

The effect of black glutinous rice bran (*Oryza sativa* L.) in diets of Nile tilapia (*Oreochromis niloticus*)

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Abstract

The effect of black glutinous rice bran inclusion used in diets of Nile tilapia was studied in fish with an initial weight of 8.76 ± 0.17 g per fish and fed with experimental diets supplemented with black glutinous rice bran at 0 (control), 50, 100, 150 and 200 g kg^{-1} for 60 days. The results of phytochemical analyses of black rice bran showed that the total anthocyanin, phenolic, tannin and phytic acid contents were 52.42 ± 0.23 , 3.77 ± 0.03 , 0.32 ± 0.13 , 0.14 ± 0.01 mg/100g, respectively. The properties of black glutinous rice bran acted as an antioxidant, and the inhibitory percentage of 1,1-diphenyl-2-picrylhydrazyl (DPPH) and thiobarbituric acid reactive substances (TBARs) was significantly different ($p < 0.05$) on storage in different conditions of 4 and 25 °C. Black glutinous rice bran stored at 4 °C showed significantly higher antioxidant activities analyzed by DPPH and TBARs tests ($p < 0.05$). The percentage of digestibility coefficients and protein digestibility in test diets ranged from 75.21-81.95 and 87.37-91.87 ($p > 0.05$), respectively. The highest average daily gain, specific growth rate and protein efficiency ratio were observed in fish fed with the diet containing 150 and 200 g kg^{-1} black rice bran ($p < 0.05$). Feed conversion ratio was slightly better in fish fed the diet supplemented with 200 g kg^{-1} black glutinous rice bran. The serum biochemical values including total protein, albumin, total bilirubin, alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity were not significantly different in the various experimental groups ($p > 0.05$). Therefore, supplementation of black glutinous rice bran in tilapia diets at a rate of 200 g kg^{-1} resulted in better growth and had no adverse effects on digestibility and serum biochemistry.

Keywords: Black glutinous, Tilapia, Phytochemical, Antioxidant, Growth, Serum biochemistry

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Introduction

Tilapia (*Oreochromis niloticus*) is a freshwater and economic fish in Thailand because it is popular for consumption and farming. The problem of intensive culture of tilapia caused by the high density of fish culture resulted in high stress of fish. In intensive fish production, the occurrence of stress situations is inevitable. During the culture period, the fish are exposed to several potential stressors (Barton and Iwama, 1991). Thus, it caused the high amount of free radicals by the process of metabolism in the fish. Moreover, an increased amount of free radicals in fish leads to cell damage or degeneration of cells, oxidation of DNA, lipids, proteins, and cell membranes that resulted in severe disease (Lander, 1997; Lionis *et al.*, 1998). Most fish feeds contain major raw materials such as fish meal and fish oil. These raw materials contain high amounts of polyunsaturated fatty acid n-3 and are susceptible to lipid peroxidation reactions. The various synthetic antioxidants including ethoxyquin, Butylated Hydroxy Toluene (BHT) and Butylated Hydroxy Anisole (BHA) had been widely used in the feed mill industry to ensure product preservation from lipid oxidation. Fish muscle is a major depot of absorbed dietary synthetic antioxidants (Bohne *et al.*, 2008). Moreover, synthetic antioxidants were recently shown to cause several unfavorable side-effect not only in fish fed diets containing this compound, but also in people (Blaszczyk and Skolimowski, 2005). With the current focus on food safety, particularly in relation to fish feeds, farmers have turned

to the use of natural antioxidants (Bohne *et al.*, 2008).

It is widely known that Thailand has been regarded as an agricultural country with a great diversity of plants such as rice. There are various breeds of rice in Thailand with different colors including white and black rice (*Oryza sativa* L.) which is one of the most important crops in Thailand. Anthocyanin in black rice contains cyanidin-3-glucoside, pelargonidin-3-glucoside, peonidin-3-glucoside and respective malonated counterparts (Abdel-Aal el and Hucl, 2003). Anthocyanin is soluble in polar solvents such as alcohol as well as in water and ranges in color from red to blue (Xu and Lin, 2003). Mazza and Maniati, 1993 reported anthocyanin solution can change its color according to its acidity (pH). In addition anthocyanin is an antioxidant that has a major role in body functions by preventing cell damage from free radical (Butsat and Siriamornpun, 2010). Zhang *et al.* (2006) reported that black rice is rich in anthocyanin and other polyphenolic compounds in much more abundance than white rice. Therefore, the black rice bran has high potential to become feedstuff in fish diets because of its high nutritional quality. To date, there is no research on the utilization of black rice bran in fish diets in Thailand. Moreover, the study on phytochemicals from black rice bran with its antioxidant activities has not been undertaken. Consequently, the present study aimed to fulfill these research gaps and limitations. The objectives of this study were 1) to evaluate phytochemicals and antioxidant properties in black

glutinous rice bran and 2) to determine protein digestibility, growth performance and serum biochemistry in tilapia supplemented with black rice bran.

Materials and methods

Black glutinous rice bran extraction

Black glutinous rice bran samples were extracted with 1 % HCl in 50 mL methanol and intermittent shaking at 20 °C at 200 rpm for 24 hr. All extracts were combined and filtered, and centrifuged at 2,500 rpm. Later, the combined extract was slowly concentrated in a rotary evaporator at 40 °C, and the total volume of the crude extract was adjusted with methanol for 5 mL (Wrolstad *et al.*, 2005). All crude extract samples were kept under nitrogen atmosphere in a dark cabinet at -20 °C before determination of phytochemical content and antioxidant properties.

Determination of phytochemicals

All phytochemicals in black glutinous rice bran extract were determined by spectrophotometric methods. Total phenolic content was determined by Folin-Ciocalteu assay (Singleton *et al.*, 1999) with calculation made from the Gallic acid standard curve. The total tannins content was determined using methods described in AOAC (2000), and calculated with the tannic acid standard curve. The phytic acid content was carried out using the modified procedure of Talamond *et al.* (1998). The method was calibrated with standard phytic acid solutions by Calcium phytate. Black rice bran extract was used for determination of total anthocyanins

content by the pH differential method (Giusti and Wrolstad, 2005). The total monomeric anthocyanin content of crude extract was calculated in terms of cyanidin-3-glucoside.

Determination of antioxidant properties

Free radical scavenging activity of black glutinous rice bran extract was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method described by Brand-Williams *et al.* (1995) with slight modifications. After 30 min, absorbance was measured at 515 nm. Scavenging of DPPH radical in percentage of inhibition was calculated by the following equation:

$$\% \text{ Inhibition} = \frac{[(\text{Abs}_{\text{DPPH}} - \text{Abs}_{\text{DPPH} + \text{sample}}) \times 100]}{\text{Abs}_{\text{DPPH}}}$$

Black glutinous rice bran extract had been analyzed for thiobarbituric acid reactive substances (TBARs) value, as a lipid oxidation index according to the method by Pegg (2005). The amount of TBARs is shown in milligrams of malondialdehyde (MDA).

Fish diets and feeding protocol

The experiment was conducted in the Department of Fisheries, Khon Kaen University (Khon Kaen Province, Thailand). Nile tilapia were acclimated in 1000 liter tanks and fed on the control diet for 7 days before the experiment. Fish with initial mean body weight at 8.76±0.17 per fish were randomly distributed into 16 cement tanks (1.0 x 1.8 x 1.4 meter) filled with 1.26 m³ of water at a rate of 20 fish per tank. Water quality parameters such as

dissolved oxygen (6.05 ± 0.38 mg L⁻¹), temperature ($22.88 \pm 0.89^\circ\text{C}$) and pH value (7.87 ± 0.27) were measured every day during the experimental period.

Four isonitrogenous and isoenergetic diets were formulated that contained approximately 300 g kg⁻¹ protein and 3,000 Kcal kg⁻¹. The experimental diets were supplemented with black glutinous rice bran at 0 (control), 50, 100, 150 and 200 g kg⁻¹ diet; each treatment was performed in four replicates (Table 1). Diets were analyzed for chemical composition including protein, moisture, fat, fiber and ash by AOAC (2000). During the trial, fish were fed *ad-libitum* by hand-fed method twice daily (08:00 and 17:00 h) for 60 days. Feed consumption was recorded weekly and fish from each tank were weighed to measure growth every two weeks until the end of the experiment; growth performance and feed utilization were calculated.

Digestibility efficiency and protein digestibility

Digestibility coefficients and protein digestibility for the test diets were calculated according to Austreng (1978) to

evaluate the digestibility of experimental diets and digestible crude protein was determined by pepsin digestibility test (AOAC, 2000).

Serum biochemistry

At the end of the feeding trial, after they were starved for 15 hrs, three fish per tank were anesthetized and blood samples were collected and placed in non-heparinized tubes. Hematocrit was determined by using microhematocrit centrifuge. The serums were separated into aliquots for analyses of serum biochemistry. Serum was analyzed for total protein, albumin, total bilirubin, alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activity. The serum biochemical indices were determined with an automatic biochemical analyser (BS-200; Mindray, Shenzhen, China)

Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) and if significant differences ($p < 0.05$) were found, Duncan's multiple range test was used to rank the groups.

Table 1: Ingredients and proximate composition of experimental diets.

Ingredients	Level of black glutinous rice bran (g kg ⁻¹)				
	0	50	100	150	200
Corn	310	275	240	210	160
Black rice bran	0	50	100	150	200
Fish meal	250	250	250	250	250
Soybean meal	290	280	270	250	250
Soya oil	40	40	40	40	40
Fish oil	40	40	40	40	40
Alpha-starch	50	50	50	50	50
Methylcellulose	10	5	-	-	-
Premix	10	10	10	10	10
Proximate composition by analysis (% dry weight on basis)					

Moisture		45.4	47.7	47.1	44.9	45.4
Protein		307.5	299.8	295.7	29.5	302.1
Table 1 continued:						
Fat		123.3	123.1	123.0	123.4	122.3
Ash		86.3	81.6	89.6	93.7	98.9
Fiber		21.6	20.6	22.1	22.7	23.1
NFE		430.9	437.2	417.4	427.4	413.2
Digestible energy		278.22	272.64	273.44	278.44	282.6
(kcal/100 g) ^a						

Note: a = data by calculated

DE = (% Protein x 4.0) + (% NFE x 2.5) + (% Fat x 8.0); NRC (1993)

Results

The study using black glutinous rice bran for tilapia diets can be divided into 2 experiments: 1) study of phytochemicals and antioxidant properties of black glutinous rice bran. 2) study of digestibility, growth performance and serum biochemistry in tilapia fed with dietary black glutinous rice bran.

Experiment 1: Study of phytochemicals and antioxidant properties of black glutinous rice bran

The analysis of phytochemicals showed that black glutinous rice bran contained total anthocyanin, phenolic, tannin and phytic acid content at 52.42 ± 0.23 , 3.77 ± 0.03 , 0.32 ± 0.13 , 0.14 ± 0.01 mg/100g, respectively. The study of the antioxidant properties of black glutinous rice bran analyzed by the DPPH-radical scavenging activity in varied quantities (including 2, 4, 6 and 8 mg) of crude anthocyanin extract found that the percentage of antioxidants in black glutinous rice bran tested with DPPH solution at 0.05 mM, was percentage 59.56 ± 2.35 , 64.61 ± 1.78 , 67.48 ± 1.61 and 69.71 ± 2.20 , respectively. The antioxidant properties of black glutinous rice bran analyzed by TBARs

found the average malondialdehyde formed by the reaction was 16.0 ± 0.69 μ M. The stability of black glutinous rice bran as an antioxidant at various storage conditions was studied by using DPPH-radical scavenging activity (Table 2) and TBARs (Fig. 1). Black glutinous rice bran was stored at 4 and 25 °C for 14 days and then samples were randomly collected on day 0, 7 and 14. The stability of black glutinous rice bran as an antioxidant at various storage conditions with DPPH-radical scavenging activity showed that inhibitory percentage of antioxidant tested with 0.05 mM DPPH solution was significantly different ($p < 0.05$). The inhibitory percentage of antioxidant increased with the quantity of crude extract from black glutinous rice bran measured in the analysis (2, 4, 6 and 8 mg, respectively). The result showed the maximum of inhibitory percentage of antioxidant was 78.93 ± 0.30 percentage at 4 °C. The lowest inhibitory activity of black glutinous rice bran was 51.14 ± 1.71 percent at 25 °C.

Table 2: Inhibitory percentage against free radical of DPPH 0.05 mM in crude anthocyanin extract of black glutinous rice bran in different storage conditions (mean \pm SD).

Time (day)	Temperature (°C)	Percentage			
		Crude extract of black glutinous rice bran (mg)			
		2	4	6	8
0	4	61.0 \pm 1.59	65.18 \pm 1.01	68.62 \pm 0.84	71.28 \pm 0.89
	25	61.0 \pm 1.59	65.18 \pm 1.01	68.62 \pm 0.84	71.28 \pm 0.89
7	4	63.69 \pm 1.02	68.49 \pm 0.38	71.19 \pm 0.23	77.42 \pm 0.84
	25	56.82 \pm 0.19	59.75 \pm 1.05	65.47 \pm 0.77	70.05 \pm 0.64
14	4	63.14 \pm 0.80	69.59 \pm 0.52	74.86 \pm 0.96	78.93 \pm 0.30
	25	51.14 \pm 1.71	58.01 \pm 1.29	59.43 \pm 0.80	64.46 \pm 1.34
<i>p</i> -value					<0.0001
Temp					<0.0001
Time					0.023
Temp*Time					<0.0001

The stability of black glutinous rice bran as an antioxidant at various conditions was analysed with TBARs. The experiment showed the antioxidant capacity decreased with temperature and storage time ($p < 0.05$). The result showed that at 4 °C, concentrations of malondialdehyde formed by the reaction of 0.01 g of black glutinous

rice bran with TBARs solution were 16.46 \pm 0.89, 19.09 \pm 1.61 and 27.26 \pm 0.78 μ M, at 0, 7 and 14 days, respectively. The minimum antioxidant capacity of black glutinous rice bran at 25 °C was 16.46 \pm 0.89, 36.32 \pm 2.25 and 56.16 \pm 2.80 μ M of malondialdehyde at 0, 7 and 14 days, respectively.

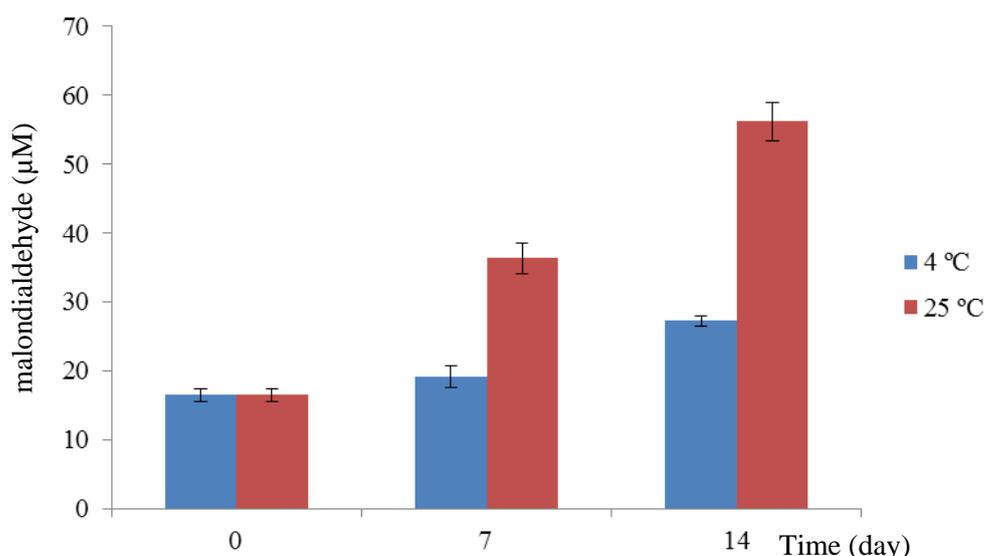


Figure 1: Malondialdehyde concentration formed by the reaction of TBARS with black glutinous rice bran at different storage conditions (mean \pm SD).

Experiment 2: Study of digestibility, growth performance and serum biochemical value in tilapia fed with dietary black glutinous rice bran

The analysis of phytochemicals in test diets containing 0, 50, 100, 150 and 200 g kg⁻¹ black glutinous rice bran showed that

the increase in the amount of black glutinous rice bran caused an increase in the amount of phytochemicals, as well. Experimental diets had low amounts of tannins, phenolic and phytic acid ranging from 0.005-0.009, 0.06-0.10 and 0.12-0.27 mg/100g, respectively (Table 3).

Table 3: Phytochemical content in experimental diets (mean \pm SD).

Phytochemicals (mg/100g)	Level of black glutinous rice bran (g Kg ⁻¹)					p-value
	0	50	100	150	200	
Total Anthocyanin	3.64 \pm 0.08 ^c	3.60 \pm 0.43 ^c	4.00 \pm 1.10 ^{bc}	4.17 \pm 0.29 ^b	4.64 \pm 0.14 ^a	0.0025
Total Phenolic	0.06 \pm 0.00 ^c	0.07 \pm 0.00 ^{bc}	0.07 \pm 0.00 ^{bc}	0.08 \pm 0.00 ^b	0.10 \pm 0.00 ^a	0.0024
Total Tannin	0.005 \pm 0.00 ^d	0.006 \pm 0 ^c	0.006 \pm 0.00 ^b	0.007 \pm 0 ^b	0.009 \pm 0 ^a	0.0010
Total Phytic acid	0.12 \pm 0.03 ^c	0.15 \pm 0.01 ^{bc}	0.18 \pm 0.00 ^{bc}	0.24 \pm 0.00 ^a	0.27 \pm 0.01 ^a	0.0023

Note: Means within the same row with different letters are significantly different ($p < 0.05$)

The study of digestibility coefficients of tilapia diets containing various levels of black glutinous rice bran found no

significant differences between digestibility coefficients and protein digestibility of test diets ($p > 0.05$). The

digestibility coefficients ranged from 75.21-81.95 percent and protein digestibility ranged from 87.37-91.87 percent.

Growth performance and feed utilization

Growth performance and feed utilization of tilapia with an initial average weight of 8.89 ± 3.25 g per fish fed diets containing 0, 50, 100, 150 and 200 g Kg^{-1} of black glutinous rice bran for 60 days is shown in Table 4. From the study of growth performance and feed utilization based on

the average daily gain, specific growth rate and protein efficiency ratio, fish fed with black glutinous rice bran at 150 and 200 g Kg^{-1} had the maximum growth performance ($p < 0.05$) compared with fish in other group. Feed conversion ratio and survival rate in fish did not have any significant difference ($p > 0.05$) among the experimental groups. Feed conversion ratio was 1.48 to 1.78 and the survival rate was 100 percent in all experimental groups.

Table 4: Growth performance and feed utilization of tilapia fed with experimental diets for 60 days (mean \pm SD).

Growth performance and feed utilization	Level of black glutinous rice bran (g Kg^{-1})					p-value
	0	50	100	150	200	
Average daily gain (g/fish/day)	0.36 ± 0.01^b	0.36 ± 0.01^b	0.39 ± 0.02^b	0.43 ± 0.00^a	0.44 ± 0.00^a	0.002
Specific growth rate (%/day)	2.21 ± 0.09^b	2.21 ± 0.03^b	2.28 ± 0.09^b	2.43 ± 0.06^a	2.46 ± 0.02^a	0.0020
Feed conversion ratio	1.78 ± 0.22	1.64 ± 0.16	1.69 ± 0.03	1.59 ± 0.08	1.48 ± 0.10	0.0976
Protein efficiency ratio	0.13 ± 0.02^c	0.14 ± 0.01^{bc}	0.14 ± 0.002^{bc}	0.16 ± 0.004^{ab}	0.17 ± 0.01^a	0.0213
Survival rate (%)	100	100	100	100	100	-

Note: Means within the same row with different letters are significantly different ($p < 0.05$)

Fish serum from all the experimental groups was analyzed for serum protein that comprised total protein and albumin. The results of protein levels shown in Table 5, indicate that the usage of black glutinous rice bran did not affect the changes in

serum protein ($p > 0.05$). The results showed that serum protein content in tilapia fed with different levels of black glutinous rice bran was normal compared with that in the control group.

Table 5: Serum biochemical parameters of tilapia fed with experimental diets (mean \pm SD).

Serum biochemical parameters	Level of black glutinous rice bran (g Kg^{-1})					p-value
	0	50	100	150	200	
Hct (%)	27.00 ± 2.64	29.33 ± 1.52	27.33 ± 6.35	27.33 ± 0.57	27.33 ± 2.51	0.9113
TP (g/dL)	5.16 ± 2.15	4.75 ± 0.21	4.93 ± 2.04	6.70 ± 4.01	5.23 ± 2.01	0.8937
ALB (g/dL)	1.56 ± 1.11	1.75 ± 0.07	1.80 ± 0.62	2.30 ± 1.41	1.70 ± 0.50	0.8888
BT (mg/dL)	5.14 ± 2.37	3.265 ± 0.33	4.24 ± 2.23	7.11 ± 6.55	4.30 ± 2.63	0.7914
AST (U/L)	161.33 ± 95.47	260.50 ± 7.77	159.00 ± 95.63	335.66 ± 252.94	232.00 ± 87.58	0.5506
ALT (U/L)	30.00 ± 21.21	37.50 ± 6.36	24.00 ± 8.48	24.33 ± 9.07	22.00 ± 11.13	0.6552
ALP (U/L)	ND	ND	ND	ND	ND	ND

Note: ND: not detected

Hematocrit (Hct), Total protein (TP), Albumin (ALB), Total bilirubin (BT), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP)

Discussion

In this study phytochemical values in black glutinous rice bran extract were determined. Total anthocyanin content was 52.42 ± 0.23 mg which was consistent with study of Ryu *et al.* (1998). But anthocyanin content in the present study was higher than total anthocyanin content in black rice (32.76 mg) as reported by Abdel-Aal and Hucl (2003). Total phenol was 3.77 ± 0.03 mg which was less than that reported in the study of Shen *et al.* (2009) and Khampang *et al.* (2009). Total tannin was 0.32 mg which was less than the values reported by Fardet *et al.* (2008) who found total phenolic content of the crude extract ranged from 0.54-3.13 mg. Total phytic acid was 0.14 ± 0.01 mg which was not in agreement with the study of Chung (2002). Nowadays, whole grain pigmented rice has been categorized as one of the potent functional foods since it contains high amounts of phenolic compounds, especially anthocyanins in pericarp (Abdel-Aal *et al.*, 2006). Rice bran is a natural resource considered to contain high amounts of nutrients and a large number of biologically active phytochemicals, such as those in groups of tocopherols, tocotrienols, oryzanols, vitamin B complex and phenolic compounds (Sookwong *et al.*, 2007; Yu *et al.*, 2007). Black glutinous rice bran is composed of phenolic compounds including ferulic acid, p -coumaric acid and synaptic acid which differed in each species (Escribano-Bailón *et al.*, 2004). Some reports showed that black glutinous rice bran from Asia has large amounts of phenolic compounds including Cyanidin-

3-glucoside with the highest level found in Kao Kham (102 ± 7.70 g/100g) (Escribano-Bailón *et al.*, 2004).

Anthocyanins are considered to be powerful natural antioxidants. Extensive studies have been reviewed on biological functions, preclinical tests and the structural determination of naturally occurring anthocyanins (Cooke *et al.*, 2005; Lee *et al.*, 2011). Finocchiaro *et al.* (2010) focused on anthocyanins contained in bran of black-coloured rice which were mainly antioxidants. Hu *et al.* (2003) indicated that the major anthocyanins found in black rice, cyanide-3-glucoside, and peonidin-3-glucoside, have strong antioxidant activities in an *in vitro* model system. But the antioxidant capacity in black glutinous rice bran increased with the increase in concentration of black glutinous rice bran and it was also found that temperature and duration of storage also affected the antioxidant capacity in black glutinous rice bran. Laokuldilok *et al.* (2011) found the greatest capability in antioxidant activity with black rice indicating that black rice had the highest potential in scavenging DPPH radicals as compared to white rice.

Feeds with a tannin content of 0.5-2.0 percent decreased growth rate in animals. However, the tannins content which is higher than 5.0 percent caused acute death of animals (Giner-Chavez, 1996). Francis *et al.* (2001) reported the use of tannin (condensed tannin) of more than 2 percent in the diet resulted in decreased growth rate and affected palatability.

The studies stated that diets with phytic acid at a rate of 5-6 g Kg⁻¹ diet may cause

low growth rates in rainbow trout and common carp (Spinelli *et al.*, 1983; Hossain and Jauncey, 1993). Satoh *et al.* (1989) reported that diets containing phytic acid of more than 2.2 percent resulted in low growth rate in channel catfish. However diets with phytic acid at 1.5 percent did not have any impact on fish (Gatlin III and Phillips, 1989; Satoh *et al.*, 1989). A high dose of phytic acid caused the low exploitation of mineral and low level of protein digestibility as well (Francis *et al.*, 2001). Therefore, the right selection of raw materials in animal feeds is important. The amount of antinutritional factors or the amount of toxins should be determined before using raw material in animal feed (Siddhuraju *et al.*, 2000).

Typically, aquatic animals have the ability to digest protein in diet ingredients ranging from 31-99 percent, lipid ranging from 76-100 percent, carbohydrate ranging from 6-92 percent, ash content ranging from 68-94 percent and energy intake ranging from 77-95 percent (Lovell, 1989). However, the results showed that the protein digestibility of tilapia fed with diet supplemented with black glutinous rice bran at 150 and 200 g Kg⁻¹, had the maximum digestibility coefficient and protein digestibility. Factors affecting the difference in digestibility included species of aquatic animals, feed ingredients, environment etc. Lovell (1989) found that the quality of proteins affected the protein content and protein digestibility which depended on raw materials and animal species.

In fish feeding, tilapia can digest the starch such as rice bran mixture up to 30-

60 percent (Smith, 1980). Small fish should not be fed on diets containing carbohydrate exceeding 35 percent because it affected digestion and caused slow growth rates (Zhao *et al.*, 1996). This study showed that black glutinous rice bran at 150 and 200 g Kg⁻¹ contained in tilapia diets resulted in more efficient use and better growth performance. Liver disorders caused a decreased level in total protein and albumin (Kaneko *et al.*, 1997). Moreover, the similar amount of total bilirubin indicated that the liver has similar performance because higher amounts of bilirubin showed liver malfunction (Kaneko *et al.*, 1997) and the hematocrit rate from an analysis showed no significant differences ($p>0.05$). In addition, the hepatic enzymes comprising AST and ALT were also studied. The study showed that fish fed on black glutinous rice bran supplement resulted in similar AST value, while the amount of ALT decreased. The amount of AST and ALT enzyme indicated a malfunction of liver. These two enzymes are synthesized by the liver, so when there is liver disorder, both enzymes will be released into the bloodstream more than from a normal liver. Hepatosomatic index ranged from 1.89 ± 0.25 - 2.30 ± 0.07 , and showed there was no difference significance ($p>0.05$). Therefore the use of black glutinous rice bran supplement at various levels in tilapia diet did not affect liver function.

Total anthocyanin, phenolic, tannin and phytic acid content in black glutinous rice bran was 52.42 ± 0.23 , 3.77 ± 0.03 , 0.32 ± 0.08 , 0.14 ± 0.01 mg/100g,

respectively. Black glutinous rice bran acted as an antioxidant based on the inhibitory percentage of DPPH and TBARs which were significantly different ($p < 0.05$). Black glutinous rice bran stored at 4°C showed significantly higher antioxidant activities analyzed by DPPH and TBARs tests. Thus, 4 °C was the ideal storage condition for anthocyanin in black glutinous rice bran. The use of black glutinous rice bran supplement in diets did not affect digestibility coefficients and protein digestibility. Fish fed with 200 g kg⁻¹ of black rice bran in the diet had better growth performance and feed utilization; which did not affect serum biochemical values as well. Therefore, the supplementation of 200 g kg⁻¹ black glutinous rice bran in tilapia diet is recommended, which results in maximum growth of tilapia while it may not affect digestibility and serum biochemistry.

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