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## The effects of ad - libitum consumption of chitosan oligosaccharide with drinking water on laying quails

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**ABSTRACT:** The aim of this study was to determine the effects of chitosan oligosaccharide on growth performance, some egg quality parameters, and some blood parameters in Japanese quails. Chitosan oligosaccharide (ChitO) was added to the drinking water of quails. A total of 192 (7 - 8 weeks old) Japanese quails (*Coturnix coturnix Japonica*) were divided into one control and 3 treatment groups, each consisting of 48 quails. Each group was divided into 6 replicates. While no additive was added to the control group, ChitO was added to the drinking water of the first, second, and third groups at the levels of 0.015, 0.030 and 0.045 g / L, respectively. They were given water and feed *ad libitum*. At the end of the study, no significant differences were observed between the control and treatment groups, except in their feed consumption and egg shell weight. In the first treatment group (given 0.015 g / L ChitO), it was observed that feed consumption significantly decreased compared to the control group; however, the difference between the other treatment groups was insignificant. The egg shell weight of the second treatment group (0.030 g / L ChitO) was found to be significantly higher ( $p < 0.05$ ) than that of the control group. In general, the addition of chitosan oligosaccharide up to 0.045 g/L to drinking water in quails tended to decrease feed consumption, without adversely affecting feed efficiency, and tended to increase eggshell weight.

**Keywords:** Quail; chitosan oligosaccharide; feed conversion ratio; egg quality.

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## INTRODUCTION

Obtaining the required yields from animals, their quality, and value for human health are directly related to animal feeding approaches. The quality and safe presentation of animal products for human consumption has resulted in the prohibition of the use of growth - promoting antibiotics in animal nutrition (Yavuz et al., 2020). The increasing need for food of animal origin due to the increase in the world population has led to the necessity of researching feed additives that can replace dietary antibiotics (Edirneli and Buğdaycı, 2020).

Prebiotics are defined as feed and foodstuffs that change the number of digestive system microorganisms in favor of beneficial microorganisms and are not digested by living thing organisms (Ferket, 2004; Gibson et al., 2004). Even cellulose, a structural polysaccharide, has some prebiotic properties. Apart from cellulose, alpha-galactooligosaccharides (GOS), inulin, fructooligosaccharides (FOS), trans-galactooligosaccharides (TOS), xylo-oligosaccharides (XOS), pyrodextrins and isomaltooligosaccharides, which are found in various foods and raw feed materials are also prebiotic. Mannanoligosaccharides (MOS) are among the oligosaccharides used for the same purpose as prebiotics; they can bind pathogenic microorganisms and remove them from the digestive system (Ergün, 2016).

Chitin and chitosan are polymers found naturally in the shells of many aquatic and terrestrial arthro-

pods, and in the cell walls of microorganisms such as mold and yeast (Crini et al., 2019; Swiatkiewicz et al., 2015). Swiatkiewicz et al., (2015) reported that chitosan oligosaccharide (as a prebiotic) reduces the number of digestive tract pathogens of young animals, that is, broiler chickens and weaned pigs. There are some studies found that chitosan oligosaccharide has effects on egg production (Meng et al., 2010), and immune system (Yan et al., 2010) in laying hens. Keser et al., (2012) declared that the use of chitosan oligosaccharides in rations has a reducing effect on blood LDL - cholesterol in broilers. It also has antioxidant properties (Crini et al., 2019; Swiatkiewicz et al., 2015). The aim of this research was to determine the effects of chitosan oligosaccharide, given via drinking water (ad - libitum) on egg production, feed efficiency, egg quality and some blood parameters on quails

## MATERIALS AND METHODS

Research on animals was conducted according to the institutional committee on animal use (2020 - 623). It was carried out in Burdur, Turkey with coordinates of 37°67'87"N and 30°31'40"E.

### Animals and trial order

A total of one hundred and ninety two female quails (*Coturnix coturnix japonica*) of 7 weeks old were used as animal materials in the study. The quails were divided into: one control and three treatment groups, with 48 in each group. All groups consumed water and feed *ad libitum*. All groups consumed the

**Table 1. Ingredients and chemical composition of basal ration.**

Ingredients (%)		Calculated composition	
Corn	53.00	ME (kcal / kg)	2885
Soybean meal	17.00	Methionine + Cystine (%)	0.70
Full fat soya	13.00	Lysine (%)	1.03
Sunflower meal	8.00	Threonine (%)	0.76
Vegetable oil	1.53	Tryptophan (%)	0.30
DCP	1.50	Calcium (%)	2.52
Limestone	5.40	Available phosphorus (%)	0.35
Salt	0.30	Chlorine (%)	0.22
Vit-Min <sup>a</sup>	0.25	Sodium (%)	0.15
dl-methionine	0.02	Potassium (%)	0.79
<b>Analyzed composition</b>		DCAB <sup>b</sup> (mEq / kg DM)	227.6
Crude protein (%)	19.25		
Ether extract (%)	4.37		
Crude fibre (%)	4.56		
Crude ash (%)	10.11		
Dry matter (%)	94.18		

<sup>a</sup> Each kilogram of vitamin-mineral mix contains 12 000 000 IU A vit, 20 000 mg E vit, 50 000 mg Mn, 50 000 mg Fe, 50 000 mg Zn, 10 000 mg Cu, 800 mg I, 150 mg Co, 150 mg Se

<sup>b</sup> DCAB (Dietary cation-anion balance): (Na<sup>+</sup> + K<sup>+</sup>) - Cl<sup>-</sup>

**Table 2.** Water pH of the control group and experimental groups to which chitosan oligosaccharide was added at different levels.

	Control	Addition levels to drinking water		
		0,015 g/L	0,030 g/L	0,045 g/L
pH	7,23	7,25	7,27	7,27

same ration (19.25% CP and ME 2885 kcal / kg) formulated according to NRC (1994) (Table 1). Each group consisted of 6 replicates of 8 quails. Chitosan oligosaccharide was added to the drinking water of the 1st, 2th and 3rd treatment groups at the concentrate of 0.015; 0.030 and 0.045 g / L, respectively. During the study, no additives were added to the drinking water of the control group. Separate water tanks for the control and trial groups were created, and the pH value of the drinking water belonging to the control and treatment groups were measured (Table 2). In the study, a photoperiod of 16 hours of light and 8 hours of darkness was applied; and quails were kept in a commercial quail cage (45 cm × 50 cm × 22 cm) (Molino et al., 2015). Chitosan oligosaccharide was obtained from Agada Food and Consulting Inc., Antalya, Turkey. The research lasted for 8 weeks (56 days). The nutrient content of the ration used in the study was determined according to AOAC (2019). Crude fiber of the ration was analyzed according to Crampton and Maynard (1938).

### Performance parameters

The quails were weighed at the beginning and end of the study. Eggs were collected separately twice a week (two consecutive days). The collected eggs were weighed the next day (Sartorius CP224S); and the average of both weighings was recorded as weekly egg weight. During the study, egg production of each replicate was recorded daily. Weekly feed consumption of each replicate was determined and daily feed consumption was calculated per quail. The feed efficiency ratios (kg feed consumed per kg egg and kg feed consumed per dozen egg) were calculated by using the parameters of egg production, egg weight and feed consumption. Mortality were recorded daily during the experiment.

### Egg quality

In order to determine the eggs' internal and external quality parameters, the eggs collected separately every two weeks were kept at room temperature for 24 hours. 2 randomly selected eggs from each replicate (48 in total) were weighed; and their width and height were measured with a digital caliper (Absolute

Digimatic). The eggs were broken on a glass table. The yolk and albumin height of the eggs were measured with a tripod micrometer (Mitutoyo No. 2050S - 19) (1 / 100) and the yolk diameter, albumin width and albumin length were measured with a digital caliper. By using these values yolk index (Y), albumin index (A) and Haugh unit (H) were calculated according to the formulas below.  $Y = (\text{yolk height} / \text{yolk diameter}) \times 100$ ;  $A = (\text{albumen height} / \text{average of albumen length}) \times 100$ ;  $H = 100 \times \log (\text{albumen height} + 7.57 - 1.7 \times \text{egg weight} \times 0.37)$  (Card and Nesheim, 1972).

In order to determine the eggshell quality, the shell weights of two randomly selected eggs from each replicate were weighed together with the egg membrane once every 2 weeks. The average of eggshell thickness of each treatment group was calculated by determining the shell thicknesses from 3 different points of the eggshells (pointed end, blunt end and side) by means of a precision micrometer (Mitutoyo, serial no: 395 - 271 - 30). Crude ash levels of the same shells were determined. At the end of the study (week 8), the calcium (Ca) and magnesium (Mg) content of the eggshell was determined using atomic absorption (Thermo Scientific, ICE 300 Series, Serial no: C103500100), and the phosphorus (P) content was determined using ICPOES - I (Thermo Scientific ICAP 6000 Series, Serial no: IC3D2a25110) (Mertens, 2005).

### Blood analysis

At the end of the experiment, blood was collected from 48 quails (2 from each replicate) during the decapitation process. Blood serum was separated by centrifuge (3000g; 10 min). Serum samples were treated by AutoAnalyser (Randox RX Monaco) to determine the albumin, total protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST), Ca, P, Mg, chlorine (Cl), sodium (Na), potassium (K), triglyceride and cholesterol levels using their accompanying commercial kits (REF AB8301; REF TP8336; REF AL8304; REF AS8306; REF Ca8309; REF PH8328; REF Mg8326; REF Cl8310; REF Na8327; REF PT8327; REF TR8332; REF CH8310).

### Statistical analyses

One way ANOVA was used to analyze the differences between the groups. Tukey test was used to check the significance of the difference between the groups (Özdamar, 2015). All parameters were given as estimated marginal means and standard error of mean. Correlation coefficients between the levels of Ca, P and Mg in the blood and the levels of the related parameters in the egg shell were determined, and statistical significance was controlled. Level of significance was taken as  $p < 0.05$ .

### RESULTS

In the study, pH value of the drinking water belonging to the control and treatment groups (0.015; 0.030 and 0.045 g / L ChitO) were measured as 7.23, 7.25, 7.27 and 7.27, respectively. Egg production of the group given 0.030 g / L ChitO was significantly reduced at 8 weeks compared to that of the control group ( $p < 0.05$ ). This difference did not affect the average egg production, that is, there was no difference between the treatment groups and the control group in terms of egg production. At the end of the study, it

was seen that the feed consumption of the 0.015 g / L ChitO group was numerically lower than the other trial groups, but the decrease in feed consumption was significant compared to the control group ( $p < 0.05$ ). ChitO given with drinking water had no effect on egg weight during the study. No statistical significance was determined between the trial groups and the control group in terms of feed conversion rates [kg feed / kg eggs and g feed / dozen eggs] (Table 3). There was no significant difference between the groups in the study in terms of their initial and final body weights.

When the effects of chitosan oligosaccharide on egg quality in the laying quails were examined, it was seen that, ChitO given with drinking water had no significant effect on shape index, albumin index, yolk index, Haugh unit and yolk color. At the end of the study, while the eggshell weight of the 0.030 g / L ChitO group was similar to the other trial groups, it was found to be significantly higher ( $p < 0.05$ ) than that of the control group. ChitO did not affect the amount of crude ash of eggshell with egg shell thickness (Table 4).

**Table 3.** The Effects of using chitosan oligosaccharide on performance of laying quails, (mean  $\pm$  s.e).

	Control	Addition levels to drinking water			p
		0.015 g / L	0.030 g / L	0.045 g / L	
Initial body Weight (g)	238.162.83	235.122.41	236.792.96	237.252.69	0.884
Final body Weight (g)	266.974.36	259.583.56	261.253.73	258.343.06	0.372
Egg production (%)	88.141.74	85.602.50	83.442.16	83.492.98	0.475
Feed consumption (g/day per quail)	32.480.23 <sup>a</sup>	31.090.18 <sup>b</sup>	31.33 0.29 <sup>ab</sup>	31.540.50 <sup>ab</sup>	0.037
Egg weight (g)	12.130.14	12.090.17	12.310.11	12.140.03	0.629
Feed efficiency (kg feed per kg egg)	2.680.03	2.570.04	2.540.03	2.600.05	0.106
Feed efficiency (kg feed per dozen egg)	451.212.4	442.416.7	458.013.9	462.920.5	0.823

<sup>a,b</sup> Means within a row followed by the different superscripts differ significantly ( $p < 0.05$ ).

**Table 4.** The effects of using chitosan oligosaccharide on egg quality parameters of laying quails, (mean  $\pm$  s.e).

	Control	Addition levels to drinking water			p
		0.015 g / L	0.030 g / L	0.045 g / L	
Shape index (%)	76.340.74	77.380.58	77.410.30	77.46 1.18	0.690
Albumen index (%)	9.510.33	10.230.33	9.350.28	9.570.26	0.231
Yolk index (%)	37.460.54	37.700.45	37.270.43	38.440.60	0.385
Haugh unit	91.410.57	92.040.82	90.400.66	90.930.54	0.348
Yolk color*	11.020.15	11.030.10	11.020.17	10.810.18	0.708
Egg shell weight (g)	1.070.02 <sup>b</sup>	1.090.01 <sup>ab</sup>	1.140.02 <sup>a</sup>	1.100.05 <sup>ab</sup>	0.030
Crude ash of egg shell (%)	81.180.48	80.190.63	79.290.57	79.160.92	0.133
Egg shell thickness (mm)	0.2100.004	0.2060.005	0.1950.002	0.1950.002	0.059

\*Egg yolk color was measured using the Roche color scale of egg yolk.

<sup>a,b</sup> Means within a row followed by the different superscripts differ significantly ( $p < 0.05$ ).

ChitO did not affect the amount of eggshell Ca, P and Mg levels also. Similarly, no significant effect of chitosan oligosaccharide was observed in terms of blood serum Ca, P and Mg levels (Table 5). There was no difference between the groups in terms of other blood parameters examined with triglyceride and cholesterol (Table 6). On the other hand, statistical and significant ( $p < 0.01$ ) negative correlation were occurred between eggshell and blood Ca levels (Table 7) in the study.

## DISCUSSION

Regarding the body weights of the quails, Kulshreshtha et al. (2014) had similar report with the present study. They showed that red seaweeds (*Chondrus*

*crispus* and *Sarcodiotheca gaudichaudii*) added to the ration of laying hen as a prebiotic feed additive had no effect on their body weight. Jiao et al. (2019) declared that chitosan oligosaccharide (EasyBio) added to the diets (0.1%; 0.2 and 0.3) of laying hens of 46 weeks old had no effect on their egg production. Besides, Meng et al. (2010) revealed that chitosan oligosaccharide (0.2 and 0.4 g / kg) added to the rations of laying hens increased their egg production numerically, but the difference in question was not statistically significant. Yan et al. (2010) acknowledged that chitosan oligosaccharide added to the ration of 27 - week - old brown layers for 8 weeks did not affect their egg production. These results support the present study.

**Table 5.** The effects of chitosan oligosaccharide on Ca, Mg and P of egg shell ash (%) and Ca, Mg, P levels of blood serum (mg / dl) of laying quails, (mean  $\pm$  s.e).

	Control	Addition levels to drinking water			p
		0.015 g / L	0.030 g / L	0.045 g / L	
Egg shell					
Calcium (%)	34.270.28	34.58 0.14	34.52 0.08	34.48 0.15	0.666
Phosphorus (%)	1.010.04	1.17 0.09	1.16 0.05	1.21 0.05	0.170
Magnesium (%)	1.290.07	1.26 0.07	1.23 0.02	1.19 0.06	0.765
Blood serum					
Calcium (mg / dl)	27.391.57	26.951.15	26.601.40	26.761.19	0.979
Phosphorus (mg / dl)	7.021.05	7.580.51	7.170.66	7.190.49	0.953
Magnesium (mg / dl)	7.290.42	7.360.31	7.58 0.31	7.900.30	0.580

**Table 6.** The effects of using chitosan oligosaccharide on blood parameters of laying quails, (mean  $\pm$  s.e).

	Control	Addition levels to drinking water			p
		0.015 g / L	0.030 g / L	0.045 g / L	
Na (mmol / L)	171.516.07	176.771.45	180.621.94	173.611.16	0.240
Cl (mmol / L)	110.582.43	113.511.52	114.772.67	112.681.43	0.549
K (mmol / L)	3.980.83	2.812.47	3.060.20	3.240.28	0.313
Total Protein (g / dL)	4.380.22	4.500.07	4.600.10	4.280.11	0.387
ALT (U / L)	7.590.67	7.580.86	7.251.07	6.920.94	0.943
AST (U / L)	226.0417.64	269.4530.79	309.7734.92	256.4213.86	0.196
Albumin (g / dL)	1.740.09	1.860.05	1.931.80	1.760.05	0.129
Triglyceride (g / dL)	1.580.13	1.620.13	1.470.14	1.520.13	0.880
Cholesterol (mg / dL)	227.186.91	250.9718.91	224.127.52	221.828.38	0.640

**Table 7.** Correlation of egg shell and blood Ca, P and Mg levels in quails consuming chitosan oligosaccharide with drinking water containing different levels, (mean  $\pm$  s.e).

	Blood Ca	Eggshell Ca	Blood P	Eggshell P	Blood Mg	Eggshell Mg
Blood Ca	1					
Eggshell Ca	-0.518**	1				
Blood P	0.366	-0.164	1			
Eggshell P	-0.218	0.177	-0.270	1		
Blood Mg	0.195	-0.090	0.627**	-0.004	1	
Eggshell Mg	-0.145	0.068	-0.188	0.422*	-0.057	1

\* $p < 0.05$ ; \*\* $p < 0.01$

Hashim et al. (2013) reported that dietary 250 ppm mannan oligosaccharide did not affect egg production in laying hens. This result is parallel with present study. Moreover, it has been reported that 1 g / kg of mannan oligosaccharide added to layer hen rations under different environmental temperatures has no effect on egg production (Bozkurt et al., 2012). However, there are also studies reporting that 1 and 1.5 g / kg mannan oligosaccharide increases egg production (Ghasemian and Jahanian, 2016), and 0.1 and 0.15% mannan oligosaccharide added to *E. coli*-infected laying hen rations positively affects egg production. Beside, Koiyama et al. (2018) found that 450 ppm yeast cell wall increased egg production in laying hens. Contrary to the research findings of the present study, just like yeast products, different levels of inulin (Shang et al., 2018) added to the diets of laying hens plus inulin oligosaccharide produced from Jerusalem artichoke (*Helianthus tuberosus*) (Park and Park, 2012) significantly increased egg production. It has been reported that all doses of inulin obtained from agave added to the drinking water of Japanese quails at the rate of 2, 4 and 6% increased egg production (Mora et al., 2014).

It has been declared that chitosan oligosaccharide added to the diet of laying hens and broilers (Soğancı ve Karşlı, 2018) has insignificant effect on their feed consumption (Yan et al., 2010). This result contradicts the present study in terms of the 0.015 g / L ChiO group. The effect of chitosan oligosaccharide given *ad libitum* to laying quails with drinking water on feed consumption contradicts that in which different levels of mannan oligosaccharide (Bozkurt et al., 2012; Jahanian and Ashnagar, 2015; Ghasemian and Jahanian, 2016) or yeast cell wall (Hashim et al., 2013; Koiyama et al. 2018) were added to the diets of laying hens. Koiyama et al. (2018) reported that yeast cell wall diet increased feed consumption, while Hashim et al. (2013) did not report so. Comparing the above studies with the present study, it is seen that the effect of mannan on the feed consumption of laying hens given oligosaccharide is insignificant. Similarly, the effect of different levels of inulin on the feed consumption of laying hens contradicts the research findings. While, some studies reported that inulin increases feed consumption (Park and Park, 2012; Shang et al., 2018), Mora et al. (2014) declared that *ad libitum* administration of agave inulin to laying quails through drinking water did not affect their feed consumption. Besides, it has been reported that red yeast rice diet (Sun et al., 2016) and xylo - oligosaccharide diet (Li et al., 2017)

as a prebiotic source have no effect on the feed consumption of laying hens.

Yan et al. (2010) declared that dietary chitosan oligosaccharide (100 and 200 mg / kg) significantly increased egg weight of laying hens. On the other hand, another study with findings similar to the present study, showed that the effect of 200 and 400 mg / kg dietary chitosan oligosaccharide on egg weight was insignificant (Meng et al., 2010). When a series of studies on prebiotics were examined, different levels of mannan oligosaccharide (Bozkurt et al., 2012; Jahanian and Ashnagar, 2015; Ghasemian and Jahanian, 2016), yeast cell wall (Koiyama et al., 2018), inulin (Shang, 2010; Mora et al., 2014), xylo oligosaccharide (Li et al., 2017), red yeast rice (Sun et al., 2016), and seaweed polysaccharide (Guo et al., 2020) had insignificant effect on egg weight, which is in line with the present study. On the contrary, it is shown that dietary inulin obtained from different sources (Park and Park, 2012; Kulshreshtha et al., 2014; Shang et al., 2018) increases the egg weights of laying hens.

Some dietary chitosan oligosaccharide studies on broilers declared that different levels of dietary chitosan oligosaccharide had no effect on feed conversion ratio (Soğancı ve Karşlı, 2018), which is in line with the present study. When the effects of other dietary prebiotics on feed conversion ratio on laying hens were evaluated, it was seen that different levels of dietary mannan oligosaccharide (Ghasemian and Jahanian, 2016), dietary yeast cell wall (Hashim et al., 2013), dietary inulin (Shang et al., 2010; Shang et al., 2018), dietary oligofructose (Swiątkiewicz et al., 2010), dietary xylo oligosaccharide (Li et al., 2017), dietary seaweed polysaccharide (Guo et al., 2020) and dietary red yeast rice (Sun et al., 2016) had no effect, which is in parallel with the present study. Prebiotics support the digestive system flora of poultry herds housed under unsuitable management and feeding conditions. The fact that the quails were housed in optimum conditions in the present study may be the reason why chitosan oligosaccharide did not have a statistically significant effect on the feed conversion ratio. Similar with the present study, Mora et al. (2014), who gave inulin via drinking water to laying quails, reported that inulin had no effect on feed conversion ratio. However, contrary to the present study, dietary (2%) red seaweed (*Sarcoditheca gaudichaudii*) (Kulshreshtha et al., 2014), 15 g / kg inulin diet (Shang et al., 2020) and different levels of mannano-oligosaccharides diets (Jahanian and Ashnagar, 2015)

positively effected the feed conversion ratio of laying hens.

Contrary to the present study, a number of studies in laying hens declared that dietary 0.1 - 0.3 % chitosan oligosaccharide or its derivatives (0.2 and 0.4 g / kg) increases Haugh unit of eggs (Meng et al., 2010; Yan et al., 2010; Jiao et al., 2019). On the other hand, there are also several prebiotic studies (seaweed polysaccharide, yeast cell wall, mannanoligosaccharide, red yeast rice, inulin), which declared an increased effect on Haugh unit, contradict the present study (Bozkurt et al., 2012; Park and Park, 2012; Sun et al., 2016; Koiyama et al., 2018; Guo et al., 2020). However, similar with the present study, several investigations performed on laying hens with dietary mannanoligosaccharide (Jahanian and Ashnagar, 2015), dietary isomaltose (Tang et al., 2015), dietary inulin (Shang et al., 2010; Shang et al., 2020) and dietary xylo oligosaccharide (Li et al., 2017) reported negligible effect on Haugh unit.

Similar with Jiao et al. (2019) and Meng et al. (2010), egg yolk color in the present study was not affected by chitosan oligosaccharide treatments. However, Yan et al. (2010) reported the opposite. On the other hand, reports on dietary mannanoligosaccharide (Jahanian and Ashnagar, 2015), dietary isomaltose (Tang et al., 2015), dietary red yeast rice (Sun et al., 2016), dietary inulin (Shang et al., 2010; Park and Park, 2012; Shang et al., 2020) and dietary xylo - oligosaccharide (Li et al., 2017) declared the opposite that they had no effect on the egg yolk color of layers. Guo et al. (2020) concluded in their study that dietary seaweed polysaccharide after molting at the late - stage of layers significantly increased the egg yolk color.

Regarding the shape index, Shang et al. (2020) reported that dietary 5, 10, 15 and 20 g / kg inulin did not affect the shape index of layer eggs, while dietary 15 g / kg inulin significantly increased the yolk index on the 28<sup>th</sup> day of laying age, contrary to our research findings. The report that there is no significant effect of dietary inulin (Shang et al., 2020) and dietary red yeast rice (Sun et al., 2016) on shell weights of hens contradicts the present study. However, the studies declaring that dietary inulin, dietary oligofructose and dietary xylo - oligosaccharide significantly increase eggshell weight of hens (Chen and Chen, 2004; Swiątkiewicz et al., 2010; Li et al., 2017) support the present study. Robertfroid et al. (2000) reported that short-chain carboxylic acids released as a result

of colonic fermentation of non-digestible polisaccharides accelerate the colonic absorption of calcium and phosphorus. Similarly, Li et al. (2007) reported that 100 mg/kg chitosan oligosaccharide added to broiler chick diets significantly increased calcium digestibility. The reason for the increase in eggshell weight of chitosan oligosaccharide given to laying quails with drinking water may be the increased absorption of calcium and phosphorus from the digestive system. The results of the present study on egg shell thickness is not in line with Jiao et al. (2019); however, it is similar to Meng et al. (2010) and Yan et al. (2010) who worked with chitosan oligosaccharide and its derivatives. The researchers who worked with several dietary prebiotics reported increased effect (Park and Park, 2012; Li et al., 2017; Guo et al., 2020; Shang et al., 2020), decreased effect (Koiyama et al., 2018), and no effect (Swiątkiewicz et al., 2010; Bozkurt et al., 2012; Jahanian and Ashnagar, 2015; Shang et al., 2020; Tang et al., 2015) on shell thickness.

Chen and Chen (2004), who studied the effects of inulin and oligofructose on mineral evaluability of laying hens at the late - stage, concluded that both prebiotics significantly increased serum Ca levels (8.02% and 7.84%, respectively) and tibia Ca and P levels. In the study, there was no significant difference between the treatment groups and the control group in terms of blood and egg shell Ca, P and Mg values. However, the negative correlation between blood and eggshell Ca levels was significant. Özpınar (1997) reported that blood ionized Ca concentration was negatively correlated with egg shell strength in laying hens.

Chitosan oligosaccharide administered with drinking water had no significant effect on serum cholesterol and triglyceride levels. This situation is compatible with research findings on chitosan oligosaccharides or their derivatives in laying hens (Jiao et al., 2019) and broilers (Mora et al., 2014). However, there were studies acknowledging that dietary mannan oligosaccharides did not affect serum cholesterol and triglyceride levels (Evrensel et al., 2009) or reduced them (Tülay Bayıbağ and Çolpan, 2007) in broilers. In addition, there were research findings reporting that inulin, oligofructose or xylo - oligosaccharide added to the diets of laying hens (Park and Park, 2012; ; Li et al., 2017) or broilers (Yusrizal and Chen, 2003) significantly reduced their serum cholesterol levels.

## CONCLUSIONS

Ad libitum administration of 0.045 g / L chitosan



oligosaccharide with drinking water did not cause any negative effects in terms of health parameters in quails. It was determined that all chitosan oligosaccharide levels added to drinking water tended to increase egg shell weight and decrease feed consumption in quails. However, 0.030 g / L level significantly increased the eggshell weight. The 0.015 g/L level of chitosan oligosaccharide added to drinking water significantly reduced feed consumption in quails. However, this situation did not decrease the egg production. New studies, which will evaluate the effects of liquid forms of chitosan oligosaccharide on layers, may contribute to the literature by including late-phase laying hens in

research, which evaluates the effect on the flora and morphology of the digestive system.

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#### **CONFLICT OF INTEREST**

The authors do not declare any conflict of interest.

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