New insights into the role of dietary marine-derived polysaccharides on productive performance, egg quality, antioxidant capacity, and jejunal morphology in late-phase laying hens

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ABSTRACT The present study was conducted to evaluate the effects of dietary marine-derived polysaccharides (MDP) from seaweed *Enteromorpha* on productive performance, egg quality, antioxidant capacity, and jejunal morphology in late-phase laying hens. A total of 240 Lohmann white laying hens (62 wk of age) were assigned to 4 dietary treatments that included MDP at concentrations of 0, 1,000, 2,500, and 5,000 mg/kg for 6 wk. Each treatment had 6 replicates with 5 cages (2 birds/cage). The results showed that dietary MDP quadratically improved egg production ($P < 0.05$) during 5 to 6 wk and 1 to 6 wk. There was a linear reduction in cracked egg rate ($P < 0.05$) with dietary MDP levels increased during 3 to 4 wk and 1 to 6 wk. After 4 wk of feeding trial, the egg shell thickness, yolk color, and Haugh unit showed a linear increase ($P < 0.05$) in response to increasing dietary MDP levels. Besides, the egg shell breaking strength, egg shell thickness, yolk color, and Haugh unit were improved linearly ($P < 0.05$) by dietary MDP at the end of the experiment. Moreover, dietary MDP showed a linear and quadratic reduction in serum malondialdehyde (MDA) content ($P < 0.05$) at the end of third week. At the end of experiment, the activity of total superoxide dismutase in serum was increased quadratically ($P < 0.05$) by dietary MDP, and dietary MDP quadratically improved the liver catalase (CAT) activity ($P < 0.05$) and linearly enhanced jejunal CAT activity ($P < 0.05$), whereas linearly decreased jejunal MDA concentration ($P < 0.05$). Furthermore, supplemental MDP linearly improved the villus height ($P < 0.05$) and quadratically increased villus height/crypt depth ratio ($P < 0.05$) of jejunum. However, dietary MDP had no effect on jejunal trypsin, amylase, and protease activity ($P > 0.10$). Taken together, these findings provided new insights into the role of MDP in improving the productive performance, egg quality, antioxidant capacity, and jejunal morphology of late-phase laying hens.

Key words: aged laying hen, egg production, egg quality, *Enteromorpha*, marine-derived polysaccharides

2020 Poultry Science 99:2100–2107
https://doi.org/10.1016/j.psj.2019.12.032

INTRODUCTION

Many factors including health status, age, nutrition, and housing system affect the productive performance and egg quality in layers (Vlckova et al., 2019). It is well known that after the end of the laying peak, egg production and quality such as laying rate, egg shell quality, and nutrients in eggs will decrease with increasing age of the laying hens (Liu et al., 2013). The decline of productive performance and egg quality during the late laying period is mainly due to the oxidative stress accumulated by long-term egg production (Liu et al., 2018). Because the use of medication is being minimized to avoid potential residues in eggs, producers rely on nutritional manipulation to improve the persistency on egg production and the resistance against oxidative stress in aged layers (Liu et al., 2013; Qu et al., 2018). As a consequence, the application of prebiotics, probiotics, and synbiotics in diets could be safe alternatives to improve poultry performance and health (Liu et al., 2019; Lv et al., 2019).

Received September 23, 2019.
Accepted December 2, 2019.

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At present, special attention has been paid to the use of polysaccharide compounds as prebiotics in poultry nutrition. Functional polysaccharides are nondigestible ingredients because of their β-1,4 linkages. Consumption of functional polysaccharides, including plant-derived and fungal-derived polysaccharides, can reportedly stimulate reproductive performance and improve egg quality in laying hens (Bozkurt et al., 2012; Abdelqader et al., 2013; Ding et al., 2018). Marine environment has rich sources of polysaccharides, and the marine-derived polysaccharides (MDP) have been confirmed to exhibit a variety of biological and pharmacological activities, including antitumor, antioxidation, hematopoiesis, immunomodulation, and gastrointestinal protection (Laurienzo, 2010). Previously, researchers have investigated the application potential of MDP for laying hens. For instance, Meng et al. (2010) reported that chito-oligosaccharide (COS) supplementation resulted in an increase in laying performance, Haugh unit of eggs, and apparent digestibility of nitrogen in laying hens during peak-laying period. Yan et al. (2010) demonstrated that supplementation of COS could promote egg weight in laying hens. Swiątkiewicz et al. (2013) confirmed that dietary COS enhanced egg production of laying hens. Although earlier studies investigated the role of certain types of MDP in improving reproductive performance of laying hens, compared with COS, the effects of dietary MDP from seaweed on performance of laying hens are not frequently reported, especially the aged laying hens.

Enteromorpha is a kind of seaweed and found to contain abundant polysaccharides, which is widely distributed in the East and South China Sea. There is increasing evidence that the MDP of seaweed *Enteromorpha* exert multiple pharmacological properties, such as anti-inflammatory, antiviral, antimicrobial, antioxidant, and immunological regulation (Wei et al., 2014; Duan et al., 2015). Based on the beneficial function, previous studies evaluated the effects of MDP from seaweed *Enteromorpha* in broilers and suggested that it could be used as natural alternative to antibiotic and stimulate the growth performance, as well as improve the intestinal health and antioxidant status (Sun et al., 2010; Du et al., 2019). However, to the best of our knowledge, there is extremely limited information about the effects of dietary supplementation with MDP from seaweed *Enteromorpha* in late-phase laying hens. Hence, the aim of the present study was to study the efficacy of supplementing graded concentrations of MDP from seaweed *Enteromorpha* on egg production, egg quality, antioxidant capacity, and jejunal morphology in laying hens during the late laying period.

MATERIALS AND METHODS

Marine-Derived Polysaccharides Products

The marine-derived polysaccharides were produced from the seaweed *Enteromorpha* and provided by Qingdao Haida Biotechnology Co., Ltd. (Qingdao, China). The content of polysaccharides is not less than 48%, and the molecular weight is 4,929 Da. According to the analysis results of polysaccharide composition by HPLC, the polysaccharide is mainly composed of rhamnose (Rha), glucuronic Acid (GlcA), glucose (Glc), galactose (Gal), and xylose (Xyl) monosaccharides. The molar percentage of monosaccharides is Rha: 40.6%, GlcA:9.3%, Glc:38.2%, Gal:5.6%, and Xyl:6.3%.

Experimental Birds, Diets, and Management

The present study was performed on the poultry experimental house of Guangdong Ocean University (Zhanjiang, Guangdong, China), and the Animal Care and Use Committee of Guangdong Ocean University approved all experimental procedures. A total of 240 Lohmann white laying hens (62-weeks-old) with average body weight 2087.42 ± 56.15 g were randomly assigned to 4 dietary treatments that included MDP levels of 0, 1,000, 2,500, and 5,000 mg/kg for 6 wk, and there was no significant difference in initial body weight between replicates and treatments. Hens were assigned to treatments based on a completely randomized block design. Each treatment had 6 replicates with 5 cages (2 birds/cage). Each treatment was uniformly distributed in the layer house to minimize environmental effects. All birds were housed with the temperature maintained at approximately 24°C. Ventilation and lighting (16L:8D) were automatically controlled in the house. The feed and water were supplied with ad libitum. The hens were fed diets in mash form during the experimental period. Basal diets (Table 1) were based on corn–soybean meal with composition and nutrient levels consistent with the National Research Council (1994).

Sampling and Measurements

During the experiment, all eggs (intact, broken, and shell-less) were collected and recorded in all replicates on a daily basis. The egg production was expressed as an average hen-day production. The feed consumption was weekly records, and then the feed efficiency was calculated. At 2 wk intervals, 30 eggs (5 eggs per replicate) from each treatment were randomly selected during 2 D for quality analysis. Soft and broken eggs were not included in the analysis. The length and width of eggs were measured by Vernier caliper, and egg shape index calculated as the formula: egg shape index = length of the longitudinal direction/width of the transverse path. Then eggs were broken, and their contents were separated and weighed individually to determine egg shell weight, thickness, breaking strength, albumin height, albumin weight, yolk color, yolk weight, and Haugh unit. Egg shell breaking strength was evaluated using an egg shell force gauge model II (Power sensor: 5 kg, detection speed: 2 mm/s. Robotmation, Tokyo, Japan). In addition, egg shell thickness was measured on the large end, equatorial region, and small end, respectively, using a dial pipe gauge (Ozaki MFG.,

Table 1. Composition of basal diets (as-fed basis).

Items	Amount
Ingredient, %	
Corn	50.85
Soybean meal, 46% CP	18.90
Wheat grain	9.85
Corn gluten meal	2.00
Wheat bran	5.00
Soybean oil	4.40
Limestone	7.00
Salt	0.30
Tricalcium phosphate (18% P)	1.40
DL-Met (50%)	0.10
Vitamin premix[1]	0.10
Trace mineral premix[2]	0.10
Calculated value[3]	
AME, kcal/kg	2,923
CP, %	15.15
Lys, %	0.84
Met + Cys, %	0.90
Ca, %	3.05
P, %	0.42

[1]Provided per kg of complete diet: 125,000 IU of vitamin A; 2,500 IU of vitamin D_3; 10 mg of vitamin E; 2 mg of vitamin K_3; 1 mg of vitamin B_1; 5 mg of vitamin B_2; 1 mg of vitamin B_6; 15 mg of vitamin B_{12}; 500 mg of folic acid; 35,000 mg of niacin; 10,000 mg of Ca-pantothenate; and 50 mg of biotin.

[2]Provided per kg of complete diet: 8 mg of Mn (as MnO_2); 60 mg of Zn (as $ZnSO_4$); 5 mg of Cu (as $CuSO_4 \cdot 5H_2O$); 40 mg of Fe (as $FeSO_4 \cdot 7H_2O$); 1.5 mg of I (as KI); and 0.15 mg of Se (as $Na_2SeO_3 \cdot 5H_2O$).

[3]Based on ingredients composition provided by National Research Council (1994).

Tokyo, Japan). Finally, the albumin height, albumin weight, yolk color, yolk weight, and Haugh unit were evaluated using an egg multitester (Tohoku Rhythm, Tokyo, Japan). The determination of Haugh units according to the formula: Haugh units = $100 \times \log (H + 7.57 - 1.7\ W^{0.37})$, where H is the albumen height and W is the weight of the egg (Card and Nesheim, 1972).

At the end of 3 and 6 wk, 6 laying hens (1 bird from each replicate) were randomly selected from each treatment, and 2 mL blood samples from each bird were collected from the wing vein by using a sterilized syringe with needle and then the samples were transferred into the vacuum tubes (5 mL, anticoagulant free. Xiangyuan Medical Ltd., Hebei, China). For serum analysis, the blood samples were centrifuged at 2,000 × *g* at 4°C for 20 min to separate the serum samples and stored at −80°C to determine the antioxidant parameters. The activities of the total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), catalase (CAT), and malondialdehyde (MDA) in serum were measured by using the kits from Jiancheng Bioengineering Institute (Nanjing, China) according to manufacturer's protocols. The commercial kits details as follows: T-SOD assay kit, WST-1 method, 96T, A001-3-2. GSH-Px assay kit, colorimetric method, 100T, A005-1-2. CAT assay kit, visible light method, A007-1-1. MDA assay kit, colorimetric method, 400T, A003-4-1.

At the termination of the experiment, 24 hens (1 per replicate) were slaughtered by cervical dislocation. The jejunum were separated, and approximately 2 g digesta from jejunum (binding terminal of jejunum-ileum) were collected. The digesta samples were diluted 10-fold with ice-cold PBS (pH 7.0), homogenized for 1 min, and sonicated for 1 min with 3 cycles at 30 s intervals. The samples were then centrifuged at 8,000 × *g* for 20 min at 4°C. The supernatants were divided into small portions and stored at −80°C for digestive enzyme activities analysis. Approximately 2 cm segments of the jejunum at the middle location were collected and fixed in 10% buffered formalin until morphology analysis. Subsequently, the liver and remaining jejunum were rinsed by ice-cold PBS (pH 7.0), and then approximately 2 g liver and jejunal samples were collected immediately for antioxidant analysis. The trypsin, amylase and protease of jejunal digesta, as well as the T-SOD, GSH-Px, CAT, and MDA of liver and jejunum were measured using commercial kits (Jiancheng Bioengineering Institute, Nanjing, China) according to manufacturer's protocols. The antioxidant kits details were described as front section, trypsin assay kit used ultraviolet colorimetry method, 50T, A080-2-2. Amylase assay kit used starch-iodine colorimetry method, 100T, C016-1-1. Protease assay kit used colorimetric method, 50T, A080-1-3. The jejunal segments were processed and stained with hematoxylin–eosin using standard histological techniques (Yamauchi et al., 2006). Villus height and crypt depth of jejunum were measured at 40× magnification using computer software (Olympus, DP72), and then villus height to crypt depth ratio was calculated.

Statistical Analyses

Data were analyzed by using GLM procedure of SAS 9.0 (SAS, 2009. SAS Institute Inc., Cary, NC) for a completely randomized block design. Replicates were the experimental units. Data were expressed as means. Differences among means were tested by using Tukey's test. Orthogonal polynomial contrasts were used to test the linear and quadratic effects of the increasing levels of dietary MDP. Variability in data was expressed as the standard error of mean, $P < 0.05$ was considered to be statistically significant, and $0.05 \leq P < 0.10$ was considered to be a tendency.

RESULTS

Productive Performance

The effects of MDP on productive performance are showed in Table 2. During 3 to 4 wk, dietary supplementation of graded levels of MDP tended to linearly and quadratically increase egg production ($P < 0.10$). During 5 to 6 wk and 1 to 6 wk, as dietary MDP concentration increased, the egg production quadratically improved ($P < 0.05$). In addition, there was a linear reduction in cracked egg rate ($P < 0.05$) with dietary MDP levels increased during 3 to 4 and 1 to 6 wk. However, MDP supplementation had no significant effect on egg weight, average daily feed intake, and feed efficiency ($P > 0.10$) throughout the experimental period.

Table 2. Effects of dietary marine-derived polysaccharides (MDP) on productive performance in late-phase laying hens.

	Dietary MDP levels (mg/kg)					*P*-value	
Items	0	1,000	2,500	5,000	SEM[1]	Linear	Quadratic
Egg production, %							
1–2 wk	60.52	63.68	63.14	61.61	1.50	0.692	0.140
3–4 wk	60.97^{a}	67.64^{b}	67.32^{b}	66.34^{a,b}	1.94	0.088	0.067
5–6 wk	63.31^{a}	69.13^{b}	67.31^{a,b}	65.79^{a,b}	1.38	0.376	0.018
1–6 wk	61.52^{a}	66.91^{b}	65.66^{b}	64.92^{b}	1.08	0.083	0.013
Cracked egg rate, %							
1–2 wk	12.07	12.24	11.44	11.43	0.53	0.273	0.864
3–4 wk	12.62^{a}	11.40^{a,b}	11.25^{b}	11.10^{b}	0.42	0.025	0.224
5–6 wk	12.47	11.52	12.41	11.79	0.40	0.532	0.678
1–6 wk	12.43^{a}	11.68^{a,b}	11.72^{a,b}	11.46^{b}	0.25	0.023	0.356
Egg weight, g							
1–2 wk	58.91	57.69	57.67	58.85	0.78	0.953	0.147
3–4 wk	59.16	56.65	58.20	58.69	0.89	0.974	0.114
5–6 wk	58.93	58.72	57.43	57.77	1.09	0.344	0.805
1–6 wk	58.99	57.59	57.73	58.34	0.69	0.570	0.166
Average daily feed intake, g							
1–2 wk	100.75	99.88	98.14	102.32	2.25	0.770	0.279
3–4 wk	99.62	98.95	100.17	99.10	3.24	0.992	0.960
5–6 wk	98.91	102.49	97.76	98.93	2.60	0.696	0.649
1–6 wk	99.76	100.43	98.69	100.15	1.57	0.934	0.809
Feed efficiency, g of feed/g of egg							
1–2 wk	2.83	2.72	2.70	2.85	0.10	0.944	0.235
3–4 wk	2.77	2.60	2.57	2.55	0.11	0.175	0.517
5–6 wk	2.66	2.53	2.54	2.61	0.12	0.777	0.383
1–6 wk	2.75	2.61	2.60	2.65	0.06	0.275	0.162

[a,b]Means in the same row with different letters are significantly different at $P < 0.05$.
[1]SEM, standard error of mean.

Egg Quality

As described in Table 3, inclusion of MDP in laying hens diets had a quadratic effect in increasing egg shape index ($P < 0.05$) and tended to linearly improve the Haugh unit ($P < 0.10$) at the end of second week. After 4 wk of feeding trial, the egg shell thickness, yolk color, and Haugh unit showed a linear increase ($P < 0.05$) in response to increasing dietary MDP levels, and supplemental MDP tended to linearly and quadratically ($P < 0.10$) increase the egg shell weight. Besides, the egg shell breaking strength, egg shell thickness, yolk color, and Haugh unit were improved linearly ($P < 0.05$) with increasing concentration of MDP in the laying hens diets at the end of the experiment.

Antioxidant Capacity

The effects of MDP on antioxidant capacity in serum, liver, and jejunum are presented in Tables 4 and 5. At the end of third week, dietary MDP showed a linear and quadratic reduction in serum MDA content ($P < 0.05$). At the end of the feeding trial, the activity of T-SOD in serum was increased quadratically ($P < 0.05$) as dietary MDP concentration increased, and dietary MDP tended to quadratically decrease serum MDA levels ($P < 0.10$). Meanwhile, increasing dietary MDP concentration quadratically improved the liver CAT activity ($P < 0.05$), whereas had a tendency to linearly and quadratically reduce MDA content in liver ($P < 0.10$). Besides, dietary supplementation of different levels of MDP linearly enhanced jejunal CAT activity ($P < 0.05$), whereas linearly decreased jejunal MDA concentration ($P < 0.05$). Also, dietary MDP tended to linearly and quadratically increase jejunal T-SOD activity ($P < 0.10$).

Jejunal Morphology and Digestive Enzyme Activity

As shown in Table 6, the results revealed that supplemental MDP linearly improved the villus height ($P < 0.05$) and quadratically increased the ratio of villus height/crypt depth ($P < 0.05$) in jejunum. However, dietary MDP did not affect jejunal digestive enzyme activity ($P > 0.10$), including trypsin, amylase, and protease.

DISCUSSION

Functional polysaccharides have been used as prebiotics and good alternatives to antibiotics in poultry nutrition during recent decades. However, no studies until today have reported the use of MDP from seaweed *Enteromorpha* in late-phase laying hens. The results of the present study first showed the potential of MDP from seaweed *Enteromorpha* to improve the egg production in laying hens during late laying period. Similar findings in regard to MDP of COS were obtained by Meng et al. (2010) and Swiątkiewicz et al. (2013), who reported that COS supplementation resulted in an increase in laying performance of hens during peak-laying period. Other sources of prebiotics

Table 3. Effects of dietary marine-derived polysaccharides (MDP) on egg quality in late-phase laying hens.

	Dietary MDP levels (mg/kg)					*P*-value	
Items	0	1,000	2,500	5,000	SEM[1]	Linear	Quadratic
2 wk							
Egg shape index	1.31[a]	1.35[b]	1.35[b]	1.33[a,b]	0.01	0.123	0.035
Egg shell breaking strength, kg/cm^2	3.17	3.38	3.18	3.47	0.16	0.339	0.793
Egg shell thickness, 10^{-2} mm	33.70	36.11	33.00	33.21	1.14	0.383	0.351
Egg shell weight, g	5.94	6.02	5.85	5.68	0.17	0.239	0.470
Albumin height, mm	3.46	3.83	3.55	3.90	0.20	0.267	0.974
Yolk color	8.28	8.09	8.53	8.42	0.31	0.542	0.905
Yolk weight, g	17.39	17.54	17.11	17.46	0.48	0.915	0.833
Albumin weight, g	38.99	37.40	35.99	36.58	1.32	0.173	0.291
Haugh unit	48.36[a]	54.72[a,b]	52.83[a,b]	56.54[b]	2.64	0.074	0.622
4 wk							
Egg shape index	1.31	1.32	1.32	1.32	0.01	0.597	0.807
Egg shell breaking strength, kg/cm^2	3.06	3.28	3.38	3.04	0.17	0.954	0.121
Egg shell thickness, 10^{-2} mm	31.68[a]	32.42[a,b]	33.87[a,b]	36.27[b]	0.91	0.002	0.378
Egg shell weight, g	5.77[a]	6.28[b]	6.03[a,b]	6.14[b]	0.10	0.087	0.079
Albumin height, mm	4.28	4.55	4.31	4.20	0.24	0.654	0.442
Yolk color	7.07[a]	7.90[a,b]	8.70[b]	8.10[b]	0.29	0.009	0.027
Yolk weight, g	17.44	16.83	17.25	16.84	0.28	0.288	0.732
Albumin weight, g	39.29	38.71	38.05	38.31	0.84	0.354	0.625
Haugh unit	54.60[a]	62.98[b]	62.50[b]	62.31[b]	2.12	0.030	0.061
6 wk							
Egg shape index	1.28	1.33	1.32	1.31	0.02	0.265	0.201
Egg shell breaking strength, kg/cm^2	2.79[a]	3.34[b]	3.33[b]	3.29[b]	0.14	0.034	0.053
Egg shell thickness, 10^{-2} mm	31.19[a]	32.60[a,b]	34.25[b]	35.36[b]	0.82	0.002	0.858
Egg shell weight, g	5.96	5.97	5.98	6.09	0.08	0.260	0.561
Albumin height, mm	3.06	3.28	3.38	3.04	0.17	0.954	0.121
Yolk color	6.87[a]	8.28[b]	8.57[b]	8.32[b]	0.25	0.001	0.005
Yolk weight, g	16.95	16.67	17.02	16.58	0.31	0.963	0.898
Albumin weight, g	37.62	36.84	35.88	37.22	0.78	0.607	0.191
Haugh unit	43.91[a]	53.95[b]	54.49[b]	53.88[b]	1.76	0.002	0.008

[a,b]Means in the same row with different letters are significantly different at $P < 0.05$.
[1]SEM, standard error of mean.

polysaccharides, including mannan-oligosaccharide and inulin have been also reported to improve the egg production of aged laying hens (61–66 wk and 64–75 wk of age) (Abdelqader et al., 2013; Jahanian and Ashnagar, 2015). These improvements might be linked to the promoting effects of prebiotics on metabolic processes and utilization of nutrients (Swiatkiewicz et al., 2015). On the other hand, previous studies have confirmed that the MDP not only has various biological activities, including protection of gastrointestinal tract and reproductive organs, but also has other pharmacological functions such as anti-inflammatory and antiviral effects, immunological regulation, and resistance to oxidation (Kim et al., 2003; Wei et al., 2014; Swiatkiewicz et al., 2015). These beneficial properties of MDP could alleviate the deleterious effects of age on reproductive performance in laying hens. However, Yan et al. (2010) observed that inclusion of 100 or 200 mg/kg MDP of COS in the layer diets had no effect on egg production, feed consumption, and egg weight. The discrepancies among these studies might be because of the differences in the polysaccharide composition, supplemental dose, and age of hens, as well as experimental conditions. Meanwhile, in this study, the findings suggested that a

Table 4. Effects of dietary marine-derived polysaccharides (MDP) on serum antioxidant capacity in late-phase laying hens.

	Dietary MDP levels (mg/kg)					*P*-value	
Items[1]	0	1,000	2,500	5,000	SEM[2]	Linear	Quadratic
3 wk							
T-SOD, U/mL	499.59	556.19	504.79	510.84	59.26	0.949	0.685
GSH-Px, U/0.1 mL	1253.51	1094.74	1523.68	1121.05	149.27	0.964	0.445
CAT, U/mL	5.79	3.76	4.02	3.46	1.19	0.253	0.561
MDA, nmol/mL	12.00[a]	6.89[b]	7.34[b]	8.05[b]	0.95	0.035	0.023
6 wk							
T-SOD, U/mL	393.53[a]	491.06[b]	493.87[b]	469.75[a,b]	25.59	0.080	0.045
GSH-Px, U/0.1 mL	1108.77	1333.35	1223.68	1212.28	97.17	0.816	0.270
CAT, U/mL	1.13	1.30	1.52	2.40	0.82	0.310	0.679
MDA, nmol/mL	8.67	5.11	5.88	7.33	1.10	0.538	0.064

[a,b]Means in the same row with different letters are significantly different at $P < 0.05$.
[1]T-SOD, total superoxide dismutase; GSH-Px, glutathione peroxidase; CAT, catalase; MDA, malondialdehyde.
[2]SEM, standard error of mean.

Table 5. Effects of dietary marine-derived polysaccharides (MDP) on antioxidant capacity of liver and jejunum in late-phase laying hens after 6 wk feeding trial.

	Dietary MDP levels (mg/kg)					*P*-value	
Items[1]	0	1,000	2,500	5,000	SEM[2]	Linear	Quadratic
Liver							
T-SOD, U/mg prot	284.30	266.77	277.94	320.80	33.00	0.445	0.396
GSH-Px, U/mg prot	4.89	9.64	8.12	18.47	5.81	0.181	0.646
CAT, U/mg prot	6.05^{a}	14.98^{b}	14.97^{b}	12.55^{a,b}	1.89	0.061	0.024
MDA, nmol/mg prot	0.74^{a}	0.38^{b}	0.40^{a,b}	0.43^{a,b}	0.09	0.082	0.088
Jejunum							
T-SOD, U/mg prot	318.03^{a}	415.48^{b}	410.88^{b}	404.44^{a,b}	25.36	0.066	0.087
GSH-Px, U/mg prot	103.84	108.93	91.93	93.09	19.62	0.595	0.924
CAT, U/mg prot	2.67	4.42	5.65	4.95	0.52	0.014	0.058
MDA, nmol/mg prot	0.46^{a}	0.25^{b}	0.24^{b}	0.26^{b}	0.05	0.026	0.054

^{a,b}Means in the same row with different letters are significantly different at $P < 0.05$.
[1]T-SOD, total superoxide dismutase; GSH-Px, glutathione peroxidase; CAT, catalase; MDA, malondialdehyde.
[2]SEM, standard error of mean.

dietary supplementation level of 1,000 mg/kg MDP of seaweed *Enteromorpha* in late-phase laying hens diets resulted in the best improvement in productive performance in terms of egg laying rate.

Egg shell quality is an important parameter which influences the economic profitability of egg productivity and hatchability. It has been estimated that eggs with damaged shells account for 6 to 10% of all eggs produced worldwide, which leads to great economic losses (Ketta and Tůmová, 2016). One of the main concerns is a decrease in egg shell quality as the hen ages because of an increase in egg weight without an increase in the amount of calcium carbonate deposited in the shell (Etches, 1998). Besides, the decrease in egg shell quality in aged laying hens was also attributed to the decline in intestinal Ca absorption efficiency compared with the younger hens (Roland, 1988). In this study, the tendency toward a decline in egg shell quality as the hens gets older was observed in control group, whereas dietary addition of MDP improved egg shell quality and reduced the rate of crack. Consistently, Swiatkiewicz (2010) demonstrated that dietary prebiotics inulin and oligofructose improved egg shell quality traits of aged laying hens (70-weeks-old). Abdelqader et al. (2013) also showed a positive effect of prebiotics inulin on egg shell weight and egg shell thickness in laying hens during 64 to 75 wk of age. On the contrary, Meng et al. (2010) and Yan et al. (2010) found that COS supplementation did not lead to significant changes in egg shell quality of peak-laying hens. The inconsistent findings suggest that the effects of dietary MDP on egg shell indices of hens could vary, and it might depend on the laying period. Based on the current experiment and previous studies, MDP and other prebiotics might increase intestinal absorption and bioavailability of calcium and thereby improving egg shell quality, but the specific mechanism warrants further investigation. This study also showed beneficial effects of MDP on yolk color and Haugh unit in late-phase laying hens. Similarly, Jahanian and Ashnagar (2015) indicated that the dietary mannan-oligosaccharides improved the egg Haugh unit of laying hens at 66 wk of age. Meng et al. (2010) and Yan et al. (2010) also confirmed that COS supplements could enhance yolk color and Haugh unit in peak-laying hens. Yolk color and Haugh unit were directly associated with the consumer preferences and shelf life (Williams, 1992; Vlckova et al., 2019). The improvements in yolk color observed in this study may be because of the brown MDP supplements was conducive to pigment deposition in the yolk. Therefore, it is interesting to explore the positive effect of MDP on yolk color and Haugh unit in this study.

It is well known that the activities of SOD, GSH-Px, and CAT serve as protective responses to eliminate

Table 6. Effects of dietary marine-derived polysaccharides (MDP) on jejunal morphology and digestive enzyme activity in late-phase laying hens after 6 wk feeding trial.

	Dietary MDP levels (mg/kg)					*P*-value	
Items	0	1,000	2,500	5,000	SEM[1]	Linear	Quadratic
Jejunal morphology							
Villus height, μm	812.65^{a}	916.02^{b}	927.82^{b}	923.93^{b}	25.00	0.021	0.076
Crypt depth, μm	292.16	262.85	262.51	297.88	16.24	0.830	0.104
Villus height: crypt depth	2.78^{a}	3.49^{b}	3.60^{b}	3.11^{a,b}	0.17	0.214	0.013
Jejunal digestive enzyme activity, U/mg prot							
Trypsin	32.97	33.76	33.84	35.52	1.40	0.265	0.766
Amylase	21.33	21.50	21.66	21.53	0.83	0.846	0.860
Protease	55.32	49.57	53.12	50.05	2.43	0.302	0.600

^{a,b}Means in the same row with different letters are significantly different at $P < 0.05$.
[1]SEM, standard error of mean.

reactive free radicals (Yin et al., 2013). As a terminal product of lipid peroxidation, the MDA is generally used as a biomarker to measure the level of oxidative stress in an organism (Yang et al., 2008). After the peak-laying period, the aged hens accumulated numerous free radicals in the body, also, free radical scavenging ability of aged hens is lower than younger hens (Etches, 1998). Excessive amounts of free radicals can affect functional proteins, unsaturated fatty acids, and nucleic acids, thus causing oxidative damage to the organism function and leading to a decline in reproductive performance (Liu et al., 2018). The current study showed that supplementation with MDP enhanced activity of T-SOD in serum, and the activity of CAT in liver and jejunum but reduced the MDA levels of serum, liver, and jejunum. The improved antioxidant capacity of aged layers by dietary MDP might also contribute to the improvement in productive performance of hens in the present study. These results were in agreement with previous report of Bozkurt et al. (2012), who observed that inclusion of mannan-oligosaccharide in hens diets could promote liver SOD activity. According to an *in vitro* study, MDP of seaweed *Enteromorpha* was found to exert excellent antioxidant properties (Duan et al., 2015). In broilers, Sun et al. (2010) also found that MDP of seaweed *Enteromorpha* improved serum SOD and GSH-Px activities, whereas reduced serum MDA concentrations. Unfortunately, little or no attention has been paid to the fact that free radical formation was reduced in body tissues when aged laying hens were fed MDP of seaweed *Enteromorpha*, and this served to highlight the lack of knowledge in this area.

Several researchers have demonstrated that feeding prebiotics, such as inulin and xylo-oligosaccharides, could improve the intestinal morphology of laying hens (Abdelqader et al., 2013; Ding et al., 2018). The prebiotics MDP, in this study, also showed positive effects on jejunal villus height and the ratio of villus height/crypt depth in aged hens. In contrast, Adhikari et al. (2018) reported that dietary inclusion of prebiotics fructo-oligosaccharide had no effect on ileal morphology in laying hens. These differences among studies may be because of several factors, including the prebiotics used, production stage, and trial conditions. Prebiotics work as substrate for the intestinal microflora and could stimulate the fermentation rate which increased the production of short-chain fatty acids (SCFA) (Rémésy et al., 1993). The SCFA benefit the differentiation and proliferation of enterocytes, thereby resulting in a better intestinal structure and nutrients utilization. However, we found that addition of MDP produced no differences in terms of jejunal digestive enzyme activity. It is suggested that the increase in productive performance may be because of the improvement of intestinal structure rather than by affecting the activity of digestive enzymes. However, research in this field is rare and deserves further study.

In summary, the present study indicated that dietary MDP of seaweed *Enteromorpha* increased egg production, egg shell quality, Haugh units, antioxidant capacity, and jejunal villus height and reduced cracked egg rate in laying hens during the late laying period. Our data suggest that the optimum concentration of MDP was 1,000 or 2,500 mg/kg in the basal diets to achieve an improvement in egg production and egg quality of aged hens after a 6 wk feeding trial.

ACKNOWLEDGMENTS

The present study was supported by the Natural Science Foundation of Guangdong Province (2018A030307023), Talent Research Start-up Project of Guangdong Ocean University (R18007, R17103), South China Sea Scholar by Guangdong Ocean University (573118025), Innovative Strong School Engineering Youth Talent Project (2017KQNCX090) and Key Platform Projects (2018302) by Department of Education in Guangdong Province, and National Research Foundation grant (2018R1C1B5086232) funded by Korean Government (MEST).

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