Effects of Methionine Supplementation in Low-Protein Diets and Subsequent Re-feeding on Growth Performance, Liver and Serum Lipid Profile, Body Composition and Carcass Quality of Broiler Chickens at 42 Days of Age

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This study was conducted to evaluate the effects of adding methionine (Met) to low-protein diets and subsequent re-feeding on the growth performance, serum lipid profile, body composition and carcass quality in male broiler chicks during 1–42 days of age. During the starter-grower period (1–21 days of age), 800 male broiler chicks (Ross 308) were divided into 5 groups and given the following diets: 1) positive control diet (all nutrients meet the requirements of the strain), 2) negative control diet (low-protein diet in which all amino acids meet the requirements of the strain, except Met), 3) negative control diet supplemented with Met to meet the total sulfur amino acid (TSAA) requirement at 100%, 4) negative control diet supplemented with Met to meet the TSAA requirement at 105%, and 5) negative control diet supplemented with Met to meet the TSAA requirement at 110%. During the finisher period (22–42 days of age), all groups were fed a diet containing the same nutrients and according to the recommendations for the strain. At Day 21, adding Met to the low-protein diet resulted in a similar body weight gain and feed conversion ratio (FCR) to that of the positive control group. Feed intake in the 105% TSAA group significantly increased compared to that in the positive control and negative control groups ($P < 0.05$). Met supplementation in the low-protein diet significantly improved the protein efficiency of chicks ($P < 0.01$), while Met deficiency increased feed cost per body weight gain ($P < 0.01$). Abdominal fat contained in chicks fed the low-protein diet was significantly increased, although Met supplementation slightly decreased the abdominal fat. In the 110% TSAA group, triglyceride in serum was significantly increased ($P < 0.01$), while low density lipoprotein-cholesterol in serum was significantly depressed ($P < 0.01$). After the re-feeding phase (at Day 42 of age), the growth performance of the negative control group was still significantly poorer than that of other groups ($P < 0.05$). Adding Met to the low-protein diet significantly improved the protein efficiency ratio compared to the positive and negative control groups ($P < 0.05$). The body compositions and abdominal fat of broiler chickens did not significantly differ among the treatments ($P > 0.05$). The carcass yield, breast meat and wings of the 110%TSAA group were significantly higher than those of the negative control group ($P < 0.05$). The results indicate that reducing the protein concentration with Met supplementation during 1–21 days of age, then re-feeding with a conventional diet is an appropriated tool for improving overall protein utilization and slightly reduces the production cost.

Key words: body composition, carcass, chicks, compensatory, low-protein, methionine

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Introduction

Feeding low-protein diets clearly reduces feed costs (Cauwenberghe and Burnham, 2001), and improves protein efficiency, whilst increasing fat accumulation (Faria Filho *et al.*, 2003) and decreasing the growth performance

of broiler chickens (Leeson and Summers, 1991). The negative effects of using a low-protein diet can be prevented by adding supplementary synthetic amino acids to the diet to improve the amino acid pattern balance (Khajali and Moghaddam, 2006; Saki *et al.*, 2007).

Methionine (Met) is the primary limiting amino acid in poultry diets, particular in a corn-soybean meal based diet (Jianlin *et al.*, 2004). Several researches have reported that the requirement for this amino acid is substantial but variable as a result of changes in the genetics, nutrition and management of broiler chicks (Kalinowski *et al.*,

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2003). Met acts as a lipotropic agent through its role as an amino acid in balancing protein, or through its role as a methyl donor and it is incorporated into carnitine and creatine (Kalinowski *et al.*, 2003). Focusing on fat metabolism, Bunchasak (1997) showed that the serum triglyceride level tends to be decreased, and the breast meat triglyceride content is significantly reduced when Met is added to diets, thus Met may have the potential to disturb the ability of lipid transporters such as lipoproteins (liver into blood).

In terms of economic returns, a low-protein diet supplemented with synthetic amino acids is still less efficient than the conventional diet (Hussein *et al.*, 2001). Improved growth performance due to amino acid supplementation can be inconsistent (Keshavarz and Austic, 2004), which may be due to several factors such as inadequate knowledge about essential amino acid requirements, the amino acid content of feed ingredients, the digestibility and bioavailability of amino acids in feed ingredients, and the proper ratio among essential amino acids in a low-protein diet. Moreover, Takahashi *et al.* (1994) reported that at 21 days of age, maximum performance cannot be achieved by feeding broilers a low-protein diet supplemented with amino acids, because young chicks are more sensitive to amino acid inadequacy than older birds. This means that sufficient amino acid intake during the starter period does not always achieve maximal growth performance, which depends on duration of feeding low-protein, age, degree of protein reduction as well as environmental conditions.

In light of the fact that many studies have reported improvements in feed efficiency during compensatory growth in restricted-refed broilers (Zubair and Leeson, 1996), the concept of feeding a low-protein diet with Met supplementation during the starter-grower period merits investigation along with associated compensatory responses in order to address the inconsistent effects of such a diet. Recently, moreover, Azarnik *et al.* (2010) recommended that supplementing a low-protein diet with amino acids during feed restriction may partially correct the depression in the growth performance of broiler after re-feeding.

Therefore, this study focused on the effects of Met supplementation in a low-protein diet during starter-grower periods and consequent re-feeding during the finishing period on the growth performance, lipid profile, body composition and carcass yields of male broiler chickens.

Materials and Methods

Animal and Managements

Eight hundred male broiler chicks (Ross 308) were used in this trial. The study was conducted in the animal research station of the Department of Animal Science (Bangkok). The chicks were divided into 5 treatment groups and each treatment group consisted of 8 replications of 20 chicks each. The chicks were kept in floor pens (0.09 m² floor space per bird) that were located in an evaporative cooling house system during 1–42 days of age. The temperature was set at 32°C at day-old and then was

decreased by 1°C every 3 days until a final temperature of 25°C was reached. The lighting management and vaccinations were provided according to commercial practice. Each pen was equipped with one hanging feeder and three nipple drinkers. The broiler chicks were allowed access to water and feed *ad libitum* throughout the experimental period.

Experimental Design and Diet

A completely randomized design was used. Five experimental diets (mash form) were provided as follows; 1) positive control diet (providing 22% CP during 1–10 days of age and 21% CP during 11–21 days of age; all nutrient requirements were adjusted as per strain recommendation), 2) negative control diet (providing 19% and 18% CP without Met supplementation, but other essential amino acids were according to strain requirements), 3) negative control diet supplemented with Met to meet TSAA requirement at 100%, 4) negative control diet supplemented with Met to meet TSAA requirement at 105%, and 5) negative control diet supplemented with Met to meet TSAA requirement at 110%. Except dietary CP and Met, all nutrients in low protein diets were formulated according to the recommendations of the strain. The broiler chicks were fed dietary treatments from 1 to 21 days of age. Subsequently, all chicks were fed a diet containing 19% CP and 3,225 ME kcal/kg of energy (according to the recommendations of the strain) during the finisher period (22–42 days of age). The feed formulas and nutrients contained in the diet are shown in Table 1. The supplemented levels of Met are presented in Table 2. The total sulfur amino acid/Lysine and Met/Lysine ratio are shown in Table 3. The quantities of Met supplementation are shown in Table 4.

Feed samples were collected and subsequently ground using a 1-mm screen in a grinder. All diets were analyzed for protein and ether extract according to AOAC (2000) methods. The amino acid composition of positive control and negative control diets in starter and grower periods was analyzed by Amino Acids Analyzer (AminoTac JEOL model JLC-500/V [JEOL Ltd., Tokyo, Japan]).

Growth Performance

The body weight and feed intake of the chicks were measured weekly. Subsequently, protein intake, protein efficiency, ADG and FCR were calculated from these data. Feed cost per body weight gain was estimated from the value of FCR multiplied by feed cost. Mortality was checked daily for calculation of the mortality rate.

Sampling Procedures

At 21 days of age, after overnight feed deprivation, all chicks were weighed. Eight chicks (one chick per pen) were randomly selected from each treatment for collection of blood from the wing vein and then sacrificed using CO₂ asphyxiation in an atmosphere of less than 2% oxygen (air displaced by CO₂) for 1.5–2.0 min. Subsequently, the liver and abdominal fat (including fat surrounding gizzard, Cabel *et al.*, 1987) of each bird were collected, weighed and stored at –20°C until used for chemical analysis.

Table 1. Composition of the experimental diets and chemical analysis

Ingredient	Amount				
	Positive control diet (1–10 days)	Negative control diet (1–10 days)	Positive control diet (11–21 days)	Negative control diet (11–21 days)	Conventional diet (22–42 days)
			(%)		
Corn	54.876	62.340	55.399	62.931	60.24
Rice bran oil	1.703	1.001	4.002	3.277	3.29
Soybean meal	36.194	28.593	34.172	26.572	25.43
Full fat soybean meal	2.000	2.000	2.000	2.000	7.00
Monodicalcium phosphate (P 21%, Ca 16%)	2.313	2.385	2.042	2.114	1.91
Lime stone	1.310	1.330	1.176	1.196	1.13
Salt	0.210	0.209	0.215	0.213	0.22
L-Lysine	0.298	—	0.103	—	—
DL-methionine	0.409	—	0.293	—	0.19
L-threonine	0.088	0.213	—	0.118	—
Premix	0.500	0.500	0.500	0.500	0.50
Sacox	0.050	0.050	0.050	0.050	0.05
Antioxidant	0.050	0.050	0.050	0.050	0.05
Corn starch	—	1.329	—	0.980	—
Total	100.00	100.00	100.00	100.00	100.00
Feed cost/kg (Thai Baht)	16.22	14.88	16.25	15.11	15.90
Chemical composition, calculated (analyzed)					
Crude protein (%)	22.00 (23.44)	19.00 (18.37)	21.00 (21.40)	18.00 (18.59)	19.00 (19.06)
Energy (ME) kcal/kg	3,010	3,010	3,175	3,175	3,225
Ether extract (%)	4.67 (4.55)	4.19 (4.00)	6.94 (7.54)	6.44 (6.21)	7.26 (8.10)
Calcium (%)	1.00	1.01	0.90	0.91	0.85
Avail. phosphorus (%)	0.50	0.51	0.45	0.45	0.42
Lysine (%)	1.44 (1.40)	1.02 (1.06)	1.23 (1.40)	0.96 (0.96)	1.02 (1.05)
Total sulfur amino acid (%)	1.09 (1.00)	0.62 (0.78)	0.95 (0.90)	0.59 (0.54)	0.80 (0.77)
* DL-methionine (%)	0.74 (0.63)	0.30 (0.25)	0.61 (0.51)	0.29 (0.24)	0.48 (0.43)
Threonine (%)	0.93 (0.84)	0.94 (0.81)	0.81 (0.75)	0.81 (0.75)	0.73 (0.68)
Arginine (%)	1.52 (1.46)	1.30 (1.22)	1.45 (1.42)	1.22 (1.15)	1.29 (1.24)
Isoleucine (%)	0.98 (0.94)	0.84 (0.80)	0.94 (0.93)	0.80 (0.72)	0.84 (0.79)
Leucine (%)	1.84 (1.81)	1.64 (1.59)	1.77 (1.77)	1.58 (1.48)	1.65 (1.60)
Valine (%)	1.07 (1.05)	0.93 (0.93)	1.02 (1.06)	0.88 (0.84)	0.93 (0.92)

Premix: vitamin A 12,000,000 IU, vitamin D3 3,000,000 IU, vitamin E 15,000 mg, vitamin K3 1,500 mg, vitamin B1 1,500 mg, vitamin B2 5,500 mg, vitamin B6 2,000 mg, vitamin B12 10 mg, nicotinic acid 25,000 mg, D-calcium pantothenate 12,000 mg, folic acid 500 mg, biotin 120 mg, manganese 80 g, zinc 60 g, iron 40 g, copper 8 g, iodine 0.05 g, cobalt 0.10 g, selenium 0.10 g; filler added to 1 ton.

* Synthetic DL-methionine was supplied by Sumitomo Chemical, Japan.

(...) Analytical analysis value.

Table 2. Dietary Met supplementation levels

	Positive control diet	Negative control diet (Low-protein diets)			
		Non-addition	100% TSAA	105% TSAA	110% TSAA
Starter period (1–10 days of age)					
Met (g/100 kg diet)	409	—	486	538	601
TSAA requirement (%)	1.09	0.617	1.09	1.14	1.20
Grower period (11–21 days of age)					
Met (g/100 kg diet)	293	—	370	419	469
TSAA requirement (%)	0.950	0.589	0.950	0.997	1.045

Table 3. Total sulfur amino acid/lysine and Met/Lysine ratios in experimental diets

	Positive control diet	Negative control diet (Low-protein diets)			
		Non-addition	100% TSAA	105% TSAA	110% TSAA
Starter period (1–10 days of age)					
TSAA/Lys ratio	0.756	0.605	0.756	0.791	0.833
Met/Lys ratio	0.512	0.294	0.539	0.574	0.618
Grower period (11–21 days of age)					
TSAA/Lys ratio	0.772	0.613	0.772	0.810	0.849
Met/Lys ratio	0.496	0.299	0.528	0.567	0.606

Table 4. Quantities of Met supplementation

	Positive control diet	Negative control diet (Low-protein diets)			
		Non-addition	100% TSAA	105% TSAA	110% TSAA
Starter period (1–10 days of age)					
Additional Methionine (g/100 kg)	409	0.00	486	538	601
Corn starch (g/100 kg)	0.00	1,329	297	245	182
Grower period (11–21 days of age)					
Additional Methionine (g/100 kg)	293	0.00	370	419	469
Corn starch (g/100 kg)	0.00	980	259	210	160

At 42 days of age, after overnight feed deprivation, all chickens were weighed. Sixteen chickens (two chickens per pen) were randomly selected from each treatment group for sacrifice using CO₂ asphyxiation for 1.5–2.0 min. Subsequently, the abdominal fat (including fat surrounding the gizzard, Cabel *et al.*, 1987) of each bird was collected and weighed. The carcass yield of a broiler chicken was defined as the carcass without blood, feathers and giblets. Breast (without skin), wings, thighs and drumsticks (with skin) were weighted and expressed as a percentage of live weight. For body composition analysis, the whole body of a broiler chicken (including feathers, abdominal fat and blood) from each replication was ground by an industrial mincer and then homogenized by a blender twice. Twenty gram samples of the homogenate were collected into a zip-lock bag and stored at –20°C until chemical analysis according to the method of AOAC (2000).

Biochemical Analyses

Liver lipid was measured according to the method of Sutton *et al.* (1984). The blood samples were centrifuged at 3,000 rpm for 15 minutes and the serum was decanted into aseptically treated vials. One gram of liver sample was taken and placed in a test tube with 5-fold ethanol: acetone (1:1 v/v), then homogenized at 20,000 rpm for 1 minute, centrifuged at 3,000 rpm for 10 minutes and the supernatant was collected in vials. The samples were stored at –20°C until further analyzed.

Serum and liver lipid samples were analyzed for triglyceride by enzyme colorimetric method (Test kits of Human Gesellschaft für Biochemica und Diagnostica

mbH, Co., Ltd, Germany). Low density lipoprotein-cholesterol (LDL-C) was measured by enzyme colorimetric method (Test kits of Roche Diagnostics GmbH, USA).

The homogenized whole body composition was subsequently analyzed for moisture, crude protein, total ash and fat according to the standard procedures of AOAC (2000). While, gross energy in the whole body was analyzed by bomb calorimeter (e2k isothermal bomb calorimeter, CAL2k, Digital Data Systems, South Africa).

Statistical Analysis

This experiment had a completely randomized design (CRD) with eight replications. Data were analyzed with analysis of variance (ANOVA) procedure using the following model. Significant difference among the mean of groups was separated by Tukey's honestly significant difference test at a 5% probability level.

$$Y_{ij} = \mu + A_i + \varepsilon_{ij}$$

When; Y_{ij} is the observed response, A_i is the effect of diets and ε_{ij} is the experimental error; $\varepsilon_{ij} \sim \text{NID}(0, \sigma^2)$.

Results and Discussion

Growth Performance

The effects of adding Met to low-protein diets during 1–21 days of age are given in Table 5. The body weight and FCR of the negative control group were significantly lower than those of other groups ($P < 0.01$), while adding Met to low-protein diets significantly improved the growth rate and FCR to levels similar to those of the positive control group. Feed intake in the low-protein diet with the 105% TSAA group was significantly higher than that of the positive and negative control groups ($P < 0.05$).

Table 5. Effects of adding Met to low-protein diets on productive performance of broiler chicks during 1–21 days of age

Item	Positive control	Low-protein			
		Negative control	100% TSAA	105% TSAA	110% TSAA
Initial body weight (g/chick)	43.86± 0.49	43.86± 0.38	43.86± 0.41	43.86± 0.42	43.86± 0.39
Body weight (g/chick)	787.51± 35.34 ^A	701.34± 19.74 ^B	785.39± 37.20 ^A	799.26± 14.63 ^A	788.46± 27.41 ^A
Body weight gain (g/chick)	743.65± 35.43 ^A	657.48± 19.88 ^B	741.52± 37.50 ^A	755.40± 14.63 ^A	744.60± 27.61 ^A
ADG (g/chick)	35.41± 1.69 ^A	31.31± 0.95 ^B	35.31± 1.78 ^A	35.97± 0.69 ^A	35.46± 1.31 ^A
Feed intake (g/chick)	1024.89± 22.23 ^b	1033.51± 29.60 ^b	1057.44± 44.42 ^{ab}	1070.57± 9.65 ^a	1051.09± 35.38 ^{ab}
Protein efficiency	3.37± 0.10 ^B	3.44± 0.07 ^B	3.79± 0.12 ^A	3.81± 0.06 ^A	3.82± 0.08 ^A
Protein intake (g/chick)	220.35± 4.78 ^A	191.20± 5.48 ^B	195.63± 8.22 ^B	198.05± 1.78 ^B	194.45± 6.54 ^B
FCR	1.38± 0.04 ^B	1.57± 0.03 ^A	1.43± 0.04 ^B	1.41± 0.02 ^B	1.41± 0.03 ^B
TSAA intake (g/chick)	10.09± 0.21 ^B	6.16± 0.17 ^C	10.41± 0.43 ^B	11.05± 0.10 ^A	11.38± 0.38 ^A
Feed cost/body weight gain (baht/kg)	23.01± 0.52 ^{AB}	23.50± 0.26 ^A	22.72± 0.53 ^B	22.70± 0.52 ^B	22.49± 0.42 ^B
Mortality rate (%)	2.50± 2.67	0.00± 0.00	1.25± 2.31	1.87± 2.58	1.87± 2.58

Mean±SD.

^a and ^b Treatment means with different superscripts in the same row are significantly different ($P<0.05$).^A, ^B and ^C Treatment means with different superscripts in the same row are significantly different ($P<0.01$).

Table 6. Effects of adding Met to low-protein diets during starter and subsequent re-feeding on growth performance of broiler chickens during 22–42 days of age

Item	Positive control	Low-protein			
		Negative control	100% TSAA	105% TSAA	110% TSAA
Body weight gain (g/chicken)	1875.41± 47.04 ^a	1790.67± 51.64 ^b	1835.27± 55.64 ^{ab}	1837.82± 63.56 ^{ab}	1829.17± 48.29 ^{ab}
ADG (g/chicken)	89.30± 2.24 ^a	85.27± 2.46 ^b	87.39± 2.65 ^{ab}	87.51± 3.03 ^{ab}	87.10± 2.30 ^{ab}
Feed intake (g)	3448.18± 102.65 ^a	3297.04± 100.74 ^b	3408.86± 80.41 ^{ab}	3376.90± 53.27 ^{ab}	3379.57± 103.62 ^{ab}
TSAA intake (g/chick)	27.58± 0.82 ^a	26.37± 0.80 ^b	27.27± 0.64 ^{ab}	27.01± 0.43 ^{ab}	27.04± 0.83 ^{ab}
Protein intake (g)	655.15± 19.50 ^a	626.44± 19.14 ^b	647.68± 15.28 ^{ab}	641.61± 10.12 ^{ab}	642.12± 19.69 ^{ab}
FCR	1.84± 0.07	1.84± 0.04	1.86± 0.04	1.84± 0.07	1.85± 0.09
Protein efficiency	2.86± 0.11	2.86± 0.07	2.83± 0.05	2.86± 0.11	2.85± 0.13
Feed cost/body weight gain (baht/kg)	29.25± 1.12	29.28± 0.73	29.54± 0.58	29.25± 1.16	29.40± 1.38

Mean±SD.

^a and ^b Treatment means with different superscripts in the same row are significantly different ($P<0.01$).

The TSAA intakes of low-protein diets in the 105% TSAA and 110% TSAA groups were higher than those in other groups. The protein intake of the positive control group was the highest ($P<0.01$), while adding Met to low-protein diets showed better protein efficiency rates (PER) than the positive and negative control groups ($P<0.01$). Moreover, feed cost per body weight gain of the negative control group was the most expensive ($P<0.01$). The mortality rate of chicks was not significantly different among the experimental groups ($P>0.05$).

Growth performances of the chickens during the re-feeding phase (22–42 days of age) are shown in Table 6. The negative control group still had significantly lower ADG, feed intake, TSAA intake and protein intake than the positive control group ($P<0.05$), although adding Met to the low-protein diet groups showed no significant difference compared to the positive control group and the negative control group. FCR, PER and feed cost per body weight gain were also unaffected by dietary treatments (P

>0.05).

Overall growth performances of chickens (1–42 days of age) are shown in Table 7. Body weight and ADG of the negative control group were significantly poorest ($P<0.05$), while adding Met to the low-protein diet groups significantly increased growth rates to levels similar to those of the positive control group. Feed intake was not significantly different among treatment groups ($P>0.05$), although the negative control group tended to decrease feed intake compared to the positive control group and low-protein diet groups with 100 and 105% TSAA ($P=0.07$). TSAA intake was lower for the negative control group than for other groups ($P<0.01$). Adding Met to the low-protein diet significantly improved the FCR of broiler chickens compared to the negative control group, and had better PER than the positive and negative control groups ($P<0.05$). The feed costs per body weight gain of the chickens fed low-protein diets supplemented with Met were slightly lower than those of the positive and negative

Table 7. Effects of adding Met to low-protein diets during starter and subsequent re-feeding on growth performance of broiler chickens during 1–42 days of age

Item	Positive control	Low-protein			
		Negative control	100% TSAA	105% TSAA	110% TSAA
Body weight (g/chicken)	2662.92 ± 50.03 ^A	2492.01 ± 55.14 ^B	2620.66 ± 82.21 ^A	2637.08 ± 60.44 ^A	2617.64 ± 40.49 ^A
Body weight gain (g/chicken)	2619.05 ± 49.87 ^A	2448.15 ± 55.01 ^B	2576.80 ± 82.57 ^A	2593.22 ± 60.41 ^A	2573.78 ± 40.73 ^A
ADG (g/chicken)	62.36 ± 1.19 ^A	58.29 ± 1.31 ^B	61.35 ± 1.97 ^A	61.74 ± 1.44 ^A	61.28 ± 0.97 ^A
Feed intake (g)	4473.07 ± 115.39	4330.55 ± 121.65	4466.30 ± 102.82	4447.47 ± 50.62	4430.66 ± 129.40
TSAA intake (g/chick)	37.68 ± 0.94 ^A	32.53 ± 0.93 ^B	37.68 ± 0.88 ^A	38.06 ± 0.40 ^A	38.42 ± 1.11 ^A
Protein intake (g)	875.51 ± 22.28 ^a	817.64 ± 23.00 ^b	843.31 ± 19.38 ^b	839.67 ± 9.63 ^b	836.57 ± 24.44 ^b
FCR	1.71 ± 0.04	1.77 ± 0.04	1.73 ± 0.03	1.71 ± 0.05	1.72 ± 0.05
Protein efficiency	2.99 ± 0.08 ^b	2.99 ± 0.06 ^b	3.05 ± 0.05 ^{ab}	3.09 ± 0.08 ^a	3.08 ± 0.09 ^a
Feed cost/body weight gain (baht/kg)	27.46 ± 0.58	27.86 ± 0.57	27.35 ± 0.29	27.01 ± 0.51	27.13 ± 0.71

Mean ± SD.

^a and ^b Treatment means with different superscripts in the same row are significantly different ($P < 0.05$).^A and ^B Treatment means with different superscripts in the same row are significantly different ($P < 0.01$).

control groups.

The low-protein induces an imbalance of the amino acid pattern in the diet (Saki *et al.*, 2007). Azarnik *et al.* (2010) showed that the body weight at 21 days of the chicks fed a low-protein diet was significantly lower than that of the groups fed a higher protein diet. Since low TSAA intake depresses the feed intake and production performance of broiler chicks (Khajali *et al.*, 2002; Bunchasak, 2009), adding Met clearly prevents this negative effect via improvement of the amino acid balance and increment of feed consumption (Takahashi *et al.*, 1994). During 1–21 days of age, therefore, a close positive correlation between TSAA intake and body weight gain of broilers was found ($r=0.98$), whereas the feed intake of the negative control was similar to that of the positive control group. However, Waldroup *et al.* (1976) stated that if a slight amino acid deficit exists, the chick will attempt to compensate by consuming more food, subsequently the growth rate may reach a maximum. In the current study, chicks fed the negative control diet have a lower Met consumption by about 40% and a lower growth rate by 11.5% compared to chicks fed the positive control diet. On the other hand, adding Met to a low-protein diet at 105% of the TSAA requirement resulted in a higher feed intake than the positive and negative control groups. Met deficiency at a level of around 60% of the TSAA recommendation (negative control group) suppresses the growth rate without any effect on feed intake, which indicates that factors affecting and the mechanism controlling feed intake involved with Met supplementation are complicated.

PER is calculated from weight gain divided by the amount of protein consumed (weight gained/protein intake) and shows the direct effect of protein quality on growth rates (Mitchell *et al.*, 1989). In this study, PER was improved in Met supplementation groups compared to the positive and negative control groups. Similarly, Bunchasak (1997) demonstrated that adding Met to a

low-protein diet improved the feed efficiency and PER value of broiler chicks. This means that feeding a low-protein diet supplemented with Met clearly promotes protein utilization better than feeding a low-protein diet alone. Additionally, feed costs per body weight gain in the Met supplementation groups tended to be decreased when compared with the positive control group. As long as feed intake is maintained by Met supplementation to the normal range, adding Met to low-protein diets may be a method of reducing the production cost of broiler chicks.

Focusing on the compensatory responses, during 22–42 days of age, it seems that chicks did not positively respond to the strategy of re-feeding by a conventional diet after feeding a low-protein diet with or without Met supplementation. Accordingly, Plavnik and Hurwitz (1990) showed that *ad libitum* feeding of a diet containing 9.4% crude protein from 8 to 14 days decreased the feed intake of broilers by some 57% and 41% growth retardation, which was not recovered after 6 weeks of realimentation.

For the overall feeding period (1–42 days), the growth rate of the negative control group was significantly poorer than that of other groups, whereas adding Met to low-protein diets still increased the growth rate and FCR to levels equal to those of the positive control group. Moreover, adding Met at the level of 105% and 110% TSAA requirement to a low-protein diet had significantly better PER and slightly lower feed cost/gain than positive and negative control diets. Since feeding a low-protein diet significantly depresses protein intake, this generally promotes better protein utilization (Hevia and Clifford, 1977; Cheng *et al.*, 1997). Taniguchi *et al.* (2008) also demonstrated that adding Met to a 10% soybean protein isolate (SPI) diet improved PER from 2.28 to 2.97. After the re-feeding phase, feeding a low-protein diet supplemented with Met during starter-grower periods promotes protein utilization and lowers production cost compared to feeding a low-protein diet or a conventional diet alone.

Table 8. Effects of methionine supplementation in low-protein diets on abdominal fat pad, lipoprotein and triglyceride in the liver and serum of broiler chicks at 21 days of age

Item	Positive control	Low-protein			
		Negative control	100% TSAA	105% TSAA	110% TSAA
Liver weight (g)	21.02 ± 1.40	21.01 ± 1.04	21.06 ± 2.22	22.23 ± 2.55	22.55 ± 2.94
Liver weight (% of live weight)	2.58 ± 0.13	2.90 ± 0.18	2.59 ± 0.25	2.72 ± 0.31	2.74 ± 0.35
Liver					
TG (mg/g)	18.08 ± 2.77	15.95 ± 2.26	17.47 ± 3.13	15.03 ± 1.82	17.44 ± 2.84
Abdominal fat pad (% live weight)	1.35 ± 0.32 ^b	2.00 ± 0.24 ^a	1.84 ± 0.40 ^a	1.95 ± 0.27 ^a	1.89 ± 0.61 ^a
Serum					
TG (mg/dl)	118.22 ± 75.43 ^B	115.50 ± 77.49 ^B	119.01 ± 40.82 ^B	151.71 ± 46.75 ^{AB}	203.90 ± 109.66 ^A
LDL-C (mg/dl)	31.86 ± 6.25 ^A	22.88 ± 2.53 ^B	23.38 ± 4.50 ^B	26.88 ± 5.49 ^{AB}	24.50 ± 3.78 ^B

Mean ± SD.

^a and ^b Treatment means with different superscripts in the same row are significantly different ($P < 0.05$).^A and ^B Treatment means with different superscripts in the same row are significantly different ($P < 0.01$).

Fat Accumulation

Measurements of abdominal fat pads, serum lipoprotein and triglyceride of chicks at 21 days of age are shown in Table 8. The weight of the abdominal fat pads of the low-protein diet groups (with or without Met supplementation) was significantly heavier than that of the positive control group ($P < 0.05$), while abdominal fat was slightly decreased by Met supplementation, and a negative correlation between TSAA intake and abdominal fat was found ($r = -0.69$). The liver of chicks fed the low-protein diet without Met supplementation seems to be bigger than that of other groups. Liver triglyceride level was not significantly affected by the treatments, but a negative correlation between liver weight (% live weight) and liver triglyceride level ($r = -0.62$) was found. The serum LDL-C level of the positive control group was significantly increased ($P < 0.05$) compared to that of other groups except for the 105% TSAA group.

Abdominal fat constitutes approximately 2–3% of the broiler live weight (Leenstra, 1986). Sex, age, feed efficiency, genotype and feeding are some of the main factors affecting abdominal fat in broilers. In the present study, the weight of abdominal fat pads of the chicks fed low-protein diets (with Met and without Met) was higher than that of the positive control group. According to Yamazaki *et al.* (2006) who demonstrated that chicks fed low-protein and amino acid supplemented diets had significantly increased abdominal fat weight compared to that of chicks fed a control diet. Aletor *et al.* (2000) and Faria Filho (2003) indicated that decreasing dietary protein generally increases the relative weights of the abdominal fat to body weight. This phenomenon may be due to high feed intake or less energy used for protein synthesis. According to several investigators, feeding low-protein with amino acid supplementation increases feed consumption, and consequently increases carcass fat deposition (Rosebrough and Steele, 1985). Moreover, the negative control group in our study had a higher energy: protein ratio than that of the positive control group. Diets with a high energy: protein ratio promote energy retention as fat

(Kamran *et al.*, 2008). One possible interpretation is that a high energy/protein ratio forces over-consumption of dietary energy, in order to meet the protein and amino acid requirements. Conversely, diets with a low calorie/protein ratio are believed to decrease carcass fat, because broilers consume less energy in order to meet the protein requirements (Aletor *et al.*, 2000). Another reason is that chicks fed a low-protein diet need less energy for nitrogen excretion as uric acid and provide more energy for lipid synthesis (Bartov, 1979). Adding Met to low-protein diets may promote growth performance and protein utilization, but high fat accumulation still remains. Therefore, supplementing low-protein diets with Met until the market weight is reached may be inappropriate because high fat deposition in the carcass is not wanted by consumers.

Lipogenesis mainly occurs in the liver of poultry. Aletor *et al.* (2000) reported that decreasing dietary protein increased liver weight, which might be caused by high activities of lipogenetic enzymes. Focusing on the effect of Met, Takahashi and Akiba (1995) reported that supplementing 0.93% of TSAA in the corn soybean meal-glutamic acid diet decreased the activity of malic enzyme, which is closely related with lipogenesis in the liver. Austic (1985) also showed that Met deficiency in the diet increased abdominal fat deposition and malic enzyme activity in the liver. The results of the present study show a low-protein diet deficient in Met tended to increase liver weight (% of body weight) and high abdominal fat accumulation was found. Conversely, adding Met to low-protein diets tended to reduce the liver weight, consequently a negative correlation between TSAA intake and liver weight per body weight gain was found ($r = -0.63$). The decrease in liver weight (as% of body weight) may be attributed to balanced amino acids by Met supplementation, since amino acid imbalance stimulates protein metabolism in the liver or increased rate of amino acid synthesis in order to provide sufficient amino acids (Hiramoto *et al.*, 1990).

There was no significant difference in the triglyceride content in the liver among the experimental groups. In

Table 9. Effects of methionine supplementation in low-protein diets during starter and grower periods and subsequent re-feeding on the body composition of broiler chickens at 42 days of age

Item	Positive control	Low-protein			
		Negative control	100% TSAA	105% TSAA	110% TSAA
Moisture (%)	65.19±1.16	65.38±1.75	64.77±1.94	64.61±1.53	65.16±1.83
Gross energy (kcal/g)	6.03±0.08	5.98±0.14	6.00±0.24	5.99±0.09	5.97±0.13
Total ash (%)	2.43±0.14	2.47±0.24	2.49±0.30	2.49±0.24	2.56±0.21
Fat (%)	14.32±0.80	13.73±0.62	14.42±0.91	13.68±1.06	13.46±0.42
Protein (%)	18.09±0.59	17.79±0.85	18.22±0.89	18.44±0.77	17.85±1.12

Mean±SD.

poultry, triglyceride is synthesized in the liver and transported by the blood system and then taken up into adipose cells by lipoprotein lipase (Bunchasak *et al.*, 1997). High triglyceride content in the liver could be caused by a nutrition imbalance such as low-protein, high energy, amino acid imbalance and amino acid deficiency (Griffith *et al.*, 1969), lipotropic (Met, choline and vitamin B₁₂) deficiency (Chen *et al.*, 1993). In laying hen studies, triglyceride and lipid in the liver were increased by supplementing the diet with Met (Bunchasak and Silapasorn, 2005). Therefore, the effect of Met on the fat metabolism of immature birds (during starter period of broiler chicks) may be different from that of mature birds (laying hens), since laying hens need more fat synthesis to support egg production (fat accumulation in yolk), which involves the activity of the hormone estrogen.

Triglyceride is produced by the esterification process of acyl-CoA and glycerol-3-phosphate in the liver, and then transported via blood circulation to extra-hepatic tissue by lipoprotein (Hermier *et al.*, 1984). Apart from *de novo* lipogenesis, fat deposition in adipose tissue depends on a change in the relative activity of lipoprotein lipase, which mediates the uptake of fatty acid from the blood and of hormone sensitive lipase, which mediates the output of fatty acid from adipose tissue (Paik and Yearick, 1978). Adding Met to a low-protein diet linearly increased the triglyceride concentration in serum ($r=0.86$) and a positive correlation between TSAA intake and serum triglyceride level ($r=0.64$) was also found.

The turnover rates of plasma cholesterol and LDL-C are higher in chickens fed a high protein diet compared with those fed a low-protein diet (Yeh and Leveille, 1973). In our study, the serum LDL-C level in chicks fed a high protein diet was higher than that in those fed low-protein diet, while serum LDL-C was decreased by Met supplementation, while positive correlation between TSAA intake and serum LDL-C levels was found ($r=0.63$) among low-protein supplemented with Met groups.

Body Composition

Whole body compositions of broiler chickens after the re-feeding phase (at 42 days of age) are presented in Table 9. Dietary treatments did not significantly affect the moisture, gross energy, total ash, fat and protein in the whole body of broiler chickens ($P>0.05$).

The body composition of broilers is affected by many factors such as strain, age, sex, quality and quantity of the dietary protein and energy, slaughter, sampling method and environmental conditions (Koide and Ishibashi, 1995). Our study indicates that the experimental diets had no effects on the whole body composition of broiler chickens after the re-feeding phase. Nevertheless, the groups with added Met tended to have decreased whole body fat. According to Bunchasak (1997) who reported that carcass compositions of broiler chicks showed no significant difference by adding Met to low-protein diets after the re-feeding period. An additional study, Auckland and Morris (1971) demonstrated that turkeys fed lower protein diets during the early rearing period resulted in weight gain and carcass composition similar to that of control-fed birds at 20 weeks of age. Pokniak and Cornejo (1982) reported no significant difference in body composition between previously restricted and full-fed chickens at 8 weeks of age. The study of Priemnguluan (2000) also reported that adding Met to low-protein diets during the starter period did not affect the whole body composition of broiler chickens after the re-feeding phase, although abdominal fat pads during the starter period were increased. This indicates that reducing the protein intake with Met supplementation during the starter-grower period followed by re-feeding with a conventional diet induces changes in the chemical composition of the bodies of chicks similar to the case of feeding a conventional diet throughout the experimental period.

Carcass Qualities

Carcass qualities of broiler chicks at 42 days of age are shown in Table 10. After the re-feeding phase, the carcass yield, outer breast, inner breast and wings of the 110% TSAA group were significantly higher than those of the negative control group ($P<0.05$). However, dietary treatments did not significantly affect the thighs, drumsticks and abdominal fat ($P>0.05$).

Since no significant compensatory response was shown during the re-feeding phase, the carcass and breast meat yields of the negative control group were still inferior to those of the other groups, which is in agreement with the findings of the study of Moran (1979), while the group of chickens fed 110% TSAA had higher breast meat yield than the negative control group and equal breast meat

Table 10. Effects of Met supplementation in low-protein diets during starter and grower periods on carcass quality and abdominal fat of broiler chickens at 42 days of age

Item	Positive control	Low-protein			
		Negative control	100% TSAA	105% TSAA	110% TSAA
Carcass yield (%)	77.99±1.49 ^{ab}	76.56±1.01 ^b	77.53±0.87 ^{ab}	76.87±1.51 ^{ab}	78.57±1.16 ^a
Outer breast (%)	14.63±1.52 ^a	12.58±1.06 ^b	14.06±1.55 ^{ab}	14.15±0.59 ^{ab}	15.24±1.14 ^a
Inner breast (%)	3.68±0.33 ^{ab}	3.30±0.25 ^b	3.71±0.32 ^{ab}	3.47±0.14 ^{ab}	3.75±0.37 ^a
Thigh (%)	12.27±1.44	11.51±0.74	11.92±0.76	11.57±1.42	12.39±1.05
Drumstick (%)	9.99±0.69	9.16±0.63	9.31±0.63	9.41±0.74	9.89±0.51
Wing (%)	7.63±0.48 ^a	6.98±0.19 ^b	7.40±0.39 ^{ab}	7.50±0.45 ^{ab}	7.71±0.38 ^a
Abdominal fat (%)	2.01±0.29	2.05±0.32	2.08±0.47	2.18±0.29	2.15±0.48

Mean ± SD.

^a and ^b Treatment means with different superscripts in the same row are significantly different ($P < 0.05$).

yield to the positive control group. This indicates that high TSAA intake (110% TSAA group; TSAA intake = 38.42 g/chick) produces high breast meat yield of broilers. A positive correlation between TSAA intake and outer breast meat yield was found ($r=0.91$). Accordingly, Moran (1994) reported that sulfur amino acid increased the mass of breast meat yield more than that of other amino acids. The study of Saki *et al.* (2007) demonstrated that Met supplementation (120% of NRC recommendation, 0.46 and 0.38%, at starter and grower stage) in a low-protein diet improved carcass quality and breast meat of broilers. Several investigators have also reported that excess Met level in the diet elevates breast meat yield (Schutte and Pack, 1995; Hickling *et al.* 1990).

The abdominal fat pads of broiler chickens at 42 days of age (Table 10) showed no significant difference among treatments, in contrast to the results at 21 days of age (Table 8). Abdominal fat may not be a good index of carcass fatness in male broilers (Rosebrough and Steele, 1985), but the results of fat accumulation in the whole body also show no significant difference among treatment groups after the re-feeding phase (Table 9). Although no compensatory response in terms of growth performance was shown, it is clear that fat accumulation was reduced after the re-feeding phase.

We suggest that feeding a low-protein diet with Met supplementation at 105% or 110% of the TSAA requirement during the starter-grower period and subsequent re-feeding is an effective tool for improving broiler chicken production.

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