Determination of the Amino Acid Requirements and Optimum Dietary Amino Acid Pattern for Growing Chinese Taihe Silky Fowls in Early Stage

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ABSTRACT: A mathematical model has been constructed to estimate the amino acid requirements for growing Taihe silky fowls in early stage. A requirement was taken as the sum of the needs for maintenance, for gain in carcass weight without feathers, and for the feathers. The maintenance requirement was considered to be the sum of the needs for replacing skin and intestinal losses and for the obligatory creatinine excretion in the urine. A comparative slaughter trial and nitrogen balance trials with growing and adult Taihe silky fowls, respectively, were conducted to estimate the parameters in the model. The amino acid requirements were then calculated with the constructed models. The results showed as following: the replacement needs for skin nitrogen loss was determined at 213.41 mg/d for adult male fowls (body weight 1.60 kg); creatinine excretion in these birds was 4.04 mg/d. when fed an nitrogen-free diets, the adult male fowls with body weight 1.60 kg excreted a total of 246.10 mg/d endogenous nitrogen. The net protein requirement for maintenance was estimated at 11.24 mg/wg^{0.75}/d. Per gram of body weight gain contained 27.18 mg carcass nitrogen for growing birds in early stage, but feathers nitrogen in per gram of body weight gain increased with age. The amino acid requirements for growing Taihe silky fowls were slightly higher than for starting and growing pullets, but lower than that of broiler chicks. The amino acid requirements patterns changed with weeks of age. (Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 12: 1782-1788)

Key Words: Taihe Silky Fowls, Amino Acids, Requirements, Model, Pattern

INTRODUCTION

Silky fowl (Gallus gallus domesticus brisson), which is also named Chinese Taihe chicken, black-bone chicken, is traceable from Taihe county of Jiangxi province. China. This bird is a special rare chicken in the poultry gene pool of China. Taihe silky fowl has special nutritive and medical values, and is also an ornamental breed due to its special conformation and appearance. With the urgent medical and mensal needs, this kind of chicken is now worldwidely being raised with great scales. However, systematic studies on the nutrients requirements, especially on amino acid requirements, just limited to the dietary energy and crude protein levels (Zhang et al., 1995; Cheng et al., 1997; Qu et al., 1999), are very scarce. The formulation of diets of silky fowls is usually based on the recommendations of starting and growing pullets or other local native birds. Taihe silky fowls, however, have their own characteristics on requirements and utilization of nutrients, because of different growth rate, body composition, nutritional physiology and biological nature from other birds.

Two main approaches to the determination of amino acid requirements have been generally used, i.e. dose-response and factorial methods. As for the dose-response method, it is based on numerical analysis obtained empirically, of the response of birds to different dietary

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amino acid levels (Combs. 1964; Fisher and Morris. 1970; Hewitt and Lewis, 1972). However, repetitious experiments for each amino acid requirement are involved in this method, and the reported values differ, because the amino acid requirements are affected by various factors as summarized to four categories, environment factors, genetic background, conditions of animals, and dietary factors (Ishibashi, 1999), the selection of response criteria also results in different requirements (Kim et al., 1997a, 1997b; Lewis et al., 1977). In the case of the later method, the amino acid requirements for growing birds are considered as the sums of the requirements for maintenance, and deposition in carcass and feather proteins (Hurwitz et al., 1978, 1980a). This separation is due to the difference in the amino acid composition among these three compartments (Hurwitz et al., 1978, 1983). Two mathematical models for calculating the amino acid requirements of layers using factorial method due to the differences in amino acid composition of maintenance, tissue and egg, have been proposed by Hurwitz and Bornstein (1973). Then the similar models for the calculation of amino acid requirements for broilers (Hurwitz et al., 1978, 1980a). turkeys (Hurwitz et al., 1983), ducklings (He et al., 1996). and laving hens (Yin and Han, 1995: Ji et al., 1999) were constructed based on the factorial method. Excellent correlation coefficients were obtained between the requirements calculated by these models and literature values in all cases. The usefulness of factorial method for the evaluation on nutrient requirements are considered biologically significant and the mathematical predictive models have more advantages because they could provide

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requirement values for birds at different ages, and environmental temperatures and to some extent also for different breeds, thus eliminating the need for repetitious experimentation (Hurwitz et al., 1983).

The objective of this study was to construct a mathematical model for estimating the amino acid requirements and pattern of Chinese Taihe silky fowls based on the theory of factorial method.

MATERIALS AND METHODS

Model construction

For the amino acid requirements of growing silky fowls, it is assumed to be the sum of the requirements for maintenance, carcass gain and feather gain (Hurwitz et al., 1978, 1980, 1983). Furthermore, the requirement for each açid excluding lyrosine-phenylalanine methionine-cystine, is assumed to be independent of others. with no interactions taken into account such as those for lysine-arginine (D'Mello and Lewis, 1970). It is also assumed that the amino acid compositions of carcass and feather do not change with age. This assumption appears to be justified in the case of feathers (Block and Weiss, 1956; Hurwitz et al., 1983). With regard to the carcass, the amino acid composition could vary with changes in the relative proportions of various organs. It can, however, be shown that muscle protein comprises the larger portion of the carcass and that changes in organs will have only a slight impact on the overall amino acid composition of the carcass (Hurwitz et al., 1978; Yin and Han, 1995; He et al., 1996). Therefore, the use of such an assumption appears to be justified, at least as an initial approximation. Based on the assumptions as stated above. A mathematical model was constructed to estimate the amino acid requirements of silky fowls, and a comparative slaughter trial in growing silky fowls and nitrogen balance trials in adult silky fowls were conducted to estimate some parameters in the model.

The net amino acid requirements for maintenance (MAA): The amino acid requirements for maintenance (MAA) were proportional to metabolic body weight to the power of 0.75, since it is believed to be proportional to the body surface area (Mitchell, 1962).

$$MAA=PRM\times AAm+C$$
 (1)

$$PRM=a \times \overline{BW}^{0.75}$$
 (2)

Where PRM is the protein requirements for maintenance; AAm is the proportions of amino acids in the protein of maintenance (i.e. amino acids pattern for maintenance); C is the losses of methionine, arginine and glycine for the excretions of creatine and creatinine; a is the coefficient of protein needed for maintenance; and BW is the body weight.

The net amino acid requirements for growth (G.44): The requirement for growth was divided into two entities, carcass and feather due to the difference in amino acid composition among these two compartments.

$$GAA = PRC \times AAc + PRF \times AAf$$
 (3)

Where PRC and PRF are the protein deposited in carcass and feathers, respectively, and obtained by multiplying the protein concentration in carcass gain by the rate of weight gain, and by multiplying feather protein as a fraction of weight gain by the rate of weight gain, respectively; AAc and AAf are the proportions of amino acids in the protein of carcass and feathers, respectively (i.e. the amino acid patterns of carcass and feathers protein, respectively).

The total amino acid requirements (TA4): The total amino acid requirements are the sum of the requirements for maintenance and growth.

$$TAA = (MAA + GAA)/0.72 \tag{4}$$

Where 0.72 (0.85×0.85) is the availability coefficient of amino acids in natural feedstuffs, and 0.85 is the digestibility coefficient of amino acids in feedstuffs and the availability coefficient of respectively absorbed amino acids from the intestine.

Experiment 1: The nitrogen and amino acid requirements for growth

Three hundred and seventy-two Taihe silky fowls (male and female fowls account for 50 percentage, respectively, initial body weight was 28.40±2.40 g) were obtained at 1 day of age from a commercial hatchery in Taihe county of Jiangxi province of China. Six fowls of each sex were killed at I day of age for the analysis of body composition. The remaining 360 fowls were divided into six groups of 60 birds each at random (males and females were equal in each group). All fowls housed in temperature-controlled room until 12 weeks of age were fed the same diets based on the recommendations as reported previously (Zhang et al., 1995: Chen et al., 1997; Qu et al., 1999), and had ad libitum access to fresh water and diets. The crude protein and metabolic energy levels of the diets for 0 to 4 weeks were 20% of the diets and 12.55 MJ/kg, respectively, for 5 to 8 weeks, 18% and 12.30 MJ/kg, respectively, and for 9 to 12 weeks, 16% and 11.95 MJ/kg. respectively.

All fowls were weighed individually at fortnightly intervals and feed intakes were measured each day to obtain average weight gain and average feed intake, and at this time, one bird of each sex which body weight was close to average body weight of each group was selected randomly from each group to be weighed and killed by an intracardiac injection of sodium pentobarbital. Their feathers and viscera

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Table 1. Composition of the experiment diets fed in the nitrogen balance trials (% of diet)

Composition	Nitrogen-low diet	Nitrogen-free diet
Soybean meal	10.00	-
Wheat bran	15.00	-
Com starch	71.90	45.28
Sucrose	-	44.20
Cellulose	-	6.00
Vitamin premix	0.02	0.02
Methionine	0.10	-
Minerals premix	0.20	0.20
Calcium carbonate	0.50	0.65
Dicalcium phosphate	1.78	1.80
Choline chloride	0.20	0.20
Sodium chloride	0.30	0.30
Magnesium carbonate	-	0.50
Potassium carbonate	-	0.70
Sodium sulfate	-	0.15

^{*} Protein content was 8.39% (analyzed value).

were removed and weighed. All samples were then sealed in polyethylene bags, where they were kept at -18°C for further analysis. Carcasses and viscera were autoclaved, pooled, and homogenized. Samples were then taken for nitrogen determination by AOAC (1990) methods. Feathers were ground and samples were used directly for nitrogen determination. Samples abtained at 0, 4, 8, 12 weeks of age were taken for amino acid determination with Hitachi 835-50 amino acid analyzer after hydrolysis under vacuum with 6N HCl at 110°C for 24 h. The measurement of tryptophan was conducted according to the method as described by Lei et al. (1997).

Experiment 2: The nitrogen and amino acid requirements of adult Taihe silky fowls for maintenance

For determination of the maintenance requirements, the net losses of amino acid must be considered: a) unabsorbed digestive secretions and sloughed off epithelial cells (endogenous losses); b) continuous loss of skin integuments in the form of macroscopic and microscopic scales; and c) catabolism of equivalent amounts of methionine, glycine and arginine involved in the obligatory synthesis of creatinine (Narayanan and Appelton, 1980), which is subsequently lost in the urine. For the measurement of the loss of protein and amino acids in the sloughed off skin integuments, it has been assumed that in the nongrowing adult bird with an unchanged nitrogen concentration in its carcass, the nitrogen balance must equal zero (Hurwitz et al., 1983).

Based on the state above, a nitrogen-low diet (Table 1) was formulated to measure the losses of skin integuments and the total excretion of creatinine with six male adult Taihe silky fowls (body weight 1,600 to 1,650 g), and a nitrogen-free diet (Table 1) to determine the excretions of endogenous nitrogen and amino acids with six

caecectomized male adult Taihe silky fowls (body weight 1,600 to 1,700 g). All fowls were individually housed in stainless steel cages. The caecectomized fowls were allowed 6 weeks to recover from surgery prior to being used in the assay. In the nitrogen-low diet experiment, the excreta were carefully collected and then homogenized for nitrogen measurement by AOAC (1990) methods and creatinine analysis. For creatinine determination, treatment and preparation of the samples were done according to the method of Hurwitz et al. (1983), the determination of creatinine was conducted by the method as described by Wang (1981). For the nitrogen-free diet experiment, birds were precisely fasted for 36 h before the experiment. Immediately, each bird was forced to feed 50 g nitrogenfree diet. Excreta were then carefully collected for 36 h on stainless steel trays placed under the cages. The excreta of each bird was used for nitrogen measurement by AOAC (1990) methods and amino acids determination with Hitachi 835-50 amino acid analyzer after hydrolysis under vacuum with 6 N HCl at 110°C for 24 h.

Based on the results of experiment 1 and 2, the total amino acid requirements of Taihe silky fowls in each period were thus calculated using the constructed model.

Statistical analysis

All results, except for the requirement values calculated by constructed model, are expressed as means±SE. The statistical analysis of data on amino acid composition of carcass and feathers proteins was performed using SAS (1999) statistical software system followed by Duncan's multiple range test. Differences were considered significant at p<0.05.

RESULTS

Experiment 1

The weight gain, weight distribution, and protein content of carcass, viscera and feathers are shown in Table 2.

As can be seen from Table 2, the relative weight of carcass and feathers increased with age and that of viscera on the contrary. The protein content of the carcass slightly increased during the first 6 weeks of life and subsequently underwent only minor changes, while the protein content of the viscera decreased with age and that of feathers increased slightly with age, and remained relatively constant after 4 weeks of age. All these results were similar to that of turkeys (Hurwitz, 1983).

The content of each amino acid in carcass increased with age before 8 weeks of age. The amino acid content was lower at 12 weeks of age than at 8 weeks of age. There were no significant differences of amino acid compositions (% of N×6.25) of carcass protein between 0, 4, 8, and 12 weeks of age (p>0.05), except for histidine, threonine and phenylalanine. There were, however, no significant

Table 2. Weight gain, distribution and protein content of carcass, viscera and feathers in each week

Week	Carcass % of body weight	Protein content (%)	Viscera % of body weight	Protein content (%)	Feather % of body weight	Protein content (%)
0	69.51±3.35	12.11±0.65	27.62±3.20	15.63±1.22	2.85±0.29	77.71±0.53
2	70.56±1.99	17.08±0.73	19.80±1.43	20.20±0.93	3.67±0.31	79.81±1.03
4	71.91±0.96	18.86±0.10	17.39±1.41	19.13±0.77	4.88±0.29	83.02±1.92
6	72.46±1.07	19.81±1.26	16.15±1.16	17.32±0.75	6.67±0.64	83.63±1.53
8	74.51±1.09	20.26±0.44	15.16±1.14	16.56±0.58	6.65±0.31	84.21±0.65
10	73.78±1.57	21.48±0.67	13.83±0.53	15.99±0.56	7.43±0.64	85.54±1.13
12	75.16±0.63	20.14±0.10	12.60±0.24	15.56±0.47	8.71±0.49	83.67±0.75

Table 3. Amino acid patterns of carcass, feather and maintenance of Taihe silky fowls (% of N×6.25)*

Amino acid	Carcass	Feather	Maintenance
Lysine	6.44 (100)	2.00 (100)	2.23 (100)
Histidine	2.44 (38)	0.61 (31)	0.76 (34)
Arginine	5.74 (84)	6.68 (334)	6.64 (298)
Threonine	3.63 (56)	4.90 (245)	5.51 (247)
Tryptophane	0.90 (14)	0.67 (34)	0.65 (29)
Sulfur amino acid	3.54 (55)	7.97 (399)	7.70 (345)
Methionine	2.47 (38)	0.51 (25)	1.15 (52)
Isoleucine	3.21 (50)	4.50 (225)	4.10 (184)
Leucine	6.81 (106)	8.29 (409)	9.13 (409)
Phenylalanine	3.41 (53)	5.00 (250)	4.84 (217)
Phenylalanine+tyrosine	6.12 (95)	7.77 (389)	8.69 (390)
Valine	3.71 (58)	6.23 (312)	6.29 (282)
Glycine+serine	12.00 (186)	20.13 (1,007)	19.58 (878)

^{*} Numbers in parentheses are specific values to lysine.

differences between 4, 8, and 12 weeks of age (p>0.05). All these data were not shown. So the mean values of 4, 8, and 12 week for each amino acid composition, were used as the amino acid requirement pattern for carcass protein deposition (AAc. Table 3). In the case of amino acid compositions of feathers protein, there were very significant differences between zero and other weeks of age (p<0.01) except for methionine, glycine and serine, but no significant difference between 4, 8, and 12 weeks of age (p>0.05). All these data were not shown. So the mean values of 4, 8, and 12 week for each amino acid composition were used as the amino acid requirement pattern for feathers protein deposition (AAf, Table 3).

Base on the data analysis (data not shown), result was obtained that the nitrogen amounts of carcass very significantly linearly correlated to body weight (p<0.01), the regression equation was expressed as follow:

Where CN is the nitrogen amounts (mg). BW is the body weight (g):

When nitrogen amount of feathers (FN, mg) was regressed on body weight (BW, g), following equation was obtained.

$$FN (mg) = 0.8688BW^{1.3892}(g)$$

$$R^2=0.9831$$
, p<0.01, n=84 (6)

According to equations (5) and (6), the following two equations could thus be obtained to calculate daily nitrogen deposition amounts of carcass (7) and feathers (8), respectively.

Where DCN and DFN are the daily amounts (mg) of nitrogen deposited in carcass and feathers, respectively; BW_1 and BW_2 are the body weight (g) in the adjacent respective week. According to equations (7) and (8), the calculated values of nitrogen deposition in each week are given in Table 4.

Experiment 2

Endogenous amino acid and nitrogen output of caecectomized adult Taihe silky fowls are given in Table 5.

Losses of skin integuments and creatinine excretion of adult Taihe silky fowls are given in Table 6. The dairy losses of skin integuments and creatinine excretion are 213.40 mg/g, and 0.0358 mmol/d, respectively. The losses of glycine, arginine and methionine are thus calculated as 2.68, 6.23, and 5.33 mg/d by multiplying their respective molecular weight with 0.0358.

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Table 4. Nitrogen	deposition of	Taihe silky	fowls in ear	ch week

Weeks of age Weig	Weight (g)	Feed intake (g/d) -	Nitrogen deposition (mg/d)				
	weight (g)	reed intake (g/d) =	Carcass	Feather	Total		
0	28.40±2.40	-	-	-	-		
0-2	79.43±12.34	8.50	99.20±40.13	20.80±6.31	120.00±18.40		
3-4	167.17±19.40	15.40	169.60±13.10	49.61±8.12	219.21±15.13		
0-4	-	12.10	134.40±20.43	35.21±4.56	169.60±21.36		
5-6	302.81±30.41	26.93	264.01±35.13	97.65±11.03	361.66±31.55		
7-8	461.57±54.34	39.45	308.82±36.08	137.64±20.38	446.46±35.23		
5-8	-	33.19	286.42±33.39	118.43±19.81	404.06±32.67		
9-10	606.59±65.42	46.34	281.65±34.11	144.03±18.16	425.68±40.43		
11-12	747.29±72.53	52.58	265.60±23.71	148.81±21.68	414.41±39.86		
9-12	-	49.96	273.63±24.46	146.42±19.89	420.05±40.13		

Table 5. Endogenous amino acids and nitrogen output for caecectomized adult Taihe silky fowls

Amino acid	Excretion value (mg/bird/d)
Lysine	10.27±1.58
Histidine	4.43±0.79
Arginine	14.54±4.53
Threonine	25.82±2.20
Tryptophane	1.85±0.21
Sulfur amino acid	21.19±1.09
Methionine	6.90±1.68
Isoleucine	1.82±0.60
Leucine	40.58±3.50
Phenylalanine	13.37±2.86
Phenylalanine+tyrosine	40.13±4.11
Valine	21.08±2.68
Glycine+serine	55.64±4.31
Endogenous nitrogen	246.10±50.62

Base on the results of Table 5, 6 and equation (2), the value of a was calculated as follow:

$$a=(246.10+213.41)\times6.25/[(1609+1634)/2]$$

=11.24 (mg/BW_g^{0.75})

So the net protein requirement for maintenance is thus expressed as:

PRM (mg/d)=
$$11.24 \text{ BW}_g^{-0.75}$$
 (9)

The amino acid requirement pattern for maintenance was determined by summing the endogenous excretion of amino acid. loss of amino acid in skin integuments and loss of glycine, arginine, and methionine which are involved in the obligatory synthesis of creatinine lost in the urine, and then dividing the protein requirement for maintenance (Table 3).

The amino acid requirements and pattern were thus estimated and summarized in Table 7 according to Table 3, 4 and equations (1), (3), (4), and (9). As shown in Table 7, the absolute requirement of each amino acid increased with age, when the requirement is expressed as a percentage of

Table 6. Losses of skin integuments and creatinine excretion for adult Taihe silky fowls

Item	Average value
Initial body weight (g)	1,609.00±67.88
Final body weight (g)	1,634.00±70.08
Feed intake (g/d)	80.00
Nitrogen intake (mg/d)	1,073.92
Nitrogen excretion (mg/d)	860.51±50.32
Nitrogen retention (mg/d)	213.41±48.93
Creatinine excretion (mg/d)	4.04 ± 0.62

diet, it decreased with age. The amino acid patterns changed with age.

DISCUSSION

It has been proved that animal amino acid requirements were highly correlated with the amino acids composition of carcass protein(r>0.90), and the amino acid pattern for growth was almost the same as that of body protein (Williams et al., 1954; for the review, see Cheng and Wang, 1999). Block and Bouing (1994) have also reported that amino acids composition of feed protein having high biological value was almost similar to that of carcass protein of animal that ingested the corresponding feed protein (for the review, see He. 1993). These results provide the theoretical proof for the determination of amino acid requirements and pattern by the composition analysis of body using comparative slaughter trials. Hurwitz et al. (1978, 1980), Hurwitz et al. (1983), He et al. (1996) have constructed similar mathematical model to estimate the amino acid requirement of broilers, turkeys and ducklings. respectively, by using comparative slaughter trial and nitrogen balance trials. The requirements calculated by these models are highly close to literature values in all cases. The amino acid requirements of growing Taihe silky fowls. calculated by our constructed model, are obviously lower than that of broilers and close to but slightly higher than that of starting and growing pullets except for tryptophane and methionine with comparison to NRC (1994).

Table 7. The amino acid requirements in each period for Taihe silky fowls

Amino Acid	Daily requirement (mg/d)	0 to 4 weeks % of diets (%)	Pattern	Daily requirement (mg/d)	5 to 8 weeks % of diets (%)	Pattern	Daily requirement (mg/d)	9 to 12 weeks % of diets (%)	Pattern
Lysine	100.94	0.84	100	223.23	0.64	100	239.98	0.48	100
Histidine	34.02	0.28	33	75.79	0.22	36	80.17	0.16	33
Arginine	119.61	1.00	119	288.75	0.87	136	347.72	0.70	146
Threonine	48.07	0.70	83	204.83	0.62	97	253.42	0.51	106
Tryptophane	16.81	0.14	17	41.86	0.12	19	49.96	0.10	21
Sulfur amino acid	103.03	0.86	102	259.67	0.78	121	332.08	0.66	138
Methionine	35.96	0.30	36	83.71	0.24	38	81.57	0.17	38
Isoleucine	71.10	0.59	70	173.84	0.52	81	211.57	0.42	88
Leucine	149.11	1.24	148	360.92	1.08	169	440.97	0.88	183
Phenylalanine	78.56	0.65	77	192.58	0.58	91	236.75	0.47	98
Phenylalanine+ tyrosine	137.33	1.14	136	333.29	1.00	156	409.60	0.82	71
Valine	92.85	0.77	92	229.58	0.69	108	287.12	0.57	119
Glycine+serine	296.56	2.47	294	733.43	2.21	354	913.90	1.83	381
N×6.25	2,430.20	20.23		5,808.90	17.50		7,541.02	15.30	

Note: The availability efficiency of diet protein was 0.58, 0.55, 0.50 in 0 to 4, 5 to 8, 9 to 12 weeks of age, respectively (values obtained by experiments).

The availability coefficient of amino acids, which ranges from 0.65 to 0.85 and changes with breed and age especially for poultry, is a critical factor affecting the determination accuracy of amino acids requirements. Recent researches have indicated that the availability of each amino acid for maintenance was probably not identical to that for protein (Herger, 1989). However, we assumed the same availability coefficient for each amino acid in the present study. which, therefore. may overestimate or underestimate the requirements of some amino acids. On the other hand, the factors affecting the amino acid requirements have different effect extent and manner on each amino acid, and each amino acid has different efficiency of utilization, turnover rate and oxidation rate in various tissues. Therefore, the amino acids composition of body is not always identical to the dietary amino acid requirements pattern. Further studies on the efficiency of utilization of feedstuffs for Taihe silky fowls, and experimental validation of the model-calculated requirements are necessary to further revise the amino acid requirement for Taihe silky fowls.

There are studies indicating that the ideal dietary amino acid profile for birds scarcely undergoes change in the whole growth period (Baker et al., 1994; William et al., 1998). However, it changed with weeks of age for growing Taihe silky fowls during the 12 weeks period posthatching, which was consistent with the results of Hurwitz et al. (1978, 1983) and He et al. (1996). The amino acid requirements for growing birds comprise three components, i.e. requirement for maintenance, carcass protein deposition, and feather protein deposition, and the amino acids composition of these three parts is different from each other (Table 3). The total requirements pattern depends on the

relative contribution of maintenance and growth requirements to it (Zhen et al., 1999). The requirement for maintenance accounts for smaller proportion to total requirements in young period, but the proportion increases with the age. On this account, the ideal amino acid profile should theoretically undergo continuous change with age.

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