

## Effects of polyphenols from sugarcane on the growth performances of farmed tilapia (*Oreochromis niloticus*)

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**Abstract.** This experiment introduces a natural bioactive additive from sugarcane (*Saccharum officinarum*), the richest source of phenolic compounds having the potential to replace some of the functions of feed additives. The experiment was conducted in the net cage where tilapia (*Oreochromis niloticus*) were stocked at 80 fish per cage and subjected to four treatments with four replications. A commercial polyphenol named 'polygain' from sugarcane was included at 0% (T<sub>0</sub>), 0.2% (T<sub>1</sub>), 0.4% (T<sub>2</sub>), and 0.6% (T<sub>3</sub>) in four diets. During stocking, initial weight and length of fishes were 2.24g and 3.21cm respectively. Final sampling showed that average weight of each treatment such as T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> were 86.64±7.0g, 93.3±7.4g, 108.71±14.0 g and 89.78±10.8g respectively. Average length of fish was higher in T<sub>2</sub> (18.25 cm) than the T<sub>0</sub> (17.34 cm), T<sub>1</sub> (18cm) and T<sub>3</sub> (18.13cm). Among four treatments, T<sub>2</sub> showed lower FCR value (1.59) than the control (2.0). The outcome of the study indicated that the addition of polyphenol in tilapia diet can improve growth performance.

**Key words:** Tilapia, Polyphenol, Sugarcane

### Introduction

Tilapia farming can bring enormous potential in Bangladesh to meet the demand of nutrition. With increasing acceptance and popularity among consumers, tilapia has become the world's second most important cultured fish after carps and easily adapt in tropical and sub-tropical regions of the world (Shelton 2002). It is regarded as the most significant fish species reducing the gap of increasing worldwide demand for protein sources from fish (Ng and Romano 2013). Aquaculture sector recently has arisen as the 2<sup>nd</sup> largest and most important contributors to export earnings of Bangladesh (Ghose 2014). Outbreak of diseases has become a major problem in fish culture system in the country due to improper management systems of culture practice (Rahman and Chowdhury 1996). Disease is the key constraint to enhancing aquaculture production including cage culture. As a result the farmers are deliberately and repeatedly applying those chemicals which are detrimental for both fish and human health (Cabello 2006). Many countries are legislating to remove both antibiotics and hormones from animal feeds which shifted farmer interest to natural plant based secondary metabolites as feed additives. If these antibiotics are removed, then new natural bioactive additives from sugarcane plant such as 'Polyphenol' has the potential to replace some of the functions

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of antibiotics. Polyphenol is a secondary plant metabolite extracted from sugarcane plant *Saccharum officinarum* which has anti-oxidative, anti-inflammatory and anti-bacterial properties beneficial for the animal feed industry (Frank *et al.* 2006). Feed additives are able to improve growth performance of animals. Polyphenol can improve the utilization of feed which increase feed efficiency as well as growth performance. The current study was designed to investigate the effects of polyphenol on growth performance, FCR, specific growth rate and condition factor of Tilapia.

### Material and Methods

The present study was carried out in 'Halda Fisheries Ltd., located in Potenga, Chittagong during the period from August to November, 2017. The cage materials such as plastic drum, nylon net, pipe etc. were collected from local market. The size of the cages was 26 x 13 feet. Experimental tilapia fry were produced in the Halda Fisheries Ltd.

**Preparation of experimental diets:** The experimental diet was prepared in the feed mill of "Halda Fisheries Ltd.", by adding appropriate amount of commercial polyphenol named "Polygain" (Australia Pty Ltd.). Polygain contains natural polyphenol, minerals and nutrients. The polyphenol content of "Polygain" used in the study was 30.40g/kg. The experiment was conducted with four treatments and each treatment had four replications. The treatments were control (T<sub>0</sub> regarded as without 'Polygain', as 0% polyphenol), T<sub>1</sub> (0.2% 'Polygain', as 60mg polyphenol/kg feed), T<sub>2</sub> (0.4% 'Polygain', as 120mg polyphenol/kg feed) and T<sub>3</sub> (0.6% 'Polygain', as 180mg Polyphenol/kg feed). The feed formulation and proximate composition of experimental diets are shown in Tables I and II. The proximate composition of the experimental diet was done in "Nutrition laboratory" of the Faculty of Fisheries following standard procedures.

**Table I. Feed ingredients with their inclusion level**

Ingredient	Inclusion (%)
Fish Meal 40%	18.75
Fish Meal 60%	18.75
Soya bean Meal	10.00
Meat & Bone Meal	15.63
Rice Bran	10.63
Wheat Bran	11.25
Mustard Oil Cake	6.25
Maize	5.00
Wheat Flour	3.75
<b>Total</b>	<b>100</b>
<b>Additives</b>	
DCP	0.5
Pellet binder	0.5
Soybean oil	0.5

**Table II. Proximate composition of feed**

Name	Percentage (%)
Moisture	14.91
Crude protein	35.41
Crude lipid	8.82
Ash	22.4

**Experimental design:** The statistical design, used for the experiment was completely randomized design (CRD). The experiment was conducted in 16 cages set in the pond where each was stocked with 80 tilapia fingerling (Table III).

**Table III. Layout of the experiment showing the distribution of 'tilapia' fishes in cages and the applied treatments**

Dietary treatment groups	Treatment × Replication (T <sub>n</sub> × R <sub>n</sub> )	No. of fishes per cage	Total no. of fish per treatments
T <sub>0</sub> (0% 'Polyphenol')	T <sub>0</sub> R <sub>1</sub>	80	320
	T <sub>0</sub> R <sub>2</sub>	80	
	T <sub>0</sub> R <sub>3</sub>	80	
	T <sub>0</sub> R <sub>4</sub>	80	
T <sub>1</sub> (60 mg 'Polyphenol'/kg feed)	T <sub>1</sub> R <sub>1</sub>	80	320
	T <sub>1</sub> R <sub>2</sub>	80	
	T <sub>1</sub> R <sub>3</sub>	80	
	T <sub>1</sub> R <sub>4</sub>	80	
T <sub>2</sub> (120 mg 'Polyphenol'/kg feed)	T <sub>2</sub> R <sub>1</sub>	80	320
	T <sub>2</sub> R <sub>2</sub>	80	
	T <sub>2</sub> R <sub>3</sub>	80	
	T <sub>2</sub> R <sub>4</sub>	80	
T <sub>3</sub> (180 mg 'Polyphenol'/kg feed)	T <sub>3</sub> R <sub>1</sub>	80	320
	T <sub>3</sub> R <sub>2</sub>	80	
	T <sub>3</sub> R <sub>3</sub>	80	
	T <sub>3</sub> R <sub>4</sub>	80	
Grand total			1280

**Sampling and calculation of growth parameters:** Sampling of the experimental fish was done in an interval of one week. The weight of fish measured by using a weight machine (RADAG-AS 220.R2) and the length was taken by using measuring scale. The following parameters were calculated:

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Total amount of feed given (g)}}{\text{Total weight gain (g)}}$$

$$\text{Specific growth rate (SGR)} = \frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{\text{Duration in days}} * 100$$

$$\text{Condition factor (CF, \%)} = \frac{\text{Weight of fish}}{(\text{Length of fish})^3} * 100 \text{ (Fulton 1902)}$$

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**Statistical analysis:** Statistical analysis were performed by using MS excel (Microsoft office excel-2007, USA) and IBM SPSS Statistics 23 Version. Values are expressed as means  $\pm$  standard deviation (SD). Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to assess statistically significant differences among the control and different treated values. Statistical significance was set at  $p < 0.05$ .

### Results

**Effects of polyphenol on fish weight and length:** The sampling was done for weekly basis and a total 16 sampling was conducted in four months. The mean final weights of fish in T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> were  $86.64 \pm 7.0\text{g}$ ,  $93.3 \pm 7.4\text{g}$ ,  $108.71 \pm 14.0\text{g}$  and  $89.78 \pm 10.8\text{g}$ , respectively. The mean lengths were  $17.34 \pm 0.25\text{cm}$ ,  $18 \pm 0.41\text{cm}$ ,  $18.25 \pm 0.29\text{cm}$  and  $18.13 \pm 0.25\text{cm}$  respectively. Treatment 2 showed better growth performance (Figs. 1 & 2) in terms of weight and length gain than the other treatments. Among dietary treatments, significant differences ( $p < 0.05$ ) were observed for final weight and length (Figs. 3 & 4). The SGR values were  $3.07 \pm 0.07$ ,  $3.13 \pm 0.07$ ,  $3.26 \pm 0.11$  and  $3.1 \pm 0.1$  in T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>, respectively (Fig. 5). Among the treatments, there had no significant differences ( $p > 0.05$ ).

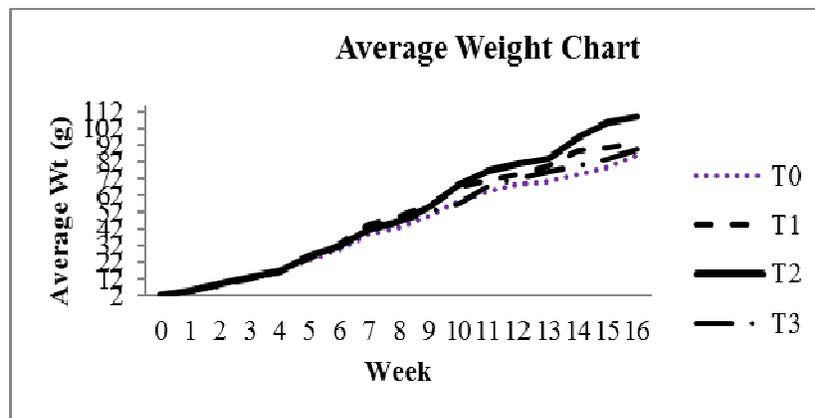
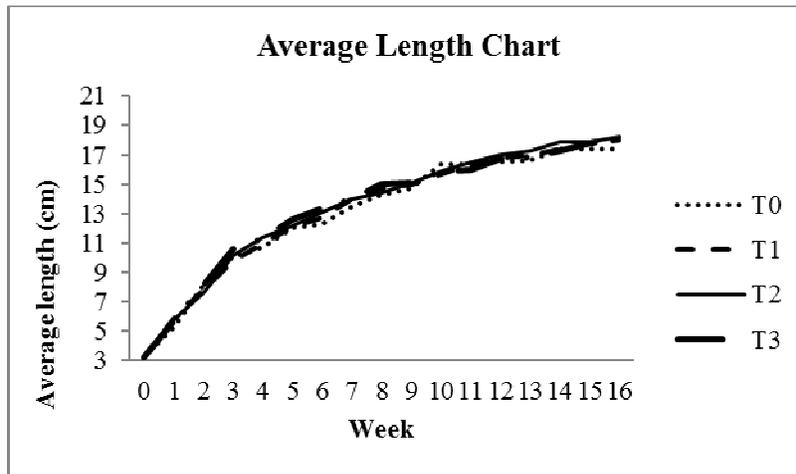
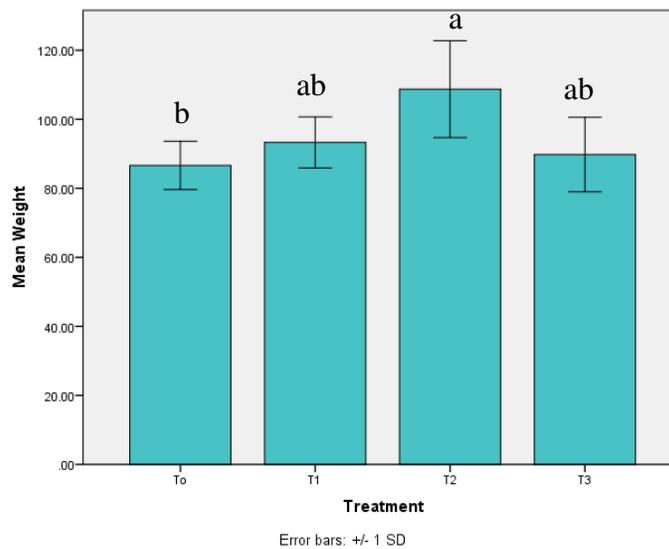


Fig. 1. Average weight chart in g of *O. niloticus* during the 16 weeks experimental period.



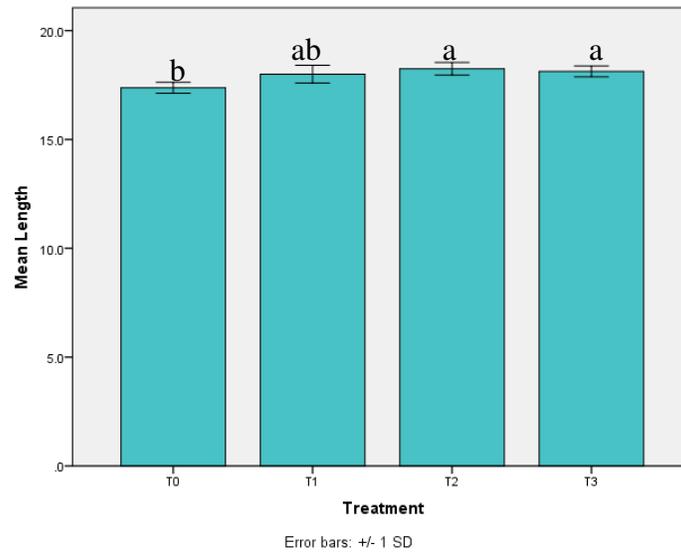
**Fig. 2.** Average length chart in cm of *O. niloticus* during the 16 weeks experimental period.

**Condition factor (K):** The values of the condition factor recorded in the present study were  $1.65 \pm 0.07$ ,  $1.6 \pm 0.06$ ,  $1.8 \pm 0.25$  and  $1.5 \pm 0.17$  in T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> respectively. Higher K value was found in T<sub>2</sub> compared to other treatments. However, no significant difference was observed among treatments ( $p > 0.05$ ) (Fig. 6).

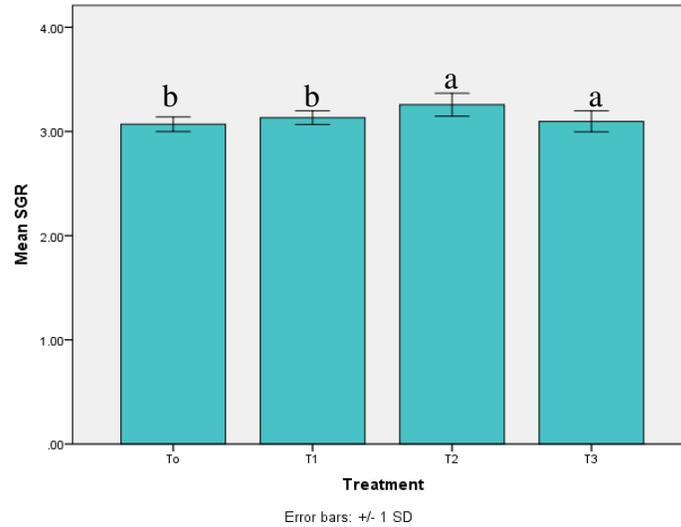


**Fig. 3.** Effects of polyphenols on weight of fish body (Mean  $\pm$  SD) after 16 weeks. The weight of fishes of T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> were compared to the control group. Values accompanied by different letters are statistically significantly different ( $p < 0.05$ )

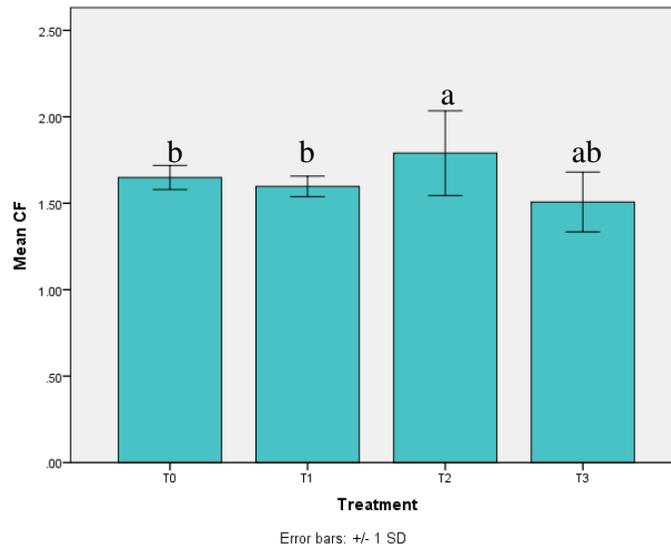
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**Fig. 4.** Effects of polyphenolon length of fish body (Mean  $\pm$  SD) after 16 weeks. The length of fishes of T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> were compared to the control group. Values accompanied by different letters are statistically significantly different ( $p < 0.05$ ).



**Fig. 5.** Effects of polyphenols on specific growth rate (Mean  $\pm$  SD) after 16 weeks experimental period. The specific growth rate of fishes of T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> were compared to the control group.



**Fig. 6.** Effects of polyphenols on condition factor (Mean  $\pm$  SD) after 4 months. The condition factor of fishes of T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> were compared to the control group.

**Feed conversion ratio (FCR):** T<sub>2</sub> showed an FCR of 1.59 whereas the FCR values of T<sub>1</sub> and T<sub>3</sub> were 1.85 and 1.93, respectively. All polyphenol treated diet showed better FCR than the control (2.0) (Table IV).

**Table IV.** FCR value of different experimental feed

Treatment	FCR
T <sub>0</sub>	2
T <sub>1</sub>	1.85
T <sub>2</sub>	1.59
T <sub>3</sub>	1.93

## Discussion

**Effect of polyphenol on weight and length:** After completion of the feeding trial, highest weight and length gain was found in T<sub>2</sub> ( $108.71 \pm 14$ ). The present work represents that fish fed without polyphenol had lower weight than the fish fed with polyphenol supplementation at 0.4% (120 mg polyphenol/kg feed) ( $p < 0.05$ ) which indicated that polyphenol had significant effects on fish growth. During the research period, 16 samplings were conducted where 1<sup>st</sup> four sampling (week 1 to week 4) showed lower growth rate because of time taken for adjustment in new environment. And week 5 to week 11 showed higher weight in all experimental fish in which T<sub>2</sub>

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showed highest weight comparing other treated fish. In week 12 to week 16 showed lower weight due to fluctuations in temperature, rainfall and weather change. Due to the low temperature, the feed intake rate also reduced inhibiting growth. No feed residues were left in polyphenol treated fish containing cage than the control because polyphenol contains good smell which easily attracts fish and consume all feed and maintain good water quality. Magrone *et al.* (2016) by feeding a polyphenol enriched diet to farmed sea bass reported that Polyphenol created lower levels of intestinal pro-inflammatory cytokines helping as an expression of a robust and protective adaptive immune response. Polyphenolic tannins show both positive and negative effects; they possess anti-nutritive properties, but are also beneficial for health due to their role as antioxidants and ability to stimulate the immune system and various effectors (Makkar *et al.* 2007; Quideau *et al.* 2011). No mortality and no outbreak of disease were observed during the study period. A continued decrease in temperature was noticed due to arrival of winter season. But fishes competed against low temperature were remained alive. This happened because of polyphenol builds strong immune system and helps sustain in extreme condition. Polyphenol had great effects on extension growth of maize (*Zea mays* L.) shoot segments, lettuce (*Lactuca sativa* L.) roots, and on radish (*Raphanus sativus* L.) seed germination (Stoms 1981). Gordon and Wareham (2010) reported that phenolic compounds have bactericidal and bacteriostatic properties. According to Viveros *et al.* (2011) Polyphenol have the capacity for minimizing adhesion of pathogenic bacteria (*Escheritia coli*, *Clostridium*), inhibit the progression of infections in the digestive tract, and improve nutrient utilization and finally animal performance. Sorsanit *et al.* (2002) conducted an experiment by using green tea extract (Polyphenol) in shrimp aquaculture. The work suggested that polyphenol of green tea in shrimps has positive effects on anti- microbial effects and has potential for use in shrimp aquaculture. The present study thus suggests that polyphenol has direct effects on increasing weight and length of farmed tilapia. The study concludes that, 0.4% Polygain (120 mg polyphenol/kg feed) containing diet has better growth performances than without polyphenol diet.

The specific growth rate is widely used dealing with the growth of aquatic organisms under experimental conditions. Specific growth rate indicates that growth rate of experimental fish during definite time period. The SGR values were recorded in present study were  $3.07 \pm .07$ ,  $3.13 \pm .07$ ,  $3.26 \pm .11$  and  $3.10 \pm 0.1$  in T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> respectively. Highest SGR was found in T<sub>2</sub> fish. But among the treated fish no significant differences ( $p > 0.05$ ) was observed. Similarly values of weight, length and condition factor were highest in fish fed 120mg polyphenol/kg feed. The result is similar to the study of Diana *et al.* (1996) who obtained SGR value of 3.1 with tilapia (*Oreochromis niloticus*) using feed and fertilizer in Thailand.

***Effect of polyphenol on condition factor:*** The condition factor of a fish is an indicator of overall health status of a fish including physical and biological condition and fluctuations by interaction among feeding conditions, parasitic infections and

physiological factors (Le Cren 1951). Therefore, information on condition factor can be vital to culture system management because they provide the producer with information of the specific condition under which organisms are developing (Araneda *et al.* 2008). The values of condition factor recorded in the present study were  $1.65 \pm .07$ ,  $1.6 \pm .06$ ,  $1.8 \pm .25$  and  $1.5 \pm .17$  in T<sub>0</sub>, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> respectively. Condition factor of greater than one showed the wellbeing of fishes fed with different experimental diets (Datta *et al.* 2013). But among treated fish no significant relation ( $p > 0.05$ ) was evident. The values of 'K' higher in T<sub>2</sub> than the T<sub>0</sub>, T<sub>1</sub> and T<sub>3</sub> suggesting that fish fed with diet containing 0.4% Polygain (120 mg polyphenol/kg feed) were much more nourished and strong than the fish fed with other treated diet. The results are equivalent with the study of Chandra and Jhan (2010) who recorded the K value of *Channa punctata* in the range of 1.05–1.89.

**Effect on Feed Conversion Ratio:** Best FCR (1.59) was found in T<sub>2</sub> treated feed fed to fish, whereas the FCR value of T<sub>1</sub> and T<sub>3</sub> treated fish were 1.85 and 1.93 respectively. All polyphenol treated fish showed better FCR performance than control fish (2.00). Broilers diet containing polyphenol (Black Tea Extract) has significantly higher weight gain, feed intake and lower FCR value (Ahmad *et al.* 2013). Tilapia has major specialties making as an ideal candidate for aquaculture. It has a wide range of benefit to meet the demand of protein source. The present study was conducted to assess the effects of plant polyphenol, a natural bioactive compound, on farmed Tilapia. The results of this research show that polyphenol has potential role on tilapia for better growth performance as well as better FCR value. Polyphenol supplementation in fish diet will ensure better profit for the farmers as well as food safety for the consumers. It is concluded that polyphenol has wide range of benefit to boosting fish growth which ultimately saves farmers' time and produce nutritionally enriched and tasty fish

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