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## Βιβλιογραφική αναφορά:

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## Effect of dietary threonine level on performance, egg quality and serum components in laying quails

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**ABSTRACT:** The current research was conducted to determine the effect of dietary threonine level on performance, egg quality, and serum components in laying quails. In the study, 120 female quails at 20 weeks of age were randomly allocated to six treatment groups with five replicates. Experimental diets were prepared to contain threonine at 0.750% (without addition, control), 0.825%, 0.900%, 0.975%, 1.050% or 1.15% levels. The performance parameters did not affected by the threonine level of the diet, except for egg production ( $P>0.05$ ). Egg production quadratically increased with the threonine level of the diet and it was maximum at 0.900% ( $P<0.01$ ). Dietary threonine level quadratically affected eggshell thickness ( $P<0.05$ ), but it did not affect other egg quality parameters in quails ( $P>0.05$ ). Eggshell thickness improved up to 0.900% level, but decreased at higher threonine levels. The serum phosphorus concentration of laying quails was linearly affected by the threonine level and increased up to 0.900%, while it decreased at higher dietary threonine levels ( $P<0.01$ ). According to the results obtained from the present study, it was determined that the threonine content of 0.900% in the laying quail diets was sufficient for optimum egg production, eggshell thickness, and phosphorus utilization.

**Keywords:** treonin; performance; egg quality; serum; quail

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## INTRODUCTION

Exactly like in other farm animals, laying quails required to be supplied with nutrients such as energy, protein, and amino acids in sufficient amounts and proportions. Although the crude protein requirement of monogastric animals is satisfied by diet, sufficient supply of amino acids, especially essential amino acids, is compulsory for optimum yield. One of these essential amino acids is threonine, which cannot be synthesized by poultry and is the third limiting amino acid after lysine and methionine (Waguespack et al., 2009). Therefore, threonine should be given with diet to poultry, including laying quails, for optimum production (Rezaeipour et al., 2012).

Threonine is also included in the structure of the mucous in the digestive system (40% of the protein in the mucous glycoprotein is threonine) as well as protein and uric acid. In other words, it is an essential amino acid for the function of the digestive system and the continuity of the intestinal barrier system (Carlstedt et al., 1993; Corzo et al., 2007; Wils-Plotz and Dilger, 2013).

The nutrient requirements reported by the NRC (1994) are widely accepted worldwide and threonine requirement of laying quails is reported as 0.74% by NRC (1994). However, it was reported that the threonine requirement of laying quails could be higher in subsequent studies (Canogullari et al., 2009; Zeweil et al., 2016). Canogullari et al. (2009) noted that the increase in threonine level from 0.74% to 1.04% in the diet, the egg production of quails improved by approximately 10%. Likewise, Zeweil et al. (2016) stated that the threonine necessity of laying Japanese quails was 1.04%. In some other studies, threonine requirement of laying quails was reported as 0.85% by Assaf et al. (2009) or 0.78% by Lima et al. (2013), and Parlat et al. (2003) additionally demonstrated that the administration of threonine to quail diets did not positively affect. Umigi et al. (2007) and Umigi et al. (2012) determined that the digestible threonine requirement of laying quails as 0.65% and 0.55%, respectively.

Considering the differences in the threonine requirement of laying quails reported in these researches, more studies are needed. In addition, the number of studies examining the effect of threonine serum components in layer quails are limited. Therefore, the aim of this research is to determine the effects of diets containing different levels of threonine on the performance, egg quality, and serum metabolic profile in laying quails.

## MATERIALS AND METHODS

### Ethics approval

The authors confirm that the European Parliament and the Council of the European Union Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

### Animals and Feed Materials

In the study, a total of 120 female Japanese quails at the age of 20 weeks were randomly distributed to six treatment groups with five replicates, according to the randomized experimental design. The basal diet was prepared isocaloric and isonitrogenic according to the NRC (1994) recommendation for laying Japanese quail (Table 1). The chemical composition of basal diet was analysed according to AOAC (2006) proceedings. Experimental diets were formed by adding L-threonine to contain 0.750% (without addition), 0.825%, 0.900%, 0.975%, 1.05%, and 1.15%. During the trial, 16 hours/day lighting and 8 hours/day darkness were applied, and water and feed were given *ad-libitum*.

### Method

#### Determination of performance to traits

The body weight gain was determined by group weighings at the initial and final of the trial. Experimental diets were given to the replicates by weighing and feed intake was calculated as g/day/quail. Eggs obtained from quails were recorded daily and egg production was calculated as %. Eggs collected in the last three days of the experiment were weighed and the egg weight was determined as g. From these data obtained, egg mass was calculated as g/day/quail with the  $(\% \text{ egg production} \times \text{egg weight}) / 100$  formula and feed conversion ratio was determined using the *feed intake / egg mass* equation.

#### Determination of egg quality to traits

Egg external and internal quality criteria were determined from all eggs collected in the last three days of the trial. Eggshell breaking strength was measured by applying supported systematic pressure to the blunt of the eggs (Egg Force Reader, Orka Food Technology, Israel). Eggs determined eggshell breaking strength were broken on a clean glass surface and after the egg residues were cleaned, the eggshells were dried at room temperature for three days and weighed, then the relative eggshell weights were calculated as

**Table 1.** Control diet and its nutrient contents

Ingredients	%	Nutrient contents	%
Maize	53.90	Metabolizable energy, kcal/kg <sup>2</sup>	2899
Soybean meal	26.40	Moisture <sup>3</sup>	12.30
Full-fat soybean	10.00	Crude fibre <sup>3</sup>	4.62
Soybean oil	2.10	Crude fat <sup>3</sup>	7.52
Limestone	5.58	Calcium <sup>3</sup>	2.51
Dicalcium phosphate	1.18	Available phosphorus <sup>2</sup>	0.35
Salt	0.35	Total phosphorus <sup>3</sup>	0.63
Premix <sup>1</sup>	0.25	Crude protein <sup>3</sup>	19.99
L-lysine	0.01	Lysine <sup>2</sup>	1.00
DL-methionine	0.16	Methionine <sup>2</sup>	0.45
Total	100.00	Cystine <sup>2</sup>	0.37
		Methionine + cystine <sup>2</sup>	0.84
		Threonine <sup>2</sup>	0.75

<sup>1</sup>Premix is supplied that per kg of diet; manganese: 80 mg, iron: 60 mg, copper: 5 mg, iodine: 1 mg, selenium: 0.15 mg, vitamin A: 8.800 IU, vitamin D<sub>3</sub>: 2.200 IU, vitamin E: 11 mg, Niacin: 44 mg, Cal-D-Pan: 8.8 mg, vitamin B<sub>2</sub>: 4.4 mg, vitamin B<sub>1</sub>: 2.5 mg, vitamin B<sub>12</sub>: 6.6 mg, folic acid: 1 mg, biotin: 0.11 mg, choline: 220 mg.

<sup>2</sup>Calculated value.

<sup>3</sup>Analyzed value.

the ratio of the eggshell weight to the egg weight (%). Eggshell thickness was calculated by taking the average of the values obtained by measuring from three points of the egg (equator, blunt and pointed parts) using a micrometer (Mitutoyo, 0.01 mm, Japan).

After the external quality characteristics of the eggs were determined, height of albumen and yolk were measured with the help of a height gauge, on the other hand, yolk diameter and albumen width and length were measured by digital calliper. From these data, albumen index did calculated with the *albumen height / ((albumen width + albumen length) / 2) × 100* formula, the yolk index from the *(yolk height / yolk diameter) × 100* formula, and the Haugh unit (Haugh, 1937) with the *100 x log (albumen height + 7.57 - 1.7 x egg weight<sup>0.37</sup>)* formula. Albumen, yolk, and wet eggshell weights were weighed on precision weighing balance and determined as % of egg weight.

### Determination of serum components

At the end of the experiment (10<sup>th</sup> week), 3 ml of blood was taken from one random quail (30 in total) of similar body weight from each subgroup to determine serum parameters. Blood were centrifuged at 4000 rpm for 10 minutes and serum did extracted. The serum were stored at -20°C until analysis, and the concentrations of glucose, triglyceride, cholesterol, total protein, albumin, globulin, urea, creatinine, calcium, and phosphorus were determined in an auto-analyser device using commercial kits in a com-

mercial laboratory (DDS® Spectrophotometric Kits, Diesis Diagnostic Systems Co., Istanbul, Turkey).

### Statistical Analysis

Data were analysed in the SPSS 22.0 software package (SPSS Inc., Chicago, IL, USA) with model of one-way ANOVA, using the group mean as an experimental unit. A probability value of P<0.05 was considered statistically significant. Orthogonal polynomial contrasts were used to evaluate the significance of linear and quadratic models to determine the response of the dependent variable to an increasing threonine level.

## RESULTS

### Performance to traits

The effect of diets containing different levels of threonine on body weight gain, egg production, egg weight, egg mass, feed intake, and feed conversion ratio of laying quails was demonstrated in Table 2. The increasing level of threonine in laying quail diets quadratically affected egg production (P<0.01), but this effect did not observed in other performance parameters (P>0.05). The increasing dietary threonine level resulted in a quadratic improve in egg production. Egg production was maximum in the group with threonine content of 0.900% in the diet, and a decrease in the increase of egg production was observed in the groups with higher threonine content (0.975% and above) than this level.

### Egg quality to traits

Effect of threonine addition to the diet on egg quality parameters in laying quails was given in Table 3. The effect of diets containing different levels of threonine on the eggshell breaking strength, eggshell weight, yolk and albumen ratio, eggshell rate, albumen index, yolk index, and Haugh unit in laying quails was statistically insignificant ( $P>0.05$ ). Eggshell thickness was quadratically affected by the supplementation of threonine to the diet ( $P<0.05$ ). Increasing dietary threonine level to 0.825% and 0.900% improved the eggshell thickness, however, a decrease in eggshell thickness was observed with the

addition of high levels (0.975% and above) of threonine.

### Serum components

Serum glucose, urea, creatinine, total protein, albumin, globulin, triglyceride, cholesterol, and calcium concentrations of laying quails did not considerably affected by the threonine level of the diet ( $P>0.05$ ). In addition, the serum phosphorus level of laying quails was linearly affected by the treatments ( $P<0.01$ ). Serum phosphorus concentration increased up to 0.900% threonine, but decreased at higher levels (0.975% and above).

**Table 2.** Effect of dietary threonine level on the performance in laying quails

Parameters	Dietary threonine levels, %						S.E.M.*	P- value of contrast	
	0.750	0.825	0.900	0.975	1.050	1.125		Linear	Quadratic
Body weight change, g	8.90	0.40	1.80	5.90	4.80	1.00	5.410	0.674	0.849
Egg production, %	88.3	88.7	92.9	91.0	88.7	89.5	0.71	0.575	0.001
Egg weight, g	12.7	12.4	12.2	12.7	12.7	12.5	0.31	0.903	0.716
Egg mass, g/day/quail	11.2	11.0	11.3	11.6	11.3	11.2	0.33	0.805	0.477
Feed intake, g/day/quail	30.1	30.3	30.2	30.1	32.1	29.6	0.70	0.521	0.052
Feed conversion ratio, g feed/g egg	2.70	2.76	2.66	2.79	2.82	2.65	0.077	0.896	0.427

\*Standard error means

**Table 3.** Effect of dietary threonine level on the egg quality parameters in laying quails

Parameters	Dietary threonine levels, %						S.E.M.*	P- value of contrast	
	0.750	0.825	0.900	0.975	1.050	1.125		Linear	Quadratic
Eggshell breaking strength, kg	1.56	1.59	1.55	1.37	1.42	1.46	0.065	0.256	0.123
Eggshell thickness, $\mu\text{m}$	229	234	232	222	220	229	3.2	0.983	0.019
Eggshell weight, % of egg weight	7.94	8.63	8.26	7.72	7.73	7.71	0.163	0.720	0.141
Yolk ratio, %	33.6	32.1	30.9	33.2	30.9	30.8	0.91	0.069	0.796
Albumin ratio, %	56.7	57.5	58.8	57.0	59.2	58.9	0.90	0.074	0.847
Eggshell rate, %	9.65	10.36	10.28	9.75	9.84	10.23	0.179	0.654	0.716
Albumen index	3.41	3.32	3.30	3.36	3.53	3.43	0.895	0.559	0.656
Yolk index	53.4	53.1	53.5	52.5	52.8	54.0	0.15	0.929	0.480
Haugh unit	94.1	94.3	94.5	94.9	96.8	95.1	1.08	0.157	0.774

\*Standard error means

**Table 4.** Effect of dietary threonine level on the serum components in laying quails

Parameters	Dietary threonine levels, %						S.E.M.*	P- value of contrast	
	0.750	0.825	0.900	0.975	1.050	1.125		Linear	Quadratic
Glucose,mg/dl	317	309	307	303	303	299	8.8	0.172	0.717
Urea,mg/dl	9.5	9.5	12.0	12.0	13.0	10.0	1.23	0.273	0.100
Creatinine,mg/dl	0.340	0.356	0.346	0.372	0.346	0.364	0.0079	0.146	0.503
Total protein,g/dl	42.0	42.4	43.2	44.6	44.2	40.8	1.87	0.983	0.203
Albumin,g/dl	14.1	13.8	13.4	15.1	14.6	13.1	0.38	0.823	0.075
Globulin,g/dl	27.9	28.6	29.8	29.5	29.6	27.7	1.67	0.942	0.300
Triglyceride,mg/dl	855	997	943	847	824	911	32.7	0.216	0.811
Cholesterol,mg/dl	173	174	197	203	190	210	19.8	0.193	0.772
Calcium,mg/dl	21.8	20.2	19.5	23.3	22.4	18.9	0.91	0.669	0.263
Phosphorus,mg/dl	6.90	7.02	9.46	6.60	6.02	5.02	0.418	0.002	0.029

\*Standard error means

## DISCUSSION

### Performance to traits

The addition of threonine to the diet quadratically increased the egg production of quails and it was maximum at the level of 0.900%. Similarly, Assaf et al. (2009) reported that the highest egg production was obtained from quails fed with diet containing 0.85% threonine, and that Canogullari et al. (2009) dietary threonine level higher than recommendation of NRC (1994) provided a numerical increase (about 10%) in egg production of quails. Lima et al. (2013), on the other hand, declared that threonine level should be 0.78% in quail diets for optimum egg production. However, Parlat et al. (2003) demonstrated that the 0.90% threonine did not affect egg production in laying quails, besides 1.10% level caused a decrease in egg production. Similar results were reported by Umigi (2007; 2012) at 0.55-0.85% digestible threonine levels in the diet. Threonine is an essential amino acid for the health of the intestinal mucosa, which is a barrier to pathogens (Peralta et al., 2018). At the same time, it positively affects the development of villi, which provides the absorption of nutrients in the intestine (Shirzadegan et al., 2015). In the current study, the advance in egg production by increasing the threonine level to 0.900% in the diet can be due to better mucosal and villi development in the intestine. In other words, dietary nutrients were better evaluated by the bird, and this can have led to increase in egg production. However, mucosal and villi development were not examined in this study.

In the current study, performance parameters did not affected by the threonine level of the diet, except for egg production. These results are partially similar to the studies conducted in previous years. Parlat et al. (2003) and Canogullari et al. (2009) reported that the body weight change, feed intake, and feed efficiency did not affected by 0.9-1.10% threonine level in laying quails, however, diets containing higher levels of threonine reduced egg weight. Similarly, Umigi et al. (2007;2012) demonstrated that the dietary digestible threonine level (0.55-0.88%) did not affect egg weight, feed intake, and feed conversion ratio in quails. On the contrary, Assaf et al. (2009) and Lima et al. (2013) stated that the optimum threonine requirement for laying quails in terms of egg weight, egg mass, and feed efficiency as 0.85% and 0.78%, respectively. The increase in egg production in the current study indicated that the threonine requirement of laying quails is higher than that reported

by the NRC (1994), and 0.900% threonine in the diet is required for optimum egg production.

### Egg quality to traits

Eggshell thickness improved up to 0.900% threonine but decreased at higher threonine levels. This result is similar to the study stated that the eggshell thickness decreased with the addition of 1, 2, and 3 g/kg L-threonine to the laying hen diets (Azzam et al., 2014). Lima et al. (2013) demonstrated that eggshell thickness in quails did not affected by the threonine level of the diet. The improvement in eggshell thickness with threonine level up to 0.900% in the diet can be due to its positive effect on calcium metabolism or eggshell formation. Therefore, further studies are needed to determine the effect of threonine on calcium metabolism and eggshell formation.

Egg internal quality parameters did not affected by the threonine levels of the diet. Similar results were reported by Umigi et al. (2007;2012) and Lima et al. (2013) in quails and by Schmidt et al. (2011) in laying hens. However, Assaf et al. (2009) reported that yolk weight and egg internal quality improved at 0.85% threonine level, Azzam et al. (2014), on the other hand, stated that the addition of L-threonine (0-3 g/kg) to the diet increased the Haugh unit. These differences could be due to the animal species and age, diet, and environmental conditions used in the studies.

### Serum components

Serum phosphorus level linearly increased up to 0.900% threonine level in the diet and linearly decreased with further levels of threonine in the diet. In the literature, studies examining the effect of dietary threonine level on serum parameters are limited. In a research conducted in laying hens, it was reported that the administration of 0-3 g/kg threonine to the diet did not change serum parameters including serum phosphorus level (Azzam et al. 2014). Jiang et al. (2019), on the other hand, stated that the dietary threonine level did not affect the serum total protein and albumin levels, but it decreased the serum urea in breeder broilers. Samuel et al. (2017) reported that increasing diet threonine level (0.67% to 1.22%) decreased serum glucose and AST levels in growing quails, also cholesterol and albumin levels decreased at 0.95% and 1.22% threonine levels, respectively. In addition, Sigolo et al. (2017) reported that threonine improves serum lipid profile. Although the study examining the effect of dietary threonine level on serum profile excluding minerals is sufficient, there are few

studies examining the effect on serum mineral levels and further studies are needed.

## CONCLUSION

The egg production improved with increasing dietary threonine level, and it was maximum at 0.900% threonine level. Eggshell thickness and serum phosphorus level increased up to 0.900% threonine in the

diet but decreased at higher threonine levels. According to the results of the current study, the optimum threonine level in the diet for laying Japanese quails was determined as 0.900%.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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