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Application of Chitosan and Omega-3 Supplementation on Blood Constituents, Immunity, and Antioxidant Enzymes in Broiler Chicks

Doaa I.M. Abdul-Rahman[1], Mostafa F.M. Hassan[2], Waleed F. Khalil[2], Eman A. Ahmed[2], Fatma M. A. Youssef[3*]

[1]Animal Health Research Institute, ARC, 41511, Ismailia, Egypt.

[2]Pharmacology unit, Faculty of Veterinary Medicine, Suez Canal University, Egypt.

[3]Animal Health Research Institute, ARC, 41511, Ismailia, Egypt.

***Correspondence**
Corresponding author: Fatma M.A. Youssef
E-mail address: fatmayousseff@yahoo.com

Abstract

This experiment investigated the impact of chitosan (CHI) and omega-3 as a delivery system for omega-3 on blood constituents, immunity, and antioxidant enzymes, in broiler chicks. Eighty (one-day-old) broiler chicks were assigned to four groups, each with 20 chicks (two replicates of 10 chicks each). The first group was a control group that did not receive any supplementation, while the other groups received different treatments, such as CHI or omega-3, either alone or in combination (over a period of 42 days). The results showed that all the supplemented groups had improved immune systems, as evidenced by higher white blood cell counts, lymphocyte percentages, and lower heterophil, and lymphocyte ratios. Additionally, the supplemented groups had higher levels of serum protein fractions, immunoglobulins, and antibody titer against ND and AI viruses. Furthermore, the study revealed that the supplemented groups had an increase in the bursa of Fabricius index, spleen index, and thymus index at both 28 and 42 days. The SOD and GSH serum levels also increased significantly in the supplemented groups. In conclusion, using CHI and omega-3 as a delivery system in broiler chicks had beneficial effects on blood constituents, immunity, and antioxidant enzymes, in broiler chicks.

KEYWORDS
Chitosan (CHI), Omega-3, Delivery system, Immunoglobulins, SOD and GSH.

INTRODUCTION

Nowadays, the application of immunomodulators is a common practice, and one of the most important branches is immunostimulants to correct the defect of the immune system, resist and prevent disease, and reinforce immunity (Rakhshan *et al*., 2010).

Chitosan (CHI) can be classified as a prebiotic since it is a biocompatible polymer of glucosamine that is derived from chitin (Yadav *et al*., 2019; Santos *et al*., 2020; Pellis *et al*., 2022). Chitin is widely distributed in nature, being found in the exoskeletons of diverse organisms, such as crustaceans, mollusks, insects, and arthropods. The process of extracting chitin typically involves several steps, including deproteinization, demineralization, and discoloration (Friedman and Juneja, 2010; Keser *et al*., 2012), all of which aim to eliminate impurities and obtain a high-quality polymer (Yadav *et al*., 2019; Santos *et al*., 2020; Pellis *et al*., 2022). The subsequent deacetylation of chitin yields chitosan, which possesses several advantageous properties such as biocompatibility, biodegradability, and low toxicity. Chitosan can be extracted through both chemical and biological methods, with the latter relying on the use of microorganisms. Due to its unique properties, chitosan has found numerous applications in various industries, including food, cosmetic, textile, chemical, biomedical, and pharmaceutical. Its biocompatibility and biodegradability make it a promising candidate for the development of eco-friendly and sustainable products (Yadav *et al*., 2019; Santos *et al*., 2020; Pellis *et al*., 2022). CHI has immune response, anti-inflammatory, and antimicrobial activities, so it used as an immunomodulator for animals (Siwicki *et al*., 1994; Yoon *et al*., 2008; Dai *et al*., 2009; Kong *et al*., 2014). Liao *et al*. (2007) reported CHI contains amino and hydroxyl groups that give CHI many natural activities, as hemostatic effect (Pusateri *et al*., 2006). Furthermore, CHI can serve as a drug delivery system, offering potential benefits in anticancer and anti-tumor chemotherapy (Toshkova *et al*., 2010).

Omega-3 fatty acids in poultry diets have gained significant popularity due to their ability to positively impact production, health, and meat quality (Alagawany *et al*., 2019). They have antioxidant and anti-inflammatory properties that help maintain the immune system by enhancing antioxidant enzyme properties and inhibiting prostaglandin production (Cândido *et al*., 2018). Chitosan serves as a drug delivery system through the process of encapsulation. Its polycationic properties enable it to interact with negatively charged mucous membranes and remain in contact with the drug for an extended period, allowing for enhanced permeation. Furthermore, Chitosan acts as an encapsulating agent for omega-3, -6, and -9 molecules, thereby facilitating their cellular uptake (Vidyashri *et al*., 2020). This work investigated the effects of chitosan and omega-3 as immunostimulants and the potential role in dipping oxidative stress in broiler chickens.

MATERIALS AND METHODS

Natural additives

Chitosan

It was purchased from the Nano Streams Company located

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in 6th of October City, Egypt, with a recommended dose of 100 mg/kg b.wt (Huang *et al*., 2007).

Omega 3

It comprises two specific groups of fatty acids - DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid). The supplement, Maxepaforte was used in this study, itis produced by Seven Seas Company with a recommended dose of 0.2 mg/kg (Andrews, 2009).

Omega 3 carried on CHI

The delivery system of CHI performed by dissolving CHI in acetic acid at 10 mg/ml in 1% (w/v) concentration. The solution was stirred overnight at room temperature to achieve complete dispersion of CHI after that, it was filtrated through Miracloth to remove impurities. Then, the encapsulated by omega3 was ready by the addition of 1% (v/v) solution containing 0.5% omega3 and 0.5% Tween 80 into chitosan solution, these processes were done according to Stoica *et al*. (2013).

Experimental design

Eighty unsexed one day old Cobb broiler chicks were obtained from the AL-Ismailia-Miser for Poultry Company, Ismailia, Egypt. The experiment was conducted for 42 days. The chicks were assigned into four groups (each of 20 chicks); Group 1 (G1): Fed on the basal diet without any feed additives and kept as control. Group 2 (G2): Fed on the basal diet and supplemented with CHI at a dose of 100 mg/kg in water. Group 3 (G3): Fed on the basal diet, supplemented with omega 3 at a dose of 0.2 mg/kg that was added in water as emulsion with tween 80. Group 4 (G4): Fed on the basal diet, supplemented with Omega 3 that was carried on CHI and added in water as emulsion with tween 80. Every group was subdivided into two replicates (10 chicks in each). The chicks were starting supplementation from the 7th day till the 28th day of experiment. All groups were kept under the same management measures and vaccination program (Yeo and Kim, 1997).

The experimental design, procedures and bird management were approved by the Research Ethical Committee of the Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt (approval no. SCVM201916).

Blood sampling

On days 28 and 42 of the experiment, six birds from each group were selected for blood collection. Two blood samples were collected into two tubes: one containing anti-coagulant (heparin) and other without anti-coagulant.

Hematological studies

Heparinized blood samples were undergone the following hematological studies; Total and differential leukocytic count (Jain, 2000). The heterophils / lymphocytes ratio (Gross and Siegel, 1983).

Immunological studies

Serum protein electrophoresis:

It was performed based on the methods of Laemmli (1970).

Lymphoid organs index

on days 28 and 42 of an experiment, where six birds from each subgroup, slaughtered and lymphoid organs (thymus, spleen and bursa of Fabricius) were dissected out, weighted. The Lymphoid organ index was calculated according to the formula of Halouzka and Jurajda (1991), by dividing the organ weight on the live body weight multiplied by 1000.

Determination of serum concentration of immunoglobulin

Serum immunoglobulins (IgA, IgG and IgM) concentrations were determined by using ELISA kits according to Schuijffel *et al*. (2005).

Haemagglutination inhibition test

It was investigated by detecting serum antibody titers against Newcastle disease virus (NDV) and avian influenza virus (AIV) according to Alexander and Chettle (1977).

Antioxidant and oxidative stress parameters were measured by analyzing hepatic antioxidant enzyme activities (Zhang *et al*., 2016), MDA content, and the activities of GSH-Px and SOD were measured using specialized kits and a semi-automated spectrophotometer (Erba-Chem7, Germany).

Statistical analysis

It was carried out using the variance method (ANOVA), with a significance level of $P < 0.05$, employing the MiniTab16© (Mini Tab 17, 2010) software.

RESULTS

Haematological studies

Data in Table 1 depicted the outcomes of examining the impact of dietary supplements on the haematological indices of broiler chicks at 28 and 42 days of age. The results demonstrated a noteworthy escalation in the counts of white blood cells and lymphocytes in all supplemented groups, as opposed to the control group. Conversely, the counts of heterophils, monocytes, and eosinophils revealed no significant distinctions between the control and supplemented groups. Additionally, the H/L ratio showed a notable decline in the supplemented groups at both time points.

Serum protein fractionation

Findings in Table 2 showed that at 28 and 42 days, all supplemented groups revealed a significantly higher total protein level than the control group, with significant increases in albumin level. Total gamma-globulin significantly increased in all supplemented groups, with the highest level observed in the omega3 carried on CHI group. Total beta-globulin significantly increased only in the omega3 carried on CHI group, while total alpha-globulin was significantly higher. Additionally, all groups exhibited a significant reduction in the A/G ratio in all supplemented groups.

Immunological studies

Results in Table 3 revealed that at 28 days, all supplemented groups (G2, G3, and G4) showed a high level in bursa of Fabricius index compared to (G1). The spleen index also significantly

increased in all supplemented groups at 28 days, with CHI and omega3 carried on CHI groups (G2 and G4) showing the highest increase. The thymus gland index significantly increased in CHI, omega3, and omega3 carried on CHI groups at 28 days, with G2 and G4 exhibiting significantly higher increases than G3.

At 42 days, no differences were noticed in the bursa of Fabricius index between groups. However, G2 and G4 showed significantly more augmentation than G1 and G3 in spleen index. The thymus gland index significantly increased in CHI, omega3, and omega3 carried on CHI groups at 42 days, with G2 and G4 exhibiting significantly higher increases than G3.

Effects of tested supplements on the serum level of Immunoglobulin production at 28 and 42 days

Table 4 shows that all supplemented groups had significantly higher levels of IgA, IgG, and IgM than the control group at 28 and 42 days. G4 had the highest increase in IgA at 28 days, while G3 had the most significant increase at 42 days. G2 had the most significant increase in IgG at both 28 and 42 days, and G2 had the highest increase in IgM at 28 days, while G3 and G4 showed significant increases at 42 days.

The humoral immune response was evaluated by measuring

Table 1. Effect of tested supplements on the differential leukocytes count (DLC) of the broiler chicks at 28 and 42 days.

DLC	Days	G1	G2	G3	G4
WBCs (10^3/ul)	28ds	10.17±1.2^{c}	16.33±0.98^{b}	19±1.58^{a}	16.83±1.8^{b}
	42ds	13.17±1.28^{b}	19.33±1.2^{a}	19.17±1.58^{a}	18.5±1.37^{a}
Heterophils (%)	28ds	44.30±0.86^{a}	41.97±0.92^{a}	42.80±0.69^{a}	42.73±0.75^{a}
	42ds	43.40±1.58^{a}	41.20±2.9^{a}	41.00±2.25^{a}	41.63±3.2^{a}
Lymphocytes (%)	28ds	51.20±0.49^{b}	54.00±0.76^{a}	53.73±0.33^{a}	53.11±0.37^{a}
	42ds	51.27±0.35^{b}	53.26±0.85^{a}	53.16±0.58^{a}	53.17±0.73^{a}
Monocytes (%)	28ds	4.17±0.37^{a}	3.73±0.92^{a}	3.17±0.48^{a}	3.83±0.48^{a}
	42ds	5.00±0.53^{a}	5.17±0.41^{a}	5.50±0.38^{a}	4.87±0.18^{a}
Eosinophils (%)	28ds	0.33±0.21^{a}	0.30±0.21^{a}	0.30±0.22^{a}	0.33±0.21^{a}
	42ds	0.33±0.05^{a}	0.37±0.06^{a}	0.34±0.06^{a}	0.33±0.08^{a}
H/L ratio	28ds	0.87±0.02^{a}	0.78±0.04^{b}	0.80±0.02^{b}	0.80±0.01^{b}
	42ds	0.85±0.04^{a}	0.77±0.02^{b}	0.77±0.02^{b}	0.78±0.01^{b}

Values are expressed as means±standard error (SE); n=6. Means within the same row with different superscripts are significantly different (P<0.05). WBCs: White Blood Cells; H/L ratio: Heterophil/Lymphocyte ratio.

Table 2. Effect of tested supplements on the serum protein fractionation (g/dl) of broiler chicks at 28 and 42 days.

Protein fractions	Days	G1	G2	G3	G4
Total protein (g/dl)	28ds	3.45±0.2^{b}	4.21±0.21^{a}	4.22±0.20^{a}	4.27±0.27^{a}
	42ds	4.31±0.39^{c}	5.12±0.42^{a}	4.88±0.3^{b}	5.18±0.92^{a}
Globulin (g/dl)	28ds	1.83±0.09^{b}	2.49±0.20^{a}	2.39±0.23^{a}	2.56±0.11^{a}
	42ds	2.14±0.2^{c}	3.13±0.22^{a}	2.93±0.15^{b}	3.18±0.48^{a}
ϒ-globulin (g/dl)	28ds	0.69±0.04^{c}	0.85±0.11^{b}	0.83±0.10^{b}	0.93±0.07^{a}
	42ds	0.81±0.1^{c}	1.46±0.12^{b}	1.47±0.11^{b}	1.54±0.27^{a}
β-globulin(g/dl)	28ds	0.6±0.05^{b}	0.69±0.07^{a}	0.68±0.15^{a}	0.75±0.28^{a}
	42ds	0.81±0.07^{b}	0.98±0.09^{a}	0.83±0.05^{b}	1.00±0.19^{a}
α-globulin (g/dl)	28ds	0.54±0.08^{b}	0.95±0.09^{a}	0.88±0.09^{a}	0.88±0.06^{a}
	42ds	0.52±0.04^{b}	0.69±0.02^{a}	0.63±0.05^{a}	0.64±0.04^{a}
Albumin (g/dl)	28ds	1.62±0.10^{b}	1.72±0.19^{a}	1.79±0.14^{a}	1.71±0.24^{a}
	42ds	2.17±0.15^{a}	1.99±0.03^{b}	1.95±0.08^{b}	2.00±0.03^{b}
Albumin/Globulin ratio	28ds	0.90±0.13^{a}	0.69±0.07^{b}	0.71±0.17^{b}	0.67±0.04^{b}
	42ds	1.01±0.2^{a}	0.64±0.03^{b}	0.67±0.04^{b}	0.63±0.04^{b}

Values are expressed as means±standard error (SE); n=6. Means within the same row with different superscripts are significantly different (P<0.05).

Table 3. Effect of tested supplements on the mean values of lymphoid organ weight index at 28 and 42 days.

Lymphoid organs	Days	G1	G2	G3	G4
Bursa Fabricius index	28ds	0.67±0.04^{b}	1.18±0.19^{a}	0.98±0.09^{a}	1.15±0.12^{a}
	42ds	0.39±0.07^{a}	0.36±0.04^{a}	0.32±0.02^{b}	0.36±0.03^{a}
Spleen index	28ds	0.36±0.03^{c}	0.58±0.04^{a}	0.45±0.02^{b}	0.62±0.04^{a}
	42ds	0.89±0.06^{b}	1.53±0.07^{a}	1.09±0.09^{b}	1.28±0.05^{a}
Thymus index	28ds	2.80±.09c	4.53±0.21a	3.41±0.14b	4.55±0.30a
	42ds	3.78±0.12c	4.91±0.27a	4.38±0.23b	5.29±0.41a

Values are expressed as means±standard error (SE); n=6.
Means within the same row with different superscripts are significantly different (P<0.05).

antibody titers of ND and AI viruses using the hemagglutination inhibition test (HIT) at 28 and 42 days are presented in Table 5. The results showed that ND and AI antibody titers were increased (P<0.05) in all supplemented groups.

Antioxidants assay

Data in Table 6 revealed that MDA levels significantly decreased in all supplemented groups than the control group at both 28 and 42 days. Superoxide dismutase (SOD) and GSH levels were significantly increased in all supplemented groups at both 28 and 42 days.

DISCUSSION

Poultry industry is using immunomodulatory agents to improve the immune system and health of birds. These agents stimulate T-cell immunity, Natural Killer cells, interferon production, and cytokine production. This approach is becoming increasingly popular in the natural health industry (Gabius, 2003; Stanilove *et al*., 2005; Lam *et al*., 2010; Yeap *et al*., 2011).

The present study compared the effects of some supplements, like chitosan (as prebiotic), omega-3 and chitosan as delivery system combined with omega-3 on haematological blood parameters, serum biochemical constituents, humoral immune response of broiler chickens and antioxidant enzymes.

The tested supplements led to an increase in total leukocytic count (TLC) and lymphocyte percentage after 28 and 42 days. Heterophils percent, monocyte, and eosinophils did not show significant changes (P<0.05), but there was a significant reduction in heterophil: lymphocyte (H/L) index in all supplemented groups. Similar results were reported by Sadeghi *et al*. (2013) who found that prebiotic supplementation increased white blood cell count and decreased H/L index in chickens. However, Shahir *et al*. (2014) found no significant effect of prebiotics on heterophil, monocyte, and eosinophil levels. The increased WBC counts were also observed by Meng *et al*. (2010) in laying hens fed with chito-oligosaccharide (COS) at 0.4% level. Also, Nuengjamnong and Angkanaporn (2017) concluded that chickens in group fed on CHI basal diet at 2 g/kg had higher WBC count than those of groups, but it was not different from group fed CHI basal diet at 1g/kg, but there was no significant difference in H/L ratio of chickens among groups. Regarding to the omega3 supplemented group the obtained results showed significant increases in WBCs and lymphocytes, and significant reduction in H/L ratio while in heterophils, monocyte and eosinophils the results showed non-significant differences. The outcome gained from omega3 carried on CHI; there were significant increment in WBCs and lymphocyte other than H/L ratio reveal significant reduction.

Blood serum proteins are crucial for maintaining homeostasis in the body. They regulate colloid osmotic pressure, provide essential amino acids, facilitate glucose production, transport minerals and hormones, build enzymes, and support the immune system. Birds have a lower concentration of blood serum proteins than mammals, with approximately 40 g/L, compared to 50-70 g/L in mammals (Kaneko, 1997).

The level of serum protein is often indicative of the body's protein metabolism and immune function status through pro-

Table 4. Effects of tested supplements on the serum level of Immunoglobulin production at 28and 42 days.

Immunoglobulins	Days	G1	G2	G3	G4
IgA (ng/ml)	28ds	203.67±15.33d	304.00±10.00b	243.00±12.0c	374.67±17.33a
	42ds	249.67±31.33c	365.00±25.00a	319.33±22.67b	381.00±21.00a
IgG (ng/ml)	28ds	105.67±14.33d	549.00±13.00a	416.00±17.00c	481.00±20.00b
	42ds	208.33±20.00^{c}	881.33±30.67^{a}	752.33±38.67^{b}	788.33±34.67^{b}
IgM ng/ml)	28ds	89.00±9.00^{c}	424.00±28.00^{a}	402.00±9.0^{b}	414.67±20.33^{a}
	42ds	151.00±12.00^{c}	478.33±29.67^{b}	537.67±32.33^{a}	510.67±22.33^{a}

Values are expressed as means±standard error (SE); n=6.
Means within the same row with different superscripts are significantly different (P<0.05).

Table 5. Effects of tested supplements on humoral immune response estimated by detecting serum antibody titers against ND and AI viruses using a hemagglutination inhibition test at 28 and 42 days.

Viruses	Days	G1	G2	G3	G4
Newcastle disease virus	28ds	4.62±0.33^{c}	6.77±0.51^{a}	5.17±0.25^{b}	6.33±0.43^{a}
	42ds	2.17±0.12^{b}	5.83±0.44^{a}	5.50±0.32^{a}	6.0±0.47^{a}
avian influenza virus	28ds	3.17±0.23^{c}	6.23±0.35^{a}	4.83±0.28^{b}	5.30±0.33^{b}
	42ds	4.32±0.36^{c}	6.33±0.29^{a}	5.33±0.34^{b}	5.17±0.43^{b}

Values are expressed as means±standard error (SE); n=6.
Means within the same row with different superscripts are significantly different (P<0.05).

Table 6. Effects of tested supplements on the serum level of antioxidant enzymes at 28 days and 42 days.

	Days	G1	G5	G3	G4
MDA (nmol/ml)	28ds	3.19±0.21^{a}	1.51±0.12^{b}	1.12±0.1^{b}	1.35±0.13^{b}
	42ds	4.07±0.30^{a}	1.33±0.11^{b}	1.46±0.08^{b}	1.44±0.1^{b}
SOD (U/ml)	28ds	86±3.00^{c}	151.67±6.33^{b}	178±9.00^{a}	193.33±12.67^{a}
	42ds	60.33±6.67^{b}	177.67±10.33^{a}	186±11.0^{a}	190.67±5.33^{a}
GSH (ng/ml)	28ds	81.33±5.67^{d}	186.33±9.67^{c}	213±10.0^{b}	249.67±14.33^{a}
	42ds	95±3.00^{c}	224.67±6.33^{b}	240.77±17.23^{a}	257.67±10.33^{a}

Values are expressed as means±standard error (SE); n=6.
Means within the same row with different superscripts are significantly different (P<0.05).

tein anabolism and proteolysis. Serum total protein is composed of albumin and globulin, which can both be used to assess the liver's protein metabolic response to dietary changes (Stoll *et al*., 1998). Duncan and Prass (1986) stated that gamma globulin is synthesized by plasma cell or B lymphocytes in spleen, bone marrow and lymph nodes. When there is an increase in the level of alpha and gamma globulins, it is typically a sign of immune system activation (Butler, 1983).

In the study, we have examined the effect of the studied supplements on serum protein fractionation at 28 and 42 days. The obtained results in group supplemented with CHI demonstrated significant rise in total protein, total globulin and significant diminished in A/G ratio these findings could be attributed to the immunostimulant effect of this additives results concord with Vytautas *et al*. (2006) who observed that, feeding broiler chicken on a prebiotic supplemented diet increased serum total protein and globulin. And Abdel-Samee *et al*. (2013) who revealed that, the prebiotic inclusion in the quail's diet caused a significant increase in the concentration of total plasma protein and total globulin. Studies by Sadeghi *et al*. (2008); Ashayerizadeh *et al*. (2009) and Shahir *et al*. (2014) have shown that the addition of prebiotics to broiler chicken diets did not affect total protein, albumin, globulin, or the albumin to globulin ratio.

The protein fractionation results in chickens that were given omega-3 supplements showed significant increases in total protein and total globulin, along with a decrease in the A/G ratio. These findings support the results of Jameel *et al*. (2015), who found that supplementing the diet with omega-3 from plant sources increased the levels of total protein, albumin, and globulin. Additionally, Al-Mayah (2009) demonstrated that including fish oil in the diet increased the production of antibodies and serum globulins, helping to maintain proper immune function. Michel (2002) also observed an increase in globulins in quail fed with fish oil compared to those fed soybean oil. At analyzing the data obtained from group of omega3 carried on CHI found the the improvement in immunity and immunoglobulin levels.

The body's lymphoid system is a sophisticated defence system, consisting of distinct organs and divided into two components based on morphology and function. The smaller lymphocytes form the thymus-dependent component and facilitate cell-mediated immunity (CMI), while the larger lymphocytes form the bursa-dependent component and transform into plasma cells in the tissue, playing a critical role in humoral immunity (Karim *et al*., 2005). Measuring the weight of immune organs is a widely used method for assessing the immune status of chickens (Heckert *et al*., 2002).

The index of immune organs reflected the growing and development of thymus, spleen and bursa of Fabricius, which used to estimate the immune status of birds. Regarding the lymphoid-organ weight indexes in this study at 28 days, there were elevated in all supplemented groups. Furthermore, the results at 42 days, there were non-significant changes among groups in a bursa of Fabricius index but there were significant increases in spleen and in thymus gland indexes in supplemented groups than to the control. Consuming CHI has been shown to have a positive impact on the development and function of immune organs, as evidenced by an increase in the weight index of the bursa and thymus. These results agreed with Zhu *et al*. (2003); Shi *et al*. (2005); Huang *et al*. (2007); Deng *et al*. (2008); Li (2009) and Shi-bin and Hong (2012). According to the study conducted by Chang *et al*. (2020), the administration of CHI resulted in an elevation in the relative weight of both the thymus and bursa of Fabricius.

While CHI had positive effect on spleen weight, this finding disagreed with those of Huang *et al*. (2007) and Shi-bin and Hong (2012), as they found no effect for CHI on spleen weight.

When examining the results from the group of chickens enriched with omega3, there was a significant increase in the index of lymphoid organs including the bursa, spleen, and thymus. This finding is consistent with Jameel *et al*. (2015), who also observed an increase in the percentage of spleen and bursa in the group supplemented with omega-3 (from plant origin).

When shedding light on omega3 carried on CHI group found that significant increase in lymphoid organ index (bursa, spleen and thymus). All supplemented groups exposed significant increment in immunoglobulin (IgA, IgM and IgG). CHI enriched group reveal significant rise this observation agreement with Huang *et al*. (2005); David *et al*., (2007) and Liu *et al*., (2007) conveyed that beneficial action of CHI on immune function was stimulation of lymphoid organ and concentration of IgG, IgA, IgM, and also, CHI acts as adjuvant that significantly increased serum IgG titres in mice, also similar result was recorded in cows when fed on a diet supplemented with CHI. Regarding to omega3 scores showed significant increase this obtained results contracted with Sugano *et al*. (2000) and Elwan *et al*. (2019). At checking the group enforced with omega3 carried on CHI found that a significant increase in immunoglobulin.

The present study provided information about the humoral immune response of chicken reared on studied supplements through detection of serum antibody titer against Newcastle disease virus (NDV) and avian influenza virus (AIV) by hemagglutination inhibition test (HIT). The result showed that, antibody titer against ND and AI were significantly increased ($P \leq 0.05$) at 28 & 42 days in all supplement groups in comparison to control.

In concern to CHI had significantly increased the antibody titer against ND and AI viruses. These results in accordance with those of Kabir *et al*. (2004); Huang *et al*. (2007); Ghahri *et al*. (2013) and Ghasemi and Taherpour (2013). However, these results incompatible with the findings of Shafey *et al*. (2001); Silva *et al*. (2009); Houshmand *et al*. (2012); Sadeghi *et al*. (2013) and Shahir *et al*. (2014). The lack of compatibility with the results of the present study with those of others could be due to differences in the type and concentration of supplements, bird health and management. The antibody titers against ND and AI were significantly increased ($P \leq 0.05$) in group received omega3, that result agreed with Abdulla *et al*. (2017) and Jameel *et al*. (2015) who reported that groups which enriched with omega3 (either plant origin or animal origin) it's result revealed that antibody titer were increased significantly ($p \leq 0.05$). AL-Mayah (2009) found that supplementing chicken diets with fish oil resulted in a significant increase in antibody production following vaccination against Newcastle disease (ND). This effect is attributed to the immunomodulatory properties of omega-3 fatty acids, which are known to be substrates for the production of prostaglandins and leukotrienes, two substances that modulate immune response. Omega-3 fatty acids can also modulate cytokine production and signal transduction in immune cells, which may contribute to the observed increase in antibody production. Furthermore, omega-3 fatty acids are important constituents of immune cell structures and are involved in eicosanoid formation. These fatty acids possess anti-inflammatory properties, which may decrease the release of pro-inflammatory eicosanoids and cytokines, thus promoting the development of a strong immune system in chickens (Stulnig, 2003). Concerning with the group had omega3 carried on CHI noted the following significant increment in antibodies titres against ND and AI viruses.

Ren (2008); Tomida *et al*. (2009) showed that, polysaccharides such as CHI augmented the action of superoxide dismutase (SOD), antioxidant capability (AOC) and enhanced the antioxidative function. Hence, CHI serves as an antioxidant to avert oxidizing harm to the cells of fauna and shields the body from peroxide injury while performing as a free radical scavenger. Oxidative stress is a biochemical imbalance that triggers the overproduction of free radicals (ROS) beyond the innate antioxidant capacity. The surplus generation of MDA is likely to occur due to the peroxidation of lipids, ultimately resulting in the rise of MDA and LDL levels, which ultimately leads to damage to cell membranes. The enzymatic countermeasure against ROS entails the utilization of GSH and SOD, with SOD being the most selective. SOD plays a pivotal function in impeding oxidative stress and serves as a crucial cellular defense mechanism against ROS. The cellular SOD works by converting O_2 to H_2O_2, which is then reduced by cata-

lase to water and molecular oxygen, thereby curtailing oxidative damage (Bo *et al*., 2016).

The present study revealed that the antioxidant effect of CHI led to a noteworthy rise in SOD and GSH levels while significantly lowering MDA levels. This result is parallel with Chang *et al*. (2020) who demonstrated that adding CHI to the diet resulted in decreased muscle MDA levels and increased muscle SOD and GSH-Px activity. Li *et al*. (2017) observed a substantial decrease in MDA levels in the COS groups, along with a high level in the activities of SOD and GSH-Px, as well as the concentration of GSH. Similarly agreed with Toz and Deger (2018) who demonstrated that the CHI groups exhibited a significant reduction in lead and MDA levels, while GSH levels and GSH-Px activity showed a marked increase ($p < 0.05$), thus establishing the protective effect of CHI against lead toxicity. Regarding to the results gained from group supplemented with omega3 were significant improvement in antioxidant effectiveness result in decrease level MDA and increase level of SOD and GSH when compared with control this outcome comparable with Avramovic *et al*. (2012); Fouad and El-Senousey (2014); Bo *et al*. (2016) and Wang *et al*. (2018) whose stated that omega3 enriched groups were reveal significant increase in SOD and GSH but show a reduction in MDA. On the topic of adding omega3 on CHI found the results as the following significant increase in SOD and GSH but show a reduction in MDA. These results were similar to Vidyashri *et al*. (2020) who reported the antioxidant activity of chitosan encapsulated with omegs3.

CONCLUSION

Chitosan (CHI) and omega 3 promote safe broiler growth and enhance immunity. Omega 3 with CHI as a delivery system is the most effective as an immunostimulant and antioxidant, with no hepato-renotoxic effects. Using CHI and omega 3 as a delivery system benefits broiler growth and overall performance.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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