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Original Article

Effect of replacement feed ingredients of *Micropterus salmoides* in exotic species

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ABSTRACT

Background: Largemouth bass (*Micropterus salmoides*) is introduced species that has caused aquatic ecology activity both *in vitro* and *in vivo* were investigated for the possibility of application of the bass extract as an alternative feed ingredient.

Methods: The bass oil was extracted using a 1-L supercritical extractor, while the protein was extracted from 250 g of bass dry matter, which was dissolved in 1 mL of H_2O at 50℃. Both oil and protein extracts were evaluated antioxidant activities and the level of DPPH radical scavenging assay and nitric oxide (NO) production assay with lipopolysaccharide response. Oral administration of 6.6 μL/g bass protein and 5.38 μL/g bass oil conducted for investigating serological and physiological effect.

Results: DPPH radical scavenging showed similar radical scavenging ability of 50 μM of ascorbic acid at 200 μg of protein and 10% of oil treatment. NO concentration was diminished by the treatment of bass oil. Oral administration of both bass oil and proteins to mice showed that the body weight increase rate of the bass oil treated group was significantly reduced by 1.55 g compared to the other groups. The number of white blood cells (WBC) was increased by 4.52 k/μL in the bass protein-treated group and 4.44 k/μL in the bass oil-treated group compared to the control group. However, the serum IgG level did not show a significant difference between the bass extract-treated groups and the control group.

Conclusions: These studies demonstrate that both bass oil and proteins extracted from the bass not only provide excellent effects of antioxidant and immune activity but can also be used as functional food supplements.

Keywords: antioxidant, diet, immune modulation, largemouth bass, *Micropterus salmoides*

INTRODUCTION

Largemouth bass (*Micropterus salmoides*) was introduced in the 1960s to South Korea, and they spread fast to native ecosystems (Jang et al., 2002). The density of *M. salmoides*' population was especially high in floodplain lakes and reservoirs directly connected to the main river channel (Jang et al., 2002). Furthermore, recent research predicts that the population of *M. salmoides*' will increase due to climate change in the future of Korean aquatic ecology (Mamun et al., 2018). These could impact a native ecosystem and cause problems including habitat alterations, introduction of diseases or parasites, hybridization with the native species, and tropic alteration and spatial

alteration (Ross, 1991). Therefore, the requirement of finding ways to consume them as nutrition forage to reduce the population of ecosystem-disturbing species has been demanded.

Fish oil, including omega-3 fatty acids, is a well-known supportive nutraceutical supplement for heart health, preventing coronary heart disease, body composition reconstitution, weight loss, and reducing inflammation factors such as eicosanoids, cytokines, reactive oxygen species (ROS), and expression of adhesion molecules (Kromhout et al., 1985; Calder, 2006; Hill et al., 2007). Recently, the consumption of freshwater fish, which contains a sufficient amount of beneficial omega-3 fatty acids, has been shown to improve human health (Jaya-Ram et al., 2018). In addition, a comparative study of lipid content between freshwater fish and marine fish has revealed that freshwater fish can serve as a replacement for marine resources in supplying omega-3 fatty acids (Li et al., 2011).

Not only does fish oil, but other components as well show physiological effects. The protein of freshwater fish is easy to digest and absorb (Steffens, 2006), and fish protein has efficiency with 96% biological value and 80% net protein utilization (Wasim, 2007). Fish protein contains abundant essential amino acids, including arginine, leucine, and lysine (Steffens, 2006). Freshwater fish meat is also a good source of minerals including calcium, phosphorus, and iron. Additionally, it has been found to contain high levels of vitamin B particularly riboflavin and niacin (Tee et al., 1989).

Dried fish is more useful than raw fish in effectively providing nutritional factors. Raw fish typically contains about 70% moisture, but drying it reduces the moisture content to about 7%, resulting in a higher proportion of protein components in the total volume (Kasozi et al., 2018). Moreover, converting dried fish into powder is a beneficial utilization method for low-value fish and fish byproducts. Powdered fish is relatively safe from microbial pathogens, reduces sugar absorption, and lowers blood pH (Abbey et al., 2016). These physiological effects could help to reduce oxidative stress, manage type 2 diabetes, and improve digestive system function (Desai et al., 2018).

Previous studies on freshwater fish have primarily focused on their nutritional importance, demonstrating their physiological effects when consumed as food. However, in the present study, the functional aspects of bass extracts were investigated, specifically examining the potential benefits of both oil and proteins derived from the largemouth bass' flesh. This study aimed to determine the optimal extraction conditions for obtaining the maximum nutritional factors from both the oil and protein components.

MATERIALS AND METHODS

Extraction and analysis of oil and protein from *Micropterus salmoides*

Largemouth bass oil was extracted from 1 kg of dried largemouth bass using a 1 L supercritical extractor from Ballys Co. (Incheon, Korea). The extraction conditions were set at temperatures of 40℃ and 50℃, and pressures of 100 bar, 200 bar, and 300 bar. The analysis of oil components was conducted at the Animal Nutrition Physiology Laboratory, Department of Animal Science and Technology, Konkuk University (Seoul, Korea). Following the oil extraction, the protein was extracted from 50 g of the remaining largemouth bass jerky using 200 mL of water as a solvent. The supernatant was collected after centrifugation at 3,000 RPM for 10 min. The extraction conditions for the protein were set at a temperature of 50℃ and pH 10. The measurement of taurine in the crude protein was carried out at the Korea Feed Ingredients Association (Seoul, Korea).

DPPH radical scavenging activity of oil and protein

Ascorbic acid, 0.25 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH), and samples were solved in 100% ethanol (CAS#64-17-5; MERCK, New Jersey, USA) and mixed at the same volume and then incubated with preventing light for 20 min at room temperature. Ascorbic acids as positive control was distributed at concentrations of 1, 5, 10, 50, 100, and 200 μM per well. The concentration of proteins was 5, 10, 20, 50, 100, and 200 μg per well and the concentration of oils was at 1, 2, 3, 4, 5 and 10% of total media volume per well. Each sample was distributed each well in 96 well plates as triple repeats. The absorbance was measured at 517 nm using a SUNRISE-BASIC TECAN Microplate reader (TECAN, Männedorf, Switzerland). Calculation of the DPPH radical scavenging formula was followed as DPPH radical scavenging ability (%) = $(1-A_s/A_c) \times 100$ (A_s = absorbance of sample, A_c = absorbance of positive control).

Nitric oxide assay of bass extraction and mixture

RAW 264.7 cells were cultured at 37℃, 5% CO_2 with DMEM medium (Welgene, Gyeongsan, Korea) and 5 × 10^5 cell/well density in 24 well plates. After 24 h of incubation, each well was washed with DPBS and replaced with phenol red-free DMEM medium plus 10% fetal bovine serum (FBS; Welgene, Gyeongsan, Korea) and 1% penicillin/streptomycin (P/S; Lonza, Basel, Switzerland) containing different concentrations of bass protein, oil and mixture. After an hour of treatment, 1 μg/mL of Lipopolysaccharide (LPS; L4130-100M; Sigma-Aldrich, St. Louis, USA) was added to each well for 24 h. Nitric Oxide (NO) production levels of supernatants were measured using the Griess assay. 100 μL supernatant was mixed with 100 μL of Griess reagent (Sigma-Aldrich, St. Louis, USA). After incubation at room temperature for 15 min, the absorbance was measured using a microplate reader at 540 nm.

Cytotoxic analysis of bass extraction in Madin-Darby canine kidney cells

The dog's normal kidney cell line, Madin-Darby canine kidney (MDCK) cells were cultured at 37℃, 5% CO_2 with MEM medium (Welgene, Gyeongsan, Korea) containing 10% FBS and 1% P/S. MDCK cells were cultured in approximately 80% of the culture dish area every 2 to 3 days and then passaged. To emulsify oil in the medium, 1.5% tween 80 detergent (Sigma-Aldrich, St. Louis, USA) was mixed in a MEM cell culture medium. The only control was cultured with MEM Media. MDCK cells were distributed to each well of 24 well plates as 5 × 10^4cell/well. Samples were filtered through a 0.45 μM pore size syringe filter (Sartorius, Göttingen, Germany). The concentration of proteins was set as 10, 50, 100, 200, 400 and 600 μg and the concentration of oil was set as 1, 2, 3, 4, 5, 10 and 20% with a volume of 12 μL per well. After MDCK cell seeding, protein and oil samples were treated each well with the 24-hour culture. For the WST-1 assay, 50 μL of EZ-Cytox reagent (DoGenBio, Seoul, Korea) was treated in each well for an hour. Absorbance was measured at 450 nm with a microplate reader.

Oral administration to mice and weight measurement

All procedures for this study were approved by the Institutional Animal Care and Use Committee of Konkuk University (approval number: KU22169). Six-week-old male ICR mice were purchased from DBL Co. Ltd (Seoul, Korea). 20 mice were set up in one group, 10 mice were sacrificed at two weeks and the other mice were sacrificed at four weeks. control groups were orally administered with D.W., and experimental groups were orally administered 6.6 μL/g bass protein and 5.38 μL/g bass oil. Mice administrated the bass extractions every Monday, Wednesday, and Friday for 4 weeks by oral administration with 0.9 × 50 mm Zonde needle (Jeung Do Bio & Plant Co., Seoul, Korea). All samples were administrated in proportion to the average body weight of the mice group. Bodyweight measurements were conducted every Monday and Thursday for 4 weeks.

Blood collection and analysis in mice

Before blood collection, mice anesthesia was performed by intraperitoneal injection of 1 mL of 20 mg/mL 2,2,2-tribromoethanol (Sigma-Aldrich, St. Louis, USA) dilute solution. After the laparotomy, blood was collected into 1.5 mL tubes for serum separation and about 1 mL of blood to ethylenediaminetetraacetic acid (EDTA) containing tube (Becton Dickinson, New Jersey, USA) from the left atrium of each mouse heart. A complete blood count (CBC) test was performed with 1 mL of complete blood at Neodin Biovet Co. (Seoul, Korea). Blood was centrifuged at 15,000 RPM for 30 min for serum separation. 500 g of trichloroacetic acid (TCA; Alfa Aesar, Massachusetts, USA) was dissolved in 350 mL D.W. for collecting serum proteins by protein precipitation. Serum and 20% TCA solution of total mixture volume were mixed in ice for 30 min. TCA and serum mixtures were centrifuged at 1,500 RPM, 4℃ for 15 min. After removing the supernatant, 100% cold ethanol was used for washing, and protein was centrifuged at 1,600 RPM, 4℃ for 5 min. After supernatant removal and the pellet dried, the protein was dissolved with 50 μL RIPA buffer (Thermo Fisher Scientific, Massachusetts, USA). These proteins were used for IgG ELISA analysis.

Enzyme-linked immunosorbent assay for Immunoglobulin G analysis

Immunoglobulin G (IgG) concentration analysis was performed by Mouse IgG ELISA Kit (CAS#E99-131; Bethyl Laboratories, Texas, USA) and followed their protocol. All samples and standards were added 100 μL to each well and repeated twice. Absorbance was measured at 450 nm wavelength by a microplate reader and calculated quality control standards defined by Bethyl Laboratories.

Statistical analysis

IBM SPSS statistics 24 ver. 15.0 (IBM Corp., New York, USA) was used for data analysis. All data were expressed as the average value. The *t*-test and Turkey's multiple comparison tests were used for statistical testing between the control and the sample. When the probability is < 0.05, the null hypothesis is rejected.

RESULTS

Oil extraction and fatty acids analysis

Largemouth bass' oil was extracted under different conditions using a supercritical extractor from the flesh of largemouth bass under conditions as shown in Table 1. The conditions 1, 2, and 3 were the same at 40℃ and the pressures were extracted at 100, 200, and 300 bar. The conditions 4, 5 and 6 were the same at 50℃ and the pressures were extracted at 100, 200 and 300 bar. As shown in Table 2, condition 1 has 20.362% palmitic acid methyl ester (C16:0), 18.908% of oleic acid methyl ester (omega-9; C18:1n9c), 11.481% of palmitoleic acid methyl ester (omega-7; C16:1), 6.334% of linoleic acid methyl ester (omega-6; C18:2n6c) and 4.993% of linolenic acid methyl ester (omega-3; C18:3n3). Condition 2 has 19.866% of palmitic acid methyl ester, 18.686% of omega-9, 12.071% of omega-7, 6.353% of omega-6 and 4.912% of omega-3. Condition 3 has 19.715% of palmitic acid methyl ester, 18.98% of omega-9, 11.915% of omega-7, 6.182% of omega-6 and 4.922% of omega-3. Condition 4 has 20.085% of palmitic acid methyl ester, 18.408% omega-9, 12.717% of omega-7, 6.181% of omega-6 and 4.972% of omega-3. Oil of condition 5 has 19.826% palmitic acid methyl ester, 18.49% omega-9, 12.516% omega-7, 6.228% omega-6 and 4.943% omega-3. Oil of condition 6 has 19.897% of

Table 1. Extraction condition of oil and protein from *Micropterus salmoides*

Extract condition	1	2	3	4	5	6
Temperature (℃)	40	40	40	50	50	50
Pressure (psi)	100	200	300	100	200	300

Table 3. Nutritional ratio of bass extract

M. salmoides meat component (%)	Moisture	Crude protein	Crude fat	Crude fiber	Crude ash
	8.93	57.25	5.92	1	16.63

Table 2. Analysis of oil from *Micropterus salmoides*

Oil component (%)	Extract condition No.					
	1	2	3	4	5	6
Lauric acid methyl ester (C12:0)	0.342	0.324	0.3	0.334	0.337	0.302
Tridecanoic acid methyl ester (C13:0)	1.218	0.825	0.781	0.744	0.738	0.838
Myristic acid methyl ester (C14:0)	2.96	2.896	2.806	3.058	3.03	2.818
Myristoleic acid methyl ester (C14:1; omega-5)	0.541	0.547	0.525	0.577	0.569	0.531
Pentadecanoic acid methyl ester (C15:0)	0.639	0.654	0.638	0.656	0.653	0.621
Cis-10-pentadecenoic methyl ester	0.259	0.272	0.269	0.274	0.272	0.261
Palmitic acid methyl ester (C16:0)	20.362	19.866	19.715	20.085	19.826	19.897
Palmitoleic acid methyl ester (C16:1; omega-7)	11.481	12.071	11.915	12.717	12.516	11.923
Heptadecanoic acid methyl ester (C17:0)	1.169	1.243	1.245	1.263	1.244	1.251
Stearic acid methyl ester (C18:0)	4.109	3.937	3.952	3.833	3.671	3.964
Oleic acid methyl ester (C18:1n9c; omega-9)	18.908	18.686	18.98	18.408	18.49	18.781
Linoleic acid methyl ester (C18:2n6c; omega-6)	6.334	6.353	6.182	6.181	6.228	6.167
Linolenic acid methyl ester (C18:3n3; omega-3)	4.993	4.912	4.922	4.972	4.943	4.869
Cis-11,14-eicosadienoic acid methyl ester (C20:2; ETA)	2.958	2.921	2.891	2.957	2.884	2.849
Erucic acid methyl ester (C22:1n9; omega-9)	1.807	1.744	1.767	1.794	1.776	1.788
Cis-13,16-docosadienoic acid methyl ester (C22:2)	2.881	2.846	2.907	2.697	2.716	2.94
Lignoceric acid methyl ester (C24:0)	4.633	4.544	4.626	4.41	4.4	4.647
Unknown fatty acids	14.403	15.358	15.576	15.041	15.706	15.553
Total fatty acids	100	100	100	100	100	100

palmitic acid methyl ester, 18.781% omega-9, 11.923% of omega-7, 6.167% of omega-6, and 4.869% of omega-3.

Soluble protein extract and taurine analysis

After the extraction of oil, the remaining components in the jerky were analyzed, and the results are presented in Table 3. The components of moisture, crude protein, crude fat, crude fiber, and crude ash in jerky were 8.93, 57.25, 5.92, 1 and 16.63%, respectively. In Table 4, taurine concentrations in the jerky after oil extraction using different extraction conditions were also measured. Among the extraction conditions, conditions 3 and 6 exhibited higher taurine concentrations, with values of 6464.57 mg/kg and 6342.49 mg/kg. Both oil and proteins from extraction condition 3 were used for further analysis.

Cytotoxic effects and antioxidant activities of largemouth bass extracts

To identify the effects of cytotoxicity of both oil and proteins, a cellular proliferation assay was conducted with MDCK cells. Protein concentrations with 10, 50, 100 μg/mL did not show a diminished proliferation rate, but the viability was significantly increased to 109% with adding 100 μg/mL (Fig. 1A). However, the viability was decreased with 98.5% with adding 200 μg/mL, and 49.4% and 30.8% with adding 400 and 600 μg/mL of extracted proteins, respectively (Fig. 1A). When MDCK cells were treated with oil extracts, all treated group showed a significant increase of the viabilities (Fig. 2B). Treatment of 5% and 10% (v/v) bass oil showed over 127% increase of cell proliferation rate (Fig. 2B). Based on these findings, maximum 200 μg/mL of bass protein concentration and 10% (v/v) of oil were applied to further functional analysis of bass extracts.

The antioxidant activities of both protein and oil extracts from largemouth bass were analyzed using the DPPH radical scavenging assay and compared to the antioxidant activity of ascorbic acid. Both oil and proteins showed strong antioxidant activities in a dose-dependent manner (Fig. 2). The antioxidant activity of 200 μg/mL of bass protein extract was found to be over 65%, which was equivalent to approximately 30 μM of ascorbic acid (Fig. 2A and 2B). This indicates that the Bass protein extract possesses significant antioxidant potential. In addition, the treatment of bass oil showed a rapid increase in antioxidant activities the radical scavenging rate was over 80% with 10% (v/v) treatment and the activity was like 50 μM of ascorbic acid (Fig. 2A and 2C). Similarly, treatment with Bass oil extract resulted in a rapid increase in antioxidant activity. The radical scavenging rate exceeded

Table 4. Taurine composition of bass protein

Extract condition	1	2	3	4	5	6
Taurine of crude protein (mg/kg)	4205.65	5645.78	6464.57	5871.61	5664.64	6342.49

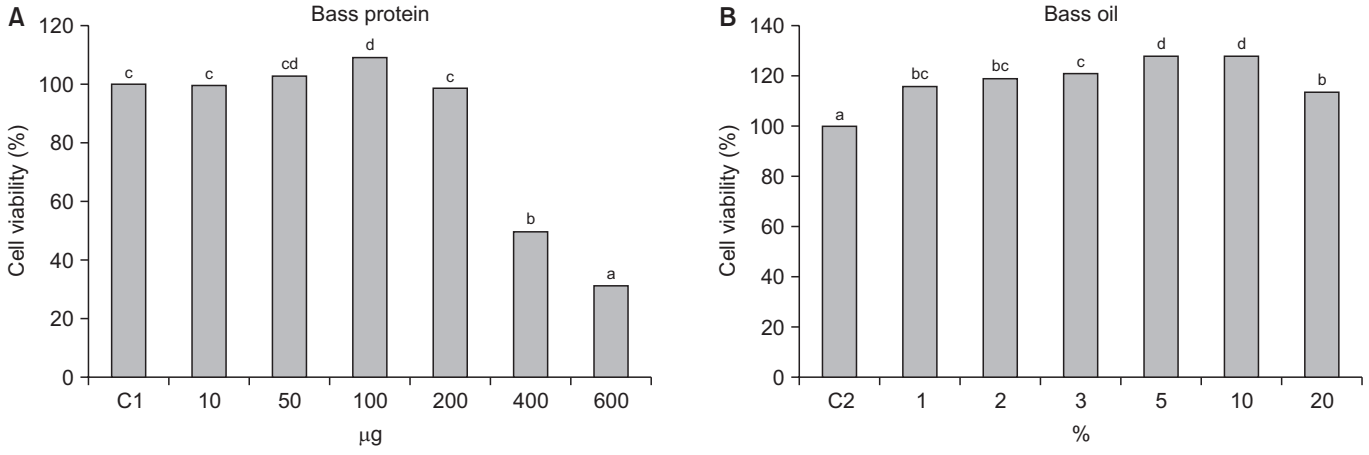

Fig. 1. WST-1 cytotoxicity assay of bass extraction with EZ-cytox reagent corresponded for an hour. (A) Cytotoxicity of bass protein and (B) cytotoxicity of bass oil. C1 is control DMEM with D.W and C2 is control DMEM including only emulsion detergent for oil. Graphs were presented as the mean of triplicate ± SEM ($p < 0.05$). Superscripts a, b, c and d indicate significant differences of cell viability with concentration of bass protein ad oil by one-way ANOVA of varience.

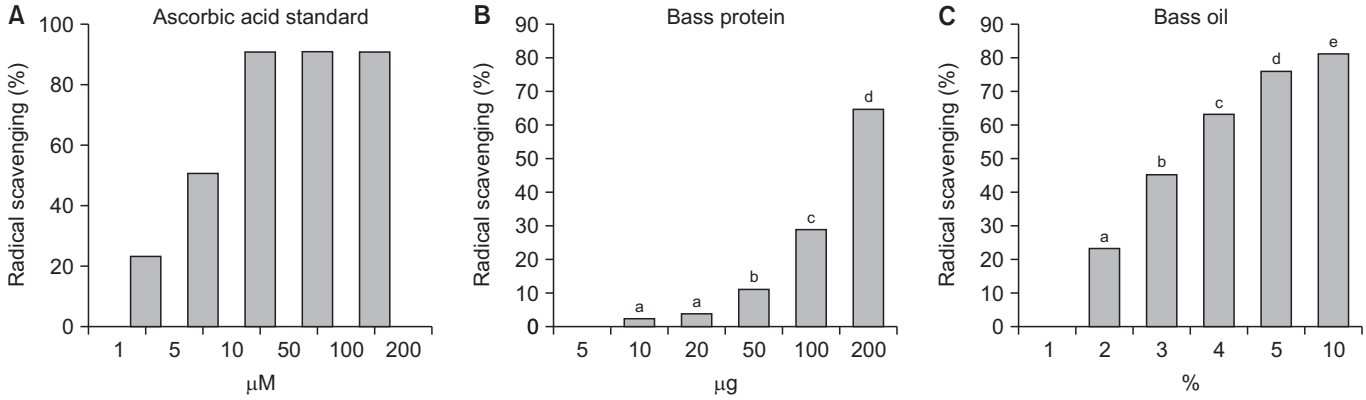

Fig. 2. Radical scavenging assay of bass extraction with DPPH (0.25 mM 2,2-diphenyl-1-picrylhydrazyl) corresponded for 30 min. (A) Ascorbic acid standard of DPPH radical scavenging assay. (B) The radical scavenging ability of bass protein. (C) The radical scavenging ability of bass oil. Graphs were presented as the mean of triplicate ± SEM ($p < 0.05$). Superscripts a, b, c, d and e indicate significant differences of radical scavenging ability depending on concentration of bass protein and oil by one-way ANOVA of variance.

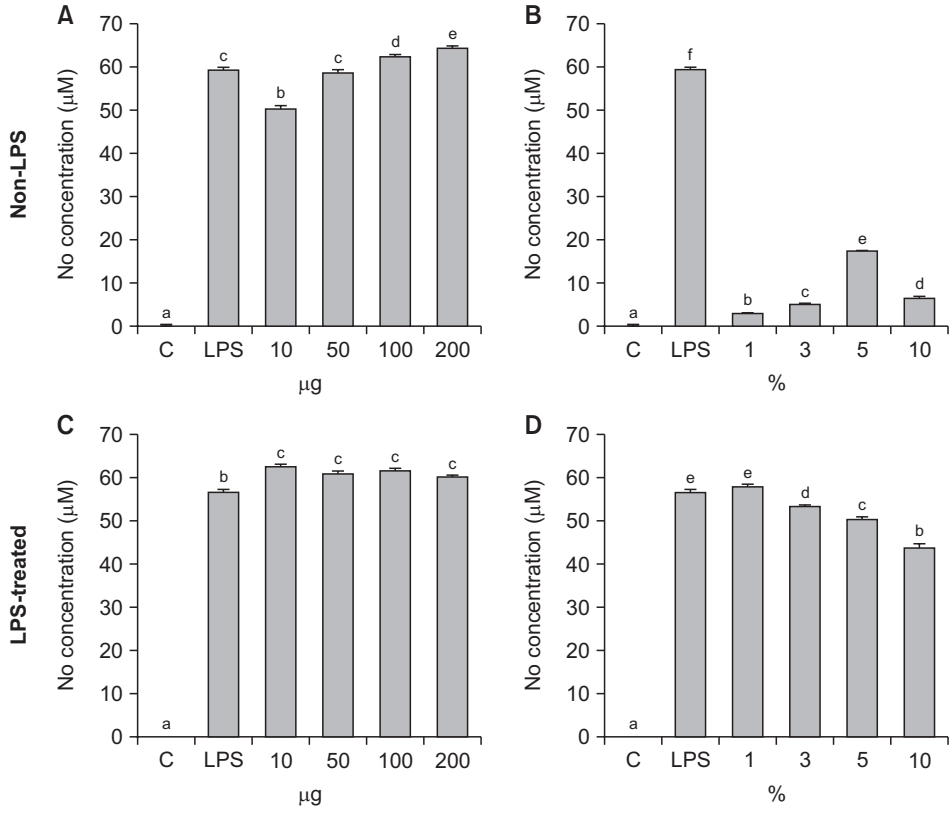

Fig. 3. NO concentration of bass protein and oil treated RAW 264.7 cells (A), (C) present NO concentration of bass protein treated RAW 264.7 cells and (B), (D) present NO concentration of bass oil treated RAW 264.7 cells. (A), (B) are non-LPS-treated on RAW 264.7 cells with bass protein and oil. (C), (D) are LPS-treated on RAW 264.7 cells with bass protein and oil. Graphs were presented as the mean of triplicate ± SEM ($p < 0.05$). Superscripts a, b, c, d, e and f indicate significant differences of NO concentration with serial concentration of bass protein ad oil by one-way ANOVA of variance.

80% with a 10% (v/v) concentration of Bass oil extract, and this activity was comparable to 50 μM of ascorbic acid (Fig. 2A and 2C).

Immune modulation effects of largemouth bass extracts

To evaluate the immune modulation effects of both largemouth bass oil and protein extracts, NO production assay was performed using RAW 264.7 macrophage cell line. The cells were treated with LPS, different concentrations of largemouth bass protein and oil. Treatment of largemouth bass protein showed a similar NO production to 1 μg/mL of LPS treatment in macrophage (Fig. 3A), However, treatment of bass oil did not increase the NO

concentrations from the cells (Fig. 3B). The concentration of NO with 1 μg/mL of LPS treatment was 59.4 μM, and treatment of 10, 50, 100 and 200 μg/mL of bass protein showed 50.4, 58.7, 62.5 and 64.5 μM, respectively. NO production mediated by largemouth bass protein was dose-dependently increased, and the concentrations were significantly higher in 100 and 200 μg/mL of largemouth bass protein treatment than LPS (Fig. 3A). Treatment of largemouth bass oil showed 2.93, 5.11, 17.3 and 7.16 μM by the treatment of 1, 3, 5 and 10% (v/v) of largemouth bass oil, and the concentrations were significantly lower than LPS treatment (Fig. 3B). To observe the inhibition activity of NO production by LPS of largemouth bass extracts, same concentrations of both oil and proteins were treated to LPS-activated macrophage cells. All bass protein-treated groups did not show any decrease in NO production but showed a significant increase in NO production of LPS-activated macrophage cells (Fig. 3C). However, 3, 5 and 10% (v/v) of bass oil-treated cells significantly and dose-dependently decreased the NO production by 5.8, 11.1 and 22.8% (Fig. 3D).

Effects of largemouth bass extracts on physiological changes in mice

Both largemouth bass oil and proteins were orally administered to mice for 4 weeks, and changes in weight gain, and blood compositions including WBC number and IgG concentration were determined. The weight gain was significantly lower in both largemouth bass protein and oil-treated groups after 2 weeks of treatment than control, the largemouth bass protein-treated group and the oil-treated group gained 0.66 ± 0.21 g and 0.525 ± 0.22 g, respectively (Fig. 4B). After 4 weeks treatment, weight gain of largemouth bass oil-treated group was significantly lower than the control group that the weight gains of control and largemouth bass oil-treated group were 2.347 ± 0.241 g and 1.55 ± 0.403 g, respectively, even the body weight of 0 weeks was higher in the control group than largemouth bass oil-treated group (Fig. 4A). However, there were no significance difference in weight gain was not found between the control and largemouth bass protein-treated group (Fig. 4B).

Body weight measurements of the mice were performed to track the weight change of the mice after oral administration. The weight measurement results were representatively expressed in weeks 2 and 4 (Fig. 4A). The control D.W.-treated ICR group shows a weight of 25.8 g at week 0, 27.1 g at week 2 and 28.5 g at week 4. The protein-treated ICR group shows a weight of 26.3 g at week 0, 27.0 g at week 2 and 28.7 g at week 4. The largemouth bass' oil treated ICR group shows a weight of 26.0 g at week 0, 26.5 g at week 2 and 27.5 g at week 4. To visually clarify the increase in body weight of mice, Fig. 3B is presented. The average weight of the mice at week 0 was set to 0 g as a control group, and the weight gains of weeks 2 and 4 were expressed. At week 2, the control D.W.-treated group showed a statistically significant increase with a weight gain of 1.33 g. The protein-treated group also shows a significant increase with a weight gain of 0.66 g and the largemouth bass oil-treated group shows an increase with a weight gain of 0.52 g. The D.W.-treated group shows also statistically significant as shown in week 2, an increase with a weight gain of 2.76 g. The protein-treated group shows a significant increase with a weight gain of 2.34 g. The edible oil-treated group shows also a significant increase with weight gain of 2.00 g. The oil-treated group shows an increase with a weight gain of 1.55 g, which is significantly the lowest increasing rate.

A

Body weight (g)	0 W	2 W	4 W
DW group	25.82 ± 0.90	27.16 ± 1.00	28.58 ± 0.91
Protein group	26.39 ± 0.58	27.05 ± 1.16	28.74 ± 0.66
Bass oil group	26.03 ± 0.83	*26.55 ± 1.22	*27.58 ± 1.16

B

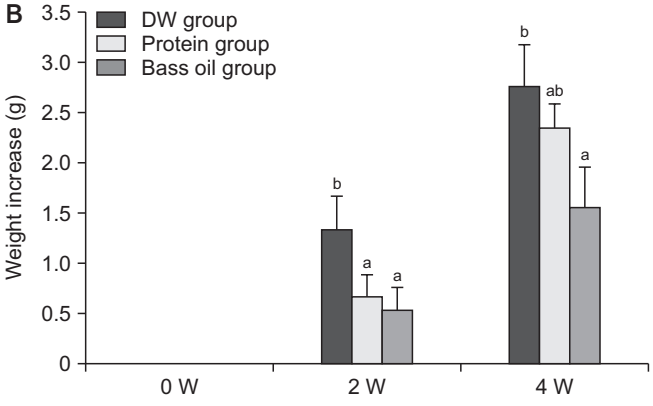

Fig. 4. A weight measurement of bass extracts treated mice was conducted every Monday and Thursday for 4 weeks. (A) Body weight sorted by each group (* < 0.05). (B) Increasing rate of body weight (p < 0.05). Graphs were presented as the mean of each group (N = 10) ± SEM. Superscripts a and b indicate significant differences of gaining of mouse weight on same week by oral administration of bass protein ad oil by one-way ANOVA of variance.

Complete blood count of mice treated with largemouth bass extracts

A complete blood count was conducted to measure the WBC count of blood. In Fig. 5A, At week 2, the control D.W.-treated group shows the most significantly lower WBC count of 1.95 k/μL. The protein-treated group shows 2.35 k/μL of WBC, and the largemouth bass-oil-treated group shows the most WBC count of 3.06 k/μL. At week 4, the control D.W.-treated group showed a WBC count of 3.11 k/μL. Protein protein-treated group shows the most significantly higher WBC count of 4.52 k/μL. The largemouth bass-oil-treated group shows a significantly high WBC count of 4.44 k/μL. The percentage contents of each type in the total WBC are presented in Fig. 5B-F. In the relative percentage of neutrophils at week 2, the protein-treated group showed a significantly highest 41.6% of WBC, however, no significant difference within about 37-40% in week 4. In the percentage of lymphocytes at week 2, the protein-treated group shows 52.1% and the Bass-oil-treated group shows 59.8%. At week 4, compared to week 2, the relative percentage of WBC decreased to a value of 36% to 47% overall, but there is no statistically significant difference. In the percentage of monocytes, the control group consistently shows 1% of WBC at both weeks 2 and 4. The protein-treated group shows 1.47% at week 2 and 1.97% at week 4. The bass-oil treated group shows 1.97% at week 2 and 1.80% at week 4. Both groups are significantly higher than the control group. In the percentage of eosinophils at week 2, the control group showed 4.93% of WBC and the protein-treated group showed 4.20%. The bass-oil-treated group shows significantly the lowest value at 2.43%. However, in week 4, all groups present a dramatically increase percentage than week 2. The control group shows 12.97% and the protein-treated group shows 17.7% but they show no significance. The bass-oil treated group shows significantly the highest 23.27% of WBC. In the percentage of basophil, only the week 2 control group shows the highest 1.63% and other

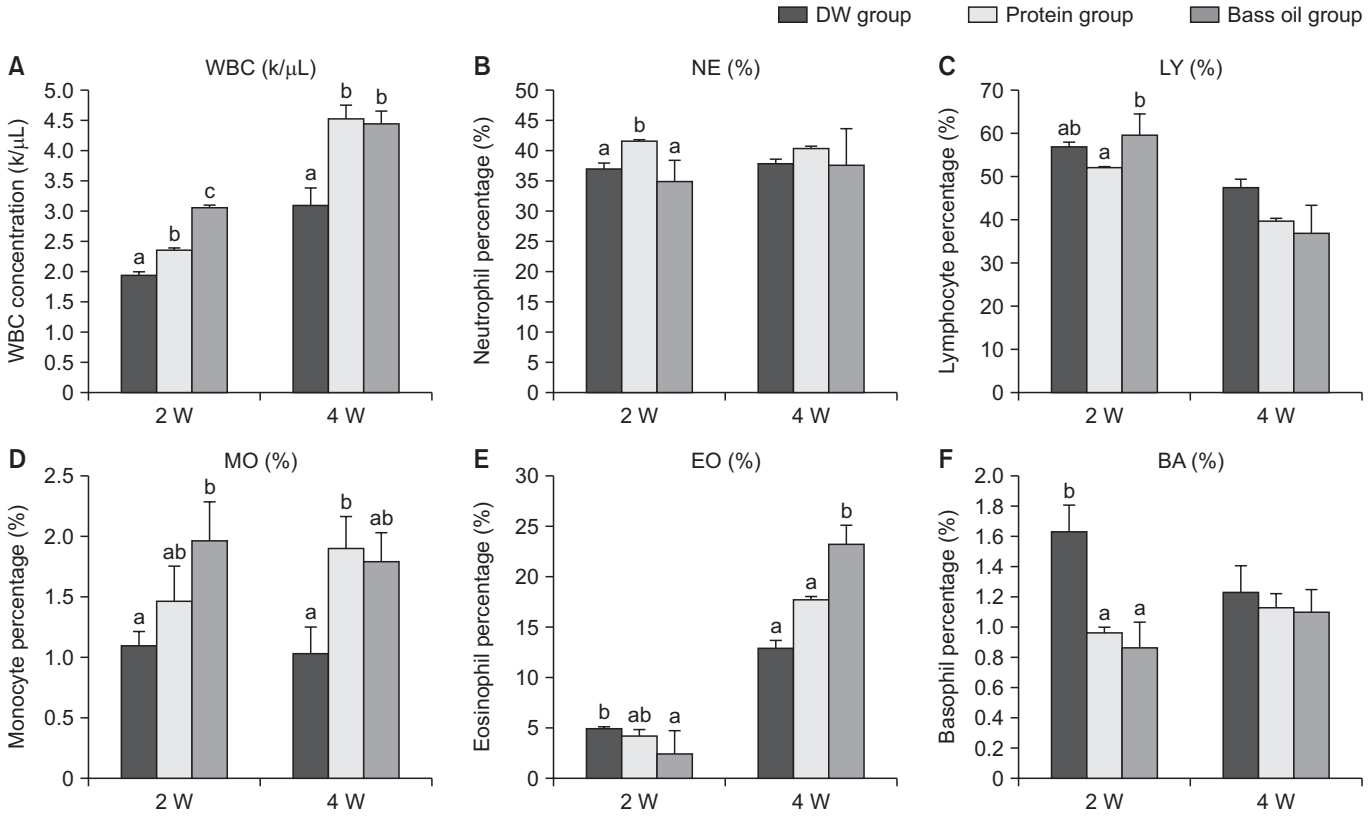

Fig. 5. Complete blood count of mice blood with oral administration of bass extraction. (A) Indicates the WBC concentration in the collected blood. (B) shows percentage of neutrophils in WBC. (C) presents the percentage of lymphocytes in WBC. (D) shows the percentage of Monocytes in WBC (E) presents the percentage of eosinophils in WBC (F) presents the percentage of basophils. Graphs were presented as the mean of each group (N = 10) ± SEM, $p < 0.05$. Superscripts a, b and c indicate significant differences of percentage of serological components on same week from oral administration by one-way ANOVA of variance.

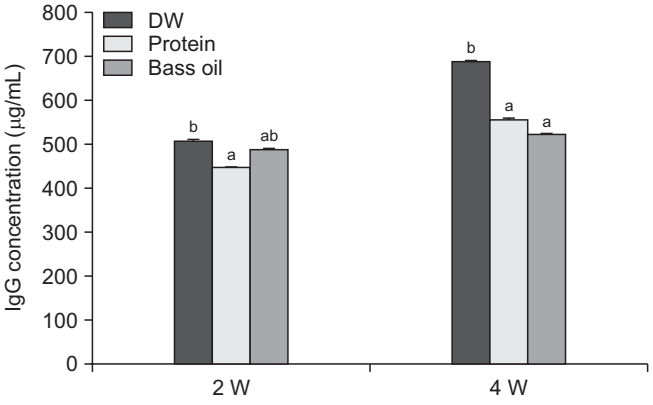

Fig. 6. IgG ELISA analysis from the serum of mice with oral administration of Bass extraction. Graphs were presented as the mean of each group (N = 10) ± SEM. Superscripts a and b indicate significant differences of IgG concentration on same week from oral administration by one-way ANOVA of variance.

groups show a consistent value between 0.87-1.23% of WBC.

IgG concentration in mice treated with largemouth bass extracts

IgG ELISA was conducted to determine the concentration of IgG in the blood. In Fig. 6, which expresses IgG concentration, the IgG concentration of the control D.W.-treated group was 507.5 μg/mL. The protein-treated group shows an IgG concentration of 446.5 μg/mL. The oil-treated group showed an IgG concentration of 487.5 μg/mL in week 2. At week 4, The IgG concentration of the control D.W.-treated group shows a concentration of 688.2 μg/mL. The protein-treated group shows an IgG concentration of 556.3 μg/mL. The oil-treated group shows an IgG concentration of 522.6 μg/mL.

DISCUSSION

This study was done to suggest a way to consume an ecosystem disruptor, largemouth bass. Before revealing the physiological effect, the composition of the oil and protein extract was examined based on the extraction method. Table 1 shows the six extraction conditions, and Table 2 shows the fat composition accordingly. According to the data in Table 2 there is no difference in the composition of oil according to the extraction conditions. Therefore, it can be suggested that there is no change in the composition of the oil in the method of extracting the oil. A study on carp oil suggests that freshwater fish may be a good alternative to supplying omega-3 fatty acids including polyunsaturated fatty acid (PUFA; Li et al., 2011). In comparison to the other study, our result suggests that largemouth bass oil contains enough omega-3 fatty acids, so it can be used for supplying nutrients with high levels of omega-3.

The analysis of the components remaining after extracting the oil has been shown in Table 2. It indicates that the remaining ingredients are largely similar, except for the presence of fats when the fish is transformed into powder (Kasozi et al., 2018). Fish powder with low moisture content is considered relatively safe from spoilage and pathogens (Abbey et al., 2016). Residues obtained from oil extraction are known to be easy to store and possess various physiological effects, such as aiding digestion and absorption (Desai et al., 2018). Among the components of these residues, protein extraction under conditions set by 50℃ distill water was performed to representatively examine the effect on the protein. Table 2 shows taurine's concentration ratio in the protein solution extracted at 50℃ D.W. It can be inferred that higher pressure in the oil extraction process leads to higher protein content.

WST-1 assay was conducted to determine the toxicity to the cells. The protein-added experimental group showed a cell viability of approximately 110% at 100 μg and showed toxicity to cells when administered at 400 and 600 μg (Fig. 1A). The oil-added experimental group showed 115% cell viability at 1% concentration, 127% viability at 5%, and toxicity to cells at 20% (Fig. 2B). Based on this, it is considered that each protein and oil itself increases the viability of cells at a specific concentration and at the same time shows the toxicity of cells when over-dosed. Based on these results, it can be concluded that both protein and oil individually increase cell viability at specific concentrations but show toxicity to cells when overdosed.

DPPH radical scavenging analysis was conducted to investigate the antioxidant effect of the oil and protein extracted with 50℃ D.W. Fig. 2A is the ascorbic acid standard and Fig. 2B and 2C show antioxidant capacity for protein and oil, respectively. 50 μM of ascorbic acid has an antioxidant capacity of over 80%. Protein has an antioxidant capacity of more than 60% when 200 μg. Oil has an antioxidant capacity of approximately 80% at 5%. The antioxidant capacity of the protein is like 20 μM ascorbic acid at 200 μg, and 50 μM at 5% oil. Both proteins and oils have antioxidant properties, and oils have great anti-

oxidant properties because they contain a higher content of fatty acids compared to proteins.

NO assay was conducted to investigate anti-inflammatory and immune abilities. Proteins and oils were treated in RAW cells to measure the concentration of NO in the LPS-treated and untreated groups. Fig. 3A and 3B show the experimental group without LPS. When compared with the control without LPS, the protein-treated group showed an increase in NO concentration. When the 5% oil-treated group showed the maximum NO concentration (17.3 μM). Increasing the concentration of NO without LPS treatment means activity against the immune response, so it is thought that the protein plays a role in activating immunity (Park et al., 2011). In the experimental group treated with LPS, Fig. 3C and 3D, showed NO concentration of the protein-treated group was higher than that of the LPS-treated control group. In the experimental group treated with oil, higher oil concentrations induced lower NO concentrations. These findings suggest that largemouth oil extract exhibits inhibitory effects on NO production in LPS-activated macrophages.

Oral administration of proteins and oils to mice was performed to measure the weight gain of mice. The group with the lowest weight gain in 4th week compared to the control group was the experimental group treated with oil. Therefore, this result suggests that the oil treatment in mice has the potential to suppress weight gain. Further research on molecular biological mechanisms will be needed to further demonstrate this.

A CBC test was analyzed in the protein and oil-treated mice. At week 2 in Fig. 5A, the number of leukocytes in the group administered D.W. was the lowest, and the number of leukocytes in the group administered the oil was 3.00 k/μL. At week 4, both the protein and oil groups were significantly higher than 4 k/μL. The number of WBC measured is all within the normal range of 2 k/μL to 10 k/μL (O'Connell et al., 2015). However, looking at the composition of WBC in Fig. 5B-F, it can be observed that the number of neutrophils of eosinophils is increased at 4 weeks. The ratio of neutrophils can be considered normal range (Forget et al., 2017). Eosinophils are known to increase due to parasitic infections, allergic reactions, and adrenal reactions (O'Connell et al., 2015), As a result of administration for a long period, the result suggests that is thought to have been stressed. which cannot be found because of dissection and should be revealed more clearly through future experiments. Finally, IgG ELISA was conducted to determine the concentrations of IgG in the blood of mice. Fig. 6 shows the results of ELISA for mouse IgG. The concentration of IgG in the blood can be said to be normal for all populations (Klein-Schneegans et al., 1990).

In conclusion, both protein and oil extraction of largemouth bass had the antioxidant ability to remove ROS and proliferate cell growth at an appropriate concentration without overdose. Through the NO assay, extracts showed suppression of excessive inflammation and indicated enhancement of the immune response. Results of oral administration of protein and oil were presented to help with weight loss. Notably, the bass-oil-treated group showed a dramatic decrease in mouse body weight that suggests the oil may contain enough docosahexaenoic acid (DHA) to replace other marine-origin oils loss (Hill et al., 2007). On the CBC test, increased WBC levels are thought to be an improvement in immunity, but increased eosinophils may indicate an infection (O'Connell et al., 2015). Nevertheless, the protein derived from largemouth bass, which disrupts the ecosystem, has been shown to have sufficient use value as an alternative substance for feed ingredients, and bass oil has shown the potential to replace PUFA obtained from marine resources.

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REFERENCES

Abbey L, Glover-Amengor M, Atikpo MO, Atter A, Toppe J. 2016. Nutrient content of fish powder from low value fish and fish byproducts. Food Sci. Nutr. 5:374-379.

Calder PC. 2006. n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. Am. J. Clin. Nutr. 83(6 Suppl):1505S-1519S.

Desai AS, Brennan MA, Brennan CS. 2018. Effect of fortification with fish (*Pseudophycis bachus*) powder on nutritional quality of durum wheat pasta. Foods 7:62.

Forget P, Khalifa C, Defour JP, Latinne D, Van Pel MC, De Kock M. 2017. What is the normal value of the neutrophil-to-lymphocyte ratio? BMC Res. Notes 10:12.

Hill AM, Buckley JD, Murphy KJ, Howe PR. 2007. Combining fish-oil supplements with regular aerobic exercise improves body composition and cardiovascular disease risk factors. Am. J. Clin. Nutr. 85:1267-1274.

Jang MH, Kim JG, Park SB, Jeong KS, Cho GI, Joo GJ. 2002. The current status of the distribution of introduced fish in large river systems of South Korea. Int. Rev. Hydrobiol. 87:319-328.

Jaya-Ram A, Fuad F, Zakeyuddin MS, Sah ASRM. 2018. Muscle fatty acid content in selected freshwater fish from Bukit Merah Reservoir, Perak, Malaysia. Trop. Life Sci. Res. 29:103-117.

Kasozi N, Asizua D, Iwe G, Namulawa VT. 2018. Nutrient composition of fish protein powder developed from Brycinus nurse (Rüppell, 1832). Food Sci. Nutr. 6:2440-2445.

Klein-Schneegans AS, Kuntz L, Trembleau S, Fonteneau P, Loor F. 1990. Serum concentrations of IgM, IgG1, IgG2b, IgG3 and IgA in C57BL/6 mice and their congenics at the nu(nude) locus. Thymus 16:45-54.

Kromhout D, Bosschieter EB, de Lezenne Coulander C. 1985. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. N. Engl. J. Med. 312:1205-1209.

Li G, Sinclair AJ, Li D. 2011. Comparison of lipid content and fatty acid composition in the edible meat of wild and cultured freshwater and marine fish and shrimps from China. J. Agric. Food Chem. 59:1871-1881.

Mamun M, Kim S, An KG. 2018. Distribution pattern prediction of an invasive alien species largemouth bass using a maximum entropy model (MaxEnt) in the Korean peninsula. J. Asia Pac. Biodivers. 11:516-524.

O'Connell KE, Mikkola AM, Stepanek AM, Vernet A, Hall CD, Sun CC, Yildirim E, Staropoli JF, Lee JT, Brown DE. 2015. Practical murine hematopathology: a comparative review and implications for research. Comp. Med. 65:96-113.

Park HJ, Suh HG, Kim JH, Jang A, Jung HJ, Lee SD, Ha WT, Lee R, Kim JH, Kim SH, Sung SH, Moon SH, Kim BK, Song H. 2011. Immune modulation effect of pig placenta extracts in a mouse model: putative use as a functional food supplement. Korean J. Food Sci. Anim. Resour. 31:701-709.

Ross ST. 1991. Mechanisms structuring stream fish assemblages: are there lessons from introduced species? Environ. Biol. Fishes 30:359-368.

Steffens W. 2006. Freshwater fish – wholesome foodstuffs. Bulg. J. Agric. Sci. 12:320-328.

Tee ES, Siti Mizura S, Kuladevan R, Young SI, Khor SC, Chin SK. 1989. Nutrient composition of Malaysian freshwater fishes. Proc. Nutr. Soc. Mal. 4:63-73.

Wasim MP. 2007. Issues, growth and instability of inland fish production in Sindh (Pakistan): spatial — temporal analysis. Pak. Econ. Soc. Rev. 45:203-230.