Recent Advances in Amino Acid Nutrition for Efficient Poultry Production[a] - Review -

T. Ishibashi* and Y. Ohta
Nippon Veterinary and Animal Science University, Musashino-shi 180-8602, Japan

ABSTRACT : The nutritional value of protein varies between feedstuffs. It is possible to feed animals using crystalline amino acids as a sole nitrogen source, but in practice only some limiting amino acids are added to the diet. In order to use feedstuffs efficiently, it is important to determine exact amino acid requirements. Reported values differ widely because the requirements are affected by various factors. In this report, therefore, the factors affecting amino acid requirements are reviewed as follows: 1) availability of dietary amino acids, conversion factors of nitrogen to protein, interaction of amino acids, and strain, sex and age of animals; 2) amino acid requirements for maximum performance and maintenance, usefulness of non-essential amino acids; 3) plasma amino acid concentration as a parameter to determine amino acid requirements; and 4) nitrogen excretion to reduce environmental pollution. These factors should be considered, it is to improve the dietary efficiency, which is to reduce excess nitrogen excretion for environmental pollution. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 8 : 1298-1309*)

Key Words : Poultry Production, Amino Acid Requirement, Broiler, Laying Hen, Plasma Amino Acid

INTRODUCTION

In the history of nutrition research, especially in vitaminology, chicks have played an important role as an experimental animal. The mechanism of protein synthesis from amino acids has been elucidated, but the mechanism of protein degradation is yet unknown. For these studies, chicks are very useful. Poultry are an economical source of high quality protein such as eggs and meat. For efficient poultry production, it is most important to clarify the CP and amino acid requirements, and the factors affecting the requirements.

Threonine was discovered by Maeda (1933) and Rose and Meyer (1936) independently as the last essential amino acid, and it has been possible to feed animals using crystalline amino acids as a sole nitrogen source. In 1979, the Illinois crystalline amino acid diet was completed after long efforts of study (Baker et al., 1978), which indicated that animals do not need dietary protein to provide amino acids. Since 1980, there have been numerous reports studying amino acid requirements of poultry from scientific and economic aspects. The reported values differ widely, because the amino acid requirements are affected by various factors as summarized by Ishibashi (1990). The factors are classified into four categories, environmental factors, genetical backgrounds, conditions of animals, and dietary factors. Environmental temperature directly affects feed intake. Even under the same environmental conditions, amino acid requirements are different among strains and lines, and affected by disease, stresses, intestinal flora, hormones, physical conditions such as molting and age. In the dietary factors various factors are involved such as dietary ME, availability of dietary amino acids, antagonism and imbalance among amino acids, deficiency and excess of amino acids, palatability, and dietary CP or metabolically related amino acid levels.

The nutritional value of dietary protein is not constant as first shown by Osborne and Mendel (1914). When whole egg protein, fish meal, and soybean meal were used as main protein sources, the maximum body weight gain of broilers was achieved at 14.5, 16.5 and 21.9% of CP, respectively (Koide et al., 1992). In 21.9% CP soybean meal, methionine requirement was satisfied, suggesting that it is not protein but amino acids which is needed for the maximum BW gain. Therefore, it is very important to determine the exact amino acid requirements for effective animal production (figure 1).

In order to evaluate nutritional values of dietary CP and amino acids, various methods have been developed, e.g., nitrogen balance, protein efficiency ratio, net protein ratio, gross protein value, nitrogen growth index, biological value, net protein utilization, nitrogen balance index, carcass analysis, enzyme activities, urinary components such as creatine and allantoin, plasma amino acid ratio, plasma amino acid index, plasma amino acid reference index and so on. These methods are laborious and expensive and require a large number of animals to be studied. Therefore, simpler methods to estimate dietary nutritional value have been developed, which do not need animals, such as chemical score, essential amino acid index,

* Address reprint request to T. Ishibashi. Fax: 81-480-93-3296, E-mail: ohta-y@infotopia.or.jp.

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available Lys and so on (Kametaka et al., 1994). In 1951, Moore and Stein developed an automatic amino acid analyzer and the study of amino acid nutrition has progressed rapidly. Now, an HPLC makes it possible to analyze amino acids within a few hours.

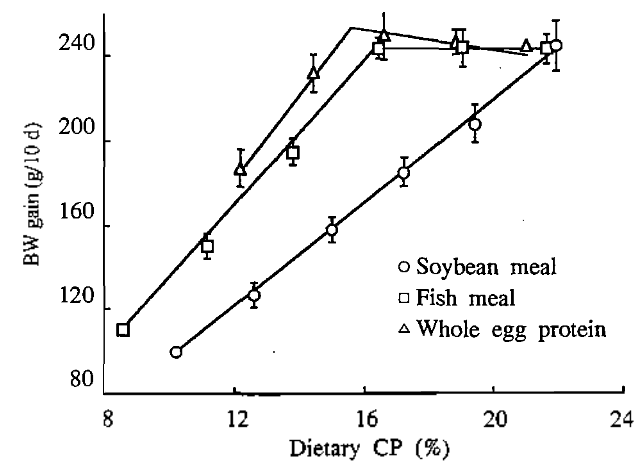

Figure 1. Effect of protein sources on body weight gain of broilers

FACTORS AFFECTING AMINO ACID REQUIREMENTS

1. Availability of amino acids

Protein has to be digested and absorbed in digestive tracts. Therefore, it is necessary to determine the digestibility of protein in order to evaluate nutritional values of protein. Because birds excrete feces and urine together from the cloaca, an artificial anus has to be attached in order to determine protein digestibility. Isshiki and Nakahiro (1988) developed an easy-handled method for attaching an artificial anus by reversing the rectum of chicken. The operated laying hens need no cannula and continue egg production normally for a long period, and the digestibility of dietary protein is determined easily.

However, some amino acids are excreted into urine. Yamazaki and Kaku (1988) developed a method to evaluate a true availability of each amino acid in a similar way to determine a true ME in feedstuffs (Sibbald, 1979). They determined the true amino acid availability of main feedstuffs in Japan, which ranged widely among feedstuffs as shown in table 1. The true availability of crystalline amino acids was estimated to be 100%, and the true amino acid availability was not affected by age of roosters used in the experiments.

When mixed feedstuffs are used, it is possible to calculate the true amino acid availability by adding each true amino acid availability of feedstuffs in pigs (Furuya and Kaji, 1991). The true amino acid availability (Y, %) is calculated as follows:

Y=(ingested amino acid-excreted amino acid+ metabolic fecal and endogenous urinary amino acid)/ingested amino acid $\times$ 100.

Yamazaki and Kaku (1988) concluded as follows : 1) the true availability of each amino acid in common feedstuffs agrees well with the digestibility determined using roosters with an aritificial anus, 2) it is impossible to determine the availability of wheat and barley by the chemical method using fluordinitrobenzene or trinitrobenzene sulphuric acid, because most Lys in excreta is determined as available Lys by the chemical method, 3) the availability of amino acids in feedstuffs supplied as a sole source is estimated to be lower than that supplied as a mixture of feedstuffs, 4) the availability of each amino acid is affected by dietary tannic acid and heat process, 5) the wide ranges of reported requirements of amino acids by many researchers are narrowed by recalculating as available amino acids instead of total amino acids.

Table 1. Composition and availability of amino acids of feedstuffs for poultry

	Composition (%)			Availability		
	Lys	Met	Thr	Lys	Met	Thr
Corn	0.26	0.21	0.33	85.7	93.2	85.8
Sorghum	0.20	0.20	0.31	78.4	85.3	80.2
Wheat	0.33	0.20	0.34	76.3	87.1	73.8
Rice	0.29	0.27	0.30	88.0	85.1	82.1
Soybean	0.28	0.65	1.83	90.1	93.3	89.4
Corn gluten meal	1.17	1.87	2.36	92.7	97.9	93.0
Fish meal	4.68	2.04	2.75	91.6	93.0	90.8
Alfalfa meal	0.70	0.24	0.65	58.9	64.6	65.1

2. Conversion factors of nitrogen to protein

As mentioned above, amino acids, but not CP, are important as a nutrient. However, it is expensive to determine dietary amino acid concentration, but it is easy and accurate to determine dietary nitrogen by the Kjeldahl method. The conversion factor of 6.25 has been used to estimate the protein content of various foods and feeds. Practically CP has been used as a parameter to estimate dietary value. Firstly the CP level is decided to formulate diets, and then the calculated limiting amino acids are added or the dietary CP is increased until all amino acid requirements are satisfied.

Dintzis et al. (1988) calculated nitrogen to protein factors for diets and fecal samples from three animal species fed low- or high-fiber diets (table 2). The conversion factors based on protein contents determined by amino acid analysis were calculated from total nitrogen determined by the Kjeldahl method and recovered amino acid plus NH_3-nitrogen determined by amino acid analysis. Those based on protein contents determined by a fluorescamine assay for amino acids were also calculated from total nitrogen by the Kjeldahl method. Those based on Kjeldahl nitrogen averaged 5.3 ± 0.7 for the diet and 4.0 ± 0.5 for the feces in six animals and diets. The largest deviations from the traditional 6.25 conversion factor occurred in the fecal samples of ruminant animals fed a corn-alfalfa meal diet at a maintenance level of intake. In contrast, conversion factors based on amino acid plus NH_3-nitrogen were quite constant. These factors averaged 5.7 ± 0.1 for feed and 5.5 ± 0.1 for feces when calculated from anhydrous amino acid residue weights. Therefore, the use of a conversion factor of 6.25 without considering the source of protein leads to an overestimation of protein contents especially in plant feedstuffs, because plant tissues may contain significant amounts of non-protein nitrogen.

Table 2. Conversion factors of nitrogen to protein of feedstuffs

Barley	5.83
Rice	5.95
Soybean products	5.71
Milk products	6.38
Whole egg powder	6.58
Corn-soybean feed	5.70
Feces	5.50

3. Interactions of amino acids

It is well known that there are interactions among amino acids, which affect each other physiologically and biologically. Harper et al. (1970) classified these interactions into four categories, deficiency, imbalance, antagonism, and toxicity.

Amino acid deficiency

When single or multiple amino acids are deficient, feed intake and performance of animals are low. Performance of animals is recovered by addition of deficient amino acids. The degree of depression in performance differs among amino acids (Okumura and Mori, 1979). Hikami et al. (1988) reported that when Met deficient diet was supplied, feed intake of some chicks did not decrease and achieved normal performance. There was no difference in enzyme activities between chicks which consumed Met deficient diet and grow normally and depressed feed intake and growth. When the amino acid deficient diet was forced- or paired-fed, there were no differences in performance between the animals fed deficient and adequate diets (Fisher and Shapiro, 1961). When Arg deficient diets were supplied, there were no differences in ME utilization rate of chicks (Sugahara et al., 1985). These results indicated that there is a possibility to achieve the maximum performance by feeding the amino acid deficient diets, namely low quality diets, if it is possible to prevent the depression of diet consumption. When the dietary amino acid is lower than the requirement, young chicks refuse to eat the diet to meet ME requirement. However, older chickens tend to eat the diet until the deficient amino acid is satisfied (unpubl. data).

Amino acid imbalance

Amino acid imbalance is a phenomenon that when the second limiting amino acid is supplied, feed intake, performance and tissue amino acid levels decrease more severely (Harper et al., 1970; Yoshida et al., 1966). Although amino acid imbalance has been studied using low CP diets, it is observed in the adequate CP diet (Kumpta et al., 1958) were supplied, depressions of feed intake and performance were recovered by forced-or paired-feeding (Fisher and Shapiro, 1960), administering amino acids (Rogers and Leung, 1973), and exposing to cold (Leung et al., 1968). These findings are the same as in the single amino acid deficiency.

Amino acid antagonism

It is well known that there are antagonisms among amino acids, especially between Arg and Lys, between Met and Thr, and among branched chain amino acids. The data reported before 1990 are reviewed by Ishibashi (1990).

Arg and Lys : When dietary Arg or Lys is in excess, the performance of chicks is depressed (Ueno et al., 1994). Table 3 shows that 1) the Lys requirements expressed as percentages of diet decrease with advancing age when the same CP diet is supplied, 2) the Lys requirements expressed as percentages of dietary CP are not constant and increase with increasing dietary CP levels, 3) the Lys requirements expressed as grams per BW gain (kg) increase with advancing age and increasing dietary CP levels, and 4) the increment of Lys requirements with increaseing dietary CP levels is not caused by dietary CP levels, but by dietary Arg levels.

The Lys requirements (Y, %) are expressed as a function of dietary Arg levels (X, %, $100 \leq X \leq 160\%$ of NRC) at 8 to 18 days of age as follows (Ueno, 1998),

$$Y = 0.00565X + 0.338 \qquad r^2 = 0.996$$

The depression of performance of chicks fed Lys excess diets is alleviated by addition of Arg. Therefore, Arg requirements (Y, %) increase with increasing dietary Lys levels, and can be expressed as a function of dietary Lys levels (X, %, $0.8 \leq X \leq 1.4$) as follows at 8 to 18 d of age (Ueno, 1998),

$$Y = 1.55\text{-}0.21 \qquad r^2 = 0.944$$

Austic and Scott (1975) analyzed Arg-Lys antagonism from various reports as follows: 1) with increasing dietary Lys until 0.75%, activities of arginase (Arg to Orn and urea) and glycine transamididase (Gly to creatinine) increase, 2) from 0.75 to 1.50% feed intake decreases, and at more than 2.0% urinary Arg excretion increases. Adversely, with increasing dietary Arg levels, the Lys requirements increased, which was partly due to the increase of lysine-ketoglutarate reductase, because this enzyme was activated by added Arg (Scott and Austic, 1978).

Table 3. Estimated lysine requirements of female broilers fed diets with different protein levels at three growing stages

Diet No.	1	2	3	4	5	6
CP (%)	12.6	15.1	17.5	20.0	22.5	24.9
Per diet (%)						
7-17 days			0.815	0.995	1.11	1.33
24-34		0.545	0.710	0.850	1.01	
41-51	0.390	0.480	0.575	0.715		
Per dietary CP (%)						
7-17 days			4.66	4.78	4.93	5.34
24-34		3.58	4.06	4.25	4.49	
41-51	3.10	3.18	3.29	3.58		
Lys per kg body weight gain (g)						
7-17 days			1.37	1.37	1.63	2.62
24-34		1.19	1.42	1.67	1.98	
41-51	1.09	1.46	1.60	1.99		

Branched chain amino acids : Three amino acids, Leu, Ile and Val, have very similar structures and are called branched chain amino acids. The antagonism among these amino acids has been established in chickens. D'Mello and Lewis (1970a, b) showed that excessive dietary Leu resulted in a retarded performance of male broilers at three weeks of age, and increasing the dietary levels of Ile and Val restored the growth rate. Similar results were obtained by Tuttle and Balloun (1976) with young turkeys. The degree of effects differs among amino acids (unpublished data).

Because the requirement of broilers for branched chain amino acids is affected by the antagonism among these amino acids, it is necessary to consider the interaction among them. However, the cited experiments to determine the chick's requirements for branched chain amino acids considered one or two of these amino acids at a time (Dobson et al., 1964; Dean and Scott, 1965; Hewitt and Lewis, 1972; D'Mello, 1974). Farran and Thomas (1990) studied the requirement of male broiler chicks during the starter period for Leu, Ile and Val simultaneously using a central-composite, rotatable design. The design yielded similar results, compared to a factorial design in which fewer treatments were employed.

Met and neutral amino acids, Thr, Gly and Ser : Most recently, the relationship between Met and Cys was reviewed by Ohta (1999). Met and Cys are used independently for protein synthesis and play independent biological roles in the body. However, Cys is converted from Met. Therefore, the replacing ratio of Met by Cys has been studied by many researchers and estimated to be about 50% (Wheeler and Latshaw, 1981). Ohta and Ishibashi (1994) reported that 0.3% of Met was absolutely required irrespective of dietary TSAA levels for maximum performance of broilers at 8 to 18 d of age. This means that the ratio of Met replaced by Cys increases with increasing TSAA levels. When the dietary Met exceed 0.7%, the excessive Met causes the depression of growth performance of broilers. However, no negative effect of growth performance was observed even when the dietary Cys was at 1.90% of diet.

This depression of performance caused by excessive Met was alleviated by addition of Thr, Gly and Ser (Ohta and Ishibashi, 1995). Excess Met caused activation of threonine-serine dehydrase activity (Sanchez and Swendseid, 1969) and glycine methyltransferase, and resulted in Thr deficiency. As shown by Edmonds and Baker (1987), dietary excess of all individual amino acids caused the depression of BW gain. Most of this depression was alleviated by addition of amino acids or CP (Yanaka and Okumura, 1981; Ueno, 1998).

Toxicity of amino acids

Edmonds and Baker (1987) and Okumura and Yamaguchi (1980) studied the effects of excess of individual essential amino acids in young chicks. In both studies, Met and Phe were most effective, and Arg, Leu and Val were most ineffective to prevent depression of BW. Compared to Met, Cys was not so toxic. In the case when a given amino acid is excessive, it is possible to assume that all other amino acids are deficient, and in the case when a given amino acid is deficient, it is also possible to assume that all the other amino acids are excessive.

4. Strains and sexes

The accumulation of muscle protein is determined by the relative rates of its synthesis and degradation. Estimation of rates of synthesis and degradation of skeletal muscle protein in domestic animals is very important for studying whole-body protein metabolism and efficiency of animal meat production. By estimating muscle protein turnover rates of various breeds, it is possible to clarify the genetic-biochemical characteristics of each breed, changes of metabolic systems resulting from selective breeding and differentiation in protein metabolism. Maeda et al. (1990) compared muscle protein metabolism among three strains of chicks. Protein synthesis was estimated to be 6.9, 8.9 and 12.6%/d, and protein degradation was estimated to be 4.6, 3.0 and 7.5%/d, for White Leghorn, broiler and Japanese native chicks, respectively. These results indicate that protein degradation rate was less in broilers.

There are many native chickens in the world. They are characterized by beautiful feather, special voice, loveliness and special taste. Muramoto et al. (1997a, b) studied the nutrient requirements of native chicken. Their growth rate was higher than that of layer pullets but lower than that of broilers. The requirements of energy, CP and amino acids of the native chicken ranged between those of layer pullets and broilers. However, there were no differences in the nutrient requirements expressed as percentages of diet between males and females. Therefore, it is impossible to obtain the nutrient requirements of native chickens by calculation from those of layer pullets and broilers. It is necessary to determine the amino acid requirement individually by experiments.

Although there are small differences in amino acid requirements among the lines of laying hens, there are differences in protein requirements between genetically lean and fat chicken (Leclercq and Guy, 1991), and in the carcass composition between male and female (Gray et al., 1983). Han and Baker (1993) studied the effects of sex, BW gain and genetic strain on dietary Lys requirement of broiler chickens, and concluded that males required a higher level of dietary Lys than females for the maximum performance.

5. Age

Broilers

As shown in many feeding standards, amino acid requirements of broilers decrease with advancing age. Ueno (1998) studied the effect of age on the Arg and Lys requirements. The Arg requirements were 1.36, 0.99, and 0.95%, and the Lys requirements were 1.10, 1.07 and 0.99% for 8 to 18, 28 to 38, and 48 to 58 d of age, respectively. The Lys requirements decreased linearly, but the Arg requirements decreased rapidly from 8 to 18 d to 28 to 38 d of age as shown in figure 2. The high Arg requirements at 8 to 18 d are caused by renewal of feathers. The Arg and Lys requirements of broilers expressed as percentages of diet decreased with advancing age, but those expressed as mg/chick/d and mg/kg BW gain increased with advancing age. As described previously, amino acid deficiencies are covered by increasing feed intake. With advancing age, the capacity of digestive tracts increases. The amino acid requirements change with advancing age as the rates of protein synthesis and degradation also change.

Laying hens

Kumagai and Ishibashi (1984) showed that egg yolk accumulated constantly, but egg albumin was synthesized mainly during three h before ovulation. At this period, the degradation of muscle increased (Hayashi et al., 1991). Hiramoto et al. (1990) showed that protein synthesis rates in tissues and whole body changed along with egg formation cycle. These observations indicate that the amino acid requirement differs along with egg formation cycle in a d.

Okazaki et al. (1994) reported that the amino acid requirements of laying hens decreased with advancing age. For the maximum egg production rate, laying hens required 130, 115, and 100% of amino acids of NRC (1994) requirements for 20 to 30, 30 to 60 and 60 to 80 wk of age, respectively. This result indicates the availability of phase feeding, although only one pattern of amino acid requirements is shown in many feeding standarads. This means that the amino acid requirements decrease with decreasing egg production rate, but not with age. Old laying hens with high egg production rate require high levels of amino acids, the same as young laying hens. There were no differences in the digestibility of CP between young and old laying hens (Ikeda et al., 1997).

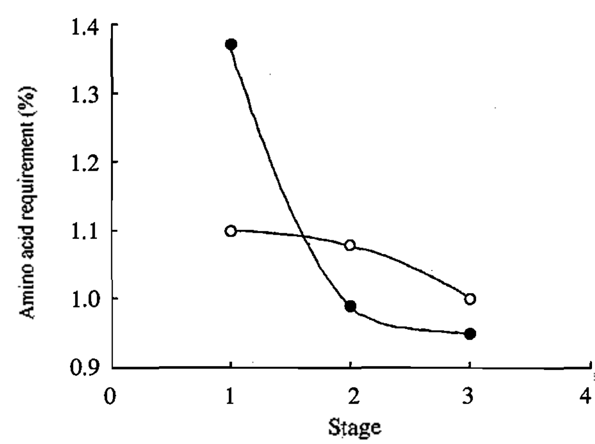

Figure 2. Effects of advancing age on arginine (●) and lysine requirement (○) of broilers

Forced-molted laying hens

Laying hens start egg production at 130 to 150 d of age. Egg production rate reaches the maximum levels at 200 d of age, and decreases linearly by

0.54%/wk. Before egg production decreases to less than 60%, laying hens are forced to molt.

Forced molting is common in the field in commercial industries around the world to extend the productive life of hens. The most popular forced molting method involves reducing BW by fasting and light control. After fasting, layers consume a low CP postmolt diet until their egg production rate reaches 5%, and then the postmolt diet until their egg production rate reaches 50%, and then the postmolt diet is switched to a layer diet. Single Comb White Leghorn hens lost about 30% of their initial BW (440 g) and 130 g of feathers by forced molting, which are recovered in two week after refeeding (Fukuma and Ishibashi, 1997). The carcass, feathers and eggs contain 0.4, 0.4 and 3.8% Met and 1.8, 6.7 and 2.4% Cys, respectively. Therefore, 0.93 g of TSAA $\{(0.60+0.23)\times440/14+(0.40+6.7)\times130/14\}/100$ has to be supplied daily, which is equivalent to TSAA in 129 g egg. Therefore, the TSAA requirement during two week post refeeding may be higher than that at the egg production phase.

Fukuma and Ishibashi (1987) compared the effect of 14.3 and 17.3 % CP diets on the performance of laying hens after forced molting and concluded that the CP requirement was less than 14.3% of diets containing 0.56% TSAA. Hoyle and Garlich (1987) reported that there were no differences in the rate of BW gain and time until restart of egg production after forced molting among hens fed the molt diets containing 12.4, 13.0, 14.8 or 17.0% CP. Because all diets were supplemented with TSAA equal to 5% of the dietary protein, TSAA requirement was estimated to be 0.62% in the 12.4% CP diet. Koelkebeck et al. (1991) studied the effects of dietary CP and Met supplementation on the performance of laying hens after molting. When 16, 13 or 10% CP molt diets were supplied, hens supplied 16 and 13% CP molt diets regained BW more rapidly, returned to egg production faster, and had no effect on postmolt performance. Because the 13% CP diet contained 0.46% TSAA, TSAA requirement of postmolt hens was estimated to be less than 0.46%.

Because plasma Met concentration responded to changes in dietary TSAA levels within two days (Yamamoto and Ishibashi, 1996), TSAA requirements of laying hens before and after restarting egg production following forced molting were determined using plasma Met concentration as a parameter (unpublished data). TSAA requirements expressed as percentage of diet determined by laying performance and by plasma TSAA agreed well after restart of egg production. Those determined by plasma Met before and after restart of egg production agreed with each other. Feed intake was highest at first week after refeeding, and then decreased and remained constant after egg production restarted. TSAA requirements expressed as milligram per hen per d before restarting egg production were estimated to be about 10% higher than those after restarting egg production. The high requirement of TSAA was compensated by increased feed intake. After forced molting, the egg quality, egg shell strength and Haugh unit are improved and decrease with advancing age in a similar manner to before molting.

AMINO ACID REQUIREMENTS

There are many reports on CP requirements for maximum performance (Worldroup et al., 1976; Parrand and Summers, 1991; Koide et al., 1992). However, the reported values range widely, which is reasonable because CP requirements are affected by many factors as discussed in section 2. As shown in figure 1, the CP requirements are decreased by improving dietary amino acid balance. Therefore, it is very important to determine exact amino acid requirements for effective poultry production.

1. Amino acid requirements for maximum performance

In the NRC (1994), only one pattern of amino acid requirement for laying hens throughout the laying period and three patterns for broilers are presented. Recently Coon (1998) presented the amino acid requirements of laying hens. As mentioned in secion 2, amino acid requirements are affected by interaction of metabolically related amino acids and age. Therefore, the requirements of each amino acid has to be expressed as a function of these factors.

2. Maintenance requirements of amino acids

The requirement of each amino acid for chickens is the sum of amino acids required for maintenance, growth, renewal of nails, skin and feather. Therefore, in order to formulate the feed of chickens for the maximum performance, it is necessary to clarify the amino acid requirement of chickens considering each factor. Although there are many reports on CP and amino acid requirements for maximum performance of broilers (Waldroup et al., 1976; Parr and Summers, 1991; Koide et al., 1992), there are no available reports on the amino acid requirements for maintenance of broilers. The amino acid requirements for maintenance of adult Single Comb White Leghorn roosters were studied by two groups (Leveille and Fisher, 1958; Ishibashi, 1973). Although it is well known that the amino acid requirement for maximum performance is affected by many factors (Ishibashi, 1990), it is believed that the amino acid requirement for maintenance is affected only by BW or metabolic body size at least after maturity and perhaps even during growing stages under the optimum state, and that it is less than that for the maximum performance, especially at the rapid growing stage. These concepts might have been one of the reasons why the amino acid requirement for maintenance of broilers has not been studied up to date.

Miyasaka et al. (1997) studied the effect of age on CP requirements for maximum performance and for maintenance at three growing stages of female broilers, at 8, 28, and 48 d of age. As shown in table 4, the CP requirements for maximum body weight gain expressed as percentages of diet decreased, and those expressed as grams per BW gain (kg) increased with increasing body weight. The CP requirements for maintenance expressed as grams per BW (kg) and per BW $(kg)^{0.75}$ decreased. Those expressed as grams per BW $(kg)^{0.75}$ decreased more slowly.

Table 4. Crude protein requirements for maximum performance and maintenance of female broiler at three growing stages

Age (d)	8 to 18	28 to 38	48 to 58
BW (g)	146	936	2,210
Maximum BW gain			
% of diet	19.0	15.7	31.0
g/BW gain (kg)	27.5	31.0	37.9
Maintenance			
% of diet	3.45	2.93	2.94
g/chick/d	0.75	2.61	4.05
g/BW (kg)	4.56	2.49	1.73
g/BW $(kg)^{0.75}$	2.91	2.52	2.14
g/BW $(kg)^{0.67}$	2.39	2.42	2.20

When the maximum feed efficiency was achieved, ME consumption was calculated to be 151, 441 and 527 kcal/chick/d and 462, 695 and 908 kcal/kg of body weight. Maintenance was calculated to be 60, 236, and 436 kcal/chick/d and 253, 246, 239 kcal/BW $(kg)^{0.75}$, respectively. The ME requirement expressed as kcal/BW $(kg)^{0.75}$ decreased slightly with increasing BW (table 4).

Ishibashi et al. (1998) determined the maintenance requirements of amino acids in young female broilers using crystalline amino acids as a sole nitrogen source (table 5). The maintenance amino acid requirements determined at zero point of BW gain and feed efficiency were higher than those of nitrogen balance as shown in table 5. These values differ from those determined by Leveille and Fisher (1958) and Ishibashi (1973) using adult roosters. The pattern of maintenance requirements of amino acids differs from those of egg, feather and muscle of broilers. Because CP requirements expressed as gram per BW and gram per BW $(kg)^{0.75}$ decreased and those expressed as g/BW $(kg)^{0.75}$ decreased slowly, amino acid requirements might decrease in the similar manner to CP requirements.

Table 5. Maintenance requirements determined at zero body weight gain, feed efficiency and nitrogen balance of chicks at 8 to 18 days of age (%)

Amino acid	BW gain	Feed efficiency	Nitrogen balance
Arg	0.175	0.150	0.084
His	<0.030	<0.030	<0.030
Ile	0.091	0.097	0.032
Leu	0.100	0.100	0.035
Lys	0.091	0.110	ND
Met	0.87	0.078	0.040
Phe	0.069	0.058	0.058
Thr	0.133	0.123	0.058
Trp	0.029	0.029	0.019
Val	0.089	0.086	0.93

3. Usefulness of non-essential amino acids

Historically, amino acids which are not synthesized in the body are called essential amino acids and those which are synthesized in the body are called non-essential amino acids. Semi-essential amino acids are synthesized, but deficient for the maximum performance and necessary to be supplied to the diet. However, recently all amino acids to be supplied to the diet are called essential amino acids, e.g., Pro, for poultry and Arg for pigs.

Rats grow on a diet containing only essential amino acids, but grow faster when are added a single or a mixture of non-essential amino acids (Rose et al., 1948) or ammonium compounds such as ammonium citrate and urea (Rose et al., 1949; Lardy and Feldott, 1949). Among non-essential amino acids, Glu is most effective, but completely replaced by other non-essential amino acids. Although each non-essential amino acid plays specific biological roles in the body, it is difficult to determine the requirement of each non-essential amino acid.

By reducing essential amino acids to the requirement level, the calorie to protein ratio increases and body fat increases. In young birds, feed intake decreases with decreasing dietary CP or specific amino acids.

However, adult birds consume more diet to compensate for a deficiency of dietary CP or specific amino acids, resulting in excess ME intake and increment of abdominal fat (unpublished data).

Francher and Jensen (1986) reported that it was possible to reduce abdominal fat by adding Glu to the diet. However, neither Glu nor ammonium citrate reduced abdominal fat significantly in our study (unpublished data). When 5% of Glu was added, plasma Glu concentration and taste of broiler meat were not affected significantly (Ueno, 1998).

These results do not deny the importance of non-essential amino acids. When non-essential amino acids are deficient, essential amino acids are used as amino-nitrogen sources for non-essential amino acids. Therefore, for the effective use of essential amino acids, non-essential amino acids have to be added. Protein of eggs and meat of poultry and feedstuffs contain about 50% of essential and non-essential amino acids, respectively.

PLASMA AMINO ACID CONCENTRATIONS AS A PARAMETER TO DETERMINE AMINO ACID REQUIREMENTS

The experiments to determine amino acid requirements are laborious and expensive, and need a large number of animals to be studied. Thus, many efforts have been made to develop a new method to determine amino acid requirements more accurately and easily. The plasma amino acid concentration is one of them. The plasma concentration of a given amino acid remians constant, and then increases with the increasing dietary levels (figure 3). The intersection of both lines agrees well with the requirement determined by feeding trials in many species including rats (Stockland et al., 1970), chicks (Zimmerman and Scott, 1965), laying hens (Ishibashi, 1985), pigs (Mitchell et al., 1968), and young men (Young et al., 1972). However, in all the cited literature, the blood samples were taken on the last day of feeding experiments. Thus it was not proper to use the plasma amino acid concentration as a parameter to determine amino acid requirements. Totsuka et al. (1992), and Yamamoto and Ishibashi (1996) reported that the plasma concentration of amino acids responded to changes in dietary levels of amino acids within a short period, and the response was kept for more than 20 d, and that it was possible to distinguish the response of plasma amino acid concentration of multiple amino acids simultaneously (Yamamoto and Ishibashi, 1997a). These values determined from the data of plasma amino acid concentration in an environmentally controlled laboratory agreed well with those determined in a practical farm using a large number of laying hens (Yamamoto and Ishibashi, 1997b). Yamamoto et al. (1997) and Kokawa et al. (1996) confirmed that the requirements of Lys and Thr determined from plasma concentration of Lys (figure 4) and Thr at 2, 4, and 20 d after changing their dietary levels agreed well, and that these values agreed well with those determined from laying performance. Yamamoto and Ishibashi (1998) confirmed that it was possible to determine the requirements of two amino acids simultaneously from the data of plasma amino acid concentration using a small number of laying hens repeatedly.

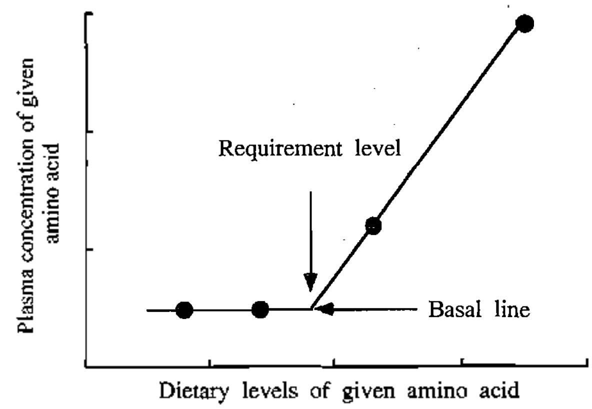

Figure 3. Relationship between dietary levels of given amino acid and its plasma concentration

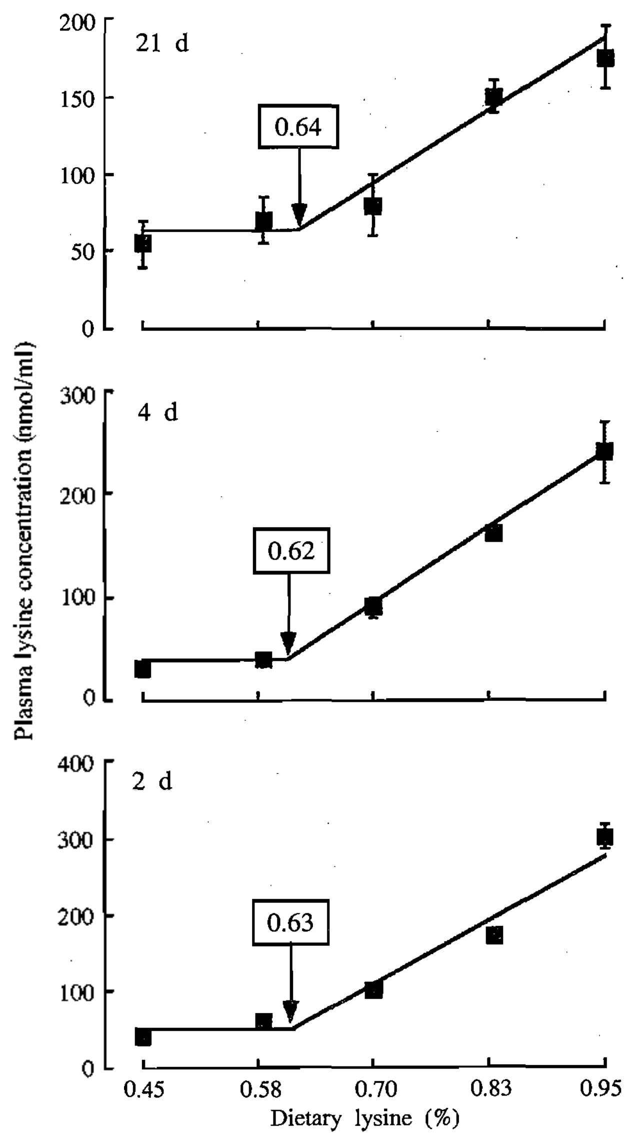

Figure 4. Lysine requirements determined from plasma concentration of laying hens at 2, 4 and 21 days after changing dietary lysine levels

These results indicate that it is possible to predict amino acid requirements of many animals. Hitherto it has been difficult to use a large number of animals and to feed them in controlled environments for a long period. Furthermore, it may be possible to judge the adequacy of dietary amino acid supply and the nutritional status of amino acids. If the plasma concentration of a given amino acid is higher than its lower constant level, the supply of the amino acid would be higher than the requirement levels. According to Watanabe (1998), the lower constant levels of plasma amino acid concentration differed between rats and laying hens. Therefore, the lower constant levels should be determined in each species.

NITROGEN EXCRETION TO REDUCE ENVIROMENTAL POLLUTION

Today, one of the most important problems in the animal industry is pollution by livestock, including water pollution and deposition of animal waste. Although there is a large demand for livestock waste as fertilizer, the most effective counterplot is to reduce the amount of waste. In order to reduce the waste of livestock, it is necessary to increase dietary efficiency, and to restrict excess feed intake. Up to the present, there have been many reports that studied the improvement of dietary efficiency and feed restriction in many species. It has been reported that dietary CP levels can be reduced by adding amino acids that are limiting in diets of broilers (Waldroup et al., 1976; Parr and Summers, 1991; Yamamoto and Ishibashi, 1997a).

By reducing dietary excess CP, it is expected that the excretory nitrogen decreases proportionally. Yamazaki et al. (1996) reported that it was possible to reduce dietary CP levels from 19 to 17% without reducing BW gain of male broiler chicks by crystalline amino acid supplementation, and that the rate of increasing excretory nitrogen was affected by age of broiler chicks. When dietary CP decreased from 19 to 17%, excretory nitrogen decreased 10.9% at 18 to 21 d of age, but only by 4.9% at 25 to 28 d of age. However, when dietary CP levels were reduced without reducing body weight gain, abdominal fat increased with depressing dietary CP levels (Summers and Leesons, 1985; Yamazaki et al., 1996). Therefore, the research on methods to reduce carcass fat invites attention, because of the need to produce lean meat. It has been demonstrated that it is possible to reduce carcass fat by restricting feed intake (Jackson et al., 1982).

Ishibashi (1996) studied the effects of dietary CP levels, feed restriction, age and strains of birds on the excretory and retained nitrogen. In the case of broilers, when the diet containing 17.9 to 27.8% CP were fed *ad libitum* at three growing stages, nitrogen excretion (Y, mg/day) increased with increasing dietary CP levels (X, % of diet) as follows.

$Y=115.4X-588$ $r^2=0.988$ for 27 to 30 d of age
$Y=235X-1298$ $r^2=0.999$ for 47 to 50 d of age

When consumption of the 27.8% CP diet at 27 to 30 d of age and the 27.8% CP diet at 37 to 40 d of age were restricted gradually to 70% of ad libitum feeding groups, excretion of nitrogen (Y, mg nitrogen/d) decreased with increasing restriction rate (X, % of *ad libitum* feeding group) as follows.

$Y=41.1X-1042$ $r^2=0.997$ for 27 to 30 d of age
$Y=44.3X-1849$ $r^2=0.998$ for 37 to 40 d of age

When the 21.2% CP diet was supplied from 20 to 50 d of age, the nitrogen excretion (Y, mg nitrogen/d) increased with advancing age (X, d of age) as follows.

$$Y=92.5X-1012 \qquad r^2=0.989$$

In the case of laying hens, the diets containning 14.0 to 19.8% CP were supplied ad libitum for 10 d from 220 to 230 d of age, the nitrogen excretion increased with increasing dietary CP levels (X, % of diet).

$$Y=116.35X-350 \qquad r^2=0.990$$

When the consumption of 21.4% CP diet was restricted to 79% of ad libitum group, the nitrogen excretion decreased with increasing restriction rate (X, % of *ad libitum* feeding group).

$$Y=44.3X-1849 \qquad r^2=0.998$$

These results indicate that it is possible to reduce the nitrogen excretion by reducing dietary excess CP and restricting feed intake both in broilers and laying hens.

Karasawa (1989) reported that amino acids and uric acids were utilized by microflora in the cecum and the synthesized ammonia was utilized for synthesis of amino acids in the liver of chicks fed low protein diets. Ishibashi et al. (1977a, b) reported that there were no differences in amino acid requirements between conventional and germ-free broiler chicks. These results indicated that intestinal microbial flora have little effect on the nitrogen excretion.

CONCLUSION

In the world, populations of human and livestock are increasing. Some feedstuffs are competing with human foods and the waste of livestock causes environmental pollution. In order to produce animal products, about six times of energy is required compared to foods directly utilized by human. Therefore, the objective of animal nutritionists has to be focused on 1) improvement of feed efficiency to reduce energy and protein required per animal production and waste for the better environmental conservation, and 2) researches on new feedstuffs which do not compete with human foods directly.

In 1956, Fisher et al. (1956) fed laying hens with the diet contained crystalline amino acids as a sole nitrogen source, and reported that the same egg production rate (56%) was obtained as that fed the commercial diet. The present egg production rate of commercial laying hens exceeds 90% at the peak egg production stage. It is possible to keep a high egg production rate on the amino acid diet (Ishibashi, 1984). However, it is too expensive to feed laying hens on the amino acid diet.

The performance of meat type chickens have been improved genetically and nutritionally as shown in table 6. By supplying limiting amino acids to the low CP diet, the same performance was achieved as that fed the high CP diet. Furthermore, by adding limiting amino acids one by one, it is possible to reduce dietary CP levels without reducing performance. Finally, when all amino acids except the last limiting amino acid are added, the utilization of dietary nitrogen reached the maximum level and nitrogen excretion reduces to the minimum level.

At that time, new problems which are not recognized now will appear and nutrition research can have no end.

Table 6. Progress of performance of broilers at 8-wk-old

Diet	Strain	BW (kg)	Feed efficiency
1930	1930	0.39	0.28
1930	1960	0.57	0.83
1950	1930	0.85	0.46
1960	1960	1.28	0.47
1999	1999	3.00	0.49

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