The Journal of Poultry Science, 43 : 150–155, 2006

Effects of Excess Essential Amino Acids in Low Protein Diet on Abdominal Fat Deposition and Nitrogen Excretion of the Broiler Chicks

Makoto Yamazaki, Hitoshi Murakami[1], Kazuki Nakashima, Hiroyuki Abe and Masaaki Takemasa[1]

National Institute of Livestock and Grassland Science, Tsukuba, Ibaraki 305–0901, Japan
[1] National Agricultural Research Center for Kyushu Okinawa Region, Nishigoshi, Kumamoto 861–1192, Japan

Two experiments were conducted to determine whether the excess essential amino acids in low protein diets affects the performance, nitrogen excretion and abdominal fat deposition of broiler chicks. Essential amino acids were classified into seven groups, ① branched-chain amino acid (BCAA ; leucine＋isoleucine＋valine) ② hydroxy amino acid (Thr ; threonine) ③ sulfur-containing amino acid (Met ; methionine) ④ aromatic amino acid (AAA ; phenylalanine＋Tyrosine) ⑤ basic amino acid (BAA ; lysine＋arginine) ⑥ glycine (Gly) ⑦ tryptophan (Trp) and were supplemented to low crude protein (19% CP) diet at the level of 150% (Experiment 1) and 200% (Experiment 2) of the Japanese Feeding Standard requirement. Seven amino acids supplemented diets, low CP and control (21% CP) diets were fed to 7-day-old chicks for 14 days.

Body weight gain of the chicks fed the low CP and amino acids supplemented diets were did not differ among the treatments. Significant reduction in feed intake compared to the control diet were not seen in excess amino acids supplemented diets. Low CP and amino acids supplemented diets significantly increased abdominal fat weight than the control diet in experiment 1, however in experiment 2, compared to the control diet, abdominal fat weight were significantly higher in chicks fed the Gly and Trp supplemented diet among the groups. Nitrogen excreted were not reduced in chicks fed the low CP diets.

These results show that the supplementation of excess amino acids to the low CP diet had little effect on abdominal fat deposition of broiler chicks in 1 to 3 wks of age.

Key words : broiler, essential amino acids, fat deposition, nitrogen excretion

Introduction

Poultry excrete vast quantities of nitrogen, which is very valuable as a source of fertilizer for most crops. However, there is a limit to the amount that can be applied per hectare of land depending on the crop to be grown and the soil type. To reduce the nitrogen content in excreta from chickens through dietary techniques, several works has been conducted to reduce the dietary protein levels of poultry diets, by the use of several synthetic amino acids that are available commercially. It has previously been shown that crude protein (CP) content in broiler diet can be reduced from 21 to 19% without lowering performance by the supplementation of crystalline amino acids during 1 to 3 weeks of age, and feeding low CP diet could reduce nitrogen excretion by about 15% (Yamazaki *et al.,* 1996).

However, lowering dietary CP may lead to greater abdominal fat weight. Yamazaki *et al.* (1998)

Received : October 6, 2005, Accepted : December 13, 2005
Correspondence to : Makoto Yamazaki
National Institute of Livestock and Grassland Science, Tsukuba, Ibaraki 305–0901, Japan
Tel. : ＋81–29–838–8657 Fax : ＋81–29–838–8657 E-mail : yamazaki@affrc.go.jp

Table 1. **Composition of experimental diets**

Dietary CP (%)	Experiment 1		Experiment 2	
	21 Control	19 Low CP	21 Control	19 Low CP
Corn	57.90	66.96	62.12	67.40
Soybean meal (46% CP)	34.34	22.78	24.40	20.65
Corn gluten meal (60% CP)	—	—	5.39	—
Fish meal (65% CP)	3.00	3.00	3.00	3.00
Soybean oil	2.00	2.00	1.95	1.95
$CaHPO_4 \cdot 2H_2O$	1.22	1.33	1.29	1.35
$CaCO_3$	0.88	0.89	0.90	0.89
Sodium chloride	0.24	0.24	0.24	0.24
DL-Methionine	0.27	0.38	0.24	0.40
L-Lysine-HCl	—	0.22	0.12	0.28
L-Arginine	—	0.35	0.20	0.41
L-Threonine	—	0.14	—	0.17
L-Isoleucine	—	0.05	—	0.09
L-Tryptophan	—	0.01	—	0.02
L-Glutamic acid	—	1.50	—	3.00
Vitamin-Mineral mixture[1)]	0.15	0.15	0.15	0.15
Calculated composition				
ME (kcal/g)	3.10	3.10	3.10	3.10
CP (%)	21.0	19.0	21.0	19.0

[1] See Yamazaki *et al*. (1996).

suggested that feeding higher rate of essential amino acids (EAA) to nonessential amino acids (NEAA) in 19% CP diets tends to prevent excess fat deposition. Geraert *et al.* (1987) observed that the genetically fat type chicken had lower plasma concentration of most glucogenic amino acids and higher levels of branched-chain and sulfur-containing amino acids than lean type chicken, thus, amino acids might be related with the fat deposition. Several studies have been investigated that certain amino acids such as Met (Huyghebaert and Pack, 1996), Arg (Leclercq *et al.,* 1994), have a potential to reduce body fat deposition in broiler chicks and genetically fat chickens, respectively. However, in those experiments, the level of those dietary amino acids were from deficiency to requirement, the effects of excessive dietary amino acids on fat deposition have not been studied sufficiently. in broiler.

The objective of this study was to determine whether the excess EAA with low-protein diet would have an effect on performance, abdominal fat deposition and nitrogen excretion in broiler chicks.

Materials and Methods

Day-old male broiler chicks (Abor Acres) obtained from a local hatchery were housed in electrically heated battery cages and had free access to water and a commercial starter diet for 7 days. They were then divided into nine groups, each of which contained three replicates of three chicks. Experimental diets were fed from 7 to 21 days of age. All experiments employed in this study followed the recommendations within the Guide for the Care and Use of Agricultural Animals in Agricultural Research of the National Institute of Livestock and Grassland Science (Tsukuba, Japan).

In experiment 1 and 2, all diets consisted mainly of corn and soybean meal and were isocaloric (3.1 kcal/g) (Table 1). In experiment 1, EAA were divided into seven groups as similarity of their structure or character, branched-chain amino acid (BCAA ; leucine + isoleucine + valine), hydroxy amino acid (Thr ; threonine), sulfur- containing amino acid (Met ; methionine), aromatic amino acid (AAA ; phenylalanine + Tyrosine), basic amino acid (BAA ; lysine + arginine), glycine (Gly), tryptophan (Trp), and supplied each synthetic amino acids to the low CP (19%) basal diet at 150% of the Japanese Feeding Standard (JFS) requirement (1992) (Table 2). L-glutamic acid (Glu) in the low CP basal diet was replaced by synthetic amino acids at each level. The low CP basal diet supplied EAA at the levels of recommendations, and the control (21% CP) diet supplied CP and EAA at

Table 2. **Dietary amino acids contents of the experimental diets (%)[1]**

	Requirement[2]	Control	Low CP	BCAA[3]	Thr	Met	AAA[3]	BAA[3]	Gly	Trp
Arg	1.40	1.39 (1.40)[4]	1.40 (1.40)	—[5]	—	—	—	2.10 (2.80)	—	—
Gly+Ser	1.45	1.96 (1.84)	1.55 (1.47)	—	—	—	—	—	2.18 (2.90)	—
Ile	0.78	0.96 (0.90)	0.78 (0.78)	1.17 (1.56)	—	—	—	—	—	—
Leu	1.31	1.86 (2.10)	1.54 (1.47)	1.97 (2.62)	—	—	—	—	—	—
Lys	1.16	1.26 (1.16)	1.16 (1.16)	—	—	—	—	1.74 (2.32)	—	—
Met+Cys	0.90	0.90 (0.90)	0.90 (0.90)	—	—	1.35 (1.80)	—	—	—	—
Phe+Tyr	1.30	1.79 (1.82)	1.41 (1.33)	—	—	—	1.95 (2.60)	—	—	—
Thr	0.77	0.81 (0.76)	0.77 (0.77)	—	1.16 (1.54)	—	—	—	—	—
Trp	0.22	0.28 (0.24)	0.22 (0.22)	—	—	—	—	—	—	0.33 (0.44)
Val	0.79	1.05 (1.01)	0.83 (0.79)	1.19 (1.58)	—	—	—	—	—	—

[1] Calculated value.
[2] Requirement of Japanese Feeding Standard (1992).
[3] BCAA, branched-chain amino acid (leucine+isoleucine+valine) ; AAA, aromatic amino acid (phenylalanine+Tyrosine) ; BAA, basic amino acid (lysine+arginine)
[4] Dietary amino acid contents of Exp.2 are shown in parentheses.
[5] Same as Low CP diet.

Table 3. **Effect of dietary excess amino acids on performance and nitrogen excretion of male broiler chicks (Experiment 1)[1]**

Dietary CP (%)	21	19								Pooled SEM
	Control	Low CP	BCAA[2]	Thr	Met	AAA[2]	BAA[2]	Gly	Trp	
Body weight gain (g/14 days)[3]	575[ab]	532[b]	611[ab]	569[ab]	574[ab]	585[ab]	615[a]	599[ab]	617[a]	24.5
Feed intake (g/14 days)[3]	854[ab]	802[b]	869[ab]	835[ab]	837[ab]	856[ab]	886[ab]	870[ab]	898[a]	27.2
Feed efficiency (%)[3]	67.2[ab]	66.3[b]	70.3[a]	68.1[ab]	68.5[ab]	68.4[ab]	69.4[ab]	68.9[ab]	68.7[ab]	1.05
Nitrogen excretion (g/3 days)[4]	2.14	1.95	1.88	2.18	1.98	1.89	2.20	2.23	2.19	0.140

[1] Each treatment consists of 3 replicates of 3 birds per pen.
[2] BCAA, branched-chain amino acid (leucine+isoleucine+valine) ; AAA, aromatic amino acid (phenylalanine+Tyrosine) ; BAA, basic amino acid (lysine+arginine)
[3] From 7 to 21 days of age.
[4] From 14 to 17 days of age.
[a,b] Means within the same rows with no common superscript are significantly different ($P<0.05$).

the levels recommended by the Japanese Feeding Standards (JFS, 1992). In experiment 2, dietary formulations were almost the same as experiment 1, except for the adding amino acids level, which had supplied each amino acids at 200% of the JFS requirement (1992).

In both experiments, lighting was provided 24 hours per day, and temperature was maintained between 25 and 28℃. From 14 to 17 days of age, a nitrogen balance trial was conducted by the total collection procedure. Excreta were collected for 3 days, dried in an oven at 55℃ and ground, and then, nitrogen content was measured by the Kjeldahl method. The birds and feed remaining in each tray were weighed at 21 days of age to determine body weight gain and feed consumption during the experiments, and the abdominal fat of all birds was weighed.

Data were analyzed using the General Linear Models procedure of SAS with Tukey's multiple range test (SAS Institute, 1988).

Results

Table 3 shows the performance of chicks in experiment 1. Chicks fed the BAA and Trp supplemented diets grew at a significantly faster rate than chicks fed low CP basal diet, but there were no significant difference among other dietary treatments. Chicks fed Trp excess had the highest feed intakes among the treatments. Feed efficiency of chicks fed the BCAA supplemented diets were significantly higher than chicks fed low CP diet. Chicks fed low CP and amino acids supplemented diets significantly increased abdominal fat weight compared to the chicks fed control diet (Fig. 1). Nitrogen excreted were no significant difference among the dietary treatments.

In experiment 2, statistical analysis indicated that

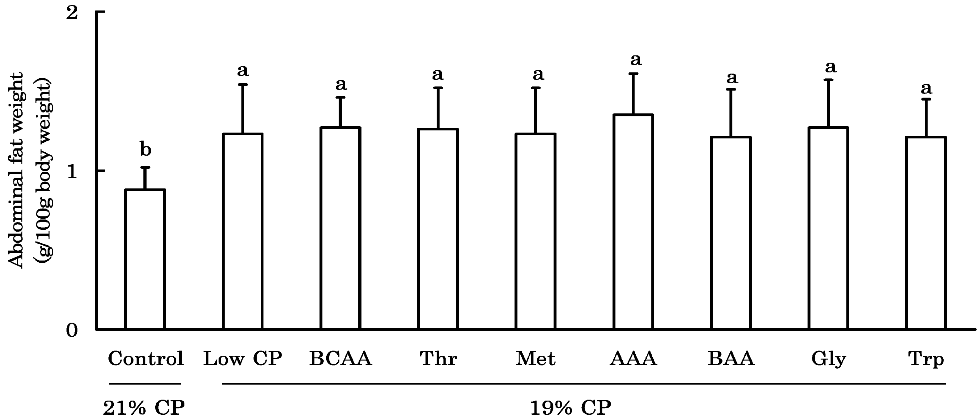

Fig. 1. **Effect of dietary excess amino acids (150% of requirement) on abdominal fat deposition of male broiler chicks (Experiment 1)**

[1] Each treatment consists of 9 birds.

[2] BCAA, branched-chain amino acid (leucine+isoleucine+valine) ; AAA, aromatic amino acid (phenylalanine+Tyrosine) ; BAA, basic amino acid (lysine+arginine)

[a,b] Means within the same rows with no common superscript are significantly different (P<0.05).

Table 4. **Effect of dietary excess amino acids on performance and nitrogen excretion of male broiler chicks (Experiment 2)[1]**

Dietary CP (%)	21	19								Pooled SEM
	Control	Low CP	BCAA[2]	Thr	Met	AAA[2]	BAA[2]	Gly	Trp	
Body weight gain (g/14 days)[3]	564[abc]	541[bcd]	649[a]	554[bc]	458[d]	508[cd]	539[bcd]	561[abc]	606[ab]	27.5
Feed intake (g/14 days)[3]	825[bc]	841[abc]	943[a]	853[abc]	744[c]	790[c]	834[abc]	839[abc]	932[ab]	35.1
Feed efficiency (%)[3]	68.4[a]	64.4[b]	68.8[a]	65.1[ab]	61.4[b]	64.1[ab]	64.5[ab]	66.7[a]	65.1[ab]	1.43
Nitrogen excretion (g/3 days)[4]	2.61[abc]	2.21[abc]	2.21[abc]	2.61[abc]	2.04[bc]	2.24[abc]	1.85[c]	2.78[ab]	2.96[a]	0.241

[1] Each treatment consists of 3 replicates of 3 birds per pen.

[2] BCAA, branched-chain amino acid (leucine+isoleucine+valine) ; AAA, aromatic amino acid (phenylalanine+Tyrosine) ; BAA, basic amino acid (lysine+arginine)

[3] From 7 to 21 days of age.

[4] From 14 to 17 days of age.

[a-d] Means within the same rows with no common superscript are significantly different (P<0.05).

body weight gain of chicks fed low CP and amino acids supplemented diets, except Met supplemented diet, were did not differ among the treatments (Table 4). Feed intake of chicks fed BCAA supplemented diet was significantly higher than chicks fed Met and AAA supplemented diets and control diet, however, there were no significant difference among other dietary treatment. Abdominal fat deposition of chicks fed Gly and Trp supplemented diets were significantly higher than chicks fed control diet, however, there were no significant difference among other treatments (Fig. 2). Nitrogen excretion was lower for the chicks fed Met and BAA supplemented diet than chicks fed Trp supplemented diet, however, there was no significant difference among other dietary treatment.

Discussion

Compared to the control (21% CP) diet, the body weight gain and feed intake on the chicks fed low CP (19%) supplemented with synthetic amino acids to meet the JFS (1992) were similar in both experiments. This is consistent with the previous study (Yamazaki *et al.* 1996), that dietary CP could be reduced from 21 to 19% without affecting body weight gain. However, feed efficiency in chicks fed the basal diet was lower than that in chicks fed the control diet in experiment 2, although such difference was not found in experiment 1. This may be due to the fact that the EAA content (Gly+Ser, Leu, Phe+Tyr and Val) of the basal diet used in experiment 2 was less excessive than that of the basal

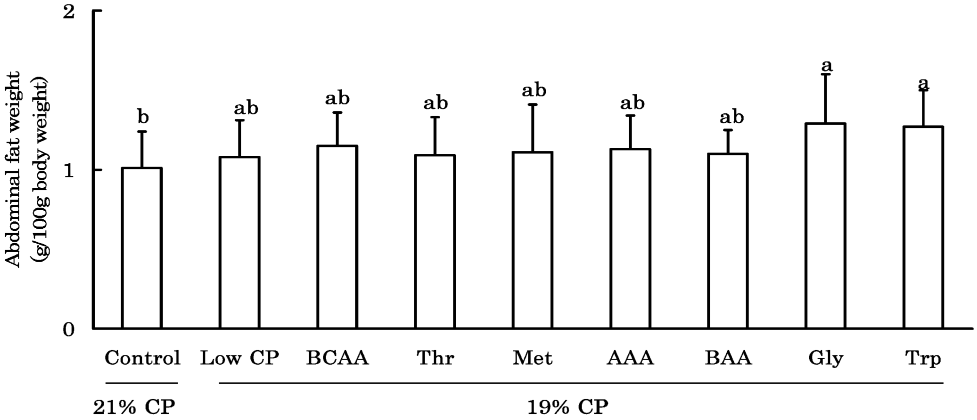

Fig. 2. **Effect of dietary excess amino acids (200% of requirement) on abdominal fat deposition of male broiler chicks (Experiment 2)**

[1] Each treatment consists of 9 birds.

[2] BCAA, branched-chain amino acid (leucine+isoleucine+valine) ; AAA, aromatic amino acid (phenylalanine+Tyrosine) ; BAA, basic amino acid (lysine+arginine)

[a, b] Means within the same rows with no common superscript are significantly different ($P<0.05$).

diet used in experiment 1. Yamazaki *et al.* (1998) suggested that optimum performance may be achieved by feeding higher EAA containing diet which provides about 120% of the amino acid requirement in low protein diet. This means that the JFS recommendation (1992) of amino acids for the age period 0 to 3 weeks appears to be low, when dietary CP content is lower than 21%.

Many amino acids, when fed in excess to growing chickens, cause toxic effects such as depressions in growth, decreases in feed intake (Han and Baker, 1993 ; Carew *et al.,* 1998). In the present experiments, excess amino acids supplementation did not influenced body weight gain, feed intake and feed efficiency. However, in experiment 2, excess Met produced the severest depression in performance, and this result agrees with Edmonds and Baker (1987), who observed that Met was the most toxic of all amino acids excesses when fed at the 4% level. In experiment 1 and 2, dietary sulfur-containing amino acids (SAA) content of Met supplemented diet was 1.35 and 1.80% (DL-Met content was 0.8 and 1.3%), respectively. Similar results have been observed previously in that excess Met up to 0.5% of the diet showed no adverse effects on either weight gain or feed efficiency, however, feed intake decreased markedly when fed the 1.0% excess Met diet (Han and Baker ,1993).

Chicks fed the low CP diets tend to excrete less nitrogen (g/3 days) than those fed control diets in both experiments, however, there were no significant difference. In our previous experiment (Yamazaki *et al.* 1998), nitrogen utilization and retention were increased when fed the higher EAA to NEAA ratio diets. However, in present experiments, excreted nitrogen expressed as percentages of nitrogen intake was not decreased when fed amino acid supplemented with excess level (data not shown), thus, excess amino acid may not be used to synthesize body protein, and excreted.

Deficiency of dietary SAA is known to increase the fat deposition in broilers. Huyghebaert and Pack (1996) observed that the fat content of drumstick were reduced with supplementation of SAA to the diet, which has low content of SAA. Bunchasak *et al.* (1996) also reported a decrease of abdominal fat deposition and triglyceride content of the liver of chicks when fed diets supplemented with Met and Cystine (Cys) to the diet containing 0.64% SAA. In their experiment, further supplementation of SAA beyond the requirement did not affect the abdominal fat content, and this observation is in line with present experiments, thus, excess SAA will not prevent the increment of abdominal fat deposition. Researches have been conducted to determine the effect of supplementing several amino acids, such as Arg (Leclercq *et al.,* 1994), Trp (Rogers and Pesti, 1990) , and these amino acids could reduce the body fat and hepatic lipid content of broiler chicks, respectively. However, the decrease was not obvious when supplemented beyond the requirement level. In our studies, excess Lys, Arg and Trp caused no changes in abdominal fat weight, thus, excess supplementation of these amino acids will not reduce fat deposition. In our previous report (Yamazaki *et al.,* 1998), feeding the diet which containing higher EAA ratio to nonessential amino acids resulted lower abdominal fat deposition. From the result of present experiments, in low CP diet fortified with EAA, further supplementation of each EAA could not reduce the fat deposition.

Han *et al.* (1992) showed that dispensable amino acid-N was necessary for optimal chick performance and body composition because amino-N (for dispensable amino acid) biosynthesis was also a limiting factor in a low protein diet. A decrease in the liver triglyceride content and abdominal fat depositon due to only 0.5% supplementation of Gly and Glu to the low protein diet, respectively, have been reported by Bunchasak *et al.* (1998). However, adding Gly and Glu to a low protein diet supplemented with synthetic EAA to the amounts in the control diet resulted in a higher carcass fat content (Deschepper and De Groote, 1995). Further studies are needed to clarify the relationship with these dietary amino acids to lipid metabolism of broiler.

In conclusion, feeding the diet that supplemented with excess EAA to the low protein diet had little effect on abdominal fat deposition of the broiler in 1 to 3 wks of age.

References

Agriculture, Forestry and Fisheries Research Council Secretariat, MAFF 1992 : Japanese Feeding Standard for Poultry. Tokyo, pp. 18–21 (in Japanese).

Bunchasak C, Tanaka K, Ohtani S and Collado CM. Effect of met+cys supplementation to a low-protein diet on the growth performance and fat accumulation of broiler chicks at starter period. Animal Science and Technology (Japan), 67, 956–966. 1996.

Bunchasak C, Tanaka K and Ohtani S. Effect of supplementing nonessential amino acids on growth performance and fat accumulation in broiler chicks fed a diet supplemented with Met+Cys. Japanese Poultry Science, 35, 182–188. 1998.

Carew LB, Evarts KG and Alster FA. Growth, feed intake, and plasma thyroid hormone levels in chicks fed dietary excesses of essential amino acids. Poultry Science, 77, 295–298. 1998.

Deschepper K and De Groote G. Effect of dietary protein, essential and non-essential amino acids on the performance and carcase composition of male broiler chickens. British Poultry Science, 36, 229–245. 1995.

Edmonds MS and Baker DH. Comparative effects of individual amino acid excesses when added to a corn-soybean meal diet : effects on growth and dietary choice in the chick. Journal of Animal Science, 65, 699–705. 1987.

Geraert PA, Leclercq B and Larbier M. Effects of dietary glucogenic amino acid supplementation on growth performance, body composition and plasma free amino acid levels in genetically lean and fat chickens. Reproduction, Nutrition and Development, 27, 1041–1051. 1987.

Han Y, Suzuki H, Parsons CM and Baker DH. Amino acid fortification of a low-protein corn and soybean meal diet for chicks. Poultry Science, 71, 1168–1178. 1992.

Han Y and Baker DH. Effects of excess methionine or lysine for broilers fed a corn-soybean meal diet. Poultry Science, 72, 1070–1074. 1993.

Huyghebaert G and Pack M. Effects of dietary protein content, addition of nonessential amno acids and dietary methionine to cysteine balance on responses to dietary sulphur- containing amino acids in broilers. British Poultry Science, 37, 623–639. 1996.

Leclercq B, Chagneau AM, Cochard T and Khoury J. Comparative responses of genetically lean and fat chickens to lysine, arginine and non-essential amino acid supply. I. Growth and body composition. British Poultry Science, 35, 687–696. 1994.

SAS Institute, 1988 : SAS/STAT™ User's guide, Release 6.03 Edition. SAS Institute Inc., Cray, NC. U.S.A.

Yamazaki M, Murakami H, Yamazaki M and Takemasa M. Reduction of nitrogen excreted from broiler chicks by feeding low-protein, amino acid-supplemented diets. Japanese Poultry Science, 33, 249–255. 1996.

Yamazaki M, Murakami H and Takemasa M. Effects of ratios of essential amino acids to nonessential amino acids in low protein diet on nitrogen excretion and fat deposition of broiler chicks. Japanese Poultry Science, 35, 19–26. 1998.