International Journal of Poultry Science 4 (5): 301-308, 2005
ISSN 1682-8356

Effects of Adding Methionine in Low-Protein Diet on Production Performance, Reproductive Organs and Chemical Liver Composition of Laying Hens under Tropical Conditions

Chaiyapoom Bunchasak[1] and Taweesak Silapasorn[2]
[1]Department of Animal Science, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand, 10900
[2]Feed Technology Office, Charoen Pokphand Co. Ltd., 313 C.P. Tower Floor 14th, Silom Road, Bangkok, Thailand, 10500
E-mail: agrchb@ku.ac.th

Abstract: This study was conducted to determine the effects of adding DL-methionine (Met) in low-protein diet (Low-CP; 14% CP) on egg production, reproductive organs, abdominal fat and chemical liver composition of laying hens under tropical conditions. Four hundred and eighty commercial laying hens (Isa-brown) during 24-44 weeks of age were fed with the Low-CP diet containing 0.26, 0.30, 0.38 or 0.44% Met. Dietary protein 16% CP with 0.38% Met was offered as the positive control group. The results showed that hen-day egg production, egg mass and feed conversion ratio of hens received Low-CP diet with 0.26% Met was the worst among experimental groups (P<0.01). Increasing Met to 0.44% could improve hen-day egg production and egg weight as same as the control group, although FCR and egg mass of all Low-CP diet with various levels of Met were still significantly poorer (P<0.05). Interestingly, adding synthetic Met to Low-CP diet resulted in significant reduction of mortality rate compared to the Low-CP diet without adding Met and the control group (P<0.05). Hens received Low-CP diet with 0.44% Met had lower yolk egg percentage than 0.26% Met group. Moreover, higher eggshell thickness, ovary weight, oviduct weight and abdominal fat weight in supplementing Met groups were observed (P<0.05). However, adding Met could not achieve development of reproductive organs compared to the control. Liver fat and abdominal fat contents were linearly increased with the Met supplementation, while the liver protein was inversely reduced (P<0.05). In conclusion, the adding Met in Low-CP diet may give advantage to the hen raised under hot climate, particularly in case of mortality rate and eggshell thickness.

Key words: Low-protein, methionine, laying-hen, production performance, tropical

Introduction

It is generally known that high environmental temperatures always negatively affect to poultry performance such as feed intake, live weight gain and feed efficiency decrease (Donkoh, 1989; Siegel, 1995), abdominal fat increase (McNaughton *et al*., 1984), egg weight decrease (Peguri and Coon, 1991) and eggshell quality decrease (Deaton *et al*., 1981; Emery *et al*., 1984; Grizzle *et al*., 1992); therefore, egg production and eggshell quality of laying hens often suffer in tropical environments, including in Thailand.

Thailand is located in tropical zone, average temperature is around 30°C. Under these conditions, heat stress is particularly a great problem when hens are kept in convention naturally ventilated houses, which have proven ineffective in many regions of country. Because of the high cost of cooling animal buildings, dietary manipulations are normally applied to alleviate the negative effects of high environmental temperature on performance of laying hens.

It is well established that dietary protein produce a high heat increment, adversely affecting hen performance during periods of high environmental temperatures. Austic (1985); Waldroup *et al*. (1976) and Waldroup (1982), therefore, have recommended a reduction in dietary protein content with suitable supplementation by essential amino acids to alleviate negative effects of heat stress. Although there were several reports indicated that increasing dietary protein level could improve growth in heat-exposed chicks (Cahaner *et al*., 1995; Temim *et al*., 1999).

The levels and balance of amino acids in the diets are all important nutritional variables that affect the economic efficiency of an egg laying enterprise (Al-Saffar and Rose, 2002). DL-methionine (Met) is normally considered to be the first-limiting amino acid in poultry diet. In general, amino acid balance and nitrogen retention are improved by Met supplementation. Recently, low dietary protein with synthetic amino acid supplementation is becoming relevant in feed formulation to minimize the nitrogen excretion and production cost (Chung *et al*., 1998).

Summers *et al*. (1991) have shown that the supplementation of a low protein (10% CP) diet with 0.32% Met resulted in a 10% increase in egg mass. Sell and Rogler (1983); Chung *et al*. (1998); Ravikiran and Devegowda (1998) also found that supplementing Met to low protein diet resulted in an improvement of egg production similar to the higher protein diet. Moreover, increasing dietary Met intake significantly increased egg weight (Harm and Russell, 1993) and decrease of abdominal fat content in broiler chicks (Bunchasak *et al*., 1997).

Hence, this study was aimed to investigate effects of adding Met in low protein on egg production, reproductive organs, abdominal fat and chemical liver composition of laying hens under tropical conditions.

Materials and Methods

Hens and diets: The study was completely randomized design. Four hundred and eighty commercial laying hens (Isa-brown) from 24 to 44 weeks of age were used. The hens were divided into 5 groups; each group consisted with 4 replications of twenty four layers each, and then hens were kept in multiple-cage located in conventional house system (an open-side poultry house). Cage dimensions were 40.5 cm × 40 cm equaling 1,620 cm^2 total floor space. With 3 hens per cage, each bird had approximately 540 cm^2 of floor space. The lighting period was provided for 16 hours from 05:00 to 21:00 daily. Water and feed in mash form were supplied *ad libitum* throughout the experiment.

To test the effects of Met, hens were fed with Low-CP diet (14% CP; 2,900 ME Kcal/kg diet) containing 0.26, 0.30, 0.38 or 0.44% Met. The diet contained 16% CP with 0.38% Met and 2,900 ME Kcal/kg diet was offered as positive control group. Amino acids pattern and other nutrients in diets were adjusted according to the recommendation of NRC (1994) (Table 1 and 2). Feed samples were collected and subsequently ground using a 1-mm screen in grinder. All diets were analyzed for protein, fat, fiber, ash, calcium and phosphorus according to the AOAC (1990) methods. Gross energy contents in diets were determined by bomb-calorimeter. Amino acids composition in both 16% CP and 14% CP diets (without supplementing synthetic amino acids) were determined using high performance liquid chromatography (HPLC) (Cohen and Michaud, 1993).

Measurements: Hen-day egg production and feed intake were recorded daily whereas egg weight were determined 4 weeks interval (5 periods), regularly in the same day of period. The hens were weighed at the beginning and final date of the experiment, gain or loss was then calculated. Egg mass was calculated by multiplying egg weight by hen-day egg production percentage. Feed conversion ratio (FCR) was calculated as gram feed consumption per day per hen divided by gram egg mass per day per hen. During 3 days of the end of each period, 16 eggs from each group were randomly taken in order to determine egg weight, egg component (percentage of egg yolk, egg albumen and eggshell), and albumen high as well as eggshell thickness.

At the end of experiment, after overnight feed deprivation, 2 hens from each replication that had body weight closed to the replicate mean were chosen and killed to evaluate the development of reproductive organs, chemical liver composition and abdominal fat content. The livers were immediately taken and stored at -20^{o}C until the chemical analysis (AOAC, 1990).

Statistical analysis: All data were statistically analyzed using analysis of variance (ANOVA). The differences between the means of groups were separated by Duncan's Multiple Range Test (Duncan, 1955). Statements of statistical significance are based on $P<0.05$. All statistical analyses were done in accordance with the method of Steel and Torrie (1980). Linear regression and correlation were also used for statistical analysis.

Results

Environmental conditions and Production Performance: During an experimental period, the temperature was gradually elevated from 29.06±6.52 to 34.60±5.71^{o}C. So, an average temperature throughout the experiment was 31.59±6.16^{o}C (Fig. 1) and relative humidity was 66.65±7.98%. Feed intake, protein intake, Met intake, production performance and mortality rate were shown in Table 3. The Low-CP diet with 0.26% Met had significantly depressed feed consumption of hens compared to other experimental groups ($P<0.01$).

The control group had highest protein intake, at the same time as protein intake of the Low-CP diet with 0.26% Met was the lowest ($P<0.01$). However, Met intake was significantly linearly increased as the supplemental levels increased ($P<0.01$). In addition, increased dietary Met intake significantly improved hen-day egg production, egg mass, egg weight, FCR and mortality of hens. Adding Met at 0.38% or 0.44% diet exhibited a less pronounced negative effect of low protein intake and had significantly lower mortality rate than the control diet ($P<0.05$), although egg mass and FCR of the control group were still significantly better than all adding Met groups.

Egg components: Egg components were significantly affected by supplemental dietary Met (Table 4). Low-CP diet tended to improve albumen high ($P=0.054$). Moreover, addition of Met to the Low-CP diet resulted in an increasing percentage of egg albumen, while inversely decreased percentage of egg yolk ($P<0.01$). Eggshell thickness of eggs from hens fed Low-CP diet without Met supplementation (0.26% of diet) was the worst ($P<0.01$). However, addition of Met also allowed an increase of the eggshell thickness. Consequently, the

Table 1: Composition of experimental diets

	Control (16% CP)	Low-protein diet (14% CP)			
	0.38% Met	0.26% Met	0.30% Met	0.38% Met	0.44% Met
Corn	69.70	72.50	72.40	72.17	72.00
Soy bean meal (44%)	17.00	16.50	16.50	16.60	16.60
Fish meal (56%)	5.50	1.68	1.69	1.70	1.70
Palm oil	0	0.42	0.46	0.50	0.57
DL-Methionine	0.07	0	0.05	0.11	0.18
DCP (P/18)	0	0.85	0.85	0.87	0.9
Oyster Shell	7.28	7.50	7.50	7.50	7.50
Salt	0.20	0.30	0.30	0.30	0.30
Premix[1]	0.25	0.25	0.25	0.25	0.25
Total (kg)	100.00	100.00	100.00	100.00	100.00

Premix: Lutavit® Mix CNK004 consist of Vitamin A 4.80 MIU; D_3 0.96 MIU; E 3.20 g; K_3 0.80 g; B_1 0.40 g; B_2 1.60 g; B_6 1.20 g; B_{12} 0.004 g; Pantothenic acid 3.80 g; Niacin 6 g; Folic acid 0.20 g; Biotin 0.036 g; Se 0.04 g; Fe 24 g; Mn 24 g Zn 16 g; Cu 2.40 g; I 0.14 g; Feed preservative substant 2.50 g; Flavor 10 g and carrier added to 1.00 kg premix.
[1]Premix was provided by BASF (Thailand).

Table 2: Nutrients contained in experimental diets based on chemical analysis

	Control (16%CP)	Low-protein diet (14% CP)			
	0.38% Met	0.26% Met	0.30% Met	0.38% Met	0.44% Met
Nutrients					
Dry matter (%)	89.56	89.52	89.54	89.54	89.57
Crude Protein (%)	16.11	14.04	14.04	14.03	14.04
Ether extract (%)	3.23	3.22	3.23	3.24	3.26
Fiber (%)	3.32	3.29	3.31	3.30	3.30
Ash (%)	10.60	10.55	10.66	10.52	10.65
Calcium (%)	3.34	3.33	3.34	3.33	3.34
Total phosphorous (%)	0.53	0.53	0.53	0.52	0.52
Gross energy ,GE (Kcal / kg)	4047.89	4034.23	4050.15	4049.67	4053.02
Amino acids					
Lysine (%)	0.87 (1.00)	0.71 (1.00)	0.71 (1.00)	0.70 (1.00)	0.70 (1.00)
Methionine (%)	0.37 (0.43)	0.25 (0.35)	0.31 (0.44)	0.38 (0.54)	0.44 (0.63)
Tryptophan (%)	0.21 (0.24)	0.19 (0.27)	0.19 (0.27)	0.19 (0.27)	0.19 (0.27)
Threonine (%)	0.59 (0.68)	0.51 (0.72)	0.51 (0.72)	0.51 (0.72)	0.51 (0.72)
Isoleucine (%)	0.82 (0.94)	0.73 (1.03)	0.73 (1.03)	0.73 (1.03)	0.73 (1.03)
Arginine (%)	0.95 (1.09)	0.83 (1.17)	0.83 (1.17)	0.83 (1.17)	0.83 (1.17)
Leucine (%)	1.23 (1.29)	1.13 (1.36)	1.13 (1.36)	1.12 (1.36)	1.13 (1.36)
Phenylalanine (%)	0.67 (0.77)	0.64 (0.90)	0.64 (0.90)	0.64 (0.90)	0.64 (0.90)
Histidine (%)	0.29 (0.33)	0.25 (0.35)	0.25 (0.35)	0.25 (0.35)	0.25 (0.35)
Valine (%)	0.79 (0.91)	0.69 (0.97)	0.69 (0.97)	0.69 (0.97)	0.69(0.97)

Values in parentheses are ratio of amino acids to lysine.

Low-CP diet with 0.30 or 0.38% Met had better in eggshell thickness than the control group (P<0.01). Although eggshell component expressed as percentage of egg was not significantly affected by all experimental diets.

Reproductive organs: Effects of diets on reproductive organs were presented in Table 5. Increasing dietary Met intake clearly improved development of ovary and oviduct weight (P<0.01). Ovary weight (% of body weight) of hens fed the Low-CP diet with 0.44% Met did not significantly differ from the control group. However, the adding Met was unable to improve the development of oviduct equal to the control group.

Chemical liver compositions and abdominal fat: Liver weight, chemical liver composition and abdominal fat weight were significantly affected by the experimental diets (Table 6). Liver weight of hens fed Low-CP diet (0.26% Met) was the heaviest (P<0.05), while adding Met resulted in the normalization of liver weight and chemical liver compositions. Supplementing Met to the Low-CP

Table 3: Effects of additional Met in Low-CP diet on production performance of laying hens from 24 to 44 weeks of age

Item	Control (16%CP)	Low-CP diet (14% CP)					
	0.38% Met	0.26% Met	0.30% Met	0.38% Met	0.44% Met	SEM	p-value
Feed intake (g/d)	98.14[a]	87.85[b]	98.21[a]	99.73[a]	99.98[a]	0.98	0.0001
Protein intake (g/d)	15.70[a]	12.30[c]	13.75[b]	13.96[b]	14.00[b]	0.26	0.0001
Met intake (mg/d)	372.94[b]	228.40[d]	294.64[c]	378.96[b]	439.93[a]	15.18	0.0001
Hen-day egg production (%)	75.60[a]	65.24[c]	69.16[b]	72.51[b]	74.76[a]	0.80	0.0001
Egg weight (g)	54.92[a]	50.80[b]	54.43[a]	55.22[a]	54.82[a]	0.38	0.0001
Egg mass (g/hen/day)	42.05[a]	33.34[d]	37.64[c]	40.41[b]	40.98[b]	0.70	0.0001
FCR (g/g egg)	2.33[c]	2.63[a]	2.60[a]	2.47[b]	2.43[b]	0.03	0.0008
Mortality (%)	8.22[b]	11.75[a]	6.07[b]	5.89[c]	5.87[c]	0.61	0.04

abc Means within a row with no common superscripts differ significantly.

Table 4: Effects of additional Met in Low-CP diet on egg components and eggshell thickness

Item	Control (16%CP)	Low-CP (14% CP)					
	0.38% Met	0.26% Met	0.30% Met	0.38% Met	0.44% Met	SEM	p-value
Yolk (%)	23.85[b]	25.01[a]	23.76[b]	23.48[b]	23.73[b]	0.14	0.0001
Albumen (%a)	66.15[a]	64.95[b]	66.19[a]	66.55[a]	66.39[a]	0.14	0.0001
Shell (%)	10.03	10.09	10.06	10.07	10.04	0.02	0.2324
Albumen high (mm)	6.43	6.99	6.86	6.55	6.66	0.70	0.0539
Shell thickness (mm)	35.03[b]	32.63[c]	36.54[a]	36.49[a]	35.72[ab]	0.36	0.0001

abc Means within a row with no common superscripts differ significantly.

Table 5: Effects of additional Met in Low-CP diet on reproductive organs

	Control (16%CP)	Low-CP diet (14% CP)					
	0.38% Met	0.26% Met	0.30% Met	0.38% Met	0.44% Met	SEM	p-value
Live weight (kg)	1.44	1.40	1.43	1.45	1.44	0.08	0.3646
Ovary weight (% live wt)	2.49[a]	1.51[c]	1.87[b]	1.97[b]	2.42[a]	0.09	0.0001
Oviduct weight (% live wt)	4.30[a]	2.66[c]	2.93[bc]	3.25[bc]	3.37[b]	0.10	0.0008
Oviduct length(cm)	74.01[a]	57.62[c]	64.93[b]	66.49[b]	69.94[b]	1.46	0.0006

abc Means within a row with no common superscripts differ significantly

Table 6: Effects of additional Met in low-protein diet on liver weight, chemical liver composition and abdominal fat weight

	Control (16%CP)	Low-CP diet (14% CP)					
	0.38% Met	0.26% Met	0.30% Met	0.38% Met	0.44% Met	SEM	p-value
Liver weight (% live wt)	1.62[b]	1.85[a]	1.68[b]	1.64[b]	1.63[b]	0.03	0.0309
Liver moisture (%)	61.51	62.84	62.63	63.25	62.57	0.15	0.3254
Liver lipid (%)	4.42[a]	2.75[d]	3.43[c]	3.66[bc]	4.14[ab]	0.15	0.0001
Liver protein (%)	21.73[b]	22.17[a]	21.61[b]	20.76[c]	20.95[c]	0.14	0.0300
Abdominal fat (% live wt)	2.14[a]	1.06[d]	1.64[c]	1.74[bc]	1.92[ab]	0.10	0.0002

abc Means within a row with no common superscripts differ significantly.

diet did negatively affect to liver protein content. On the other hand, the increase of liver lipid and abdominal fat content due to supplementing Met was found (P<0.01). The Low-CP diet contained 0.26, 0.30 or 0.38% Met had significantly lower abdominal fat content than the control group (P<0.01).

Discussion

Average house temperature in this study was over than 30^{o}C, the average highest and lowest was 35.16 and

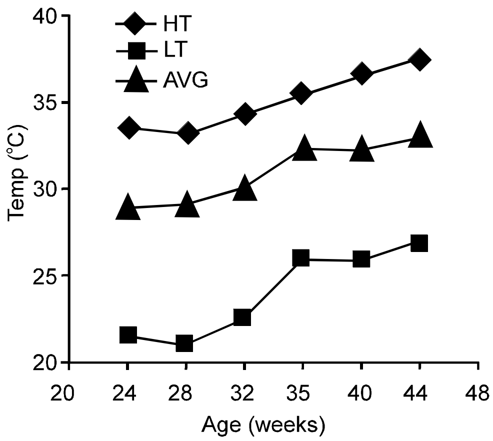

Fig. 1: Environmental temperature (°C) of the experimental house.

Average highest temperature (HT)	=	35.16ºC
Average lowest temperature (LT)	=	23.98ºC
Average temperature (AVG)	=	31.59ºC

23.98°C, respectively. This high ambient temperature did absolutely induce heat stress to the hens, since the thermoneutral zone of various types of poultry is around 18.3 to 23.9ºC (Keshavarz, 1990). Because high environmental temperature may induce a thermoregulatory response from the thermally sensitive loci in the anterior hypothalamic area that depress feed intake (Peguri and Coon, 1991). Lowering amount of feed intake in present study (87.00-99.98 g/hen/day) compare to average commercial feed intake (110-120 g/hen/day) indicated a clear effect of heat stress. Accordingly, several researches also reported the effect of high ambient temperature on lowering feed intake (Austic, 1985; Howlider and Rose, 1987; Emery *et al.*, 1984; Savory, 1986; Miller and Heath, 1970). However, only one temperature was involved in this study, it was not possible to identify the specific effects of temperature that were involved in hens' performance.

Among the study groups, it was shown that Low-CP diet without adding Met group had the lowest feed consumption. Consequently, Met and protein intake of this group were also the lowest compared to other groups. These lowering nutrients intake might be due to amino acids imbalance because Harper and Rogers, (1965) had reported that feed intake in animals consuming an imbalanced diet is normally reduced. In addition, lowering feed intake of broiler chicks due to inadequate Met consumption has been reported by Bunchasak *et al*. (1996; 1997). Two types of imbalance many be recognized that caused by the addition of a relatively small quantity of an amino acid to a Low-CP diet, and that precipitated by an incomplete mixture of amino acids (D' Mello, 1994). Moreover, Keshavarz and Jackson (1992) and Penz and Jensen (1991) had reported that decreasing protein level and supplemented Met in poultry diets usually did not depress feed intake. So, in this study, supplementing Met in Low-CP diet could prevent negative effect of amino acids imbalance on feed consumption.

Met intakes increased significantly with increases in dietary Met concentration and this was positively reflected in significant increases in hen-day egg production. Moreover, there was positive relationship between Met intake and egg weight (r = 0.82). This was agreed with Shafer *et al*. (1996) who reported that Met resulted in an improvement of egg weight. Furthermore, Penz and Jensen (1991); Keshavarz and Jackson (1992) had also shown that supplementing Met to Low-CP diet (14% CP) improved egg productions similar to the 16% CP diet. Although, Summers *et al*. (1988) has reported that supplemental Met and/ or increase in dietary protein did not affect early egg size of hens.

We found that Met intake of 439.93 mg/hen/day (Low-CP; 0.44% Met) was able to improve hen-day egg production and egg weight similar to the control group (16% CP; 0.38% Met) which had Met intake of 372.94 mg/hen/day. The study of Met requirement of Isa-brown laying hens strain in Australia also reported that Met concentration of 2.83 g/kg, a mean daily Met intake of 370 mg/hen and 433 mg/hen satisfied the Met requirements of the hens housed in single cages and multiple-caged hens (5 hens/cage), respectively (Annual report, 2000). In present study conditions, the hens were kept in multiple-caged (3 hens/cage) and high environmental temperature, it is indicated that hens may need higher Met intake for maximum egg production when lowering dietary protein was offered, although egg mass (g/hen/day) and FCR were still poorer than the control. Nevertheless, the production advantages of increasing the protein diet from 14 to 16% were limited to a small, significant improvement in FCR due to a slight decrease in feed consumption of 16% CP group compared to Low-CP with high Met supplementation (14% CP, 0.44% Met).

Interestingly, rate of mortality was dramatically increased in Low-CP diet without adding Met (0.26%) and then spectacularly decreased as increasing Met levels. Furthermore, hens received Low-CP diet with 0.38 or 0.44% Met had significantly lower in mortalities rate than the control group. In general, during summer period of Thailand (~ 35ºC), increasing incidence of sudden dead syndrome due to heat stress is always found. Thus, lowering dietary protein supplemented with Met significantly showed the prevention of this problem, although such supplementation may not offer protection against heat stress-related depression in all parameters observed. This result also confirmed what Pequri and Coon, (1991) had reported that high protein intake caused an increase of heat increment under heat stress conditions.

Egg component was significantly affected by lowering Met intake. Low-CP diet with 0.26% Met significantly resulted in an increment of yolk percentage and a reduction of albumen percentage. Penz and Jensen (1991) found that decrease of egg size due to decreasing protein intake was mainly resulted from decrease of egg albumen. Similarly, this study showed that there was high positive relationship between percentage of egg albumen and egg weight (r = 0.96), whereas albumen height was tended to decline when Met was supplemented. Moreover, albumen height and serum total immunoglobulin titres declined with increasing dietary Met concentration (Annual Report, 2000).

The eggshell is formed during the passage of the egg through the oviduct, where the various layers of the eggshell are assembled sequentially (Novak *et al*., 2004). Eggshell thickness was significantly improved when Met at 0.30 or 0.38% was added to Low-CP diets. On the other hand, the thickness was slightly decreased in the highest level of Met group (0.44% Met). In this case, the tendency of negative effect of high supplementing Met (0.44%) may be due to the fact that eggshell thickness and eggshell weight reduces with increase in egg size (Roland, 1988; Jackson *et al*., 1987). Another reason is that, in general, the foundation of a shell consists of a protein matrix, and it may be possible that increasing the TSAA intake may influence protein synthesis of shell membranes (Novak *et al.*, 2004).

Increasing Met intake from 228.4 to 439.93 mg/hen/day could improve eggshell thickness. Similarly, Carey *et al*. (1991) reported increased eggshell weight when increasing the level of Met from 330 to 450 mg/hen/day. While, Shafer *et al*. (1996) reported that there was no effects of TSAA level on eggshell weight or percentage of eggshell when increasing the TSAA intake from 624 to 822 mg/hen/day. Thus, it is suggested that Met intake higher than 620 mg/hen/day will not give any benefit to eggshell quality. Unfortunately, we could not find the research which conducted effect of Met intake from 450 to 620 mg/hen/day on eggshell quality. On the other hand, when eggshell quality was indicated as percentage of egg, effect of supplementing Met on eggshell was not significantly found. This was in agreement with Scheideler and Elliot (1998) who also reported a lack of response of wet eggshell percentage to TSAA intakes.

Mongin (1968) has noted that the first restrictive factor of the eggshell formation was the Ca and the second was the carbonate ions, during heat stress, decreasing CO_2 in blood caused by panting could induce lowering eggshell quality. Nevertheless, increasing dietary Ca did not improve eggshell quality under heat stress has been reported (de Andrade *et al*., 1977; Tanor *et al*., 1984). Hence, adding Met to Low-CP diet may be an advantageous way to improve eggshell quality under this high environmental temperature conditions.

It is well known that development of the reproductive organs (ovary and oviduct) require both protein (amino acids) and energy (fat) accretion. Hens that are underweight may cause poor reproductive tract development. The results showed that the lowest Met consumption (Low-CP; 0.26% Met group) deprived reproductive organs size (ovary and oviduct; expressed as percentage of body weight). In addition, final body weight of this group was slightly lighter than other groups due to a significant reduction of feed intake. This is also agreed with Muramutsu *et al*. (1987) who reported that an insufficiency of amino acid or protein consumption or amino acids imbalance resulted in poor improvement of both ovary and oviduct of the hens due to lowering the protein synthesis.

The liver weight of hens received Low-CP diet with 0.26% Met was significantly heavier than those other groups. Supplementing Met significantly induced reduction of liver size. This phenomenon is not surprised since a principle role of Met in the prevention of condition such as liver enlargement in poultry is already well known. The result showed that there was no significant difference among experimental groups for liver moisture content. Supplementing Met did directly affect to the proportion of liver lipid and protein content. Surprisingly, supplementing Met resulted in decrease of liver protein content (r = -0.82), while liver fat content was positively related with Met intake (r = 0.81). The result of liver fat was tended to disagree with general knowledge that deficiency in methyl donor intake always induces high liver fat deposition. Fatty liver syndrome is a condition that mainly affects to the hens. The basic cause is thought to be excessive dietary energy intake. However, since the feed consumption of all study hens were lower than the commercial recommendation of feed intake and liver fat of hens fed Low-CP with Met supplementation were still lower than the control group, thus it can be said that an incidence of fatty liver syndrome was not appeared in this study.

Three major reasons might be given for the changing proportion of liver fat and protein content are that; 1) Low-CP (0.26% Met) without adding Met caused an imbalance of amino acid and then induced high protein synthesis in the liver (Harper and Rogers, 1965); 2) addition of Met improved energy utilization in body (Brody, 1994) because supplementing Met resulted in lowering FCR and elevation of abdominal fat content; and 3) supplementing Met stimulated high fatty acid synthesis in the liver (Smith *et al*.,1983). In the liver, fat synthesis and accumulation were mainly affected by hormone estrogen which synthesized from an ovary (Akiba *et al*., 1982). In the supplementing groups, increasing liver fat content may be caused by the effect of high estrogen synthesis in ovary in order to support high egg production. Thus, in fat metabolism aspect, it is noted that effects of supplementing Met on laying hens may be significantly different from broiler chickens or other animals.

Acknowledgements

The authors gratefully acknowledge that vitamins and minerals supplementation was supported from BASF (Thailand) LTD. We also appreciate the support of several staff from Department of Animal Science at Bangkhen Campus, Kasetsart University, Thailand.

References

Akiba, Y., L.S. Jensen and M.S. Lilburn, 1982. Effect of estrogen implants on hepatic lipid deposition in chicks fed different isonitrogenous and caloric diets. J. Nutr., 112: 189-196.

Al-Saffar, A.A. and S.P. Rose, 2002. The response of laying hens to dietary amino acids. World's Poult. Sci., 58: 209 -234.

Annual Report, 2000. Poultry research foundation, The University of Sydney, Australia.

AOAC, 1990. Official Method of analysis. 15th ed. Association of Official Agricultural Chemists, Inc., Verginia, 1422 p.

Austic, R.E., 1985. Feeding poultry in hot and cold climates. Pages 123-136 *in:* Stress Physiology in Livestock. Youssef M. K. eds.123-136 CRC Press Boca Raton, FL.

Brody, T., 1994. Nutritional Biochemistry. Academic Press, Inc., USA, 658 p.

Bunchasak, C., K. Tanaka, S. Ohtani and C.M. Collado, 1996. Effect of Met+Cys supplementation to a low-protein diet on the growth performance and fat accumulation of broiler chicks at starter period. Anim. Sci. Tec. (Jpn.), 67: 956-966.

Bunchasak, C., U. Santoso, K. Tanaka, S. Ohtni and C.M. Collado, 1997. The effect of supplementing methionine plus cystine to a low-protein diet on the growth performance and fat accumulation of growing broiler chicks. AJAS, 10: 185-191.

Cahaner, A., Y. Pinchasov, I. Nir and Z. Nitsan, 1995. Effects of dietary protein under high ambient temperature on body weight, breast meat yield and abdominal fat deposition of broiler stocks differing in growth rate and fatness. Poult. Sci., 74: 968-975.

Carey, J.B., R.K. Asher, J.F. Angel and L.S. Lowder, 1991. The influence of methionine intake on egg composition. Poult. Sci., 70: (Suppl. 1) 151.

Chung, H.J., L. Chung-Yi and P. Wen-Shyg Chiou, 1998. Effects of ambient temperature and methionine supplementation of a low protein diet on the performance of laying hens. Anim. Feed Sci. Tec., 74: 289-299.

Chohen, S.A. and D.P. Michaud., 1993. Synthesis of a fluorescent derivatizing reagent, 6-aminoquinolyl-*N*-Hydroxysuccinimidyl carbamate, and its application for the analysis of hydrolysate amino acids via High-Performance Liquid Chromatography. Anal. Biochem. 211: 279-287.

Deaton, J.W., F.N. Reece, J.L. McNaughton and B.D. Lott, 1981. Effect of differing temperature cycles on egg shell quality and layer performance. Poult. Sci., 60: 733-737.

de Andrade, A.N., J.C. Rogler, W.R. Featherston and C.W. Alliston, 1977. Interrelationships between diet and elevated temperatures (cyclic and constant) on egg production and shell quality. Poult. Sci., 56: 1178-1188.

Donkoh, A., 1989. Ambient temperature: a factor affecting performance and physiological response of broiler chickens. Int. J. Biometeorol., 33: 259-265.

Duncan, D.B., 1955. Multiple Range Test. Biometrics. 11: 1-42.

D'Mello, J.P.F., 1994. Amino acid Imbalances, antagonisms and toxicities. Page 63-97, In: Amino acids in farm animal nutrition, Edited by J.P.F. D'Mello, CAB International.

Emery, D.A., D. Vohra and R.A. Ernst, 1984. The effect of cyclic and constant ambient temperature on feed consumption, egg production, egg weight, and shell thickness of hens. Poult. Sci., 63: 2027-2035.

Grizzle, J., M. Iheanacho, A. Saxton and J. Broaden, 1992. Nutritional and environmental factors involved in eggshell quality of laying hens. Br. Poult. Sci., 33: 781-794.

Harm, RH. and G.B. Russell, 1993. Optimizing egg mass with amino acid supplementation of a low-protein diet. Poult. Sci., 72: 1892-1896.

Harper, A.E. and Q.R. Rogers, 1965. Amino acid imbalance. Proc. Nutr. Soci., 24: 173-190.

Howlider, M.A.R. and S. P. Rose, 1987. Temperature and the growth of broilers. World's Poult. Sci., 72: 701-708.

Jackson, M.E., H.M. Hellwig and P.W. Waldroup, 1987. Shell Quality: Potential for Improvement by dietary means and relationship with egg size. Poult. Sci., 66: 1702-1713.

Keshavarz, K. and M.E. Jackson, 1992. Performance of growing pullets and laying hens fed with low-protein amino acid-supplemented diets. Poult. Sci., 71: 905-918.

Keshavarz, K., 1990. Managing in hot weather. Broiler Industry. September 1990, pp: 24-32.

McNaughton, J.L., J.D. May and F.N. Reece, 1984. Response of broiler chickens to dietary energy and lysine levels in warm environment. Poult. Sci., 63: 1170-1174.

Miller, S.H. and J.E. Heath, 1970. Thermo-responsiveness of the preoptic region of the brain in house sparrows. Science, 168: 1008-1010.

Mongin, P., 1968. Role of acid-base balance in the physiology of egg formation. World's Poult. Sci. J., 24: 200-230.

Muramutsu, T., K. Hiramoto, I. Tasaki and J.I. Okumura, 1987. Effect of protein starvation on protein turnover in liver, oviduct and whole body of laying hens. Comp. Biochem. Physiol., 87B: 227.

National Research Council, 1994. Nutrition Requirement of Poultry. 9th ed. National Academy of science. Washington, D.C., 155 p.

Novak, C., H. Yakout and S. Scheideler, 2004. The Combined Effects of Dietary Lysine and Total Sulfur Amino Acid Level on Egg Production Parameters and Egg Components in Dekalb Delta Laying Hens1. Poult. Sci., 83: 977-984.

Peguri, A. and C. Coon, 1991. Effect of temperature and dietary energy on layer performance. Poult. Sci., 70: 126-138.

Penz, A.M. and L.S. Jensen, 1991. Influence of protein concentration, amino acid supplementation and daily time of access to high-or low-protein diets on egg weight and components in laying hens. Poult. Sci., 70: 2460-2466.

Ravikiran, D. and G. Devegowda, 1998. Effects of DL-methionine supplementation in the ration of commercial layers during summer. Ind. J. Poult. Sci., 33: 279-283.

Roland, D.A., 1988. Egg Shell Problems: Estimates of incidence and economic impact. Poult. Sci., 67: 1801-1803.

Savory, J.C., 1986. Influence of ambient temperature on feeding activity parameters and digestive function in domestic fowls. Physiol. Behav., 38: 353-357.

Scheideler, S.E. and M.A. Elliot, 1998. Total sulfuric amino acid (TSAA) intake to maximize egg mass and feed efficiency in young layers (19-45 weeks of age). Poult. Sci., 77: (Suppl 1).

Sell, D.R. and J.C. Rogler, 1983. The effect of sorghum tannin and methionine on the performance of laying hens maintained in two temperature environments. Poult. Sci., 63: 109-116.

Shafer, D.J., J.B. Carey and J.F. Prochaska, 1996. Effect of dietary methionine intake on egg component yield and composition. Poult. Sci., 75: 1080-1085.

Siegel, H.S., 1995. Stress, strains and resistance. Br. Poult. Sci. 36: 3-22.

Smith, J.T., V.A. Robert, B.B. Joyce and L.G. Mary, 1983. A metabolic comparison of cysteine and methionine supplements in the diet of a rat. J. Nutr., 113: 222-227.

Steel, R.G.D. and J.H. Torrie, 1980. Principle and procedures of statistics. Mc Graw Hill Publishers. New York. 2nd edn.

Summers, J.D., D. Spratt and S. Leeson, 1988. Response of laying hens to mash and pellet diet portions containing various nutrient combinations. Nutr. Rep. Inter., 37: 311-318.

Summers, J.D., J.L. Atkinson and D. Spratt, 1991. Supplementation of a low protein diet in an attempt to optimize egg mass output. Can. J. Anim. Sci., 71: 211-220.

Tanor, M.A., S. Leeson and J.D. Summers, 1984. Effect of heat stress and diet composition on performance of White Leghorn hens. Poult. Sci., 63: 304-310.

Temim, S., A.M. Chagneau, S. Guillaumin, J. Michel, J. Peresson, P.A. Geraert and S. Tesseraud, 1999. Effects of chronic heat exposure and protein intake on growth performance, nitrogen retention and muscle development in broiler chickens. Reprod. Nutr. Dev., 39: 145-156.

Waldroup, P.W., R.J. Mitchell, J.R. Payne and K.R. Hazen, 1976. Performance of chicks fed diets Formulated to minimize excess levels of essential amino acids. Poult. Sci., 55: 243-253.

WaIdroup, P.W., 1982. Influence of environmental temperature on protein and amino acid needs of poultry. Fed. Proc., 41: 2821-2823.