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Full Length Research Paper

Thyroid function and egg characteristics of laying hens in response to dietary methionine levels

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This experiment was conducted to evaluate the effects of dietary methionine (met) levels on thyroid function, egg characteristics and protein and energy efficiency of laying hens. A total number of 216 commercial laying hens at 23 weeks of age were randomly allocated to six treatments (four replicates with nine birds in each), and fed 6 dietary have deficiency in met supplement (0.24, 0.29, 0.34, 0.39, 0.44 and 0.49% met). With increasing dietary met level from 0.24 to 0.49%, plasma triiodothyronine (T_3) showed an inverted U relationship. Plasma thyroxin (T_4) was similar between all treatments. Protein and energy efficiency were increased by increasing dietary Met levels. Egg shape index was significantly higher and lower with 0.29 and 0.39% met levels in diet, respectively. Egg surface area and unit surface shell weight increased by increasing dietary met level from 0.24 to 0.49%, and shell ratio decreased linearly. Shell weight increased significantly by increased met level of diet to 0.34%. Albumin index and Haugh unit were significantly lower in hens which fed the three higher levels of met (0.39 to 0.49%). It is concluded that met deficiency alters normal thyroid hormone metabolism, but the effect was dependent on the degree of deficiency. Furthermore, optimal supplementation of met in diets deficient in met, could improve egg quality of laying hens.

Key words: Metionine, egg characteristics, triiodothyronine, thyroxine, laying hens.

INTRODUCTION

In regions where soybean meal is the primary protein source of poultry rations, methionine (met) and lysine (lys) are generally considered as the first and second limiting essential amino acids for laying hens (Carew et al., 2003). Therefore, supplemental met is routinely used in layer feeds to solve lower dietary protein content problem, resulting in reduction of feed cost and a reduction in Feed Consumption (FC), as dietary energy increases. Many reports have been published on the met requirement of poultry. But, there is a wide variation in recommendations due to factors that influence its dietary requirement (NRC, 1994; Ishibashi and Yonemochi, 2003). The importance of met is indicated by three major functions in poultry: a metyl donor, in protein synthesis, and as a precursor to cysteine (Cys) (Graber and Baker, 1971). Furthermore, sulfur amino acids (SAA) are essential for growth, metylation reactions (including the synthesis of metabolites such as phosphatidyl Choline and creatine), feather synthesis and are important pre-cursors for synthesis of glutathione, taurine, Coenzyme A, selenoenzymes, and polyamines (Larbier and Leclercq, 1992; Lumpkins et al., 2007).

Several studies have shown that low dietary protein intakes influence thyroid function, especially circulating levels of thyroid hormones. Plasma triiodothyronine (T_3) is often elevated in protein-deficient chicks (Alster and Carew, 1984; Keagy et al., 1987; Buyse et al., 1992) accompanied frequently by depressions in plasma thyroxin (T_4) (Alster and Carew, 1984; Keagy et al., 1987). An important question concerning changes in thyroid function in protein-deficient chicks is what extent low intakes of the different Essential Amino Acids (EAA)

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may be responsible for these effects. A study of the effects of deficiencies of EAA on thyroid function has shown that dietary deficient of individual EAA have different effects on blood levels of T_3 and T_4 in growing chicks (Carew et al., 1997).

Many aspects of met deficiency on avian metabolism have been studied, such as interaction with choline, betaine, folic acid, and vitamin B_{12} (as the metyl donors) (McDevitt et al., 2000) as well as effects on the immune system. However, very few studies were carried out on the effects of dietary met levels on the avian endocrine system, especially in laying hens. Research on nutritional effects on Liquid Egg (LE) yield and composition may provide the egg producer an effective management tool for customized egg shell production specially managed to maximize the yield of LE product (Shafer et al., 1998). Albumin and yolk are the valued products, whereas shell is treated as a low value by-product, or wastage, by breaking operations. Increasing proportional liquid component yield will allow processors to produce greater liquid mass from an equivalent number of eggs (Shafer et al., 1998). Genetic potential of the commercial layers is continuously changing to support both the liquid egg and egg shell markets. The objective of current study is designed to focused on the effects of excessive and deficient of dietary met on thyroid function and egg characteristics of Hy-Line W36 laying hens.

MATERIALS AND METHODS

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

A total of 216 commercial white Leghorn layer hens were obtained from the local parent stock supplier. Hens were moved into the production house (14 × 6 m^2) at 18 wk of age. Artificial light was arranged in 16 h/d, and maintained until the end of experimental period. The air temperature was controlled within the range of 22 to 28.3°C to obtain a constant feed intake. At 22 week of age all hens were individually weighed and allocated randomly into six treatments with four replicates and nine birds in each replication. Replicates were equally distributed into upper and lower cages to minimize cage level effect. Three hens were housed in a 42 × 40 × 48 cm^3 cage, and 3 adjoining cages consists a replicate. Cages were equipped with nipple drinkers and trough feeders.

Basal diet was formulated based on corn, wheat, and soybean meal (Table 1) and contained 15.33% Crude Protein (CP), 2832.71 k cal metabolizable energy (ME)/kg, and 0.24% met. Synthetic DL-Met was added to the deficient (basal) diet in increments of 0.05% (0, 0.05, 0.10, 0.15, 0.20 and 0.25) at the expense of soybean meal to meet the desired met levels in experimental diets. Supplemental L-Lys was included in the diet to a total of 0.88% (a concentration found to be adequate for a high performance of laying hens (NRC, 1994) to ensure that met was the first limiting amino acid. Ingredients were analyzed for CP, Ether Extract (EE), ash and Crude Fiber (CF) before formulating the diet according to the procedures of the Association of Official Analytical Chemists (AOAC, 1990). Energy value for these feeding stuffs was calculated based on NRC (1994), and similar density was maintained in all diets. The amino acids compositions of all ingredients (corn, wheat, and soybean meal) were analyzed by Chemical lab of Bu-Ali Sina University (Tecator apparatus (optilab 5931 liquid chromatograph), C18 column) by the method described by Ravindran et al. (1999). The experimental diets were prepared and mixed freshly at intervals of about four weeks. Feed (in mash form) and water were provided *ad libitum* for the entirety of the experiment.

Thyroid function

At 27 and 32 weeks of age, 1 ml of blood was drawn from the brachial vein of the 8 hens from each treatment. Blood samples were collected at the same time in the morning and transferred to vial tubes containing heparin as anticoagulant. Plasma was prepared by centrifuging (5,000 × g for 20 min) and stored at -20°C for analysis. Plasma T_3 and T_4 levels were analyzed by radioimmunoassay RIA (TECAN, Magellan apparatus), which had been validated as described by Carew et al. (1997). Specificity of the assays for T_3 and, T_4 had been established by the supplier (Pishtaz Teb, Tehran).

Efficiency of protein and metabolizable energy and egg characteristics

Protein Efficiency Ratio (PER) and ratio of met intake (mg)/ ME intake (Kcal/kg) were determined on replicate basis. Egg characteristics were evaluated on a biweekly basis. On each predetermined sample day, all eggs from each replicate were collected during a 24 h period. Cracked eggs, dirty, misshapen, or of extreme size were culled. The short and long diameters of the eggs were measured by a caliper with a sensitivity of 0.01 mm to determine the shape index. Eggs were weighed individually, and then broken out on a flat surface, with a waiting period of 5 min. The shells were washed with distilled water to release albumin residues, allowed to air dry, and were weighed. The heights of the yolk and albumin, the long and short diameters of the albumin, and the diameter of the yolk were measured using the caliper. From the values obtained, the following data were calculated according to (Yannakopoulos and Tserveni-Gousi, 1986) using the formulates shown below:

Shell ratio = shell weight/egg weight × 100
Shape index = short edge/long edge × 100
Yolk index = yolk height/yolk diameter × 100
Albumin index = albumin height/ (long diameter of albumin + short diameter of albumin/2) × 100
Egg specific gravity = egg weight/ (0.968 × egg weight – 0.4759 × shell weight)
Egg surface area = $3.9782 \times \text{egg weight}^{0.75256}$
Haugh unit = $100 \pm \log (\text{albumin height} + 7.57 - 1.7 \times \text{egg weight}^{0.37})$ (Nesheim et al., 1979).

Experimental design and statistical analysis

The experiment was conducted as a Completely Randomized Design (CRD). The data were subjected to ANOVA with the General Linear Model procedure of SAS (2004). The general linear model employed for statistical analysis was the following:

$$X_{ij} = \mu + \delta_j + \varepsilon_{ij}$$

Homogeneity of variance was determined by Bartlett's test. Duncan's multiple range test (1955) was used to determine the

Table 1. Composition and nutrient content of 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
Analyzed values[7] (%)	
Crude protein	15.33
Ether extract	2.65
Crude fiber	2.79
Ash	2.25
Met	0.24
Cys	0.28
Met+Cys	0.52
Lys	0.88
Calculated values[8]	
ME[9] (Kcal/kg)	2832.71
Ca (%)	3.50
NPP[10] (%)	0.35
Na (%)	0.17

[1]Based on 100 g/h per day. [2]Contained 18.7% P and 22% Ca. [3]Contained 38% Ca. [4]Contained 39% Na. [5]Supplies per kilogram of diet: copper, 10 mg; ethoxyquin, 65 mg; iodine, 2 mg; iron, 60 mg; manganese, 90 mg; selenium, 0.2 mg; and zinc, 80 mg. [6]Supplies 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; and vitamin K, 2 mg. [7]Based on analysis of corn, soybean meal and wheat. [8]Calculated from tabular values (NRC, 1994).[9] Metabolizable energy. [10] None phytate phosphorous.

significance of differences among treatments means. Statements of significance were based on $P < 0.05$.

RESULTS

Thyroid function

Plasma T_3 concentration of laying hens was influenced by met levels (Table 2). Plasma T_3 showed an inverted U relationship by increasing dietary met levels from 0.24 to 0.49%. The lowest and highest plasma T_3 concentration were respectively related to hens which consumed 0.24 (1.00 ng/ml) and 0.29% (1.69 ng/ml) dietary met. There were inconsistent and no significant differences in plasma T_4 of laying hens by met levels from 0.24 to 0.49% (Table 2).

Efficiency of protein and metabolizable energy and egg characteristics

Adding 0.1% DL-Met to diet significantly increased PER ($P < 0.05$, Table 3) However, further Met supplementation in diet had no more effect on PER. The ratio of met intake (mg)/ ME intake (Kcal/kg) increased significantly ($P <$

Table 2. Plasma thyroid hormones concentrations by various levels of dietary met in laying hens.

Parameter	Dietary metionine (%)						P	MSE[1]
	0.24	0.29	0.34	0.39	0.44	0.49		
T_3 (ng ml^{-1})	1.00^{b}	1.69^{a}	1.55^{ab}	1.37^{ab}	1.24^{ab}	1.37^{ab}	0.3078	0.307
T_4 (µg dl^{-1})	1.97^{a}	1.53^{a}	1.70^{a}	1.59^{a}	1.46^{a}	1.67^{a}	0.8976	0.724

PER (Protein Efficiency Ratio). Means within a row without a common superscript differ significantly ($P < 0.05$).[1] Mean square error.

Table 3. Protein and energy efficiency in response to dietary met levels.

Parameter	Dietary methionine (%)						P	MSE[1]
	0.24	0.29	0.34	0.39	0.44	0.49		
PER	8.53^{b}	11.84^{ab}	14.09^{a}	14.60^{a}	14.54^{a}	14.02^{a}	0.0467	8.042
Met intake (mg)/ ME intake (Kcal/kg)	0.85^{f}	1.02^{e}	1.20^{d}	1.38^{c}	1.55^{b}	1.73^{a}	<0.0001	0.000

Means within a row without a common superscript differ significantly ($P < 0.05$). [1] Mean square error.

Table 4. Laying hens egg characteristics in response to dietary met levels.

Parameter	Dietary methionine (%)						P	MSE[1]
	0.24	0.29	0.34	0.39	0.44	0.49		
Shape index (%)	76.39^{ab}	76.87^{a}	76.03^{ab}	74.43^{c}	75.14^{bc}	76.16^{ab}	0.0067	10.350
Shell weight (g)	4.76^{b}	4.85^{b}	5.08^{a}	5.13^{a}	5.22^{a}	5.22^{a}	0.0001	0.235
Shell ratio (%)	10.06^{a}	9.74^{b}	9.53^{bc}	9.56^{bc}	9.58^{bc}	9.47^{c}	0.0002	0.608
Albumin index (%)	22.89^{a}	22.02^{a}	22.07^{a}	19.69^{b}	20.33^{b}	19.26^{b}	0.0008	3.392
Yolk index (%)	43.26^{a}	41.99^{a}	42.78^{a}	43.96^{a}	41.23^{a}	41.64^{a}	0.2058	5.486
Egg specific gravity (%)	1.09^{a}	1.09^{a}	1.08^{a}	1.09^{a}	1.08^{a}	1.08^{a}	0.4667	0.001
Egg surface area (cm^2)	72.50^{e}	75.32^{d}	78.22^{c}	79.85^{bc}	80.55^{ab}	81.36^{a}	0.0001	6.780
Unit surface shell weight (mg/cm^2)	0.65^{e}	0.66^{d}	0.67^{c}	0.674^{bc}	0.68^{ab}	0.69^{a}	0.0001	0.001
Haugh unit (%)	71.44^{a}	70.65^{a}	70.04^{a}	66.24^{b}	66.62^{b}	64.92^{b}	0.0001	2.641

Means within a row without a common superscript differ significantly ($P < 0.05$). [1] Mean square error.

0.05) by each step of Met supplementation (Table 3). Shape index (SI) (as an important factor in egg marketing) was significantly ($P < 0.05$) influenced by dietary met levels (Table 4). SI was significantly decreased at 0.39% met compared to all other levels. Increasing dietary met level to 0.34% significantly ($P < 0.05$) increased Shell Weight (SW), but there were no significant differences between these higher levels. Even though SW increased as a result of increasing dietary met content, Shell Ratio (SR) significantly decreased ($P < 0.05$, Table 4).

Albumin Index (AI) was significantly lower from hens fed the three higher levels of met than those fed three lower levels ($p < 0.05$, Table 4). Haugh Unit (HU) exhibited a similar trend as AI (Table 4), and was significantly higher from hens fed the three lower levels of met ($P < 0.05$).

No significant differences were observed on Yolk Index (YI) and Egg Specific Gravity (ESG) between treatments by dietary met levels. A graded addition of dietary met content from 0.24 to 0.49% led to a progressive increase in Egg Surface Area (ESA) from 72.50 to 81.36 cm^2. In a similar manner, increasing dietary met resulted in a increase ($P < 0.05$) in Unit Surface Shell Weight (USSW) (Table 4) from 0.65 to 0.69 mg/cm^2 for rations contained 0.24 to 0.49% Met, respectively.

DISCUSSION

With increasing dietary met levels from 0.24 to 0.49%, plasma T_3 showed an inverted U relationship. The lowest and highest plasma T_3 concentration were respectively related to hens which consumed 0.24 (1.00 ng/ml) and 0.29% (1.69 ng/ml) dietary met. It seems from the results that severe deficiency of met in laying hens redounds to sopping reduction in plasma T_3 concentration. On the other hand, trivial deficiency leads to increment in plasma

T_3 concentration. Plasma T_4 was not affected by met levels. Carew et al. (2003) reported that plasma T_3 was higher in all deficient chicks compared with the free-fed control, which was significant only with 0.3% met. Also, in their experiment plasma T_4 was minimally affected by the met deficiency. These observations are corresponding with results of previous study conducted by these investigators (Carew et al., 1997) on chicks and results achieved in current experiment in laying hens. It has been shown in several species including chickens (Alster and Carew, 1984; Keagy et al., 1987) and rats (Glass et al., 1978; Tyzbir et al., 1981) that plasma T_3 will decrease in response to restricted feed intake or fasting. We observed this decrease in the present study with the most severe met deficiency. It seems that the met deficiency increases the production or release of T_3 into the blood or inhibits its normal removal compared to avian consumed sufficient amounts of dietary met. This may operate through inhibited synthesis of a key protein involved in the metabolism or turnover of T_3 due to lack of sufficient met for polypeptide synthesis (Carew et al., 2003). From reported studies with protein deficiencies in chicks or rats, it has been suggested that elevated T_3 may be a consequence of increased secretion rate and activity of the thyroid gland (March et al., 1964; Tulp et al., 1979), slower clearance of T_3 from the blood (Hutchins and Newcomber, 1966), or alterations in plasma-binding capacity of the blood and changes in receptor binding or affinity (Refetoff et al., 1970; Smallridge et al., 1982; Rouaze-Romet et al., 1992), among others. Enhanced conversion of T_4 to T_3 due to increased hepatic or renal 5'-deiodinase activity does not seem to occur with a protein deficiency in rats (Smallridge et al., 1982) or chicks (Weyland, 1993). However, in the absence of direct data with met and in view of many other roles of met in animals (Finkelstein et al., 1982; Hawrylewicz and Huang, 1992), other mechanisms of an unknown nature may be involved.

Shape Index was significantly decreased at 0.39% met compared to all other levels except 0.44%. These inconsistent but significant differences are difficult to explain, but generally reflect little effect of met on SI. Increasing dietary met level to 0.34% significantly ($P < 0.05$) increased SW. Even though SW increased as a result of increasing dietary met content, shell ratio significantly decreased ($P < 0.05$). Increase in average egg weight (EW) influenced by met supplementation can explain this phenomenon. These findings showed that the met requirement for shell protein matrix synthesis needs to be considered to optimize shell quality. Simkiss and 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 shell percentage and overall shell quality.

Albumin Index was significantly lower from hens fed the three higher levels of met than those fed three lower levels ($P < 0.05$). In contrast, Narváez-Solarte et al. (2005) who reported that increasing dietary TSAA (via met supplementation) had no significant effect on AI. Haugh unit exhibited a similar trend as AI. Our results are in congruence with previous researches (Narváez-Solarte et al., 2005; Wu et al., 2005) as HU declined as dietary met content increased. Wu et al. (2005) reported that reduction in HU with met supplementation in diet is probably related to simultaneous increase in EW, which previously we mentioned that EW increased with increasing dietary met in our study. Dietary met level did not affect YI and ESG. Novak et al. (2006) reported that as dietary met decreased, there was a linear decrease ($P < 0.05$) in ESG. Such a finding is in discrepancy with our result. On the other hand, the effect of met level on YI was consistent with the Narváez-Solarte et al. (2005) reported that stated increasing dietary met content had no significant effect on YI. The number of studies conducted to evaluate the effects of dietary met content on egg characteristics are limited and existence of different findings in respects to egg characteristics between literatures indicate the need for more future investigations in this case.

IMPLICATIONS

According to results of current study concluded that met deficiency alters normal thyroid hormone metabolism. However, further studies are necessary to clarify the effects of dietary met level on egg characteristics and thyroid function. With the exception of YI and ESG, dietary met levels can significantly affect other egg quality parameters.

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