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Effect of Different Levels of Methionine, Protein and Tallow on the Productive Performance and Egg Quality of Laying Hens in the Late-phase Production

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ABSTRACT

An experiment was conducted to determine the effects of different levels of methionine, protein and tallow on productive performance and egg quality of laying hens in the late phase of production. A completely randomized design with a 3×2×2 factorial arrangement, with three levels (0.34, 0.31, and 0.27%) of methionine (MET), two levels (12.8 and 14.7%) of protein (PRO) and two levels (1 and 3%) of tallow (TAL) with constant level of linoleic acid (1.55 ± 0.02%), was used. A number of 144 Hi-Line W-36 layers from 70 to 76 wk of age was randomly distributed into 12 treatment groups with 4 replicates of 3 hens each. Egg production and egg weight were daily recorded and feed intake and egg quality traits were recorded every 2 wk. There was a significant interaction between PRO levels and TAL for egg weight. Low levels of TAL and PRO decreased egg weight throughout the experiment. High levels of MET and TAL with concomitant reduced PRO, increased eggshell thickness, and a significant interaction between levels of MET, PRO and TAL was observed during the experiment (70 to 76 wk). Low level of protein (12.8%) significantly decreased albumen weight in the third 2-wk period. Yolk color increased when hens were fed low levels of PRO and TAL. Results of this experiment indicated that the simultaneous reduction of dietary PRO and MET in diets of Hi-Line W-36 laying hens in the late phase of production, reduced egg weight ($P<0.05$). Productive performance and egg quality were not affected by 12 and 20% reduction of PRO and MET, respectively. It seems that decreasing the levels of MET and PRO to lower than the recommended values can decrease egg weight without negative effects on productive performance and egg quality of laying hens in the late phase of production.

INTRODUCTION

In spite of preferences for large eggs by consumers, a very large increase in egg size in old hens might not be of benefit because the incidence of shell quality problems and the proportion of broken eggs increase (Bennett, 1992; Abdallah *et al*., 1995). As hens grow older, the nutrient requirements decrease. It is important for commercial laying breeders to know the nutritional requirements of laying hens at different ages (Wu *et al*., 2005b). Methionine is the first limiting amino acid for egg weight (Al-Saffar & Rose, 2002).

Some studies have shown that calcium absorption decreases with ages in layers (Roland *et al*., 1975; Keshavarz & Nakajima, 1993). It was shown that absolute daily retention of Ca (Keshavarz, 2003) and shell weight (Roland *et al*., 1975) remain constant as hens age. The reason for reduced shell quality is increasing in egg size, distributing a constant amount of shell over a larger egg. Consequently, limiting egg size should also prevent loss of shell thickness (Keshavarz, 2003). Increase in egg size has resulted in reduced of eggshell thickness and eggshell weight (as a percentage of egg weight) (Roland, 1988; Jackson *et al*., 1987). Thus, researchers have been interested in reducing egg size during the late stages of the egg production cycle by dietary manipulation of nutrients for increasing eggshell quality.

MET (Sohail *et al*., 2002), linoleic acid (LIN) (Harms & Russell, 2004), and fat (Grobas *et al*., 1999b,c) are three factors that affect egg size. Also, Keshavarz (2003) observed that, by reducing dietary level of protein (PRO) to 13% and omitting supplemental MET from the diet, egg production, egg weight, egg mass, feed consumption, body weight gain, and extra-large plus large-sized eggs were reduced and feed conversion and the number of small plus tiny sized eggs increased. Accordingly, a reduction in the levels of these factors in the diet may decrease egg size and increase eggshell quality. Keshavarz (2003) pointed that reducing the dietary MET level in laying hens from 54 to 72 wk of age from 0.36 to 0.23% decreased productivity, including lower egg production and smaller egg size. Jackson *et al.* (1987) observed that egg weight was reduced and shell strength increased by reducing dietary MET. However, these changes were obtained at the expense of lower egg production. Petersen *et al.* (1983) reported that reducing dietary MET reduced egg weight and improved shell quality without affecting egg production. Safaa *et al.* (2008) reported that a decrease in MET content of the diet from 0.36 to 0.31% and of LIN from 1.60 to 1.12% did not affect hen performance at any age. Reducing the level of added supplemental fat from 3.0 to 1.1% impaired egg production (79.3 vs. 77.0%; $p < 0.05$), egg weight (66.3 vs. 64.9 g; $p < 0.001$), egg mass (52.5 vs. 49.8 g; $p < 0.001$), and feed conversion ratio (FCR) (2.26 vs. 2.36 kg/kg of eggs; $p < 0.001$) (Safaa *et al*., 2008). 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 83);), Chung *et al*. 98);), Ravikiran and Devegowda (1998) also found that supplementing MET to a 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 & Russell, 1993). Many studies have shown that a decrease in dietary fat significantly decreases egg size (Keshavarz & Nakajima, 1995; Bohnsack *et al*., 2002; Sohail *et al*., 2003). Supplemental fat increased both yolk and albumen weights (Safaa *et al*., 2008), but in some study, the improvement was proportionally greater for the albumen than for the yolk (Grobas *et al*., 1999b). The results of many studies have shown that the requirement of LIN for hen productivity and egg size ranged from 1% to 2% of the diet (Scragg *et al*., 1987). Hence, this study was aimed at investigating the effects of dietary levels of MET, PRO and tallow (TAL) or the combination of these nutrient at a constant level of LIN (1.12%) on the performance and eggshell quality in aged laying hens.

MATERIAL AND METHODS

All experimental procedures used in this experiment were approved by the Animal Care Committee of the Ferdowsi University of Mashhad. A completely randomized experimental design was applied in a 3×2×2 factorial arrangement, with three dietary MET levels (0.35, 0.31 and 0.27%), two dietary PRO levels (14.3 and 12.87%) and two dietary TAL levels (3 and 1%), with a constant level of linoleic acid (1.55 ± 0.02%). One hundred forty-four of 70-wk-old hens layers (Hi-line W36) with initial body weight (BW) of 1,687 ± 15.8 g and similar egg production, egg weight, and egg specific gravity, were used in the experiment. The hens were randomly divided in to 12 combinations of MET, PRO and TAL (4 replicates of 3 hens per treatment). Each three hens were housed in a battery cage (40.6 × 45.7 cm) in a house with temperature maintained as close to 21°C as possible and a 16L: 8D lighting program. All hens were fed an experimental diet from 70 to 76 wk of age. They were supplied with feed and water *ad libitum*. The experimental diets were formulated to have simila AMEs and minerals levels, according to the nutritional requirements suggested in the Hi-line W36 Commercial Managemnt Gguide (Hy-Line International, 2009-2011). Ingredients and the nutrient composition of the experimental diets are shown in Table 1.

Feed consumption was recorded biweekly for calculation of average daily feed consumption. Egg production and egg weight were recorded daily. Total eggs from each unit were collected during the last 3 d of every-14 d interval, weighed and graded as indicated by the European Council Directive (2006). The four categories registered for egg size were extra large (>73g), large (73 to 63 g), medium (63 to 53 g) and small (<53g). Weighed and graded eggs were assessed for quality by specific gravity shortly after collection that same day. The specific gravities of the eggs were determined using the formula referring to the Archimedes method (Hempe *et al*., 1988):

Table 1 - Ingredient composition and nutrient content of laying hen diets.

	0.34% MET[1]				0.31% MET				0.27% MET			
Ingredient (%)	14.3 % PRO[2]		12.87 % PRO		14.3 % PRO		12.87 % PRO		14.3 % PRO		12.87 % PRO	
	3% TAL[3]	1% TAL	3% TAL	1% TAL	3% TAL	1% TAL	3% TAL	1% TAL	3% TAL	1% TAL	3% TAL	1% TAL
Corn	59.13	66.47	58.00	65.20	59.11	66.55	58.00	65.20	59.10	66.61	58.00	65.20
Soybean meal	18.94	19.26	13.90	14.43	19.00	19.31	13.95	14.46	19.04	19.40	13.99	14.53
Barley	3.15	0.50	8.00	6.42	3.15	0.40	8.00	6.42	3.16	0.30	8.00	6.39
Wheat bran	3	0	4.15	0	3	0	4.13	0	3	0	4.13	0
Salt	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38
Tallow	3.00	1.00	3.00	1.00	3.00	1.00	3.00	1.00	3.00	1.00	3.00	1.00
Limestone	10.30	10.30	10.30	10.30	10.30	10.30	10.30	10.30	10.30	10.30	10.30	10.30
Dicalcium phosphate	1.44	1.44	1.46	1.48	1.44	1.44	1.46	1.48	1.44	1.44	1.46	1.48
Methionine	0.11	0.10	0.13	0.12	0.08	0.07	0.10	0.09	0.03	0.03	0.06	0.05
Lysine	0.05	0.05	0.18	0.17	0.04	0.05	0.18	0.17	0.05	0.04	0.18	0.17
Mineral premix[4]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix[5]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Calculated analysis												
Energy (kcal ME/kg)	2756	2754	2756	2762	2755	2750	2755	2762	2755	2754	2755	2761
Protein (%)	14.3	14.3	12.87	12.87	14.3	14.3	12.87	12.87	14.3	14.3	12.87	12.87
Methionine (%)	0.34	0.34	0.34	0.34	0.31	0.31	0.31	0.31	0.27	0.27	0.27	0.27
Linoleic acid (%)	1.53	1.57	1.54	1.57	1.53	1.57	1.54	1.57	1.53	1.57	1.54	1.57
Calcium (%)	4.29	4.29	4.29	4.29	4.29	4.29	4.29	4.29	4.29	4.29	4.29	4.29
Non-phytate P (%)	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38

[1]MET= methionine
[2]PRO= protein
[3]TAL= Tallow
[4]Vitamin premix provided per kilogram of diet: vitamin A (retinyl acetate), 8,800 IU; cholecalciferol, 2,200 IU; DL-α-tocopheryl acetate, 11 IU; menadione sodium bisulfite, 2.2 mg; riboflavin, 4.4 mg; D-calcium pantothenate, 8.8 mg; nicotinic acid, 44 mg; pyridoxine hydrochloride, 2.2 mg; d-biotin, 0.11 mg; thiamine hydrochloride, 2.5 mg; ethoxyquin, 125 mg.
[5]Mineral premix provided per kilogram of diet: MnSO4: H2O, 185 mg; ZnO, 62 mg; FeSO47H2O, 149 mg; CuSO4: 5H2O, 19.6 mg; KI, 1.4 mg; Na2SeO3, 0.22 mg.

Specific gravity = weight in air (g)/ [weight in air (g) - weight in water (g)]

A random sample of total eggs per replicate was taken from the collection of last 3 d of each 14 d interval. The eggs used for specific gravity were weighed and broken. The yolks were separated from the albumen. Before determining yolk weight, the chalaza was removed by using a forceps. Each yolk was rolled on a blotting paper towel to remove adhering albumen. The shells were cleaned of any adhering albumen and dried for 5 d. Albumen weight was calculated by subtracting the yolk and shell weights from the whole egg weight. Subsequently, specific gravity measurement was savedt of determining shell weight. Shell weight per unit of surface area (SWUSA) was calculated via by dividing the shell weight (mg) the egg surface area (cm^2). Egg surface area was determined according to Carter (1975), applying the equation: 3.9782 × egg weight (g) $^{0.7056}$. Shell thickness the average of measurements made at three part regions of the egg (air cell, equator, and sharp end) using a shell-thickness measuring gauge (Seri 500, Mitutoyo, Tokyo, Japan). Yolk color and Haugh units were measured (in 4 eggs from each replicate) at the end of the experiment using an egg multi-tester (EMT-5200, Robotmation Co. Ltd., Tokyo, Japan). Haugh units were calculated based on albumen

Table 2 - The effect of methionine (MET, %), protein (PRO, %), and tallow (TAL, %) levels on egg production, egg weight and egg mass.

Treatment			Egg production (%)				Egg weight(g)				Egg mass(g/day)			
MET (%)	PRO (%)	TAL (%)	70 to 72 wk	72 to74 wk	74 to76 wk	70 to76 wk	70 to 72 wk	72 to74 wk	74 to76 wk	70 to76 wk	70 to 72 wk	72 to74 wk	74 to76 wk	70 to 76 wk
0.34	14.7	3	78.3	77.3^{ab}	76.7	77.5	69.1^{ab}	68.9^{ab}	70.1	69.4^{a}	54.0	53.4	53.8	53.7
0.34	14.7	1	78.8	81.5^{a}	78.5	79.6	69.9^{a}	69.1^{a}	67.3	68.7^{ab}	55.0	56.3	52.8	54.7
0.34	12.8	3	75.0	67.8^{b}	78.5	73.8	69.6^{a}	69.5^{a}	68.3	69.1^{a}	52.1	47.1	67.8	51.0
0.34	12.8	1	80.5	79.1^{ab}	79.1	79.6	66.9^{ab}	68.0^{bc}	67.6	67.5^{ab}	53.9	53.9	53.6	53.8
0.31	14.7	3	82.2	81.5^{a}	72.0	78.5	66.1^{b}	67.4^{bc}	66.7	66.7^{b}	54.4	55.0	48.0	52.4
0.31	14.7	1	77.2	77.3^{ab}	73.8	76.1	68.1^{ab}	68.4^{bc}	68.3	68.3^{ab}	52.6	52.9	50.4	52.0
0.31	12.8	3	76.6	75.0^{ab}	73.8	75.1	68.6^{ab}	69.3^{a}	68.7	68.9^{ab}	52.5	51.8	50.6	51.7
0.31	12.8	1	79.4	79.7^{a}	77.3	78.8	67.0^{ab}	66.5^{b}	67.7	67.1^{ab}	53.3	53.0	52.4	52.9
0.27	14.7	3	76.1	73.2^{ab}	75.0	74.7	67.1^{ab}	68.0^{bc}	67.7	67.6^{ab}	51.0	49.7	50.7	50.5
0.27	14.7	1	76.6	70.8^{ab}	75.0	74.1	67.7^{ab}	67.9^{bc}	67.8	67.8^{ab}	52.0	48.1	50.7	50.2
0.27	12.8	3	76.1	72.6^{ab}	72.0	73.5	68.1^{ab}	68.9^{ab}	68.8	68.6^{ab}	51.9	50.0	49.6	50.5
0.27	12.8	1	81.1	79.1^{ab}	79.1	79.8	67.6^{ab}	67.1^{b}	65.3	66.7^{b}	54.8	53.2	51.8	53.3
SEM			3.13	3.50	3.68	2.98	0.903	0.894	1.05	0.747	2.15	2.44	2.55	1.84
Main effect														
MET (%)		0.34	78.1	76.4	78.2	77.6	68.9	68.9	72.6	70.1	53.8	52.7	57.0	54.5
		0.31	78.8	78.4	74.2	77.1	67.5	67.9	67.89	67.7	53.2	53.2	50.4	52.2
		0.27	77.5	73.9	75.2	75.5	67.6	68.0	67.4	67.7	52.4	50.2	50.7	51.1
PRO (%)		14.7	78.2	76.9	75.1	76.8	68.0	68.3	68.0	68.1	53.1	52.5	51.1	52.3
		12.8	78.1	75.5	76.6	76.8	68.0	68.2	70.6	68.9	53.1	51.5	54.3	53.0
TAL (%)		3	77.4	74.6	77.1	75.5	68.1	68.7	71.2	69.3	52.7	51.2	53.4	52.4
		1	78.9	77.9	74.7	78.0	67.9	67.8	67.3	67.7	53.6	52.9	51.9	52.8
								P						
MET			0.719	0.210	0.290	0.548	0.067	0.248	0.513	0.117	0.883	0.202	0.187	0.263
PRO			0.977	0.492	0.488	0.998	0.961	0.898	0.684	0.793	0.786	0.462	0.564	0.934
TAL														
			0.458	0.104	0.251	0.130	0.657	0.115	0.097	0.112	0.566	0.231	0.555	0.276
PRO × TAL			0.084	0.046	0.548	0.091	0.014	0.024	0.292	0.017	0.480	0.166	0.777	0.309
MET× PRO			0.867	0.151	0.918	0.577	0.283	0.959	0.699	0.755	0.843	0.140	0.894	0.704
MET× TAL			0.515	0.305	0.899	0.686	0.649	0.982	0.230	0.323	0.690	0.292	0.801	0.951
MET× TAL × PRO			0.809	0.978	0.722	0.912	0.594	0.603	0.401	0.746	0.864	0.973	0.920	0.900

a-c Means within each column with no common superscript differ ($p < 0.05$).

height and egg weight using the formula: $HU = 100 \log_{10} (H - 1.7 W^{0.37} + 7.56)$, where HU = Haugh unit, H = height of the albumen (mm) and W = egg weight (g). Moreover, shell strength was measured using an egg force gauge (Sanovoeng Co. Ltd., Tokyo, Japan).

The experiment was conducted as a completely randomized design with 12 treatments arranged in a factorial arrangement. Data were analyzed by means of GLM (SAS Institute, 2003). Significant differences of means among treatments were tested by Duncan's multiple range tests. Variation within each treatment was expressed as the P value and SEM (standard error of the mean) at 5% probability level.

RESULTS AND DISCUSSION

The effects of different dietary treatments on egg production, egg weight and egg mass are shown in Table 2. The interaction between dietary treatments was not significant for egg production, egg weight and egg mass with exception of PRO × TAL; therefore only the main effects are presented. The Significant interactions were observed between PRO and TAL for egg production (72 to 74 wk) and egg weight (70 to 72, 72 to 74 and 70 to 76 wk). A protein level by TAL interaction ($p < 0.05$) was observed for the entire experimental period, indicating that the impact of 1% TAL on egg weight reduction was more pronounced in hens fed 12.8% PRO than in hens fed 14.7% PRO. Egg production can be affected by protein (36 weeks of age) (Liu *et al*., 2005), lysine (85 weeks of age) (Wu *et al*., 2005a), and supplemental fat (65 weeks of age) (Grobas, 1999b). There have been contradictory results about the effect of supplemental fat on egg production. Jackson *et al*. (1987) observed that egg weight decreased by reducing dietary MET. Likewise, Petersen *et al*. (1983) reported that reducing dietary

Table 3 - The effect of methionine (MET, %), protein (PRO, %), and tallow (TAL, %) levels on feed intake and feed conversion.

Treatment			Feed intake(g/day)				Feed conversion(g/100 g egg mass)			
MET (%)	PRO (%)	TAL (%)	70 to 72 wk	72 to74 wk	74 to76 wk	70 to76 wk	70 to 72 wk	72 to74 wk	74 to76 wk	70 to76 wk
0.34	14.7	3	127.15	142.51	131.99	133.88	2.35	2.67[ab]	2.45	2.49
0.34	14.7	1	117.18	125.67	119.55	120.80	2.13	2.23[b]	2.26	2.21
0.34	12.8	3	122.11	132.62	134.75	129.83	2.34	2.81[a]	1.99	2.55
0.34	12.8	1	117.37	135.39	127.11	126.62	2.17	2.51[ab]	2.37	2.35
0.31	14.7	3	119.95	132.14	124.69	125.59	2.20	2.40[ab]	2.60	2.39
0.31	14.7	1	121.12	128.54	118.17	122.61	2.30	2.43[ab]	2.34	2.36
0.31	12.8	3	133.00	131.42	124.78	126.40	2.53	2.53[ab]	2.46	2.44
0.31	12.8	1	122.41	138.54	130.20	130.38	2.30	2.61[ab]	2.48	2.46
0.27	14.7	3	117.77	130.94	127.13	125.28	2.31	2.63[ab]	2.50	2.48
0.27	14.7	1	129.02	135.65	128.79	131.15	2.48	2.82[a]	2.54	2.61
0.27	12.8	3	114.46	121.86	122.60	119.64	2.20	2.44[ab]	2.47	2.37
0.27	12.8	1	116.91	133.08	133.51	127.83	2.13	2.50[ab]	2.58	2.40
SEM			4.39	6.630	6.507	5.209	0.120	0.179	0.246	0.154
Main effect										
MET (%)		0.34	120.95	134.05	128.35	127.78	2.60	3.16	2.90	2.89
		0.31	121.62	132.66	124.46	126.25	2.61	3.10	3.07	2.93
		0.27	119.54	130.38	128.01	125.98	2.67	3.23	3.14	3.02
PRO (%)		14.7	122.03	132.58	125.05	126.55	2.66	3.14	3.05	2.95
		12.8	119.38	132.15	128.83	126.79	2.59	3.19	3.02	2.93
TAL (%)		3	120.74	131.92	127.66	126.77	2.65	3.20	3.05	2.97
		1	120.67	132.81	126.22	126.57	2.60	3.13	3.02	2.92
						P				
MET			0.830	0.512	0.716	0.872	0.763	0.587	0.601	0.493
PRO			0.215	0.566	0.416	0.734	0.351	0.679	0.977	0.826
TAL			0.861	0.542	0.607	0.580	0.430	0.481	0.537	0.562
MET × TAL			0.090	0.247	0.312	0.173	0.153	0.040	0.634	0.317
MET × PRO			0.312	0.323	0.719	0.460	0.221	0.066	0.999	0.431
PRO × TAL			0.677	0.110	0.292	0.274	0.328	0.945	0.511	0.727
MET × TAL× PRO			0.732	0.883	0.901	0.895	0.458	0.830	0.866	0.718

[a-b] Means within each column with no common superscript differ ($p < 0.05$).

MET declined egg weight without affecting egg production. In one study, Keshavarz (2003) found that, after reducing dietary level of protein from 15 to 13% and omitting supplemental MET, egg production, egg weight, egg mass, feed consumption, body weight gain and extra large-sized eggs were reduced and FCR was increased. Similarly, Harms & Russell (2003) reported that a reduction in MET from 0.36 to30% of in the diet of laying hens from 45 to 54 wk of age did not affect body weight, egg production, egg weight, and feed intake. In fact, the authors reported that, when MET content was greater than 0.30%, egg production and egg weight were numerically rduced, indicating that MET was not limiting performance. Shafer *et al*. (1996) found that egg production was not significantly different due to MET treatments when laying hens were fed diets containing MET from 0.283 to 0.4%. Egg weight can be increase by increasing dietary protein (Liu *et al*., 2005 & Wu *et al*., 2005a), MET (Keshavarz, 1995), lysine (Novak *et al*., 2004 and Liu *et al*., 2005), supplemental fat (Grobas, 1999a, 1999b; Sohail *et al*., 2003) and energy (Bryant *et al*., 2005).

The impacts of protein and amino acids have been well understood. However, there are discrepant reports about the impact of supplemental fat or dietary energy on egg weight. In our experiment, a significant interaction was obtained between PRO × TAL on egg weight. This outcome was similar to that of Sohail *et*

Table 4 - The effect of methionine (MET, %), protein (PRO, %), and tallow (TAL, %) levels on specific gravity and eggshell thickness.

Treatment			Specific gravity				Eggshell thickness (mm)			
MET (%)	PRO (%)	TAL (%)	70 to 72 wk	72 to74 wk	74 to76 wk	70 to76 wk	70 to 72 wk	72 to74 wk	74to76 wk	70 to76 wk
0.34	14.7	3	1.054[ab]	1.066	1.068[ab]	1.063	0.371	0.374	0.388	0.377[ab]
0.34	14.7	1	1.049[b]	1.068	1.067[ab]	1.062	0.379	0.394	0.396	0.390[ab]
0.34	12.8	3	1.056[ab]	1.070	1.071[a]	1.065	0.392	0.391	0.306	0.396[a]
0.34	12.8	1	1.050[ab]	1.069	1.068[ab]	1.062	0.369	0.389	0.383	0.380[ab]
0.31	14.7	3	1.049[b]	1.064	1.067[ab]	1.060	0.365	0.382	0.301	0.383[ab]
0.31	14.7	1	1.053[ab]	1.067	1.069[ab]	1.063	0.365	0.371	0.380	0.372[ab]
0.31	12.8	3	1.050[ab]	1.063	1.065[b]	1.060	0.352	0.348	0.395	0.365[b]
0.31	12.8	1	1.051[ab]	1.067	1.069[ab]	1.062	0.374	0.380	0.386	0.380[ab]
0.27	14.7	3	1.052[ab]	1.067	1.064[b]	1.061	0.363	0.373	0.369	0.368[b]
0.27	14.7	1	1.053[ab]	1.067	1.071[a]	1.064	0.392	0.377	0.399	0.389[ab]
0.27	12.8	3	1.054[ab]	1.067	1.069[ab]	1.063	0.382	0.388	0.395	0.388[ab]
0.27	12.8	1	1.059[a]	1.067	1.068[ab]	1.065	0.374	0.376	0.384	0.378[ab]
SEM			0.002	0.002	0.001	0.001	0.010	0.011	0.010	0.007
Main effect										
MET (%)		0.34	1.052	1.068	1.069	1.063	0.378	0.387	0.393	0.386
		0.31	1.051	1.065	1.068	1.061	0.364	0.379	0.391	0.375
		0.27	1.055	1.067	1.068	1.063	0.378	0.370	0.387	0.381
PRO (%)		14.7	1.052	1.067	1.068	1.062	0.373	0.378	0.389	0.380
		12.8	1.053	1.067	1.068	1.063	0.374	0.379	0.391	0.381
TAL (%)		3	1.053	1.066	1.067	1.062	0.371	0.376	0.392	0.380
		1	1.053	1.068	1.069	1.063	0.376	0.381	0.388	0.382
					P					
MET			0.136	0.150	0.564	0.144	0.185	0.126	0.691	0.126
PRO			0.224	0.681	0.563	0.117	0.530	0.969	0.689	0.757
TAL			0.906	0.239	0.221	0.273	0.520	0.417	0.511	0.650
MET × TAL			0.044	0.414	0.032	0.054	0.531	0.606	0.291	0.795
MET × PRO			0.774	0.593	0.265	0.410	0.769	0.374	0.936	0.586
PRO × TAL			0.919	0.657	0.181	0.507	0.408	0.921	0.113	0.187
MET × PRO × TAL			0.697	0.762	0.181	0.937	0.137	0.102	0.200	0.016

[a-b] Means within each column with no common superscript differ ($p < 0.05$).

Table 5 - The effect of methionine (MET, %), protein (PRO, %), and tallow (TAL, %) levels on shell weight and SWUSA.

Treatment			Shell weight(g)				SWUSA(mg/cm²)			
MET (%)	PRO (%)	TAL (%)	70 to 72 wk	72 to74 wk	74 to76 wk	70 to76 wk	70 to 72 wk	72 to74 wk	74 to76 wk	70 to76 wk
0.34	14.7	3	5.40	5.54	5.66	5.53	68.5	70.3	70.9	69.8
0.34	14.7	1	5.82	5.76	5.84	5.81	72.9	72.9	75.5	73.7
0.34	12.8	3	5.86	5.50	5.62	5.66	73.8	69.4	64.8	68.5
0.34	12.8	1	5.57	5.63	5.59	5.60	72.1	72.1	71.8	72.0
0.31	14.7	3	5.35	5.57	5.76	5.56	69.8	71.7	74.7	72.1
0.31	14.7	1	5.44	5.43	5.65	5.51	69.6	69.1	72.1	70.3
0.31	12.8	3	5.56	5.16	5.66	5.46	70.7	65.3	71.9	69.3
0.31	12.8	1	5.58	5.67	5.54	5.60	72.2	73.8	71.1	72.3
0.27	14.7	3	5.66	5.55	5.18	5.46	73.1	71.0	66.3	70.1
0.27	14.7	1	6.04	5.54	5.57	5.71	77.4	70.9	71.4	73.2
0.27	12.8	3	5.55	5.67	5.58	5.60	70.9	71.9	70.9	71.3
0.27	12.8	1	5.37	5.46	5.37	5.40	69.1	70.6	70.6	70.1
SEM			0.219	0.179	0.204	0.153	2.69	2.25	3.21	2.13
Main effect										
MET (%)		0.34	5.66	5.61	5.68	5.65	71.8	71.2	70.8	71.0
		0.31	5.56	5.46	5.65	5.53	70.6	70.0	72.4	71.0
		0.27	5.48	5.56	5.42	5.54	72.6	71.1	69.8	71.2
PRO (%)		14.7	5.62	5.56	5.61	5.60	71.9	71.0	71.8	71.6
		12.8	5.58	5.52	5.56	5.55	71.5	70.5	70.2	70.6
TAL (%)		3	5.56	5.50	5.58	5.55	71.1	69.9	69.9	70.2
		1	5.64	5.58	5.59	5.60	72.2	71.6	72.1	71.9
						P				
MET			0.423	0.486	0.237	0.803	0.554	0.705	0.510	0.989
PRO			0.780	0.654	0.732	0.660	0.787	0.701	0.380	0.430
TAL			0.568	0.435	0.485	0.242	0.491	0.214	0.250	0.163
MET × PRO			0.154	0.889	0.494	0.920	0.102	0.905	0.329	0.926
MET × TAL			0.991	0.432	0.407	0.821	0.976	0.447	0.261	0.537
PRO × TAL			0.091	0.555	0.358	0.648	0.261	0.212	0.919	0.966
MET × PRO × TAL			0.568	0.203	0.539	0.149	0.497	0.119	0.631	0.328

al. (2003), who reported that increasing fat affected egg weight. In contrast, Zou & Wu (2005) reported that increasing supplemental fat had no significant effect on egg weight. The differences among results in literatures may be due to differences in bird strain, body weight, age and fat composition. Significant interactions were observed between the levels of MET and TAL for FCR from 72 to 74 wk of age. Other dietary treatments did not have a noteworthy influence on FCR. An increase in MET content of the diet from 0.27 to 0.34% with constant level of 1%TAL resulted to best FCR (2.82 vs. 2.23) (Table 3). Safaa *et al.* (2008) reported that egg production and FCR per a dozen eggs were impaired at low levels of MET (0.31%) and supplemental fat (SFAT) (1.1%) in the diet. Soheil *et al*. (2003) indicated that SFAT enhanced feed efficiency and egg weight in laying hens. Some researchers showed that increased dietary MET intake significantly improved egg production, egg mass, egg weight and FCR (Bunchasak & Silapasorn, 2005), which also agrees with our results. Indeed, increasing fat content has the influence of slowing passage rate, allowing more time for the contact between enzymes and dietary components, which may lead to added digestibility of the nutrients such as protein and amino acids (Ewan, 1991). There was an interaction of MET × TAL for specific gravity in during the periods of 70 to 72 and 74 to 76 wk. Specific gravity dropped from 1.059 to 1.049 when hens were fed additional MET (0.34 vs.27%) at the level of 1%TAL. Keshavarz (2003) indicated that a reduction in MET content in the diet of laying hens from 0.32 to 0.27% did not

Table 6 - The effect of methionine (MET, %), protein (PRO, %), and tallow (TAL, %) levels on yolk weight and albumen weight.

Treatment			Yolk weight(g)				Albumen weight(g)							
MET (%)	PRO (%)	TAL (%)	70 to 72 wk	72 to74 wk	74 to76 wk	70 to76 wk	70 to 72 wk	72 to74 wk	74to76 wk	70 to76 wk	Haugh unit	Yolk color	Shell strength (kg force)	Large eggs (%)
0.34	14.7	3	20.0ab	20.3	19.9	20.0	40.8ab	42.6	44.9	42.8	51.0	7.000	2.465abc	87.1
0.34	14.7	1	19.9b	20.1	20.4	20.1	43.3ab	43.2	44.6	43.7	41.9	7.375	2.123abc	84.7
0.34	12.8	3	20.8a	19.4	19.1	19.8	43.1ab	40.7	40.3	41.4	51.9	7.625	2.679a	66.1
0.34	12.8	1	19.5ab	20.3	20.5	20.1	42.0ab	42.2	43.9	42.7	55.5	7.375	2.350abc	72.0
0.31	14.7	3	19.6ab	20.1	20.1	19.9	40.7ab	43.1	43.9	42.6	38.1	6.750	2.185abc	75.5
0.31	14.7	1	20.1ab	19.8	20.8	20.2	42.1ab	41.1	43.9	42.4	50.4	7.875	2.452abc	79.5
0.31	12.8	3	20.8a	21.3	19.7	20.6	45.3a	44.0	44.6	44.7	50.4	7.375	2.197abc	73.3
0.31	12.8	1	20.1ab	20.1	20.2	20.1	42.0ab	42.7	41.9	42.2	47.8	8.000	2.523ab	71.5
0.27	14.7	3	21.0a	20.5	20.2	20.6	43.7ab	41.6	41.9	42.4	49.5	7.125	1.947c	73.0
0.27	14.7	1	20.7a	20.2	19.8	20.3	42.5ab	41.6	41.5	41.9	56.4	7.000	2.323abc	74.1
0.27	12.8	3	19.5ab	19.7	19.8	19.6	39.6b	42.3	41.3	41.0	57.3	7.125	2.455abc	69.3
0.27	12.8	1	18.8ab	19.6	20.0	19.5	44.6ab	42.4	41.6	42.9	57.4	7.250	2.055bc	66.1
SEM			0.462	0.422	0.525	0.389	1.506	1.012	1.207	0.858	5.716	0.254	0.167	7.86
Main effect														
MET (%)		0.34	20.0	20.0	20.0	20.0	42.3	42.2	43.5	42.7	50.1	7.34	2.40	76.7
		0.31	20.1	20.3	20.2	20.2	42.5	42.7	43.6	43.0	46.7	7.50	2.33	75.0
		0.27	20.0	20.0	20.0	20.0	42.6	42.0	41.6	42.1	55.2	7.12	2.19	68.1
PRO (%)		14.7	20.2	20.2	20.2	20.2	42.8	42.2	43.5a	42.6	47.9	7.18b	2.24	76.8
		12.8	19.9	20.1	19.9	20.0	42.2	42.4	42.3b	42.5	53.4	7.45a	2.37	69.7
TAL (%)		3	20.3	20.2	19.8	20.1	42.2	42.4	42.8	42.5	49.7	7.16b	2.32	74.6
		1	19.8	20.0	20.3	20.1	42.8	42.2	42.9	42.6	51.6	7.47a	2.30	71.92
								p						
MET			0.895	0.477	0.828	0.669	0.960	0.553	0.069	0.325	0.121	0.127	0.210	0.444
PRO			0.270	0.708	0.299	0.299	0.510	0.808	0.045	0.768	0.104	0.033	0.197	0.060
TAL			0.121	0.526	0.119	0.888	0.529	0.728	0.950	0.777	0.573	0.030	0.860	0.706
MET × PRO			0.002	0.053	0.870	0.129	0.324	0.152	0.378	0.221	0.928	0.497	0.753	0.546
MET × TAL			0.669	0.201	0.386	0.717	0.412	0.173	0.224	0.115	0.607	0.047	0.039	0.764
PRO × TAL			0.074	0.713	0.454	0.801	0.679	0.643	0.411	0.863	0.656	0.588	0.234	0.675
MET × PRO × TAL			0.813	0.239	0.794	0.632	0.027	0.965	0.104	0.174	0.226	0.369	0.156	0.487

a-c Means within each column with no common superscript differ ($p < 0.05$).

influence the percentage of large eggs or the specific gravity of the eggshell from 56 to and 68 wk of age. However, in a second experiment, this author observed that a further reduction of MET to 0.23% decreased the percentage of large eggs from 37.6 to 22.2% and increased eggshell specific gravity from 1.0749 to 1.0788 in laying hens from 54 to 72 wk of age, which is almost identical to our experiment. Novak *et al.* (2006) showed that by feeding low-protein diets, specific gravity was linearly reduced, indicating that shell quality was being reduced. Increasing the total sulfur amino acids (TSAA): lysine rate increased shell quality, indicating that, in order to optimize shell quality, the sulfur amino acid requirements for the synthesis of shell synthesis of shell protein matrix needs to be considered. Simkiss & Taylor (1957) reported that the shell protein matrix is comprised of 70% protein. Also, increasing the sulfate groups present in the shell matrix significantly increases the Ca binding ability, which in turn, may increase both shell percentage and specific gravity, as well as overall shell quality. Other researchers have also indicated that decreasing dietary protein will decrease shell quality (Keshavarz & Nakajima, 1995; Keshavarz & Jackson, 1992). Eggshell thickness was reduced due to decreasing the MET content of the diet from 0.34% to 0.31% when the dietary TAL was 3% (p<0.05), independently of PRO level. Eggshell thickness decreased significantly due to the reduction of dietary Met content from 0.34 to 0.27% (p<0.05). 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 a*004). Same Consistent with our experiment, Bunchasak & Silapasorn (2005) reported that eggshell thickness was significantly improved when MET was added at 0.3 or 0.38% to low crude protein diets. Similarly, Carey *et al. (1991)* observed increasing eggshell weight when increasing Met level from 330 to 450 mg/hen/day. However, Shafer *et al*. (1996) reported that increasing TSAA intake from 624 to 822 mg/hen/day had no effect on eggshell weight or eggshell percentage at 52 wk of age. Thus, it is suggested that MET intake higher than 620 mg/hen/dayd would not have any benefit on eggshell quality. Another reason is that, in general, the foundation of a shell consists of a protein matrix, and it may be possible that increasing the total sulfur amino acid intake may influence the protein synthesis of the shell membranes (Novak *et al.*, 2004).

Yolk weight (Table 6) was affected by different levels of MET × PRO from 70 to 72 wk. The hens fed the highest level of MET and PRO had the lowest yolk weight. There was interaction (p<0.05) among MET, PRO and TAL for albumen weight in the first 2-wk period. Albumen weight diminished as the level of MET (0.31 to 0.27) decreased at a constant level of PRO (12.87%) and TAL (3%) in the diet (45.38 vs. 39.66 g). Albumen weight was significantly (p<0.05) affected by dietary PRO level from 74 to 76 wk (Table 6). Hens receiving the diet with 14.7% PRO produced heavier albumen than those receiving diet with 12.8% PRO. At the level of 3% TAL and 12.8% protein of diet, albumen weight was reduced when the MET level was reduced from 0.31 to 0.27% (p<0.05). Novak *et al.* (2006) indicated that, as protein intake declined, dry albumen and yolk weights linearly decreased and increased, respectively. On the other hand, protein intake influenced yolk components. As protein intake was decreased from 16.3 to 13.8 g/day, there was a linear rise in percentage of yolk from 27 to 27.4%, respectively. According to Table 6, yolk color was more intense at the end of experimental for layers fed 12.8% PRO than those fed 14.7%. Contrary to the results obtained by Gunawardana *et al*. (2008), increasing TAL levels from 1 to 3% in the diet significantly weakened egg yolk color (Table 6). Corn, corn gluten meal, and alfalfa meal are the main xanthophyll sources used in poultry feeds. In laying hens,the muscles and skin xanthophyll supplies are transferred to the ovaries at the onset of sexual maturity, and part of them are excreted in the egg yolk (Gouveia *et al*. 1996). It is likely that reduction of corn level in diet contained 3%, compared with 1% TAL, resulted to paler yolk color. Gunawardana *et al*. (2008) reported that yolk color depends on fat soluble carotenoids present in dietary fats. There are contradictory results relative to the effect of added fat on yolk color. Madiedo & Sunde (1964) reported that added dietary fat had no effect on egg yolk color. In contrast, Mackay *et al*. (1963) found that supplemental fat had a significant effect on egg yolk color. A significant interaction of MET × TAL was observed for yolk color (Tabl 6). The supplementation of 3% TAL significantly depressed yolk color compared to with 1% TAL. Omara & Romeilah (2009) found that egg color did was not statistically different between 0.35 and 0.40% MET levels. Differences in egg yolk color were significant considering the energy × MET × folic acid interaction. These discrepancies may be due to the xanthophyll content of the main feed ingredients rather than to the actual energy levels, for the xanthophyll levels were not controlled within the diets. This could be due to high carotenoid content of corn (Ciftci *et al*, 2003). Significant MET × TAL interaction was also observed for shell strength (Table 6). The highest shell strength value was obtained in hens fed diets with high levels of MET and TAL plus 12.8% protein. In contrast to this experiment, Novak *et al*. (2006) reported that dietary treatments varying in the total sulfur amino acids and protein had no effect on eggshell breaking strength. Egg size was not influenced by dietary treatments. In conclusion, the results of the present experiment showed that decreasing the levels of MET and PRO to lower levels than the recommended values can decrease egg weighgt with no negative effects on the productive performance and egg quality of laying hens in the late phase of production.

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