

## Protein Quality and Amino Acid Utilization in Chickens

Ji-Hyuk Kim

*Poultry Science Division, National Institute of Animal Science, RDA, Cheonan 331-808, Republic of Korea*

**ABSTRACT** It is well known that dietary protein affects the growth performance and carcass composition of poultry. Over the last several decades, numerous studies have been carried out to investigate to optimize the level of dietary protein since the protein is an important and expensive constituent in poultry feed. It is generally accepted that dietary protein should represent a balance of amino acids supporting the requirements for growth and maintenance of birds. A protein with balanced essential amino acids that matches a bird's requirement and sufficient non-essential amino acid nitrogen to enable the synthesis of all of the non-essential amino acids, is referred to as an 'ideal protein'. Feeding of excess protein or amino acids may result in an amount of nitrogen emission. Most common method to reduce nitrogen emission is using diet formulation which has lower dietary crude protein level and higher concentration of amino acid supplements. However, there are conflicting reports whether low protein diets supplemented with synthetic amino acids can obtain the growth performance equal to high protein diets. Excessive nitrogen excretion caused by amino acid imbalance also may influence the environment of poultry house due to ammonia production from uric acid. These environmental conditions may increase the incidence of skin problem or respiratory diseases of chickens. Various strategies based on comprehensive understanding should be tested to optimize nitrogen utilization and reduce nitrogen emission while maintaining the performance in poultry production.

(Key words : protein quality, amino acid balance, nitrogen, chicken, welfare)

### INTRODUCTION

As poultry industries throughout the world increase in size, the importance of feed production also continues to increase. One of the main objectives of animal nutrition is to formulate diets that allow a predetermined rate of production to be achieved at least cost. The supply of balanced nutrients in animal feed is one of the most critical requirements for achieving optimal growth. Protein, especially, is the major nutrient that determines the performance of animals, assuming that energy is non-limiting. It is also an expensive dietary constituent and there is much concern about optimizing its dietary concentration in commercial practice. Dietary protein must be judged as a source of individual amino acids which are needed for synthesis of protein by the animal.

Ever since Almquist (1947) pointed out the importance of the ratio of the individual amino acids in the diet, many scientists have shown that dietary proteins which do not contain adequate amounts of essential amino acids to satisfy the animal's requirement cannot be used efficiently for their growth and maintenance and eventually cause growth failure and

deficiency symptoms. For these reasons, the terms 'protein quality' and 'ideal protein' have been introduced.

Protein quality also influences the environment, because of nitrogen excretion. One of the ways of minimizing nitrogen excretion in the waste is through diet composition, rather than dealing with it after the waste has been produced (Blair et al., 1999). Reduction in nitrogen excretion and improvement in the efficiency of nitrogen deposition can be obtained by matching the amino acid composition of the diet with the amino acid requirements of the bird for maintenance and production (Deschepper and de Groote, 1995). This is a protein blend which comes as close as possible to providing amino acids in the exact ratios required by the bird. Therefore, it is environmentally and economically beneficial to provide an ideal blend of protein in diets.

Additionally, excretion of nitrogen from excess amino acids can cause welfare problems; carcass defects such as breast blister and hock burn are often attributable to high uric acid excretion rates caused by poor quality protein. Breast blister can also be exacerbated by poor feather growth due to amino acid deficiency.

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<sup>†</sup> To whom correspondence should be addressed : [jihyuk@korea.kr](mailto:jihyuk@korea.kr)

The aim of this article is to review about background knowledge of protein metabolism and the effects of amino acid balance of feed on the performance and nitrogen utilization of chickens. It also briefly covers the nitrogen utilization and its effects on the environment and welfare related issues.

## PROTEIN QUALITY AND AMINO ACID BALANCE

### 1. Protein Quality for Poultry Feed

The dietary protein requirement is actually a requirement for the amino acids contained in the protein. Amino acids are organic compounds containing both an amine group and a carboxylic acid part. It is in the form of the constituent amino acids that protein is absorbed from the intestine. However, not all amino acids within intact proteins need to be released as such during digestion, because there is some absorption of peptides. Uptake of free amino acids as peptides may be advantageous to the birds, relative to processing of free amino acids from within the gut lumen. Boorman and Ellis (1996) suggest that one advantage of a bird utilizing peptides is that there will be less bacterial degradation within the digesta and that this activity should not be underestimated.

The protein quality refers to the assortment and proportions of amino acid: the more complete the assortment and the more nearly the proportions approach the physiological needs of animals for amino acid, the higher the quality of protein (Lloyd et al., 1978). However, the amino acid composition of a foodstuff is not the final measurement for protein quality because some of the amino acids may not be biologically available to animals. We also have to consider a reasonable ratio of essential amino acids (EAA) to non-essential amino acids (NEAA). Overall, the protein quality of diet is a function of its quantity, its digestibility and amino acid balance. Boorman (1999) stated that relative to the animal's requirements one essential amino acid is always likely to be in shorter supply than the others. It is referred to as the first limiting amino acid.

### 2. Ideal Amino Acid Balance

In recent decades, 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. Ishibashi (1990) summarized that the factors are classified into four categories, environmental factors, genetic background, condition of animals and dietary factors. In the dietary factors, various factors are involved such as dietary metabolizable energy, availability of dietary amino acids, antagonism and imbalance among amino acids, deficiency and excess of amino acids, palatability, and dietary crude protein or metabolically related amino acid levels. The statement of a requirement is the rate at which a bird needs to be supplied with energy or a nutrient in order to carry out a set of functions such as maintenance, protein synthesis, or egg production at certain rates (Emmans and Fisher, 1986).

There have been many reports suggesting the requirements or recommendations for crude protein content and individual amino acid concentrations. Although recommendations by various researchers differ to some degree, NRC (1994) recommendations have been used as a standard for formulating poultry diets for many years. However, slightly higher concentrations of some amino acids are being used in practice for better productivity. For example, dietary lysine concentration can have a large influence on breast meat yield since breeders have selected the broiler for increased meat yield, breast meat represents a large portion of total carcass meat, and breast muscle contains a high concentration of lysine (Kerr et al., 1999). Kerr et al. (1999) reported that breast meat weight and proportional yield were significantly increased by increasing dietary lysine up to 121% of NRC recommendation. Leclercq's review (1998) also suggested that lysine exerts specific effects on body composition at dietary levels higher than that required for maximum growth rate, which also results in an improved feed conversion ratio. Wallis (1999) found that dietary supplements of methionine increased breast meat yield and decreased abdominal fat in growing broilers. A protein with balanced EAA that matches a bird's requirement and sufficient NEAA nitrogen to enable the synthesis of all of the NEAA, is referred to as an 'ideal protein' (Baker and Han, 1994 a; Cole and van Lunen, 1994).

A number of research has been carried out to assess the amino acid requirements of chickens and to define optimal dietary amino acid balance. Grau (1948; cited by Morris et

al., 1999) stated that the requirement for an amino acid expressed as a proportion of the diet increases in direct proportion to the protein content. He reported that the lysine requirement in chicks fed on diets based on sesame meal formulated to supply 50 to 300 g CP/kg diet was higher in the 300 g CP/kg diet than in the 200 g CP/kg diet, even though the growth rate achieved with an optimal supplement of lysine was essentially the same at these two protein levels. Further research confirmed this principle. Nelson et al. (1960) reported that the requirement for methionine + cystine was a constant proportion of the protein in diets containing from 212 to 275 g CP/kg. Boomgaard and Baker (1971, 1973) reported that lysine and tryptophan requirements for maximum growth are constant proportions of the dietary protein content ranging from 140 to 230 g CP/kg diet. Robbins (1987) reported similar results for threonine. More reports (e.g. Mendonca and Jensen, 1989; Barbour et al., 1993; Morris et al., 1987, 1999) have dealt with the relationship between dietary protein content and amino acid requirement and all of these authors have concluded that the requirement increases as a linear function of dietary protein content.

### 3. Imbalancing Effect and Amino Acid Utilization

An animal's response to dietary protein is a response to the most limiting amino acid in that protein. Theoretically, therefore, it should be possible to achieve the same maximum response with any protein, irrespective of its quality, provided sufficient protein is fed (Boorman and Ellis, 1996). Carpenter and de Muelenaere (1965) firstly tested this hypothesis by feeding chicks groundnut flour (which contains low methionine, lysine and threonine) with lysine supplement to ensure sulphur-containing amino acid deficiency. They concluded that under certain conditions, higher levels of poor quality protein will result in nearly as good growth as can be obtained with practical diets containing good quality protein. However, later experiment by Wethli et al. (1975) using high content of groundnut meal and soybean meal as protein supplements to a cereal-based diet did not result in maximum growth. They stated that 'The only explanation which fits the evidence is that the amino acids supplied by these low-quality proteins are in such disproportion, compared with the animal's needs, that the utilization of the first limiting amino acid(s) is impaired'.

Morris et al. (1987) stated that at very high concentrations of dietary protein, the excess load of absorbed amino acids may exert an imbalancing effect, requiring an increased supply of the first limiting amino acid if maximum growth is to be achieved. This effect of excess amino acids has been demonstrated many times, using free amino acids. The term imbalance has been defined by Harper (1964) who investigated this effect using growing rats. Harper and Rogers (1965) stated that dietary amino acid imbalance precipitates its adverse effects by reducing food intake while efficiency of amino acid utilization remains unimpaired. The issue of amino acid imbalance assumed practical significance in poultry nutrition with studies of Wethli et al. (1975). They invoked this phenomenon to explain the inferior utilization of the first limiting amino acid in low quality protein sources. Some reports (Morris et al., 1987; Abebe and Morris, 1990, Morris and Abebe, 1990; Mendonca and Jensen, 1989) indicated that imbalance may increase the requirement of the chick for the first limiting amino acid, but this effect has not been corroborated by D'Mello (1990). Most experiments with imbalancing mixtures of amino acids report a depression in food intake but show no reduction in utilization of the first limiting amino acid when growth rate is regressed on amino acid intake (Fisher et al., 1960; Harper et al., 1970; Boorman and Ellis, 1996).

There is good quantitative evidence for the interdependence of lysine and arginine and of valine, leucine and isoleucine in chick diet (D'Mello and Lewis, 1970). Antagonism usually occurs because of interactions between structurally similar amino acids. Lysine specifically antagonises the utilization of arginine. Leucine impairs the utilization of isoleucine and valine; these three compounds are branched-chain amino acids. In practice, the antagonisms can be reduced by supplementation of the diet with arginine, isoleucine or valine. Since their main excretory product is uric acid, poultry cannot synthesize arginine. This makes them particularly sensitive to lysine antagonism. There is increased activity of kidney arginase in chicks receiving excess lysine, leading to increased arginine breakdown (D'Mello, 1994).

As mentioned earlier, much research has shown that, when diets varying in CP were fed to chicks, the level of lysine needed to maximize growth at each protein concentration was a fixed proportion of the protein (e.g. Morris et al., 1987). As

stated by Abebe and Morris (1990), this proportionality rule is well established for diets with limiting protein contents and can be explained by the hypothesis that, as the supply of a first-limiting amino acid is increased, the limit to response is determined by the supply of the second-limiting amino acid. It is important, however, to recognize that such statements may not be true in situations of amino acid oversupply and/or poor amino acid balance.

Fisher et al. (1960) used sesame meal as the sole source of protein in the diet, varying the protein and lysine concentrations and the degree of imbalance by adding a mixture of amino acids lacking lysine. They observed the typical effects of imbalance such as low food intake and growth rate on the more imbalanced diets, especially at low protein concentrations. Their conclusion was confirmed by Netke et al. (1969) who used purified diets limiting in lysine, leucine or isoleucine and this imbalance effect was comprehensively reviewed by Harper et al. (1970) later.

D'Mello (1990) compared lysine responses in three diets, one containing 225 g CP/kg and two others containing 315 g CP/kg, obtained by adding unbalanced mixtures of free amino acids. The results showed no reduction in lysine utilization in the presence of excess amino acids. However, the lysine additions to the 'high protein diets' were not sufficient to yield an estimate of optimum response.

More recently, Boorman and Ellis (1996) performed the experiment with protein mixtures of different quality by mixing maize gluten meal and soybean protein concentrate in constant proportions, supplementing with tryptophan, threonine and arginine to adequacy and varying amino acid score by varying additions of free lysine. They reported that there is no evidence, on deficient intakes, that the utilization of the limiting amino acid is affected adversely by poor protein quality (amino acid imbalance) and it is not possible to elicit maximum response to the limiting amino acid by feeding large amounts of poor-quality protein. MacLeod (1997) stated that the strongly negative correlation between protein retention per g of lysine consumed and lysine: CP ratio may have resulted from a greater catabolism of lysine when it is present in greater concentration relative to other amino acids.

Sterling et al., (2006) reported significant interaction between dietary protein level and lysine for body weight and

feed efficiency during starter period of broiler chicks showing the best performance at the highest lysine level at 23% CP. Abdel-Maksoud et al. (2010) reported that maximum body weight could be achieved with 21% CP diet supplemented by EAA to the same level as that of the chicks fed 23% CP diet. They also stated that optimum lysine level was not a constant proportion of CP levels with or without amino acid supplementation.

Overall, it appears unlikely that amino acid imbalance is a satisfactory explanation for the protein effect on lysine utilization and the issue raised by the data of Morris et al. (1987) and others (Mendonca and Jensen, 1989; Abebe and Morris, 1990) remain essentially unresolved.

## IDEAL AMINO ACID PROFILE

Amino acid requirements of animals depend on various factors such as sex, strain, diet, body composition and environment. In swine nutrition, researchers have been using ideal ratio (to lysine) for different weight categories as a basis for formulating swine diets to solve this problem (Wang and Fuller, 1989; Chung and Baker, 1992). As Mack et al. (1999) stated, this method is based on the fact that, although the amino acid requirements change because of the factors mentioned above, the ideal ratio of EAA to lysine will be only marginally affected within a certain age range. The benefit of applying the ideal amino acid pattern is that once an ideal ratio of EAA to lysine is established for a certain age range, one can concentrate on determining the lysine requirement accurately under a variety of conditions and can calculate the requirement for all other indispensable amino acids by applying their ideal ratio to lysine (Mack et al., 1999). This concept in poultry is supported by work reported by Morris et al. (1987) where it was found that for chickens over the range of 140 to 280 g CP/kg, lysine requirement could be expressed as a constant proportion of the protein (5.4%). Other reports (e.g. Boomgaardt and Baker, 1973; Robbins, 1987) also confirmed this hypothesis. However, this may not be true in cases where there are excesses of amino acid and/or poor amino acid balance (Cole and van Lunen, 1994). Amino acid profile, therefore, should be considered assuming that appropriate levels of high-quality protein are being used.

According to Baker and Han (1994), lysine was selected as the reference amino acid for three primary reasons: 1) its analysis in feedstuffs, unlike those of tryptophan and sulphur amino acids, is relatively simple and straight forward; 2) a considerable body of data exists for digestible lysine needs of poultry; and 3) unlike several other amino acids (e.g. methionine, cystine and tryptophan), absorbed lysine is used only for protein accretion.

Tables 1 and 2 show ideal amino acid patterns for broilers aged 0 to 3 wk and 3 to 6 wk, respectively, calculated as a percentage of lysine from various studies (NRC, 1994; Han and Baker, 1994; Rhone-Poulenc Nutrition Guide, 1993; Uzu, 1993; Mack et al., 1999). After 3 wk old, the ratio for some amino acids such as sulphur amino acids, threonine and tryptophan have to be increased because of changing maintenance requirements.

Ideal amino acid patterns for laying hens and broiler chickens are shown in Tables 3 and 4. It appears that the ideal amino acid pattern for laying hens is slightly different from broilers. The laying hen has been selected for high egg production and low muscle mass while the broiler has been selected for rapid weight gain and high muscle mass. This may explain the differences in pattern between these two types during growth; a greater proportion of dietary amino acids is required for muscle production in the broiler chickens. When the layer reaches its laying period, amino acid requirements

**Table 2.** Amino acid profiles expressed as percentage of lysine for broiler chickens 3~6 wk old by some research groups

	Baker & Han <sup>1</sup> (Digestible)	RPAN <sup>2</sup> (Digestible)	Mack <i>et al.</i> <sup>3</sup> (Digestible)
Lysine	100	100	100
M+C <sup>4</sup>	75	81	75
Methionine	37	48	-
Arginine	105	108	112
Valine	77	85	81
Threonine	70	67	63
Tryptophan	17	19	19
Isoleucine	67	75	71
Histidine	32	-	-
Leucine	109	144	-

<sup>1</sup> Baker and Han (1994).

<sup>2</sup> RPAN Rhone-Poulenc Animal Nutrition (1993).

<sup>3</sup> Mack et al.(1999).

<sup>4</sup> Methionine + cysteine.

are mainly for maintenance and egg production. Since maintenance has a relatively small amino acid requirement compared to egg production, the ideal amino acid pattern will be governed to a large extent by the requirement for egg production. Table 5 shows the amino acid composition of protein in poultry carcass, feather and egg. The amino acid composition

**Table 1.** Amino acid profiles expressed as percentage of lysine for broiler chickens 0~3 wk old by some research groups

	NRC <sup>1</sup> (Total AA)	RPAN <sup>2</sup> (Total AA)	RPAN <sup>2</sup> (Digestible AA)	Baker & Han <sup>3</sup> (Digestible AA)
Lysine	100	100	100	100
Methionine	46	47	51	36
Arginine	113	110	117	105
Valine	82	83	79	77
Threonine	73	64	65	67
Tryptophan	18	19	19	16
Isoleucine	73	75	78	67
Histidine	32	-	-	32
Leucine	109	140	150	109

<sup>1</sup> National Research Council (1994).

<sup>2</sup> Rhone-poulenc Animal Nutrition (1993).

<sup>3</sup> Baker and Han (1994).

**Table 3.** Amino acid profile and requirement for broiler calculated from NRC (1994)

	0~6 wk		6~12 wk		12~18 wk		18~laying	
	Profile	% of diet	Profile	% of diet	Profile	% of diet	Profile	% of diet
Lysine	100	0.85	100	0.60	100	0.45	100	0.52
Arginine	118	1.00	138	0.83	149	0.67	144	0.75
Histidine	31	0.26	43	0.26	38	0.17	38	0.20
Isoleucine	71	0.60	83	0.50	89	0.40	87	0.45
Leucine	129	1.10	142	0.85	156	0.70	154	0.80
M+C <sup>1</sup>	73	0.62	87	0.52	93	0.42	90	0.47
Methionine	35	0.30	42	0.25	44	0.20	42	0.22
Threonine	80	0.68	95	0.57	82	0.37	90	0.47
Tryptophan	20	0.17	23	0.14	24	0.11	23	0.12
Valine	73	0.62	87	0.52	91	0.41	88	0.46

<sup>1</sup> Methionine + cysteine.

**Table 4.** Amino acid profile and requirement for layer calculated from NRC (1994)

	0~6 wk		6~12 wk		12~18 wk		18~laying	
	Profile	% of diet	Profile	% of diet	Profile	% of diet	Profile	% of diet
Lysine	100	0.85	100	0.60	100	0.45	100	0.52
Arginine	118	1.00	138	0.83	149	0.67	144	0.75
Histidine	31	0.26	43	0.26	38	0.17	38	0.20
Isoleucine	71	0.60	83	0.50	89	0.40	87	0.45
Leucine	129	1.10	142	0.85	156	0.70	154	0.80
M+C <sup>1</sup>	73	0.62	87	0.52	93	0.42	90	0.47
Methionine	35	0.30	42	0.25	44	0.20	42	0.22
Threonine	80	0.68	95	0.57	82	0.37	90	0.47
Tryptophan	20	0.17	23	0.14	24	0.11	23	0.12
Valine	73	0.62	87	0.52	91	0.41	88	0.46

<sup>1</sup> Methionine + cysteine.

of carcass and egg is similar due to the similarity in the balance of amino acids in tissues and eggs. Consequently, the ideal balance of amino acids required for growth and for egg production are similar.

## LOW PROTEIN DIET AND NITROGEN UTILIZATION

It is generally recognized that reducing the dietary protein level is the most efficient way to lower nitrogen pollution. As mentioned in the introduction, the reduction of nitrogen excretion and efficiency of nitrogen deposition can be controlled by matching the amino acid composition of the diet with the amino acid requirement of the bird for growth, maintenance and production. In some regions, environmental pollution places a severe limitation on the expansion of animal agriculture,

**Table 5.** Amino acid composition (g/kg protein) of protein in poultry carcass, feathers and egg

	Carcass	Feather	Egg
Alanine	69 <sup>c</sup>	35 <sup>c</sup>	54 <sup>a</sup>
Arginine	69 <sup>cd</sup>	61 <sup>c</sup>	68 <sup>a</sup>
Asparagine	0	68	0
Aspartic acid	93 <sup>c</sup>	62 <sup>c</sup>	107 <sup>a</sup>
Cystine	11 <sup>bc</sup>	67 <sup>c</sup>	18 <sup>a</sup>
Glutamic acid	136 <sup>c</sup>	87 <sup>c</sup>	120 <sup>a</sup>
Glutamine	0	67	0
Glycine	82 <sup>c</sup>	68 <sup>c</sup>	62 <sup>a</sup>
Histidine	29 <sup>bcd</sup>	8 <sup>cde</sup>	24 <sup>a</sup>
Hydroxyproline	20	20	0
Isoleucine	43 <sup>bd</sup>	50 <sup>de</sup>	56 <sup>a</sup>
Leucine	73 <sup>bc</sup>	81 <sup>de</sup>	83 <sup>a</sup>
Lysine	80 <sup>bd</sup>	20 <sup>de</sup>	62 <sup>a</sup>
Methionine	27 <sup>bd</sup>	7 <sup>de</sup>	32 <sup>a</sup>
Phenylalanine	41 <sup>bcd</sup>	50 <sup>de</sup>	51 <sup>a</sup>
Proline	61 <sup>c</sup>	92 <sup>c</sup>	38 <sup>a</sup>
Serine	44 <sup>c</sup>	91 <sup>c</sup>	78 <sup>a</sup>
Threonine	42 <sup>bc</sup>	48 <sup>de</sup>	51 <sup>a</sup>
Tryptophan	10 <sup>b</sup>	7 <sup>d</sup>	18 <sup>a</sup>
Tyrosine	30 <sup>bcd</sup>	27 <sup>de</sup>	40 <sup>a</sup>
Valine	47 <sup>bcd</sup>	76 <sup>de</sup>	75 <sup>a</sup>

<sup>a</sup> Lunven et al. (1973), <sup>b</sup> Hakansson et al. (1978), <sup>c</sup> Nitsan et al. (1981), <sup>d</sup> Hurwitz et al. (1983), <sup>e</sup> Blair et al. (1981).

because of the implications for the contamination of soil and water. Growing pigs, for example, excrete approximately 70% of the nitrogen present in their feed and broilers excrete 58% (Table 6). This shows that a substantial proportion of the consumed nitrogen is excreted in feces and urine.

There are several ways to reduce nitrogen excretion from poultry production. Most common method is using diet formulation which has lower dietary crude protein level and higher concentration of amino acid supplements. Use of supplemental amino acids allows the total dietary protein to be reduced while still meeting the bird's requirements. Estimates of the reduction in nitrogen excretion possible with laying

**Table 6.** Fate of nitrogen consumed in the feed by different livestock

	Feed protein (g/kg DM)	Body (%)	Feces (%)	Urine (%)
Beef cattle	150	22	30	48
Piglet	184	40	10	50
Growing pig	170	32	15	53
Laying hen	170	32	12	56
Broiler	217	42	10	48

Adapted from Tamminga (1992).

hens range from 20% (Blair et al., 1976) to over 50% (Summers, 1993). More recent study showed that lysine and methionine supplementation to lower dietary protein by 4% reduced nitrogen excretion in laying hen by 30% (Latshaw and Zhao, 2011). For broilers, the estimates range from 10% (Han et al., 1992) to 30% (Parr and Summer, 1991). Blair et al. (1999) showed results that reduction in dietary CP content caused a 10~27% reduction in the total amount of nitrogen excreted during the 6-week broiler rearing period and with layers, there was a 30~35% reduction in daily nitrogen output.

### 1. Effect of Low Protein Diet on Performance

Although it is possible to reduce dietary CP in poultry diet, there are biological limits to the amount of protein that can be replaced by synthetic amino acids. It is, therefore, important to determine the ideal balance between amino acids in order to maximize the performance level and minimize the nitrogen excretion, especially on a low protein diet. There is conflicting evidence as to whether low protein diets with amino acids supplementation can support maximum productivity.

Twining et al. (1974) reported that broiler chicks receiving a low protein starter diet had lower body weights and food conversions at 4 week-old compared to those receiving the required amount. However, when they subsequently consumed an adequate finisher diet, they showed compensatory growth, with final performance almost equal to controls. Schutte (1987) fed diets containing either 20 or 16% CP to chicks from 7 to 28 days of age and obtained equal weight gain and feed efficiency with 16% protein diet supplemented with all EAA equivalent to the level present in the 20% protein diet. Parr

and Summers (1991) and Stilborn and Waldroup (1989) also obtained optimal performance with low protein diets supplemented with amino acids. There is general agreement that supplementing diets with methionine, the first limiting amino acid, and lysine, the second limiting amino acid, allows the reduction of CP levels to a point (Lipstein et al., 1975; Uzu, 1982, 1983). By removing the dietary CP minimum and supplementing with methionine and lysine, Waldroup et al. (1976) found that the best broiler performance could be obtained during 1 to 8 week old by meeting EAA needs without considering a particular dietary CP minimum. Ciftci and Ceylan (2004) reported that chicks fed the diet containing 21.3% CP showed poorer performance than those fed lower CP (19.13 and 17.97%) supplemental amino acid during starter period.

However, other scientists have reported inferior performance of broiler chickens fed low protein, amino acid-supplemented diets than that of birds receiving higher protein diets. Edmonds et al. (1985) demonstrated an inferior growth of chicks fed an amino acid-supplemented, 16% protein diet in comparison with those fed 24% protein diet. Fancher and Jensen (1989) and Mendonca and Jensen (1989) also reported an inferior performance for chickens fed low protein, amino acid-supplemented diets compared with performance of chickens fed a more conventional protein concentration. Fancher and Jensen (1989) stated that chicks fed the diet containing 17.8% protein supplemented with synthetic amino acids to meet the requirements suggested by NRC (1984) were significantly lower in body weight gain and feed efficiency than chicks fed the diet with 24.5% protein. Waldroup et al. (2005) reported that decreasing CP levels lower than 22% significantly decreased body weight gain and increased feed conversion ratio. They also stated that EAA or NEAA supplementation to the low CP diets significantly improved the performance but did not completely overcome the adverse effect of low CP diets. More scientists (Pinchasov et al., 1990; Holsheimer and Janssen, 1991; Moran et al., 1992; Bregendahl et al., 2002; Jiang et al., 2005, Waldroup et al., 2005) claimed that maximum performance cannot be achieved by feeding broilers low protein diets supplemented with amino acids.

Dean et al. (2006) reported that broiler chicks fed amino acid fortified 16% CP diet achieved optimal growth equal to the chicks reared in 22% CP diet control group when glycine

+ serine were additionally supplied to low CP diet. They stated that the requirements for glycine seems to be higher in low CP diets than in high CP diets and the estimated requirements for glycine + serine is 2.44% of diet.

Namroud et al. (2008) stated that limitation on replacing intact protein with crystalline amino acids is partly due to increase of blood ammonia. These authors also suggested the possibility that the pattern or concentration of plasma-free amino acids may be detected in the hypothalamus and its likely regulatory effects on appetite.

Reasons for the reduction in performance of birds fed low protein diets have not been clearly explained yet. This may be due to differences in basal diets, assay length, age of chicks or other factors (Waldroup et al., 2005). More research is needed to be done on the requirements for individual amino acids in low protein diet.

## 2. Effect of Low Protein Diet on Carcass Composition

Although compensatory gain can restore performances to those obtained under optimal circumstances, possible carcass quality alterations such as increased body fat and reduced protein content may occur (Moran, 1994; Si et al., 2001). Lipstein et al. (1975) reported similar results with low protein diet fed to birds 5 to 9 wk of age. Later research also found increased carcass fat in the birds fed on low protein diet supplemented with synthetic amino acids although they obtained same performances as birds fed on commercial control diet (Summers and Leeson, 1985; Parr and Summers, 1991; Deschepper and de Groote, 1995). These authors concluded that birds increase their feed intake compensatorily in an attempt to obtain the limiting amino acid required for optimal growth rate, and this results in increased carcass fat deposition.

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). Uzu (1983) stated that addition of non-essential synthetic amino acids such as glutamic acid reduces the fat deposition of broilers fed on low protein diet with amino acids supplementation. Research by Fancher and Jensen (1989) also reported that the addition of glutamic acid to a low protein diet significantly reduced



abdominal fat deposition in male broilers.

However, it is still questionable whether dietary energy intake plays a role in regulating lipogenesis in birds fed at a suboptimal regimen of protein nutrition (Kamran et al., 2008). Collin et al. (2003) stated that the macronutrients content, especially the dietary protein content regulates protein and fat deposition in chickens. Generally, diets with high energy : protein ratio promote energy retention as fat. Kamran et al. (2008) reported that low CP diet with a constant ME:CP ratio has adversely affected growth performance of broilers even when standard levels of critical AA were maintained but carcass parameters were unaltered.

## AMINO ACID BALANCE AND WELFARE PROBLEMS

With each moult the protein in the old feathers is lost and new feather growth requires a supply of amino acids. Each successive juvenile feather coat is heavier than the previous one and will therefore require an increased input of amino acids. Dietary amino acids play a critical role in feather development since 89~97% of the feather dry matter is protein (Fisher et al., 1981). Cystine and methionine, the sulphur-containing amino acids, are the major amino acids involved in the synthesis of feather keratin (Wheeler and Latshaw, 1981). Cystine and methionine are required for general maintenance and growth in addition to their role in feather synthesis. The relative proportion of the sulphur containing amino acids is much greater in the integument than in muscle tissue and marginal dietary deficiencies of these amino acids will often be initially manifested as abnormal feathering (Deschutter and Leeson, 1986). Cystine and methionine are frequently the first limiting amino acids and accurate determinations of the sulphur containing amino acid requirements during various stages of growth, therefore, are necessary to ensure optimum body and feather growth (Leeson and Walsh, 2004). The sulphur amino acids are the most important for feather growth, although feathers showing signs of amino acid deficiency have been noted in chicks fed rations deficient in arginine, valine, leucine, isoleucine, phenylalanine and tyrosine (Anderson and Warnick, 1967). Although amino acid deficiency can be indicated by

abnormal feathering, antagonisms or imbalances of various amino acids can often result in similar abnormalities of plumage (Deschutter and Leeson, 1986).

The excretion of excess nitrogen caused by amino acid imbalance also may influence breast blister formation because of the high uric acid excretion rates produced by poor quality protein. Birds on wet litter have a high incidence of breast skin lesions and breast blisters, with the breast area caked with litter and feces (Greene et al., 1985; Gonder, 1987).

In practice, the use of synthetic amino acids and enzyme supplementation are suggested to reduce nitrogen emission (Nahm, 2007). Namroud et al. (2008) reported that reduction of CP to 19% decreased nitrogen, ammonia, and pH of excreta that may improve upon litter and air quality. Sequential feeding is another strategy to improve nitrogen utilization and welfare condition of chickens. Sirri and Meluzzi (2012) reported positive repercussion of sequential feeding on environmental and animal welfare by reduced nitrogen emission and decreased the incidence of foot pad lesions in broilers.

In poultry manure, nitrogen in uric acid can be quickly converted to ammonia by hydrolysis, mineralization, and volatilization (Oenema et al., 2001). Improper management of poultry house can lead to increase of ammonia concentration and this may influence on not only performance but also poultry and worker health. Miles et al. (2002) reported that broilers showed lower body weight gains when exposed to ammonia level of 25 ppm or greater. Continuous exposure to 20 ppm has resulted in various disorders including increased Newcastle disease susceptibility and respiratory tract damage (Anderson et al., 1964). Maintaining low ammonia concentrations is a difficult challenge for commercial poultry production systems without the potential of putting excessive financial burden on poultry producers. Poultry health and welfare problems related to ammonia emission are reviewed in detail by some researchers (e.g. Carlile, 1984; Kristensen and Wathes, 2000; Al Homidan et al., 2003).

## CONCLUSION

Dietary strategies aimed at nutrient reduction, particularly dietary protein content, can result in a reduction of ammonia

formation (Ritz et al., 2004). Feeding reduced protein diets can reduce nitrogen excretion and subsequent ammonia volatilization (Sutton et al., 2001). Other dietary manipulation strategies that can optimize nitrogen digestion and reduce nitrogen emission include feed formulation based on amino acid requirements rather than CP, optimizing the dietary amino acid profile with requirements, phase feeding for current growth and production, selection of feed ingredients with low nutrient variability to reduce protein margins of safety, and use of feed enzymes and additives.

It may also be possible to improve efficiency of nitrogen utilization by genetic selection. However, the extent to which birds vary in nitrogen utilization efficiency and the extent to which this variation is heritable are not well known. Energy and amino acid requirement studies should be conducted to accomplish genetic improvement in nitrogen utilization efficiency along with growth rate in commercial broilers.

Dawson (2006) has reported on the future benefit of nutrigenomics, studies which will enable a better understanding of the interaction between genes and nutrition at the molecular level, to evaluate the effects of nutrition on animal health and production. There is a growing awareness of the need to balance the rate of genetic improvement with improvement in feed availability, health care and general management (Sonaiya and Swan, 2004). Therefore, proper integration of nutrition, genetics, management, and genomic tools should be employed for comprehensive research on protein metabolism and utilization in modern poultry production system.

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