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Estimates of Methionine and Sulfur Amino Acid Requirements for Laying Hens using Different Models

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ABSTRACT

This experiment was conducted to evaluate the effects of dietary methionine (Met) content on the performance of white commercial laying hens and to determine Met and total sulfur amino acids requirements (TSAA). These requirements were estimated using three statistical models (broken-line regression, exponential and second order equations) to evaluate their abilit to determine amino acid requirements. A total of 216 laying hens (23 wks of age) was used in a completely randomized design (CRD) with six treatments with four replicates of nine birds each. The basal diet contained 15.25% crude protein, 2830.16 kcal/kg ME and 0.24% Met. Synthetic DL-Met was added to the deficient (basal) diet in 0.05% increments to make the other five experimental diets (0.29, 0.34, 0.39, 0.44 and 0.49% Met). Increasing Met level from 0.24 to 0.34% significantly increased egg production, egg weight, egg mass, egg content, and feed intake and decreased feed conversion ratio ($p<0.05$). However, further Met increases, from 0.34 to 0.49%, no longer influenced these parameters. Out of the three models, the broken-line regression model presented better estimates of AA requirements. Based on broken-line equations, average Met and TSAA requirements of the laying hens were 0.31 and 0.60% (245.50 and 469.25 mg/hen/day) from 22 to 36 wks of age, respectively.

INTRODUCTION

The efficiency of dietary protein utilization depends on the amount, composition, and digestibility of the amino acids (AA) in the diet. Laying hen requirements should be expressed on digestible amino acid basis, rather than on protein. Therefore, it is important to formulate diets according to accurate values of amino acid requirements. Methionine (Met), lysine (Lys), and tryptophan (Trp) are considered as the most limiting AA in practical layer diets based on corn and soybean meal (Harms & Russell, 2000).

The use of linear programming techniques has shown that both the protein level and the cost of practical rations are very much affected by the requirements of specific essential AAs, particularly Met and Lys. Therefore, estimates of these requirements must be reliable (Fisher & Morris, 1970). Indeed, Lopez & Leeson (1995) reported that since chickens can only utilize about 40% of the dietary protein, it seems logical to decrease dietary protein level in the diet, which would also minimize nitrogen excretion. However, synthetic AA need to be added to the diet in order to meet the requirements of limiting AA due to AA dilution when dietary protein is reduced. Formulating diets based on the ideal protein concept is one of the methods to reduce dietary protein, which, in turn, will decrease fecal nitrogen excretion, while maintaining egg production parameters (Novak *et al*., 2006).

Results of studies on Met and total sulfur amino acids (TSAA) requirements of laying hens widely vary. Moran (1969) reported 2.9 g Met/kg requirement in a diet containing 3.4 g Cys and 12.35 MJ metabolizable energy (ME)/kg, which is equivalent to an intake of 788 mg TSAA/h/d, out of which 362 mg correspond to Met. Met requirement recommended by Harms and Damron (1969) was 275 mg available Met/h/d during production peak. In other studies, the recommended Met requirement varied between 300 and 320 mg/h/d (Carlson & Guenthner, 1969; Novacek & Carlson, 1969; Jensen *et al*., 1974; Sell & Johnson, 1974). The NRC (1971) recommended a daily intake of 280 mg Met/d, but the requirement was increased to 300 mg/d in 1977, and to 350 mg/d in 1984. In the NRC revision of 1994, the requirement was reduced to 300 mg/d.

These requirements were suggested assuming the hen needed a certain amount of TSAA and a portion of his amount was to satisfy a need for approximately 280 mg of Cys. The recommendation of ARC (1975) for young laying pullets producing 50 g egg mass (EM) per hen/d was 350 mg available Met of a total of 470 mg available TSAA/h/d. Rostango (1990) suggested that hens with daily feed intake (FI) of 105 g required 0.311% Met and 0.567% TSAA or 327 and 595 mg/d of Met and TSAA, respectively. Ahmad *et al*. (1997) reported that TSAA levels ranging from 580 to 660 mg/h/d had no effect on performance of laying hens.

A large number of reports has been published on Met and TSAA requirements and their supplementation. However, there is a wide variation in recommended requirement of Met and TSAA for laying hens. Therefore, this experiment was conducted to determine Met and TSAA requirements for laying hens.

MATERIAL AND METHODS

This study was carried out at animal research station of Bu-Ali Sina University in August, 2009, in Hamadan-Iran. All experimental protocols were approved by the Animal Welfare Committee of the Agricultural School of Bu-Ali Sina University.

A total of 216 commercial laying hens were obtained from a local supplier. After 18 wks of age, birds received increasing stimulation of up to 16 hours of light, which was maintained until the end of the experiment. At 22 wks of age, hens were individually weighed and allocated in to six treatments with four replicates of nine birds each (three hens in each conventional cage, measuring 42×40×50 cm^3). The basal diet was based on corn, wheat, and soybean meal (Table 1) and contained 15.33% crude protein (CP), 2832.71 kcal ME/kg, 0.24% Met, and 0.52% TSAA. This diet was formulated to meet or to exceed the NRC (1994) requirements of layers in lay for all nutrients, except for Met and TSAA. Synthetic DL-Met was added to the deficient (basal) diet in 0.05% increments (0, 0.05, 0.10, 0.15, 0.20, 0.25) at the expense of soybean meal to meet the desired Met and TSAA levels in the experimental diets. Feedstuffs and feeds were analyzed for crude protein (CP), ether extract (EE), ash, and crude fiber (CF), according to the procedures of the Association of Official Analytical Chemists (AOAC, 1990; Table 1). Energy values were based on NRC recommendation (1994). Feedstuff AA composition was analyzed at the Chemical lab of Bu-Ali Sina University (Tecator apparatus, Optilab 5931 Liquid Chromatograph, C18 column) using the method described by Ravindran *et al*. (1999). The AA composition of the basal diet, except for Met and TSAA, covered the ideal amino acid profile suggested by Schutte & De Jong (1996).

Table 1 – Composition and nutrients content of the basal diet.

Ingredients (%)	Basal diet[1]
Corn (8.72%)	41.90
Soybean meal (44.19%)	20.00
Wheat (11.35%)	25.00
Soybean oil	2.47
Dicalcium phosphate[2]	1.28
Oyster shell[3]	8.37
Sodium chloride[4]	0.37
Mineral mix[5]	0.25
Vitamin mix[6]	0.25
DL- Met	-
L-Lys-HCl	0.11
Total	100
Analyzed values[7] (%)	
Crude protein	15.33
Ether extract	2.65
Crude fiber	2.79
Ash	1.25
Met	0.24
Cys	0.28
Met+Cys	0.52
Lys	0.88
Calculated values[8]	
ME (Kcal/kg)	2832.71
Ca (%)	3.50
NPP (%)	0.35
Na (%)	0.17

1Based on 100 g/h per day. 2Contained 18.7 % P and 22 % Ca. 3Contained 38% Ca. 4Contained 39% Na. 5Per kg mineral premix supplied the following: Mn, 64 g; Zn, 44 g; Fe, 100 g ; Cu, 16 g ; I, 0.64 g; Co, 0.2 g; Se, 3 g. 6Supplied per kilogram of diet: biotin, 0.2 mg; cholecalciferol, 2,200 IU; choline, 500 mg; ethoxyquin, 65 mg; folic acid, 1 mg; niacin, 60 mg; pantothenic acid, 15 mg; pyridoxine, 5 mg; riboflavin, 5 mg; thiamin, 3 mg; vitamin A, 8,000 IU; vitamin B12, 0.02 mg; vitamin E, 20 IU; vitamin K, 2 mg.

The experimental diets were freshly prepared and mixed at four-week intervals. Feed (in mash form) and water (via nipple drinkers) were supplied *ad libitum* throughout the experiment.

The experiment was conducted for 14 wks (22 to 36 wks of age), but the first 2 wk were considered as a depletion period (Harms & Russell, 1996b). The hens were individually weighed at the beginning and at the end of the experiment, and body weight gain (BWG) was calculated. Feed intake, egg production (EP), egg weight (EW), EM, feed conversion ratio (FCR), egg content (EC) and shell weight (SW) were recorded on a replicate basis. Egg content was calculated by multiplying EP by EW minus SW. The experiment was conducted according to a completely randomized design (CRD).

Data were submitted to analysis of variance (ANOVA), using the linear model procedure of the statistical package of SAS Institute (2004). Variance homogeneity was determined by Bartlett's test. Duncan's multiple range test (1955) was used to compare treatment means (p<0.05).

Met and TSAA requirements of laying hens were determined submitting BW change, EP, EW, EM, EC, FCR and FI data to regression analysis using the broken-line model (Robbins *et al*., 2006), exponential model or second order equations.

The equation of the broken-line model was:

$$y = L + U (X_{LR} – R),$$

where y = performance parameter (e.g., body weight, feed intake, etc.), L = the ordinate of the breakpoint in the curve; R = the abscissa of the breakpoint in the curve (requirement estimate); X_{LR} = value of X less than R; and U= slope of the line for X less than R. In the broken-line model, dietary Met and TSAA concentration was calculated as the concentration required to achieve maximum performance, according to the parameter.

The equation of the exponential model was:

$$y = a + b (1 – e^{–c(x–d)}),$$

where y = performance parameter (e.g., body weight, feed intake, etc.), a = intercept (performance of the basal diet), b = maximum response to Met or TSAA concentrations, e = nepher value, c = slope, d = Met or TSAA concentrations in the basal diet, and x = Met or TSAA concentration in the experimental diet. In the exponential model, the dietary Met and TSAA concentrations were calculated that were required to achieve 95% of the maximum of the performance parameter considered.

The equation of the second order model was:

$$y = a + bx +cx^2$$

where y = performance parameter (e.g., body weight, feed intake, etc.), and x = Met or TSAA concentration in the diet.

RESULTS

Methionine and TSAA requirements estimated by exponential equations for EM, EC, FCR and FI were higher than maximum dietary Met and TSAA supplied in this experiment (0.49 and 0.77%, respectively; Tables 2 and 3). Methionine and TSAA requirements estimated by second-order equations, irrespective of the results, were very variable (Tables 4 and 5). In this respect, broken-line regression showed better ability to estimate AA requirements. On the other hand, highest

Table 2 – Exponential equations of Met requirements

	Met		
Measurement	Equation	$^aR^2$	Requirement (%)
Body weight change	$y = 83.91 + 98.87 (1 – e^{-21.87(x-0.24)})$	0.62	0.377
Egg production	$y = 50.45 + 23.51 (1 – e^{-0.31(x-0.24)})$	0.56	0.303
Egg weight	$y = 48.72 + 6.14 (1 – e^{-11.65(x-0.24)})$	0.85	0.490
Egg mass	$y = 26.79 – 187.40 (1 – e^{-47.47(x-0.24)})$	0.24	> 0.490
Egg content	$y = 42.42 + 8.07 (1 – e^{-10.62(x-0.24)})$	0.92	> 0.490
Feed conversion ratio	$y = 2.77 + 11.05 (1 – e^{-0.19(x-0.24)})$	0.43	> 0.490
Feed intake	$y = 65/70 – 346.50 (1 – e^{-0.25(x-0.24)})$	0.21	> 0.490

[a] Coefficient of determination.
y, performance parameters; e, nepher value; x, Met or TSAA concentration in the diet.

Table 3 – Exponential equations of TSAA requirements

Measurement	TSAA		
	Equation	[a]R^2	Requirement (%)
Body weight change	$y = 83.91 + 98.87 (1 - e -21.87(x-0.24))$	0.62	0.730
Egg production	$y = 50.45 + 23.51 (1 - e -0.31(x-0.24))$	0.56	0.618
Egg weight	$y = 48.72 + 6.14 (1 - e -11.65(x-0.24))$	0.85	0.760
Egg mass	$y = 26.79 - 187.40 (1 - e -47.47(x-0.24))$	0.24	> 0.770
Egg content	$y = 42.42 + 8.07 (1 - e -10.62(x-0.24))$	0.92	> 0.770
Feed conversion ratio	$y = 2.77 + 11.05 (1 - e -0.19(x-0.24))$	0.43	> 0.770
Feed intake	$y = 65/70 - 346.50 (1 - e -0.25(x-0.24))$	0.21	> 0.770

[a] Coefficient of determination.
y, performance parameter; e, nepher value; x, Met or TSAA concentration in the diet.

coefficient of determination values of the evaluated performance parameters (except for EC) among the three statistical models were obtained with broken-line regression. Thus, Met and TSAA requirements estimated by broken-line regression equations are discussed below.

Increasing dietary Met content from 0.24 to 0.29% significantly increased BWG ($p<0.05$; Table 6). However, BWG was not significantly different when hens were fed diets containing 0.29 to 0.49% Met (0.57 to 0.77% TSAA). Broken-line regression equations of BWG estimated Met and TSAA requirements of 0.323 and 0.603%, respectively (Table 7). Based on FI data and analysis of feedstuffs samples, these values are equivalent to 255 and 475 mg/h/d, respectively.

EP of hens receiving the diet with 0.24% Met (0.52% TSAA) was significantly lower compared with hens receiving all other diets ($p<0.05$, Table 6). Met supplementation in the basal diet significantly ($p<0.05$) increased EP when 0.34% Met (0.62% TSAA) was fed. In contrast, increasing Met level from 0.34 to 0.49% (0.62 to 0.77% TSAA) did not improve EP. Broken-line regression of EP indicated that Met and TSAA requirements were 0.316 and 0.596% (249 and 470 mg/h/d), respectively (Table 7).

No significant EW reduction was detected when Met content was reduced from 0.49 to 0.34% (0.77 to 0.62% TSAA, Table 6). On other hand, EW was significantly reduced when Met levels were below 0.34% ($p<0.05$). Based on broken-line regression equations, Met and TSAA requirements for EW were 0.303 and 0.620% (239 and 489 mg/h/d), respectively (Table 7).

Egg mass significantly increased ($p<0.05$) from 23.24 to 44.00 g/h/d when Met level increased from 0.24% (0.52% TSAA) to 0.34% (0.62% TSAA) (Table 6).

Table 4 – Second-order equations of Met requirement for performance parameters

Measurement	Met		
	Equation	[a]R^2	Requirement (%)
Body weight change	$y = -404.30 + 2869.40x - 3458.10x^2$	0.61	0.415
Egg production	$y = -85.52 + 837.50x - 1071.30x^2$	0.51	0.391
Egg weight	$y = 29.99 + 106.60x - 116.00x^2$	0.84	0.459
Egg mass	$y = -57.25 + 478.40x - 563.30x^2$	0.69	0.425
Egg content	$y = 18.41 + 136.10x - 147.40x^2$	0.92	0.462
Feed conversion ratio	$y = 6.44 - 21.31x + 24.97x^2$	0.74	0.427
Feed intake	$y = -17.22 + 496.10x - 604.80x^2$	0.64	0.410

[a] Coefficient of determination.
y, performance parameter; x, AA concentration in the diet.

Table 5 – Second-order equations of TSAA requirement for performance parameters

Measurement	TSAA		
	Equation	$^{a}R^2$	Requirement (%)
Body weight change	$y = -1384.90 + 4529.00x – 3256.00x^2$	0.61	0.695
Egg production	$y = -404.00 + 1437.50x – 1071.30x^2$	0.51	0.671
Egg weight	$y = - 8.95 + 171.60x – 116.00x^2$	0.84	0.740
Egg mass	$y = -235.40 + 793.90x – 563.30x^2$	0.69	0.705
Egg content	$y = - 184.40 + 629.00x – 441.90x^2$	0.58	0.712
Feed conversion ratio	$y = 14.36 – 35.29x + 24.97x^2$	0.74	0.707
Feed intake	$y = -203.50 + 834.80x – 604.80x^2$	0.64	0.690

[a] Coefficient of determination.
y, performance parameter; x, AA concentration in the diet.

Differences in EM were inconsistent and not significant when dietary Met ranged from 0.34 to 0.49% (0.62 to 0.77% TSAA). According to the broken-line regression equations, Met and TSAA requirements for EM were 0.316 and 0.595% (249 and 469 mg/h/d), respectively (Table 7).

Egg content followed the similar trend as EM (Table 6). A graded reduction of dietary Met content from 0.34 to 0.24% (0.62 to 0.52% TSAA) led to a progressive and significant ($p<0.05$) decrease in EC (40.49 vs. 21.50 g, respectively). Egg content decreased and then increased, albeit not statistically, as dietary Met content increased from 0.34 to 0.49% (0.62 to 0.77% TSAA). Broken-line regression equations for EC showed that Met and TSAA requirements were 0.324 and 0.604% (255 and 476 mg/h/d), respectively (Table 7).

Feed conversion ratio was not affected by dietary Met level until it was higher than 0.34% (0.62% TSAA) (Table 6). However, further reductions in dietary Met steadily increased FCR whenever dietary Met was reduced ($p<0.05$). Based on broken-line regression equations, Met and TSAA requirements were 0.309 and 0.585% (244 and 461 mg/h/d), respectively (Table 7).

Increasing dietary Met content from 0.24 to 0.34% significantly increased FI ($p<0.05$; Table 6). Feed

Table 6 – Performance of commercial laying hens in response to different Met and TSAA levels

Dietary Met (TSAA) (%)	Initial body weight (g)	Body weight change (g)	Egg production (%)	Egg weight (g)	Egg mass (g/h/d)	Egg content (g)	FCR (g feed/g egg)	Feed intake (g/h/d)
0.24 (0.52)	1061.47^{a}	84.97^{b}	50.52^{c}	48.80^{c}	23.24^{c}	21.50^{c}	2.87^{a}	64.53^{c}
0.29 (0.57)	1081.94^{a}	143.22^{a}	70.40^{b}	51.02^{b}	35.96^{b}	31.40^{b}	2.22^{b}	78.93^{b}
0.34 (0.62)	1079.36^{a}	180.97^{a}	81.11^{a}	53.63^{a}	44.00^{a}	40.49^{a}	1.94^{c}	84.42^{a}
0.39 (0.67)	1069.78^{a}	181.31^{a}	74.95^{ab}	53.40^{a}	40.24^{ab}	35.60^{ab}	2.10^{bc}	81.53^{ab}
0.44 (0.72)	1088.19^{a}	179.48^{a}	68.73^{b}	54.18^{a}	41.89^{ab}	35.84^{ab}	1.97^{c}	80.63^{ab}
0.49 (0.77)	1091.97^{a}	178.22^{a}	72.16^{b}	54.62^{a}	43.95^{a}	40.45^{a}	1.90^{c}	83.20^{a}
MSE	60.05	34.90	27.15	0.76	17.52	19.58	0.02	16.38
P	0.8640	0.0016	0.0006	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Means in the same column without a common superscript significantly differ ($p<0.05$).

Table 7 – Broken-line regression equations of Met and TSAA requirements for performance parameters[a]

Measurement	Met		TSAA	
	Requirement (%)	$^bR^2$	Requirement (%)	R^2
Body weight change	y = 181.8 + 1.165 (x – 0.323)	0.63	y = 181.8 + 1.165 (x – 0.603)	0.63
Egg production	y = 80.78 + 0.397 (x – 0.316)	0.64	y = 80.78 + 0.397 (x – 0.596)	0.64
Egg weight	y = 53.95 + 0.044 (x – 0.303)	0.85	y = 53.31 + 0.045 (x – 0.620)	0.87
Egg mass	y = 42.52 + 0.254 (x – 0.316)	0.77	y = 42.21 + 0.254 (x – 0.595)	0.77
Egg content	y = 38.07 + 0.198 (x – 0.324)	0.68	y = 38.07 + 0.198 (x – 0.604)	0.68
Feed conversion ratio	y = 1.98 + 0.013 (x – 0.309)	0.83	y = 2.03 + 0.013 (x – 0.585)	0.84
Feed intake	y = 83.44 + 0.288 (x – 0.306)	0.76	y = 83.44 + 0.288 (x – 0.586)	0.76
Average requirement	0.31		0.60	

[a] Determined by the procedure of Robbins *et al.* (2006).
[b] Coefficient of determination.
y, performance parameter; X, a value of X less than R.

intake was not significantly different among hens that received diets containing 0.34 to 0.49% Met (0.62 to 0.77% TSAA). According to the broken-line regression equations, Met and TSAA requirements for FI were 0.306 and 0.586% (241 and 462 mg/h/d), respectively (Table 7).

DISCUSSION

There are two main advantages of using the broken-line method compared with the exponential and the second-order methods. Firstly, the broken-line method estimates AA requirements based on the best response, not taking into account safety margins or economic aspects. Secondly, the broken-line method applies to real conditions and objective cases, whereas the other methods are based on hypotheses and subjective cases, such as economic issues. Non-liner exponential and second-order models use a confidence interval (in most cases 95%) with probability of error (5 %), which reduces the accuracy of these two models (Mack *et al.*, 1999; Baker *et al.*, 2002). Also, the highest coefficient of determination of each performance parameters (except for EC) was obtained with broken-line regression. Thus, broken-line regression provided the best estimates of AA requirements. Many researchers reported that broken-line regression is the best method for estimating amino acid requirements (Mack *et al.*, 1999; Baker *et al.*, 2002; Bregendahl *et al.*, 2008), which is consistent with the findings of the present experiment.

In this experiment, Met supplementation resulted in an increase in BWG. This result is in agreement with the reports of Harms & Russell (1998) and Narváez-Solarte *et al*. (2005) with laying hens. However, Shafer *et al*. (1998) reported that dietary Met content did not affect average BWG of layers. It should be mentioned that the minimum dietary Met level used by Shafer *et al*. (1998) was 0.18% higher than the minimum level used in our study, and possibly, Met deficiency effects were not evaluated by Shafer *et al*. (1998). McDevitt *et al*. (2000) analyzed body mass data using feed intake as a covariate (because FI was different among treatments) in their experiment, and observed that chick BWG was still highly influenced by the addition of DL-Met to the diet. The TSAA requirement estimated by Narváez-Solarte *et al*. (2005) for maximum BWG (0.683%) was 8% higher than the value obtained in the present study (0.603%). Also, the TSAA requirements estimated by Narváez-Solarte *et al*. (2005) for each production parameter were higher those obtained here, and the reasons for these differences are discussed below.

The increase in EP due to increasing dietary Met level observed in the present study has already been reported in literature (Keshavarz, 2003; Harms & Russell, 2003; Liu *et al*., 2005; Narváez-Solarte *et al*., 2005; Wu *et al*., 2005a; Novak *et al*., 2006). Harms & Russell (2003) recommended 245.6 mg Met/h/d, which is very close to our estimate (249 mg/h/d). However, the TSAA requirements reported by Narváez-Solarte *et al*. (2005) for maximum EP (0.658%) was 6.3% higher than our estimate (0.596%).

In the present experiment, the heaviest eggs were produced by hens fed diets containing more than 0.34% Met. This observation is consistent with the findings of Keshavarz (2003), Harms & Russell (2003), Liu *et al*. (2005), Narváez-Solarte *et al*. (2005), and Wu *et al*. (2005a). Met requirement for EW in our study was 239 mg/h/d, which is 72.2 mg lower than the value estimated by Harms & Russell (2003) (311.2 mg/h/d). Hens in the study conducted by Harms & Russell (2003) were approximately 23 wks older than those in the present study; therefore, their hens produced larger eggs. Layer age may be lead to differences in recommended Met requirements. On the other hand, the TSAA requirement determined by Narváez-Solarte *et al*. (2005) for EW (0.681%) was higher than values estimated in present study.

Egg mass was increased in 22.76 g/h/d when 0.1% DL-Met was supplemented to the basal diet. The negative effect of low Met diets on EM has been also reported by other researchers (Liu *et al*., 2005; Narváez-Solarte *et al*., 2005; Wu *et al*., 2005a; Novak *et al*., 2006). In the current experiment, TSAA requirement for maximum EM was 0.595%, which is 6.9% lower than the requirement reported by Narváez-Solarte *et al*. (2005).

Egg content exhibited a similar trend as EM, with a decline as dietary Met decreased. This reduction was expected as reduced Met levels resulted in lower EP and EW. This observation agrees with the results reported by Carey *et al*. (1991) and Harms & Russell (1998, 2003).

Met supplementation in the basal diet significantly decreased FCR up to the 0.34% level (0.62% TSAA). The results of many studies have led to the assumption that FCR of laying hens improves when Met is supplemented in the diets (Novak *et al*., 2004; Narváez-Solarte *et al*., 2005; Wu *et al*., 2005a). The explanation for the improved FCR with increasing Met and TSAA levels may be attributed to a better AA balance (Narváez-Solarte *et al*., 2005). It is also possible that hens become more efficient in utilizing the available dietary Met. There are few studies determining Met and TSAA requirement for FCR.

Increasing dietary Met content from 0.24 to 0.34% significantly increased FI. A similar effect of dietary Met content on layer FI was also reported by Harms & Russell (2003), Narváez-Solarte *et al*. (2005) and Novak *et al*. (2006). In contrast, Schutte & Pack (1995b) suggested that TSAA has no effect on FI. It seems that Met and TSAA levels may regulate layer FI. Harper *et al*. (1970), Austic (1986), and Hurwitz *et al*. (1998) reported that Met and TSAA may modify plasma AA profile in order to stimulate appetite. Feed intake reduction when highly deficient Met diets are fed also reduces the intake of non-essential AA, such as glutamic acid, cystine and glycine, which are important N sources. These AA may become limiting or essential AA may be used for non-essential purposes, which may limit protein (egg) synthesis.

In the current experiment, based on broken-line regression equations, the average Met and TSAA requirements for maximum BWG, EP, EW, EM, EC and FI, and minimum FCR was 0.31 and 0.60% from 22 to 36 wks of age, respectively. According to the average FI calculated in this experiment (78.87 g/h/d), these values are equivalent to 245.50 and 469.25 mg/h/d, respectively. Martin *et al*. (1969) and Novacek & Carlson (1969) reported that laying hens required 250 mg Met and 460 mg TSAA/h/d, which are close to the requirements estimated in the current experiment. Met and TSAA requirements estimated by Rostango (1990) (0.310 and 0.567%, respectively) also are very close to those of present study. The Met requirement determined in this study (0.31%) was similar to the 0.30% reported by Jensen *et al*. (1974) and Sell and Johnson (1974). The TSAA requirements for each production parameter and their mean value (0.67%) determined by Narváez-Solarte *et al*. (2005) were higher than values determined in the present study. Narváez-Solarte *et al*. (2005) used about 25% sorghum in diet. Investigations conducted by Rhone-Poulenc Animal Nutrition (1993) showed that if sorghum tannin content is higher than 0.50%, AA digestibility is reduced, which may lead to requirement overestimation. Met and TSAA requirements estimated in present study (0.30 and 0.60%, respectively) are very close to those of 0.30 and 0.58%, respectively reported by NRC (1994).

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