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A Review on Recent Findings on Amino Acids Requirements in Poultry Studies

Review Article

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ABSTRACT

Amino acids (AA) are important in poultry nutrition not only for protein deposition, but also for other metabolic functions; such as improving immune and gut functions. Although, some table values are available on amino acids requirement, AA requirements may vary due to changes in dietary composition and breeding selection. Thus amino acids requirements have remained an ongoing research topic. Formulating diets with optimal amino acids will not only reduce the feed costs and improve the production's efficiency, but also have environmental benefits. Recent studies reported the requirements of some essential amino acids and their optimal ratios. This paper is aimed at reviewing the studies presented at XIIIth European Poultry Conference which provided the most recent information on amino acids requirement in poultry. These studies showed that the commonly used table values might underestimate amino acids requirements in poultry.

KEY WORDS amino acids, protein deposition, improving immune, gut functions.

INTRODUCTION

Amino acid requirement is an ongoing topic with overwhelming importance for poultry. It is well known that imbalance in AA profile will result in reduced growth rate and feed utilization efficiency. A deficiency in dietary amino acids impairs immune function. Protein and amino acids are some of the most expensive nutrients in feeds formulation; thus selecting the correct level of amino acids needed in feed formulation has economical impact. In addition, diets formulated with balanced AA profile will reduce nitrogen losses in animal waste and lead to an environmental benefit.

The amino acids (AA) requirements are influenced by many factors including dietary, environmental, genetics and productive purposes. Although, general information on ideal AA ratios is available in feed tables, measurements of AA requirement are ongoing research area due to, among others, changes in feed formulations (e.g. removal of antibiotics growth promoter from the feed) and the breeding selections.

Threonine requirement

Threonine is one of the limiting essential amino acids in poultry; its requirement may increase under challenging conditions and this is confirmed by researchers in Netherlands. Star *et al*. (2010) investigated threonine requirement of broilers during sub-clinical intestinal clostridium infection. Coccidioses and *Clostridium perfringens* infections can cause intestinal damage and increase mucus production. The mucus layer is important for the non-immune gut barrier. Threonine is important for the intergrity of the mucus since this essential amino acid is the main component of mucine. Thus under infection conditions, threonine requirement may be increased. In this study, threonine re-

quirements were assessed in response to Thr:Lys ratio (0.65 vs. 0.70 SID) in two situations: challenged or non-challenged with *Eimeria maxima* and *Clostridium perfringens* in a sub-clinical Clostridium infection model.

In this study, 1152 one day old Ross 308 male broilers were used. Each of the 4 treatments consisted of 12 replicates. Birds in experimental group were inoculated with a pathogenic *E.maxima* at day 9 to cause necrotic enteritis and inoculated with a *C.perfringens* strain at day 14 of age. Birds in control group were inoculated with saline and liver broth at day 9 and 14. No lesions were observed in the uninfected treatments. Incidence of birds with lesions was on average 67 and 68% in the infected treatments with a thr:lys ratio of 0.65 and 0.70, respectively.

Table 1 Effect of Thr/Lys ratio on growth performance of uninfected and infected broilers* (Star *et al*. 2010)

	Uninfected Thr:Lys 0.65	Uninfected Thr:Lys 0.70	Infected Thr:Lys 0.65	Infected Thr:Lys 0.70
Day 8-20				
BWG, g	623[a]	602[a]	431[c]	465[b]
Feed Intake, g	848[a]	825[a]	672[c]	700[b]
FCR,g/g	1.37	1.37	1.561	1.521
Day 0-37				
BWG, g	2309[a]	2303[a]	1963[c]	2107[b]
Feed Intake, g	3858	3837	3263	3422
FCR,g/g	1.67	1.645	1.665	1.63

*The means within the same row that have at least one common letter, do not have significant difference (P>0.05).

The infection significantly reduced weight gain. However, a higher Thr:Lys ratio (0.70) in the infected treatment improved body weight gain (BWG) and feed intake compared to a Thr:Lys ratio of 0.65 (Table 1).

This study reflected that threonine requirement increased during *C.perfringens* infection.

The recommendations from NRC tables may underestimate the threonine requirement for young broilers, as demonstrated by Nassiri Moghaddam *et al*. (2010). Effect of threonine levels on performance of Ross broiler chicks during 0 to 10 days of age was evaluated. Diets were based on corn and soybean meal (SBM), formulated to provide minimum 100% NRC amino acids recommendations . Four dietary treatments contain 0.8, 0.87, 0.94 and 1.01% total threonine, respectively. The optimal threonine inclusion level was observed at 0.87%, based on feed intake, weight gain and feed efficiency data. The authors suggested that NRC recommendation for threonine is inadequate.

Brown-egg laying hens responded positively to increasing Thr/Lys ratio. Lelis *et al*. (2010) used a total of 180 Dekalb brown layers (25 to 37 wk old) in a trial to determine optimal Thr/Lys ratio. Three dietary Thr/Lys ratios of 66, 72 and 78%, respectively were tested during three periods of 28 days, with 10 replicates of 6 birds per treatment. A linear response was observed with increasing Thr/Lys ratio for egg production, egg mass and feed conversion per egg mass. The authors recommended that the dig Thr/Lys ratio for brown-egg laying hens is 78%. Although, this was the highest level tested in this study, it cannot be excluded that a higher ratio may further improve the performance; therefore, further research is needed to determine the optimal Thr/Lys ratio in brown-egg laying hens.

Lysine requirement

In several studies, the digestive lysine requirement in broilers was determined. These studies demonstrated that digestive lysine requirement differs for different strains, ages of animals and techniques used.

Abdallah *et al*. (2010) carried out two experiments to determine the optimal digestible lysine and metabolizable energy (ME) requirements in corn and SBM based diets formulated according to ideal amino acids profile concept. Different dig Lys and metabolizable energy levels were tested with different strains; details are given in Table 2.

The results from first experiment with Ross 308 and Hubbard broilers showed that increasing dietary dig Lys levels from 1.1 to 1.2 during 0-14 days, from 1.0 to 1.05 during 15 to 24 days, significantly improved feed intake and weight gain, while no significant effect on feed conversion was observed. Increasing ME level had also positive impact on growth performance of broilers. Ross 308 showed better weight gain and feed conversion compared to Hubbard.

In the second experiment with Cobb 500 broilers, it was observed that the highest dig lysine level of 1.3% resulted in the best weight gain (P<0.05). It seems that there is a linear increase in weight gain; however, the linear response was not reported by the authors. Increasing dig Lys level from 1 to 1.05% during 15-24 days, from 0.85 to 0.9% during 25 to 38 days significantly improved weight gain. Feed conversion was significantly improved by increasing dig lysine level from 0.85 to 0.9% during 25 to 38 days. Increasing ME levels had also significant positive impact on growth rate. Increasing dig Lys levels significantly increased dressing weight (Table 2). The authors concluded that increasing dig lysine level in broiler diets is beneficial since it results in an increased growth rate and thus reduces the days required to reach market weight.

Sakomura *et al*. (2010) estimated lysine requirements of 8 to 22 days old Cobb 500 broilers with two different feed formulation techniques; i.e. diets formulated by supplementation or dilution techniques. For supplementation

Table 2 Performance of broiler chicks fed diets containing different digestible lysine and ME levels* (Abdallah *et al*. 2010)

a, With two strains, Ross 308 and Hubbard, exp. 1

	Dig Lys, %			ME, kcal/kg			Strain	
1-14 d	1.1	1.2	1.3	2950	3000	3050	Ross308	Hubbard
WG, g	361^{b}	377^{a}	375^{a}	340^{b}	380^{a}	383^{a}	379^{a}	356^{b}
FI, g	448^{b}	479^{a}	465^{a}	425^{b}	479^{a}	471^{a}	459	459
FC, g/g	1.24	1.27	1.24	1.25	1.26	1.23	1.21^{a}	1.29^{b}
	Dig Lys, %			ME, kcal/kg			Strain	
15-24 d	1.0	1.05	1.1	3000	3050	3100	Ross308	Hubbard
WG, g	515^{b}	544^{a}	550^{a}	523^{b}	531^{b}	555^{a}	560^{a}	512^{b}
FI, g	917^{c}	995^{b}	1023^{a}	962	972	994	986^{a}	963^{b}
FC, g/g	1.78	1.83	1.86	1.84	1.83	1.79	1.76^{a}	1.88^{b}

b, With Cobb 500 broiler chicks, exp. 2

	Dig Lys, %			ME, kcal/kg	
1-14 d	1.1	1.2	1.3	2950	3050
WG, g	310^{b}	317^{b}	335^{a}	313^{b}	329^{a}
FI, g	332	335	342	334	339
FC, g/g	1.07	1.0	1.08	1.06	1.03
	Dig Lys, %			ME, kcal/kg	
15-24 d	1.0	1.05	1.1	3000	3100
WG, g	656^{b}	683^{a}	678^{a}	652^{b}	692^{a}
FI, g	1060^{b}	1161^{ab}	1125^{b}	1112	1117
FC, g/g	1.62	1.71	1.66	1.71	1.62
	Dig Lys, %			ME, kcal/kg	
25-38 d	0.85	0.90	0.95	3150	3250
WG, g	941^{b}	1073^{a}	1075^{a}	1019	1040
FI, g	2031	2113	2164	2070	2135
FC, g/g	2.17^{b}	1.97^{a}	2.02^{a}	2.03	2.07
	Dig Lys, %			ME, kcal/kg	
1-38 d	1.1/0.85	1.2/0.90	1.3/0.95	2950/3150	3050/3250
WG, g	1907^{b}	2073^{a}	2088^{a}	1984^{b}	2061^{a}
FI, g	3423	3609	3631	3516	3592
FC, g/g	1.8	1.73	1.76	1.77	1.75
Dressing percentage	69.34^{b}	69.77^{ab}	71.16^{a}	69.82	70.36

*The means within the same row that have at least one common letter, do not have significant difference (P>0.05).

technique, a corn and SBM based diet were supplemented with L-lysine HCl to obtain digestible lysine levels of 0.84, 0.932, 1.024, 1.116 and 1.208% in the tests diets. For dilution technique, basal diet was formulated with high level of protein and lysine (108% of dig Lys requirement) and contained other amino acids enough to exceed the ideal protein ratios by at least three percentage points. The basal diet was diluted with a protein free isoenergetic diet to get the same levels of dig lysine as the diets formulated with supplementation technique.

requirements are in general higher than the recommendations from NRC table.

Silva *et al*. (2010) determined the methionine+cystine (M+C) requirements for laying type pullets (Dekalb White pullets) in different phases (14 to 42 days; phase I, 56 to 84 days; phase II, 98 to 126 days; phase III). For each phase, 8 different dietary treatments (8 dig M+C levels) were tested, with 6 replications per treatment. M+C requirement was determined by the first intercept of the broken-line on the quadratic curve.

Table 3 Effect of dietary digestible Met+Cys levels on body weight gain (BWG, g/bird/d) and feed conversion (FC, g/g) in pullets (Silva *et al*. 2010)

	Digestible M+C, %*							
Phase I**	0.302	0.403	0.504	0.605	0.706	0.907	1.008	0.443
BWG	2.54	5.02	6.92	9.63	10.84	11.61	11.49	6.04
FC	6.89	4.52	3.6	2.93	2.67	2.54	2.56	4.06
Phase II	0.253	0.338	0.422	0.506	0.591	0.760	0.844	0.378
BWG	5.82	7.8	10.3	10.78	11.21	12.23	12.35	9.804
FC	7.8	6.12	4.71	4.33	4.06	3.56	3.69	5.05
Phase II	0.197	0.276	0.355	0.434	0.513	0.630	0.790	0.306
BWG	4.21	7.46	8.49	10.39	10.85	11.12	11.24	7.85
FC	12.93	8.13	6.78	5.60	5.04	4.81	4.69	7.48

* Dietary dig M+C significantly affected BWG and FC in all phases (P<0.01).
** Phase I: 14 to 42 days; phase II: 56 to 84 days; phase III: 98 to 126 days.

Feed intake, weight gain, feed conversion, protein, fat and lysine accretion and efficiency of lysine utilization were evaluated. In general, similar response was observed with increasing dig lysine in the diets, regardless of feed formulation techniques used. Only the response in feed conversion was significantly different between the two feed formulation techniques. The optimal dig lysine level was determined by quadratic response as 1.171%, based on weight gain data. While the optimal lysine levels were 1.140 and 1.187% respectively for supplementation and dilution techniques, when using feed conversion as a criterion. The authors concluded that dilution technique improved the response in feed conversion and reduced fat accretion compared to supplementation technique, being more appropriate for evaluating response to dig Lys. The estimated dig Lys in broilers diet from 8 to 22 days was 1.187%, based on feed conversion data. This level correspondents to the consumption of 11.16 g or 797 mg/d of lysine.

Methionine/cystine requirement

A study showed that methionine+cystine (M+C) requirements for laying type pullets differ at different age, and the

The estimated optimal dig M+C levels for pullets based on body weight gain and feed conversion were respectively, 0.835 and 0.644% for phase I, 0.625 and 0.56% for phase II and 0.524 and 0.494% for phase III (Table 3). These levels are higher than those recommended by NRC (1994) and the Brazilian Tables for poultry and pigs cited by Silva *et al*. (2010).

Isoleucine requirement

The three first limiting amino acids in broilers are well known as methionine, lysine and threonine, the 4th limiting amino acid is still under discussion. Some studies showed that isoleucine could be the 4th limiting amino acid for corn SBM based diet or wheat SBM based diet.

Lowering dietary protein levels are beneficial to environment and animal welfares; however, the 4th limiting amino acid becomes important in feed formulated with low dietary protein levels. A recent study reported that optimal Ile/Lys ratio is related to assessment criteria (e.g. weight gain, feed conversion ratio). Helmbrecht *et al*. (2010) investigated the optimal digestible Ile/Lys ratio in broilers at different ages. A corn, SBM and corn gluten meal based diet were used.

Table 4 Apparent animal acid digestibility in 10 weeks old turkeys as compared to roosters and young chickens* (Lessire *et al*. 2010)

	Rooster	Chickens (3 week)	Turkeys (10 week)
N	78[b]	79.4[a]	73.6[c]
Lys	85.3[a]	85.9[a]	83.8[b]
Met	89.8[b]	90.7[a]	87.7[c]
SAA	86.3[a]	85.6[a]	81.8[b]
Trp	84.6[a]	81.4[b]	78.0[c]
Thr	80.9[a]	79.2[b]	73.9[c]
Ile	85.1[a]	84.3[a]	80.8[b]
Leu	87.1[a]	85.9[a]	82.4[b]
Val	83.7[a]	82.8[a]	78.1[b]
Arg	86.2[a]	86.6[a]	85.0[b]
His	86.1[a]	86.2[a]	84.3[b]
Mean AA	86.0[a]	85.2[a]	82.0[b]

*The means within the same row that have at least one common letter, do not have significant difference (P>0.05).

There was a quadratic increase in weight gain (Figure 1) and quadratic decrease in feed conversion with increasing dig Ile/Lys ratios. The optimal Ile/Lys ratios were related to regression model used as well as assessment criteria. When using quadratic regression model, the authors estimated that the optimal Ile/Lys ratios were 68% and 72% for starter and finisher respectively, based on weight gain data. While the optimal ratios were 68 and 75% for starter and finisher respectively, based on feed conversion data.

The AWT recommendations for Ile/Lys ratios are 67% and 67-71% for starter and finisher, respectively.

Tryptophan requirement

Some recent studies showed that both layers and broilers may have higher Trp/Lys requirement than the recommendations from literature or Feed Table.

In brown-egg laying hens, a total of 180 Dekalb brown layers (25 to 37 wk old) were used in a trial to determine optimal Trp/Lys ratio (Lelis *et al*. 2010).

Three dietary treatments (three Trp/Lys ratios, 17, 20 and 23%) were tested during three periods of 28 days, with 10 replicates of 6 birds per treatment. A linear re sponse was observed with increasing Trp/Lys ratio for egg production, egg mass and feed conversion per egg mass. The authors concluded that the dig Trp/Lys ratio recommended for brown-egg laying hens was 23%. This is in agreement with NRC recommendations. However, as this was the highest level tested in this study, it is possible that a higher ratio may further improve the performance. Further research is needed to determine the optimal Trp/Lys ratio in brown-egg laying hens.

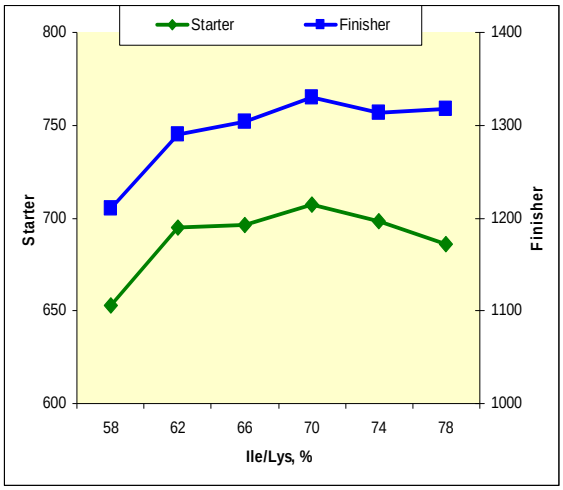

Figure 1 Effect of dig Ile/Lys ratios on weight gain of broilers. Starter: 7 to 21 days of age; Finisher: 30 to 43 days of age

In broilers, two experiments were conducted to determine optimal dig Trp/Lys ratios for male Cobb 500 broilers during starter (7-21 days) and finisher (28 to 40 days) periods (Campos *et al*. 2010). Five dig Trp/Lys ratios were tested, e.g. 15, 16, 17, 18 and 19%, during starter period and 14, 16, 18, 20 and 22% during the finisher period. Diets were formulated to meet minimal nutritional requirements except

for lysine, dig lysine levels were 1.08 and 0.98% respectively in starter and finisher diets. During the starter period, it was observed that increasing Trp/Lys ratios had quadratic impact on feed conversion and breast fillet yield. During the finisher period, feed intake, weight gain and feed conversion were influenced quadratically with increasing dietary dig Trp/Lys ratios (Figure 2). The authors recommended that Trp/Lys ratios for starter and finisher phases were 17% and 18%, respectively, i.e. 16 and 17% respectively for starter and finisher phases.

Amino acids digestibility in turkeys

Amino acids digestibility values determined in turkeys are scarce and nutritionists use tabulated values traditionally derived from roosters. However, the AA digestibility in turkey may differ significantly from that of roosters. Lessire *et al*. (2010) determined amino acids digestibility in turkey (BUT9) in comparison to roosters and young chickens (ROSS PM3). Diets were for mulated with different protein sources (soybean meal, soy protein concentrate and soy alternative; e.g. wheat DDGs, rapeseed meal, pea and potato protein) and with different protein to energy ratios (98 g/1000 kcal and 73 g/1000 kcal).

It was observed that there were large differences in amino acids digestibility due to animal species and dietary composition. Amino acid digestibility in soy protein concentrate was by far higher than that of soybean meal and soy alternatives, without interaction of animal species. Lysine digestibility in soy protein concentrate was 87.6%; significantly higher than soybean meal (83.6%) and soy alternatives (83.7%). Protein to energy ratio did not significantly influence AA digestibility. Mean apparent amino acids digestibility values obtained on 10 weeks old turkeys were significantly lower than that of roosters and chicken, while no differences were observed on AA dig between rooster and chicken (Table 4). The authors concluded that AA dig data determined with rooster or young chickens should not be used in formulating diets for 10 weeks old turkeys.

However, Dublecz *et al*. (2010) reported that there were no significant differences in apparent digestibility of lysine and methionine between 6 wks old Ross-308 broiler chicks and 8 week old BIG BUT turkeys (Table 5). In this study, diet was formulated based on corn, wheat and soybean meal, containing 22% crude protein and 12 MJ/kg ME.

The inconsistent results reported in different studies may be related to differences in diet compositions and methods used in AA digestibility determination. Further studies are needed.

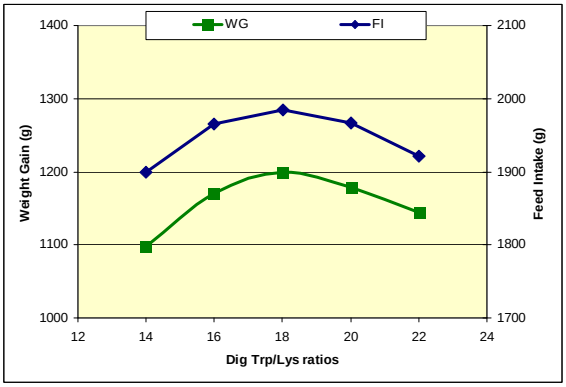

Figure 2 Effect of dig Trp/Lys ratio on feed intake and weight gain of broilers finisher from 28 to 40 days of age

In ovo AA supplementation

Early access to amino acids may be beneficial to newly hatched broilers by supplementing it to the embryo. Pandey *et al*. (2010) carried out a study to determine the effect of supplementing amino acids to the developing embryo on gene expression, in order to maximize post-hatch growth and development. Nine hundred fertile eggs were distributed into 9 groups; i. eone control group and 8 test groups with injection of 8 amino acids (Lys, M+C, Arg, Thr, Ile, Leu, Gly and Ser), respectively. Administration of AA (10% of first 7 days requirement) was done on 14th day of incubation into the yolk sac/amnion of the embryo. The growth related genes, e.g. the relative hepatic expression levels of cGH and IGF-1 genes, was derived through real time PCR quantification of cDNA samples of in ovo injected and control eggs at different interval till 14th day post hatching.

Table 5 Apparent lysine and methionine digestibility (%) in different poultry species*

	Guinea-fowl	Quail	Pheasant	Pigeon[1]	Broiler	Turkey	Duck[2]	Goose[3]
Age, weeks	10	10	55	24	6	8	10	8
Lys	89.2^{c}	88.4^{bc}	88.2^{bc}	84.4^{ab}	81.1^{a}	85.9^{bc}	85.7^{bc}	87.1^{bc}
Met	91.0^{bc}	94.5^{c}	93.8^{c}	90.3^{abc}	87.7^{ab}	86.6^{a}	88.6^{ab}	93.5^{c}

1, Pannon Texan pigeons; 2, Peking Duck; 3, Landesian geese.
*The means within the same row that have at least one common letter, do not have significant difference (P>0.05).

Based on the observations of this study, the authors concluded that the amino acids which enhanced the mRNA expression of IGF-1 and cGH are Lys, Thr, Arg, Met and Gly during pre and postnatal development of broilers.

CONCLUSION

The recent studies presented at EPC have demonstrated that AA requirement is related to genetics (e.g. strains), age of animals, feed formulations, determination methods and assessment criteria. Most of the studies showed that current feed tables may underestimate the AA requirements. Further research is needed to determine the AA requirements for different used poultry species and strains, considering modern feed formulation.

Take home messages from the studies presented in EPC conference are:

Threonine is important for the integrity of the mucus. Under infection conditions, threonine requirement increased.

The optimal threonine level was observed at 0.87% for 0-10 days Ross broilers, suggesting that NRC recommendation for threonine is inadequate.

The dig Thr/Lys ratio recommended for brown-egg laying hens is 78%.

Increasing dig lysine level in broiler diets is beneficial as these results in an increased growth rate and thus reduces the days required to reach market weight.

The optimal dig Lys level in the diet for broilers differs for different strains and it may be higher than the recommendation from table values.

Methionine+cystine (M+C) requirements for laying type pullets may be higher than the recommendation from NRC table.

The optimal Ile/Lys ratios were reported as 68% and 72-75% for broiler starter and finisher, respectively.

Broilers may have higher Trp/Lys requirement than the recommendations from Feed Tables.

Lys, Thr, Arg, Met and Gly supplementation to the developing embryo may enhance the mRNA expression of IGF-1 and cGH during pre and postnatal development of broilers.

ACKNOWLEDGEMENT

This paper is aimed to review some most recent studies on AA requirements and optimal AA ratios from the studies presented at XIIIth European Poultry Conference (August 2010).

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