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Investigating of Antioxidant Protective Effects of Shrimp Shells Extracted Chitosan in Broiler Chickens

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Abstract

Chitin is the most abundant natural biopolymer that can be converted to chitosan, which has various biomedical applications. This study was conducted to investigate the antioxidant activity of chitosan extracted from shrimp shells on body weight of broiler chickens. The study comprised three experiments. In experiment 1, shrimp (*Penaeus merguiensis*) shells were demineralized using HCL (1N), deproteinized using NaOH (1N), decolorized using acetone, and deacetylated using 50% NaOH. The antioxidant potencies of chitosan and vitamin C were compared by their abilities to scavenge 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radicals. The DPPH free radical scavenging activity of chitosan at varying concentrations (1-20 mg/ml) ranged between 34.59 and 87.11%, whereas that of 1 mg/ml of vitamin C was 91.1%. In experiment 2, six dietary treatments containing varying concentrations of chitosan (0, 0.125, 0. 25, 0.5, 1, and 2 g/kg) were allocated randomly to 60 male broiler chicks from 10 to 42 days of age. Chitosan at varying concentrations had no significant effect on the body weight, but it significantly decreased the MDA level in breast meat ($p<0.05$). In experiment 3, a total of 20 male broiler chickens were divided into two groups (n=10) and were fed a corn-soybean basal diet with or without chitosan for 42 days. On days 35 and 38, five chickens from each group were treated with carbon tetrachloride (CCL4) at 1 mL/kg body weight to induce oxidative stress. Administration of CCL4 significantly decreased body weight and increased AST and ALT activities, which are indicators of liver damage, on day 42, whereas chitosan improved and normalized body weight and enzyme activity ($p<0.05$). The results suggested that chitosan extracted from shrimp shells exhibits antioxidant effects by increasing the shelf life of the meat and normalizing the body weight of the broilers as well as serum AST and ALT enzyme activities.

Introduction

Chitin is the most abundant and natural straight-chain biopolymer after cellulose. Composed of ß-1, 4-N-acetylglucosamine, chitin is found in crustacean shells, insect cuticles, and fungal cell walls (Yen *et al*., 2008). Although shrimps are considered highly important aquaculture products, one of the major components of their waste is chitin, known to cause environmental pollution on the coastal area because of its slow degradation (Kandra *et al*., 2012). Therefore, shrimp waste is typically used for recovering chitin for its commercial applications, with chemicals (Kumari *et al*., 2015) and enzymatic treatments (Younes *et al*., 2014) being the frequently used extraction methods. The basic process used for chitin extraction typically induces demineralization (use of acid to remove calcium carbonate), deproteinization (use of bases to remove proteins), and decolorization (use of acetone to remove the color) (Hafsa *et al*., 2016). Chitin is highly insoluble

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in most solvents, because of its highly compact structure, and this insolubility is a major concern in its use in biological systems. However, when the degree of deacetylation (DD) of chitin is more than 50%, the product is known as chitosan, which is soluble in dilute acid liquid (pH<6), has greater antibacterial (Benhabiles *et al*., 2012), antifungal (Rooler *et al*., 1999; Kong *et al*., 2010), antitumor (Qin *et al*., 2006), and hypochlostromy (Gallaher *et al*., 2000) properties, and has wide applications in food, agriculture, biotechnology, and pharmaceutical industries (Rinaudo *et al*., 2006; Yen *et al*., 2008). Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and Scanning electron microscope (SEM) are the main methods used to recognize chitin and chitosan (Kumari *et al*., 2015). Some studies have shown the use of chitosan as a natural antioxidant in biological systems (Yen *et al*., 2007; Yen *et al*., 2008), positively affecting poultry health and performance (Huang *et al*., 2005; Li *et al*., 2007; Zhou *et al*., 2009). Conversely, few studies have reported no major effects of using chitosan in broiler diets on performance (Khambualai *et al*., 2008; Li *et al*., 2016).

Oxidative reactions, which are naturally occurring essential metabolic processes in live aerobic cells, result in the production of reactive oxygen species or free radicals. These radicals, which are highly reactive molecules because of the presence of unpaired electrons, are known to cause damage to important biomolecules such as lipids, proteins, and nucleic acids. Oxidative stress is defined as an imbalance between the formation of free radicals and the ability of the body to scavenge these reactive molecules (Delles *et al*., 2014). In the poultry industry, the oxidation rate may increase as a result of a high oxidant diet, heat stress, transport system, and fast growth rates (Lin *et al*., 2004; Estevez *et al*., 2015). On the contrary, the use of high polyunsaturated fatty acids ($PUFA_S$) in poultry diets (such as plant oils), results in the meat being relatively rich in PUFA and hence more susceptible to oxidative damages (Tavarez *et al*., 2011). Feed supplementation with antioxidant compounds such as vitamin C, vitamin E, selenium and natural essential oils is a simple preslaughter strategy to reduce the negative effects of oxidative stress on broiler performance and to improve the meat quality (Ferreira *et al*., 2015; Lu *et al*., 2014).

In the present study, we aimed to investigate the antioxidant activity of chitosan on 1, 1- diphenyl-2-picrylhydrazyl free radicals and body weight of broiler chickens. The study comprised three experiments. First, chitosan was extracted from shrimp shells using chemical processes. FTIR was used to identify the functional groups and determine the DD. In vitro, antioxidant activity was analyzed using DPPH radical scavenging method. Second, the effects of chitosan at varying concentrations in broiler diet on the body weight and meat malondialdehyde (MDA) concentration, an index of lipid peroxidation, was investigated. Finally, we examined the effects of using chitosan on body weight, relative liver weight, and some serum enzyme activity of broiler chickens which have been challenged by carbon-tetrachloride (CCL4).

Materials and Methods

All experimental procedures used in this study were approved by the Animal Ethics Committee of the Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

Experiment 1: Preparation, extraction, and characterization of chitosan, and its scavenging activity against DPPH radicals Preparation, extraction, and characterization of chitosan

Fresh shrimp shells were obtained from a local market, in Iran, washed under running water, followed by boiling for 2 h to remove tissue and other impurities. The samples were dried in an oven for 12 h at 105°C and ground using a mill hammer (IKA, M20 Universal) to obtain a coarse powder. The coarse powder was treated according to the method described by Kumari *et al*., (2015) with some modifications. Briefly, the shells powder was treated with 1.0 M hydrochloric acid (1/10, w/v) for 6 h at room temperature to remove the calcium carbonate (demineralization) and then deproteinized using 0.5 N NaOH (1/10, w/v) for 18 h at ambient temperature. The solid materials were filtered, washed with distilled water to neutral, dried, and decolored using acetone 1:5 (w/v) for 10 min, washed, and dried. Chitin was treated with a 40% NaOH solution at 105°C for 120 min to obtain soluble chitosan in acidic conditions. The residues were washed with deionized water and dried. The DD of chitosan was analyzed using the data obtained by FTIR and calculated using to the following formula:

$$DD\ (\%) = 100 - [(A1650/A3447) \times 100/1.33],$$

Where;

$A1655\ cm^{-1}$ is the absorption of the band at 1655 and $A3450\ cm^{-1}$ is the absorption of the band at 3450.

The obtained shrimp shells chitosan was compared structurally to commercial chitosan (Sigma-Aldrich Chemical Co, St. Louis, MO) as standard.

Chitosan scavenging activity against DPPH radicals The DPPH radical scavenging activity of the prepared chitosan was determined, according to the method described by Hafsa *et al*. (2016). Chitosan sample, (1 mL) at different concentrations (1-20 mg/mL) in 1% acetic acid, was mixed with 3 mL of DPPH solution in methanol (Sigma-Aldrich; 300 M). The mixture was vortexed and incubated for 30 min in a dark room. The absorbance of the solution was measured at 517 nm. Four samples were prepared for

each chitosan concentration. Vitamin C (1 mg/mL) was purchased from Sigma Chemical Co (St. Louis, MO) and used for comparison. The inhibitory percentage of DPPH was calculated using the following formula:

$$\text{Scavenging activity (\%)} = [1 - (Abs_{sample}/Abs_{control})] \times 100.$$

Experiment 2: Impact of chitosan on broiler body weight and MDA concentration

In total, 180 day-old male Ross 308 broiler chickens were supplied by a commercial local hatchery. Chickens were housed in a deep litter floor and fed a mashed corn-soybean meal starter diet up to 10 days. On day 10, all birds were wing banded and weighed individually. Then, sixty birds with similar body weight were selected and received randomly one of six experimental treatments so that each treatment had 10 birds. The treatments included a basal corn-soybean meal diet without chitosan and any feed additives (control), and five chitosan diets prepared by adding 0.125, 0.25, 0.5, 1, and 2 g/kg chitosan to the basal diet. The feed ingredients and nutrient composition of basal diet in starter (1–10 d of age), grower (11–24 d of age), and finisher (25–42 d of age) periods are presented in Table 1.

Table 1. Ingredients and chemical analyses of the basal diet (Experiments 2 and 3)

Ingredients (g/kg)	Starter (1-11 d)	Grower (11-24 d)	Finisher (25-42 d)
Corn	552.4	574.7	626.1
Soybean meal	392.8	363.1	309.8
Soybean oil	13.0	22.6	27.5
Dicalcium phosphate	15.5	14.4	13.0
Calcium carbonate	11.8	10.3	9.6
Vitamin premix[1]	2.5	2.5	2.5
Mineral premix[2]	2.5	2.5	2.5
DL-Methionine	3.2	3.0	2.7
L-Lysine	2.4	1.8	1.9
L-Theronine	1.0	0.6	0.4
Sodium chloride	2.9	4.5	4.0
Chemical analysis			
ME (MJ/kg)	11.92	12.34	12.76
Crude protein (g/kg)	218	205	186
Calcium (g/kg)	9.0	8.2	7.5
Available phosphorus (g/kg)	4.5	4.2	3.9
Methionine (g/kg)	6.6	6.2	5.7
Methionine + Cystine (g/kg)	10.2	9.7	8.9
Lysine (g/kg)	13.8	12.6	11.4

[1]Vitamin premix provided the following per kilogram of diet: vitamin A, 11,500 IU; cholecalciferol, 2,100 IU; vitamin E, 22 IU; vitamin K3, 1.50 mg; thiamine, 3 mg; riboflavin, 4.4 mg; pantothenic acid, 25 mg; niacin, 40 mg; choline chloride, 560 mg; biotin, 0.1 mg; folic acid, 0.8 mg; pyridoxine 10 mg; vitamin B12, 0.060 mg.

[2]Trace mineral premix provided the following in milligrams per kilogram of diet: iron, 50 mg; zinc, 55 mg; manganese, 75 mg; iodine, 1.8 mg; copper, 8 mg; selenium, 0.3 mg; cobalt, 0.2 mg.

Diets were formulated to meet minimum nutritional requirements defined by the Aviagen nutrition specifications (Aviagen, 2014). The temperature was set at 32°C brooding day 1 and decreased gradually by 2°C per week until 24°C was reached, which was kept constant up to the end of the experiment. Feed and water were provided *ad libitum* and constant lighting was used throughout the experiment. Birds were weighed individually on days 11, 18, 24, 28, 35, and 42. At the end of the experiment, all the birds were killed and breast meat samples were immediately wrapped in air-permeable plastic bags and stored for 90 days in a freezer at −20°C ± 1°C. The thiobarbituric acid reactive substance was used to determine the MDA levels in fresh and frozen breast meat samples, according to the method described by Buge and Auost (1978).

Experiment 3: Impact of chitosan on broilers challenged with CCL4

Twenty day-old male broiler chickens (Ross 308 strain) were wing tagged, divided into two groups (n = 10), and fed a basal diet without chitosan or a basal diet supplemented with 0.125 g/kg chitosan from 1 to 42 d of age. Birds were kept in a deep litter floor with a constant lighting program and were provided feed and water *ad libitum*. On 35 and 38 days of age, five chicks from each group were injected intraperitoneally with CCL4 (1 mL/kg body weight) of body weight (mixed with olive oil in a ratio of 1:1), whereas the other chicks were injected with 1 mL of NaCl solution (0.9%/kg body weight) (Sharma *et al*., 2006). All birds were weighed individually on 11, 24, 35, and 42 days. Blood samples were collected from all birds in nonheparinized tubes from the brachial vein on day 42. Sera were obtained by centrifuging the samples at 1500 × g for 7 min at 4°C and were stored at −20°C until biochemical analysis. Serum liver enzymes, including alanine aminotransferase (ALT) and aspartate aminotransferase

(AST), were measured using the Technicon RA-1000 autoanalyzer. Also, relative liver weight and appearance were analyzed on day 42.

Statistical Analysis

Data from all three experiments were analyzed as a completely randomized design using the General Linear Model procedure of SAS (2003) software. Differences in the means of treatments were compared using the Tukey test at the 0.05 level of probability. Also, a regression analysis was performed for finding the relationship between chitosan concentration and DPPH scavenging activity (experiment 1) or the body weight of broilers (experiment 2).

Results

Experiment 1

The FTIR spectra of chitosan isolated from shrimp shells and commercial chitosan are presented in Figure 1, and both show a nearly similar peak. The DPPH radical scavenging activities of different concentrations of chitosan (1, 5, 10, 15, and 20 mg/mL) and vitamin C are presented in Figure 2. Although the free radical scavenging activity of chitosan at different concentrations was significantly lower than vitamin C ($P < 0.05$), all the concentrations of chitosan had an appreciable free radical scavenging ability, which increased in a dose-dependent manner (from 34.59 to 87.11).The linear regression graph between DPPH scavenging activity and chitosan concentration had an intercept equal to 11.59% and a slope equal to 18.19.

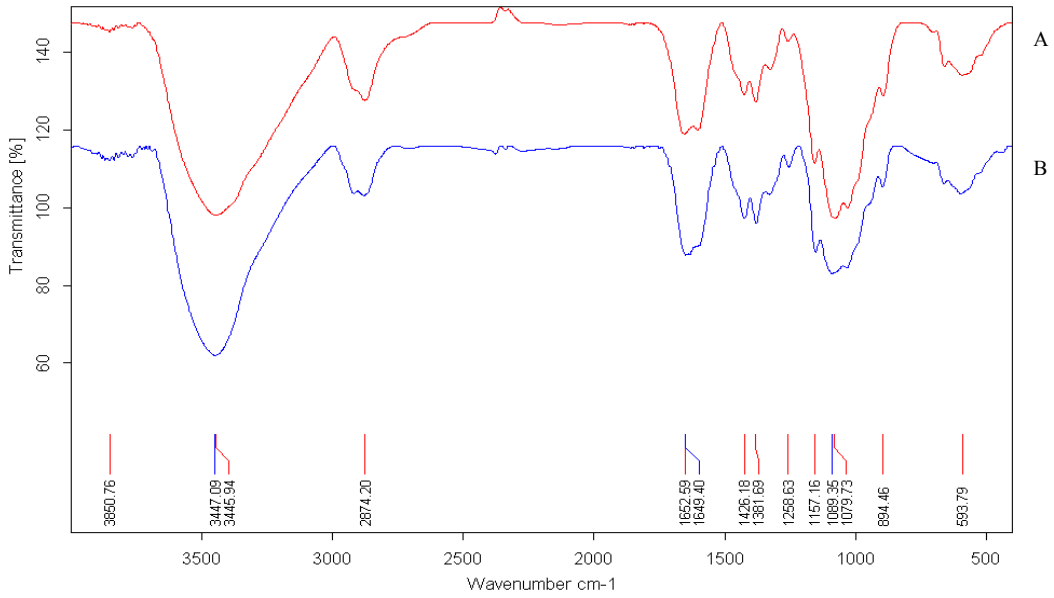

Figure 1. FTIR spectra of commercial chitosan (A) and isolated chitosan from shrimp shell (B).

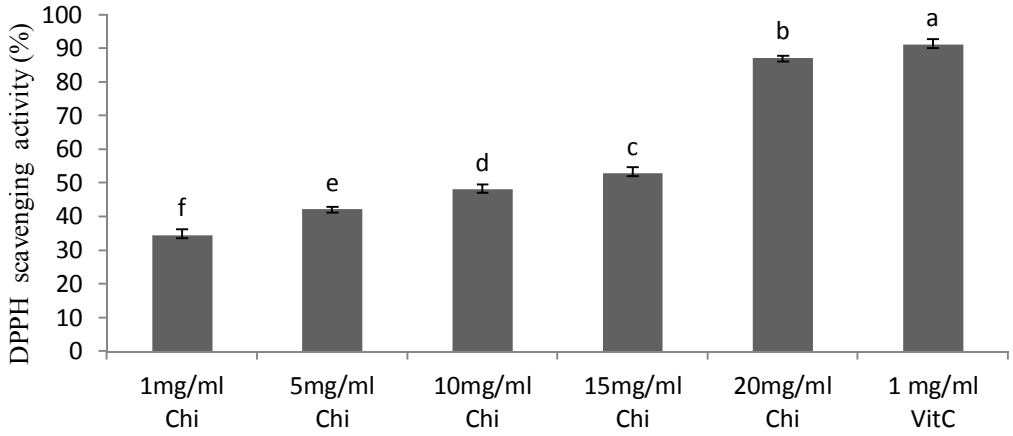

Figure 2. DPPH free radical-scavenging activities of chitosan (Chi) sample at different concentrations in comparison by vitamin C (VitC).Data are presented as mean and error bars shown standard deviation. (n=4). Means without similar letters differ significantly ($P < 0.05$) (Experiment 1)

Experiment 2

There was a nonsignificant difference between body weight as well as body weight gain of broilers received different levels of chitosan and the body weight, as well as the body weight gain (Table 2). The effects of chitosan on breast meat MDA concentration are presented in Figure 3. The MDA concentration in breast meat was significantly lower in the chitosan group than the control group ($P < 0.05$).

Table 2. Body weight (BW) and body weight gain (BWG) of broilers receiving different levels of chitosan at different ages (Experiment 2)

Chitosan level (g/kg)	BW11d (g)	BW18d (g)	BW24d (g)	BW28d (g)	BW35d (g)	BW42d (g)	BWG 11-42d (g)
0 (Control)	178.1	364.3	682.6	928.3	1391.1	2050.6	1872.4
0.125	182.0	393.8	717.7	978.7	1418.1	2123.3	1941.3
0.25	181.4	375.0	688.1	955.5	1459.0	2120.0	1938.6
0.5	177.6	355.5	677.6	935.5	1445.0	2146.5	1968.9
1	173.8	376.4	713.6	927.2	1358.3	2039.2	1865.4
2	181.7	401.0	747.5	1007.2	1530.5	2232.8	2051.0
P-value	0.712	0.319	0.621	0.703	0.319	0.422	0.103
SEM	4.63	16.39	31.03	42.43	52.86	73.36	71.68
CV	7.84	14.01	14.15	14.30	12.81	13.28	14.21
F value[1]	0.34ns	1.35ns	1.01ns	0.99ns	1.91ns	2.12ns	2.10ns

[1]Reprent of analysis regression, ns=not significant

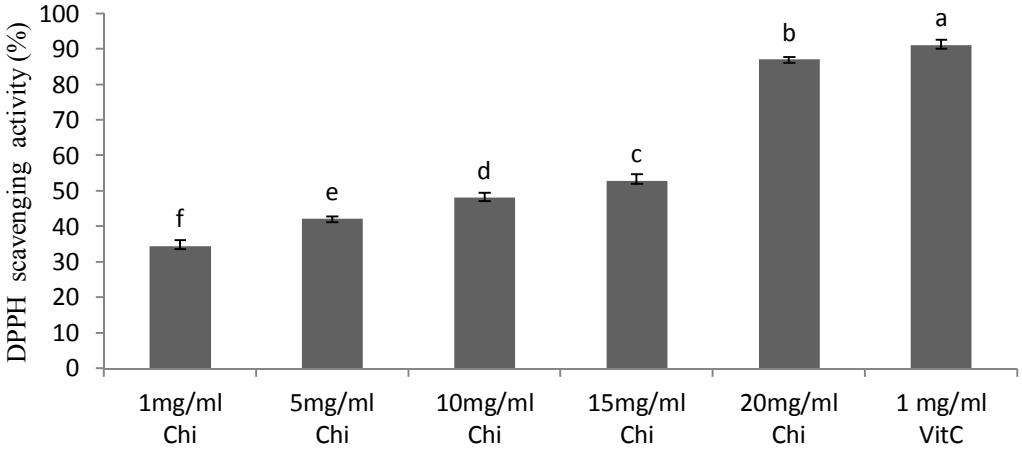

Figure 3. DPPH free radical-scavenging activities of chitosan (Chi) sample at different concentrations in comparison by vitamin C (VitC).Data are presented as mean and error bars shown standard deviation. (n=4). Means without similar letters differ significantly ($P < 0.05$) (Experiment 1)

Experiment 3

Chitosan had no significant effect on broiler body weight on days 11, 24, and 35; however, after CCL4 administration on day 35, the effects of chitosan were significant on broiler body weight, relative liver weight, and serum AST and ALT activities ($P < 0.05$; Table 3).

Table 3. Effects of chitosan and CCL4 on broiler body weight, relative liver weight (% live body weight), and sera enzyme activity (Experiment 3)

Treatments	BW11 (g)	BW24 (g)	BW35 (g)	BW42 (g)	Liver weight (% LBW[1])	ALT (IU/L)	AST (IU/L)
Control	189.9	762.4	1543.1	2110^{a}	2.31^{b}	4.15 ^{b}	186 ^{c}
Chitosan	200.4	841.1	1525.7	2085^{a}	2.37^{b}	4.30 ^{b}	189 ^{c}
CCL4	-	-	-	1406^{b}	3.50^{a}	8.05 ^{a}	327^{a}
Chitosan+CCL4	-	-	-	2039^{a}	2.67^{b}	4.46 ^{b}	282 ^{b}
P-value	0.421	0.219	0.813	0.042	0.056	0.011	0.053
Pooled *SEM*	9.5	44.1	48.8	124.02	0.17	0.6	11.2

Means within a column without similar letters differ significantly ($P < 0.05$).
[1]Live body weight

Discussion

FTIR is the most frequently used technique to characterize the chemical structure of chitosan. In the present study, the FTIR spectrum showed the typical infrared absorption bands of chitosan. The absorption peak at 3447 cm^{-1} corresponded to OH stretching vibrations of water and hydroxyl molecules, and NH stretching vibrations of free amino groups. The band observed at 2878 cm^{-1} corresponded to CH stretching vibrations. The peak bands were around 1649 (amide I band, C=O stretch), 1420 (C-H bending), 1380 (amide III band, C-H stretch), 1259 (C-N stretch), 1153 (bridge C-O-C stretch) and 1083 (C-H stretch). The characteristic peaks and their intensities were nearly the same as those reported by Kumari *et al*. (2015) and Hafsa *et al*. (2016). According to Rinaudo *et al*. (1993), the DD of isolated chitosan from shrimp shells is 66.62%. DD can be between 50% and 98% and depends on the concentration of NaOH solution, time and temperature in the deacetylation process of chitin (Rahman *et al*., 2015) and when the DD is more than 50%, the polymer will be called, chitosan (Rinaudo *et al*., 1993).

DPPH free radical scavenging activity is a fast and simple method that is widely accepted by researchers to investigate the antioxidant activity of different antioxidants (Hafsa *et al*., 2016). Similar to the results of the present study, Younes *et al*. (2014) and Hafsa *et al*. (2016) reported the dose-dependent free radical scavenging activity of chitosan. Xing *et al*. (2005) reported that sulfated chitosan exhibited a high scavenging ability of 83.4%. The primary and the most important reason for the radical scavenging ability of chitosan is because of the presence of ammonium ion groups ($NH_3^{+)}$ formed by the reaction of amine groups (NH_3) of chitosan and hydrogen ions (H^+) of the acidic solution. The unstable free radical molecules can react with the hydrogen ion from the $NH3^+$ and then change to a stable form of the molecule (Xie *et al*., 2001).

The nonsignificant difference between different levels of chitosan on body weight, as well as body weight gain, in experiment 2 is following the findings reported by Kobayashi *et al*. (2002, 2006), and Li *et al*. (2016). However, some studies have indicated that chitosan or Chito-oligosaccharides (produced by depolymerization of chitosan) improves the performance of broilers (Huang *et al*., 2005; Zhou *et al*., 2009). On the contrary, Razdan and Pettersson (1994) reported that chitosan decreases broiler performance because of the increasing viscosity of materials in the gastrointestinal tract and decreasing the digestibility of nutrients.

MDA is a 3-carbon compound and the main index of lipid oxidation (Tamura *et al*., 1991). We could not find any reports regarding the effect of feeding chitosan to broiler chickens on the meat quality. However, Marwa *et al*. (2017) showed the coating meat with chitosan increases lipid oxidative stability and inhibits the growth of bacteria during storage. It is generally accepted the lipid oxidation is the primary reason for the decrease in the shelf-life of the meat. It has been shown that supplementing broiler diets with antioxidant compounds converts hydroxyl and lipid radicals to stable molecules and decreases the MDA level in thigh samples (Luna *et al*., 2010). Goni *et al*. (2007) also reported that supplementing broiler diets with vitamin E decreases MDA formation in breast and thigh meat after 1, 4, and 7 days of refrigeration. The considerable decline of the MDA level in breast meat of broilers in the present study could be related to the antioxidant activity of chitosan, which has been shown in Experiment 1.

CCL4 is typically used to induce oxidative stress and liver injury in mice and rats (Amin and Ghoneim, 2009; Cui *et al*., 2009; Kim *et al*., 2010). Recently Baradaran *et al*. (2019) have been reported CCL4 induce oxidative stress in broiler chickens. Similar to the findings of Sonkusale *et al*. (2011), birds exposed to CCL4 exhibited lower body weight because of oxidative damage. CCL4 is a toxic substance that is converted to the free radicals trichloromethyl ($CCl_3^{\cdot}$) and proxy-tri-chloromethyl ($CCL_3OO^{\cdot}$) that attach to the hepatocytes (Mahmoodzadeh *et al*., 2017) and damage them. Administration of chitosan after CCL4 treatment prevented the change in broiler body weight, which is an indication of the normal growth rate.

In our study, broilers challenged with CCL4 and not receiving chitosan had higher relative liver weight and greater serum ALT and AST activities compared with other broilers. These findings are following those reported by Sonkusale *et al*., (2011), Wang *et al*., (2013) and Bradaran *et al*., (2019), who showed that inclusion of CCl4 in broilers diets increases hepatic enzymes levels. Increased relative liver weight in the CCL4 group observed in this study is in agreement with previous study findings on rats (Domitrovic *et al*., 2009; Bak *et al*., 2016). However, supplementing the broiler diet with chitosan declined the detrimental effects of CCL4 on relative liver weight and enzyme activities.

The liver is the primary target organ for CCL4 toxicity (Domitrovic *et al*., 2009; Bak *et al*., 2016). The increase in the relative liver weight in CCL4-treated birds may be attributed to the CCL4-induced impaired fat metabolism in the liver increasing the hepatocyte fat content (Boll *et al*., 2001). Ismali *et al*. (2009) reported oil supplemented with vitamins E and C decreases the percentage weight of liver and AST and ALT activities in CCL4-treated rats. The mechanism of increasing ALT and AST activity after CCL4 treatment is related to lipid peroxidation in liver cells, which causes hepatocyte swelling the release of these enzymes into the blood circulation (Recknagel *et al*, 1989). Interestingly, the

hepatoprotective activity of chitosan against CCL4-induced liver injury in rats has been reported previously (Chen *et al*., 2005). Furthermore, some studies have indicated that chitosan can be used as a protective agent because of its antioxidant properties against toxicity caused by various hepatotoxins such as CCl4 in rats (Subhapradha *et al*., 2014; Ramasamy *et al*., 2014; Subhapradha *et al*., 2017).

These results of the present study demonstrated that chitosan extracted from shrimp shells has an antioxidant activity against DPPH free radicals. Moreover, the use of chitosan in diets of broiler chickens improves the shelf-life of the meat and performance of the birds, especially in the oxidative stress conditions. The natural antioxidant effects of chitosan make it a preferred additive for broiler diets.

References

Amin A & Ghoneim D. 2009. Zizyphus spinachristi protects against carbon tetrachloride-induced liver fibrosis in rats. Food and Chemistry Toxicology. 47, 2111-2119. DOI: 10.1016/j.fct.2009.05.038

Aviagen., 2014. Ross308 Broiler: Nutrition Specifications. http://en.aviagen.com/assets/Tech Center/Ross_Broiler/Ross308BroilerNutritionSpe s2014-EN.pdf.

Bak J, Nam KJ, Hae YC, Takako Y, Sik Y & Jeon-ok M. 2016. Oligonol Ameliorates CCl4-Induced Liver Injury in Rats via the NF-Kappa B and MAPK Signaling Pathways. Oxidative Medicine and Cellular Longevity. Article ID 3935841, 12 pages. DOI: 10.1155/2016/3935841

Benhabiles MS, Salah R, Lounici H, Drouiche N, Goosen MFA & Mameri N. 2012. Antibacterial activity of chitin, chitosan and its oligomers prepared from shrimp shell waste. Food Hydrocolloids. 25: 48-56. DOI: 10.1016/j.foodhyd.2012.02.013

Boll M, Lutz W, Weber D, Eberhard B & Stampfl A. 2001. Hepatocyte damage induced by carbon tetrachloride: Inhibited lipoprotein secretion and changed lipoprotein composition. Zeitschrift für Naturforschung. 56: 283-290. DOI: 10.1515/znc-2001-3-419

Buege JA & Aust SD. 1978. Microsomal lipid Peroxidation. Methods in Enzymology. 52: 302-310. DOI: 10.1016/S0076-6879(78)52032-6

Chen AS, Taguchi T, Sakai K, Matahira Y, Wang MW & Miwa I. 2005. Effect of chitobiose and chitotriose on carbon tetrachloride-induced acute hepatotoxicity in rats. Biological and Pharmaceutical Bulletin. 28, 1971-1973. DOI: 10.1248/bpb.28.1971

Cui CP, Wei P, Liu Y, Zhang DJ, Wang LS & Wu CT. 2009. The protective role of hepatopoietin Cu on liver injury induced by carbon tetrachloride in rats. Hepatology Research. 39: 200-206. DOI: 10.1111/j.1872-034X.2008.00447.x

Delles RM, Xiong YL & Dawson A. 2014. Dietary antioxidant supplementation enhances lipid and protein oxidative stability of chicken broiler meat through promotion of antioxidant enzyme activity. Poultry Science. 93: DOI: 10.3382/ps.2013-03682

Domitrovic R, Jakovak H, Tomac J & Sain I. 2009. Liver fibrosis in mice induced by carbon tetrachloride and its reversion by luteolin. Toxicology and Applied Pharmacology. 241: 311-321. DOI: 10.1016/j.taap.2009.09.001

Estevez M. 2015. Oxidative damage to poultry from farm to fork. Poultry Science. 94: 1368-1378. DOI: 10.3382/ps/pev094

Ferreira IB, Junior JBM, Sgavioli S, Vicentini TI, Morita VS & Boleli IC. 2015. Vitamin C prevents the effects of high rearing temperatures on the quality of broiler thigh meat. Poultry Science. 94: 841-851. DOI: 10.3382/ps/pev058

Gallaher CM, Munion J, Hesslink JR, 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. Journal of Nutrition. 130: 2753-2759. DOI: 10.1093/jn/130.11.2753

Goni I, Brenes A, Centeno C, Viveros A, Saura-calixto F, Rebole A, Arija I & Estevez R. 2007. Effect of dietary grape pomace and vitamin E on growth performance, nutrient digestibility, and susceptibility to meat lipid oxidation in chickens. Poultry Science. 86: 508-516. DOI: 10.1093/ps/86.3.508

Hafsa J, Smach MA, Charfeddi B, Limem K, Majdoub H & Rouatbi S. 2016. Antioxidant and antimicrobial proprieties of chitin and chitosan extracted from Parapenaeus Longirostris shrimp shell waste. Annales Pharmaceutiques Françaises. 74: 27-33. DOI: 10.1016/j.pharma.2015.07.005

Huang RL, YinYL, 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. Poultry Science. 84: 1383-1388. DOI: 10.1093/ps/84.9.1383

Ismail RSA, El-Megeid AAA & Abdel-Moemin AR. 2009. Carbon tetrachloride-induced liver disease in rats, the potential effect of supplement oil with vitamins E and C on the nutritional status. Archive of German Medical Science. 2: 295-302. DOI: 10.3205/000064

Kandra P, Challa MM & Jyothi HK. 2012. Efficient use of shrimp waste: present and future trends. Applied Microbiology and Biotechnology. 93: 17-29. DOI: 10.1007/s00253-011-3651-2

Khambualai O, Yamauchi KE, Tangtaweewipat S & Cheva-Isarakul B. 2008. Effects of dietary chitosan diets on growth performance in broiler

chickens. Journal of Poultry Science. 45: 206–209. DOI: 10.2141/jpsa.45.206

Kim HY, Kim JK, Choi JH, Jung JY, OH WY, Kim DC, Kang SS, Lee SH & Lee SM. 2010. Hepatoprotective effect of pinoresinol on carbon tetrachloride-induced hepatic damage in mice. Journal of Pharmacological Sciences. 112: 105-112. DOI: 10.1254/jphs.09234FP

Kobayashi S, Terashima Y & Itoh H. 2002. Effects of dietary chitosan on fat deposition and lipase activity in digesta in broiler chickens. British Poultry Science. 43: 270-273. DOI: 10.1080/00071660120121490

Kobayashi S, Terashima Y & Itoh H. 2006. The effects of dietary chitosan on liver lipid concentration in broiler chickens treated with propylthiouracil. Journal of Poultry Science. 43: 162-166. DOI: 10.2141/jpsa.43.162

Kong M, Chen XG, Xing K & Park HJ. 2010. Antimicrobial properties of chitosan and mode of action: A state of the art review. International Journal of Food Microbiology. 144: 51-63. DOI: 10.1016/j.ijfoodmicro.2010.09.012

Kumari S, Rath P, Sri-Hari-kumar A & Tiwari TN. 2015. Extraction and characterization of chitin and chitosan from fishery waste by chemical method. Environmental Technology and Innovation. 3: 77-85. DOI: 10.1016/j.eti.2015.01.002

Li QP, Gooneratne SR, Wang RL, Zhang R, AN LL, Chen JJ & Pan W. 2016. Effect of different molecular weight of chitosans on performance and lipid metabolism in chicken. Animal Feed Science and Technology. 211: 174-180. DOI: 10.1016/j.anifeedsci.2015.11.013

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. Poultry Science. 86: 1107-1114. DOI: 10.1093/ps/86.6.1107

Lin H, Decuypere E & Buyse J. 2004. Oxidative stress induced by corticosterone administration in broiler chickens (Gallus gallus domesticus) 1. Chronic exposure. Comparative Biochemistry and Physiology – Part B: Biochemistry and Molecular Biology. 139: DOI: 10.1016/j.cbpc.2004.09.013

Lu T, Harper AF, Zhao J, Corl BA, Leroith T & Dalloul RA. 2014. Effects of a dietary antioxidant blend and vitamin E on fatty acid profile, liver function, and inflammatory response in broiler chickens fed a diet high in oxidants. Poultry Science. 93: 1658-1666. DOI: 10.3382/ps.2013-03827

Luna A, Labaque MC, Zygadlo AJ & Marin RH. 2010. Effects of thymol and carvacrol feed supplementation on lipid oxidation in broiler meat. Poultry Science. 89: 366-370. DOI: 10.3382/ps.2009-00130

Mahmoodzadeh Y, Mazani M & Rezagholizadeh L. 2017. Hepatoprotective effect of methanolic Tanacetum parthenium extract on CCl4-induced liver damage in rats. Toxicology Reports. 4: 455-462. DOI: 10.1016/j.toxrep.2017.08.003

Marwa RS, Abdollah MA, Mohmaed HMH & Emara MT. 2017. Improving the sensory, physicochemical and microbiological quality of pastirma (A traditional dry cured meat product) using chitosan coating. Journal of Food Science and Technology. 86: 247-253. DOI: 10.1016/j.lwt.2017.08.006

Qin C, Li H, Xiao Q, Liu Y, Zhu J & Du Y. 2006. Water-solubility of chitosan and its antimicrobial activity. Carbohydrate Polymers. 63: 367-374. DOI: 10.1016/j.carbpol.2005.09.023

Rahman RM, Radim H, Abdel-Mohsen AM, Fouda MG, Solimand AY, Mohamed FK, Kazi M & Tiago DP. 2015. Chitin and chitosan from Brazilian Atlantic Coast: Isolation, characterization and antibacterial activity. International Journal of Biological Macromolecules. 80: 107-120. DOI: 10.1016/j.ijbiomac.2015.06.027

Ramasamy P, Subhapradha N, Shanmugam V & Shanmugam A. 2014. Protective effect of chitosan from Sepia kobiensis (Hoyle 1885) cuttlebone against CCL4 induced hepatic injury. International Journal of Biological Macromolecules. 65: 559-563. DOI: 10.1016/j.ijbiomac.2014.02.009

Razdan A & Pettersson D. 1994. Effect of chitin and chitosan on nutrient digestibility and plasma lipid concentrations in broiler chickens. British Journal of Nutrition. 72: 277-288. DOI: 10.1079/BJN19940029

Recknagel RO, Glende JREA, Dolak JA & Waller RL. 1989 Mechanisms of carbon tetrachloride toxicity. Pharmacology and Therapeutics. 43: 39-54. DOI: 10.1016/0163-7258(89)90050-8.

Rinaudo M. 2006. Chitin and chitosan: Properties and applications. Progress in Polymer Science. 31: 603-632. DOI: 10.1016/j.progpolymsci.2006.06.001

Rinaudo M, Milas M & Le-dung P. 1993. Characterization of chitosan. Influence of ionic strength and degree of acetylation on chain expansion. International Journal of Biological Macromolecules. 15: 281-285. DOI: 10.1016/0141-8130(93)90027-J

Roller S & Covill N, 1999. The antifungal properties of chitosan in laboratory media and apple juice. International Journal of Food Microbiology. 47: 67-77. DOI: 10.1016/S0168-1605(99)00006-9

SAS (Statistical Analysis System). 2003. SAS/STAT®. User's Guide. SAS Institute Inc. Cary,

North Carolina.
Sharma A, Sharma MK & Kumar RM. 2006. Protective effect of *Mentha piperita* against arsenic-induced toxicity in liver of swiss albino mice. Basic and Clinical Pharmacology and Toxicology. 100: 249-257. DOI: 10.1111/j.1742-7843.2006.00030.x
Sonkusale P, Bhandarker AG, Kurkare NV, Ravikanth K & Sood D. 2011. Hepatoprotective activity of superliv liquid and repchol in CCL4 induced FLKS syndrome in broilers. International Journal of Poultry Science. 10: 49-55. DOI: 10.3923/ijps.2011.49.55
Subhapradha N, Shanmugam V & Shanmugam A. 2017. Chitosan nanoparticles from marine squid protect liver cells against N-diethylnitrosoamine-induced hepatocellular carcinoma. Carbohydrate Polymers. 171: 18-26. DOI: 10.1016/j.carbpol.2017.04.097
Subhapradha N, Saravanan R, Ramasamy P, Srinivasan A, Shanmugam V & Shanmugam A. 2014. Hepatoprotective effect of β-Chitosan from Gladius of Sepioteuthis lessoniana against carbon tetrachloride-induced oxidative stress in Wistar rats. Applied Biochemistry Biotechnology. 172: 9-20. DOI: 10.1007/s12010-013-0499-1
Tamura H, Kitta K & Shibamoto T. 1991. Formation of reactive aldehydes from fatty acids in a Fe^{+2}/H2O2 oxidation system. Journal of Agricultural and Food Chemistry. 39: 439-442. DOI: 10.1021/jf00003a002
Tavarez MA, Boler DD, Bess KN, Zhao J, Yan F, Dilger AC, Mckeith FK & Killefer J. 2011, Effect of antioxidant inclusion and oil quality on broiler performance, meat quality, and lipid oxidation. Poultry Science. 90: 922-930. DOI: 10.3382/ps.2010-01180
Wang C, Zhang T. Cui X, Li S, Zhao X & Zhong X. 2013. Hepatoprotective effects of a Chinese herbal formula, longyin decoction, on carbon-tetrachloride-induced liver injury in chickens. Evidence – Based Complementary and Alternative Medicine. 10: 392-401. DOI: 10.1155/2013/392743
Xie W, Xu P & Liu Q. 2001. Antioxidant activity of water-soluble chitosan derivatives. Bioorganic and Medical Chemistry Letters. 11: 1699-1701. DOI: 10.1016/S0960-894X(01)00285-2
Xing R, Yu H, Liu S, Zhang W, Zhang Q, Li Z & Li P. 2005. Antioxidative activity of differently regioselective chitosan sulfates in vitro. Bioorganic and Medicinal Chemistry. 13: 1387-1392. DOI: 10.1016/j.bmc.2004.11.002
Yen MT, Tseng YH, Li RC & Mau JL. 2007. Antioxidant properties of fungal chitosan from shiitake stipes. Food Science and Technology. 40: 255-261. DOI: 10.1016/j.lwt.2005.08.006
Yen MT, Yang JH & Mau JL. 2008. Antioxidant properties of chitosan from crab shells. Carbohydrate Polymers. 74: 840-844. DOI: 10.1016/j.carbpol.2008.05.003
Younes I, Hajji S, Farchet V, Rinaudo M, Jellouli K & Nasri M. 2014. Chitin extraction from shrimp shell using enzymatic treatment. Antitumor, antioxidant and antimicrobial activities of chitosan. International Journal of Biological Macromolecules. 69: 489-498. DOI: 10.1016/j.ijbiomac.2014.06.013
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. Poultry Science. 88: 593-600. DOI: 10.3382/ps.2008-00285