inclusion of 2 pounds per ton in place of the filler). Subsequent diets contain Primalac at an inclusion of 1 pound per ton and diet series 1 represents control; 2 represents adjusted control; and 3 represents adjusted and reduced control

negative effects of reduced amino acid levels (Waldroup *et al*., 1976). It must be pointed out that the reduced diet density in Experiment 1 did not follow balance in terms of ideal protein ratios to amino acids. It may be that the reduction in some of the less limiting amino acids caused the decrease in performance (Kidd and Hackenhaar, 2005).

Primalac has been shown to increase metabolic efficiencies by improving intestinal health (Chichlowski *et al*., 2007). Main effects due to Primalac were not observed in this research (Tables 3 and 4). However, contrast analysis of breast meat yield (Table 5) demonstrated that reduced diets showed a trend to improve fillet yield (p = 0.12) in the presence of Primalac (16.04 vs 16.68%), indicating that some nutrient absorption changes might have occurred.

In experiment 2, as L-Thr entered formulation and CP was reduced, the less limiting amino acids were decreased, but were not allowed to become deficient as digestible minimums (i.e., Ile/Lys, 66; Val/Lys, 75; Arg/Lys, 1.10) were set. Differences in live production performance or breast meat yield did not occur (Table 6), as expected, because all amino acid needs were met However, the diet containing one pound per ton of L-Thr

Table 5: Contrast analyses of breast yields in birds fed reduced amino acid diets (treatments 1 and 2) with and without Primalac at day 48 (yields relative to live body weight)

	- Primalac	+ Primalac	Probability
Fillet yield, %[1]	16.04	16.68	0.122
Total breast yield, %[2]	19.60	20.35	0.182

[1] =Pectoralis major. [2]=Pectoralis major and Pectoralis minor

Table 6: Live performance, N excretion and processing results of broilers fed diets varying in L-Thr from 25-43 d of age, experiment 2

Treatments	BW gain[1] (kg/bird)	Feed conversion (kg/kg)	N excretion[2] (%)	Breast meat[3]	Abdominal fat[3]
				---------------------- (%)	----------------------
0 L-Thr	1.499	1.84	5.53	20.89	2.54
0.5 pounds L-Thr/ton	1.574	1.84	4.99	20.52	2.64
1.0 pounds L-Thr/ton	1.519	1.85	5.21	20.61	2.94
SEM	0.109	0.05	0.18	0.39	0.11
Probability	0.799	0.978	0.133	0.763	0.736

[1]Final body weights for the three treatments did not differ (p = 0.76) and averaged 2.82 kg/bird, [2]Represents a 2.5 g sample of excreta analyzed in duplicate. Percent N excretion = (N excreted/N intake) x 100. [3]Relative to live processing BW

resulted in higher (p = 0.07) percentage abdominal fat than did the diet devoid of L-Thr. Although the ME of the diets was formulated to be equal, this increase in abdominal fat may be due to an increase in the diet's net energy.

The ability to minimize broiler house ammonia is heavily dependent upon good grower management, but the impact of dietary N should not be overlooked. Kidd *et al*. (2001) showed that reducing CP decreased N excretion in 5 to 15 d old chicks. The former experiment indicated that for each 1% decrease in dietary CP a 14% reduction in N excretion was observed (Kidd *et al*., 2001). In experiment 2, birds fed the diet containing 0.5% L-Thr had reduced (p = 0.13) percentage N excretion vs birds fed the control. A further decrease in N excretion in birds fed one pound of L-Thr was not noted. It may be that the former lack of response was a feed intake function to compensate for an unaccounted nutrient limitation (no L-Thr, 162 g/bird/d; 0.5 pounds L-Thr, 163 g/bird/d; 1.0 pounds L-Thr, 168 g/bird/d).

In conclusion, integrators using feeding programs with minimal regulated feed additives and reduced diet density should evaluate Primalac and its potential effect on breast meat yield. Furthermore, integrators using L-Thr may be benefiting from lower N excretion and subsequently reduced broiler house ammonia levels in addition to reduced feed costs.

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