YUCCA SCHIDIGERA EXTRACT DIETARY SUPPLEMENTATION AFFECTS GROWTH PERFORMANCE, HEMATOLOGICAL AND PHYSIOLOGICAL STATUS OF EUROPEAN SEABASS

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Abstract

The study herein evaluated the effects of dietary supplementation with different yucca (*Yucca schidigera*) extract levels on rearing water quality, growth performance, protein utilization, hematological and immunological status, and economic benefits of cultured European seabass (*Dicentrarchus labrax*) juveniles. Yucca extract (YE) was incorporated in an experimental diet at levels of 0, 0.25, 0.50, and 1 g YE kg⁻¹, and offered to fish reared in 70-L glass aquaria (15 fish per aquarium; three replicates each). Dietary YE supplementation significantly ($P<0.05$) decreased ammonia-nitrogen levels in water. Compared with the control, groups fed 0.50 and 1 g YE kg⁻¹ diets showed improved growth performance (by 26.02% and 36.98%, respectively) and protein efficiency ratio (by 31.39% and 37.29%, respectively). In addition, hematological parameters (red blood cells, hemoglobin, hematocrit, white blood cells and neutrophil) and innate immune parameters (globulin fractions and lysozyme activity) improved with increasing dietary YE levels. Growth, hematological, and immunological parameters increased with a polynomial second-order regression models, with curves peak close to 1 g YE kg⁻¹ diet. The cortisol level decreased significantly ($P<0.05$) with increasing dietary YE levels. The groups fed the 0.50 and 1 g YE kg⁻¹ diets generated significantly ($P<0.05$) higher revenue than groups fed the 0.25 g YE kg⁻¹ and the control diets. The using of YE is recommended as a dietary feed additive at a level of 1 g kg⁻¹ for reducing the environmental footprint of fish protein production, accelerating growth, and improving health status of cultured seabass in a short-term feeding period.

Key words: *Dicentrarchus labrax*, growth performance, hematological, immunological status, water quality, *Yucca schidigera* extract

European seabass (*Dicentrarchus labrax*) is one of the economically important marine fish species in temperate areas, including the Mediterranean Sea, the Black Sea, and the Atlantic coastline from Norway to Morocco (Eroldogan et al., 2004; Altan and Korkut, 2010). In Egypt, European seabass production is second to gilthead sea bream, the estimated yield is 30720 tons with the expectation to be duplicated in near future due to intensive culture (GAFRD, 2017). Intensification of fish farming is the best way in the aquaculture industry to meet the human demand for protein and reduce the pressure on natural fishery resources (Aubin et al., 2019). However, with the intensification of aquaculture, both consumed feed and excreted nitrogen will increase, which may affect fish metabolism and ambient water bodies (Besson et al., 2016; Aubin et al., 2019). Therefore, the improvement of protein metabolism and subsequent reduction of nitrogen excretion are crucial for sustainable development in this sector (Kelly and Kohler, 2003). Natural feed additives such as phytogenics are suitable for cleaner production (Adegbeye et al., 2019) and improving growth, feed utilization, general physiology, and disease resistance of cultured fish (Mansour et al., 2017; Acar, 2018; Mansour et al., 2018 b; Acar et al., 2019).

Yucca schidigera (Mojave yucca) grows in the deserts of southwestern USA and Mexico (Tenon et al., 2017). The ability of yucca to grow in arid regions with high temperature and low water availability maximizes its potential use as a future sustainable feed additive, particularly in the regions that are likely to experience a new dimension of global warming (Adegbeye et al., 2019). The commercial products of *Y. schidigera*, including yucca powder which is dry powder of yucca trunk and yucca extract which is concentrated juice produced by mechanical squeezing of plant logs are approved for human use by the FDA (Piacente et al., 2005). Yucca extract (YE) is a rich source of steroidal saponins, resveratrol and polyphenolics (Ayasan et al., 2005; Tenon et al., 2017).

The extract of yucca has been used in the aquaculture, poultry, and livestock industries, primarily to mitigate pollution by minimizing ammonia excretion and methane emission (Headon and Dawson, 1990; Hristov et al., 1999; Sarkar, 1999; El-Saidy and Gaber, 2004). Direct application of yucca to both fresh- and saltwater aquatic rearing systems reduced ammonia levels in a dose-dependent manner (Santacruz-Reyes and Chien, 2010; Khalil et al., 2015; Fayed et al., 2019). In addition, YE enhanced protein metabolism, reduced ammonia excretion, and improved growth performance of different fish species (Kelly and Kohler, 2003; El-Saidy and Gaber, 2004; Gaber, 2006; Güroy et al., 2014; Fayed et al., 2019).

The potential effect of YE on controlling ammonia levels relies on its content of steroidal saponin fractions, which have surface active properties and can bind to ammonia via glycol-component fractions (Cheeke, 2000). Moreover, YE can be absorbed and enter the blood stream (Yang et al., 2015; Hassan et al., 2017). The systemic effect of YE in the animal body is varied, whereas it has anti-inflammatory (Cheeke, 2000), immunostimulatory, antioxidant (Su et al., 2016), antistress (Cigerci et al., 2009; Ince et al., 2013), antiviral and antitumor effects (Piacente et al., 2005). Moreover, YE can contribute to the regulation of energy metabolism and hormonal activity in animals, and the treatment of nutritional disorders (Kucukurt et al., 2016). The YE has been firstly used for environmental considerations of controlling

global warming greenhouse gases, however it also has several desirable biological effects in the animal body (Adegbeye et al., 2019). Taking this previous knowledge into consideration, the present study was designed to investigate and evaluate the effectiveness of dietary supplementation of YE on water quality, zootechnical performance, protein utilization, hematological and immunological status, cortisol level, and economic revenue in cultured European seabass, *D. labrax*, juveniles.

Material and methods

Fish and experimental facilities

A total of 180 apparently healthy *D. labrax* fingerlings with an average body weight of 5.0 ± 0.5 g were used. The feeding experiments were performed in an indoor wet laboratory at El-Shreif Fish Farm and Hatchery (Mariut valley, Alexandria, Egypt). Prior to starting the experiments, the fish were acclimated to the experimental conditions for 2 weeks in four indoor circular fiberglass tanks (1 m^3) and were fed the control basal diet (Table 1).

The fish were randomly divided into 12 glass aquaria ($70 \times 40 \times 30 \text{ cm}^3$; $\sim 70 \text{ L}$ each; 15 fish per aquarium), representing four experimental treatments (three replicates per treatment). Each aquarium was supported with artificial aeration through an air blower and 5-cm sandstone. The water source was groundwater of a saline water well, with 32‰ salinity and pH (7.0 ± 0.5). Throughout the experimental period, the lighting regime was a 12:12-h light: dark cycle and the dissolved oxygen (DO) level was $6.01 \pm 0.2 \text{ mg L}^{-1}$. Fish excreta were removed by manual siphoning, and the water exchange rate was 30% per day. The Institutional Animal Care and Use Committee of Alexandria University have approved the fish care used in the present study.

Experimental design and diets

Fish in the four experimental treatments were fed one of the following isonitrogenous ($\sim 45\%$ crude protein) and isocaloric ($\sim 20.32 \text{ MJ kg}^{-1}$) diets (Table 1) as follows: (1) control diet without any YE supplementation (YE_0); (2) experimental diet supplemented with $0.25 \text{ g YE kg}^{-1}$ ($\text{YE}_{0.25}$); (3) experimental diet supplemented with 0.5 g YE kg^{-1} ($\text{YE}_{0.5}$); and (4) experimental diet supplemented with 1 g YE kg^{-1} (YE_1) (Table 1). The YE levels were selected according to previous studies (Kelly and Kohler, 2003; Gaber, 2006). The experiment lasted 45 days.

The fish were fed the experimental diets to apparent satiation two times daily (9:00 a.m. and 2:00 p.m.). Apparent satiation was considered to have occurred 30 min after introduction of the diet, after which the remaining pellets were collected, dried, and weighed for accurate determination of actual feed intake. The diets were prepared by finely grinding solid ingredients to powder and manually mixing in a plastic container for ~ 15 min to ensure homogeneity. Oil and additives were slowly added to the mixture. Warm distilled water was gradually added until the diet began to clump and form a dough-like paste, which was then passed through a commercial meat grinder to form a spaghetti-like prepared diet (0.3 mm in diameter). The result-

ant pellets were dried in a forced-air drying oven at 40–45°C to a moisture level of <10% and stored at –20°C until further use.

The proximate chemical composition (%) of the formulated diets was analyzed (AOAC, 2000) and presented in Table 1.

Measured parameters

Water quality parameters

Water quality parameters were determined according to the standard guidelines (A.P.H.A., 1998). Dissolved oxygen was measured with a digital oxygen meter (HI-9142, Hanna Instruments, US), temperature, and pH were measured with a digital pH meter (Research Model 211 Digital pH Meter, Hanna Instruments, US). Salinity was measured with a salinity meter (YSI EcoSense EC300 Conductivity/Salinity151, US). Total ammonia levels were measured with a portable digital multi-meter (Crison Model MM41, Spain) and were daily recorded (at 12:30 p.m.) for all aquaria throughout the experiment. Non-ionized ammonia (NH₃) levels were calculated as the percentage of total ammonia based on water temperature and pH according to the US Environmental Protection Agency (EPA) guidelines (U.S. EPA, 1989).

Growth performance and survival

Mean final body weight (FBW) in each experimental treatment was determined by dividing the total fish weight in each aquarium by the number of fish. Weight gain (WG), specific growth rate (SGR), and survival (%) were calculated using the following equations (Castell and Tiewes, 1980):

$$WG\ (g\ fish^{-1}) = W_2 - W_1$$

where:

W_1 is the initial weight of the fish (g) and W_2 is FBW of the fish (g).

$$SGR\ (\% \ day^{-1}) = 100 \times (\ln W_2 - \ln W_1)/days$$

where:

$\ln$ is the natural log.

Survival (%) = $100 \times$ (the final number of the fish/initial number of the fish).

Table 1. Ingredients and chemical composition (on dry matter basis) of the experimental diets

Ingredients (%)	Yucca extract levels (g kg ⁻¹)			
	0.0	0.25	0.50	1.00
1	2	3	4	5
Fish meal (68% CP)	30.00	30.00	30.00	30.00
Soybean meal (47% CP)	37.50	37.50	37.50	37.50
Corn gluten (60% CP)	9.00	9.00	9.00	9.00
Rice bran (12% CP)	6.50	6.50	6.50	6.50
Wheat middling (13% CP)	7.10	7.08	7.05	7.00
Soybean oil	4.00	4.00	4.00	4.00

Table 1 – contd.

1	2	3	4	5
Fish oil	4.88	4.88	4.88	4.88
Dicalcium phosphate	0.80	0.80	0.80	0.80
Vitamins and minerals mixture*	0.20	0.20	0.20	0.20
Vitamin C	0.02	0.02	0.02	0.02
Yucca, <i>Yucca schidigera</i> , extract [‡]	0.00	0.025	0.050	0.10
Chemical composition (g 100 g ⁻¹ DM)				
Dry matter (DM)	93.80	93.75	93.18	93.77
Crude protein (CP)	44.86	44.88	44.97	44.60
Ether extract (EE)	12.56	13.10	12.98	12.89
Nitrogen free extract (NFE) [†]	28.34	25.70	26.65	27.86
Crude fiber (CF)	3.24	3.32	3.40	3.20
Ash	11.00	12.00	12.00	11.45
Gross energy (GE; MJ kg ⁻¹ DM) [‡]	20.41	20.17	20.31	20.39

*Composition of vitamins and minerals mixture for 1 kg: Vitamin A: 5,000,000 IU; vitamin D₃: 10,000,000 IU; vitamin B₂: 2.0 g; vitamin E: 750 U; vitamin K: 1.0 g; calcium pantothenate: 2.5 g; nicotinamide: 10.0 g; vitamin B₁₂: 6.0 g; choline chloride: 150.0 g; manganese sulphate anhydrous: 27.5 g; potassium iodide: 1.0 g; ferrous sulphate: 7.5 g; zinc sulphate heptahydrate: 15.0 g; copper sulphate: 2.0 g; sodium selenite, 0.1 g; cobalt chloride: 0.45 g; and calcium carbonate: up to 1000 g.

[‡]Yucca (*Yucca schidigera*) extract: Vime-Yucca (P)[®] containing 33% yucca extract with 12% saponin, produced by Vemedim Corporation, Vietnam.

[†]NFE: Nitrogen-free extract calculated using the following equation: NFE = 100 – (crude protein + ether extract + crude fiber + ash) (National Research Council, 2011).

[‡]GE: Gross energy calculated on the basis of 23.6, 39.4, and 17.2 kJ gross energy g⁻¹ protein, ether extract, and NFE, respectively (National Research Council, 2011).

Feed and protein utilization

Feed intake for each group was calculated by dividing the total feed intake (g) of the group by the number of fish in that group. Feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated as follows:

$$FCR = \text{feed intake (g)} / \text{weight gain (g)}$$

$$PER = \text{weight gain (g)} / \text{protein intake (g)}$$

Hematological and immunological parameters

At the end of the experiment, the fish were anesthetized with clove oil (50 mg L⁻¹) and blood samples were collected from 10 fish per aquarium using sterile, disposable 1-ml syringes containing 50 µL heparin (IU mL⁻¹, Amoun Pharmaceutical Co. S.A.E., Egypt). The collected blood was divided into two tubes; the first was used for hematological assays and the second was centrifuged (1075 × g, 5 min) to obtain the plasma. The plasma samples were stored at –80°C until being used for biochemical and immunological assays.

Total red and white blood cell counts (RBC, 10⁶ mm⁻³ and WBC, 10³ mm⁻³, respectively) were determined using a standard Neubauer hemocytometer with Shaw's solution as a diluent. Hematocrit (Ht; %) was determined by filling hematocrit cap-

illary tubes, which were centrifuged ($8400 \times g$, 10 min) in a microhematocrit centrifuge (Krebs, Bunsen, EU). Hematocrit values were recorded using a centrifuge combo-reader. Hemoglobin level (Hb; $g\ dL^{-1}$) was determined colorimetrically using commercial kits (Diamond, Egypt) according to the cyanmethemoglobin method (Van-Kampen and Zijlstra, 1961). The differential leukocyte count was determined by observing Giemsa-stained smears through light microscopy at 100x magnification (B-293 LD 1.50, Optika, Via Rigla, Ponteranica, Italy).

Total plasma protein level ($g\ dL^{-1}$) was determined using the biuret method (Gornal et al., 1949). Albumin level ($g\ dL^{-1}$) was determined using the bromocresol green method (Reinhold, 1953), and globulin level ($g\ dL^{-1}$) was calculated as the difference between total protein and albumin levels. Globulin fractions were determined in plasma samples using capillary zone electrophoresis (Ihara et al., 2001).

Plasma lysozyme activity ($U\ mg^{-1}\ protein$) was determined using turbidimetric assay (Swain et al., 2007). Briefly, 20 μL of plasma was placed in 96-well flat-bottomed plates with 180 μL of freeze-dried *Micrococcus lysodeikticus* ($0.2\ mg\ mL^{-1}$, Sigma-Aldrich) in 40 mM sodium phosphate (pH 6.2). As blanks of each sample, 20 μL of plasma was added to 180 μL of sodium phosphate buffer. The OD was measured at 450 nm after 20 min at $35^{\circ}C$ in a microplate reader. The amounts of lysozyme present in plasma were obtained from a standard curve made with hen egg white lysozyme (HEWL, Sigma). Cortisol level ($pg\ mL^{-1}$) was determined using a commercial ELISA kit based on manufacturer instructions (IBL®, Germany) (Gomez-Sanchez et al., 1977).

Economic evaluation

A simple economic evaluation was done by calculating the cost of the feed in Egyptian pounds (LE) needed to produce 1 kg live weight of fish. Additionally, the cost of protein production was calculated as follows:

$$Feed\ cost* (kg^{-1}\ WG) = FCR \times cost\ kg^{-1}\ feed$$

*The costs of feed ingredients were as follows: fishmeal: LE 19, soybean meal: LE 5.8, corn gluten (60%): LE 8, rice bran: LE 1.8, wheat middling: LE 2.3, soybean oil: LE 14, herring oil: LE 16, vitamins: LE 18, dicalcium phosphate: LE 4.8, and YE [Vime-Yucca (P)®]: LE 300. 1 US \$= 17.68 LE at the time of calculation.

Statistical analysis

All data were subjected to one-way analysis of variance and when a significant difference was found among treatments ($P \leq 0.05$). Duncan's multiple range test was performed to rank the groups (Duncan, 1955) using SPSS (Standard Version 17.0 SPSS Inc. Chicago, Illinois). The associations of YE supplementation levels with specific growth, hematological and immunological parameters were investigated to determine the fit of regression models and the optimum dietary level of YE was identified using GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California USA.

Results

Water quality parameters

The water quality parameters of aquaria harboring *D. labrax* fingerlings fed diets supplemented with different levels of YE are illustrated in Table 2 and Figure 1. Increasing YE supplementation level significantly decreased levels of total ammonia nitrogen as well as ionized and unionized ammonia nitrogen in a dose-dependent manner. However, DO and pH did not differ significantly across treatments.

Table 2. Water quality parameters of European seabass (*Dicentrarchus labrax*) fingerlings fed diets supplemented with different levels of *Yucca schidigera* extract (g kg⁻¹ diet); average salinity: 32 ppt; temperature: 28 ± 1.0°C

Treatments	Yucca extract levels (g kg ⁻¹)			
	0.0	0.25	0.50	1.00
pH	7.54±0.29	7.63±0.17	7.55±0.58	7.88±0.26
Dissolved oxygen (mg L ⁻¹)	5.41±0.23	5.54±0.07	5.81±0.18	5.28±0.18
Total-ammonia nitrogen (mg L ⁻¹)	1.06±0.07 a	1.13±0.07 a	0.81±0.01 b	0.37±0.10 c
NH ₄ -N (mg L ⁻¹)	0.83±0.05 a	0.73±0.01 b	0.41±0.01 c	0.07±0.01 d
NH ₃ -N (mg L ⁻¹)	0.019±0.001 a	0.021±0.001 a	0.011±0.001 b	0.005±0.01 c

Means in the same row bearing different letters differ significantly (P≤0.05).

Figure 1. Heatmap of aquarium water quality changes with dietary supplementation of *Yucca schidigera* extract (g kg⁻¹ diet) in European seabass (*Dicentrarchus labrax*) fingerlings diets

Growth performance

Growth performance data of *D. labrax* fed different dietary levels of YE are presented in Table 3 and Figure 2 A. Final body weight was significantly increased with YE_{0.5} and YE₁ compared to that with control (YE₀). Moreover, WG and SGR were significantly improved with all YE-supplemented diets in a quadratic regression fit model. Moreover, no mortality was reported during the experimental period.

Feed and protein utilization

The effects of YE supplementation on feed intake and protein utilization are illustrated in Table 3 and Figure 2 B. Feed intake did not significantly differ across

treatments. Meanwhile, FCR was significantly improved with increasing YE supplementation levels compared to that with control in a polynomial second-order regression model. Protein efficiency ratio was significantly increased with higher supplementation levels of YE (0.5 and 1 g kg⁻¹) compared to that with control.

Table 3. Growth performance and survival of European seabass (*Dicentrarchus labrax*) fed diets supplemented with different levels of *Yucca schidigera* extract (g kg⁻¹) (n = 3; mean ± SE)

Treatments	Yucca extract levels (g kg ⁻¹)			
	0.00	0.25	0.50	1.00
Initial weight (g fish ⁻¹)	5.53±0.09	5.30±0.06	5.43±0.09	5.43±0.15
Final body weight (g fish ⁻¹)	12.87±0.22 c	13.92±0.27 c	16.22±0.41 b	17.63±0.42 a
Weight gain (g fish ⁻¹)	7.34±0.13 d	8.62±0.22 c	10.78±0.49 b	12.20±0.28 a
Specific growth rate (% day ⁻¹)	2.52±0.02 d	2.60±0.02 c	2.75±0.03 b	2.83±0.02 a
Survival (%)	100±00	100±00	100±00	100±00
Feed intake (g fish ⁻¹)	11.97±0.99	14.17±1.59	13.30±0.10	13.20±0.60
Feed conversion ratio	1.63±0.03 a	1.65±0.04 a	1.24±0.06 d	1.08±0.02 c
Protein efficiency ratio	1.37±0.02 c	1.36±0.04 c	1.80±0.08 b	2.07±0.05 a

Means in the same row bearing different letters differ significantly (P≤0.05).

Figure 2. Polynomial second-order regression curve of specific growth rate and feed conversion ratio of European seabass (*Dicentrarchus labrax*) fingerlings fed diets supplemented with *Yucca schidigera* extract (g kg⁻¹)

Table 4. Hematological parameters of European seabass (*Dicentrarchus labrax*) fed diets supplemented with different levels of *Yucca schidigera* extract (g kg⁻¹) (n = 3; mean ± SE)

Treatments	Yucca extract levels (g kg ⁻¹)			
	0.0	0.25	0.50	1.00
Red blood cells (10 ⁶ mm ⁻³)	1.62±0.22 b	2.14±0.32 b	2.94±0.43 a	2.97±0.37 a
Hematocrit (%)	25.08±1.15 b	26.19±1.26 b	31.12±1.35 a	29.33±1.31 a
Hemoglobin (g dl ⁻¹)	7.23±0.72 b	8.86±0.72 b	11.62±0.89 a	11.39±0.85 a
White blood cells (10 ³ mm ⁻³)	24.33±1.31 c	31.67±1.37 b	41.11±1.69 a	37.18±1.64 a
Lymphocyte (%)	35.33±2.03	35.67±2.08	38.33±2.09	39.33±2.09
Monocyte (%)	2.67±0.33	2.00±0.58	2.50±0.33	2.97±0.33
Neutrophil (%)	40.67±2.91 c	41.67±2.91 bc	49.67±1.76 ab	52.67±2.91 a

Means in the same row bearing different letters differ significantly (P≤0.05).

Hematological parameters

The results of hematological examination showed a significant increase in RBC, Hb, Ht, and WBC values with the highest level of YE supplementation (Table 4). Furthermore, WBC differential showed a significant increase in neutrophil count (%) with increasing YE supplementation level (Table 4). However, lymphocyte and monocyte (%) counts were not significantly affected by YE supplementation. In the polynomial second-order regression model of increasing YE level, the highest Ht and WBC values were 0.72 and 0.69, respectively (Figure 3 A and B).

Immunological parameters

Total protein, globulin, and globulin fraction (alpha, beta, and gamma globulin) values were markedly increased with increasing YE supplementation levels. In addition, albumin level and lysozyme activity increased with YE_{0.5} and YE₁ (Table 5). The association of YE supplementation level with globulin level and lysozyme activity showed a polynomial second-order regression curve, with peak lysozyme activity at 0.82 g YE kg⁻¹ (Figure 3 C and D). Meanwhile, cortisol levels decreased with increasing YE supplementation levels in a dose-dependent manner.

Table 5. Immunological parameters and cortisol levels of European seabass (*Dicentrarchus labrax*) fed diets supplemented with different levels of *Yucca schidigera* extract (g kg⁻¹) (n = 3; mean ± SE)

Treatments	Yucca extract levels (g kg ⁻¹)			
	0.00	0.25	0.50	1.00
Total protein (g dL ⁻¹)	4.30±0.17 bc	3.85±0.17 c	4.67±0.12 b	5.08±0.11 a
Albumin (g dL ⁻¹)	2.57±0.18 a	1.96±0.18 b	2.51±0.12 a	2.64±0.10 a
Globulin (g dL ⁻¹)	1.73±0.19 b	1.89 ±0.19 b	2.16 ±0.12 ab	2.44±0.12 a
Alpha globulin (g dL ⁻¹)	0.41±0.02 d	0.47±0.01 c	0.55±0.02 b	0.78±0.02 a
Beta globulin (g dL ⁻¹)	0.30±0.01 d	0.39±0.01 c	0.53±0.02 b	0.68±0.01 a
Gamma globulin (g dL ⁻¹)	0.59±0.01 d	0.76±0.01 c	0.87±0.03 b	0.96±0.05 a
Lysozyme (U mg ⁻¹ protein)	0.10±0.01 b	0.12±0.03 b	0.59±0.06 a	0.49 ±0.03 a
Cortisol (Pg mL ⁻¹)	457.0±9.95 a	380.0±6.95 b	280.43±2.01 c	266.43±4.01 d

Means in the same row bearing different letters differ significantly (P≤0.05).

Revenue

The production cost of feeding *D. labrax* fingerlings with diets supplemented with different levels of YE was significantly reduced for YE_{0.5} and YE₁ (by 23.34% and 32.72%, respectively) compared to that of the control (Figure 4).

Figure 3. Polynomial second-order regression curve of different hematological and immunological parameters of European seabass (*Dicentrarchus labrax*) fingerlings fed diets supplemented with *Yucca schidigera* extract (g kg⁻¹)

Figure 4. Cost of feed to obtain 1 kg live weight of European seabass (*Dicentrarchus labrax*) fingerlings fed diets supplemented with different levels of *Yucca schidigera* extract (g kg⁻¹ diet) (mean $\pm$ SE).

Discussion

Reducing the environmental footprint of animal protein production is a global concern parallel to sustainable livestock development (Patra, 2012; Adegbeye et al.,

2019). Consequently, improving protein metabolism and controlling nitrogenous wastes in aquaculture systems are necessary to meet effluent standards (Kelly and Kohler, 2003) and maximize protein utilization which is the most expensive feed component (Goda et al., 2019). In aquaculture systems, particularly those involving carnivorous marine species, the use of high-protein diets increases the excretion of nitrogenous wastes (Efole-Ewoukem et al., 2013). Therefore, supplementing the diet with plants or their extracts may be a practical strategy in aquaculture nutrition to minimize ammonia production and promote growth and immunity (Awad and Awaad, 2017; Mansour et al., 2017, 2018 a; Adegbeye et al., 2019; Kesbiç et al., 2020).

The present study showed that dietary YE supplementation significantly decreased ammonia levels in fish-rearing water in a dose-dependent manner. Similarly, YE supplementation (0.5 and 1 g kg⁻¹) reduced ammonia excretion and increased the final weight of cultured channel catfish (*Ictalurus punctatus*) (Kelly and Kohler, 2003). In addition, dietary YE successfully controlled ammonia nitrogen levels in rearing waters of different aquatic animal species, including white shrimp (*Litopenaeus vannamei*), striped catfish (*Pangasianodon hypophthalmus*), and Nile tilapia (*Oreochromis niloticus*) (Güroy et al., 2014; Yang et al., 2015; Hassan et al., 2017). The YE addition to fish-rearing water decreased ammonia levels and improved water quality (Santacruz-Reyes and Chien, 2009; Fayed et al., 2019). This reduction in ammonia levels could be due to the adsorption of ammonia by active surface components of YE, including steroidal saponins and glycol (Cheeke, 2000; Ayasan et al., 2005), hence it will be more available for synthesis of another nitrogenous compound (Hertrampf and Piedad-Pascual, 2000). Controlling ammonia excretion may facilitate aquaculture intensification with decreasing nutrient losses to the environment, thereby lowering the occurrence of large point source of environmental pollution (Sutton et al., 2013).

In this study, FBW of the treated fish was improved by 8.16%, 26.02%, and 36.98% with YE_{0.25}, YE_{0.5}, and YE₁, respectively, compared to that of the control fish. These results are consistent with those of *I. punctatus* fed YE-supplemented diets at a level of 1 g kg⁻¹, which gained significantly more weight (Kelly and Kohler, 2003). Moreover, the growth performance of *O. niloticus* improved significantly when fed a diet supplemented with YE (El-Saidy and Gaber, 2004), and YE at a level of 0.75 mg kg⁻¹ improved the growth of fish fed plant protein-based diets to a level equal to that of fish fed fishmeal-based diets (Gaber, 2006). In addition, the growth of striped catfish (*P. hypophthalmus*) improved significantly with dietary supplementation of YE and *Y. saponaria* extracts (Güroy et al., 2014). Furthermore, the positive effects of YE on growth have been confirmed in several animal species, including white shrimp (Yang et al., 2015), Arbor Acres broilers (Su et al., 2016), and growing rabbits (Amber et al., 2004; Földešiová et al., 2017).

Regarding feed and protein utilization, our findings showed an improvement in feed intake in YE-supplemented groups, indicating that YE did not negatively affect diet palatability. Moreover, the increased feed intake noted in the present study may be due to hormonal regulation by YE (Kucukkurt and Dundar, 2013), whereas YE increased leptin and insulin levels in the plasma of treated rats. Furthermore,

FCR improved significantly with YE supplementation at higher levels (by 39% with YE_{0.5} and 55% with YE₁) compared to that with the control treatment. Protein efficiency ratio also increased significantly by 43% and 70% in the groups fed YE_{0.5} and YE₁, respectively. Likewise, YE improved feed and protein utilization in channel catfish (Kelly and Kohler, 2003), Nile tilapia (El-Saidy and Gaber, 2004; Gaber, 2006), striped catfish (Güroy et al., 2014), white shrimp (Yang et al., 2015), broiler chickens, and rabbits (Amber et al., 2004; Su et al., 2016; Földešiová et al., 2017). Nitrogen utilization improved by 24.3% in rabbits fed a diet supplemented with YE (0.25 g kg⁻¹) compared to that in rabbits fed a control diet (Amber et al., 2004). In contrast, in the present study, no improvement in PER to the same level was noted with YE supplementation at 0.25 g kg⁻¹, although PER improved with higher levels of YE supplementation; this may be attributed to differences in experimental animals and stark differences in digestive system anatomy and physiology.

The improvement of protein utilization with increasing YE level in the present study may be attributed to enhance activities of digestive enzymes associated with YE supplementation (Liu et al., 2005). Additionally, an increase in PER could be due to the reduction in ammonia nitrogen levels in the rearing water as a result of reduced excretion of ammonia in fish fed diets supplemented with YE (Figure 1). In parallel with the present findings, there was a reduction in ammonia excretion and fecal nitrogen concentrations with YE, as reported in *I. punctatus* (Kelly and Kohler, 2003). Moreover, YE supplementation reduced blood urea and cecal ammonia concentrations in rabbits (Amber et al., 2004), possibly reflecting the strong adsorption capacity of YE active components for volatile compounds, including ammonia and hydrogen sulfide (Cheeke, 2000).

Furthermore, improvements in growth performance, FCR, and PER in the present study may be attributed to the active components of YE including steroidal saponins and resveratrol (Cheeke, 2000; Ayasan et al., 2005). The presence of steroidal saponins can improve nutrient absorption (Yang et al., 2015) by altering the enterocyte membrane structure, reducing surface tension (Goetsch and Owens, 1985), and increasing the permeability of intestinal membranes to dietary nutrients (Francis et al., 2002). In addition, YE can improve the intestinal wall integrity by increasing thickness of the intestinal mucosa, preventing the invasion of certain types of viruses, improving the animal's immunity, and suppressing intestinal bacterial growth (Huang et al., 2005).

In the present study, the increase in RBC, Hb, Ht, and WBC values indicated an improvement of hematopoietic organs activities and health status. In striped catfish, *P. hypophthalmus*, dietary supplemented with YE at a level of 0.15% significantly improved PCV (%), Hb, and lymphocyte percentage compared with the non-supplemented diet (Güroy et al., 2014). Additionally, increasing YE addition in the liquid form to rearing water significantly improved the hematological and physiological status of seabass fingerlings (Fayed et al., 2019). However, increasing YE supplementation level to 1 g kg⁻¹ tended to decrease some hematological and immunological parameters in the present findings, as indicated by the polynomial second-order regression fit models with curves peak in the range of 0.72–0.82 g YE kg⁻¹. Therefore, the optimum YE supplementation level should not exceed 1 g kg⁻¹ in seabass

diets. Limitations such as this may be attributed to increasing saponin levels in supplemented diets, which may affect the function of specific cells and increase hemolysis, leading to improper functioning of certain fish organs (Tidwell et al., 1992).

Yucca extract supplementation improved innate immune responses with increasing total protein, albumin, globulin, and globulin fraction (alpha, beta and gamma globulin) levels and lysozyme activity in YE-supplemented group. The plasma total protein is an indicator of general health status of the animal reflecting the functions of liver, kidney, and the immune system (Patra et al., 2011). The major components of total protein are globulin and albumin, therefore improving protein synthesis in the liver could improve the globulin level. The globulin fraction consists of alpha and beta acting as acute-phase proteins and gamma globulin fraction level represents the antibody (Jolles et al., 2014). Accordingly, increasing the globulin fraction in the present study means an improvement in immune response with dietary YE supplementation.

In agreement with the present findings, YE supplementation increased non-specific immune defenses in *L. vannamei* (Yang et al., 2015). Moreover, saponins as the main components of YE could stimulate cytokine secretion, which in turn regulates humoral and cellular immune responses (Palatnik de Sousa et al., 2004; Song and Hu, 2009). Saponin supplementation maximized the efficacy of immunization in chickens and increased protection against challenge (Berezin et al., 2010). Furthermore, YE promoted leptin hormone secretion, which participates in controlling the immune system function in several animals (Kucukkurt and Dundar, 2013).

In addition, YE has been shown to exhibit a wide variety of immunological characteristics (Mimaki and Sashida, 1996), such as anti-inflammatory, antiallergic, antiviral, and molluscicidal activities (Hassan et al., 2017). Moreover, YE improved antioxidant balance in broiler chickens (Su et al., 2016) and showed a strong radical scavenging activity (Sobia et al., 2013). Resveratrol as one of the YE components can protect animals from diseases because of its antioxidant, antiviral, anti-inflammatory, and iNOS inhibitory properties (Piacente et al., 2005). In addition, YE showed a potential stress relief effect in biological systems (Cigerci et al., 2009; Ince et al., 2013). In this study, cortisol levels were significantly reduced with YE supplementation in a dose-dependent manner.

Finally, the major proportion of operating costs for aquaculture projects arises from the feed. Therefore, increasing feed utilization or decreasing feed costs would help in maximizing the revenue margin (Goda et al., 2019; Khalil et al., 2019; Allam et al., 2020). In this study, dietary supplementation with higher doses of YE reduced the cost of fish production (by 23.34% with YE_{0.5} and by 32.72% with YE₁). In the same vein, the increasing of dietary supplementation levels, including prebiotics, probiotics, and symbiotics in fresh and marine culture fish diets can increase revenue (Azevedo et al., 2015; Khalil et al., 2019).

Conclusion

The present study demonstrated multiple benefits of dietary YE supplementation in terms of water quality, zootechnical performance, health status, and revenue. Yucca extract supplementation reduced ammonia nitrogen levels in fish-rearing wa-

ter and improved growth performance, FCR, and PER of the cultured fish in a dose-dependent manner, with a maximum response noted at 1 g YE kg⁻¹ diet as confirmed by quadratic regression models. In addition, there were significant improvements in hematological parameters and innate immune status with increasing dietary YE levels. In conclusion, dietary YE supplementation may contribute to the optimal water use and cleaner production in the aquaculture industry. However, the long-term effect of YE supplementation needs more investigation.

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Conflicts of Interest

There is no conflict of interests among authors and organizations.

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