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Some Productive, Egg Quality and Serum Metabolic Profile Responses Due to L-threonine Supplementation to Laying Hen Diets

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ABSTRACT
This study evaluated the effects of different levels of L-threonine on some productive performances, egg quality and serum metabolic profile of the late laying period. A total of one hundred and twenty Hy-Line Brown laying hens (68-week-old), were assigned to the basal control diet or the basal diet supplemented with 0.2, 0.4 and 0.6% of L-threonine (n = 6 per diet, each replicate consists of 5 birds). Production performance was measured for 8 weeks and egg quality characteristics and blood parameters were determined at 76 weeks of age. Hy-Line Brown laying hens showed significantly linear and quadric ($p<0.01$) responseon egg production, egg weight and egg mass. Feed intake increased linearly and quadrically ($p<0.001$) with the increasing levels of L-threonine. Feed Conversion Ratio (FCR) significantly decreased with the increasing L-threonine in the laying diets. The level of 0.4% L-threonine resulted in overall best performance. No differences were observed for shell percentage, shell thickness and albumin height among the treatments. However there were significant quadratic response ($p = 0.028$) on Haugh Unit (HU) among treatments. Serum cholesterol decreased linearly ($p<0.001$) and quadrically ($p = 0.032$) with supplemental threonine levels. Serum glucose significantly increased ($p<0.002$) with increased L-threonine, whereas no changes were observed on serum calcium and phosphorus. It could be stated that L-threonine used in this study had beneficial effects on productive and HU as well as to decrease serum cholesterol. The threonine level of 0.4% gives the best result than other levels and this threonine level could be recommendable during late laying period.

Key words: Laying hens, threonine, performance, egg quality, serum biochemistry

INTRODUCTION
During the recent years most of the laying hen strains have shown considerable increases in the productivity of the birds and consequently increase income by advances in genetics and nutrition. Despite these developments, there are still issues related to nutrition that require more study (Figueiredo *et al*., 2012). Moreover, there is a need to update the amino acid recommendations in laying hen diets, in view of the genetic progress and the possibility of different bird response, different environments and changes in production systems (Filho *et al*., 2006).

The essential amino acid levels in the diet stand out among the nutritional factors that influence characteristics such as egg size, yolk and albumen deposition, total solids percentage and internal egg quality (Martinez-Amezcua *et al*., 1999; Figueiredo *et al*., 2012).

Since threonine is next limiting amino acid after methionine and lysine in poultry diets. L-threonine allows nutritionists to formulate more precise feeds, thereby lowering crude protein levels (Ojano-Dirain and Waldroup, 2002). L-threonine is added to the diet of pigs and poultry in order to exactly match the dietary amino acid balance to the unique nutritional requirements of the animal (Baylan *et al.*, 2006). There are many reports on the threonine requirements of poultry (Rosa *et al.*, 2001; Shan *et al.*, 2003; Ahmadi and Golian, 2010) but revealed contradictory results. Positive effects on egg production, egg weight and feed conversion ratio of laying hens were observed when threonine was added to layer diets (Martinez-Amezcua *et al.*, 1999). However, body weights were not significantly different among hens that received diets containing 0.45-0.53% threonine (Faria *et al.*, 2002).

Dietary threonine exceeded the requirement level, egg mass and feed conversion ratio decreased with increasing dietary threonine levels (Ishibashi *et al.*, 1998). While, Azzam *et al.* (2011) did not observe improvements in feed conversion ratio resulting from increased threonine. To our knowledge, only limited data exist on late period of laying hens in the second cycle of production effects of different levels of L-threonine in contrast to data of essential amino acids. Therefore, the aim of this study was to evaluate the potential levels of L-threionine in laying diets, using higher inclusion levels.

MATERIALS AND METHODS

Animal housing and management: The present study was conducted in the breeding farm of Animal and Poultry Production Department, Agriculture faculty, South Valley University, Qena, Egypt. A total of one hundred and twenty Hy-Line Brown laying hens (average weight = 1.810 g, 68 weeks age) were randomly housed in a battery type metal wire cages. The dimensions of each cage were 60×60×40 cm each cage (5 layers cage^{-1}). The experimental diets (Table 1) and drinking water were offered for *ad libitum*. The hens were exposed to 17 h of light a day.

Experimental design and dietary treatments: The experiment was conducted as a completely randomized block design with 6 replicates of 5 hens in each. Treatments included 4 levels of L-threonine (0, 0.2, 0.4 and 0.6%) to achieve 0.47, 0.67, 0.87 and 1.07% threonine, respectively (NRC, 1994). Hens, were fed experimental diets from 68-76 weeks of age. Composition of diet ingredients and analyzed chemical composition are presented in Table 1. The hens were fed mash form diets during the experiment (68-76 week). The diets were formulated to meet or exceed NRC (1994) recommendations.

Laying performance parameters: Hen-day egg production, feed intake, egg weight and hen mortality were recorded daily on a replication basis. Feed Conversion Ratio (FCR) was calculated as grams of feed intake per gram of egg mass produced.

Egg quality parameters: The parameters relative to egg quality were evaluated at 76 weeks of age. Fifteen eggs were randomly collected per treatment to determine these parameters. The collected eggs were weighed and each egg was then exposed to a pressing force by using an eggshell strength meter. On breaking, the egg contents were poured. Eggshell thickness (without the shell membrane) was measured at the middle part of the egg. The Haugh Unit (HU) was measured for the internal quality of the eggs. The height, correlated with the weight, determines the HU. The test was introduced by Haugh (1937) and is considered the most significant measure of egg quality. Shell percentage was calculated.

Table 1: Ingredient composition and chemical analysis (g kg^{-1}) of the basal diet

Parameters	Diets
Ingredients (g kg^{-1})	
Corn	679.00
Soybean meal	178.00
Soybean oil	8.00
Corn gluten meal	25.00
Di-calcium phosphate	19.00
Limestone	82.00
Vitamin and mineral mix[a]	2.00
DL-Methionine	2.50
Sodium chloride	4.00
Choline	0.50
L-Threonine	0.00
Chemical compositions (g kg^{-1})	
Dry matter	895.00
Ash	114.00
Crude protein	182.00
Ether extract	42.40
ADFom[b]	41.20
aNDFom[c]	102.00
Calcium	37.50
Phosphorus	5.44
Lysine	0.95
Methionine	0.38
Threonine	0.47
Gross energy (MJ kg^{-1})	16.00

[a]Provided the following per kg of diet: Vitamin A, 12,000 IU: Vitamin D_3, 7200 IU: Vitamin E, 20 IU: Vitamin B_1, 2.5 mg: Vitamin B^2, 5 mg: Vitamin K, 3 mg: Vitamin B_{12}, 1.5 µg: Pyridoxine, 0.225 µg: Pantothenic acid, 10 mg: Niacin, 35 mg: Folic acid, 1.5 mg: Biotin 125 mg: Mn, 90 mg: Cu, 7.5 mg: Zn, 65 mg: Fe, 50 mg: Se, 0.1 mg, [b]ADFom: Acid detergent fibre basis on an organic matter, [c] aNDFom: Neutral detergent fibre basis on an organic matter and assayed with a heat stable amylase

Blood sampling and laboratory analyses: At the end of the experiment (week 76), 12 h after feed withdrawal, blood samples were drawn into Eppendorf tubes (10 mL) from the axillary vein of 12 hens (2 birds per replicate). After the serum was separated naturally it was centrifuged for 10 min (3,000×g) at room temperature. Pure serum samples were aspirated by pipette, stored in 1.5 mL Eppendorf tubes at -70°C until analyses and thawed at 4°C before analysis. Serum concentrations of glucose, cholesterol, calcium (Ca) and phosphorus (P) were measured spectrophotometrically by using commercial kits (spectrum chemical company, PO Box 30 Obour City-Cairo, Egypt).

Chemical analysis: The diets were analyzed for moisture by oven drying (930.15), ash by incineration (942.05), protein by Kjeldahl (984.13) and ether extract by Soxhlet fat analysis (920.39), as described by the AOAC (2000). Calcium was measured using atomic absorption spectrometry and phosphorus was analysed colorimetrically (method 10.6.1; VDLUFA, 2007). The contents of neutral detergent fibre (assayed with a heat stable amylase, aNDFom) and Acid Detergent Fibre (ADFom), both expressed exclusive residual ash, were determined sequentially without sodium sulphite (method 6.5.1 for aNDFom and 6.5.2 for ADFom; VDLUFA, 2007). Amino acids (after oxidation) were analysed using an amino acid analyser after hydrolysis (6 M HCl) of the diets using HPLC. The Gross Energy (GE) contents of the diets were measured using an adiabatic bomb calorimeter (model C 200; IKA, Heitersheim, Germany).

Statistical analysis: The statistical analysis was performed using a completely randomized design and the General Linear Model (GLM) procedure of SAS 9.2 (SAS, 2005). The model only included the level of supplementation. Pens were the experimental units for all analysis. Orthogonal polynomial contrasts were used to determine the linear and quadratic effects of the increasing levels of inclusion and Duncan multiple range test was used to compare means. Significance was declared at $p<0.05$; p-values less than 0.001 are expressed as "<0.001" rather than the actual value.

RESULTS AND DISCUSSION

Productive performance: The productivity data of laying hens was summarized in Table 2. Egg weight, egg mass, egg production (%) and feed intake were significantly increased with the increasing L-threonine levels but average daily FCR decreased significantly ($p<0.01$) with increasing levels of supplementation. Overall L-threonine at 0.4% resulted in the best performance.

In the present study, the general health status of laying hens was good during the experimental period (68-76 weeks of age). Generally, the birds given diets containing L-threonine had the greatest overall egg production, egg mass and egg weight compared with the birds given control diet proving that L-threonine had beneficial action in the productive performance, imperative on the conversion of digested feed into eggs (Table 2). Suggesting that threonine affects intestinal functionality and maintenance and therefore improve nutrient digestibility and performances. It was found that threonine supplementation affected villus height, epithelial thickness, goblet cell number and the crypt depth (Zaefarian *et al*., 2008; Azzam *et al*., 2012). It could be expected that, increased the dietary threonine level of laying hens at late period may result in economic improved due to decreased feed conversion and increased productive performance. Others have reported different conclusions concerning the effect of threonine on feed intake, egg production, egg weight and egg mass. Our result is in agreement with the results of Martinez-Amezcua *et al*. (1999) reported that egg production, egg weight and feed conversion ratio improved as threonine was increased above the NRC (1994) suggested threonine recommendation. In addition, Faria *et al*. (2002) reported that egg production, egg mass and daily threonine intake were significantly increased with increasing dietary threonine levels. However, our results disagree with those of others who reported no effects of supplemental L-threonine on egg weight, egg production and feed conversion ratio in laying hens (Azzam *et al*., 2011; Ishibashi *et al*., 1998; Gomez and Angeles, 2009).

Table 2: Effect of L-threonine on productive performance of laying hens

	L-Threonine (%)					p-value	
Items	0	0.2	0.4	0.6	SEM[1]	Lin[2]	Quad[3]
Egg weight (g/hen/day)	58.90^{b}	63.50^{a}	65.4^{a}	63.80^{a}	0.95	0.004	0.012
Hen-day production (%)	75.60^{b}	81.90^{a}	83.2^{a}	82.00^{a}	1.03	0.002	0.006
Egg mass (g/hen/day)	50.40^{b}	55.80^{a}	57.0^{a}	54.90^{a}	1.17	0.023	0.013
Feed intake (g/hen/day)	95.50^{c}	100.00^{b}	104.0^{a}	101.90^{a}	0.85	<0.001	0.003
Feed conversion ratio	2.15^{b}	1.93^{a}	1.91^{a}	1.94^{a}	0.04	0.009	0.015

[a-d]Means not sharing a common superscript are significantly different ($p<0.05$), [1]Standard error of the means, [2,3]Linear and quadratic responses, respectively, to dietary inclusion levels

Quality of eggs: In relation to egg quality (Table 3), this study showed no significant differences in shell percentage and shell thickness as well as albumen height among treatments. However, there were significant effects on HU of laying hens at 76 weeks of age.

These improvements in HU are important for the egg-food industry, because threonine may enhance the safety and stability of eggs. In the present study, the highest value of the HU was observed on the group received 0.4% threonine. The higher the number, the better the quality of the egg (fresher, higher quality eggs have thicker whites). The HU score is known as an indicator of egg freshness and is related to shelf life (Williams, 1992; Ozek *et al.*, 2011). Our findings are in disagreement to those obtained by Gomez and Angeles (2009) found that eggshell percentage decreased significantly by increasing dietary threonine and methionine levels during the second cycle of production. Moreover, Cupertino *et al.* (2009) verified no effects of threonine levels for the HU measurement. The explanation for this result is unknown.

Serum biochemical parameters: The blood parameters in this study were summarized in Table 4. Serum cholesterol concentration decreased linearly with increasing L-threonine supplementations and serum cholesterol of L-threonine group was 1.47, 5.91 and3.94% lower compared to the control group respectively ($p<0.001$).

To our knowledge, no publication exists on threonine effects on serum biochemical parameters aying periods in hens (76 weeks of age). In this study, the serum glucose concentrations lineally increased ($p<0.002$) with increasing level of L-threonine. There was no significant effect of any of these treatments on serum calcium and phosphors ($p = 0.05$) and the values remained within the normal physiological range (Azzam *et al.*, 2011; Weber *et al.*, 2013). These results agree with those of Azzam *et al.* (2011) that reported supplementation with L-threonine, had no effect ($p>0.05$) on calcium, phosphorus and alkaline phosphatase of laying hens at 48 weeks of age.

Table 3: Effect of L-threonine on egg quality parameters of laying hens

	L-Threonine (%)					p-value	
Items	0	0.2	0.4	0.6	SEM[1]	Lin[2]	Quad[3]
Shell (%)	8.10	8.95	9.25	8.90	0.55	0.698	0.665
Shell thickness (mm)	0.34	0.34	0.35	0.34	0.02	0.701	0.774
Haugh unit (score)	79.70[b]	80.30[ab]	81.60[a]	80.00[b]	0.40	0.284	0.028
Albumen height (mm)	6.55	6.70	6.85	6.70	0.07	0.122	0.089

[a-d]Means not sharing a common superscript are significantly different ($p<0.05$), [1]Standard error of the means, [2,3]Linear and quadratic responses, respectively, to dietary inclusion levels

Table 4: Effect of L-threonine on serum metabolites of laying hens

	L-Threonine (%)					p-value	
Items	0	0.2	0.4	0.6	SEM[1]	Lin[2]	Quad[3]
Calcium (mmol L^{-1})	2.47	2.51	2.52	2.48	0.03	0.358	0.072
Phosphorus (mmol L^{-1})	2.40	2.44	2.45	2.41	0.02	0.799	0.074
Cholesterol (mg dL^{-1})	203.00[a]	200.00[a]	191.00[b]	195.00[b]	1.29	<0.001	0.032
Glucose (mg dL^{-1})	227.00[b]	231.00[ab]	236.00[a]	234.00[a]	1.59	0.002	0.549

[a-d]Means not sharing a common superscript are significantly different (p0.05), [1]Standard error of the means, [2,3]Linear and quadratic responses, respectively, to dietary inclusion levels

CONCLUSION

The effects of different dietary levels of L-threonineat 0, 0.2, 0.4 and 0.6% on productive performance, egg quality and serum biochemistry showed that 8 weeks administration of these threonine levels have beneficial effects on egg production, egg weight, egg mass, feed intake, FCR, HU and serum cholesterol during the late laying period in Hy-Line brown hens. The threonine level of 0.4% gives the best result than other levels and this threonine level could be recommendable during late laying period.

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