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INFLUENCE OF DIETARY CHITOSAN-OLIGOSACCHARIDES SUPPLEMENTATION ON PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF LAYING HENS

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Abstract

This experiment aimed to ascertain whether adding chitosan-oligosaccharides (COS) to the diet will affect Mandarah laying hens' productivity and reproductive efficiency. At the age of 34 weeks, 120 laying hens and 12 cocks of the Mandarah chicken strain were separated into four groups, each consisting of 30 hens and three cocks. The 1st group served as a control group, receiving only a basic diet. The 2nd, 3rd, and 4th experimental groups received 0.1, 0.2, and 0.5 g/kg of chitosan-oligosaccharides as an addition to the base diet, respectively. According to the findings of this study, the majority of the analyzed attributes differed significantly between treatment groups. Comparing the COS-treated birds to the control birds, the COS-treated birds significantly increased egg production, improved feed conversion, Haugh unit score, shell thickness, fertility, hatchability, ovary weight, and the number of yellow ovarian follicles as well as blood estradiol-17β (E_2) and testosterone concentrations. Furthermore, compared to the other treated groups, introducing COS at a level of 0.1 g/kg diet resulted in the best laying rate, egg mass, and feed conversion. The highest percentage of fertility, hatchability of hatching eggs, ovary weight, number of yellow ovarian follicles, and serum E2 concentration was also found in birds fed COS at a dose of 0.2 g/kg feed. In addition, compared to the control diet, COS treatment at 0.1, 0.2, and 0.5 g/kg enhanced relative economic efficiency by 464.43, 457.41, and 352.75%, respectively. It is concluded that chitosan improves economic efficiency, reproductive performance, productivity in laying hens, and overall health when used at 0.1 g/kg diet.

Key words: hatchability, Mandarah strain, chitosan-oligosaccharides, egg mass, estradiol-17β

Egg production is one of the most important poultry industries. Improving layer hens' productivity and egg quality by using natural feed substances is very critical nowadays in light of the scarcity and the rising cost of conventional diet ingredients (El-Sabrout et al., 2022 a, b; El-Saadany et al., 2022; El-Sabrout et al., 2023). Also, using certain growth promoters, including probiotics, prebiotics, and phytobiotics as feed additives, is a strategy to boost the gut immune defense (Syanya et al., 2023). They boost host mucosa immunity and resistance to harmful bacterial colonization, which has a good impact on animal health conditions (Li et al., 2021). Chitosan-oligosaccharides (COS), a deacetylated form of chitin, are widespread throughout nature. Arthropods in the Crustacean family, which includes crabs, shrimp, insects, and other marine organisms, have exoskeletons that are good sources of chitosan (Li et al., 2015; Abd El-Hack et al., 2020; Abd El-Aziz et al., 2022; Kamal et al., 2022). COS is one of the most extensive and naturally happening polymers and is a naturally positive-charged alkaline polysaccharide (Xu and Wang, 2005). Since COS has reactive functional collections such as amino acids and hydroxyl groups, it has antibacterial, anti-inflammatory, antioxidant, immune-stimulating, and hypocholesterolemic characteristics (Xia et al., 2011; Li et al., 2022; Youssef et al., 2023).

Chitosan revealed a growth-promoting impact and can be utilized as a successful replacement for growth-promoting antibiotics based on the previously established positive effects. Moreover, oligosaccharides have been used as prebiotics to increase immunological performance in broilers (Wang et al., 2003), minimize the occurrence of infections in the intestine (Uyanga et al., 2023), and enhance the productivity of laying hens (Meng et al., 2010; Sheoran et al., 2018; Youssef, 2022).

Chitosan may also be added to feed to improve the reproductive efficiency of cattle and poultry (Wan et al., 2016). Improved ovarian function in sows was associated with the positive effects of COS on reproductive efficiency (Xu et al., 2018 a). Under normal physiological settings, COS can increase production efficiency and egg quality when utilized in place of antibiotics for laying hens (Xu et al., 2020). Moreover, COS may reduce intestinal in-

flammation and oxidative stress in laying hens (Gu et al., 2022). In broilers' liver, spleen, and stomach, COS may help lessen inflammation and oxidative stress (Lan et al., 2020). Compared to the control group, Xu et al. (2020) reported that feeding Fengada-1 laying hens COS at 75 and 125 mg/kg diet for ten weeks increased hen-day egg output, egg mass, FCR and shell thickness.

Based on these characteristics, COS may be useful as an antibiotic-free alternative to in-feed feed supplements for agricultural animals. Osho and Adeola (2020) noted that dietary COS enhanced broiler chickens' immune systems and growth performance. It was predicted that COS would improve the local laying hens' blood profile, egg output, and quality through increased egg production rates and decreased blood cholesterol and egg shape index. Therefore, this experiment was conducted to study the effect of chitosan-oligosaccharides administration in the diet on productivity, reproductive performance and economic efficiency of Mandarah strain laying hens.

Material and methods

Birds and management

At the age of 34 weeks, 120 laying hens and 12 cocks of the Egyptian chickens Mandarah strain (Mandarah is a breed of chickens suitable for the conditions of the Egyptian environment as a result of the mating of males of Alexandrian breed with females of Dokki 4 for four generations and selected for eight years to produce eggs. This name comes from the Mandara neighborhood in Alexandria. Strain specifications: the average body weight of a female at the age of 21 weeks is 1490 g, the number of eggs produced in 52 weeks is 195 eggs, the age at the peak of production is 30 weeks, the average weight of the egg is 55 g, the incubation period of this strain is 21 days and the hatching rate is 86%) were selected and at random allotted into four groups, each group divided into three replicates (10 hens and 1 cock each). The hens in each group had comparable average body weights and daily egg production (1428.56 g and 46.45%, respectively). The same administrative and sanitary practices were used to raise the birds, and they were fed a basal laying diet created following the requirements of NRC (1994). Table 1 lists the basal laying ration's feed components and chemical analysis. The photoperiod was maintained throughout the experiment at 16 hours of light and 8 hours of darkness daily. The experiment's average inside ambient temperature and relative humidity were 23.51°C and 58.81°C, respectively. With a density of 5 birds per m^2, all birds were kept in a semi-closed housing system on floor-laying pens (2 × 1.5 m). The experimental duration extends from 34 weeks to 50 weeks of age (16 weeks of egg production).

Experimental design

Individually weighed birds were allocated into four equal experimental groups at random (30 hens and 3 cocks each). Each group represented one treatment; each treatment was randomly assigned to 3 replicate pens. Each pen represented an experimental unit and contained 10 hens and 1 cock. A basal diet was consumed by the 1st group, which served as a control group. The 2nd, 3rd and 4th experimental groups were fed 0.1, 0.2, and 0.5 g/kg of COS in addition to a base diet. Egypt's Chitosan Egypt Agriculture Corporation, located in Dokki, Giza, provided the chitosan powder from shrimp shells with a degree of deacetylation of 92% ±5.

Table 1. Ingredients and the basal diet's determined chemical composition

Ingredients	%
Yellow corn (8.5%)	63.14
Soybean meal (44%)	27.10
Limestone ($CaCO_3$)	7.60
Di-Ca^{++}phosphate	1.50
Dl-Methionine 99%	0.06
(NaCl)	0.3
Vit + Min. premix*	0.3
Overall	100.00
Calculated analysis**	
Crude protein (%)	17.33
ME, Kcal/Kg	2722.00
Ca^{++} (%)	3.35
Available phosphorus (%)	0.40
Lysine (%)	0.88
Methionine (%)	0.34
Methionine plus Cystine (%)	0.64
Zinc (mg/Kg)	22.21

*Vitamins and minerals premix: Given per kg of diet: vit. A, 12000 IU; vit. E, 10 mg; vit. D_3, 2200 ICU; vit. K_3, 2 mg; vit. B_1, 1 mg; vit. B_2 5 mg; vit. B_6 1.5 mg; vit. B_{12} 10 mcg; Pantothenic acid 10 mg; Nicotinic acid 30 mg; Folic acid 1 mg, Biotin 1.5 mcg; Choline 250 mg; Copper 10 mg; Selenium 0.1 mg; Cobalt 0.1 mg; Manganese 60 mg; Iron 30 mg; Zinc 50 mg; Iodine 1 mg.

**Calculated according to NRC (1994).

Measurements

Live body weight and mortality rate

At the start of the experiment, when the birds' age was 34 weeks, and at the end, when they were 50 weeks old, each bird was weighed individually. The body weight changes of birds of all experimental groups were calculated. Also, birds were observed daily and the mortality rates were recorded during the experimental time.

Egg production traits

Egg number (EN) was daily recorded and laying intensity was calculated for each pen within each experimental group during the experimental period. Also, eggs were individually weighed (EW) twice a week to the nearest gram during the experimental period. Each experimental group's egg mass (EM) was calculated throughout the experiment. Feed consumption (FC) and feed conversion ratio (FCR) for each pen were recorded weekly throughout the experiment.

Table 3. Effects of dietary chitosan oligosaccharides supplementation on egg quality characteristics of Mandarah laying hens from 34 to 50 weeks of age (means ± SE)

Traits	Treatments (g chitosan/kg diet)				P-value
	Control	COS 0.1	COS 0.2	COS 0.5	
Egg shape index	75.48±0.76	77.10±0.72	74.58±0.50	77.06±0.78	0.088
Yolk index	44.24±0.82	44.48±0.28	45.06±0.03	43.49±0.55	0.269
Haugh unit score	84.86±0.67 c	91.78±0.21 a	91.04±1.40 ab	88.69±0.37 c	0.001
Shell thickness (μm)	275.00±0.01	289.01±0.50	290.32±0.01	269.21±0.20	0.085

a, b, c – means within the same row with different letters are significantly different (P≤0.001).

Table 4. Effects of dietary chitosan oligosaccharides supplementation on fertility, hatchability, embryonic mortality and chick weight at the hatching of Mandarah laying hens from 34 to 50 weeks of age (means ± SE)

Traits	Treatments (g chitosan/kg diet)				P-value
	Control	COS 0.1	COS 0.2	COS 0.5	
Fertility (%)	86.11±0.32 d	89.07±0.18 c	91.66±0.00 a	90.37±0.18 b	0.000
Hatchability (Fertilized eggs %)	75.06±0.91 b	82.55±1.27 a	83.03±0.35 a	82.38±0.39 a	0.000
Total embryonic mortality (%)	24.93±0.91 a	17.44±1.27 b	16.96±0.35 b	17.61±0.39 b	0.000
Early dead embryos (%)	11.17±0.18 a	9.13±0.19 b	8.48±0.34 b	8.60±1.06 b	0.036
Late dead embryos (%)	10.52±0.77 a	6.66±1.46 b	6.26±0.40 b	6.75±0.36 b	0.027
Pipped (%)	3.22±0.01	2.07±0.20	2.21±0.40	2.25±0.40	0.096
Chick weight at hatching (g)	30.45±0.58	32.32±0.23	32.22±0.64	32.19±0.45	0.086

a, b, c – means within the same row with different letters are significantly different (P≤0.001).

Egg quality characteristics

In total, 432 eggs were used to examine various aspects of egg quality at ages 38, 42, 46, and 50 weeks (4-week intervals) and Table 3 reflects the average of the four periods. Fresh eggs were taken from each treatment, weighed, and separated. Using a digital caliper, the yolk height and diameter, the egg's width and length and the albumen height were all measured. Yolk weight and shell weight were measured using a sensitive scale. Using a micrometer (Mitutoyo Series 102, Misumi (Thailand) Co., Ltd.), the thickness of the shell (including the membranes) was measured. The ratio of the maximum egg width to the maximum egg length × 100 was calculated as the egg shape index (ESI) (Anderson et al., 2004). The yolk index was calculated as height/diameter × 100 (Kondaiah et al., 1983). Haugh unit (HU) score for each egg was calculated according to Moula et al. (2013) as follows: $HU = 100 \log (H + 7.57 - 1.7 W^{0.37})$ where H = albumen height (mm); W = egg weight (g).

Fertility, hatchability and embryonic mortality

One thousand eighty eggs from the Mandarah chicken strain were used at 38, 44 and 50 weeks of age and Table 4 reflects the average of the three periods. Daily egg collections were made before being put in the incubator, and they were kept at room temperature for up to five days. Assessments were made on fertility, hatchability, and embryonic mortality. Upon hatching, all hatchlings, both alive and dead, were counted. After examination, the unhatched eggs were classified as infertile or having experienced embryonic death. Yakimenko et al. (2002) stated that embryonic mortality was split into three categories: pipped embryos, late-dead embryos, and early-dead embryos. The ratio of fertile eggs to all eggs laid was used to calculate fertility. Hatchability was evaluated as the ratio of the total number of hatchlings to the total number of fertile eggs.

Female and male reproductive organs and hormonal levels

After the experiment (age 50 weeks), nine birds from every experimental group (6 hens and 3 cocks) were slaughtered by cutting their jugular vein. Each bird's blood was taken in non-heparinized tubes, and blood serum was centrifuged to separate it at 3000 rpm for 15 minutes. Blood serum was kept until analysis at –20°C (Dhurat and Sukesh, 2014). Serum concentrations of estradiol-17β (E_2) and testosterone (T) were analyzed by ELISA kits (DiaMetra, Spello-Perugia, Italy). After slaughtering, the abdominal cavity of each bird was opened. The ovary and oviduct were immediately removed and weighed. Each female's oviduct was measured for length, and the number of yellow ovarian follicles was counted. Each male's right and left testicles were also taken out and weighed individually (Gongruttananun, 2011).

Economic efficiency (EE)

The input/output examination was used to determine COS supplementation's economic efficiency (EE) in diet and egg production based on the cost of the experimental diets and eggs produced. The values of EE were calculated as the net revenue per unit of total costs. Net revenue and economic efficiency were calculated as follows:

- Net revenue (Egyptian pound) equals the price of all eggs produced minus all feed costs

- Economic efficiency is calculated as net revenue/cost of feed intake.

The price of the birds that were bought, the cost of veterinary care, the cost of housing, and the cost of labor were not included because they were the same for all treatments.

Statistical analyses

The General Linear Model (GLM) technique was applied to examine the data procedure of SPSS (SPSS, 2019). Duncan's multiple-range test evaluated mean differences (Duncan, 1955). Using the following model, a one-way analysis of variance (ANOVA-test) was performed:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where:

Y_{ij} = the observation on the jth individual from the ith treatments.

μ = the overall mean.

T_i = the fixed effect of the ith treatments (control and COS treatments).

e_{ij} = the random error connected with the individual ij.

Results

Live body weight, productive performance and total mortality rate

Initial body weight (IBW) at 34 wks of age, final body weight (FBW) at 50 weeks of age, body weight change (BWC), egg production parameters and total mortality rate (TMR) as influenced by dietary COS supplementation are presented in Table 2. Variance analysis presented greatly significant differences (P≤0.0001) due to the treatments in the number of eggs (EN), egg mass (EM), laying rate (LR), feed conversion ratio (FCR), the total mortality rate (TMR) and feed consumption (FC), among treatment groups. However, treatments in all groups did not show significant differences between IBW, FBW, BWC, and egg weight. The results demonstrated that compared to the control group, using COS at various doses considerably improved EN, EM, LR, FCR, and lowered TMR in Mandarah laying hens.

Also, in comparison to the control group, adding COS at a dosage of 0.1, 0.2, and 0.5 g/kg diet increased EN by 37.60, 38.68, and 35.87%, EM by 42.17, 40.24, and 36.80%, and LR by 37.61, 38.69, and 35.88% at a dosage of 0.1, 0.2, and 0.5 g/kg diet, respectively. However, in comparison to the control group, FC decreased by –3.93, –3.69, and –1.66% and FCR by –31.84, 31.00, and –27.11% at a dosage of 0.1, 0.2, and 0.5 g/kg diet, respectively. Moreover, using COS at a dosage of 0.1, 0.2, and 0.5 g/kg diet decreased mortality rates by –66.66, –83.33, and –83.33%, respectively, relative to the control group (Figure 1).

Table 2. Effects of dietary chitosan oligosaccharides supplementation on live body weight, productive performance and total mortality rate of Mandarah laying hens (means ± SE)

Traits	Treatments (g chitosan/kg diet)				P-value
	Control	COS 0.1	COS 0.2	COS 0.5	
Body weight (g)					
Initial (34 wks old)	1434.66±7.41	1423.43±1.59	1423.60±2.66	1430.10±2.43	0.174
Final (50 wks old)	1668.73±29.66	1633.83±29.74	1582.53±18.71	1591.10±21.80	0.065
Change	234.06±30.37	210.40±28.93	158.93±18.38	161.01±21.92	0.098
Egg production performance					
Egg number/hen	53.40±3.11 b	73.00±0.05 a	73.76±2.05 a	71.83±2.23 a	0.000
Egg weight (g)	47.17±0.63	48.67±0.34	47.65±0.32	47.54±1.02	0.448
Egg mass (g /hen)	2523.23±181.10 b	3553.26±23.91 a	3515.00±90.91 a	3410.96±47.22 a	0.000
Laying rate	47.67±2.78 b	65.17±0.05 a	65.86±1.83 a	64.13±1.99 a	0.000
Feed consumption (g/bird)	13850.25±15.74 a	13306.06±79.35 b	13338.63±144.97	13619.54±26.27 a	0.005
Feed conversion (feed/egg mass)	5.54±0.39 a	3.74±0.03 b	3.80±0.11 b	3.99b±0.04 b	0.001
Total mortality rate	18.18±0.00 a	6.06±3.03 b	3.03±3.03 b	3.03±3.03 b	0.010

a, b – means within the same row with different letters are significantly different (P≤0.05).

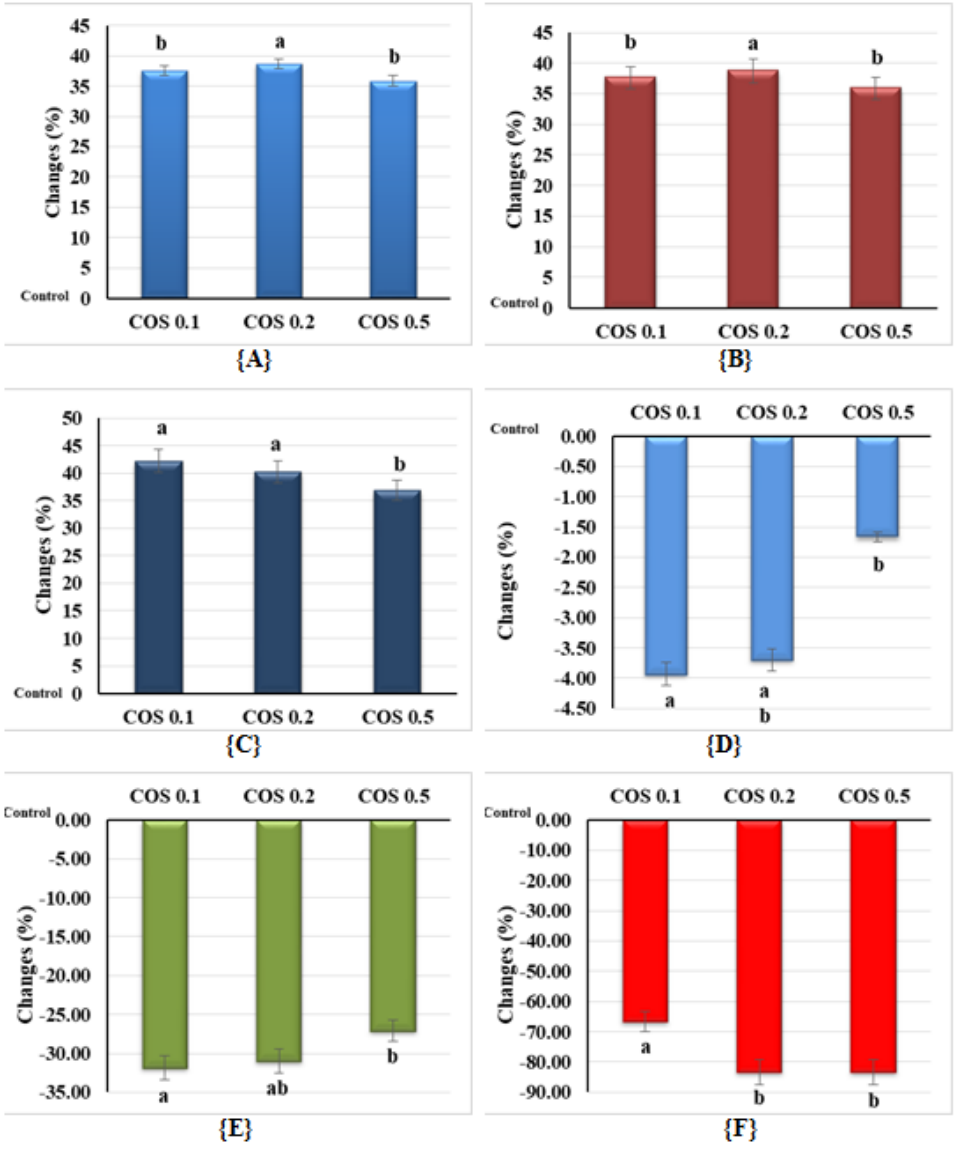


Figure 1. Changes' percent in egg number (A), laying rate (B), egg mass (C), feed consumption (D), feed conversion ratio (E) and total mortality rate (F) of Mandarah laying hens between control and COS treatment groups

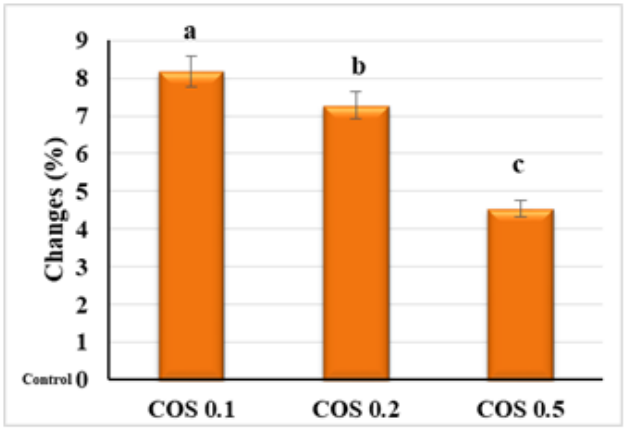


Figure 2. Changes' percent in Haugh unit of Mandarah laying hens between control and COS treatment groups

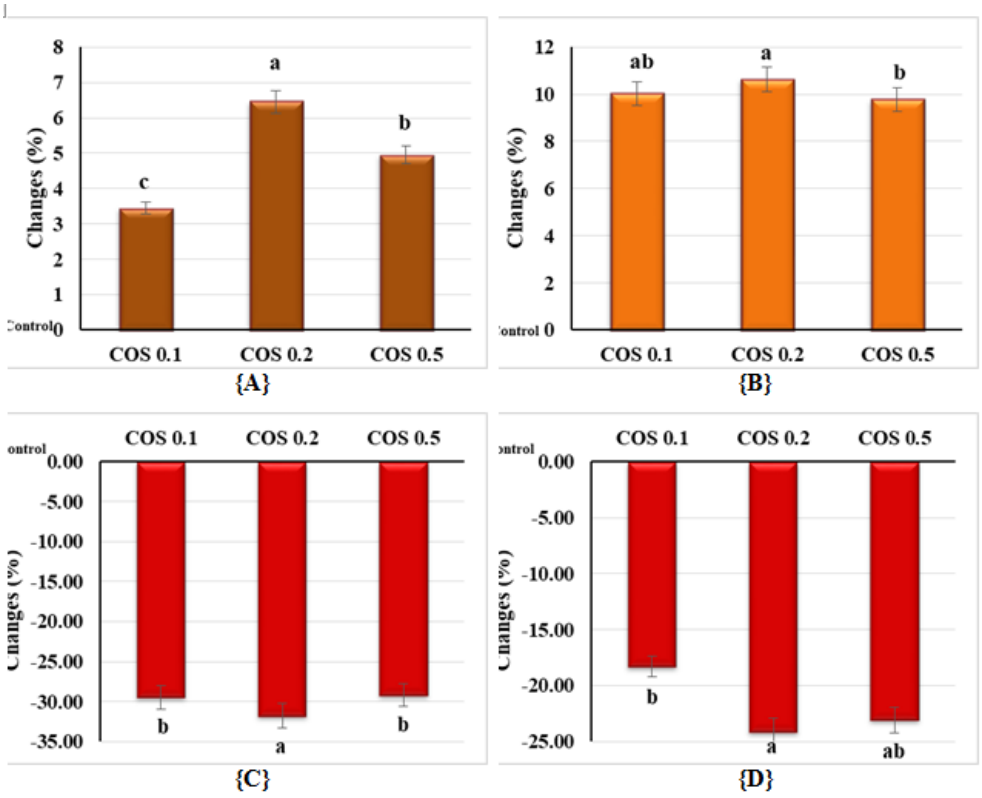


Figure 3. Changes' percent in fertility (A), hatchability (B), total embryonic mortality (C) and early dead embryos (D) of Mandarah laying hens between control and COS treatment groups

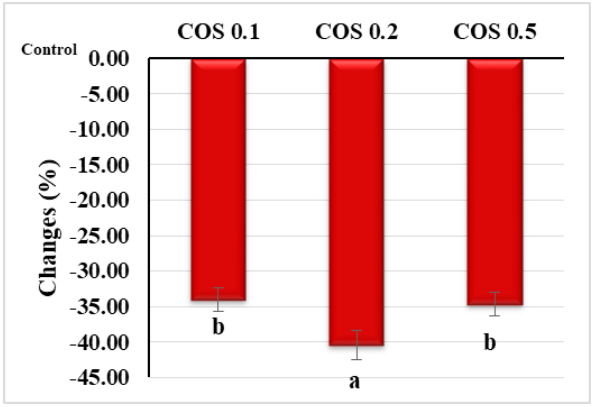


Figure 4. Changes' percent in late dead embryos of Mandarah laying hens between control and COS treatment groups

Egg quality characteristics

Table 3 presents the influences of dietary COS supplementation on egg quality characteristics in Mandarah laying hens, including egg shape index, yolk index, Haugh unit score, and shell thickness (μm). Only in the Haugh unit did the analysis of variance reveal significant differences between treatments. Eggs laid from hens fed COS at a rate of 0.1 g/kg of diet had the greatest Haugh unit scores, but eggs laid from control and 0.5 g/kg COS of diet had the lowest values.

Figure 2 also shows the percent changes in egg quality attributes influenced by treatments. Comparatively to the control group, using COS raised the percent of change in the Haugh unit by 8.17, 7.27, and 4.54% at rates of 0.1, 0.2, and 0.5 g/kg diet, respectively.

Fertility, hatchability and embryonic mortality

The results in Table 4 show the impacts of dietary COS supplementation on fertility, hatchability and embryonic mortality percentages (early dead embryos, late dead embryos and pipped) and chicks' weight at the hatching of the Mandarah chicken strain. Variance analysis has shown significant differences among treatment groups ($P \leq 0.001$) in fertility, hatchability, total embryonic mortality, and early and late dead embryos.

Table 5. Effects of dietary chitosan oligosaccharides supplementation on female and male reproductive organs, estradiol-17β and testosterone levels of Mandarah laying hens at 50 weeks of age (means ± SE)

Traits	Treatments (g chitosan/kg diet)				P-value
	Control	COS 0.1	COS 0.2	COS 0.5	
Females					
Female body weight (g)	2069.33±94.74	1838.33±84.35	1884.67±26.59	1776.67±92.67	0.212
Ovary (g)	35.57±2.05 b	41.86±0.25 ab	50.38±5.23 a	40.85±0.70 ab	0.036
Ovary (%)	1.73±0.17 b	2.30±0.17 ab	2.66±0.24 a	2.31±0.13 ab	0.045
Ovarian yellow follicles (No.)	3.66±0.16 c	5.83±1.09 ab	7.00±0.28 a	4.66±0.44 bc	0.023
Oviduct (g)	57.28±11.92	36.70±6.32	42.41±4.65	49.06±10.19	0.433
Oviduct (%)	2.72±0.46	1.97±0.27	2.24±0.21	2.71±0.41	0.415
Oviduct length (Nogueira et al.)	62.66±4.91	55.66±3.28	61.50±2.56	66.66±1.45	0.209
E_2 (pg/ml)	182.66±3.17 c	251.66±4.05 ab	262.66±6.76 a	244.01±3.78 b	0.000
Males					
Male body weight (g)	2170.66±158.35	2239.33±113.08	1991.33±87.66	2240.01±147.05	0.519
Total testes weight (g)	19.89±1.39	25.67±1.10	34.86±3.01	41.22±16.3	0.341
Total testes weight (%)	0.92±0.08	1.15±0.09	1.76±0.21	1.78±0.60	0.232
Testosterone (ng/ml)	2.62±0.29 b	5.42±0.25 a	4.17±0.77 a	5.07±0.16 a	0.008

a, b, c – means within the same row with different letters are significantly different (P≤0.001)

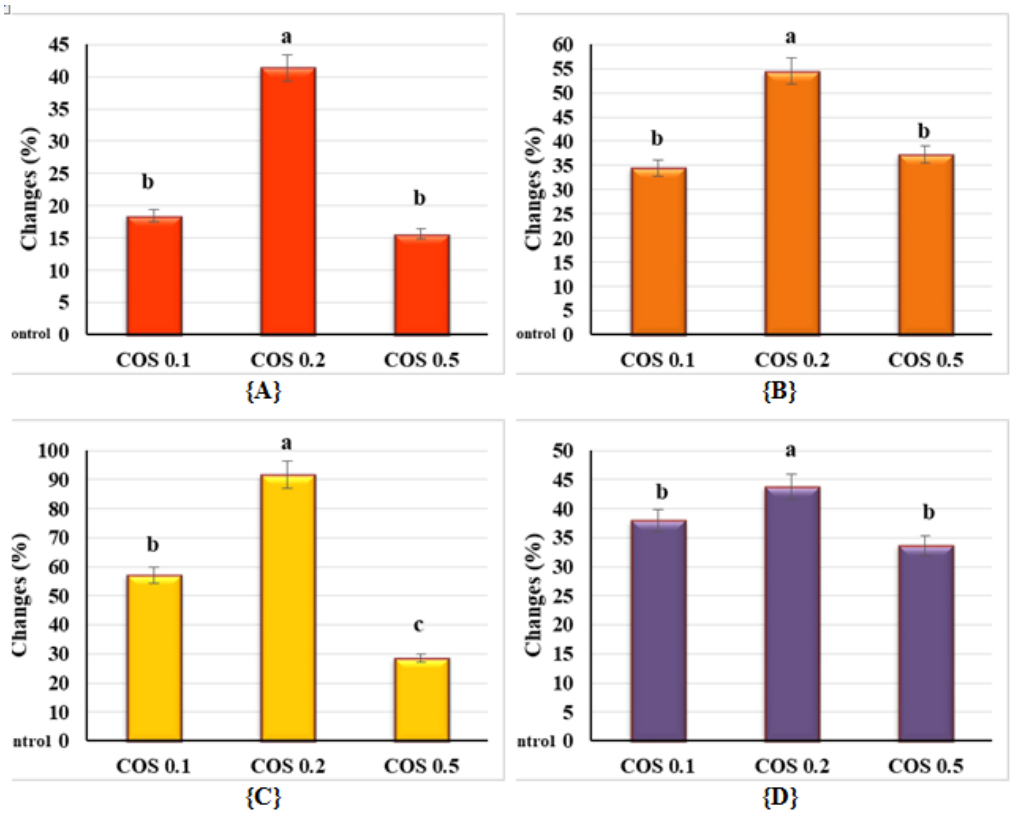


Figure 5. Changes' percent in ovary weight (A), ovary percent (B), number of ovarian yellow follicles (C) and E2 levels (D) of Mandarah laying hens between control and COS treatment groups

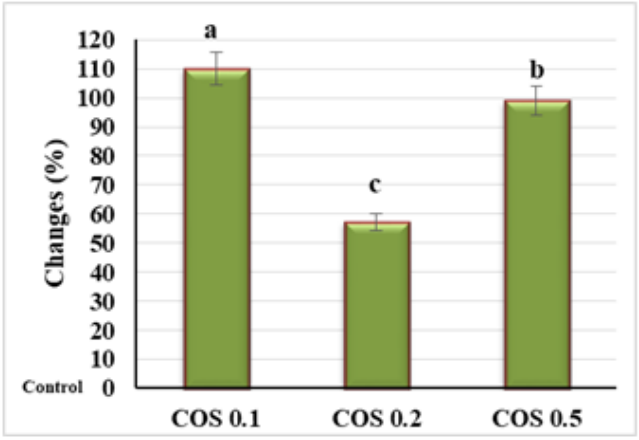


Figure 6. Changes' percent in testosterone levels of Mandarah laying hens between control and COS treatment groups

The eggs laid by birds fed COS at a rate of 0.2 g/kg diet had the greatest percentages of fertility and hatchability, whereas the eggs laid by control birds had the lowest percentages. Yet, compared to the control group, birds who received COS at various dosages had significantly decreased early and late dead embryos and total embryonic mortality.

The percent changes in fertility, hatchability and embryonic mortality are illustrated in Figures 3 and 4. Using COS increased the percent change of fertility by 3.45, 6.45 and 4.95%, and hatchability by 10.04, 10.63 and 9.77%. It decreased total embryonic mortality by –29.45, –31.77 and –29.18% and early dead embryos by –18.28, –24.12 and –23.05% and late dead embryos by –34.05, –40.42 and –34.66% at rates of 0.1, 0.2 and 0.5 g/kg diet, respectively compared to the control group.

Female and male reproductive organs and hormone levels

The genital organs, serum estradiol-17β and testosterone levels of birds as influenced by COS treatments are represented in Table 5. In terms of absolute and relative ovary weight, the number of yellow ovarian follicles, serum concentration of estradiol-17β, and testosterone levels, variance analysis showed significant differences ($P<0.05$) across treatment groups.

Females fed COS at a dose of 0.2 g/kg diet had the greatest absolute and relative ovary weight, number of yellow ovarian follicles, and serum estradiol-17β concentration ($P<0.05$), but control females had the lowest values. Furthermore, cocks fed COS at a rate of 0.1 g/kg diet were estimated to have the maximum serum testosterone concentration, whereas control cocks had the lowest value. Figures 5 and 6 also show the percentage changes in the birds' reproductive organs, serum estradiol-17, and testosterone levels. Using COS increased the percent change of the number of ovaries (g) by 18.43, 41.34 and 15.58%, ovary (%) by 34.46, 54.46 and 37.26%, ovarian yellow follicles by 57.14, 91.67 and 28.57%, E_2 level by 37.91, 43.75 and 33.67% and serum testosterone level by 110.02, 57.17 and 98.92% at levels of 0.1, 0.2 and 0.5 g/kg diet, respectively in comparison to the control group.

Economic efficiency

Table 6 states that the net revenue above the feed cost is how economic efficiency is calculated. Economic efficiency increased and relative economic efficiency improved in COS at all levels in the current study compared to the control group. Moreover, utilizing COS at 0.1 g/kg diet was more economically efficient than using the entire COS. Comparatively to the control group (100%), the relative economic efficiency was 464.43, 457.41, and 352.72% for COS at levels 0.1, 0.2, and 0.5 g/kg diet, respectively, as shown in Figure 7.

Table 6. Input-output analysis and economic efficiency of the experimental groups

Traits	Treatments (g chitosan/kg diet)			
	Control	COS 0.1	COS 0.2	COS 0.5
Number of eggs/hen	53.4	73	73.76	71.83
Eggs price (LE)[1]	93.45	127.75	129.08	125.7
Feed consumption (kg)	13.85	13.31	13.34	13.62
Feed cost (LE)[2]	83.1	80.93	82.23	87.34
Net revenue/hen (LE)[3]	10.35	46.816	46.847	38.367
Economic efficiency (EE)[4]	12.45	57.84	56.96	43.93
Relative economic efficiency (%)[5]	100	464.43	457.41	352.72

[1]total egg number × 1.75 (one egg 1.75 LE).

[2]total feed consumption × price (the price of 1 kg of control feed was 6.00 pounds. The price of one kilogram of chitosan was 825 pounds. The price of a kilogram of diet plus 0.1 grams, 0.2 grams, and 0.5 grams of chitosan was 6.083, 6.165, and 6.413 pounds, respectively).

[3]Net revenue/hen (LE) = price of all eggs/hen (LE) minus total feed cost/hen.

[4]Economical efficiency = net revenue / feed intake price × 100.

[5]economic efficiency for treatment / economic efficiency of control × 100.

The economic study was conducted in 2020.

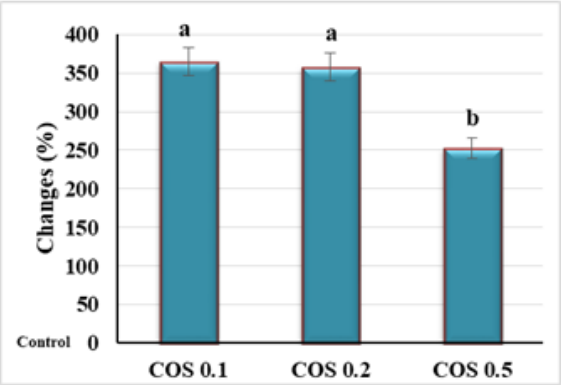


Figure 7. Changes' percent in the relative economic efficiency of Mandarah laying hens between control and COS treatment groups

Discussion

The current study's findings demonstrated that using COS at various levels improved the productive performance of laying hens, as it seems from an increase in egg production and laying rates as well as an enhancement in feed consumption and feed conversion to the control group. This improvement can be due to COS's contribution to improved gastrointestinal health, increased feed digestibility, and improved absorption and metabolism (Walsh et al., 2012; Adli et al., 2023). Moreover, COS enhanced bioavailability in the extra-intestinal tissues by minimizing oxygen consumption in addition to animal portal vein absorption of dietary amino acids, due to its variable fluidity, so it remains on the cell surface and forms a polymer to function as a barrier against oxygen and the low oxygen permeability of chitosan can decrease oxygen (Yin et al., 2010; Büyükyörük, 2021). Moreover, COS boosted the capacity for absorption, increased cell proliferation, and the digestibility of the ileal contents, demonstrating the potential use of chitosan as a feed additive to enhance the efficiency of the digestive process and stimulate nutrient absorption (Suthongsa et al., 2017). Our findings align with those of Xu et al. (2020) and Świątkiewicz et al. (2013), who found that adding COS to the laying hen diet enhanced laying rate, egg quantity, egg mass, FCR, and reduced feed consumption. Also, these findings are consistent with those published by Nogueira et al. (2003) and Hamady and Farroh (2020), who reported that chitosan supplements had no impact on egg weight. In this context, chitosan decreased dietary intake, is associated with increased serum leptin concentrations and decreased CRP concentrations. Hence, the results of this study highlight novel nutritional endocrine mechanisms by which chitosan exhibits anti-obesity properties *in vivo* (Walsh et al., 2013). Also, chitosan dissolves in the stomach, emulsifying fat and forming a gel, which binds with the fat in the intestine, interfering with fat absorption in the intestine (Gades and Stern, 2003). This insoluble complex then passes undigested through the large intestine and is naturally excreted. It has been proposed in other studies that the decreased body weight gain achieved by chitosan supplementation is related to its fat-binding properties (Zhang et al., 2008). Also, chitosan increased ileal villus height to crypt depth in birds. The intestinal mucosal architecture can reveal useful information on intestinal function. Increasing the villus height suggests an increased surface area capable of greater absorption of available nutrients, which may cause an increase in the productive performance of birds (Awad et al., 2008; Youssef et al., 2023).

In this respect, numerous investigations have shown that altering nutrient transport and permeability of bacterial membranes and cytoplasmic membrane barriers were the mechanism. Chitosan's antibacterial action often depends on some variables, including molecular weight, type of bacteria, pH, concentricity of active components associated with chitosan and its derivatives, and other physicochemical properties along with the type of microorganism (Gram-negative or Gram-positive). Positively charged chitosan can attach to or adsorb into the microbes' cell wall throughout the microorganisms' negatively charged constituents existing in the microbial cell (Liaqat and Eltem, 2018). Furthermore, given the prebiotic influences of COS, the improved digestibility of nutrients can be described by the enhanced intestinal environment (reduced number of pathogenic bacteria and improved number of beneficial bacteria), decreased intestinal pH and promotion of the activities of digestive enzymes in broiler chickens (Liu et al., 2018).

The results also indicated that the treatments had statistically significant differences in the mortality rate. Jan et al. (2012) indicated that chitosan has a powerful potential to enhance immunity, for instance, by increasing the titer of Newcastle disease antibodies and $CD4^+$ T-cell levels and controlling the disease such as decreased respiratory and diarrhea symptoms, as well as a decrease in bird mortality, indicating that it may be utilized as a feed supplement.

The Haugh unit scale is the most widely used today for measuring egg albumen quality and, therefore, for judging egg freshness (Sehirli and Arslan, 2022). Re-

sults showed a greater Haugh unit score when laying hens were fed COS-supplemented diets; albumin quality moreover increased numerically in COS treatment, which may suggest the contribution of COS to egg freshness. These results are related to those obtained by Meng et al. (2010), Hamady and Farroh (2020) and Tao et al. (2022) at chitosan levels of 400, 200 and 800 mg/kg diet, respectively, who indicated that COS supplement in laying hens resulted in an improvement in the Haugh unit score. These improvements in egg quality characteristics can be linked to the role of COS in improving digestive enzyme action, intestinal microflora and intestinal morphology, thereby boosting digestion and assimilation of nutrients (Gurbuz et al., 2011)

Results also showed that COS reduced total embryonic mortality and increased fertility and hatchability percentage. These findings were confirmed by Kim et al. (2006), who indicated that COS significantly increased the fertility and hatchability rate. The increase in spermatozoa density and sperm quality in the treated groups may be responsible for this improvement (Shashidhara and Devegowda, 2003). Comparatively, fertility was higher in the COS groups compared to the control group. Differences in fertility across the treatments appeared primarily caused by variations in the amount of sperm in the oviduct. A substantial positive link between the number of spermatozoa inseminated, perivitelline spermatozoa, and fertility was also observed by Bramwell et al. (1995).

Also, the results recorded that the use of COS, especially on 0.2 g/kg of diet, resulted in an improvement in female reproductive traits, as evidenced by an increase in the absolute and relative ovary weight, the number of yellow follicles and the concentration of E_2 and testosterone hormones in the blood. This boost in reproductive efficiency may be attributed to COS's role in promoting genital organ growth and sexual hormone concentration in both males and females. Estradiol-17β was very responsible for female reproductive and sexual behavior. The levels of secretion were not stable during different physiological statuses (Pfaus et al., 2003). Ilés et al. (2013) also described a high correlation between female reproductive characteristics and enhanced E_2 levels. These findings match those of Irawan et al. (2020), who established that the COS developed the histological condition of the reproductive system cells. Additionally, they noted that this is shown by an increase in the ovarian follicle's diameter, an increase in the number of endometrial arterioles, and an increase in the thickness of the endometrial. Moreover, Huang et al. (2020) observed that COS enhanced ovarian germ cell proliferation and improved ovarian function by enhancing the microenvironment.

These results indicated that using COS at various levels improved reproductive efficiency compared to the control group. Studies showed that chitosan improved reproductive efficiency by lowering free radicals and acting as an antioxidant. This improvement may help to improve the testis histological structure. Moreover, prebiotics' potential to decrease spreading endotoxins and advance related sequelae, for instance, inflammation and/or negative health indicators, may cause a rise in plasma testosterone levels (Daniel et al., 2003). Also, according to Xu et al. (2018 b), COS uses enhanced the antioxidative function of weaned pigs and elevated serum antioxidant enzyme activities, which are indicative of the body's enzymatic antioxidants. Additionally, they noted that chitosan could chelate metal ions and scavenge free radicals by donating an atom of hydrogen or a single pair of electrons.

In this study, the relative economic efficiency was improved at all rates of COS, especially at the rate of 0.1 g/kg feed compared with the control group. The increased egg quantity, egg mass, and FCR may be responsible for this improvement. Our findings agree with those of Kamal et al. (2023) in rabbits and Jasim and Nafea (2021), who reported that the addition of COS to the diet of broiler chicks would increase their economic efficiency.

Conclusion

The results of this experiment indicated that using COS in the diets of the Mandara chicken strain had positive and significant impacts on productive and reproductive performance and economic efficiency compared to the control birds. This study's findings suggest that COS, at a 0.1 g/kg diet, is a good substitute for antibiotics as a growth promoter to enhance the performance of laying hens.

Conflict of interest

The authors declare no conflict of interest.

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