Partition of Amino Acids Requirement for Maintenance and Growth of Broilers II. Methionine

J. H. Kim, W. T. Cho, C. J. Yang, I. S. Shin[1] and In K. Han[2]
Department of Animal Science and Technology, College of Agriculture and Life sciences,
Seoul National University, Suweon 441-744, Korea

ABSTRACT : Purified diets containing five levels of methionine with 0.4% cystine were fed to growing chicks (8 days old male Arbor Acre strain) to evaluate methionine requirements for growth and maintenance. A model was developed to separate methionine requirement for maintenance from requirement for growth. From this model the daily methionine requirement for growth was 4.22 mg/g gain, and the daily methionine requirement for maintenance was 0.034 times metabolic body size ($W^{0.75}$). Based on nitrogen gain response, the methionine requirement for growth was 0.162 mg/mg N gain, and the daily maintenance requirement was 0.037 times metabolic body size. The plateau of plasma methionine concentration reached at 117.16 mg intake per day. The total methionine requirement determined based on weight gain response was 138.29 mg/day or 0.33% of the diet and the one determined based on nitrogen gain response was 141.7 mg/day of 0.34% of the diet, respectively. As a percentage of protein, methionine was calculated to be 2.6%; the reported methionine content of carcass CP was 1.76%.
(**Kew Words** : Methionine, Requirement, Growth, Maintenance, Broiler Chicks)

INTRODUCTION

There is a great deal of controversy concerning sulfur amino acids requirement for growing chicks. Willis and Baker (1980) reported that the total sulfur amino acids requirement was 0.6% of the diet, using 19.78% of free amino acid mixture in their purified diet. Graber et al. (1970) found the similar results using purified diet with 18.58% amino acid mixture were obtained; 0.63%, 0.65%, 0.70% of the diet for the 2, 5, and 8 week old chicks, respectively. They also indicated that sulfur amino acids requirements remained relatively constant through the growth stages. However, Baker et al. (1982) depicted 0.51% of the diet as the sulfur amino acids requirement, which agreed with the report by Klain et al. (1960). For methionine per se, the variation among estimated requirements is great ranging from 0.18% to 0.57%, but when consider the methionine requirement, it should be carefully considered the cystine status in the basal diets. Klain et al. (1960) reported that the methionine requirement with 0.4% cystine is 0.18% of the diet. Hurwitz et al. (1978) reported that the methionine requirement was 0.39% and 0.34% of the diet for 7-14 day and 14-21 day old chicks, respectively, using their computer model. However, Almquist (1947) and Waldroup et al. (1979) reported much higher value (0.50% and 0.57%, respectively) as the methionine requirements.

Little information about the maintenance needs of methionine is available. However, considering the direct relationship between the amino acid composition of keratoid tissue and the amino acid needs for maintenance of the adult chicken (Leveille et al., 1960), the maintenance needs for methionine or the combination of methionine and cystine should be considered. Owens et al. (1985) reported that the maintenance requirement for methionine was 332 mg/kg weight/day. They determined the maintenance needs for amino acids where the animal showed zero response to the limiting amino acids.

The requirement of methionine for growth and maintenance would be expected to vary with various factors that influence maximum growth and feed intake. Those factors include dietary nutrients, age, sex, performance potentials, physiological status, and environmental conditions.

The objective of this study was to partition the methionine requirement into two portions; one for growth and another for maintenance. Weight gain and nitrogen retention were evaluated with 0.4% added cystine of the purified diet. Estimated methionine requirements were compared with the previous data.

[1] Present address: American Soybean Association, Seoul, Korea.
[2] Address reprint requests to In K. Han.
Received October 29, 1996; Accepted February 3, 1997

MATERIALS AND METHODS

A total of 125 Arbor Acres male chicks of a broiler strain were used in this experiment. Five groups of 5 chicks each housed in battery cages made of wire in a room with constant light and air ventilation, were fed each of five test diets. These diets contained either 25, 50, 75, 100, 125% of NRC (1994) estimated methionine requirement for growing chicks with 100% requirement for all other amino acids including 0.4% of added cystine. The composition of basal diet is shown in table 1.

Table 1. Composition of basal diet for broiler

Ingredient	% of diet	Amino acid mixture[c]	% of diet
Corn starch	59.148	L-Arginine	1.250
Amino acid mixture	23.397	L-Histidine · HCl · H_2O	0.473
Mineral mixture[a]	5.250	L-Isoleucine	0.800
Refined soy oil	5.000	L-Leucine	1.200
Cellulose	5.000	L-Lysine · HCl	1.374
$NaHCO_3$	1.500	L-Methionine	Variable
Vitamin mixture[b]	0.500	L-Cystine	0.400
Choline chloride	0.200	L-Phenylalanine	0.720
Tocopheryl acetate	0.002	L-Tyrosine	0.620
BHT	0.003	L-Threonine	0.800
		L-Tryptophan	0.200
Total	100.000	L-Valine	0.900
		L-Proline	0.600
		L-Glycine	0.750
		L-Glutamic acid	Variable
		Total	23.397

[a] Mineral mixture provided per kilogram of diet: $CaCO_3$, 3 g; $Ca_3(PO_4)_2$, 28 g; KH_2PO_4, 9 g; NaCl, 8.8 g; $MgSO_4 \cdot H_2O$, 2.2 g; $MnSO_4 \cdot H_2O$, 0.65 g; $FeSO_4$, 0.5 g; $ZnSO_4$, 0.15 g; $CuSO_4 \cdot H_2O$, 0.142 g; H_3BO_3, 9 mg; $Na_2MoO_4 \cdot H_2O$, 9 mg; KI, 40.6 mg; $CoSO_4 \cdot H_2O$, 0.6 mg; $Na_2(SeO_3)_2$, 0.215 mg.

[b] Vitamin mixture provided per kilogram of diet: thiamin, 20 mg; niacin, 50 mg; riboflavin, 16 mg; Ca-pantothenate, 30 mg; Vit B_{12}, 0.04 mg; pyridoxine, 6 mg; biotin, 0.9 mg; folic acid, 4 mg; inositol, 20 mg; menadione, 2 mg; Vit. C, 50 mg; Vit. A, 5,200 IU; Vit. D, 600 IU.

[c] Patterned after NRC (1994).

DL-methionine was substituted for L-glutamic acid on an equal weight basis so that all diets had the same amounts of amino acid mixture. The chicks were fed commercial diet for 1 week before the study, from the eighth day posthatching the chicks were fed experimental diets. The chicks had *ad libitum* access to fresh water and test diets. Initial body weight was 115.3 g. Body weights were measured each week and feed intakes were measured each day on replication basis during the experiment to obtain average weight gain and average feed intake. Since the experimental diets were purified, the feeds were stored at −4℃ throughout the experimental period and changed daily to avoid any deterioration. On the final day of the experiment, blood plasma samples were collected from the cervical arteries of five chicks selected randomly from each treatment. Samples were centrifuged (Hanil, Korea) in the heparinized tube. Blood plasma was obtained and deproteinized with sulfosalycilic acid (Terrlink et al., 1994) and then analyzed for amino acid concentration (LKB 4150, Pharmacia Instrument Co., England). In addition, five chicks from each treatment were sacrificed by cervical dislocation. After removing the intestinal contents, the carcasses including internal organs and feather were stored at −20℃ until body composition (total body water and N content) was determined. Carcass samples were freeze dried (Ilsin Engineering, Korea), ground and analyzed by AOAC (1990) methods.

The mathematical equation for this model to partition the methionine requirement into a maintenance fraction based on metabolic body size and into a growth fraction based on weight gain or nitrogen gain was developed as described by Shin et al. (1991).

$I = 1/a\ (R\text{-}bW^{0.75})$

where I = amino acid intake,

R = response,

$-b/a'W^{0.75}$ = maintenance requirement per metabolic body size

1/a = growth requirement per g gain or mg nitrogen gain.

All the parameters were determined at the point of residual sum of square minimized by the Nonlinear Least Squares method (SAS, 1985). The equation covering the plateau portion was not included in the equation estimating the methionine requirement because genetic potential, energy or some nutrients other than amino acids was limiting response in that region of the curve (figure 1). The relationship between plasma methionine concentration and daily methionine intake was described by a broken-line model and its breakpoint was determined by solving the two equations.

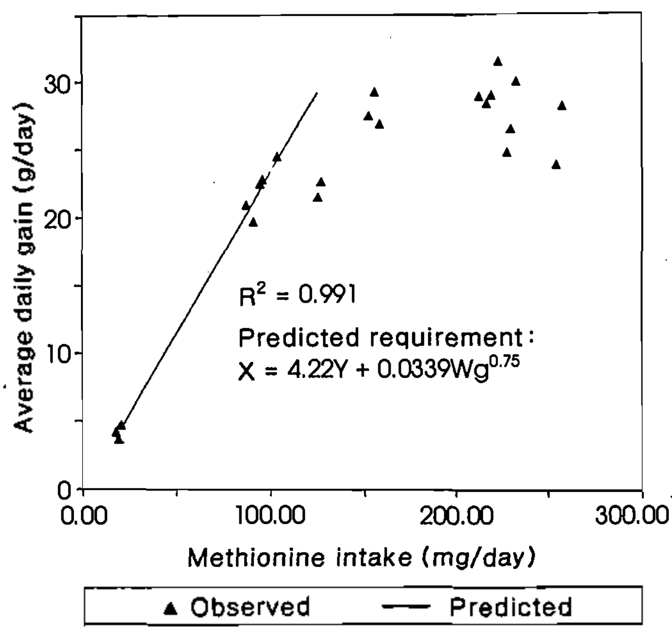

Figure 1. Weight gain responses to daily methionine intake.

RESULTS AND DISCUSSION

Responses of chicks fed diets containing five different levels of methionine are presented in table 2. Average daily feed intake, average daily gain, nitrogen gain, and gain to feed ratio increased remarkably up to 0.25% methionine, beyond 0.25% methionine the responses remained relatively constant.

Water content of chicks carcasses increased in the same manner as protein content increased up to 0.5% methionine as shown in table 3. But the protein gain to water gain ratio remained relatively constant, which means that body composition of chicks was not affected significantly by moderate deficiency or excess of methionine in the diet.

Daily methionine intakes were plotted against daily weight gain in figure 1 together with prediction line and equation. From the equation, the estimated growth requirement of methionine was 4.22 mg/g weight gain, while the estimated requirement for maintenance was 0.034 mg/unit of metabolic body size. Owens et al. (1985) reported that the methionine requirement for growth was 7.5 mg/g gain and methionine plus cystine was 4.4 mg/g gain. Present estimates were determined with 0.4% cystine in the basal diet, thus it could be compared with the value 4.4 mg/g gain and those values are in very close agreement (Owens et al., 1985). Assuming chicks weighing 376 g, with 41.43 g/day of feed intake and 32 g/day of weight gain as suggested by NRC (1994), from the equation in figure 1, the growth requirement of broilers for methionine was 135.40 mg/day and the maintenance requirement was 2.89 mg/day. The sum of these requirement is the total methionine requirements and converted to be 0.33% of the diet. At this rate of gain, about 2.1% of the total requirement would be utilized for maintenance. This value was compared with previous estimates of methionine requirements for growing chicks (Almquist, 1947; Dean and Scott, 1965; Klain et al., 1960; Hewitt and Lewis, 1972; Hurwitz et al., 1978; Waldroup et al., 1979; Robbins and Baker, 1980; Moran, 1981). The mean value of previous estimates is 0.42±0.12% of the diets as is summarized in table 5. Thus, these estimates are in the range of experimental error of the previously reported estimates of methionine requirement. Since the experimental diet used in this study contained crystalline amino acid mixture, the requirements estimated from equation 1 and 2 might be lower than previous estimates due to the differences in availability of amino acids in the experimental diets. Also, it should be considered that considerable amount of cystine may be released (when deficient) from endogenous glutathione (Baker, 1977).

Daily methionine intake was plotted against daily nitrogen gains in figure 2, including prediction line and equation. The estimated methionine need for growth was 0.162 mg/mg nitrogen gain and the estimated requirement for maintenance was 0.037 times metabolic body size. The total requirement is a sum of growth and maintenance requirements. A chick weighing 376 g and gaining 855 mg nitrogen/day would require 138.5 mg of methionine/day for growth and 3.2 mg of methionine/day for maintenance. Thus, the total methionine requirement would become 141.7 mg/day. About 2.24% of the total requirement was available for maintenance. If this daily amount is converted to percentage of the diet with 41.43 g of feed intake, the dietary percentage would be 0.34%. The maintenance needs for methionine of 1,000 g chick would be 6.03 mg/day, 6.62 mg/day based on weight gain and nitrogen gain responses, respectively. This value is extremely lower than the estimates (332 mg/kg body weight/day) for broilers by Owens et al. (1985), and the estimates (90 mg/kg body weight/day) for adult roosters by Leveille et al. (1960). But in this study, since the basal diet contained 0.4% cystine, the direct comparison is not meaningful. Moreover, Owens et al. (1985) reported that the maintenance need for methionine plus cystine was 141 mg/kg body weight/day, which was much lower than the maintenance need for methionine only. This would mean that a combination of methionine and cystine is used more efficiently than the use of methionine as a main sulfur amino acid source. In this study, the maintenance needs for cystine and methionine plus cystine were not determined. However, it is considered that substantial that substantial amount of cystine in the basal diet was used for the maintenance need for chicks.

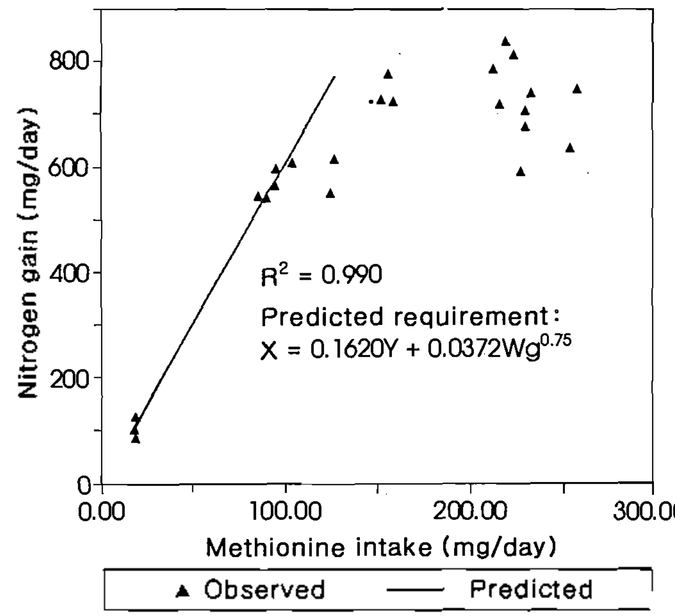

Figure 2. Nitrogen gain responses to daily methionine intake.

Table 2. Responses of broilers fed diets containing five different levels of methionine[1,4]

Dietary methionine	Mean[2] $W^{0.75}$	Average daily feed intake	Average daily gain	Methionine intake	Nitrogen[3] retention	Gain / feed
%	g	g/day	g/day	mg/day	%	
0.125	41.71 ± 0.33	15.07 ± 0.32	4.12 ± 0.24	18.83 ± 0.39	3.17 ± 0.22	0.27 ± 0.02
0.250	64.63 ± 1.15	37.89 ± 1.14	21.95 ± 0.81	94.73 ± 2.84	6.55 ± 0.08	0.58 ± 0.01
0.375	68.44 ± 1.96	38.41 ± 1.96	25.41 ± 1.51	144.05 ± 7.34	7.66 ± 0.16	0.66 ± 0.01
0.500	73.07 ± 0.79	44.17 ± 0.71	29.39 ± 0.55	220.84 ± 3.53	7.66 ± 0.26	0.67 ± 0.01
0.625	68.64 ± 0.82	38.40 ± 1.07	25.32 ± 0.76	240.01 ± 6.69	7.62 ± 0.29	0.67 ± 0.02

[1] Average initial weight was 116.1 g.
[2] $W^{0.75}$ is {(initial weight + final weight)/2}$^{0.75}$.
[3] Values are means ± S.E. of 5 chicks of each treatment.
[4] Values are means ± S.E. of 25 chicks of each treatment except nitrogen retention.

Table 3. Body composition of broilers fed five levels of methionine

Methionine	Live body weight	Daily body weight	Water gain	Protein gain	Protein gain /water gain
%	g	g	g	g	
0.125	53.53 ± 3.13[d]	17.15 ± 2.20[c]	36.72 ± 1.92[d]	8.92 ± 0.63[d]	0.242 ± 0.01
0.250	285.40 ± 10.45[c]	96.25 ± 5.47[b]	191.36 ± 6.13[c]	46.34 ± 1.10[c]	0.243 ± 0.00
0.375	330.29 ± 19.58[b]	102.77 ± 4.75[ab]	227.69 ± 14.57[b]	55.04 ± 3.33[b]	0.242 ± 0.00
0.500	382.10 ± 7.19[a]	114.64 ± 4.30[a]	266.57 ± 4.64[a]	63.16 ± 1.80[a]	0.239 ± 0.01
0.625	335.62 ± 9.88[b]	109.04 ± 4.92[ab]	227.83 ± 6.20[b]	54.59 ± 2.16[b]	0.240 ± 0.01

[abc] Means with different superscripts in the same column differ significantly ($p < 0.05$).

Table 4. Blood plasma amino acid concentration of broilers fed five graded levels of methionine (mg/100 ml)

AA	Dietary methionine (%)					SE[1]
	0.125	0.250	0.375	0.500	0.625	
THR[ℓ]	29.180[a]	27.168[a]	14.496[c]	18.283[ab]	14.637[b]	2.02
VAL[q]	6.070	7.284	8.566	8.423	6.783	0.39
MET	1.334[c]	1.322[c]	3.426[b]	12.287[a]	11.203[a]	1.03
ILE	3.140[b]	2.794[b]	6.546[a]	2.773[b]	3.257[b]	0.50
LEU	4.384[b]	3.768[b]	6.894[a]	4.007[b]	3.967[b]	0.43
PHE	3.756[b]	2.862[b]	7.790[a]	2.907[b]	5.273[ab]	0.58
HIS	7.082[a]	4.282[b]	7.114[a]	6.300[ab]	5.253[ab]	0.42
LYS*	18.966	19.328	12.614	12.930	10.380	1.70
ARG[ℓ]	10.298	10.538	14.146	14.213	13.750	0.67
EAA:	84.21	79.34	81.60	82.12	74.50	3.03
ASP	4.402	4.822	3.500	5.083	5.123	0.27
SER[ℓ]	14.568	14.824	9.680	11.520	10.247	0.84
GLU[ℓ]	13.222[c]	20.332[abc]	16.694[bc]	28.300[a]	24.290[ab]	1.65
PRO	6.418	8.968	4.620	8.497	5.807	0.69
GLY[ℓ]	14.198[a]	16.922[a]	8.392[b]	8.603[b]	9.187[b]	0.96
ALA[q]	9.100[b]	18.674[a]	20.184[a]	25.183[a]	22.093[a]	1.62
TYR[q]	4.508[b]	4.518[b]	9.138[a]	5.593[b]	4.297[b]	0.61
NEAA:	66.42[b]	89.06[ab]	72.21[ab]	92.78[a]	81.04[ab]	3.76
Total	150.63	168.40	153.80	174.90	155.54	5.61

[1] Standard error of the mean.
[abc] Means with different superscript in the same column differ significantly ($p < 0.05$).
[ℓ] Linear relationship among treatment means ($p < 0.05$).
[q] Quadratic relationship among treatment means ($p < 0.05$).
* Linear relationship among treatment means ($p < 0.01$).

Table 5. Comparison of methionine requirements of growing chicks

References	Age (days)	Response criteria	Breed	Requirement (%)
Almquist, 1947	10-20	Growth	Not specified	0.50
Dean and Scott, 1965	7-14	Growth, feed efficiency	New hampshire × columbian	0.45
Klain et al. 1960	7-14	Growth, feed efficiency	Not specified	0.18
Hewitt and Lewis, 1972	7-21	Growth, feed efficiency	Brolier strain	0.39
Hurwitz et al. 1978	7-14	Computer model	Not specified	0.39
Hurwitz et al. 1978	14-21	Computer model	Not specified	0.34
Waldroup et al. 1979	1-21	Growth, feed efficiency	Cobb	0.57
Robbins and Baker, 1980	8-21	Growth, feed efficiency	New hampshire × Columbian	0.44
Moran, 1981	1-14	Growth, feed efficiency, feathering	White mountain × hubbard	0.46
Mean	–	–	–	0.42

The nitrogen content of weight gain would be 2.6%, which equals 16.25% CP in gained weight. As a percentage of retained CP, the methionine requirement was calculated to be 2.6%, which was higher than the values (1.76%, 1.98%) of methionine contents of whole muscle CP of chicks reported by Williams et al. (1954) and Price et al. (1953), respectively. This might be due to high contents of methionine and cystine in feathers which were not excluded from the samples analyzed for the carcass composition in this study.

Plasma amino acid concentrations of growing chicks fed five levels of methionine are shown in table 4 and the response of plasma methionine to daily methionine intake is presented in figure 3. The plasma methionine concentration remained relatively constant up to the breakpoint (117.2 mg intake/day), then it increased abruptly. As a percentage of the diet, the plasma methionine concentration would be 0.28% of the diet which is much lower than the values obtained from weight gain and nitrogen gain responses. Since Zimmerman and Scott (1965) first suggested that the plasma technique could be used to determine the chick's requirement for amino acids, it has become conventional to use the breakpoint in the plasma response curve to estimated the dietary requirement of an amino acid (Lewis et al., 1977). However, in most experiments, the break in the plasma response curve has occurred at an intake below the requirement indicated by growth curve. The exactly same results were found in this experiment. Lewis et al. (1977) also has suggested that the requirement is higher than indicated by the break on the plasma response curve. The fact that the plasma samples were collected at the end of this experiments also could be a reason for the lower estimates (Shin et al., 1991).

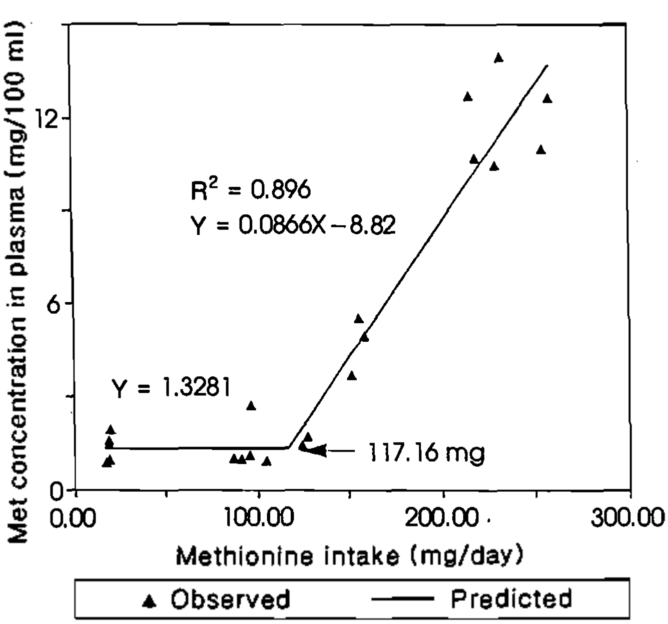

Figure 3. Plasma methionine responses to daily methionine intake.

The model equation tested in this study was already successfully tested with other species (Shin et al., 1991, 1994a,b; Yang et al., 1997a,b,c) and with chicks (Kim et al., 1997). In this study, based on the comparison between the estimated value and the values of previous studies, the model equation appears to have practical importance to estimate a single amino acid requirement for maintenance and growth of broilers. However, to be more practical, more study was needed to get the amino acid requirement for female chicks, since only male chicks were used in this experiment. Amino acid requirement can be different between male and female growing chicks (Freeman, 1979).

In conclusion, with the model equation, the total methionine requirement of growing broiler determined based on weight gain response was 138.29 mg/day or 0.33% of the diet and the one determined based on nitrogen gain response was 141.7 mg/day of 0.34% of the diet, respectively. About 2.1 to 2.24% of these requirements were utilized for maintenance.

ACKNOWLEDGEMENTS

The authors are grateful to the Ajinomodo Co. for their donation of the amino acids and to Korea Chemical for their donation of the vitamin and mineral mixture. Without their donation, this research may not be completed.

REFERENCES

Almquist, H. J. 1947. Evaluation of amino acid requirements by observations on the chick. J. Nutr. 34:543.

AOAC. 1990. Official method of analysis (15th ed.). Association of Official Analytical Chemists, Washington, D. C.

Baker, D. H. 1977. Amino acid nutrition of the chick. pp. 299-335. In: (H. H. Draper. Eds.) Advances in nutrition research. Plenum Press. New York.

Baker, D. H., K. M. Halpin, G. L. Czarnecki and C. M. Parsons. 1982. The choline-methionine interrelationship for growth of the chick. Poult. Sci. 62:133.

Dean, W. F. and H. M. Scott. 1965. The development of an amino acid reference diet for the early growth of chicks. Poult. Sci. 44:803.

Freeman, C. P. 1977. The tryptophan requirement of broiler chicks. Br. Poult. Sci. 20:27.

Graber, G., H. M. Scott and D. H. Baker. 1971. Sulfur amino acid nutrition of the growing chick: Effect of age on the dietary methionine requirement. Poult. Sci. 50:854-858.

Hewitt, D. and D. Lewis. 1972. The animo acid requirements of the growing chick. 1. Determination of amino acid requirements. Br. Poult. Sci. 13:449.

Hurwitz, S., D. Sklan and I. Bartov. 1978. New formal approaches to the determination of energy and amino acid requirements of chicks. Poult. Sci. 57:197.

Kim, J. H., W. T. Cho, I. S. Shin, C. J. Yang and In K. Han. 1997. Partition of amino acids requirements into requirements for maintenance and growth of broilers I. Lysine. Asian Australasian J. Anim. Sci. 10:178.

Klain, G. J., H. M. Scott and B. C. Johnson. 1960. The amino acid requirement of the growing chick fed a crystalline amino acid diet. Poult. Sci. 39:39.

Leveille, G. A., R. Shapiro and H. Fisher. 1960. Amino acid requirements for maintenance in the adult rooster. IV. The requirements for methionine, cystine, phenylalanine, tyrosine and tryptophan; the adequacy of the determined requirements. J. Nutr. 72:8.

Lewis, A. J., E. R. Peo, Jr., P. J. cunningham and B. D. Moser. 1977. Determination of the optimum dietary proportions of lysine and tryptophan for growing rats based on growth, food intake and plasma metabolites. J. Nutr. 71:1361.

Moran, E. T. Jr. 1981. Cystine requirement of feather-sexed broiler chickens with sex and age. Poult. Sci. 60:1056.

NRC. 1994. Nutrient requirements of poultry. Ninth revised edition. National Reserach Council. Washington, D.C.

Owens, F. N., J. E. Pettigrew, S. G. Cornelius and R. L. Moser. 1985. Amino acid requirements for growth and maintenance of rats and chicks. J. Anim. Sci. 61(suppl. 1):312.

Price, W. A. Jr., M. W. Taylor and W. C. Russell. 1953. The retention of essential amino acids by the growing chick. J. Nutr. 51:413.

Robbins, K. R. and D. H. Baker. 1980. Effect of high-level copper feeding on the sulfur amino acid need of chicks fed corn-soybean meal and purified crystalline amino acid diets. Poult. Sci. 59:1099.

SAS. 1985. User's guide: Statistics. 5th ed.

Shin, I. S., F. N. Owens, J. E. Pettigrew and J. W. Oltjen. 1991. Apportioning histidine requirements for maintenance versus growth. Nutr. Res. 11:1451.

Shin, I. S., F. N. Owens, J. E. Pettigrew and J. W. Oltjen. 1994a. Apportioning methionine requirements for maintenance versus growth of rats. Nutr. Res. 14:229.

Shin, I. S., F. N. Owens, J. E. Pettigrew and J. W. Oltjen. 1994b. Apportioning threonine requirements for maintenance versus growth of rats. Nutr. Res. 14:229.

Terrlink, T., P. A. M. van-Leeuwen and A. Hondijk. 1994. Plasma amino acids determined by liquid chromatography within 17 minutes. Clin. Chem. 40:245.

Waldroup, P. W., C. J. Cabray, J. R. Blackman and Z. B. Johnson. 1979. The influence of copper sulfate on the methionine requirement of the young broiler chick. Nutr. Rep. Intl. 20:303.

Williams, H. H., L. V. Curtin, J. Abraham, J. K. Loosli and L. A. Maynard. 1954. Estimation of growth requirements for amino acids by assay of the carcass. J. Biol. Chem. 208:277-286.

Willis, G. M. and D. H. Baker. 1980. Lasalocid-sulfur amino acid interrelationship in the chick. Poult. Sci. 59:2538-2543.

Yang, C. J., D. W. Lee, I. B. Chung, Y. M. Cho, I. S. Shin, B. J. Chae, J. H. Kim and In K. Han. 1997a. Developing model equation to subdivide lysine requirements into requirements for growth and maintenance in pigs. Asian Australasian J. Anim. Sci. 10:54.

Yang, C. J., D. W. Lee, I. B. Chung, Y. M. Cho, I. S. Shin, B. J. Chae, J. H. Kim and In K. Han. 1997b. Developing model equation to subdivide methionine + cystine requirements into requirements for growth and maintenance in pigs. Asian Australasian J. Anim. Sci. 10:86.

Yang, C. J., D. W. Lee, I. B. Chung, Y. M. Cho, I. S. Shin, B. J. Chae, J. H. Kim and In K. Han. 1997c. Developing model equation to subdivide threonine requirements into requirements for growth and maintenance in pigs. Asian Australasian J. Anim. Sci. 10:122.

Zimmerman, R. A. and H. M. Scott. 1965. Interrelationship of plasma amino acid levels and weight gain in the chick as influenced by suboptimal and superoptimal dietary concentrations of single amino acids. J. Nutr. 87:13.