Arginine Requirements and Optimum Balance between Dietary Arginine and Lysine in Broiler Chicks

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Abstract The reported arginine (Arg) requirements range widely by investigators, and Arg requirements are affected by dietary lysine (Lys) levels. Therefore, this experiment was conducted to ascertain the interrelationship between Arg requirements and dietary Lys levels using body weight gain and plasma concentration (PC) of Arg as parameters. Female broiler chicks at 8 days of age were divided into 20 dietary groups of 10 chicks, housed individually, and given free access to the experimental diets for 10 days. The Arg- and Lys-deficient basal diets were consisted of corn, corn gluten meal, soybean meal and wheat bran, and contained 0.65% Arg, 0.80% Lys, 18.7% crude protein and 3.20 kcal metabolizable energy (ME)/g of diet. To the basal diet, four graded levels of Lys were added, and to each Lys diet, graded five levels of Arg were added. The body weight (BW) gain of chicks increased with increasing dietary Arg levels and then decreased in all dietary Lys levels. The PC of Arg increased slowly and then linearly with increasing dietary Arg levels. The Arg requirements (Y, % of diet) determined from BW gain and PC of Arg increased linearly with increasing dietary Lys levels (X, % of diet, $0.80 \leq X \leq 1.40$ for BW gain and $0.80 \leq X \leq 1.70$ for PC of Arg) as follows. $Y = 1.15X - 0.21$ ($r^2 = 0.944$) for BW gain, and $Y = 1.10X - 0.21$ ($r^2 = 0.991$) for PC of Arg. When BW gain was plotted against dietary levels of Lys and Arg, the maximum BW gain was achieved at 1.10% Lys and 1.36% Arg and the ratio of Arg/Lys was estimated to be 1.24.

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Key words : Arginine requirement, Broiler chick, Crude protein, Lysine, Plasma arginine

In the practical low crude protein (CP) broiler diet supplemented with crystalline lysine (Lys) and methionine, arginine (Arg) is liable to be one of the limiting amino acids (AA) at young age. Therefore, Table 1 shows the many reports on Arg requirements of broilers at a young age. The values in the cited reports and NRC are shown in Table 1, which range widely from 0.80⁷⁾ to 1.40%³⁾ of diet. This wide range of Arg requirements is not unreasonable, because AA requirements are affected by

many factors, such as dietary levels of CP^{10,20)} or metabolically related AA³²⁾, age^{2,15)}, strain of chicks²³⁾ and so on.

From the data except NRC (1994)²²⁾ in Table 1, Arg requirements (Y, % of diet) increase with increasing dietary CP levels (X, % of diet), and are expressed as a function of dietary CP levels as follows. However, the obtained coefficient was low.

$$Y = 0.046X + 0.31 \quad r^2 = 0.410 \quad 14.5 \leq X \leq 23.0 \quad (1)$$

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It is well known that there is an antagonism between Arg and Lys^{1,7,8,17}. D'Mello and Lewis^{7,8} clearly showed that the Arg requirements increased with increasing dietary Lys levels. From their data in Table 1, the Arg requirements (Y, % of diet) are expressed as a function of dietary Lys levels (X, % of diet) as follows.

$$Y = 0.47X + 0.29 \quad r^2 = 1.00 \quad 1.10 \leq X \leq 1.85 \quad (2)$$

However, the calculated ratios of Arg/Lys are lower than the others in Table 1. The Arg requirements are expressed as follows

$$Y = 1.13X - 0.03 \quad r^2 = 0.710 \quad 0.83 \leq X \leq 1.18 \quad (3)$$

Ueno *et al.*³² confirmed that the depression of performance caused by Arg deficiency was intensified by addition of Lys, but not of other AA individually to the Arg deficient diet. When a large amount of glutamic acid was added to the diet as a source of nitrogen, the requirement of Arg was not affected (not yet published). These observations indicate that the Arg requirements is affected by dietary Lys, but not by other AA and CP.

It is well documented that AA requirements determined from plasma concentration (PC) of AA agree well with those by performance in many species, including rats²⁹, chicks^{7,36}, laying hens^{16,34}, pigs¹⁹, and young men³⁵.

The present study was conducted to ascertain the Arg requirements and to estimate optimum balance between dietary Arg requirements and Lys in young female broilers using growing performance and PC of AA as parameters.

Materials and Methods

Animal and design of experiment

One-day-old Cobb strain female broiler chicks were obtained from a commercial hatchery and group-fed a stock diet containing 23% CP and 3.20 kcal metabolizable energy (ME)/g of diet. On day 8 posthatching, the chicks were divided into 20 dietary groups of 10

chicks with the same average body weight (BW) of 150 ± 1 g, housed individually in wire cages and given free access to the experimental diets and water for a 10 day experimental period in an environmentally controlled room ($23 \pm 3^\circ\text{C}$, and lights on 4 : 00 to 20 : 00).

At the termination of the experiment, body weight and feed intake were recorded, and blood samples were taken from the wing vein with heparinized syringes. The PC of AA was determined with an HPLC (Simadzu LC 6 A) as described by Fujimura *et al.*⁹.

Experimental diet

The basal diet consisted mainly of corn, corn gluten meal, soybean meal and wheat bran. To meet the AA requirements by NRC (1994)²², five essential AA and glutamic acid (Glu) were added (Table 2). The basal diet contained 18.7% CP, 0.65% Arg, 0.80% Lys, 6.85% Glu and 3.20 kcal ME/g of diet, and the AA composition of basal diet is shown in Table 2. To the basal diet, 0, 0.3, 0.6 or 0.9% crystalline Lys was added by balancing the same amount of Glu to make 0.80, 1.10, 1.40 and 1.70% Lys diets. To each Lys diet, crystalline Arg was added to make five diets with graded Arg levels, 0 to 0.40, 0 to 0.80, 0 to 1.20, or 0 to 1.60%, respectively, by balancing the same amount of Glu.

Statistical analysis

For statistical analysis, one-way analysis of variance was employed using the General Linear Model procedure in SAS[®] software²⁷. Statistical significance of differences between treatment means was determined with the least significance difference test²⁸. Statements of significance are based upon $P < 0.05$ unless otherwise stated.

Response curves for performance (Y, BW gain, % of diet or mg/chick/day) to Arg intake (X, % of diet or mg/chick/day) were fitted with an exponential equation presented by Toyomizu *et al.*³¹ :

$$Y = (A - BX) (1 - e^{-C(X-D)}) \quad (4)$$

The Arg requirements for the maximum performance were estimated as lower X level at

95% of the maximum response.

The calculated response surface was also conducted by a following equation³⁰⁾.

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_1^2 + b_4 X_2^2 + b_5 X_1 X_2 \quad (5)$$

Where Y represents the BW gain, X_1 is dietary Arg levels (%) and X_2 is dietary Lys levels (%).

The Arg requirements were also estimated from the PC of Arg using a broken line model by Ohtsuka and Yoshihara²⁴⁾.

Results

The body weight gain and feed intake increased with increasing dietary Arg levels and then decreased at all dietary Lys levels. The maximum BW gain achieved on the respective Lys diets increased and then decreased with increasing dietary Lys levels (Table 4 and Fig. 1).

The Arg requirements were estimated to be 0.69, 1.08, 1.38 and 1.14% for the maximum BW gain, and 0.66, 1.00, 1.35 and 1.23 for feed efficiency (FE) on the 0.80, 1.10, 1.40, and 1.70% Lys diets, respectively.

When the maximum BW gain was achieved, the feed intake was estimated to be 52.8, 52.5, 525 and 52.4 g/chick/day, respectively. Therefore, the Arg requirements were estimated to be 364, 567, 725 and 597 mg/chick/day, respectively, for the 0.80, 1.10, 1.40 and 1.70% Lys diets.

Except the values on the 1.70% Lys diets, the Arg requirements (Y, % of diet or mg/chick/day) were expressed as a function of dietary Lys levels (X, % of diet or mg/chick/day, $0.80 \leq X \leq 1.40$) as follows.

$$Y(\%) = 1.15 X - 0.21 \quad r^2 = 0.944 \quad (6)$$

$$Y(\text{mg/chick/day}) = 602 X - 110 \quad r^2 = 0.995 \quad (7)$$

The maximum BW gain estimated by formula (5) was achieved at 1.36% Arg and 1.10% (Fig. 1).

The PC of Arg increased slowly and linearly with increasing dietary Arg levels on all dietary Lys diets (Table 4). From the PC of Arg, the Arg requirements were estimated to be 0.65,

Fig. 1. Response surfaces depicting relationships between BW (body weight) gain and dietary levels of Lys and Arg.

0.89, 1.31 and 1.65%, respectively, for the 0.80, 1.10, 1.40 and 1.70% Lys diets. The Arg requirements (Y, % of diet) were expressed as a function of dietary Lys levels (X, % of diet, $0.80 \leq X \leq 1.70$) as follows.

$$Y(\%) = 1.10 X - 0.21 \quad r^2 = 0.991 \quad (8)$$

Since the feed intake was not recorded everyday, the Arg requirements were not expressed as mg/chick/day in the case of PC of Arg.

The PC of Lys decreased linearly, and remained constant with increasing dietary Arg levels on all Lys diets. The break points were obtained at < 0.65, 0.93, 1.34 and 1.70% dietary Arg levels, respectively, on the 0.80, 1.10, 1.40, and 1.70% Lys diets.

To avoid complexity, only the PC of threonine are presented in Table 4. The PC of threonine decreased and remained constant with increasing dietary Arg levels at all dietary Lys levels as similar to other AA.

Discussion

To make diets with graded levels of Lys and Arg, both AA were added by balancing the same amount of Glu. Nitrogen and ME contents of Glu (9.5% and 2.88 kcal/g) were lower

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Table 1. Reported arginine (Arg) requirements of growing chicks (%)

References	Arg requirement	Dietary		Diet	Strain	Sex	Age (day)
		Lys	CP				
NRC ⁽²²⁾	1.25	1.01	23.0	Compilations	—	—	0-21
Klain <i>et al.</i> ⁽¹⁸⁾	1.08	1.01	23.0	Purified	N C	M	7-14
Dean and Scott ⁽⁵⁾	1.10	1.12	17.7	Purified	N C	M	8-14
Zimmerman and Scott ⁽³⁶⁾	1.00	0.83	14.5	Purified	N C	M	15-22
Sasse and Baker ⁽²⁸⁾	0.95	0.91	14.8	Purified	N C	M	8-21
Myung <i>et al.</i> ⁽²¹⁾	1.24	1.10	23.0	Purified	Cobb	M	8-21
Hurwitz <i>et al.</i> ⁽¹⁴⁾	1.33	1.18	19.2	Computer	—	—	—
Calet ⁽⁹⁾	1.05	0.95	20.0	Conventional	—	—	—
Grigor'ev ⁽¹¹⁾	1.20	1.00	20.0	Conventional	—	—	—
Hewitt and Lewis ⁽¹³⁾	0.86	0.85	18.0	Conventional	Cobb	M	7-21
Packham and Payne ⁽²⁵⁾	0.92	0.90	15.0	Conventional	—	—	—
Burton and Waldroup ⁽³⁾	1.40	1.10	20.9	Conventional	—	M, F	1-28
Cuca and Jensen ⁽⁵⁾	1.25	1.20	23.0	Conventional	P A	M	1-21
D'Mello and Lewis ⁽⁷⁾	0.80	1.10	20.1	Conventional	—	—	7-21
	0.92	1.35	20.1				
	1.04	1.60	20.1				
	1.15	1.85	20.1				

Lys ; Lysine, CP ; crude protein, N ; New Hampshire, C ; Columbian, P ; Petersen, A ; Arbor Acre

than those of Lys (19.2% and 4.60 kcal/g) and Arg (32.2% and 3.06 kcal/g). Therefore, the diets were not isonitrogenous and isocaloric. The maximum differences of CP and ME between the lowest and highest Lys-Arg diets were calculated to be 2.8% and 0.02 kcal/g of diet. From formula 1, the Arg requirement increased only 0.11% by 2.8% CP. The obtained Arg requirements were 0.65% on the lowest Lys diet and 1.65% on the highest Lys diet, respectively. Therefore, the effect of difference of dietary CP might not be a factor to increase Arg requirements.

As shown in Table 1, the reported Arg requirements ranged widely from 0.80 to 1.40% of diet, which might be attributed to the wide range of dietary Lys and not to dietary CP levels. When 6.0% Glu was replaced by the same amount of corn starch, the same Arg requirements were obtained from the diets

Table 2. Composition of basal diet (%)

Ingredients	
Corn	69.91
Soybean meal (44.0% CP)	3.55
Corn gluten meal (60.2% CP)	13.40
Wheat bran	3.00
Soybean oil	1.15
Calcium carbonate	1.20
Tricalcium phosphate	2.40
Sodium chloride	0.30
Choline chloride	0.10
Vitamin-mineral premixture ¹	0.35
Agar	0.81
Amino acid mixture ²	3.83
Analytical composition	
CP, (%)	18.70
ME, (kcal/g)	3.20

CP ; crude protein, ME ; metabolizable energy

¹ Ueno *et al.*⁽³²⁾

² See Table 3.

with or without addition of 6% Glu (unpublished data), which indicated that the dietary CP *per se* was not the direct cause of increment of Arg requirement with increasing dietary CP levels.

The Arg requirement determined from PC of Arg on the 1.70% Lys diets was fitted on the linear line as shown in formula 6, but those from performance of broilers were less than that from the PC of Arg. Because the Arg : Lys ratios ranged almost the same width in all Lys diet, the depression of performance on the 1.70% Lys diet might not be caused by Arg deficiency and might be caused by Lys toxicity which was not alleviated by Arg.

The Arg requirements determined from PC of Arg were less than those from performance. As shown in the feeding standard²²⁾, the Arg requirements decrease 0.15 points of percentage from 0 to 3, to 3 to 6 weeks of age (0.05%/weeks). Because the PC of AA responds within a short period after changing dietary AA levels³²⁾, the Arg requirements determined from the PC of Arg represents the requirement on the blood sampling day. However, those determined from performance are means for experimental period, especially for earlier period. Therefore, the former is less on than the latter.

As shown in formulas 6 and 8, the Arg requirements increased at the rate 1.10 to 1.15 per percentage of dietary Lys levels. Following two reasons for the Arg requirements increased with increasing dietary Lys levels are suggested. As shown in Table 3, first, the maximum BW gain increased with increasing dietary Lys levels until 1.40%. The BW gain accompanied the protein syntheses. Therefore, the Arg requirements increased with increasing of protein syntheses rate. The second reason is when the dietary Lys increased, the kidney arginase, which degraded Arg, was induced, and supply of more Arg was needed¹⁾.

From the data obtained in this study, it was

Table 3. Composition of amino acids (AA) in mixture, diet and NRC requirement (%)

	AA		NRC requirement ²²⁾
	AA mixture	Diet ¹⁾	
Arg ²	0 to 1.60	0.65 to 2.25	1.25
His	—	0.37	0.35
Ile	0.18	0.80	0.80
Leu	—	2.19	1.20
Lys ²	0.57 to 0.90	0.80 to 1.70	1.10
Met	0.15	0.60	0.50
Cys	—	0.30	0.40
Phe	—	0.83	0.72
Tyr	—	0.70	0.72
Thr	0.26	0.80	0.80
Trp	0.08	0.20	0.20
Val	0.15	0.90	0.90
Glu ²	0 to 2.50	4.35 to 6.85	—

¹ AA except Met, Cys and Trp were calculated from analytical data of feedstuffs.

² Met, Cys and Trp were calculated from Table by NRC (1994)²²⁾.

L-Arg and L-Lys were substituted by the same amount of Glu.

clear that the Arg requirements increased with increasing dietary Lys levels (Formulas 6, 7 and 8) as observed by D'Mello and Lewis⁷⁾ (Formula 2) and other investigators (Formula 3). The Arg requirements increased 1.15% from BW gain and 1.10% from PC of Arg per percentage of dietary Lys, respectively in this study. However, the Arg requirements increased only 0.48% per percentage of dietary Lys in Formula 2 by D'Mello and Lewis⁷⁾. As indicated by them, the Arg requirements were lower compared to those determined at same dietary Lys levels by others. The lower Arg requirement was partly due to the method of calculation. Although the Arg requirements for the maximum performance were estimated as lower X level at 95% of the maximum response in this study, D'Mello and Lewis⁷⁾ estimated at 90% of maximum response. However, the reasons of lower ratio of Arg/Lys was unclear.

Han and Baker¹²⁾ studied the effects of sex,

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Table 4. Effects of dietary lysine (Lys) and arginine (Arg) on the performance and plasma amino acid (AA) concentration of female broiler at 8 to 18 d of age

Dietary		Feed intake (g/10 d)	Feed efficiency (g/g)	Plasma concentration		
Lys	Arg			Arg	Lys	Thr
(%)				(nmol/ml)		
0.80	0.65	523	0.530	49 ^d	173	2809 ^a
	0.75	538	0.548	97 ^c	144	1619 ^b
	0.85	539	0.555	191 ^b	108	1553 ^b
	0.95	534	0.560	245 ^a	106	1180 ^c
	1.05	545	0.561	282 ^a	90	1612 ^b
Pooled SE		16	0.011	5	25	34
1.10	0.65	459 ^c	0.488 ^c	62 ^d	814 ^a	2288 ^a
	0.85	495 ^b	0.561 ^b	87 ^d	408 ^b	1626 ^a
	1.05	521 ^a	0.611 ^a	159 ^c	282 ^{bc}	771 ^b
	1.25	529 ^{ab}	0.624 ^a	250 ^b	248 ^c	859 ^b
	1.45	535 ^a	0.615 ^a	331 ^a	225 ^c	772 ^b
Pooled SE		14	0.010	9	25	56
1.40	0.65	456 ^a	0.371 ^d	58 ^d	1386 ^a	1981 ^a
	0.95	522 ^b	0.496 ^c	86 ^d	846 ^b	1705 ^a
	1.25	533 ^b	0.585 ^b	127 ^c	637 ^b	789 ^b
	1.55	532 ^b	0.636 ^a	207 ^b	621 ^b	695 ^c
	1.85	528 ^b	0.626 ^a	324 ^a	567 ^b	459 ^c
Pooled SE		13	0.012	10	57	51
1.70	0.65	346 ^c	0.321 ^d	55 ^d	1463 ^a	2589 ^a
	1.05	522 ^a	0.523 ^c	108 ^c	1174 ^{ab}	1788 ^b
	1.45	525 ^a	0.593 ^a	143 ^c	937 ^b	613 ^d
	1.85	479 ^b	0.569 ^{ab}	301 ^b	774 ^b	787 ^c
	2.25	409 ^c	0.556 ^b	532 ^a	749 ^b	522 ^d
Pooled SE		16	0.016	17	67	47

^{a-d} Means within the same columns in the same dietary Lys levels with no common superscripts differ significantly ($P < 0.05$).

Means $\pm$ SE for 10 chicks with average initial body weight of 150 ± 1 g.

BW gain and genetic strain on the Lys requirement of broiler chickens, and concluded that males grew faster and required more Lys than females for maximum performance, which was compensated by increasing feed intake. The values expressed as mg/chick/day obtained in this study showing females were lower than those for males, agreed with the findings of Han and Baker¹²⁾ but the values expressed as percentages of diet may be applied to males.

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ブロイラーにおけるアルギニン要求量と飼料中アルギニン およびリジンの最適バランス

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報告されているブロイラーのアルギニン (Arg) 要求量は研究者により大きな差があり, これは飼料中のリジン (Lys) 含量の影響を受けているものと考えられる. そこで, 本試験は増体量および血漿アミノ酸濃度を指標としてアルギニン要求量と飼料中リジン含量の関係を確認するために行った. 8日齢の雌ブロイラーを1区10羽ずつの20区にわけ, 単飼し, 10日間試験飼料を給与した. Arg-Lys欠の基礎飼料は主としてトウモロコシ, コーングルテンミール, 大豆粕, フスマからなり, 粗タンパク質18.7%, Arg 0.65%, Lys 0.80%, 代謝エネルギー 3.2kcal/gであった. Lys 4段階, それぞれ Arg 5段階の飼料を調製した. 各 Lys 飼料で鶏の増体量は飼料中の Arg 含量の増加にともない増加し, その後減少した. 血漿 Arg は飼料中の Arg 含量の増加にともない緩やかに増加し, その後急激に増加した. 両者から得られた Arg 要求量 (Y, 飼料中%) は飼料中の Lys 含量 (X, 飼料中%) の関数として次式のように求められた. 増体量 : $Y = 1.55X - 0.21$, $r^2 = 0.944$, $0.80 \leq X \leq 1.40$, 血漿 Arg : $Y = 1.10X - 0.26$, $r^2 = 0.991$, $0.80 \leq X \leq 1.70$. Lys, Arg に対する増体量の関係から, 最大増体量のための Lys, と Arg の要求量は 1.10% と 1.36% で, Arg/Lys 比は 1.24 であった.

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