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Dietary Effects of *Quillaja saponaria* and *Yucca schidigera* extract on Rearing Performance of Nile Tilapia *Oreochromis niloticus* L. and its Antioxidant Capacity and Metabolic Response Following Hypoxic Stress

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This study evaluated the effects of Quillay *Quillaja saponaria* and/ or Yucca *Yucca schidigera* extract on growth and survival of Nile tilapia *Oreochromis niloticus* L. and their antioxidant capacity and metabolic response to hypoxic challenge. Fish were fed diet supplemented with 150 mg kg^{-1} Quillay (QS), 150 mg kg^{-1} Yucca (YS), combination of 150 mg kg^{-1} Quillay and 150 mg kg^{-1} Yucca (M) or control diet (C) without addition of Quillay or Yucca for six weeks. Growth and survival were monitored periodically. After rearing, fish were subjected to hypoxic challenge and after a week, antioxidant capacity (superoxide dismutase, glutathione peroxidase and glutathione reductase) and metabolic response (glucose, triglycerides and lactate) were analyzed. Final weight, weight gain and specific growth rate of M-fish were significantly higher than that of C-fish. However, no significant difference was found on survival after 6-week rearing. Among antioxidant capacity and metabolic response, significant effects were found only on superoxide dismutase and glucose level. Superoxide dismutase of M-fish was 48% lower than that of C-fish. Furthermore, glucose level of QS-, YS- and M-fed fish was 21, 30 and 37% lower than that of C-fish, respectively. Interestingly, percentage survival of YS- and M-fish was increased by 11 and 22% as compared to that of C-fish after 1-week hypoxic challenge, respectively. Overall, combination of Quillay and Yucca improved growth performance, and demonstrated favorable antioxidant activity (superoxide dismutase), metabolic response (glucose) and resistance of fish to hypoxic environment.

Keywords: Antioxidant capacity; Metabolic response; *Oreochromis niloticus*; Quillay; Yucca

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Introduction

In recent years, increasing attention has been paid to the role of natural plant functional products in animal husbandry. These products provide consumers safer and more acceptable alternatives than synthetic compounds (Rajasekaran *et al.,* 2008). Many synthetic antioxidants, such as butylated hydroxyanisole and butylated hydroxytoluene, are available, however, their use is under strict regulation due to their potential health hazards (Branen, 1975). Therefore, the search for natural antioxidants as alternatives to synthetic products is of great interest, particularly in the aquaculture industry, since they have significant effects on growth, feed intake and reproduction of fish (Francis *et al.,* 2001; Francis *et al.,* 2002b). Natural and renewable plant resources, such as Quillay, *Quillaja saponaria* (Molina) and Yucca, *Yucca schidigera* (Roezl ex Ortgies), could be considered because of their substantial biological effects on fish and animals (Francis *et al.,* 2002a).

Quillay, a tree native to China, Peru, and the arid zones of Chile, is rich in saponins which are steroids or triterpene glycosides (Francis *et al*. 2002a). The bark of Quillay is one of the major sources of industrial triterpenoid saponins that play a role in promoting permeabilization of plasma membranes (Leung, 1997). The saponins possess a wide variety of activities, such as anti-inflammatory, anti-allergic, anti-viral, molluscicidal (Lacaille-Dubois *et al.*, 1996; Hassan *et al.,* 2010) and other immunological importance (Mimaki and Sashida, 1996).

Yucca, a plant native to southwest US and Mexico (Cheeke, 2000), is a potential antioxidant because of its phenol fractions (Piacente *et al.,* 2004). The main active compounds of Yucca are steroidal saponins and glycocomponents. Steroidal saponins are naturally non-ionic surfactants, thus, due to their strong surfactant power, the cell membranes of the intestinal wall become more capable for nutrient absorption in ruminant (Oleszek *et al.,* 1994). These also increase the intestinal flora activity, improving the digestive process. On the other hand, glycocomponents are molecular structures which are highly thermo stable that have the ability to capture ammonia molecules in the digestive tract and in the metabolic processes. These neutralize noxious effects and convert compounds into another type of non-toxic nitrogen compounds, thus improving the metabolic process (Johnston *et al.,* 1981).

Under normal conditions, the generated reactive oxygen species (ROS) are detoxified by the antioxidants present in the body and the generated ROS and the present antioxidants are in equilibrium. However, due to ROS overproduction or inadequate antioxidant defense, this equilibrium is hampered favoring the increase of ROS that leads to oxidative stress. Physico-chemical parameters and metabolic changes can induce distinct responses in fish

antioxidant defense and fish exposed to hypoxia or hyperoxia showed marked antioxidant defense alterations (Guerriero *et al.,* 2002; Ritola *et al.,* 2002; Wilhelm Filho *et al.,* 2005).

Objectives: Therefore, this study investigates if dietary Quillay and/or Yucca could improve growth and survival of Nile tilapia *Oreochromis niloticus* L. and enhance the antioxidant capacity and metabolic response under hypoxic environment.

Materials and methods

Diet preparation

This study had four treatments with three replicates in each treatment. Control diet (C) was a commercial freshwater fish feed (B-Meg, San Miguel Foods, Incorporated, Bulacan, Philippines) containing 13 % moisture, 30 % crude protein, 8 % fat, 7 % fiber and 16 % ash. Treated diets were based on the control diet supplemented with 150 mg kg^{-1} Quillay extract (product no. S-7900, Sigma Chemical Company, Saint Louis, Missouri, USA, from *Quillaja* bark, containing 11.l% sapogenin) (QS), 150 mg kg^{-1} Yucca extract (Dessert King International, San Diego, California, USA) (YS) or 150 mg kg^{-1} QS and 150 mg kg^{-1} YS (M). QS and/ or YS at 150 mg were diluted in 100 ml of distilled water. The solution was then sprayed with a gardening sprayer evenly onto 1 kg feed spread on an aluminum pan. The control diet was sprayed with only 100 ml distilled water. The four experimental diets were brought to an oven and dried at 35 ^{o}C overnight. Experimental diets were then stored in a dry plastic container at room temperature.

Fish rearing, feeding and sampling

Fish were obtained from a commercial farm in San Mateo, Isabela, Philippines, were randomly weighed and distributed in 12 glass aquaria (44 cm x 33 cm x 21.5 cm) with 30 fish in each aquarium. The initial weight of the fish (1.9 g $\pm$ 0.08) in all treatments had no significant differences ($p > 0.05$). Fish were acclimated to aquarium conditions for one week prior to the 6-week rearing during which they were fed a daily ration of 6% of their body weight twice at 0800 and 1500 h. Each aquarium was aerated. Feces and uneaten feed were siphoned out and one-third of the water was replaced daily. Water quality parameters were monitored and kept within safe levels: DO 5 - 7 mg l^{-1}, temperature 24 - 29 ^{o}C and pH 6.7 - 7.8.

Growth and survival

Fish were weighed every 2 weeks with a digital scale. The quantity of feed given was readjusted after each weight sampling and survival in each tank was monitored daily.

Weight gain (WG) and specific growth rate (SGR) were used as indices for the growth performance of fish.

$WG\ (\%) = 100 \times ((W_f - W_i)/W_i)$

$SGR\ (\%\ day^{-1}) = 100 \times ((\ln(W_f) - \ln(W_i)) /T$

Where W_i is the initial body weight (g), W_f the final body weight (g), ln the natural logarithm and T the length of culture period (days).

% Survival= final count/ initial count *100

Hypoxic stress

This experiment was conducted to find out if different experimental diets could affect the survival, antioxidant capacity and metabolic response of fish under hypoxic environment. At the completion of the previous experiment, ten fish from each treatment were randomly selected and immediately transferred to one of the twelve 10-l glass aquaria (four treatments with three replicates) right after filled up with water from deep well pump. To retain the hypoxic condition (DO at 0.1 mg l^{-1}), no aerator was installed and a cover placed on each aquarium. The DO level was from 0.1-0.3 mg l^{-1} while the temperature from 20-28 ^{o}C. Mortality was recorded daily for one week.

Antioxidant capacity and metabolic response

Blood samples were taken after one week of hypoxic stress. Sampled fish were quickly anesthetized with tricaine methanesulphonate (MS-222) at 100 mg l^{-1}. Approximately 200 µl heparinized blood was withdrawn from the caudal vessel of 3 fish per aquarium using 1-ml sterile syringe with 25 gauge needles. Heparin was used in order to avoid the blood coagulation. Blood was then centrifuged for 5 min at 1800 *g* and the plasma was drawn and immediately frozen (-4^{o}C) for later evaluation of antioxidant capacity and metabolic response.

The antioxidant capacity was analyzed with Bio-Tek Synergy HT enzyme linked immunosorbent assay (ELISA) reader (BioTek Instruments Taiwan, Inc., Taipei, Taiwan) for superoxide dismutase (SOD) and SP-830 plus metertech spectrophotometer (Hitachi Ltd, Tokyo, Japan) for glutathione

peroxidase (GPx) and glutathione reductase (GR). The volumes of plasma used were 10, 10 and 20 µl for SOD, GPx and GR analysis, respectively.

SOD activity was measured by its ability to inhibit superoxide radical dependent reactions. The reaction mixture (1.7 ml) contained xanthine (0.05 mM) and 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT, 0.025 mM) dissolved in 50 mM CAPS (pH 10.2) and 0.94 mM EDTA. In the presence of xanthine oxidase (80 U l^{-1}, 250 µl), superoxide and uric acid were produced from xanthine. The superoxide radical was then reacted with INT to produce a red formazan dye. The optical density was measured at 505 nm, 37 ℃, and the rate of reaction was estimated from the absorbance readings at 30 s and 3 min after adding xanthine oxidase. A reference standard SOD was supplied with the Randox Kit (Crumlin, Co. Antrim, UK). One unit of SOD was defined as the amount required to inhibit the rate of xanthine reduction by 50% (Biagini *et al.,* 1995). One unit of activity was expressed in U ml^{-1}.

GPx activity was measured based on the method described by Paglia and Valentine (1967). GPx catalyses the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized form of glutathione was immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance at 340 nm was measured. Briefly, 15 µl diluted plasma mixture was added to the reaction mixture containing 40 µl cumene hydroperoxide and 10 mM buffer. The optical density of NADPH was measured at 340 nm, 37 ℃, and the rate of reaction was estimated from the absorbance readings at the first 3 min after adding cumene hydroperoxide. One unit of activity was expressed in U ml^{-1}.

Finally, GR catalyses the reduction of glutathione in the presence of NADPH, which is oxidized to NADP+. The decrease in absorbance at 340 nm was measured. This assay was carried out using Randox laboratories kit according to manufacturer's instructions (Biagini *et al.,* 1995). One unit of activity was expressed in U ml^{-1}.

ELISA reader with individual Randox kits was used for determination of Gluc (Randox, GOD-PAP), Trig (Randox-GPO-PAP) and Lac (Randox, PAP). Methods were adapted to a 96-well plate using 3 µl samples and 300 enzyme reagents (Palacios *et al.,* 2000). Gluc, Trig and Lac levels were expressed in mg dl^{-1} plasma.

Statistical Analysis

An arcsine transformation was used before processing percentage data. Levene and Kolmogorov–Smirnov's test for homogeneity of variance and

normality were applied on the data of growth, survival, antioxidant capacity and metabolic response. The data were homogenous and showed normal distribution. One-way analysis of variance (ANOVA) was then performed to find out the effects of Quillay and/or Yucca on growth, survival, antioxidant capacity (SOD, GPx and GR) and metabolic response (Gluc, Trig and Lac). Two-way ANOVA was performed to determine the main effects of Quillay and Yucca and their interaction effects on growth, survival, antioxidant capacity and metabolic response. Duncan's Multiple Range Test (DMRT) was then carried out to compare differences between levels of each factor. Correlation analyses were applied on growth, survival, antioxidant capacity and metabolic response. SAS v.9 was the software used in all the analysis. The significant level applied to all analysis was set at 5%.

Results

Feed intake and behavior

From 1st to 3rd week of feeding experiment, fish consumed the treated diets completely. However, on the 4th week, fish did not consume entirely the daily ration and hence it was adjusted to 3% after several back and forth feeding trials. This feeding rate was adopted since then until the end of the experiment.

Growth and survival

Only after the 6th week the treatments showed their effect on growth (Table 1). The W_f of M-fish was significantly higher than C-fish but comparable to QS- and YS-fish. Furthermore, WG and SGR of M-fish were higher than C- and QS-fish but comparable to YS-fish. Disregarding Yucca effect, fish fed diets with Quillay had higher SGR as compared to those fish fed diets without Quillay. On the other hand, disregarding Quillay effect, fish fed diets with Yucca had higher W_f, WG and SGR than those fish fed diets without Yucca. No significant difference was found on survival after six weeks of rearing. None of the interaction effects were found on W_f , WG, SGR and Survival (Table 1).

Fish behavior on hypoxic stress

The hypoxic stress eventually leads to hyperactivities, surfacing, discolorations of the skin, erratic swimming, changes in behavior and

increasing opercula ventilation and movement. Some of the test fish showed increase in weakness, motionless and gasp for air with slow opercula movement and death. Mortality was observed from 2^{nd} to 5^{th} day of stress test.

Antioxidant capacity

After hypoxic stress, only SOD showed significant difference among the tested parameters (Table 2). YS- and M-fish had lower SOD activity than the C-fish. Disregarding Quillay effect, fish fed diets with Yucca had lower SOD than those fish fed diets without Yucca. None of the interaction effects were found on SOD, GPx and GR.

Metabolic Response

After hypoxic stress, only Gluc showed significant difference among the tested parameters (Table 3). QS-, YS- and M-fish had lower Gluc level than the C-fish. Disregarding Quillay effect, fish fed diets with Yucca had lower Gluc than those fish fed diets without Yucca. None of the interaction effects were found on Gluc, Trig and Lac.

Survival on hypoxic stress

After hypoxic stress, M-fish had higher survival than QS- and C-fish (Table 4). Disregarding Quillay effect, fish fed diets with Yucca had higher survival than those fish fed diets without Yucca. None of the interaction effects were found on survival.

Correlation Analysis

Correlation analysis was conducted among growth, antioxidant capacity, metabolic response and survival of fish after six weeks of rearing (Sur A) and after exposure to low DO (Sur B) (Table 5). Growth parameters (W_f, WG and SGR) and Sur B were negatively correlated to Gluc and SOD. In addition, SOD was positively correlated to Gluc but negatively correlated to Sur A. Sur B was also but positively correlated to Sur A and W_f.

Table 1. Mean initial weight, final weight, weight gain, specific growth rate and survival of Nile tilapia *Oreochromis niloticus* L. fed four experimental diets for six weeks.

Means (±S.E) in the same row without a common superscript are significantly different ($p \leq 0.05$).

Parameter[1]	One-way ANOVA				Two-way ANOVA					MSE[17]
					Main Effect				Interaction	
	Treatment[7]				Quillay (Q)		Yucca (Y)		Q x Y	
	C[8]	QS[9]	YS[10]	M[11]	No[12]	Yes[13]	No[14]	Yes[15]	(P> F)[16]	
W_i[2]	1.94 [a]	1.94 [a]	1.96 [a]	1.89 [a]	1.95[a]	1.92 [a]	1.95[x]	1.92 [x]	0.54	3.3 X 10 $^{-3}$
	(0.34)	(0.08)	(0.04)	(0.04)	(0.04)	(0.03)	(0.02)	(0.04)		
W_f[3]	6.61 [b]	6.85 [ab]	7.06 [ab]	7.30 [a]	6.83 [a]	7.08 [a]	6.73 [y]	7.17 [x]	0.99	8.3 X 10^{-6}
	(0.24)	(0.14)	(0.05)	(0.05)	(0.14)	(0.06)	(0.15)	(0.12)		
WG[4]	240 [b]	253 [b]	261 [ab]	287 [a]	250 [a]	270[a]	247[y]	274[x]	0.52	1.3 X 10 ^{2}
	(12)	(10)	(5)	(12)	(7.69)	(7.97)	(7.45)	(12.35)		
SGR[5]	0.507 [b]	0.527 [b]	0.533 [ab]	0.566 [a]	0.520 [b]	0.547 [a]	0.517 [y]	0.550 [x]	0.57	1.3 X 10 $^{-4}$
	(0.015)	(0.012)	(0.006)	(.011)	(0.01)	(0.01)	(0.01)	(0.01)		
Survival[6]	91 [a]	94 [a]	94 [a]	94 [a]	93 [a]	94 [a]	93 [x]	94 [x]	0.37	3.3 X 10^{1}
	(0.00)	(1.73)	(1.73)	(1.73)	(1.76)	(2.18)	(1.76)	(1.36)		

[1]/Parameter: [2]/Wi-Initial weight; [3]/W_f- Final weight; [4]/WG-Weight gain (%) = $(W_f - W_i)/ W_i) \times 100$; [5]/SGR-Specific growth rate = ((ln mean final weight) − (ln mean initial weight)/no. of days) × 100; [6]/Survival (%) = no. of fish survived at the end of 6-week rearing/no. of fish at stocking.

[7]/Treatment: [8]/C- Control diet; [9]/QS-Diet supplemented with 150 mg kg^{-1} Quillay *Quillaja saponaria* (product no. S-7900, Sigma Chemical Company, Saint Louis, Missouri, USA, from *Quillaja* bark, containing 11.l% sapogenin) ; [10]/YS-Diet supplemented with 150 mg kg^{-1} Yucca *Yucca schidigera* (Dessert King International, San Diego, California, USA) ; [11]/M- Diet supplemented with 150 mg kg^{-1} Quillay and 150 mg kg^{-1} Yucca .

[12]/Fish were fed control diet and diet supplemented with 150 mg kg^{-1} Yucca only.

[13]/Fish were fed diet supplemented with 150 mg kg^{-1} Quillay and combination of 150 mg kg^{-1} Quillay and 150 mg kg^{-1} Yucca.

[14]/Fish were fed control diet and diet supplemented with 150 mg kg^{-1} Quillay only.

[15]/Fish were fed diet supplemented with 150 mg kg^{-1} YS and combination of 150 mg kg^{-1} Quillay and 150 mg kg^{-1} Yucca.

[16]/Interaction between Quillay and Yucca

[17]/Mean standard error

Table 2. Mean activities of activities of plasma antioxidant capacity of Nile tilapia *Oreochromis niloticus* after fed four experimental diets for six weeks and then subjected to hypoxic challenge for one week.

Parameter[1]	One-way ANOVA				Two-way ANOVA					MSE[15]
					Main Effect				Interaction	
	Treatment[5]				Quillay (Q)		Yucca (Y)		Q x Y	
	C[6]	QS[7]	YS[8]	M[9]	No[10]	Yes[11]	No[12]	Yes[13]	(P> F)[14]	
SOD[2]	0.44[a] (0.04)	0.31[ab] (0.06)	0.27[b] (0.05)	0.23[b] (0.02)	0.36[a] (0.07)	0.28[a] (0.05)	0.38[y] (0.06)	0.25[x] (0.04)	0.36	5.6×10^{-3}
GPx[3]	2.79[a] (0.08)	2.72[a] (0.15)	2.98[a] (0.33)	3.02[a] (0.51)	2.88[a] (0.22)	2.87[a] (0.35)	2.76[x] (0.11)	3.00[x] (0.38)	0.86	9.9×10^{-3}
GR[4]	0.028[a] (0.005)	0.030[a] (0.005)	0.042[a] (0.011)	0.040[a] (0.010)	0.035[a] (0.009)	0.035[a] (0.008)	0.029[x] (0.004)	0.041[x] (0.009)	0.78	2.2×10^{-5}

Means (±S.E) in the same row without a common superscript are significantly different ($p \leq 0.05$).

[1] /Parameter: [2]/SOD- Superoxide dismutase; [3]/GPx-Glutathione peroxidase; [4]/GR-Glutathione reductase

[5]/Treatment: [6]/C- Control diet; [7]/QS-Diet supplemented with 150 mg kg^{-1} Quillay *Quillaja saponaria* (product no. S-7900, Sigma Chemical Company, Saint Louis, Missouri, USA, from *Quillaja* bark, containing 11.l% sapogenin); [8]/YS-Diet supplemented with 150 mg kg^{-1} Yucca *Yucca schidigera* (Dessert King International, San Diego, California, USA); [9]/M- Diet supplemented with 150 mg kg^{-1} Quillay and 150 mg kg^{-1} Yucca.

[10]/Fish were fed control diet and diet supplemented with 150 mg kg^{-1} Yucca only.

[11]/Fish were fed diet supplemented with 150 mg kg^{-1} Quillay and combination of 150 mg kg^{-1} Quillay and 150 mg kg^{-1} Yucca.

[12]/Fish were fed control diet and diet supplemented with 150 mg kg^{-1} Quillay only.

[13]/Fish were fed diet supplemented with 150 mg kg^{-1} Yucca and combination of 150 mg kg^{-1} Quillay and 150 mg kg^{-1} Yucca.

[14]/Interaction between Quillay and Yucca

[15]/Mean standard error

Table 3. Mean plasma metabolic response of Nile tilapia *Oreochromis niloticus* after fed four experimental diets for six weeks and then subjected to hypoxic challenge for one week.

Parameter[1]	One-way ANOVA				Two-way ANOVA					MSE[15]
					Main Effect				Interaction	
	Treatment[5]				Quillay (Q)		Yucca (Y)		Q x Y	
	C[6]	QS[7]	YS[8]	M[9]	No[10]	Yes[11]	No[12]	Yes[13]	(P> F)[14]	
Gluc[2]	88.62[a]	70.44[b]	62.32[b]	55.51[b]	75.47[a]	62.97[a]	79.53[y]	58.92[x]	0.32	9.7x 10^{1}
	(4.6)	(4.9)	(6.7)	(5.3)	(9.8)	(6.6)	(7.2)	(5.8)		
Trig[3]	160.77[a]	178.97[a]	165.75[a]	138.02[a]	163.26[a]	158.49[a]	169.87[x]	151.88[x]	0.07	1.5x 10^{3}
	(15.5)	(7.3)	(12.8)	(7.0)	(12.8)	(14.4)	(12.3)	(12.7)		
Lac[4]	46.66[a]	51.01[a]	51.40[a]	48.2[a]	49.63[a]	49.03[a]	48.83[x]	49.82[x]	0.10	4.2 x 10^{1}
	(2.0)	(0.7)	(1.1)	(3.3)	(2.1)	(2.3)	(1.9)	(2.4)		

Means (±S.E) in the same row without a common superscript are significantly different ($p \leq 0.05$).

[1]/Parameter: [2]/Gluc- Glucose; [3]/Trig- Triglycerides; [4]/Lac-Lactate.

[5]/Treatment: [6]/C- Control diet; [7]/QS-Diet supplemented with 150 mg kg^{-1} Quillay *Quillaja saponaria* (product no. S-7900, Sigma Chemical Company, Saint Louis, Missouri, USA, from *Quillaja* bark, containing 11.l% sapogenin); [8]/YS-Diet supplemented with 150 mg kg^{-1} Yucca *Yucca schidigera* (Dessert King International, San Diego, California, USA); [9]/M- Diet supplemented with 150 mg kg^{-1} Quillay and 150 mg kg^{-1} Yucca.

[10]/Fish were fed control diet and diet supplemented with 150 mg kg^{-1} Yucca only.

[11]/Fish were fed diet supplemented with 150 mg kg^{-1} Quillay and combination of 150 mg kg^{-1} Quillay and 150 mg kg^{-1} Yucca.

[12]/Fish were fed control diet and diet supplemented with 150 mg kg^{-1} Quillay only.

[13]/Fish were fed diet supplemented with 150 mg kg^{-1} Yucca and combination of 150 mg kg^{-1} Quillay and 150 mg kg^{-1} Yucca.

[14]/Interaction between Quillay and Yucca

[15]/Mean standard error

Table 4. Mean survival of Nile tilapia *Oreochromis niloticus* after fed four experimental diets for six weeks and after hypoxic challenge for one week.

Parameter[1]	One-way ANOVA				Two-way ANOVA					MSE[13]
					Main Effect				Interaction	
	Treatment[3]				Quillay (Q)		Yucca (Y)		Q x Y	
	C[4]	QS[5]	YS[6]	M[7]	No[8]	Yes[9]	No[10]	Yes[11]	(P> F)[12]	
Survival [2]	63[b]	67[b]	70[ab]	77 [a]	67 [a]	72 [a]	65 [x]	73 [y]	0.52	1.3×10^{-3}
	(3.3)	(3.3)	(0.0)	(3.3)	(9.8)	(6.6)	(7.2)	(5.8)		

Means (±S.E) in the same row without a common superscript are significantly different ($p \leq 0.05$).

[1]/Parameter: [2]/Survival- Survival of fish after fed four experimental diets for six weeks and after hypoxic challenge for one week;

[3]/Treatment: [4]/C- Control diet; [5]/QS-Diet supplemented with 150 mg kg^{-1} Quillay *Quillaja saponaria* (product no. S-7900, Sigma Chemical Company, Saint Louis, Missouri, USA, from *Quillaja* bark, containing 11.l% sapogenin); [6]/YS-Diet supplemented with 150 mg kg^{-1} Yucca *Yucca schidigera* (Dessert King International, San Diego, California, USA); [7]/M- Diet supplemented with 150 mg kg^{-1} Quillay and 150 mg kg^{-1} Yucca.

[8]/Fish were fed control diet and diet supplemented with 150 mg kg^{-1} Yucca only.

[9]/Fish were fed diet supplemented with 150 mg kg^{-1} Quillay and combination of 150 mg kg^{-1} Quillay and 150 mg kg^{-1} Yucca.

[10]/Fish were fed control diet and diet supplemented with 150 mg kg^{-1} Quillay only.

[11]/Fish were fed diet supplemented with 150 mg kg^{-1} Yucca and combination of 150 mg kg^{-1} Quillay and 150 mg kg^{-1} Yucca.

[12]/Interaction between Quillay and Yucca

[13]/Mean standard error

Table 5. Correlation matrix among survival, antioxidant capacity and metabolic response of Nile tilapia *Oreochromis niloticus* after fed diet supplemented with 150 mg kg^{-1} Quillay *Quillaja saponaria*, 150 mg kg^{-1} Yucca *Yucca schidigera*, combination of 150 mg kg^{-1} Quillay and 150 mg kg^{-1} Yucca or control diet for six weeks and then subjected to hypoxic challenge for one week.

Parameters[1]	**Sur A**	**W_f**	**WG**	**SGR**	**SOD**	**GPx**	**GR**	**Gluc**	**Lac**	**Sur B**
	Sur				-0.72*					0.57*
		W_f	0.75*	0.75*	-0.73*			-0.71*		0.62*
			WG	0.99*				-0.70*		
				SGR	-0.56*			-0.73*		
					SOD		-0.59*	0.82*		-0.74*
						GPx				
							GR			
								Gluc		-0.64*
									Lac	

The correlation between two parameters is shown by correlation coefficient (r) value; * significant ($p \leq 0.05$); Blank- not significant.
[1]/Parameters: Sur-Survival; SOD-Superoxide dismutase; GPx-Glutathione peroxidase; GR-Glutathione reductase; Gluc-Glucose; Trig-Triglycerides;Lac-Lactate

Discussion

Plant containing saponins, like Quillay and Yucca enhanced the growth performance of an animal. In this study, W_f of M-fish was increased by 10 % as compared to the C-fish. In other studies, positive effect of Quillay on growth of common carp *Cyprinus carpio* L. was also observed at a dietary level of 150 mg kg^{-1} (Francis *et al*., 2001; Angeles and Chien, 2014). In addition, Yucca has also been found to improve growth, feed efficiency, health and even water quality by lowering the ammonia concentration in rearing systems of aquatic species (Kelly and Kohler, 2003; Santacruz-Reyes and Chien, 2009; Santacruz-Reyes and Chien, 2012; Güroy *et al.*, 2014; Yang *et al.*, 2015). The improved growth performance can be attributed to the capacity of dietary saponins to increase permeability of intestinal wall and nutrient absorption (Francis *et al*., 2002b).

Low oxygen availability may result in oxidative stress which is characterized by excessive production of reactive oxygen species (ROS) (Storey, 1996) and the increased of antioxidant defense (Lushchak *et al.*, 2001). In this study, SOD of M-fish was 48% lower than that of C-fish which indicated stability of antioxidant defense. It has been reported, that lower SOD may indicate higher cell protection (Hartog *et al.*, 2003) and could therefore due to the development of the antioxidant defense system within a particular period of time. In other study, SOD activity in the gill, liver and muscle tissue and GPx activity in the brain of *C. carpio* were increased under hypoxia stress (Lushchak *et al*., 2001). Astaxanthin, a powerful antioxidant, gave lowest value of GPx when fed to characins, *Hyphessobrycon callistus* (Boulenger) which indicated that astaxanthin could be effective in removing H_2O_2 induced by hypoxia (Pan *et al.*, 2010). Increased SOD and GPx activity under hypoxia stress showed a synergistic relationship between the two antioxidant capacity indicators (Lushchak *et al.*, 2001).

The increased level of antioxidant defenses in anoxic/hypoxic fish suggests that preparation for oxidative stress is a biochemical adaptive mechanism for anoxia/hypoxia tolerance (Vág and Nemcsók, 1989; Lushchak, 2001) in a certain period of time. The antioxidant defense had the ability to augment under hypoxic condition indicated their active role in adaptation. This study shows that perturbation of hypoxic condition is accompanied by modification of free radical processes in fish. However, the antioxidant enzyme activities in the blood did not return to initial values, showing that osmoregulatory processes caused substantial physiological changes in the fish (Lushchak, 2011). Thus, in this study, the oxidative stress brought by hypoxic stress can be minimized with the supplementation of Quillay and Yucca in the diet as reflected by more stable SOD activity.

For metabolic response, Gluc, Trig and Lac serve as indicator to stress (Barton and Iwama, 1991). These responses to stressors are considered adaptive and important for the fish to regain homeostasis (Mommsen *et al.*, 1999). In this study, QS-, YS- and M- fish had 21, 30 and 37 % lower Gluc level as compared to the C-fish, respectively. Plasma Gluc level in fish increases during stress probably as a result of catecholamine action on stored glycogen in liver and other tissues (Pottinger, 1998). It has been reported that the elevation in plasma Gluc of wolfish, *Anarhichas minor* (Olafsen) after 4 h exposure was due to hypoxic stress which indicated hyperglycemic response (Lays *et al.*, 2009). While in another study, yucca powder supplementation decreased serum Gluc, cholesterol and Trig level in laying quails, *Coturnix coturnix japonica* (Temminck and Schlegel) (Kaya *et al.*, 2003). The elevated plasma Gluc level observed in C-fish is likely due to cortisol stimulation of glyconeogenesis, especially from amino acids mobilized from peripheral stores (Mommsen *et al.*, 1999). This study demonstrated that diets containing Quillay and Yucca contributed in stabilizing the metabolic response to attain homeostasis.

The metabolic parameters can respond prior treatments and various stresses differently in terms of responding time and extent. Lac level of fish varies and can be affected by several factors. Increase of Lac levels in several species was observed after subjecting the fish to stress (Arends *et al.*, 1999). On the contrary, in this study, no significant differences were observed in plasma Lac level among treatments. It has been also reported that Eurasian perch *Perca fluviatilis* L. did not show significant changes in Lac level after being subjected to transport and acute handling stress (Acerete *et al.*, 2004). In other study, red porgy *Pagrus pagrus* L. fry reared in seawater at high and low density for 45 d showed no difference in Gluc, Trig and Lac (Vargas-Chacoff *et al.*, 2011). However, when reared in brackish water, the fish at high density showed higher Gluc and Trig than at low density. In this study, Trig and Lac were not affected by dietary treatments and hypoxic stress. This indicates that either Trig or Lac was less sensitive responding to the altered conditions, namely, dietary treatments and hypoxic stress, or the metabolic activities associated with Trig and Lac of the fish had adapted to the stress.

Antioxidant capacity and metabolic response that make up the antioxidant defense system and metabolic processes are expected to increase under stress in order to detoxify ROS and stabilize the overall metabolism, respectively. The antioxidant enzymes are intrinsically linked and dependent upon the activity of one another as well as in metabolic response. One would therefore expect to see correlative changes among the tested parameters. Significant correlations among growth, survival, antioxidant capacity (SOD) and metabolic response (Gluc) could indicate that the growth and survival were

related to the reaction of superoxide radicals by SOD and the stability of metabolic response of Gluc. The survival of YS- and M-fish was increased by 11 and 22% as compared to that of C-fish after 1-week hypoxic stress, respectively. The elicited correlations among survival, antioxidant capacity and metabolic response might be either due to the beneficial effect of the combination of Quillay and Yucca or double dosage effect of saponins.

Overall, combination of Quillay and Yucca improved growth performance, and demonstrated favorable antioxidant activity (superoxide dismutase), metabolic response (glucose) and resistance of fish to hypoxic environment. These natural and renewable resources could be therefore considered as potential alternatives to synthetic antioxidant products used in aquaculture industry. However, further investigations are still needed to maximize their functions as growth promoter and antioxidant .Optimizing the dosage and ratio of Quillay and Yucca is worth to be studied. Since the response in the administered treatments might be species-specific and varies by elapsed time, further investigations on the effect of combination of Quillay and Yucca on metabolic response and antioxidant capacity to different species, stressors and sampling times are recommended.

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