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Research Article

Digestible Methionine and Lysine Requirements, Ratio and Interactions in Lohmann Egg-Type Cockerels

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Abstract: Metabolism and growth trials were conducted to study true digestible methionine (tMet) and lysine (tLys) requirements for growing Lohmann egg-type cockerels and to evaluate the effects of 2 dietary tMet levels and 4 tLys levels in a factorial arrangement on growth, nitrogen balance and serum biochemical indices. In the metabolism trial, 20 caecectomised and anus-stitch-surgery healthy adult male birds were used to determine the true digestibility of Met and Lys. In the growth trial, 30 Lohmann egg-type cockerels were allocated to each of 32 floor pens (total 960 birds). During the 0-6 week starting period, 2 levels of tMet (0.37, 0.42%) and 4 levels of tLys (0.84, 0.92, 1.00, 1.08%) were fed to birds, while during the 7-10 week finishing period tMet levels were 0.29% and 0.33% and tLys levels 0.73%, 0.80%, 0.87% and 0.94%, respectively. The results showed that the survival rate in all treatments was 100% during the whole period, indicating that levels of tMet and tLys in the experiment had no influence on the rate. Considering the average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), nitrogen retention ratio (N-retention), and serum uric acid (UA) content of all treatments together with Met and Lys digestibility, optimal tMet and tLys levels were 0.42%/1.00% (T7) for the starter diet and 0.33%/0.94% (T8) for the finisher diet. Although serum growth hormone (GH), thyroxine (T_4) and triiodothyronine (T_3) contents of chicks indicated significant differences among the treatments during the whole period, the relationship between dietary tMet and tLys levels and serum GH, T_4 and T_3 contents seemed irregular. The results also showed that there were interactions between tMet and tLys, and that this interaction had influenced ADG, ADFI, FCR, N-retention, serum UA and hormone contents significantly during the whole period.

Key Words: Digestible methionine, digestible lysine, nitrogen balance, hormone, egg-type cockerels

Introduction

Billions of egg-type cockerels are hatched every year in the world. The related breeding industry has become prosperous for egg-type cockerels in terms of meat flavour in comparison to fast-growing-type broilers. Much research has been done to evaluate the nutritional requirements of broilers (1-4); however, little research has been conducted on the nutritional parameters of egg-type cockerels. At present, most diets formulated are based on NRC (5) recommendations, which are uneconomical for the producers. In earlier studies, energy, protein, calcium and phosphorus requirements of egg-type cockerels were determined (6-8). Met and Lys are considered to be the first and second limiting essential amino acids in chicks fed on corn-soybean meal diets in a preliminary study conducted on Met and Lys levels in cockerel diets (9). Our experiment was conducted to further investigate the requirements of tMet and tLys of egg-type cockerels and the effects of interactions between tMet and tLys on growth performance and serum hormone contents.

Materials and Methods

Basal Diet

A practical basal diet (Table 1) was formulated to meet the requirements of chicks recommended by the Chinese Nutrition Standard of Broilers (10) in terms of all nutrients except for Met and Lys for the 0-6 and 7-10 week periods. Met and Lys added to the basal diet were provided as feed grade methionine and L-lysine-HCl (78.8% lysine), assuming both of them to be 100% bioavailable (11).

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Table 1. Composition and nutritional concentration of the basal diet.

Ingredients (%)	Diets	
	0-6 weeks	7-10 weeks
Corn	55.30	60.00
Extruded soybean	8.33	10.00
Soybean meal	20.00	13.80
Cottonseed meal	3.00	3.00
Rapeseed meal	3.00	2.10
Soybean oil	1.50	2.40
Corn gluten meal	5.00	5.00
Limestone	1.50	1.35
Dicalcium phosphate	1.50	1.50
Salt	0.37	0.35
Premix[1]	0.50	0.50
Nutritional concentration		
ME (MJ/kg)	12.13	12.59
CP	21.02	19.00
Calcium	1.01	0.94
Available P	0.45	0.43
Methionine	0.35	0.32
Lysine	0.93	0.80
Methionine+Cystine	0.72	0.66

Values of CP, Ca, P, amino acid contents were results of chemical analysis.

[1] Supplied per kg of diet: 12 mg of Cu; 98 mg of Fe; 50 mg of Zn; 80 mg of Mn; 0.35 mg of I; 8000 IU of vitamin A; 200 IU of vitamin D_3; 40 IU of vitamin E; 4.5 mg of vitamin B_1; 9 mg of vitamin B_2; 9 mg of vitamin B_6; 0.04 mg of vitamin B_{12}; 85 mg of niacin; 27 mg of d-pantothenic acid; 0.4 mg of biotin; 1.2 mg of folic acid.

Metabolism Trial: Met and Lys Digestibility Assay

The true digestibility of Met and Lys in the basal diet was determined using 20 caecectomised and anus-stitch-surgery applied healthy adult male birds with uniform age and similar weight according to the Sibbald method (12). The birds were confined to individual collection cages. After 48 h fasting (supplying 50 g glucose via drinking water) 10 birds were selected randomly to receive a 50 g crop intubation of basal diet, and another 10 birds received a 50 g crop intubation of nitrogen-free diet to estimate the endogenous excretion of amino acids. The nitrogen-free diet was composed of cornstarch (45.50%), sucrose (45.50%), cellulose (5.00%), dicalcium phosphate (3.13%), salt (0.37%) and premix (0.50%).

Excreta were collected using plastic bags over a 48 h collection period. The collected excreta were dried at 70 °C and ground for amino acid analysis using an amino acid autoanalyser. The formula utilised to calculate true amino acid digestibility was as follows:

True amino acid digestibility of basal diet % = (AA intake – AA excreted + endogenous AA)/AA intake x 100

Growth Trial: Growth Assay

During the 0-6 week starter period, 30 Lohmann egg-type cockerels 1-day old (average initial weight 37.5 ± 0.02 g) were allocated to each of 32 floor pens (total 960 birds). A methionine-lysine-fortified basal diet (21% CP, 12.13 MJ AME/kg) with 2 levels of tMet (0.37%, 0.42%) and 4 levels of tLys (0.84%, 0.92%, 1.00%, 1.08%) was fed to the birds (Table 2).

Thirty Lohmann egg-type cockerels 6-weeks old (average initial weight 602.0 ± 0.50 g) were weighed individually after 12 h fasting and then allocated to each of 32 floor pens. A methionine-lysine-fortified finisher diet (19% CP, 12.59 MJ AME/kg) with 2 levels of tMet (0.29%, 0.33%) and 4 levels of tLys (0.73%, 0.80%, 0.87%, 0.94%) was given to the birds from 7 to 10 weeks of age (Table 2).

The experiment consisted of 8 treatments according to a 2 x 4 factorial arrangement, each containing 4 replicates. Feed and fresh water were offered ad libitum. The birds were also supplied with 5% sugar in the water throughout the experiment. The temperature was maintained at 35 ± 1 °C for the first 3 days, and then decreased by 2 °C each week until reaching 25 °C. Regular management and immunisation programmes were conducted daily. At the end of each period, the birds were weighed and feed intake was recorded.

At the end of each period, 3 birds from each replicate were selected randomly for blood sampling after fasting in the morning via jugular venipuncture; serum was separated at 3500 rpm over 10 min and stored at -20 °C before analysis.

In order to determine the nitrogen balance, feed consumption during the last 3 days of each period was recorded and excreta were collected in plastic bags. After removing feather and skin fragments, the excreta were sprayed with 10% H_2SO_4 to prevent nitrogen from volatilising, and then dried at 70 °C before grinding for nitrogen analysis.

Table 2. Amino acid concentration in the experimental diets (%).

	Items	Treatments							
		T1	T2	T3	T4	T5	T6	T7	T8
Starter period	tMet	0.37	0.37	0.37	0.37	0.42	0.42	0.42	0.42
	tLys	0.84	0.92	1.00	1.08	0.84	0.92	1.00	1.08
	tMet / tLys	0.44	0.40	0.37	0.34	0.50	0.46	0.42	0.39
Finisher period	tMet	0.29	0.29	0.29	0.29	0.33	0.33	0.33	0.33
	tLys	0.73	0.80	0.87	0.94	0.73	0.80	0.87	0.94
	tMet / tLys	0.40	0.36	0.33	0.31	0.45	0.41	0.38	0.35

tMet: true digestible methionine
tLys: true digestible lysine

Chemical Analysis and Calculation

N analysis: Nitrogen was analysed using the Kjeldahl method (13), and the formula used to calculate the nitrogen retention ratio was as follows:

Nitrogen retention ratio % = (nitrogen intake – nitrogen excretion)/nitrogen intake x 100

AA analysis: With the exception of the sulphur-containing AA, the samples were hydrolysed with 6 N HCl at 110 °C for 24 h, and then analysed using an amino acid autoanalyser (Hitachi L-8800, Japan). Methionine and cysteine were determined as methionine sulphone and cysteine acid after oxidation with performic acid. The oxidation process was carried out according to the AOAC method (13).

Uric acid, GH, T_4 and T_3: Serum uric acid content was assayed using an Olympus-640 biochemical autoanalyser, while a ^{125}I RIA kit was utilised for GH, T_4 and T_3 analysis (Shanghai Bioproduct Institution).

Statistical Analysis

All data were subjected to statistical analysis using the General Linear Models Procedure of the Statistical Analysis System (14). The significance of the differences among treatments with variable means was determined by Duncan's new multiple range test. All statements of significance were based on probability $P \leq 0.05$, with very significant defined as $P \leq 0.01$.

Results

Amino Acid Digestibility

True digestibility of Met and Lys in the basal diet (Table 3) for cockerels was 90.83% and 90.46% during weeks 0-6, and 91.58% and 91.51% during weeks 7-10, respectively. These figures indicated that the basal diet provided 0.32% and 0.29% tMet, and 0.84% and 0.73% tLys for the consecutive periods, respectively.

Survival Rate

The survival rate in all treatments was 100% for the whole period, indicating that Met and Lys levels in the diets were within a reasonable range and had no detrimental effects on the birds.

Growth Trial (weeks 0-6)

According to the data given in Table 4, increasing dietary tMet from 0.37% to 0.42% failed to affect ADG, N-retention, serum UA, GH or T_4 contents ($P > 0.05$); however, significant differences occurred in ADFI and FCR ($P < 0.05$), with a very significant decrease in T_3 content ($P < 0.01$).

tLys levels ranging from 0.84% to 1.08% had significant effects on ADG ($P < 0.05$), and very significant effects on ADFI, FCR and serum GH and T_4 contents ($P < 0.01$), but no significant effects on N-retention and serum UA or T_3 contents ($P > 0.05$).

There were significant interactions between tLys and tMet calculated in terms of ADG and UA content ($P < 0.05$), and very significant interactions in terms of ADFI,

Table 3. True digestibility of Met and Lys.

Period	Apparent digestibility (%)		True digestibility (%)	
	Met	Lys	Met	Lys
0-6 weeks	82.93 ± 1.23	81.40 ± 0.40	90.83 ± 1.02	90.46 ± 0.38
7-10 weeks	82.80 ± 0.38	80.53 ± 1.14	91.58 ± 0.29	91.51 ± 1.11

serum GH and T_4 contents (P<0.01), but not for FCR, N-retention or serum T_3 content (P > 0.05). With 0.37% tMet, the best ADG and FCR values and the lowest UA content were obtained at 0.92% tLys, with the highest ADFI and GH data at 0.84% and 1.08% tLys, respectively. Varying tLys levels did not affect N-retention, or serum T_4 or T_3 contents. At 0.42% tMet, the highest ADG and T_4 values and the lowest UA content were obtained with 1.00% tLys, while the highest GH content was at 0.84% tLys. Varying tLys levels did not have any significant effect on ADFI, FCR, N-retention or T_3 data.

Growth Trial (weeks 7-10)

Although, according to the data given in Table 5, no significant differences due to tMet levels occurred in ADG, FCR, N-retention or UA content (P > 0.05), increasing dietary tMet from 0.29% to 0.33% caused very significant changes in ADFI, serum GH, T_4 and T_3 contents (P < 0.01). Furthermore, serum T_4 content elevated, but serum GH and T_3 contents decreased with a dietary tMet increase (P < 0.01).

tLys levels ranging from 0.73% to 0.94% affected ADFI, FCR and serum GH content very significantly (P < 0.01); however, differences in ADG, N-retention, serum UA, T_4 and T_3 contents were not significant (P > 0.05).

Interactions between tLys and tMet affected ADG, ADFI, FCR, N-retention, serum UA and GH contents very significantly (P < 0.01), and affected serum T_3 content significantly (P < 0.05). No significant effects of the interactions on T_4 content were observed (P > 0.05). With 0.29% tMet, the best FCR, the highest N-retention and GH content, and the lowest UA content were obtained at 0.80% tLys, while the highest ADFI was at 0.94% tLys. Varying tLys levels did not affect ADG, or serum T_4 or T_3 contents. At 0.33% tMet, the highest ADG and feed conversion, and N-retention, T_3 and the lowest UA data were obtained from the treatment with 0.94% tLys, and the highest ADFI and GH content with 0.73% tLys and the highest T_4 content from the 0.80% tLys treatment.

Discussion

In terms of ADG and FCR, the optimal tMet/tLys ratio in diets was 0.42 (0.42/1.00) for the starting period, and 0.35 (0.33/0.94) for the finishing period. Considering the whole period, it could be stated that imbalanced amino acid ratios (0.34 and 0.50 during the starter period, and 0.31 and 0.45 during the finisher period) hinder cockerel growth performance. This indicated that the balance of tMet and tLys was more important than their level in diets of cockerels, in agreement with preceding research (15,16). An appropriate tMet/tLys ratio could increase feed efficiency and promote production, decrease cost and improve profit (15,17).

Scarcity of amino acid will restrain protein synthesis, enhance protein decomposition, and increase catabolism and heat loss, thus increasing nitrogen excretion, which is the theoretical basis of evaluating animal protein and amino acid requirements via nitrogen balance (18). As shown in Tables 4 and 5, variation of N-retention in all treatments was in agreement with growth performance data. This could be attributed to increased nutrient availability and N-retention due to appropriate amino acid content and a suitable balance in the diets of these amino acids. In contrast, nutrient availability would be lowered when Met or Lys was insufficient or excessive or their ratio was unfavourable (19).

Serum UA reflects the decomposition metabolism of protein in the animal body directly. Miles and Featherston (20) reported that serum UA as a criterion of amino acid requirements had potential value. Research by Amnbode (21), Luo et al. (22), and Wu et al. (3,4) also pointed out that it could be utilised as a referential criterion for amino acid requirements of broilers. As shown in Tables 4 and

Table 4. Effects of dietary tMet and tLys levels during 0-6 weeks of age.

Treatments		ADG (g)	ADFI (g)	FCR	N-retention ratio (%)	UA (mmol/l)	GH (ng/ml)	T_4 (ng/ml)	T_3 (ng/ml)
tMet	tLys								
0.37	0.84	13.56 ± 0.22[ab]	31.34 ± 0.26[a]	2.31 ± 0.04[ab]	54.35 ± 4.52	1.16 ± 0.13[b]	2.06 ± 0.14[b]	54.39 ± 9.67[b]	3.49 ± 0.40[a]
0.37	0.92	13.67 ± 0.25[a]	30.31 ± 0.38[b]	2.22 ± 0.03[c]	57.53 ± 3.80	1.13 ± 0.20[b]	2.03 ± 0.24[b]	54.08 ± 7.29[b]	3.20 ± 0.38[ab]
0.37	1.00	13.27 ± 0.21[ab]	29.99 ± 0.34[b]	2.26 ± 0.03[bc]	52.91 ± 3.19	1.38 ± 0.14[ab]	2.00 ± 0.44[b]	54.33 ± 6.68[b]	3.05 ± 0.29[ab]
0.37	1.08	13.15 ± 0.24[b]	31.21 ± 0.26[a]	2.37 ± 0.07[a]	52.72 ± 3.58	1.66 ± 0.29[a]	2.79 ± 0.23[a]	47.88 ± 2.00[bc]	3.03 ± 0.15[ab]
0.42	0.84	13.13 ± 0.27[b]	30.01 ± 0.37[b]	2.29 ± 0.05[bc]	51.22 ± 3.25	1.65 ± 0.24[a]	2.76 ± 0.11[a]	43.05 ± 2.34[c]	2.61 ± 0.36[b]
0.42	0.92	13.67 ± 0.22[a]	30.49 ± 0.40[b]	2.23 ± 0.06[bc]	56.36 ± 3.21	1.33 ± 0.18[ab]	2.33 ± 0.33[b]	50.00 ± 2.14[bc]	2.99 ± 0.20[ab]
0.42	1.00	13.71 ± 0.21[a]	30.24 ± 0.31[b]	2.21 ± 0.02[c]	57.05 ± 4.06	1.11 ± 0.28[b]	1.96 ± 0.12[b]	66.39 ± 2.16[a]	2.72 ± 0.39[b]
0.42	1.08	13.38 ± 0.29[ab]	30.61 ± 0.27[b]	2.29 ± 0.03[bc]	53.54 ± 3.82	1.38 ± 0.18[ab]	2.08 ± 0.21[b]	64.41 ± 2.94[a]	2.69 ± 0.17[b]
Source of variation					probability				
Met		> 0.05	< 0.05	< 0.05	> 0.05	> 0.05	> 0.05	> 0.05	< 0.01
Lys		< 0.05	< 0.01	< 0.01	> 0.05	> 0.05	< 0.01	< 0.01	> 0.05
Met x Lys		< 0.05	< 0.01	> 0.05	> 0.05	< 0.05	< 0.01	< 0.01	> 0.05

Values within a column with the different superscript letters differ significantly ($P < 0.05$).
Values in the table were Means ± SD
tMet: true digestible methionine
tLys: true digestible lysine

Table 5. Effects of dietary tMet and tLys levels during 7-10 weeks of age.

Treatments		ADG (g)	ADFI (g)	FCR	N-retention ratio (%)	UA (mmol/l)	GH (ng/ml)	T_4 (ng/ml)	T_3 (ng/ml)
tMet	tLys								
0.29	0.73	26.38 ± 0.80^{ab}	65.97 ± 0.59e	2.50 ± 0.06c	74.97 ± 3.95^{abc}	1.59 ± 0.20^{ab}	2.45 ± 0.06^{a}	44.61 ± 3.43^{cd}	2.94 ± 0.17^{a}
0.29	0.80	26.48 ± 0.72^{ab}	63.31 ± 0.37f	2.39 ± 0.08 d	78.54 ± 4.42^{a}	1.37 ± 0.15b	2.47 ± 0.13^{a}	41.79 ± 4.47^{cd}	2.59 ± 0.23^{ab}
0.29	0.87	25.81 ± 0.35^{bc}	71.08 ± 0.49^{ab}	2.75 ± 0.05^{a}	74.17 ± 3.38^{abc}	1.82 ± 0.17^{a}	2.31 ± 0.06^{a}	46.29 ± 4.32^{bcd}	2.66 ± 0.34^{ab}
0.29	0.94	25.67 ± 0.38^{bc}	71.74 ± 0.35^{a}	2.80 ± 0.05^{a}	70.95 ± 2.12bc	1.78 ± 0.15^{a}	1.90 ± 0.16^{b}	39.34 ± 2.25^{d}	2.49 ± 0.16^{abc}
0.33	0.73	25.05 ± 0.64c	70.81 ± 0.55^{b}	2.83 ± 0.07^{a}	69.08 ± 3.92^{c}	1.62 ± 0.16^{ab}	1.92 ± 0.07^{b}	44.41 ± 4.13^{cd}	1.84 ± 0.31^{d}
0.33	0.80	26.11 ± 0.33^{ab}	68.05 ± 0.39^{c}	2.61 ± 0.03^{b}	73.45 ± 3.21^{abc}	1.78 ± 0.10^{a}	1.51 ± 0.13^{c}	53.53 ± 2.18^{a}	2.33 ± 0.20^{bc}
0.33	0.87	26.20 ± 0.35^{ab}	67.15 ± 0.32^{d}	2.56 ± 0.02bc	77.06 ± 4.21^{ab}	1.61 ± 0.13^{ab}	1.50 ± 0.06^{c}	52.94 ± 5.44^{ab}	2.08 ± 0.12^{cd}
0.33	0.94	26.98 ± 0.39^{a}	63.16 ± 0.43f	2.34 ± 0.05^{d}	79.68 ± 2.85^{a}	1.48 ± 0.13^{b}	1.81 ± 0.16^{b}	48.86 ± 2.19^{abc}	2.35 ± 0.30^{bc}
Source of variation		Probability							
Met		> 0.05	< 0.01	> 0.05	> 0.05	> 0.05	< 0.01	< 0.01	< 0.01
Lys		> 0.05	< 0.01	< 0.01	> 0.05	> 0.05	< 0.01	> 0.05	> 0.05
Met x Lys		< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	> 0.05	< 0.05

Values within a column with the different superscript letters differ significantly ($P < 0.0$ 5).
Values in the table were Means ± SD
tMet: true digestible methionine
tLys: true digestible lysine

5, an appropriate tMet/tLys ratio could increase protein synthesis, decrease protein decomposition in the body, and improve cockerel growth performance.

Phenylalanine and tyrosine are necessary for T_4 and T_3 synthesis. In theory, Met and Lys deficiency or excess may result in a disproportionate ratio among essential amino acids and will probably affect T_4 and T_3 synthesis through phenylalanine and tyrosine metabolism (23). Tables 4 and 5 show that although serum GH, T_4 and T_3 contents in all treatments had significant/very significant differences during both the starter and finisher periods, the relationships between dietary tMet and tLys levels and serum GH, T_4 and T_3 contents were quite irregular. This may be explained by 2 factors: first, poultry serum hormone level varied significantly among birds; second, the GH secretion is impulsive. Therefore, the GH assay in this study could not reflect the true GH level in the body. This result was in agreement with the reports by Carew et al. (24), Carew et al. (25) and Govaerts et al. (26), which demonstrated that decreasing tMet level elevated plasma T_3 level and lowered or maintained T_4 level.

Based on the data obtained, it could be concluded that the optimal tMet and tLys levels for egg-type cockerel diets were 0.42%/1.00% (T7) during the 0-6 week starting period and 0.33%/0.94% (T8) during the 7-10 week finishing period. During the whole growing period, variations in tMet and tLys levels had significant effects on growth performance as mentioned in previous studies conducted on typical fast-growing broilers (5,27). However, the optimal tMet and tLys levels found in this study (0.42% and 1.00% for the starting period, and 0.33% and 0.94% for the finishing period) were lower than those in broilers. This may be attributed to the lower growth rate of Lohmann egg-type cockerels compared to typical fast-growing broilers. In fact, optimal dietary levels of total Met and Lys for egg-type chicks during weeks 0-6 and 6-12 recommended by the NRC (5) are 0.28% and 0.80%, and 0.23% and 0.56%, respectively. Certainly, essential amino acid requirements of egg-type cockerels raised for meat production should be elevated; however, in any case requirements would be lower than for fast-growing broilers since they grow more slowly.

The effects of different tMet and tLys levels on carcass quality traits, which are considered important factors by modern poultry farms, have not been determined. Selection of the suitable requirements of essential amino acids is a matter of economics, and all factors such as chicken meat price and carcass quality should be taken into consideration. Precise determination of the requirements of egg-type cockerels needs further research.

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