OPEN ACCESS
Journal of Animal Health and Production

Research Article

Effect of Dietary Chitosan, Nano-Chitosan Supplementation and Different Japanese Quail Lines on Growth Performance, Plasma Constituents, Carcass Characteristics, Antioxidant Status and Intestinal Microflora Population

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Abstract | This study aimed to examine the effects of dietary chitosan (CH) and nano-chitosan (NCH) supplementation as prebiotics on growth performance, carcass characteristics, plasma constituents, antioxidant status and microbial counts of two lines of Japanese quail. A total of 840 unsexed selected Japanese quail (SJQ) and Japanese quail (JQ) (one day old) with an average initial body weight of 11.65 ±0.03 g and 9.11± 0.04 g, respectively were used in 10 treatment groups, each group has 3 replicates having 28 quail chicks in each. The experimental groups for each line were as follows: The 1st group was fed the basal diet and served as the control; the 2nd and 3rd groups were fed the basal diet supplemented with CH at 50 and 70mg/kg diet; the 4th and 5th groups were fed the basal diet supplemented with NCH at 30 and 50 mg/kg diet. The results showed that SJQ group had a higher (P<0.0001) live body weight, body weight gain, feed intake and better feed conversion ratio. The group of SJQ birds fed diet supplemented with 70 mg CH had the highest (P<0.05) live body weight. The diet supplemented with 70 mg CH gave the highest plasma total protein (P<0.05) for SJQ and JQ groups. Diets supplemented with 70 mg CH or 50 mg NCH exhibited the highest albumin level and TAOC (P<0.0001). The lowest cholesterol content (P<0.05) was obtained by SJQ groups fed diet supplemented with 70 mg CH or 50 mg NCH and JQ groups having 70 mg CH or 50 mg NCH. The lowest LDL cholesterol content (P<0.0001) of plasma was for SJQ fed diet supplemented with 70 mg CH and JQ fed diet supplemented with 30 mg NCH. Supplementation of 30 and 50 mg NCH recorded higher (P<0.0001) *Lactobacillus* count and the lowest (P<0.0001) *E.coli* and *Salmonella* counts for SJQ and JQ. In conclusion, the dietary supplementation of CH and NCH in different quail lines diets could be used as an antioxidant and antibacterial additive without causing any negative effects on growth performance, carcass characteristics and plasma constituents.

Keywords | Chitosan, Nano-chitosan, Japanese quail lines, Growth, Antioxidant, Intestinal microbial

Received | July 15, 2020; **Accepted** | August 05, 2020; **Published** | February 17, 2021
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Citation | Hassan FA, Abd El-Maged MH, El-Halim HA, Ramadan GS (2021). Effect of dietary chitosan, nano-chitosan supplementation and different japanese quail lines on growth performance, plasma constituents, carcass characteristics, antioxidant status and intestinal microflora population. J. Anim. Health Prod. 9(2): 119-131.
DOI | http://dx.doi.org/10.17582/journal.jahp/2021/9.2.119.131
ISSN | 2308-2801

INTRODUCTION

A wide variety of feed additives like prebiotics are recently used in poultry diets as better alternative to antibiotic growth promoters, chitosan is one of those prebiotics. Chitosan is a natural bio-polyaminosacharides, biodegradable and non-toxic biopolymer (Vimal et al., 2013) derived from alkaline deacetylation of chitin from shrimp wastes and fungal biomass (Darwesh et al., 2018). Chitosan has specific bioactivities such as immune-enhancing properties and antibacterial activities (Alishahi, 2014). Many of studies have been performed on chitosan

as poultry feed supplementation. Xu et al. (2013) previously stated that dietary supplementation of chitosan may enhance the growth performance of animals. Furthermore, Hernawan et al. (2017) demonstrated that the addition of chitosan at 150 ppm/g diet had beneficial impact on reducing the levels of blood cholesterol and malondialdehyde in the diet of laying hens at the age of 28 weeks. Also, Anraku et al. (2018) stated that chitosan has antioxidant properties so can be used as a powerful source of antioxidant for broiler chicken. The dietary chitosan oligosaccharides may be helpful for mitigating the adverse effects of stress on the gut health of broiler chickens (Osho and Adeola, 2020). Nano-particles (NPs) are particles which range from one to 100 nm (Khan et al., 2019). It has been proposed that NPs could be used as an alternative to chemo-prophylactic medicines in poultry. To date, various types of NPs have been studied for feeding, watering or other ways to improve poultry health for use in the poultry industry (Anwar et al., 2019). Chitosan nanoparticles may be formulated and used as a natural antifungal agent (Ing et al., 2012). Several applications of nano-chitosan as a natural material with excellent physicochemical properties were conducted for the improvement of growth performance, immune status and microflora of commercial poultry birds. Abdeltwab et al. (2019) reported that nano-chitosan was the most effective in delaying fungal activity at concentrations between 3.0 and 4.5µg/ml.

As well, Darwesh et al. (2018) used chitosan and nano-chitosan at 100 or 200 mg kg/ BW for rats and found that they had a high level of antimicrobial activity and are not toxic. The present study was designed to evaluate the impact of chitosan and nano- chitosan supplementations as prebiotics on growth performance, carcass characteristics, plasma constituents, antioxidant status and microbial counts of two different lines of Japanese quail.

MATERIALS AND METHODS

Preparation of Chitosan Nanoparticles

The nano chitosan (NCH) solution was prepared by using an ionic gelation method described by Tang et al. (2007). In brief, 0.5g chitosan was dissolved in 90 ml of distilled water contains 2 ml acetic acid. Then, 10 ml Sodium tri-polyphosphate (STPP) solution was added drop by drop wisely into the chitosan beaker at room temperature. After that, the chitosan solution was magnetically stirred at room temperature for 45 min in order to obtain chitosan nanoparticles. These chitosan nanoparticles could be stored at cold for use.

Birds, experimental design, diets and management

All applicable international, national, and institutional guidelines for the care and use of animals were followed. A total of 840 one day old, selected line of Japanese quail (selected for high live body weight at 30 days of age for 42 generations) and Japanese quails (*Coturnix coturnix japonica*) (one day old) with an average initial body weight at hatch was 11.65 ±0.03 g and 9.11± 0.04 g, respectively were used in a completely randomized design experiment with 10 treatment groups. Each group was subdivided into 3 replicates with 28 unsexed chicks each. The basal diet was formulated to meet SJQ and JQ requirements according to NRC (1994). The basal diet was supplemented with chitosan (CH) at 50 and 70 mg/kg diet or nano-chitosan (NCH) at 30 and 50 mg/kg diet. The experimental groups were for each line as follows: The 1st group was fed the basal diet and served as control; the 2nd and 3rd groups were fed the basal diet supplemented with CH at 50 and 70mg; the 4th and 5th groups were fed the basal diet supplemented with NCH at 30 and 50 mg. Composition and calculated analysis of the basal diet are presented in Table 1. The birds were housed in a conventional type cage (50 × 30 × 50 cm^3) with feed and fresh water provided *ad libitum* throughout the experimental period. Birds were maintained in 24h light period throughout the trial. All chicks were kept un

Table 1: Composition and calculated analysis of the basal diet

Ingredients	%
Yellow corn (8.5%)	35.00
Soybean meal (44%)0	55.54
Corn gluten meal (62%)	6.50
Dicalcium phosphate	0.80
Limestone	1.35
Salt NaCl	0.35
Vit-min premix[1]	0.30
DL-Methionine	0.05
L-Lysine	0.11
Total	100
Calculated analysis[2]	
Crude Protein %	24.02
Crude fiber%	3.87
Metabolizable energy, Kcal/kg	2900
Calcium%	0.81
Phosphorous (available)%	0.30
Methionine+Cystine %	0.75
Methionine %	0.50
Lysine%	1.30

[1]each kg contain Vit. A, 12000.000 IU; Vit. D3, 2000.000 IU; Vit E,10g; Vit. K2, 1g; Vit. B1, 1g, Vit. B2, 4g, Vit. B6, 1. 5g; Vit. B12, 10g; Pantathenic acid, 10g; Nicotinic acid, 20g; Folic acid, 1000 mg; Biotin, 50g; Choline chloride, 500g; Copper, 10g; Iodine, 1g; Iron, 30g; Manganese, 55g; Zinc, 55g; Selenium, 0.1g. [2]Calculated according to NRC (1994).

Table 2: Least square means of live body weight and body weight gain of two lines of Japanese quail supplemented by dietary chitosan and nano-chitosan.

Items		Live body weight (g)			Body weight gain (g)		
		1 day	14 Day	35 day	1-14 day	14-35 day	1-35 day
Quail's line effect							
SJQ		11.65[a]	113.18[a]	336.55[a]	101.52[a]	223.37[a]	324.9[a]
JQ		9.11[b]	68.82[b]	204.44[b]	59.70[b]	135.61[b]	195.32[b]
SEM		0.06	1.8	3.1	1.8	3.3	3.16
P-value		0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Supplementation effect							
Control		10.29	90.35	272.42	80.06	182.07	262.13
50 mg CH		10.25	93.02	266.30	82.95	173.10	256.05
70 mg CH		10.45	89.14	275.52	78.67	186.39	265.07
30 mg NCH		10.54	95.25	267.83	84.71	172.57	257.29
50 mg NCH		10.38	87.07	270.40	76.65	183.33	260.01
SEM		0.1	2.84	5	2.8	5.2	4.9
P-value		0.29	0.30	0.7	0.31	0.26	0.71
Quail's line X Supplementation							
SJQ	Control	11.49[a]	111.53[b]	335.64[ab]	100.1[b]	224.2[a]	324.2[ab]
	50 mg CH	11.45[a]	109.85[b]	322.54[b]	98.3[b]	212.6[a]	311.6[b]
	70 mg CH	11.78[a]	111.40[b]	346.48[a]	99.7[b]	254.9[a]	334.7[a]
	30 mg NCH	11.85[a]	124.45[a]	336.32[ab]	112.6[a]	211.8[a]	324.5[ab]
	50 mg NCH	11.75[a]	108.60[b]	341.79[ab]	96.8[b]	233.9[a]	330[ab]
JQ	Control	9.17[b]	69.24[c]	209.21[c]	60.5[c]	139.9[b]	200[c]
	50 mg CH	9.03[b]	76.55[c]	210.06[c]	67.5[c]	133.5[b]	201[c]
	70 mg CH	9.13	66.73[c]	204.57[c]	57.6[c]	137.8[b]	195.4[c]
	30 mg NCH	9.22[b]	66.06[c]	199.34[c]	56.8[c]	133.2[b]	190[c]
	50 mg NCH	9.02[b]	65.53[c]	199.01[c]	56.6[c]	133.5[b]	189.9[c]
SEM		0.14	4.0	7.1	4.1	7.4	7.1
P-value		0.04	0.007	0.02	0.008	0.004	0.02

[a,b,c] Means within the same column for each main effect with different superscripts are significantly different (P<0.05).
CH: chitosan, NCH: Nano-chitosan, SJQ: selected Japanese quail, JQ: Japanese quail

der the same managerial, hygienic, and environmental conditions. Body weight (BW) and feed intake (FI) of each group were recorded weekly, and then body weight gain (BWG) and feed conversion ratio (FCR) were computed. Dead quails were recorded daily and were expressed as mortality (%).The trial lasted for 5 weeks.

Slaughter procedure and carcass traits

At the end of the experimental period (35 days of age), three fasted birds from each group were randomly taken for slaughter, fasted for 12 hours, individually weighed and slaughtered to complete bleeding then liver, heart, gizzard, were separated then weighed and their relative weights were calculated as a percentage of live body weight. Carcasses were manually eviscerated and weighed.

Biochemical Analysis

Blood samples were collected during slaughtering using 3 quail from each group. Plasma was separated by centrifugation at 5,000 rpm (3,354 g force) for 15 min and stored at -20°C until biochemical analysis. Plasma total protein, albumin, total cholesterol, Low Density Lipoprotein (LDL-cholesterol), High Density Lipoprotein (HDL-cholesterol), Triglycerides, total antioxidant capacity (TAOC) and Catalase (CAT) were colorimetrically determined using commercial kits (purchased from Bio-Diagnostic, Cairo, Egypt), according to the manufacturers' guidelines. Plasma total protein was determined according to Orsonneau et al. (1989). Albumin was determined according to the method of Doumas et al. (1971). Plasma globulin concentration was calculated by the difference between plasma total protein and albumin, and then the albumin/globulin ratio was calculated. Plasma cholesterol,

Table 3: Least square means of feed intake, FCR and mortality of two lines of Japanese quail supplemented by dietary chitosan and nano-chitosan.

Items		Feed intake (g/bird)			FCR(g feed/g gain)			Mortality (%)		
		1-14 day	14-35 day	1-35 day	1-14 day	14-35 day	1-35 day	1-14 day	14-35 day	1-35 day
Quail's line effect										
SJQ		192.39[a]	550.3[a]	742.7[a]	1.90[b]	2.46[b]	2.29[b]	5.2	6.1	10.9
JQ		121.45[b]	373.1[b]	494.5[b]	2.06[a]	2.77[a]	2.54[a]	5.0	8.6	10.7
SEM		2.2	11.4	12.1	0.05	0.09	0.06	1.16	2.0	2.4
P-value		0.0001	0.0001	0.0001	0.05	0.03	0.01	0.8	0.4	0.9
Supplementation effect										
Control		157.00	457.14	614.14	1.96	2.52	2.35	3.5	5.5	8.9
50 mg CH		161.20	440.59	601.79	1.96	2.57	2.35	3.5	4.7	8.2
70 mg CH		160.27	464.65	624.95	2.07	2.51	2.38	4.7	4.5	9.2
30 mg NCH		153.45	463.09	616.48	1.88	2.75	2.46	4.9	4.7	9.6
50 mg NCH		152.67	483.09	635.77	2.01	2.73	2.51	3.7	5.3	9.0
SEM		3.6	18.2	19.1	0.008	0.15	0.10	1.8	3.2	3.9
P-value		0.35	0.5	0.7	0.6	0.6	0.7	0.4	0.5	0.7
Quail's line X Supplementation										
SJQ	Control	194.0[a]	552.9[a]	746.9[a]	1.93[ab]	2.5	2.30[ab]	4.7	4.9	9.5
	50 mg CH	196.5[a]	530.6[a]	727.2[a]	2.0[ab]	2.5	2.34[ab]	4.5	4.7	9.2
	70 mg CH	193.4[a]	571.4[a]	764.8[a]	1.93[ab]	2.4	2.28[ab]	4.7	4.8	9.5
	30 mg NCH	189.0[a]	524.2[a]	713.2[a]	1.69[b]	2.4	2.19[b]	4.7	3.8	8.3
	50 mg NCH	188.9[a]	572.2[a]	761.1[a]	1.95[ab]	2.4	2.30[ab]	4.9	4.1	9.0
JQ	Control	119.9[b]	361.3[b]	481.3[b]	1.99[ab]	2.6	2.40[a]	4.3	5.2	9.5
	50 mg CH	125.8[b]	350.4[b]	476.3[b]	1.93[ab]	2.6	2.37[ab]	4.5	4.7	9.2
	70 mg CH	127.1[b]	379.9[b]	485.1[b]	2.2[a]	2.6	2.59[ab]	4.3	5.5	9.8
	30 mg NCH	117.8[b]	401.8[b]	519.6[b]	2.1[a]	3.0	2.73[a]	3.5	5.5	9.0
	50 mg NCH	116.4[b]	393.9[b]	510.3[b]	2.1[ab]	3.0	2.72[a]	4.5	4.5	9.0
SEM		5.1	25.6	26.9	0.11	0.22	0.14	2.6	4.6	0.5
P-value		0.009	0.005	0.005	0.003	0.006	0.003	0.5	0.4	0.5

[a,b,c] Means within the same column for each main effect with different superscripts are significantly different (P<0.05).
CH: chitosan, NCH: Nano-chitosan, SJQ: selected Japanese quail, JQ: Japanese quail

LDL- and HDL-cholesterol were determined according to the method of Lopez-Virella et al. (1977). Triglycerides were determined according to Wahlefeld (1974). Plasma Total antioxidant capacity (T-AOC) was determined according to Koracevic et al. (2001) and catalase (CAT) activity was measured according to Aebi (1984).

Microbiological determination

The contents of the gastrointestinal tract of each bird were collected and stored in the freezer at -4°C. The cultures were prepared. The ileal contents from the gut were serially diluted. From the prepared intestinal content, 0.1 ml was inoculated in suitable medium and then incubated in aerobic and anaerobic conditions. Each sample was serially diluted from 10^{-1} to 10^{-6}. Dilutions were subsequently plated on duplicate selective agar media for enumeration of selective bacteria (*Lactobacillus*, *E.coli* and *Salmonella*) using MRS agar, Mackonkey and *Salmonella Shigella* agar (SS agar), respectively. The plates were then incubated at 37°C for 48 to 72 h aerobically and colonies were counted. Results were expressed as $\log_{10}$ colony-forming units per gram of ileum digesta ($\log_{10}$ CFU/g) (Hartemink and Rombouts, 1999).

Statistical Analysis

The obtained data was subjected to two-way analysis of

Table 4: Effect of dietary chitosan and nano-chitosan supplementation on carcass traits of two lines of Japanese quails.

Items		Carcass weight (g)	Carcass%	Liver%	Heart%	Gizzard%	Total edible parts%[1]
Quail's line effect							
SJQ		246.86[a]	69.67	2.65	0.97	2.61[a]	75.01
JQ		151.30[b]	70.04	2.72	0.99	2.24[b]	76.91
SEM		4.11	2.4	0.13	0.05	0.08	2.50
P-value		0.0001	0.9146	0.7001	0.8351	0.0088	0.9788
Supplementation effect							
Control		192.20[ab]	69.8	2.72	1.1	2.29	75.95
50 mg CH		211.83[a]	70.9	2.66	0.99	2.37	76.98
70 mg CH		185.83[b]	66.4	2.82	0.91	2.62	72.83
30 mg NCH		198.58[ab]	69.2	2.66	0.85	2.26	74.98
50 mg NCH		206.66[a]	72.8	2.59	1.1	2.61	79.06
SEM		9.67	3.80	0.21	0.08	0.14	3.90
P-value		0.0001	0.8262	0.9448	0.2244	0.2	0.8492
Quail's line X Supplementation							
SJQ	Control	237.66[a]	68.97	2.77	0.93[ab]	2.62[ab]	75.29
	50 mg CH	243.67[a]	70.24	2.35	0.92[ab]	2.43[abc]	75.95
	70 mg CH	242.33[a]	69.76	2.54	1.05[ab]	2.77[ab]	76.13
	30 mg NCH	251.00[a]	69.89	2.86	0.86[b]	2.30[abc]	75.91
	50 mg NCH	259.67[a]	69.49	2.73	1.12[ab]	2.92[a]	76.27
JQ	Control	147.33[bc]	70.71	2.66	1.28[a]	1.95[bc]	76.61
	50 mg CH	180.00[b]	71.65	2.96	1.08[ab]	2.31[abc]	78.00
	70 mg CH	129.33[c]	63.18	3.1	0.76[b]	2.46[abc]	69.52
	30 mg NCH	146.17[bc]	68.54	2.46	0.85[b]	2.21[bc]	74.05
	50 mg NCH	153.67[bc]	69.49	2.43	0.99[ab]	2.30[abc]	81.85
SEM		9.19	5.40	0.29	0.11	0.19	5.60
P-value		0.049	0.8078	0.2921	0.0498	0.004	0.8535

[a,b,c] Means within the same column for each main effect with different superscripts are significantly different (P<0.05).
CH: chitosan, NCH: Nano-chitosan, SJQ: selected Japanese quail, JQ: Japanese quail
[1]Total edible parts%= (Carcass%+Giblets%); Giblets%= (Liver%+Gizzard%+Heart%)

variance to detect the effects of quail's line (L), supplementation (S) and the interaction (L*S) using a completely randomized design with General Linear Model (GLM) procedures of XIstat (2014). All data are reported as least square means (LSM) ± standard errors (SE). Differences between values were separated, when significance existed, using Duncan's multiple range test (Duncan, 1955). Significance level was set at 5%. All results were analyzed using the following statistical model was as follows:

$Y_{ijk}= \mu+ L_i+ S_j+LS_{ij}+e_{ijk}$ Where: Y_{ijk}= the k^{th} observation of j^{th} treatment within the i^{th} line; μ = the overall mean; L_i= The effect of the i^{th} line; S_j= The effect of the j^{th} supplementation; LS_{ij}= The interaction between the i^{th} line and the j^{th} supplementation and eij= Experimental random error.

RESULTS

Growth performance

The effect of dietary CH and NCH supplementations on the growth performance of two different lines of quail is presented in Table 2 and 3. The obtained results revealed that SJQ group had a significant increase (P<0.05) in BW, BWG and FI compared to the JQ group at all experimental periods. Moreover, the SJQ recorded the best (P<0.05) FCR compared to the JQ at all experimental periods. Concerning the dietary supplementation effect, there were insignificant differences between the different dietary supplementation of CH at levels of 50 or 70 mg and NCH at levels of 30 or 50 mg in BW, BWG, FI and FCR. In regard to the interaction effect between the quail's line and dietary supplementation, the group of SJQ fed diet supplemented with 70 mg CH had higher (P<0.05) live body weight than the group of SJQ fed diet contained 50 mg CH, the group

of JQ birds fed diets supplemented with CH at 50 or 70 mg, those having NCH at 30 or 50 mg and the control group of JQ at 35 days of age (Table 2).

It could be observed that the SJQ fed the basal diet and diets supplemented with 50 or 70 mg CH and 30 or 50mg NCH achieved higher (P<0.05) feed intake than the corresponding treatments of JQ at all tested levels. The SJQ fed diet supplemented with 30 mg NCH significantly improved FCR compared to the JQ fed the control diet and diets supplemented with 30 or 50 mg NCH during the whole periods. Regarding mortality%; the investigated factors did not change mortality% (Table 3).

Carcass characteristics

The effect of CH and NCH addition to the quail diets on the carcass characteristics is shown in Table 4. The present results indicated that the carcass weight significantly affected by quail's line and the SJQ group recorded higher (P<0.05) carcass weight than the JQ group. The dietary supplementation of 50 mg CH and 50 mg NCH increased (P<0.05) the carcass weight compared to 70 mg CH supplementation. Moreover, the groups of SJQ fed diets supplemented with CH and NCH at tested levels and the control group had higher (P<0.05) carcass weight than the SJ groups fed CH and NCH and the control diet. Otherwise, carcass %, liver %, Heart % and total edible parts% did not significantly differ among the experimental groups as a result of the effect of quail's line and dietary supplementation of CH and NCH. However, the SJQ exhibited better (P<0.05) gizzard% than the JQ. With regard to the interaction, SJQ birds fed diet supplemented with 50 mg NCH increased gizzard% compared to the JQ fed the basal diet and diet supplemented with 30 mg NCH. On the other hand, the SJQ fed diet contained 30mg NCH and the JQ fed diet supplemented with 70 mg CH and 30 mg NCH tended to be lower (P<0.05) in heart% than the JQ fed the control diet. Total edible parts% was not significantly influenced by the interaction effect.

Plasma biochemical constituents

Table 5 shows data of plasma biochemical constituents. It is clear to notice that there was no effect of quail's line on plasma total protein, globulin, A/G ratio and total cholesterol concentrations. As well as, the SJQ had higher (P<0.05) plasma albumin, triglycerides and HDL levels than JQ group. On the other hand, SJQ tended to be lower (P<0.05) in LDL level than the JQ group. Concerning the supplementation effect, quail fed diet supplemented with CH at level of 70 mg had higher (P<0.05) plasma total protein and albumin levels than the other experimental groups. The highest A/G ratio and plasma HDL concentrations were recorded for quail received diet supplemented with 50 mg NCH/kg. On the other hand, plasma total cholesterol and LDL concentrations were significantly reduced (P<0.05) with dietary addition of CH and NCH compared to the control group. The dietary supplementation of CH and NCH had no significant impact on plasma triglycerides levels. While, supplementation of 70 mg CH, 30 or 50 mg NCH increased (P<0.05) HDL concentration compared to the control and 50 mg CH groups.

The interaction between the quail's line and dietary supplementations significantly (P<0.05) affect all the plasma constituents. Moreover, SJQ group fed diet contained 70 mg CH increased (P<0.05) total protein concentration. In contrast, SJQ group fed the basal diet, 50 mg CH, JQ fed diet including 50 mg CH and 50 mg NCH decreased (P<0.05) total protein compared to the other groups. As well as, dietary supplementation of CH and NCH increased (P<0.05) albumin level compared to the control groups of SJQ and JQ. The JQ fed the control diet was the highest (P<0.05) in globulin level. While, the JQ fed diet supplemented with 50mg NCH recorded the lowest (P<0.05) value of globulin concentration.

In regard of the interaction effect on lipids profile, plasma LDL levels were decreased (P<0.05) with dietary supplementation of CH and NCH for SJQ and JQ groups. However, the group of JQ fed the control diet had the highest (P<0.05) LDL level compared to the other tested group. Further, dietary supplementation of CH and NCH reduced (P<0.05) plasma total cholesterol. An opposite effect was noticed with the group of SJQ fed the basal and 50 mg CH diets whereas, the levels of total cholesterol were significantly (P<0.05) increased. Additionally, JQ group fed diets supplemented with 50 or 70 mg CH, 30 or 50 mg NCH had lower (P<0.05) triglycerides and higher (P<0.05) HDL levels.

Antioxidant status and intestinal microflora population

As shown in Table 6, significant increase in TAOC and catalase levels were observed in the group of JQ compared to the SJQ group. The supplementation level of CH and NCH had significant (P<0.05) effect on TAOC and catalase levels. It is clear to notice that CH at level of 70mg recorded higher (P<0.05) TAOC followed by NCH 50 mg and the control group was significantly the lowest. While, the dietary supplementation of 70 mg CH recorded the highest (P<0.05) catalase level, on the other hand, the control group had the lowest (P<0.05) catalase level. Concerning the interaction effect, the groups of SJQ fed diets supplemented with 70 mg CH and 50 mg NCH had higher (P<0.05) TAOC than the other tested groups. However, the group of SJQ fed diets included 70 mg CH achieved higher (P<0.05) catalase activity. In contrast, the control group of both SJQ and JQ recorded the lowest (P<0.05)

Table 5: Effect of dietary chitosan and nano-chitosan supplementation on plasma constituents of two lines of Japanese quail.

Items		Protein profile				Lipid profile			
		Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Quail's line effect									
SJQ		6.17	3.61^{a}	2.56	1.42	181.70	90.91^{a}	74.38^{b}	90.13^{a}
JQ		6.16	3.57^{b}	2.59	1.40	180.12	79.79^{b}	88.16^{a}	75.93^{b}
SEM		0.02	0.01	0.02	0.01	1.12	1.49	0.19	1.60
P-value		0.6662	0.0047	0.4039	0.2663	0.3321	0.0001	0.000	0.0001
Supplementation level effect									
Control (zero level)		6.07^{b}	3.19^{d}	2.87^{a}	1.12^{e}	198.79^{a}	84.95	94.53^{a}	77.28^{b}
50 mg CH		5.94^{c}	3.39^{c}	2.55^{c}	1.33^{d}	195.97^{b}	86.50	89.85^{b}	77.89^{b}
70 mg CH		6.53^{a}	3.83^{a}	2.70^{b}	1.42^{c}	172.21^{b}	87.28	70.74^{e}	83.86^{a}
30 mg NCH		6.16^{b}	3.72^{b}	2.44^{d}	1.53^{b}	170.71^{b}	84.43	72.83^{d}	86.65^{a}
50 mg NCH		6.13^{b}	3.82^{a}	2.31^{e}	1.66^{a}	166.87^{b}	83.60	78.40^{c}	89.44^{a}
SEM		0.03	0.01	0.04	0.02	1.78	2.42	0.30	2.01
P-value		0.0001	0.0001	0.0001	0.0001	0.0001	0.8150	0.0001	0.0012
Quail's line X Supplementation									
SJQ	Control	5.95^{de}	3.22^{e}	2.73^{b}	1.18^{g}	202.74^{a}	96.85^{a}	91.66^{b}	68.03^{d}
	50 mg CH	6.01^{cde}	3.41^{d}	2.60^{bc}	1.63^{ab}	198.42^{ab}	91.63^{ab}	88.93^{c}	68.03^{d}
	70 mg CH	6.57^{a}	3.84^{a}	2.73^{b}	1.41^{de}	167.09^{d}	86.46^{abc}	54.35^{g}	77.56^{c}
	30 mg NCH	6.16^{bc}	3.76^{b}	2.40^{de}	1.57^{bc}	173.18^{cd}	91.50^{ab}	68.77^{f}	82.13^{bc}
	50 mg NCH	6.17^{ab}	3.83^{a}	2.34^{de}	1.64^{ab}	167.07^{d}	88.10^{abc}	68.21^{f}	83.44^{bc}
JQ	Control	6.19^{ab}	3.18^{e}	3.01^{a}	1.06^{h}	194.83^{b}	86.46^{abc}	97.39^{a}	86.54^{ab}
	50 mg CH	5.87^{e}	3.36^{d}	2.50^{cd}	1.35^{ef}	193.52^{b}	81.36^{bcd}	90.76^{b}	87.31^{ab}
	70 mg CH	6.50^{a}	3.82^{a}	2.68^{b}	1.43^{de}	177.33^{c}	73.05^{d}	87.13^{d}	90.17^{ab}
	30 mg NCH	6.16^{bc}	3.68^{c}	2.48^{cd}	1.49^{cd}	168.25^{d}	77.36^{cd}	76.90^{e}	91.18^{ab}
	50 mg NCH	6.08^{bcd}	3.81^{ab}	2.27^{e}	1.68^{a}	166.67^{d}	79.10^{cd}	88.59^{c}	95.45^{a}
SEM		0.05	0.02	0.05	0.03	2.51	2.96	0.42	2.84
P-value		0.032	0.043	0.007	0.028	0.015	0.0281	0.0001	0.378

^{a,b,c} Means within the same column for each main effect with different superscripts are significantly different (P<0.05).
CH: chitosan, NCH: Nano-chitosan, SJQ: selected Japanese quail, JQ: Japanese quail, LDL: low density lipoprotein (LDL-cholesterol), HDL: high density lipoprotein (HDL-cholesterol), A/G ratio: albumin/globulin ratio

catalase activity.

Regarding the intestinal microflora population, the quail's line had no significant effect on intestinal microflora of quails. On the other hand, the supplementation affected positively intestinal microflora. The supplementation of NCH at levels of 30 and 50 mg were the highest (P<0.05) in *Lactobacillus* bacteria count, while, the control group was the lowest (P<0.05) one. Conversely, the addition of CH and NCH significantly decreased the counts of *E.coli* and *Salmonella* compared to the control group. Concerning the interaction effect, It is noteworthy that the groups of SJQ and JQ fed the control diet had the lowest (P<0.05) count of *Lactobacillus*. Meanwhile, SJQ and JQ fed diets supplemented with 30 or 50 mg NCH recorded the highest (P<0.05) *Lactobacillus* count. However, the groups of SJQ and JQ fed diets supplemented with CH and NCH tended to decrease (P<0.05) the counts of *E.coli* and *Salmonella* compared to the control groups.

DISCUSSION

In the current study, SJQ group had an improving effect (P<0.05) on BW and BWG. This improvement may be due to the strain characteristics such as the profile of gen

Table 6: Effect of dietary chitosan and nano-chitosan supplementations on antioxidant status and intestinal microflora population of two lines of Japanese quail.

		Antioxidative status		Intestinal microflora		
Items		TAOC (mM / L)	Catalase (U/ml)	*Lactobacillus*	E.coli	*Salmonella*
Quail's line effect						
SJQ		4.65^{b}	0.72^{b}	6.52	8.72	8.33
JQ		4.68^{a}	0.74^{a}	6.50	8.71	9.12
SEM		0.003	0.002	0.05	0.045	0.014
P-value		0.0001	0.0001	0.7825	0.9258	0.9745
Supplementation level effect						
Control (zero level)		4.58^{d}	0.46^{e}	5.18^{d}	9.14^{a}	9.12^{a}
50 mg CH		4.64^{c}	0.71^{d}	6.22^{c}	8.82^{b}	8.47^{b}
70 mg CH		4.72^{a}	0.88^{a}	6.65^{b}	8.72^{b}	8.22^{c}
30 mg NCH		4.67^{b}	0.81^{b}	7.27^{a}	8.30^{c}	7.98^{d}
50 mg NCH		4.71^{a}	0.79^{c}	7.23^{a}	8.62^{b}	7.87^{e}
SEM		0.004	0.003	0.08	0.07	0.02
P-value		0.0001	0.0001	0.0001	0.0001	0.0001
Quail's line X Supplementation						
SJQ	Control	4.51^{f}	0.48^{g}	5.20^{f}	9.15^{a}	9.10^{a}
	50 mg CH	4.60^{e}	0.54^{f}	6.30^{de}	8.92^{ab}	8.43^{b}
	70 mg CH	4.73^{a}	0.89^{a}	6.80^{bc}	8.62^{bdc}	8.24^{c}
	30 mg NCH	4.67^{cd}	0.87^{c}	7.13^{ab}	8.40^{de}	8.02^{d}
	50 mg NCH	4.73^{a}	0.83^{d}	7.17^{a}	8.52^{cd}	7.86^{e}
JQ	Control	4.66^{cd}	0.45^{h}	5.17^{f}	9.13^{a}	9.13^{a}
	50 mg CH	4.68^{bc}	0.88^{ab}	6.13^{e}	8.71^{bcd}	8.50^{b}
	70 mg CH	4.70^{b}	0.87^{c}	6.50^{cd}	8.81^{bc}	8.20^{c}
	30 mg NCH	4.66^{cd}	0.75^{e}	7.40^{a}	8.20^{e}	7.94^{de}
	50 mg NCH	4.70^{b}	0.76^{e}	7.30^{a}	8.72^{bcd}	7.87^{e}
SEM		0.006	0.005	0.11	0.10	0.03
P-value		0.0001	0.0001	0.0001	0.0001	0.0001

[a,b,c] Means within the same column for each main effect with different superscripts are significantly different (P<0.05).
CH: chitosan, NCH: Nano-chitosan, SJQ: selected Japanese quail, JQ: Japanese quail, TAOC: total antioxidant capacity

otype of SJQ because the selection for growth was very effective on increasing overall body weight at a specific age (Mahmoud et al., 2019). The dietary supplementation of CH and NCH with SJQ increased (P<0.05) BWG compared to JQ. The results agreed herein with Suk (2004) who suggested that genetic interaction factors with supplementation of chitosan may affect growth performance and feed conversion ratio of broiler chickens. As well as, Osho and Adeola (2019) stated that the basal diet supplemented with 1.0 g chitosan/kg of feed for broiler chickens improved body weight gain. Besides, Nuengjamnong and Angkanaporn, (2018) showed that the addition of 2 g chitosan/kg diet tended to enhance FCR of broiler diet. Furthermore, Shi et al. (2005) demonstrated that broiler chickens fed diet supplemented with either 0.5 or 1.0 g chitosan /kg diet tended to have better growth and FCR. Conversely, Razdan and Pettersson (1994) observed that feeding broiler chickens diets containing chitosan at an inclusion level of 30 g/kg lowered body weights and daily feed intake which resulted in poorer feed conversion compared with the basal diet.

Growth performance enhancement in quail fed diets supplemented with either CH or NCH may be attributed to an important role for CH in controlling intestinal microflora including improving digestion and absorption of protein (Shi et al., 2005). Moreover, Osho and Adeola (2020) suggested that dietary supplementation of chitosan could

be used to enhance the negative impact of stress on broiler chickens' intestinal health. In addition, chitosan could be used as an efficient alternative to antibiotics in broiler diets with an improvement of gut function and microbial populations (Nuengjamnong and Angkanaporn, 2018). Chitosan has been a new growth promoter for farm animals (Zaki et al., 2015), it can stimulate the secretion of digestive enzymes from stomach, pancreas, and intestinal walls (Hou and Gao, 2001). Ravi et al. (2018) suggested that using dietary chitosan could improve the overall growth of animals, enhance nutrients absorption and feed efficiency, possess immune response, antioxidant effect, antimicrobial activity and improves gut microflora. The positive effects of chitosan on the gut health may be related to its antimicrobial activity against pathogenic microorganisms particularly the gram-negative bacteria like *E. coli* because chitosan interacts electrostatically with wall and membrane of bacterial cell (Kong et al., 2010). In addition, Darwesh et al. (2018) concluded that shrimp and Rhizopus stolonife chitosan and nano-chitosan had strongly antimicrobial activity and were not harmful in the same time, and they could be used as feed ingredients. It has been shown that applications of nanotechnologies and incorporation of nano-particles into poultry feeds are growing because they improved feed quality and availability of nutrients and elimination of pathogens (Amenta et al., 2015). Furthermore, nanotechnology had an ability to minimize microbial populations without creating drug residues in products of poultry, thereby enhancing the growth performance and immune response of poultry (Anwar et al., 2019).

The effect of dietary supplementations of CH at levels of 50 or 70 mg and NCH at levels of 30 or 50 mg did not have any impact on BW, BWG, FI and FCR**.** This result is consistent with finding of Keser et al. (2011) who found that chitosan supplementation to broiler chickens diets did not affect growth performance. Similarly, Huang et al. (2005) reported that 50 or 150 mg/kg chitosan supplementation to broiler diet did not show a significant effect on body weight, weight gain and feed intake at the overall period of the experiment. In another side of view, there are reports that CH supplementation to broilers diet improved growth performance (Li et al., 2007; Zhou et al., 2009; Pramujo et al., 2019). The contemporary findings related to mortality % are reliable with the observations of Razdan and Pettersson (1996) who suggested that mortality was not varied by dietary CH supplementation. Conversely, Pramujo et al. (2019) reported that broiler fed diet supplemented with 100 mg CH/ kg diet reduced mortality%.

Concerning carcass characteristics, the group of SJQ seemed to increase (P<0.05) the carcass weight compared to the group of JQ. This increase may be attributed to the genotype of SJQ which is selected for the high live body weight (Mahmoud et al., 2019). The effect of quail's line and dietary supplementation of CH and NCH had no significant effect on dressing %, liver %, Heart % and total edible parts%. The findings of carcass characteristics are in line with other results revealed that no significant effects of chitosan supplementation were detected on slaughter body weight, carcass weight, carcass ratio, heart, spleen and gizzard ratio to carcass weight of broiler chickens (Arslan and Tufan, 2018). Additionally, Miao et al. (2020) reported that dietary chitosan did not have significant influence on the dressing percentage, eviscerated carcass percentage and halt-eviscerated carcass percentage in growing Huoyan geese. Besides, Lokman et al. (2019) noted that the dietary addition of chitosan had no impact on the carcass and organ characteristics of broiler chickens. However, the percentage dressed weight was lowest at 0.5 g/kg each of shrimp chitosan. Moreover, Tufan et al. (2015) evaluated the effect of chitosan addition at levels of either 75 or 150 mg/kg to the Japanese quail diets on carcass traits and found that carcass weight, carcass, heart, liver and gizzard percentages did not differ among the tested groups. However, the present findings are disagreed with those reported by Tufan and Arslan (2012) who observed that 50 or 100 mg/kg of chitosan added to the broiler chickens diet increased the carcass ratio, leg and wing ratio and decreased the liver ratio. In this direction, Zhou et al. (2009) found that the addition of 14 or 28 g/kg of chitosan to broiler chicken diets improved the weight of liver but did not affect the breast meat ratio. Furthermore, Pramujo et al. (2019) demonstrated that broiler fed diet contained 100 mg chitosan/kg diet had increased (P<0.05) carcass weight (g).

Dietary supplementation of 70 mg CH increased (P<0.05) plasma total protein and albumin levels which may be related to an improvement of body protein anabolism in quail. The plasma total protein concentration usually reflects the protein metabolism and immunity function situation at *in vivo* condition (Li et al., 2007). In this direction, Arslan and Tufan (2018) reported that broiler chickens fed diet supplemented with 100 mg chitosan did not change serum total protein and albumin concentrations. Also, Zhou et al. (2009) found that dietary supplementation of chitosan at level of 14 and 28 g in broiler chickens diets had no effect on the serum total protein, albumin. In the present study, the dietary supplementation of CH and NCH significantly lowered the plasma total cholesterol and LDL concentrations owing to the effect of CH supplementation. Besides, chitosan appears to lower cholesterol through reduced cholesterol absorption (Gallaher et al., 2000). Also chitosan has been shown to reduce digestibility of ileal fat in broiler chickens (Razdan and Pettersson, 1996). In addition, it is possible that the cholesterol-lowering effect of chitosan can be caused by a rise in the intestinal viscosity

of its contents which is strongly associated with decreased plasma and liver cholesterol (Gallaher et al., 1993). Han et al. (1999) stated that chitosan inhibits pancreatic lipase activity that may reduce the plasma cholesterol. Likewise, Zong et al. (2012) reported that chitosan stimulated the hepatic LDL-R expression in mice and led to reduce LDL cholesterol. On the contrary, Zhou et al. (2009) and Nuengjamnong and Angkanaporn (2018) found that dietary supplementation with chito-oligosaccharide or chitosan in broilers did not influence plasma cholesterol and LDL concentrations.

In the current study, the dietary supplementation of CH and NCH had no impact on triglycerides level. In agreement with other results reported by Yao and Chian (2002) who demonstrated that no significant difference in plasma triglycerides level was observed when rats fed diet contained 7% chitosan and suggested that chitosan may play an important role in the regulation of lipoprotein metabolism in rats. Also, Nuengjamnong and Angkanaporn (2018)reported that there was no influence on serum triglycerides level with chitosan supplementation at level of 1 and 2 g/kg diet in broilers. Conversely, Zhou et al. (2009) found that triglycerides level of birds fed diet contained 0.2% chitosan was lowered by 10.7% than that of birds fed the basal diet. From the interaction point of view, the concentration of plasma triglycerides decreased in response to treatment with CH an NCH for JQ group because triglycerides are secreted by triglyceride-rich lipoproteins from the liver into the blood therefore, impaired hepatic lipogenesis which resulting in decreased triglycerides concentrations in plasma (Zhou et al., 2009).

Regarding HDL level, the supplementation of 70 mg CH, 30 mg NCH and 50 mg NCH caused significant increase ($P<0.05$) in HDL concentration. These results were consistent with Chiu et al. (2020) who postulated that rats fed high-fat diet contained 5% of high, low molecular weight chitosan and oligosaccharide for 8 weeks elevated the ratio of TC/HDL-C plasma and confirmed that 5% chitosan and its derivatives effectively exert the mitigation of the imbalance of circulated cholesterol and lipoprotein levels in HF diet-fed rats. Further, Zhou et al. (2009) observed that 0.4% chitosan addition in broiler diet resulted in greater ($P<0.05$) HDL level by 27.5%. Therefore, these results are consistent with our present results which suggested that CH and NCH have significant potential for cholesterol lowering properties. The present findings were confirmed by those of Xia et al. (2011) who stated that chitosan had hypocholesterolemic properties. As well as chitosan as nano-particles can easily transported via the gastrointestinal tract into the blood stream with increased bioavailability (Gopi et al., 2017). Thus, our results suggested that both CH and NCH are more effective in decreasing cholesterol absorption, interfering with bile acid absorption and they should be explored further as a means to reduce intestinal fat absorption. On the other hand, the degree of deacetylation and the amount of dietary chitosan ingested are the two main factors that may affect the plasma lipids and protein profile (Yao and Chian, 2002).

As observed in the present study, CH and NCH supplementation enhanced the antioxidative status of quail and increased the activity of catalase as an antioxidant enzyme which is representative enzymatic antioxidants in poultry. This might be due to the reaction of chitosan or nano-chtiosan with free radicals due to the active hydroxyl and amino groups present on their chains. The hydroxyl and amino group in chitosan can be used as hydrogen donors to the proxy radicals and react with unstable free radicals, hence protecting cells from damage (Fenga et al., 2008). The present results are in harmony with those reported by Osho and Adeola (2020) who suggested that chitosan might contribute to the improvement of antioxidative functions when broiler fed diet supplemented with 2 g chitosan/ kg diet. Furthermore, it has been documented that dietary chitosan improved the anti-oxidative status and mitigate stress (Fenga et al., 2008; Swiatkiewicz et al., 2015). A well-known function of the antioxidative systems is its capacity of inhibiting the reactive oxygen species, which consists of an enzymatic system and non-enzymatic antioxidants. The enzyme defense system consists of SOD, CAT, and GPx. Hydrogen peroxide is reduced to oxygen and water by catalase enzyme (Ahmad et al., 2012). In contrast to the present study, Darwesh et al. (2018) speculated that adding either chitosan or nano-chitosan in rats diets at 100 and 200 mg kg^{-1} body weight showed no significant effects in antioxidant enzymes such as CAT, TAC. As observed in the current study, it is worthy to note that NCH at level of 70 mg recorded higher ($P<0.05$) TAOC followed by NCH 50 mg and the control group was the lowest ($P<0.05$) one. This effect may be linked to that the addition of nanoparticles to the feed that enables better absorption of nutrients because the nano scale particles offer a very larger surface (Gangadoo et al., 2016). As well as, nanoparticles have the ability to transport various components under environmental conditions (Gopi et al., 2017). Finally, these results clearly showed that CH and NCH improved the antioxidative status expressed by TAOC and the activity of catalase enzyme.

Regarding the microflora, the dietary CH and NCH supplementation reduced the pathogenic bacteria such as *E. coli*, *Salmonella* sp. and promoted the count of *Lactobacillus* as beneficial bacteria. It is well known that *Lactobacillus* index is an indicator of healthy gut. Such results agreed with the findings obtained by Nuengjamnong and Angkanaporn (2018) who mentioned that broilers fed diet included

chitosan at level of 1 and 2 g/kg diet improved the gut microflora such as *Bacillus* sp. numbers, while, the population of *E.coli* reduced (P<0.001), so, this may enhance the gut function. Also, Tufan et al. (2015) reported that the concentration of *E. coli* was significantly lower when quail fed diet contained 150mg chitosan oligosaccharides /kg diet compared to the quail fed the basal diet. Additionally, Simunek et al. (2006) reported the dietary addition of 75 or 150 mg/kg of chitosan decreased the intestinal pathogen microorganism populations (*E. coli*). Likewise, Li et al. (2007) found that the addition of 100 mg chitosan to broiler chicken diets reduced the cecal *E. coli* count. Several studies showed that dietary chitosan supplementation increased *Lactobacillus* population and inhibited the counts of *E.coli* in the guts of poultry (Spring et al., 2000; Xu et al., 2013; Tufan et al., 2015). On the contrary, Tufan et al., (2015) demonstrated that chitosan oligosaccharides addition to quail diets at levels of 75 or 150 mg/kg diet resulted in decreasing *Lactobacillus* spp. compared to the control group. The chitosan is considered to be a bacteriocidal that can kill the live bacteria or bacteriostatic that means it may prohibit the growth of bacteria but does not kill the bacteria (Goy et al., 2009). In this respect, one of the mechanisms for the chitosan as antimicrobial potential is the binding of chitosan with microbial DNA, which activate the inhibition of the mRNA and protein synthesis through the penetration of chitosan into the microorganism's nuclei (Sebti et al., 2005). The present findings are confirmed by those of Shaltout et al. (2019) who stated that NCH exhibited higher antimicrobial activity than CH.

CONCLUSION

It can be concluded that the dietary supplementations of chitosan and nano-chitosan supplementations in selected lines of Japanese quail diets are helpful in improving antioxidative status, lowering the plasma cholesterol, decreased gut population of pathogenic microorganisms such as *E.coli* and *Salmonella* and increased *Lactobacillus* bacteria count. Furthermore, chitosan and nano-chitosan supplementation's interaction with genotype can also positively affect growth, carcass characteristics and plasma constituents of quail.

CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

AUTHORS CONTRIBUTION

All authors contributed equally according to their tasks and approved the final manuscript.

REFERENCES

- Abdeltwab WM, Abdelaliem YF, Metry WA, Eldeghedy M (2019). Antimicrobial effect of Chitosan and Nano-Chitosan against some Pathogens and Spoilage Microorganisms. J. Adv. Laborat. Res. Biol. 10 (1):8-15.
- Aebi H (1984). Catalase in vitro. Methods Enzymol. 105:121–126.
- Ahmad H, Tian J, Wang J, Khan MA, Wang Y, Zhang L, Wang T (2012). Effects of dietary sodium selenite and selenium yeast on antioxidant enzyme activities and oxidative stability of chicken breast meat. J. Agric. Food Chem. 60:7111–7120.
- Alishahi A (2014). Antibacterial effect of chitosan nanoparticle loaded with nisin for the prolonged effect. J. Food Safety. 34**:** 111–118.
- Amenta V, Aschberger K, Arena M, Bouwmeester H, Moniz F B, Brandhoff P, Gottardo S, Marvin H J P, Mech A, Pesudo L Q P, Rauscher H, Schoonjans R, Vettori MV, Weigel S, Peters R J (2015). Regulatory aspects of nanotechnology in the agri/feed/food sector in EU and non-EU countries. Regulat. Toxicol. Pharmacol. 73: 463-476.
- Anraku M, Gebicki JM, Iohara D, Tomida H, Uekama K, Maruyama T, Hirayama F. Anraku M, Otagiri M (2018). Antioxidant activities of chitosans and its derivatives in in vitro and in vivo studies. Carbohydrate Polymers. 199:141-149.
- Anwar MI, Awais MM, Akhtar M, Navid MT, Muhammad F (2019). Nutritional and immunological effects of nanoparticles in commercial poultry birds. World's Poult. Sci. J. 261-272. https://doi.org/10.1017/S0043933919000199
- Arslan C, Tufan T (2018). Effects of chitosan oligosaccharides and L-carnitine individually or concurrent supplementation for diets on growth performance, carcass traits and serum composition of broiler chickens. Revue Méd. Vét.169, 4-6: 130-137.
- Chiu CY, Yen TE, Liu SH, Chiang MC (2020). Comparative effects and mechanisms of chitosan and its derivatives on hypercholesterolemia in high-fat diet-fed rats. Int. J. Mol. Sci., 21: 92-103. https://doi.org/10.3390/ijms21010092
- Darwesh OM, Sultanb YY, Seif MM, Marrez DA (2018). Bio-evaluation of crustacean and fungal nano-chitosan for applying as food ingredient. Toxicol. Rep. 5: 348–356. https://doi.org/10.1016/j.toxrep.2018.03.002
- Doumas BT, Watson WA, Biggs HG (1971). Albumin standards and the measurement of serum albumin with bromcresol green. Clin. Chem. Acta. 31:87–96.
- Duncan DB (1955). Multiple range and multiple F-tests. Biometrics. 11: 1-42.
- Fenga T, Du Y, Li J, Hu Y, Kennedy JF (2008). Enhancement of antioxidant activity by irradiation. Carbohydr. Polym. 73:126–132.
- Gallaher CM, Munion J, Hesslink Jr R, Wise J, Gallaher DD (2000). Cholesterol reduction by glucomannan and chitosan is mediated by changes in cholesterol absorption and bile acid and fat excretion in rats. J. Nutr. 130: 2753–2759.
- Gangadoo S, Stanley D, Hughes RJ, Moore RJ, Chapman J (2016). Nanoparticles in feed: Progress and prospects in poultry research. Trends Food Sci. Technol. 58: 115-126.
- Gopi M, Pearlin B, Kumar RD, Shanmathy M, Prabakar G (2017). Review Article, Role of nanoparticles in animal and poultry nutrition: Modes of action and applications in formulating feed additives and food processing. Int. J. Pharmacol. 13 (7): 724-731. https://doi.org/10.3923/

ijp.2017.724.731
- Goy RC, de Britto D, Assis OBG (2009). A review of the antimicrobial activity of chitosan. Polimeros. 19:241–247.
- Han LK, Kimura Y, Okuda H (1999). Reduction in fat storage during chitin-chitosan treatment in mice fed a high-fat diet. Int. J. Obes. Relat. Metab. Disord. 23: 174-179.
- Hartemink R, Rombouts FM (1999). Comparison of media for the detection of bifiobacteria, lactobacilli and total anaerobes from faecal samples. J. Microbiol. Meth. 36: 181-192.
- Hernawan E, Adriani L, Mushawwir A, Cahyani C, Darmawan (2017). Effect of dietary supplementation of chitosan on blood biochemical profile of laying hens. Pakistan J. Nutrit. 16(9):696-699.
- Hou QL, Gao QS (2001). Chitosan and medicine. Shanghai Science Technology Press, Shangai, China.
- Huang RL, Yin YL, Wu GY, Zhang YG, Li TJ, Li LL, Li MX, Tang ZR, Zhang J, Wang B, He JH, Nie XZ (2005). Effect of dietary oligochitosan supplementation on ileal digestibility of nutrients and performance in broilers. Poult. Sci. 84:1383–1388.
- Ing LY, Zin NM, Sarwar A, Katas H (2012). Antifungal activity of chitosan nanoparticles and Correlation with their Physical Properties. Int. J. Biomat. https://doi.org/10.1155/2012/632698.
- Keser O, Bilal T, Kutay HC, Abas I, Eseceli, H (2011). Effects of chitosan oligosaccharide and/or beta-glucan supplementation to diets contained organic zinc on performance and some blood indices in broilers. Pakistan Vet. J. 32: 15-19.
- Khan I, Saeed K, Khan I (2019). Review: Nanoparticles: Properties, applications and toxicities Arabian J. Chem. 12: 908–931. http://creativecommons.org/licenses/by-nc-nd/4.0/.
- Kong M, Chen XG, Xing K, Park HJ (2010). Antimicrobial properties of chitosan and mode of action: a state of the art review. Int. J. Food Microbiol. 144:51e63.
- Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V (2001). Method for the measurement of antioxidant activity in human fluids. J. Clin. Pathol. 54:356–361.
- Li XJ, Piao XS, Kim SW, Liu P, Wang L, Shen YB, Jung SC, Lee HS (2007). Effects of chito-oligosaccharide supplementation on performance, nutrient digestibility, and serum composition in broiler chickens. Poult. Sci., 86: 1107-1114. https://doi.org/10.1093/ps/86.6.1107
- Lokman H, Ibitoye EB, Hezmee MNM, Goh YM, Zuki ABZ, Jimoh AA (2019). Effects of chitin and chitosan from cricket and shrimp on growth and carcass performance of broiler chickens. Trop. Anim. Health Prod. 51:2219–2225. https://doi.org/10.1007/s11250-019-01936-9
- Lopez-Virella MF, Stone P, Ellis S, Colwell JA (1977). Cholesterol determination in high-density lipoproteins separated by three different methods. Clin. Chem. 23:882–884.
- Mahmoud BYF, Semida DAM, El-Full EA, Emam AM (2019). Effect of selection for fast growth rate on Japanese quail laying performance and fitness traits. Egypt. Poult. Sci. 40(I): 15-27.
- Miao Z, Guo L, Liu Y, Zhao W, Zhang J (2020). Effects of dietary supplementation of chitosan on carcass composition and meat quality in growing Huoyan geese. Poult. Sci. 99:3079–3085. https://doi.org/10.1016/j.psj.2020.03.025
- NRC (1994). Nutrient Requirements of Poultry: Ninth Revised Edition. National Academy Press, Washington, D.C., USA.
- Nuengjamnong C, Angkanaporn K (2018). Efficacy of dietary chitosan on growth performance, haematological parameters and gut function in broilers. Italian J. Anim. Sci. 17(2): 428–435. https://doi.org/10.1080/1828051X.2017.1373609
- Osho SO, Adeola O (2019). Impact of dietary chitosan oligosaccharide and its effects on coccidia challenge in broiler chickens. Brit. Poult. Sci. 60,(6): 766-776.
- Osho SO, Adeola O (2020). Chitosan oligosaccharide supplementation alleviates stress stimulated by in-feed dexamethasone in broiler chickens. Poult. Sci. 99:2061–2067. https://doi.org/10.1016/j.psj.2019.11.047
- Orsonneau JL, Douet P, Massoubre C, Lustenberger P, Bernard S (1989). An improved pyrogallol red-molybdate method for determining total urinary protein. Clin. Chem. 35:2233–2236.
- Pramujo M, Mutia R, Wijayanti I (2019). Effect of chitosan oligosaccharide (COS) and l-arginine supplementation on broiler performance. Earth Environ. Sci. 251: 012060 https://doi.org/10.1088/1755-1315/251/1/012060
- Ravi A, Reddy RKP, Chakravarthi KM (2018). Chitosan as an alternative to antibiotic feed additive in poultry and pig production. 18th Indian veterinary congress, xxv Annual conference (23-24 february, 2018). 92-101p.
- Razdan A, Pettersson D (1994). Effect of chitin and chitosan on nutrient digestibility and plasma lipid concentrations in broiler chickens. Brit. J. Nutrit. 72: 277-288.
- Razdan A, Pettersson D (1996). Hypolipidaemic, gastrointestinal and related responses of broiler chickens to chitosans of different viscosity. Brit. J. Nutrit. 76: 387-397.
- Sebti I, Martial-Gros A, Carnet-Pantiez A, Grelier S, Coma V (2005). Chitosan polymer as bioactive coating and film agaist Aspergillus niger contamination. J. Food Sci. 70 (2): 100-104.
- Shaltout FA, El-Diasty EM, Hassan AMA (2019). Effect of nano-chitosan and onion extract as coating materials on the quality properties of chicken fillet meat during refrigeration. Global Vet. 21(6):368-372. https://doi.org/10.5829/idosi.gv.2019.368.372.
- Shi BL, Li DF, Piao X S, Yan SM (2005). Effects of chitosan on growth performance and energy and protein utilisation in broiler chickens. Br. Poult. Sci. 46(4):516-9. https://doi.org/10.1080/00071660500190785
- Simunek J, Tishchenko G, Hodrová B, Bartonová H (2006). Effect of chitosan on the growth of human colonic bacteria. Folia Microbiol. (Praha). 51: 306-308.
- Spring P, Wenk C, Dawson KA, Newman KE (2000). The effects of dietary mannanoligosaccharides on cecal parameters and the concentration of enteric bacteria in the ceca of *Salmonella* challenged broiler chicks. Poult. Sci. 79: 205-211, 2000. https://doi.org/10.1093/ps/79.2.205
- Suk YO (2004). Interaction of breed-by-chitosan supplementation on growth and feed efficiency at different ages in broiler chickens. Asian-Aust. J. Anim. Sci. 17(12): 1705-1711.
- Swiatkiewicz S, Swiatkiewicz M, Arczewska-Wlosek V, Jozefiak D (2015). Chitosan and its oligosaccharide derivatives (chito-oligosaccharides) as feed supplements in poultry and swine nutrition. J. Anim. Physiol. Anim. Nutrit. 99: 1–12. https://doi.org/10.1111/jpn.12222
- Tang ZX, Qian JQ, Shi LE (2007). Preparation of chitosan nanoparticles as carrier for immobilized enzyme. Appl. Biochem. Biotechnol. 136: 77-96.
- Tufan T, Arslan C (2012). Broyler rasyonlarına kitosan

oligosakkarit ilavesinin besi performansı, karkas özellikleri, besin madde sindirilebilirlikleri, serum lipidleri ve göğüs eti yağ asidi profiline etkileri. Doktora Tezi, Kafkas Üniv. Sağlık Bil. Enst.

- Tufan T, Arslan C, Sari M, Önk K, Deprem T, Çelik E (2015). Effects of chitosan oligosaccharides addition to Japanese Quail's diets on growth, carcass traits, liver and intestinal histology, and intestinal microflora. Kafkas Univ. Vet. Fak. Derg. 21 (5): 665-671. https://doi.org/10.9775/kvfd.2015.13010
- Vimal S, Abdul Majeed, S, Taju GK, Nambi SN, Sundar RN, Madan N, Faroo MA, Rajkumar T, Gopinath D, Hameed AS (2013). Chitosan tripolyphosphate (CS/TPP) nanoparticles: Preparation, characterization and application for gene deliver in shrimp. Acta Trop. 128:486-493.
- Wahlefeld AW (1974). Triglycerides determination after enzymatic hydrolysis. In: Bergmeyer HU, editor. Methods of enzymatic analysis. New York (NY): Academic Press; p. 1831–1835.
- Xia W, Liu P, Zhang J, Chen J (2011). Biological activities of chitosan and chitooligosaccharides. Food Hydrocoll. 25: 170–179.
- XLSTAT (2014). Data analysis and statistics with Microsoft Excel, Paris, France, 2014.
- Xu Y, Shi B, Yan S, Li T, Guo Y, Li J (2013). Effects of chitosan on body weight gain, growth hormone and intestinal morphology in weaned pigs. Asian Australas. J. Anim. Sci. 26 (10): 1484-1489. http://dx.doi.org/10.5713/ajas.2013.13085
- Yao HT, Chiang MT (2002). Plasma lipoprotein cholesterol in rats fed a diet enriched in chitosan and cholesterol. J Nutr. Sci. Vitaminol. 48, 379-383.
- Zaki MA, Salem M El-S, Gaber MM, Nour AM (2015). Effect of chitosan supplemented diet on survival, growth, feed utilization, body composition and histology of sea bass (Dicentrarchuslabrax). World J. Eng. Technol. 3: 38– 47.
- Zhou TX, Chen YJ, Yoo JS, Huang Y, Lee JH, Jang HD, Shin SO, Kim HJ, Cho JH Kim IH (2009). Effects of chitooligosaccharide supplementation on performance, blood characteristics, relative organ weight, and meat quality in broiler chickens. Poult. Sci. 88, 593-600. https://doi.org/10.3382/ps.2008-00285.
- Zong C, Yu Y, Song G, Luo T, Li L, Wang X, Qin S (2012). Chitosan oligosaccharides promote reverse cholesterol transport and expression of scavenger receptor BI and CYP7A1 in mice. Exper. Biol. Med. 237: 194–200. https://doi.org/10.1258/ebm.2011.011275.