
Performance of Red Tilapia (*Oreochromis* sp.) Fed Diet with Fermented Banana (*Musa Acuminata* × *Balbisiana*) Peel at Different Stages of Ripeness Following *Aeromonas Hydrophila* Infection

Grace H. Mones¹ and Isagani P. Angeles Jr.^{1,2*}

¹Provincial Institute of Fisheries, Isabela State University-Roxas Campus, Roxas, Isabela, Philippines 3320; ²Institute of Fisheries, Isabela State University-Echague Campus, Echague, Isabela, 3309 Philippines.

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This study evaluated the dietary effect of fermented banana (*Musa acuminata* × *balbisiana*) peel at different stages of ripeness on growth, antioxidant capacity, metabolic response and survival of Red tilapia (*Oreochromis* sp.) reared for nine weeks following *Aeromonas hydrophila* infection. The fermented banana peel (FBP) with 100 ml DW was sprayed unto one kg of commercial tilapia feed; 200 ml immature FBP (iFBP), 200 ml ripe FBP (rFBP), 200 ml over ripe FBP (oFBP), while 300 ml DW for the control diet (C). Significant effects of treatments on growth were observed from 3rd to 9th week of rearing. Final weight, specific growth rate and protein efficiency ratio of oFBP-fish were significantly higher and FCR was significantly lower than that of C-fish. WG of oFBP, rFBP, iFBP-fish was increased by 80%, 43% and 29% than that of C-fish, respectively. On the other hand, disregarding ripeness, percentage survival of FBP-fish was significantly higher than that of C-fish after nine weeks of rearing. SOD of oFBP, rFBP and iFBP- fish was decreased by 59, 43 and 35 % as compared to that of C, respectively. GPx and GR activity of oFBP-fish were higher than that of the C-fish. oFBP and rFBP-fish had 52 and 44 % lower Gluc level as compared to that of C-fish, respectively while Lac level of oFBP-fish was lower than that of C-fish. However, no significant difference was found on Trigs. Interestingly, all FBP-fed groups exhibited higher percentage survival than that of C-fish group and the highest post-challenge survival (70%) was recorded in the oFBP-fish group. Disregarding ripeness, higher extrapolated fish yield per 1000 m² was obtained in FBP-fish than that of C-fish, oFBP>rFBP>oFBP>C while the results for cost-benefit ratio was as follow: oFBP<rFBP<oFBP<C. Overall, these results indicated that FBP at different stages of ripeness, especially oFBP enhances growth performance, stabilizes both antioxidant capacity and metabolic response, improves resistance of Red tilapia against *A. hydrophila* infection and provides better cost-benefit ratio. FBP could be therefore considered as potential alternative to synthetic growth promoter and antioxidant products used in aquaculture industry.

Keywords: *Aeromonas hydrophila*; Fermented Banana Peel, Tilapia

* **Coressponding Author:** Isagani P. Angeles Jr. **E-mail:** ipangelesjr_15@yahoo.com

Introduction

Tilapia is the Philippines' third largest aquaculture product in terms of volume (after seaweeds and milkfish) and value (after milkfish and tiger prawn). In recent years, the hybrid red tilapia has been opted as one of the most popular candidate species for modern aquaculture practices (Watanabe *et al.*, 2002). Red hybrid tilapia are gaining popularity among culturists due to their resemblance to premium marine species such as sea bream (*Chrysophrys major*) and red snapper (*Lutjanus campechanus*) and excellent growth, feed conversion rates in freshwater and also gives it higher market value (Popma and Masser, 1999).

The exponential growth of the aquaculture sector during the past two decades is a result of the progressive intensification of production systems and use of quality feeds, which meet the nutritional requirements of cultured fish (FAO, 2006). Fish feeds account for the highest operational costs in aquaculture with protein being the most expensive diet (Munguti *et al.*, 2012). Intensive rearing of aquaculture fish species generates environmental stress to fish, which can increase susceptibility to various pathogens such as viruses, bacteria, fungi, and parasites (Wang *et al.*, 2015); this has led to a huge economic loss. The most common and frequently encountered bacterial pathogen in freshwater aquaculture is *Aeromonas hydrophila* (Maiti *et al.*, 2012). Potential antioxidants such as carotenoids help to inactivate free radicals produced from normal cellular activity and biological and environmental stress (Chew, 1995). The need for antioxidants becomes even more critical with increased exposure to free radicals. In the past two decades, it has become increasingly clear that oxidative stress plays a major role in the pathogenesis of a number of diseases (Mates *et al.*, 1999). It is the cellular toxicity caused by overproduction of free radicals. Aquatic organisms have developed defenses to protect against ROS-induced damage including antioxidant enzymes such as GPx and SOD (Eyckmans *et al.*, 2011) and GR was observed under different stress (Halliwell and Gutteridge, 1989). Therefore, measurement of these antioxidant parameters may provide a hint of the antioxidant status in fish, and these parameters can serve as biomarkers for oxidative stress (Zhang *et al.*, 2013).

The search for natural antioxidants as alternatives to synthetic products is of great interest, particularly in the aquaculture industry (Francis *et al.*, 2001). Plant extracts are known to promote growth, stimulate appetite, and enhance tonicity and immunostimulation. Moreover, plant extracts facilitate maturation of cultured species, and possess stress reduction, sexual stimulation, and antipathogenic properties in fish (Reverter *et al.*, 2014).

Banana (*Musa* spp.) is the second leading fruit produced after citrus, contributing to approximately 17% of the world's total fruit production; it is

cultivated in over 130 countries (FAO, 2013). Saba banana (*Musa acuminata* × *balbisiana*) is a climacteric fruit made up of peel and edible pulp that has a high nutritional value (Amarnath and Balakrishnan, 2007). It is rich in dietary proteins, essential amino acids, vitamins, polyunsaturated fatty acids, fibre, and potassium (Emaga *et al.*, 2007), that make it favorable as a feed for livestock and poultry (Mohapatra *et al.*, 2010). Bioactive compounds like flavonoids, tannins, phlobatannins, alkaloids, glycosides, anthocyanins, and terpenoids were found in banana peels, and these compounds have been reported to exert various biological and pharmacological effects (antibacterial, antihypertensive, antidiabetic, and anti-inflammatory activities) which possess various beneficial effects on human health (Pereira and Maraschin, 2015). Further, antioxidant compounds (e.g., prodelphinidins, polyphenols, catecholamines, and carotenoids) (Rebello *et al.*, 2014) and high amount of micronutrients (Sundaram *et al.*, 2011) were found in the peels of genus *Musa*.

Fermentation is being developed worldwide as supplemental diet for aquaculture production. Fermented Fruit Juice (FFJ), the process of breaking down complex organic substance of fruit through the process of fermentation, can be considered as feed ingredient. The fruit is extracted using raw sugar or molasses through osmotic pressure. It was reported that FFJ had been identified as one of the less expensive means of increasing the protein quality of fruit wastes (Ubalua, 2007). The use of microorganisms to convert carbohydrates, lignocelluloses and other industrial waste into food stuffs that are rich in protein is possible due to the inherent nature of microorganisms; hence, there is a need to assess the potential of non-conventional raw ingredients such as BP. It would therefore be more economical to explore other ways like fermentation of BP to make cheaper but still efficient fish diet.

Objectives: This study generally aims to evaluate the effect of FBP at different stages of ripeness (immature, ripe and over ripe) on growth, antioxidant capacity, metabolic response and survival of Red Tilapia following *A. hydrophila* infection.

Specifically, this study aims to: evaluate the rearing performance of Tilapia fed with FBP after nine weeks of rearing experiment; determine the antioxidant capacity and metabolic response of Tilapia fed with FBP after nine weeks of rearing experiment and one day after *A. hydrophila* infection; determine which from the different stages of ripeness (immature, ripe, over ripe) gave best effect on rearing performance, antioxidant capacity and metabolic response of Tilapia; establish the correlation among growth, antioxidant capacity, metabolic response and survival of Tilapia fed with FBP after nine weeks of rearing experiment and one day after *A. hydrophila* infection and; evaluate the cost- benefit ratio of FBP at different stage of ripeness.

Materials and methods

Banana peel fermentation

The BP was collected from Ramon Public Market, Ramon, Isabela, Philippines. The BP was washed, sliced in small pieces and weighed. The one kg BP was then mixed with 200 ml molasses in a plastic container covered with cheesecloth and store in a cool dry room. The juice of FBP was then collected after 30 days of fermentation.

Diet preparation

There were four treatments in this study. The commercial diet was sprayed with FBP at different stages of ripeness (immature, ripe or over ripe). The FBP was diluted to DW and was sprayed into one kg feed with a gardening sprayer on a plastic pan and spread evenly; 200 ml immature FBP + 100 ml DW (iFBP), 200 ml ripe FBP + 100 ml DW (rFBP), 200 ml over ripe FBP + 100 ml DW (oFBP). The control one kg diet was also sprayed with 300 ml distilled water (DW). The four experimental diets were air dried overnight and were then stored in a dry plastic container and store in a refrigerator (4 °C).

Proximate analysis

Twelve fish were sampled at the beginning of the trial and three fish per treatment per replicate were sampled to determine carcass composition. Fish was dried in an air oven at 105 °C until a constant weight was achieved to determine moisture content. Dried fish was pooled (three fish per sample) and ground for composition analysis according to AOAC (1995) protocols; all samples were analyzed in triplicate. The proximate analysis of the experimental diets is shown in Table 1.

Table 1. Proximate analysis of the experimental diets.

	<i>Proximate analysis (% dry matter basis)</i>			
	C	iFBP	rFBP	oFBP
Moisture	86.73	86.99	87.44	87.79
Crude protein	33.92	36.51	37.54	37.84
Crude lipid	3.28	3.74	3.94	4.38
Ash	12.08	14.07	13.30	12.94

Fish rearing, feeding and sampling

The 240 fish were assigned to 12 glass aquaria following complete randomized design (CRD) where each treatment was triplicated with 20 fish in each aquarium. Fish were obtained from Central Luzon State University-National Freshwater Technology Center (CLSU- NFFTC), Science City of Muñoz, Nueva Ecija, Philippines, were weighed and distributed in each aquarium. Fish were acclimated in an aquarium conditions for one week prior to the nine weeks of rearing experiment during which they were fed a twice daily a ration of 6% of their body weight 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 such as temperature (25-28 °C), dissolved oxygen (>5 mg l⁻¹) and pH (6.9-7.2) were monitored and kept within safe levels.

Growth and survival

Weight sampling was conducted every 21 days with a digital scale. The quantity of feed given was readjusted after each weight sampling, while the survival in each plastic container was monitored daily.

Weight gain (WG), specific growth rate (SGR) and feed conversion ratio (FCR) was used as indices for the growth performance of fish.

Weight gain (WG) = final weight- initial weight

Specific growth rate (%) = $100[(\ln W2 - \ln W1) / (\ln W1)] / T$; where W1 and W2 are initial weight and final weight, and T is the number of days in the feeding period;

Feed conversion ratio (FCR) = feed intake / weight gain;

Survival (%) = final count – initial count*100

Collection of blood samples

Fish blood samples were collected after nine weeks of rearing and one day after *A. hydrophila* infection. Blood samples were taken from the gills into tubes containing EDTA as anticoagulant.

Bacterial challenge test

After rearing, fish were challenged with 0.2 ml of 1×10^5 cfu ml⁻¹ *A. hydrophila* through intraperitoneal injection. The set-up was consisted of eight aquaria arranged in CRD with two replicates per treatment. Manifestations of *A.*

hydrophila infections in the fish in each treatment were evaluated daily after the infection. During this period, fish was continually fed with experimental diets.

Antioxidant capacity

Blood samples were taken after rearing and one day after *A. hydrophila* infection. Approximately 200 µl heparinized blood was withdrawn from the caudal vessel of 3 fish per plastic container using 1-ml sterile syringe with 23 gauge needles. Heparinized blood was then centrifuged for 5 min at 1800 g and the plasma was drawn and immediately frozen (-4 °C) for later evaluation of antioxidant capacity.

The antioxidant capacity was analyzed with enzyme linked immunosorbent assay (ELISA) reader for superoxide dismutase (SOD) and SP-830 plus metertech spectrophotometer 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 was 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 °C, 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 inhibiting 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 & 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 were 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 °C, 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), Trigs (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, Trigs and Lac levels were expressed in mg dl⁻¹ plasma.

Cost-benefit analysis

The production cost was estimated in the grow-out production up to marketable size to determine the cost of diets with FBP at different stage of ripeness. Likewise, a simple cost and return analysis was made on the production phase to compare the cost benefits among treatments. Cost-modeling did not take any account fixed costs, such as the cost of establishing and operating grow-out facilities. Similarly, labor costs were not factored into the comparison. Consequently, our cost-modeling considers only the direct costs of production, such as feed, fingerlings and additional molasses.

Statistical analysis

One-way analysis of variance (ANOVA) was performed to determine the effect of experimental formulated diet among growth (Wf, WG, PER, SGR and FCR), antioxidant capacity (SOD, GPx and GR), metabolic response (Gluc, Trigs and Lac) and percentage survival. Tukey's test was then performed to determine the difference among treatments. Correlation analysis was also carried out to evaluate the correlation among growth, antioxidant capacity, metabolic response and survival. The significant level applied to all analyses was set at 5%. SAS software version 9.0 (SAS Institute, Inc., Cary, NC) was used for statistical analysis.

Results

Feed intake and behavior of Red tilapia

All the experimental fish consumed the feed ration completely. Leaching of supplement was in little chances due to the immediate response of fish during feeding.

Growth and survival

There was a significant difference in triweekly weight sampling of fish fed diet with FBP at different stages of ripeness or a reference diet for nine weeks as presented in Figure 1. The oFBP- fish had significantly higher weight compared to that of C-fish from 3rd to 6th week of rearing. Furthermore,

weight of oFBP-fish was significantly higher than that of rFBP, iFBP and C-fish after six weeks of rearing.

Significant effects of treatments on growth performance were observed (Table 2). Disregarding ripeness, the Wf of FBP-fish was significantly higher than that of C-fish. Furthermore, WG and SGR of oFBP-fish were higher than that of iFBP-fish and C-fish but comparable to rFBP-fish. In addition, PER of oFBP-fish was higher than that of C-fish but comparable to that of rFBP-fish and iFBP-fish while FCR of oFBP-fish was lower than that of iFBP-fish and C-fish but comparable to rFBP-fish. On the other hand, disregarding ripeness, percentage survival of FBP-fish was significantly higher than that of C-fish.

Antioxidant capacity

After bacterial infection, significant effects of treatments on plasma SOD, GPx and GR of fish are presented in Table 3. oFBP -fish had lower SOD activity than that of iFBP-fish and C-fish but comparable to rFBP -fish. Furthermore, oFBP -fish had higher GPx activity than iFBP-fish and C-fish but comparable to rFBP-fish. In addition, oFBP- fish had highest GR activity among treatments.

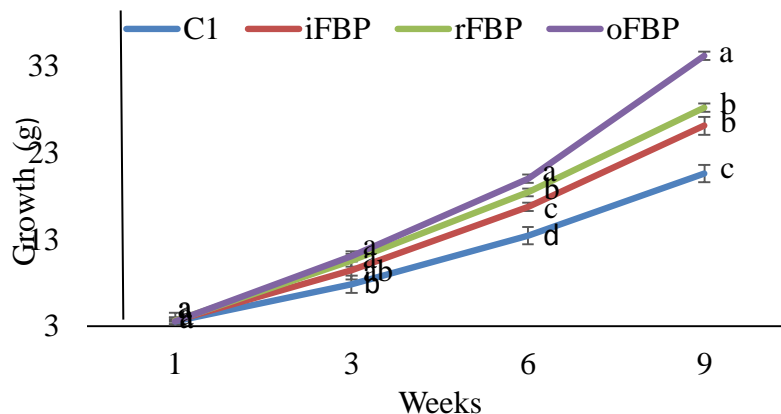


Figure 1. Mean weight (\pm S.E) of Red tilapia *Oreochromis sp.* fed diet with fermented banana (*Musa acuminata* \times *balbisiana*) peel at different stages of ripeness for nine weeks.

Metabolic Response

After bacterial infection, the plasma Gluc, Trigs and Lac of fish are presented in Table 4. The oFBP-fish had lower Gluc level than that of iFBP-fish and C-fish but comparable to rFBP-fish. Furthermore, lower Lac level was observed in oFBP-fish than that of rFBP-fish, iFBP-fish and C-fish. However, Trigs level among treatments were comparable.

Survival of Red tilapia after *Aeromonas hydrophila* infection

After 2-3 days of infection, majority of the experimental fish had coloration changes on the peritoneal part. Disease manifestation such as reddening of gill cover and fins, ulceration disease and lesion were observed. These manifestations were observed mostly at the C-fish (Fig. 2 and 3).

Table 2. Mean initial weight (W_i), final weight (W_f), weight gain (WG), protein efficiency ratio (PER), specific growth rate (SGR), feed conversion ratio (FCR) and survival (SUR) of Red tilapia *Oreochromis sp.* fed diet with fermented banana peel at different stages of ripeness or a reference diet for nine weeks.

Trt ¹	W_i (g)	W_f (g)	WG (%)	PER	SGR (%)	FCR	SUR (%)
Control	3.56 ^a	20.58 ^c	481.84 ^b	14.21 ^b	4.18 ^c	2.44 ^a	75.93 ^b
	(±0.22)	(±0.27)	(±29.53)	(±0.87)	(±0.12)	(±0.07)	(±1.85)
iFBP	3.62 ^a	26.07 ^b	620.78 ^b	17.00 ^{ab}	4.70 ^{bc}	2.18 ^b	85.18 ^a
	(±0.08)	(±0.28)	(±18.93)	(±0.52)	(±0.06)	(±0.04)	(±1.85)
rFBP	3.61 ^a	28.20 ^b	690.40 ^{ab}	18.39 ^{ab}	4.90 ^{ab}	2.12 ^{bc}	87.03 ^a
	(±0.23)	(±0.61)	(±70.13)	(±1.87)	(±0.20)	(±0.06)	(±1.85)
oFBP	3.56 ^a	34.15 ^a	865.97 ^a	22.89 ^a	5.39 ^a	1.87 ^c	90.74 ^a
	(±0.18)	(±0.67)	(±61.31)	(±1.62)	(±0.15)	(±0.04)	(±1.85)

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

¹/Treatment: C-Control, iFBP-200 ml kg⁻¹ immature fermented banana peel, rFBP- 200 ml kg⁻¹ ripe fermented banana peel, oFBP- 200 ml kg⁻¹ over ripe fermented banana peel.

Table 3. Average activities of plasma antioxidant capacity of Red tilapia *Oreochromis sp.* fed diet with fermented banana peel at different stages of ripeness or a reference diet after *Aeromonas hydrophila* infection.

Treatment ¹	Antioxidant capacity ² (U ml ⁻¹)		
	SOD	GPx	GR
Control	0.51 ^a (±0.01)	1.62 ^b (±0.00)	0.01 ^b (±0.00)
iFBP	0.33 ^b (±0.02)	3.63 ^b (±0.72)	0.01 ^b (±0.00)
rFBP	0.29 ^{bc} (±0.02)	5.59 ^{ab} (±1.31)	0.02 ^b (±0.01)
oFBP	0.21 ^c (±0.02)	8.86 ^a (±0.44)	0.03 ^a (±0.00)

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

1/Treatment: C-Control, iFBP-200 ml kg⁻¹ immature fermented banana peel, rFBP- 200 ml kg⁻¹ ripe fermented banana peel, oFBP- 200 ml kg⁻¹ over ripe fermented banana peel.

2/Antioxidant parameters: SOD-Superoxide dismutase, GPx-Glutathione peroxidase and GR-Glutathione reductase

There was significant difference on survival of fish after *A. hydrophila* infection. Disregarding ripeness, the FBP had significantly higher percentage survival from 3rd to 7th day of infection as compared to that of C-fish (Figure 4).

Table 4. Average activities of plasma metabolic response of Red tilapia *Oreochromis sp.* fed diet with fermented banana peel at different stages of ripeness or a reference diet after *Aeromonas hydrophila* infection.

Treatment ¹	Metabolic response ² (mg dl ⁻¹)		
	Gluc	Trigs	Lac
Control	195.98 ^a (±7.72)	185.58 ^a (±8.13)	44.19 ^a (±1.34)
iFBP	152.50 ^{ab} (±14.02)	185.25 ^a (±5.20)	39.03 ^a (±2.91)
rFBP	109.68 ^{bc} (±9.45)	190.45 ^a (±14.30)	30.00 ^b (±1.61)
oFBP	95.00 ^c (±0.65)	177.78 ^a (±2.28)	12.90 ^c (±0.88)

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

1/Treatment: C-Control, iFBP-200 ml kg⁻¹ immature fermented banana peel, rFBP- 200 ml kg⁻¹ ripe fermented banana peel, oFBP- 200 ml kg⁻¹ over ripe fermented banana peel.

2/Metabolic Parameters: Gluc-Glucose, Trigs-Triglycerides and Lac-Lactate



Figure 2. Coloration changes on the peritoneal part of Red tilapia *Oreochromis* sp. after subjecting to *Aeromonas hydrophila* infection (circled).

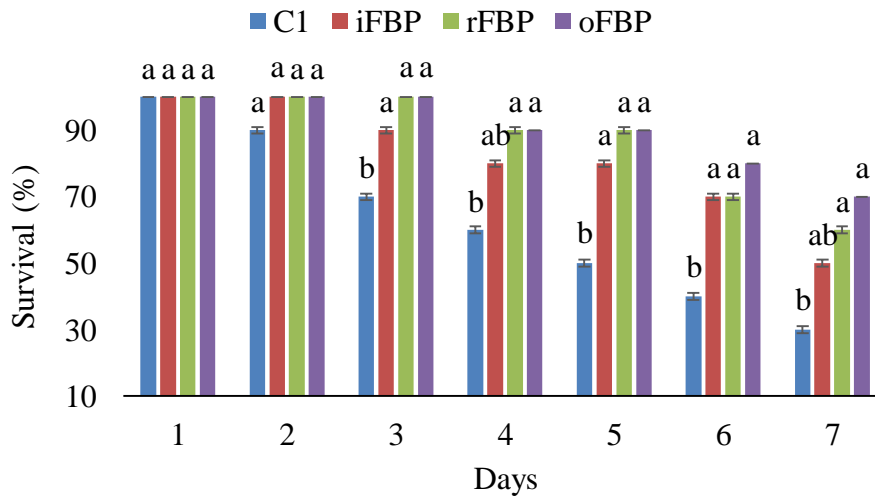


Figure 4. Mean percentage survival (\pm S.E) of Red tilapia *Oreochromis* sp. fed diet with fermented banana (*Musa acuminata* \times *balbisiana*) peel at different stages of ripeness after *Aeromonas hydrophila* infection for one week.



Figure 3. Ulceration, lesion, and reddening of gills and fins of Red tilapia *Oreochromis* sp. infected with *Aeromonas hydrophila*.

Correlation Analysis

Correlation analysis was conducted among growth, antioxidant capacity, metabolic response and percentage survival of fish after nine weeks of rearing and one week after *A. hydrophila* infection (Table 5). WG was positively correlated to SGR, PER, SUR, SOD1, GR1, Gluc1, Trigs1, Lac1, GPx2 and GR2 but negatively correlated to FCR, SOD2, Gluc2 and Lac2. Moreover, SGR was positively correlated to PER, SUR, SOD1, GPx1, GR1, Gluc1, Trigs1, Lac1, GPx2 and GR2 but negatively correlated to FCR, SOD2, Gluc2 and Lac2. FCR was also negatively correlated to PER, SUR, SOD1, GPx1, GR1, Gluc1, Trigs1, Lac1, GPx2 and GR2 but positively correlated to SOD2, Gluc2 and Lac2. On the other hand, PER was positively correlated to SUR, SOD1, GR1, Gluc1, Trigs1, Lac1 and GPx2 but negatively correlated to Lac2.

Moreover, SUR was positively correlated to SOD1, GPx1, GR1, Gluc1, Trigs1, Lac1, GPx2 and GR2 but negatively correlated to SOD2, Gluc2 and Lac2. Furthermore, SOD1 was positively correlated to GPx1, GR1, Gluc1, Lac1, GPx2 and GR2 but negatively correlated to SOD2, Gluc2 and Lac2. GPx1 was also positively correlated to GR1, Gluc1, Lac1, GPx2 and GR2 but negatively correlated to SOD2, Gluc2 and Lac2. Moreover, GR1 was positively correlated to Gluc1, Lac1 and GPx2 but negatively correlated to Gluc2 and Lac2. On the other hand, Gluc1 was positively correlated to Trigs1, Lac1, SOD2, GPx2 and GR2 but negatively correlated to Gluc2 and Lac2. Trigs1 was positively correlated to Lac1, GPx2 and GR2 but negatively correlated to SOD2, Gluc2 and Lac2. Furthermore, Lac1 was positively correlated to GPx2 and GR2 but negatively correlated to SOD2, Gluc2 and Lac2. On the other hand, SOD2 was positively correlated to Gluc2 and Lac2 but negatively correlated to GPx2 and GR2. GPx2 was also positively correlated to GR2 but negatively correlated to Gluc2 and Lac2. Lastly, GR2 was negatively correlated to Lac2.

Cost-benefit ratio

A simple cost-benefit analysis of Red tilapia fed diet with FBP at different stage of ripeness reared for nine weeks and after *A. hydrophila* infection is presented in Table 6. Disregarding ripeness, higher extrapolated fish yield per 1000 m² was obtained from fish fed with FBP than that of Control (Commercial feeds), $\text{oFBP} > \text{rFBP} > \text{oFBP} > \text{C}$ while the results for cost-benefit ratio was as $\text{oFBP} < \text{rFBP} < \text{oFBP} < \text{C}$.

Table 5. Correlation matrix among growth, antioxidant capacity, metabolic response and survival of Red tilapia *Oreochromis sp.* fed diet with fermented banana peel or a reference diet for nine weeks and one week after *Aeromonas hydrophila* infection.

	Growth parameters ¹				Day 1 ²						Day 2 ³					
	SGR	FCR	PER	Sur ⁴	SOD1	GPx1	GR1	Gluc1	Trig1	Lac1	SOD2	GPx2	GR2	Gluc2	Trig2	Lac2
WG	0.99	-0.95	0.99	0.76	0.82		0.66	0.89	0.78	0.89	-0.76	0.87	0.79	-0.74		-0.85
	SGR	-0.96	0.98	0.78	0.85	0.87	0.70	0.89	0.74	0.88	-0.81	0.88	0.77	-0.78		-0.87
		FCR	-0.93	-0.83	-0.89	-0.91	-0.76	-0.93	-0.77	-0.93	0.83	-0.91	-0.83	0.80		0.92
			PER	0.72	0.75		0.59	0.85	0.81	0.87	0.83	0.77				-0.81
				S5	0.90	0.97	0.85	0.91		0.85	-0.90	0.91	0.72	-0.82		-0.82
					SOD1	0.94	0.95	0.90		0.90	-0.95	0.95	0.81	-0.95		-0.95
						GPx1	0.87	0.93		0.88	-0.93	0.94	0.74	-0.85		-0.87
							GR1	0.79		0.81	0.87	0.91		-0.90		-0.87
								Gluc1	0.81	0.97	0.87	0.91	0.92	-0.84		-0.93
									Trig1	0.86	-0.45	0.68	0.84	-0.42		-0.72
										Lac1	-0.81	0.92	0.95	-0.81		-0.96
											SOD2	-0.86	-0.72	0.95		0.86
												GPx2	0.79	-0.87		-0.90
													GR2			-0.92
														Gluc2		
																Trig2

¹/Growth parameters of tilapia fed diet with fermented banana peel or a reference diet for nine weeks; WG- Weight Gain; SGR- Specific Growth Rate; FCR- Feed Conversion Ratio; PER-Protein Efficiency Ratio.

²/Antioxidant and metabolic level of Red tilapia fed diet containing fermented banana peel or a reference diet for nine weeks.

³/Antioxidant and metabolic level of Red tilapia fed diet containing fermented banana peel or a reference diet for nine weeks and after one d exposure to *A. hydrophila* infection.

⁴/Survival of Red tilapia fed diet containing fermented banana peel or a reference diet for nine weeks and after one d exposure to *A. hydrophila* infection.

Blank- not significant

Table 6. Simple cost-benefit analysis of extrapolated grow-out production of Red tilapia *Oreochromis* sp. fed diet with fermented banana peel or a reference diet reared up to marketable size under normal and *Aeromonas hydrophila* infection.

Item	Treatments ¹			
	C	iFBP	rFBP	oFBP
Cost				
Feeds ²	31,500	31,500	31,500	31,500
Fingerlings ³	2,500	2,500	2,500	2,500
Molasses ⁴	0	3,150	3,150	3,150
Total cost ⁵	34,000	37,150	37,150	37,150
Under normal condition ⁶				
Survival %	76	85	87	91
Gross Income ⁷	114,000	127,500	130,500	136,500
Total Net income ⁸	80,000	90,350	93,350	99,350
Cost-benefit ratio ⁹	0.30	0.29	0.28	0.27
Under <i>A. hydrophila</i> infection ¹⁰				
Survival %	30	50	60	70
Gross Income	45,000	75,000	90,000	105,000
Total Net income	11,000	37,850	52,850	67,850
Cost-benefit ratio	0.76	0.50	0.41	0.35

¹/Treatment: C-Control; iFBP-200 ml kg⁻¹ immature fermented banana peel; rFBP- 200 ml kg⁻¹ ripe fermented banana peel; oFBP- 200 ml kg⁻¹ over ripe fermented banana peel.

²/ Feeds (Php): 750 cavan⁻¹ x 42 cavans: 4 months⁻¹

³/ Fingerlings (Php): .50 cent pc⁻¹ x 5 pcs m²⁻¹ x 1000m²

⁴/Molasses (Php): 200 ml kg⁻¹ x 25 kg cavan⁻¹ x 42 cavans

⁵/Total cost (Php): Feeds+ Fingerlings + Molasses

⁶/Under normal condition: percentage survival of Red tilapia fed diet containing fermented banana peel or a reference diet for nine weeks.

⁷/Gross Income (Php): 5000 pcs of tilapia x no. of survival fish x 120 pesos kg⁻¹

⁸/Total Net income (Php): Gross Income – Total cost

⁹/Cost-benefit ratio: Gross Income- Total Net income Gross Income⁻¹

¹⁰/Under *A. hydrophila* infection: percentage survival of Red tilapia fed diet containing fermented banana peel after *A. hydrophila* infection for one week.

Discussion

Optimum level of fermented plant products helps to enhance the growth performance of fish. In this study, WG of oFBP, rFBP and iFBP-fish was increased by 80%, 43% and 29% as compared to the C, respectively. In addition, SGR and PER of oFBP-fish was increased by 29% and 61 % as compared to the C, respectively. Moreover, FCR of oFBP-fish was 23% lower than the C. The differences in growth observed among experimental diets are indication of the variation in the feed utilization. Ability of an organisms to convert nutrients especially protein will positively influence its growth performance. This was justified by the best PER and growth performance in oFBP meal inclusion diet. Lower FCR indicates better utilization of the feed by the fish. In other studies, dietary supplementation with 5% BP flour for 60 days significantly increased WG and SGR in Rohu *Labeo rohita* (Giri *et al.*, 2016).

Banana are very digestible fruits, rich in components that stimulate digestion, as well as appetite stimulant. The higher feed intake observed with the group fed over ripe banana may be due to the sweet nature of banana fruit and the ability of banana to replenish nutritional deficiencies (Forster *et al.*, 2002). The decreased feed intake observed in the unripe group may be due to the presence of tannin; varying levels of active tannins, the factor that is responsible for the astringency of raw green banana (Babatunde, 1992). The level of nutrient utilization in the test diets may not be unconnected with starch converted to simple sugar which makes digestion and utilization simple especially in the over ripe banana. Maturation of banana involves, increase in soluble sugar, decrease in starch and hemicelluloses, and slight increase in protein and lipid content (Emaga *et al.*, 2007). In addition, improvement of growth response observed in fish fed with FBP can be due to the improved nutritional quality of BP by fermentation where in fermentation completely removes hydrocyanic compounds which significantly reduce the phytates, tannin and oxalate and increased the phosphorus content of fermented products. Furthermore, fermentation improves the nutritional of weaning foods which converts insoluble proteins to soluble components and increases the levels of lysine as well as of vitamins B and C. (Fassasi *et al.*, 1999). This implies that different stages of ripeness of FBP especially the oFBP in the diet of tilapia enhance growth performance.

SOD activity is affected by the feed supplementation and biological stressors. In this study, SOD of oFBP, rFBP and iFBP- fish was decreased by 59, 43 and 35 % as compared to the C, respectively. It has been reported that lower SOD may indicate higher cell protection (Hartog *et al.*, 2003). These results are in agreement with Pan *et al.* (2010a;b) in characins *Hyphessobrycon callistus* (Steindachner). Moreover, SOD decreased with increasing dietary

carotenoid concentrations. It is speculated that after feeding with AX, the increase in body AX content can result in better oxidation-reduction buffer capacity within the cells. The lower values recorded in each antioxidant capacity indicator could therefore be due to the development of the antioxidant defense system within a particular period of time. Consequently, the need to produce SOD to scavenge superoxide radicals was lessened (Wang *et al.*, 2006).

In other study, SOD and GPx activity were increased under hypoxia stress showing their synergistic relationship (Lushchak *et al.*, 2001). Despite such correlations, there was still discrepancy between SOD and GPx in antioxidant capacity. In some antioxidants, it has been reported that dietary carotenoid reduced SOD activity but no effect in GPx under hypoxia stress (Pan *et al.*, 2010a). GPx activity of oFBP- fed fish was increased by 82% than that of the C-fed fish. The upregulated transcription of the GPx gene may be induced by the increasing H₂O₂ resulting in an increase in GPx activity. The enhancement of GPx is considered to be associated with increasing protection to diminish the harm from H₂O₂ after the invasion by a pathogen. Therefore, under stressful conditions, the content of glutathione related enzymes decreased due to its use by antioxidant mechanisms. Decrease in GPx and GR activities after bacterial infection maybe a sign of oxidative stress.

The decline in the glutathione activity makes cellular and subcellular membranes more sensitive to oxidative damage. Significant reduction in the activities of GPx might lead to the formation of O²⁻ and H₂O₂, which in turn form hydroxyl radical (OH) and bring about a number of reactions harmful to cell membranes (Mathew *et al.*, 2007). Therefore, the increased GPX activity in hepatopancreas protected the organ from formation of lipid peroxides by reducing H₂O₂ levels which in turn attenuates OH generation (Dandapat *et al.*, 2000).

The decrease in the activity of GR may lead to formation of O₂ and H₂O₂ (Yu, 1994) and hydroxyl radical (OH) that bring harmful to cell membrane. Furthermore, it has been reported that there was a decrease in GR activity in rainbow trout *Onchorynchus mykiss* could be somehow indicative of a failure antioxidant defense. Some research showed that inhibition of GR activity could be somehow indication of failure in antioxidant defense due to oxidative damage (Hermes-Lima and Storey, 1993) and an increase in GR activity reflects the ability to regenerate glutathione was enhanced (Noctor *et al.*, 2002). In our study, GR activity of oFBP-fish was increased by 67% than that of the C-fed fish that indicates the activation of higher antioxidant defense in response with the bacterial infection.

Antioxidants enable to decrease or inhibit oxidation can be found naturally or synthesized from fermentation of *Rhushirta*, *Quercus*

alba, and *Cornus stolonifera* (Ceriello, 2006; Chertow, 2004; Evans *et al.*, 2003). Natural antioxidants such as polyphenolic compounds and their effects against free-radical scavenging and oxidative stress have been studied (Kim *et al.*, 2004; Huang *et al.*, 2007). Biologically fermented plant products appear as a clear brown liquid with a sour taste due to the fermentation of plants, herbs, vegetables, or fruits with sugar in a closed environment with lactic producing bacteria or probiotic bacteria. A previous study indicated that they are rich in antioxidants with antioxidative activity similar to butylated hydroxyanisole and green tea (Schubert *et al.*, 1999). Furthermore, studies indicate that the antioxidative activity increased upon fermentation, which dissolves the ingredients and bacteria to release useful chemicals and phytochemicals in the process. During *Lactobacillus* fermentation organic acids are formed and accumulated, possibly leading to protein hydrolysis and solubilisation of antioxidant ferulic acid from cell wall plant materials (Kroon *et al.*, 1996). Fermentation of grain food with *Aspergillus oryzae* possesses strong antioxidant and free-radicals scavenging activities (Minamiyama *et al.*, 2007). Antioxidative and anti-inflammatory activities of the biologically fermented plant products can be utilized alternatively for improving health (Deiana *et al.*, 2002).

In the other study, supplementation of 5% BP flour for 60 days resulted in the highest SOD and CAT activities. In addition, supplementation of BP flour significantly enhanced GPx for up to 30 days (Giri *et al.*, 2016). The results revealed that BP at an appropriate concentration could stimulate the secretion of antioxidant enzymes as well as antioxidants, which can efficiently eliminate excess free radicals and regulate the balance of free radical in the body, resulting in improved antioxidant ability (Zhang *et al.*, 2013). Bioactive compounds such as phenolic compounds in banana peel may be responsible for the antioxidant activity (Rebello *et al.*, 2014). Antioxidant potential of banana peels has also been reported in previous studies (Rebello *et al.*, 2014; Rattanavichai and Cheng, 2015).

Elevation of plasma Gluc, Lac and Trigs concentrations are used as indicators of the secondary response of fish (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, oFBP and rFBP had 52 and 44 % lower Gluc level as compared to the C-fish, respectively. Plasma Gluc level in fish increases during stress probably as a result of the increased level of catecholamines and cortisol as they considered the principle hormones in controlling carbohydrate metabolism (El-Khaldi, 2010). These results are in agreement with the study who proved that stress might increase secretion of catecholamines which initially suppressed insulin secretion (Pickering *et al.*,

1982) and subsequently increasing plasma levels of glucose. It has been reported that plasma Gluc of *O. niloticus* increase quickly after exposure to hypoxia, overcrowding and starvation stress (El-Khaldi, 2010). While in another study, groups treated with banana peels showed significant decrease in glucose level at 150 min as compared to control group in wistar rats for antihyperglycemic effects (Navghare and Dhawale, 2016). It is a systemic metabolic disorders characterized by increased blood glucose, triglyceride and hypo insulinemia that may lead to decrease in both insulin action and insulin secretion (Maiti *et al.*, 2004 and Wadkar *et al.*, 2008).

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). In this study, oFBP-fish and rFBP-fish had 71 and 32 % lower Lac level as compared to the C-fish, respectively. In other study, Lac dehydrogenase also called lactic dehydrogenase (LDH) is responsible for converting muscle lactic acid in to pyruvic acid, an essential step in producing cellular energy. Tissue breakdown releases LDH and, therefore, LDH can be used as a marker of tissue breakdown. The mean LDH level of control fish was almost doubled during stress condition (Pakhira *et al.*, 2015) in common carp *Cyprinus carpio* (Dobsikova *et al.*, (2006, 2009) and in *L. rohita* juveniles (Chatterjee *et al.*, 2004). The increase in LDH activity could be explained by the elevation in anaerobic catabolism of blood cortisol and due to the damage of the liver and muscle tissues (Schram *et al.*, 2001).

Dietary supplementation FBP for nine weeks enhanced the resistance of Red tilapia after *A. hydrophila* infection. All FBP-fed groups exhibited higher survival than the control and the highest post challenge survival (70%) was recorded in the oFBP-fish group. Our results indicated that FBP assisted in the control of microbial pathogens as well as infections. It was reported that active components present in banana peel have higher antibacterial activities against gram-positive and gram-negative pathogens and hydroxyapatite nanoparticles derived from banana peel pectin had strong antibacterial activity against *S. aureus* and *E.coli* (Gopi *et al.*, 2014).

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, GPx and GR) and metabolic response (Gluc and Lac) 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 elicited correlations among survival, antioxidant capacity and metabolic response might be either due to the beneficial effect of FBP.

Overall, these results indicated that FBP at different stages of ripeness, especially oFBP enhances growth performance, stabilizes both antioxidant capacity and metabolic response, improves resistance of Red tilapia against *A. hydrophila* infection and provides better cost-benefit ratio. FBP could be therefore considered as potential alternative to synthetic growth promoter and antioxidant products used in aquaculture industry.

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