Effect of Vegetable Tanning By-product on the Growth Performance of Caged Nile Tilapia (*Oreochromis niloticus*) as a Fishmeal Replacement

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ABSTRACT

The effect of vegetable tanning by-product (VTBP) on the growth performance of Nile tilapia (*Oreochromis niloticus,* Linnaeus) under cage culture system was evaluated. The chemical composition of vegetable tanning by-product (VTBP) and Fishmeal (FM) were analyzed. The dry matter (DM), crude protein (CP), ether extracts (EE) and ash content of VTBP were found 90.63, 77.02, 2.83 and 7.19%, respectively. The dry matter content of Fishmeal was recorded 87.3%. Ether extract content of Fishmeal (4.3%) was found slightly higher than the VTBP. The ash content of fishmeal was found 29.5%. But protein content of the Fishmeal (51.9%) was found much lower than the VTBP. Amino acid profile of both VTBP and Fishmeal were analyzed where the former showed higher amounts of some essential amino acids. The presence of aflatoxin in VTBP was found to be negative. The average temperature of pond water recorded during experimental period was 28.37, 27.69 and 28.64°C for treatment 1, 2 and 3 respectively. Dissolve oxygen level was similar to all the treatments and ranged from 6.1 to 6.7 ppm. The pH value was found between the ranges of 7.3 to 7.6. However, there was no significant difference among the survival rate, temperature, dissolved oxygen and pH. The fishes under various feeding treatments showed some difference in growth performance. The initial live weights of fishes were average 3 gm fish^{-1}. The fish productions in cage were 8.8 kg 10.2 kg 12.9 kg in treatment 1, 2 and 3 respectively. The production showed that treatment 3 significantly different from other treatments. Treatment 3 (VTBP) showed the highest production (12.9 kg/cage) and Treatment 1 obtained the lowest production (8.8 kg cage^{-1}). Mean weight gain and daily weight gain of fishes also showed similar results where the highest value for Mean weight (177.6 ± 6.65 g) gain and Daily weight gain (2.9 ± 0.11 g) were recorded in treatment 3. The survival rate was more or less similar to all the treatments which were around 97%.

Keywords: Vegetable tanning by-product, *Oreochromis niloticus,* fishmeal, growth, amino acid

INTRODUCTION

The industrial development of many developing countries over the last two decades has featured the tanning industry as an important component of their industrial structure emerging as a significant source of export income as well as a base for a growing domestic leather products industry. Tanning Industry is considered to be a major source of pollution as tanning by-products posses serious environmental impact on water (with its high oxygen demand, discolouration and toxic chemical constituents) terrestrial and atmospheric systems (Ros and Ganter, 1998; Song et al., 2000). Given its specific environmental impact, especially on the utilization and degradation of land and water resources, environmental concerns pertaining to the tanning industry have been increasing over the last couple of decades. This has also been promoted by the enforcement of new regulations worldwide, which are increasingly restrictive, relative to the control of waste and waste disposal.

Tanneries are regarded as the oldest industries in Bangladesh and considered as one most potentially rich manufacturing sectors in terms of both financial return and social benefits

(Ahmed, 2005). According to the statistics of the Export Promotion Bureau of Bangladesh, the leather sector grew by 17.5 percent in financial year 2011-12 and earned $765 million in revenue (BBS, 2014). Of this $434.8 million was attributed from leather products, accounting for approximately 57 percent of the total revenue. At present there are 277 tanneries operating in Bangladesh and 95% of these tanneries are concentrated mainly in Hazaribagh industrial zone of Dhaka (Karmaker, 2010). But environmental matters cannot be taken in isolation from leather making, as every facet of pollution or residual material is a direct function of manufacture (Richard, 2004). Salam and Billah (1998) reported that tanneries of Hazaribagh alone produce a total production of 8.47 million liter liquid and 98 metric tons solid wastes every day. Barton (2011) estimated dumping of 22,000 liters of toxic waste including cancer-causing chromium into nearby river Buriganga every day. Among these wastes raw trimmings, fleshing/pelt trimmings, chrome shaving and finished leather cuttings comprises of 10, 30, 10, 1 and 1.4%, respectively.

In the chrome and other inorganic tanning process, hides are treated with inorganic tannins such as chromium, aluminum, titanium, iron and zirconium basic salts (Krishnamoorthy et al., 2012). Among these most common tanning agent is chromium sulfate. This results in presence of large quantity of heavy metals including hexavelant chromium in the process wastes and by product. Cassano et al. (2001) found that about 30% of the initial chromium salt remains in the out-coming stream of a chrome tanning process. In another study, chromium content of 4.15 to 4.33 % in leather waste was reported by Malek et al. (2009).

Chromium is non-biodegradable and tends to accumulate in living organisms, causing serious diseases and disorders (Bailey et al., 1999; Mohan et al., 2006). Chromium recovery and detoxification are practiced in some developed countries (Alexander et al. 1992; Covington and Alexander, 1993). The precipitation, oxidation/reduction, lime neutralization have been most commonly used but very complex and expensive (Boddu et al., 2003; Mohan et al., 2006). Although biosorption appears as an economically feasible means for the removal and/or recovery of heavy metals from industrial wastewaters (Volesky, 1994; APHA, 1999) but like many other developing countries, Bangladesh still not in a position to imply these sophisticated treatment process due to weak socio-economic condition and lack of awareness. Therefore, in the present study wastes from tanneries that follow vegetable tanning process were only used to avoid heavy metal linked health hazards.

Except for a few big leather producers, most of the firms in Hazaribagh use vegetable tanning processes which is today's most classic and recognizable technique compared to other types of tanning processes and capable of imparting unique properties to leather and respects the environment as well. This eco-friendly technique uses natural tannins obtained from different part of plants including woods, barks, fruits, fruit pods and leaves. Since the scraps after vegetable tanning contain up to 7% N and 7-20% fat and 30-60% moisture (Kowalski and Piwowarski, 1996), on proper processing, it may play an important role in nutrition of fish and livestock nutrition as an alternate source of protein, which ultimately help reduce feed related production cost in fish and livestock sector.

In aquaculture, although the commercially manufactured pelleted feed is the most effective way to cultivate fish, but it appears as a major constraint as feed costs are extremely high. Moreover, previous surveys indicated the inefficient use of expensive inputs in intensive culture system is a major problem due to high cost of feeds and limited protein sources (Liti et al., 2005). Even most farmers cannot afford supplementary feeds. Most of the poor framers (traditional farmers) reported that higher production costs as well as lack of money was the most important constraint for fish farming It is therefore, suggested that a preferable option of decreasing production costs could be by using farm-made quality feed in order to increase profits. In Bangladesh like other countries, feed costs generally constitute the highest single operational cost, accounting for 76, 69 and 59% of total costs in intensive, semi-intensive and traditional feeding practices, respectively (Ahmed, 2007). Cost of fish feed ingredients including fishmeal and other animal protein sources has been showing an ever-increasing trend in Bangladesh and at present high price and shortage of feed ingredients, especially cheap protein source is one of the main constraints for profitable fish production in Bangladesh. Certain amount of protein form animal source must be added to the diet in order to satisfy essential amino acid requirements (Scott et al., 1976). In this circumstance, fish farmers are in desperate search for a cheap and balanced protein source. Since wastes of vegetable tanning is a by-product of leather industry and has very high protein content it can be a good alternative of animal protein source.

The present study, therefore, investigates the suitability of protein concentrate prepared from byproduct of vegetable tanning process as a Fishmeal replacement in aqua-feed and its effect on growth performance of caged tilapia.

MATERIALS AND METHODS

The experiment was conducted at the field laboratory of Department of Fisheries, Sher-e-Bangla Agricultural University, Dhaka to study the suitability of tanning by-product for its utilization as aqua-feed and to investigate growth performance of fish species. Before starting the experiment, a survey was conducted in the tanneries located at Hazaribagh, Dhaka. Some chemical analyses like crude protein, crude fibre, lipid, nitrogen free extract, crude ash, energy and presence of aflatoxin were done at the laboratory of Bangladesh Livestock Research Institute (BLRI) and Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka.

Preparation of vegetable tanning by-products (VTBP)

The tanning by-products were collected through contract basis from a number of tanneries of the Hazaribagh industrial area. The shaving or splits were collected only from vegetable-tanning units but not from wet blue leather to avoid of high amount of chromium contamination. Fleshings which are the excess of fat, fascia and flesh removed from the flesh side of the hides and skins were collected from hides and skins store house. Trimmings were collected by cutting the unnecessary portions of the hides and skins during processing. Shavings or splits were collected from the smoothing and thickness adjusting stage of dried tanned leather. The method described by Blazej et al. (1969) was used for the preparation of vegetable tanning by-product (VTBP) in the present experiment with necessary modification (Fig. 1).

After collecting the tanning by-products, fleshings and trimmings were properly washed 2-3 times with clean tap water to remove salt and dust or other foreign materials. The shavings or splits from vegetable tanned leather were not washed as they did not contain any undesirable matter. Then washed and dried fleshings and trimmings were cut in small pieces and mixed thoroughly at a ratio of 17:83. Then mixed fleshings and trimmings were taken into a hot water bath with same volume of water and boiled at 100°C for 4-5 hours (Fig. 1). Again shavings or splits were taken into a hot water bath with about 50% water and boiled at 100°C for 1-1.5 hours. They became soft after boiling. Then boiled fleshings and trimmings were properly mixed with soft shavings or splits on the clean cemented floor at a ratio of 60:40 as shavings contain more protein (Fig.1). Then the samples were properly sun-dried after mixing and the dried mixture was ground properly by a special grinding machine. Finally the ground mixture was sieved by a sieve fitted with net of 2 mm pores to remove the undesirable particles and hairs. The prepared finely sieved protein concentrate was packed in the hessian/gunny bag for storage purpose.

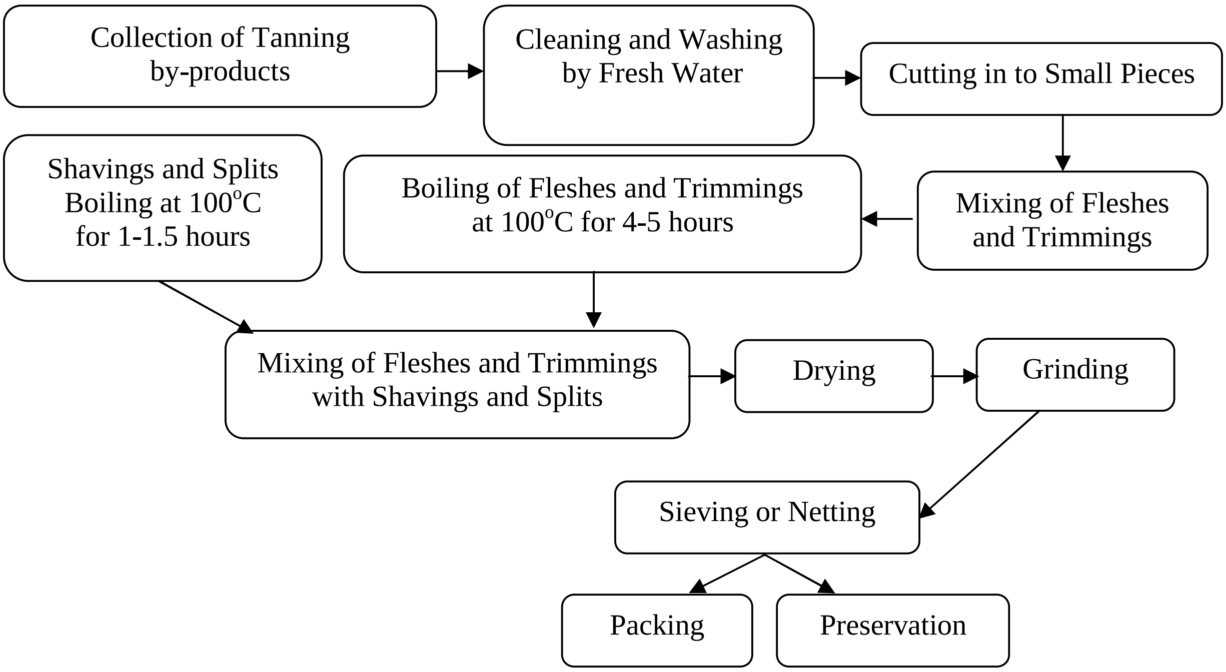


Fig. 1. Schematic Diagram of Vegetable tanning by-product Preparation

Experimental diets and feeding regime

Locally available commercial feed ingredients like rice bran, molasses, soybean oil and vitamin-mineral premix were used for formulating test diets. Composition of diets used for different treatments were presented below in Table 1.

Table 1: Composition of Diets used in different treatments

Feed ingredients	Present (%) in diet for T_1	Present (%) in diet for T_2	Present (%) in diet for T_3
Fishmeal	42	21	0
VTBP	0	21	42
Rice bran	46	46	46
Molasses	8	8	8
Soybean oil	3	3	3
Vitamin-mineral premix	1	1	1

All fishes in the cage were supplied pelleted feed according to the proportion mention above. Feeding rate of 10, 9, 8 and 6% of body weight were followed during 1st -2nd, 3rd -4th, 5th -6th and 7th - 8th week, respectively. Feeds were supplied everyday at 9.00 am and 3.00 pm in two equal splits.

Experimental Design

The feeding trial was conducted in nine 1m^3 net cages, housed in a 8000 m^2 earthen pond, Bamboo framed nylon net cage were set on bamboo stales and attached to wooden structures. Ten cages (one for control treatment) were placed in equal distance in the pond. Feeding rings (50-cm diameter) were placed in each cage to avoid feed mixing among experimental units. A walkway was extended to each cage to supply prepared feed and to collect sample for laboratory analyses. Nile tilapia fingerlings were obtained from a commercial fish hatchery and were acclimated in 8 m^3 net enclosure (hapa) before starting the feeding trial. From this group, 180 fish (average weight 3.2 ± 0.94g) were randomly distributed into ten 1 m^3 net cages. A completely randomized design was adopted, where three diets were fed to triplicate groups of 75 fingerlings.

Collection of experimental organism

The most appropriate species or strains of tilapia for cage culture are Nile Tilapia (*Oreochromis niloticus*), blue Tilapia (*O. aureus*) and hybrids between these species and strains. The choice of a species for culture depends mainly on availability, legal status, growth rate and temperature tolerance. As it has been culturing in Bangladesh for long time and its growth performance are better than the other strains for this reason Nile tilapia was selected for the cage culture for the experiment. All male Nile tilapia (*Oreochromis niloticus*) fry were collected from established mono sex tilapia. The average weight of the fish was 0.2 gram and all the fishes were reared in a nylon hapa (small netted enclosure) for 30 days to make them suitable for cage culture. It was found that fishes reached average 3 gm after rearing. Commercially available nursery feed (mash) were supplied during the nursing period.

Water quality parameters

Major water quality parameters like temperature, pH, dissolved oxygen (DO) of experimental ponds were determined. Physical quality parameters of water except temperature were determined in the Animal science and Fisheries laboratory, SAU. Water samples (250 ml) were collected carefully in clean plastic bottles and mouth of the bottles were closed with stopper and airtight cap and placed into insulated box. Water samples were collected in the morning, between 9.00 and 10.00 am, and the bottles were filled up with water from various spots and at various depths in each pond. In the laboratory water samples from each bottle was filtered through glass fibre filter paper (Whatman GF/C) and used for analysis. All the analyses were done in triplicate and the means are presented.

The temperature (°C) of pond water was recorded with the help of thermometer between 9.00 and 10.00 am. The pH of water sample was measured by a portable pH meter (CORNING-445, USA). Dissolved oxygen was measured by DO meter (YSI 85 Oxygen conductivity meter, Model 85-10, USA). The total alkalinity of the experimental ponds' water was determined with EDTA titrimetric method (APHA, 1980).

Biochemical Analysis

Prepared feeds were analyzed in the laboratory of Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka for the determination of Dry Matter (DM), Crude Protein (CP), Crude Fiber (CF), Ether Extract (EE), Ash and Metabolizable Energy (ME). All these are determined according to the method followed by AOAC (1990). Aflatoxin was tested in the toxicity laboratory of BCSIR, Dhaka by ALISA kit (AOAC, 1990). Amino acids composition was determined with an automatic amino acid analyser (LKB 4151 plus, Biochrom Ltd., Cambridge, UK) according to Bidlingmeyer et al. (1987) in the Analytical Laboratory of Institute of Food Science and Technology (IFST), BCSIR, Dhaka.

Growth performance

All cages were monitored every day. Cages were cleaned weekly by using hard brush. Fish growth performances were monitored bi-weekly. At the end of the experiment all fishes in the cage were counted and weighted to measure the individual weight, total weight of fish, and survival rate. Weight of the fish are taken weekly by electronic balance. Twenty fishes are collected from each cage on random sampling basis. Then the mean weights are calculated for further analysis.

Statistical analysis

The statistical analyses were performed using Student's t-test and one way ANOVA. Multiple comparisons of means were done by least significant difference (LSD) test. A probability value of $p<0.05$ was considered to be significant. All data were analyzed by the statistical software SPSS (version 11.0). The data were presented as mean ± SD of three determinations.

RESULTS AND DISCUSSION

Chemical composition of VTBP and Fishmeal

The chemical composition of vegetable tanning by-product (VTBP) and Fishmeal are shown in Table 2.

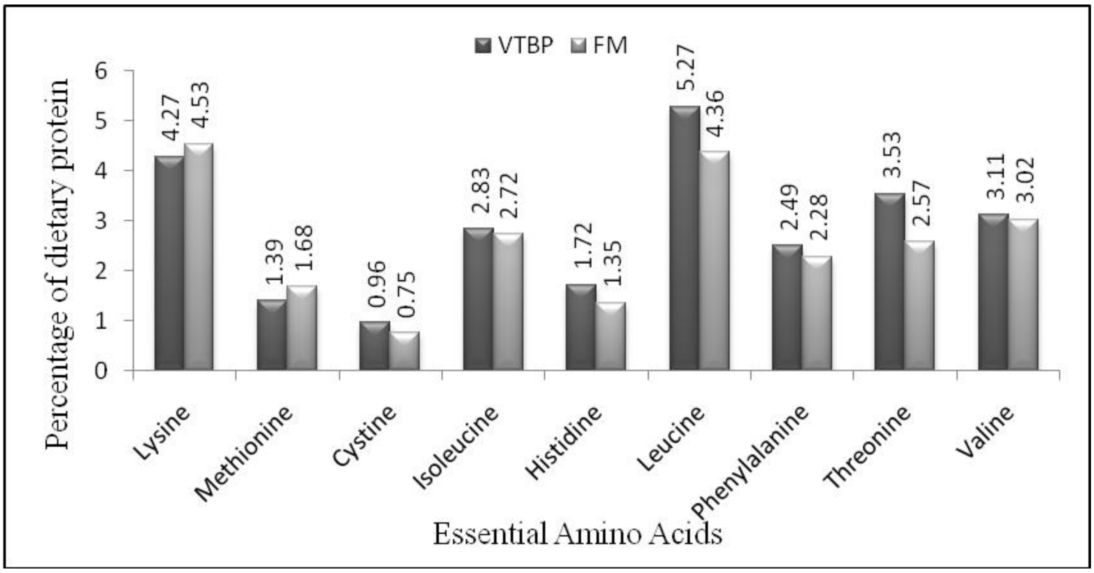
Table 2: Chemical composition of VTBP and Fishmeal used in formulated diets

Parameters	VTBP	FM
Dry matter (%)	90.63	87.3
Crude protein (%)	77.02	51.9
Ether extract (%)	2.83	4.3
Ash (%)	7.19	29.5
Metabolizable energy (Kcal kg^{-1})	3450	2776

FM= Fishmeal, VTBP= Vegetable tanning by-product

The chemical composition of VTBP (on DM basis) contains more CP than that of Fishmeal (FM). The crude protein content in Fishmeal is 51.9% where as in VTBP was 77.02%. The ash content was found 7.19 and 29.5 % for VTBP and FM respectively. The high ash content in fishmeal can be attributed due to the presence of bone during milling whole dried fish. The metabolizable energy per unit weight varied considerably where VTBP and FM supplied 3450 and 2776 Kcal/kg respectively. The moisture content was found more or less similar of the two samples and FM contained slightly higher ether extract (4.3%) than that of VTBP (2.83%) The composition of VTBP is close to the values reported by Hoque et al. (1992). They reported that sun dried tanning by-products contain 69.8% and 68.8% crude protein. The contamination of aflatoxin in VTBP was found to be negative.

Essential amino acid contents of vegetable tanning by-product (VTBP) and fishmeal were showed in Figure 2. In general, amino acid concentration of samples was directly proportional to the crude protein content. Furthermore, the ratio of individual amino acid to crude protein was inconsistent among samples.



FM= Fishmeal, VTBP= Vegetable tanning by-product

Fig 2: Comparison between essential amino acids of VTBP and Fishmeal

The amino acid content of fishmeal may largely depend on the fish or group of fish from which it was produced. One kind of fish contained higher in one amino acid than the others, but was lower in other amino acids. From the result of amino acid analyses, it was found that EAA profile of VTBP was a better match with the dietary essential amino acid requirement of *O. niloticus* than that of Fishmeal mirroring the recommended amino acid requirement for tilapia by Santiago and Lovell (1988). Essential amino acid requirements are therefore not absolute values rather an indication of concentration range which must be present in fish diet to enhance adequate performance of fish.

Growth Performance of Fish

A sample of fish was taken for every two weeks to obtain the average body weight form each cage which was used to monitored the weight gain for a given period of time and to manipulated the feed budget as well as for comparison of the specific growth rate of fishes. The final growth rate, the specific growth rare and survival rate were calculated for each cage.

The summary of growth performance of caged Nile tilapia in different treatments was presented in Table 3. It was observed that the fishes showed significant growth performance in different treatments. The initial live weights of fishes were average 3 gm fish^{-1}. The fish productions in cage were 8.8 kg 10.2 kg 12.9 kg in treatment 1, 2 and 3 respectively. The production showed that treatment 3 significantly different than other treatments.

Table 3: Different culture parameters of caged Nile tilapia in experimental treatments

Culture parameters	Treatment 1	Treatment 2	Treatment 3
Stocking			
Total weight (kg $cage^{-1}$)	0.23	0.23	0.23
Mean weight (g $fish^{-1}$)	3±0.13	3±0.21	3±0.16
Water quality			
Average Temperature (°C)	28.37	27.69	28.64
Dissolve Oxygen (mg L^{-1})	6.3	6.7	6.1
pH	7.6	7.3	7.5
Growth Performance			
Survival (%)	97 ± 1.53	97 ± 1.00	97 ± 1.15
Total weight (kg $cage^{-1}$)	8.8 ±0.47a	10.0 ± 0.31b	12.9 ± 0.30c
Mean weight gain (g $fish^{-1}$)	120.6 ± 5.32a	136.5 ± 3.26b	177.6 ± 6.65c
Daily weight gain (g $fish^{-1}$)	1.96 ± 0.09a	2.4 ± 0.19b	2.9 ± 0.11c

Values are mean ± SD (n=3), mean values with different letters in superscript in the same row were significantly different (P<0.05).

The result showed that the treatments are significantly different from each other. Treatment 3 (VTBP) showed highest growth performance among the treatments. Highest production (12.9 kg $cage^{-1}$) was found in treatment 3 and Treatment 1 showed the lowest production (8.8 kg $cage^{-1}$) of fish (Fig. 2). Mean weight gain and Daily weight gain of fishes also significantly different among the treatments. The survival rate was more or less similar to all the treatments. Mean weight gain varies from 120.6 to 177.6 gm. Highest daily weight gain was observed in treatment 3.

Dissolve oxygen level was similar to all the treatments and ranged from 6.1 to 6.7 ppm. The pH value was found in between the ranges of 7.3 to 7.6. The dissolved oxygen and pH value was within the suitable range for fish culture. The average temperature of pond water recorded was 28.37, 27.69 and 28.64°C for treatment 1, 2 and 3 respectively. The temperature recorded in experimental pond was within the acceptable range for pond fish culture (Ali et al, 1982). Michael (1969) concluded that the quantity of DO present in the ponds was dependent on the various biological processes taking place in the medium. Banerjee (1967) reported that 7.0 mg L^{-1} dissolved oxygen and pH value ranging from 6.5 to 7.5 of water body was good for production. However, there was no significant different among the survival rate, temperature, dissolved oxygen and pH.

As the protein level was higher in tanning by-product so it has positive impact on growth of fish. The proportion of fishmeal can be easily replaced by tanning by-product as a cheaper source of protein. The production was higher than the normal cage culture of fish in pond ecosystem. The production by using the fish meal was similar to the research finding done by Beveridge (1984).

From the result it was found that tanning by-product has significant effect on the growth performance of fish than the fish meal. So it can be concluded that fish meal can be successfully replaced by vegetable tanning by-product.

CONCLUSION

Result from the caged fish experiment showed that the treatments are significantly ($P<0.05$) different from each other. Diet in which 100% fishmeal was replaced by vegetable tanning by-product showed highest growth performance among all the treatments. Highest production (12.9 kg $cage^{-1}$) was found from highest tanning by-product containing feed. Mean weight gain and Daily weight gain of fishes were also found to be significantly different among the treatments. The survival rate was more or less similar to all the treatments. Current cost of vegetable tanning by-product and fishmeal (Grade A) is 5-5.5 and 50-55 tk kg^{-1}, respectively [Taka (tk) = Bangladeshi currency; $1=80 taka]. So complete or partial replacement of Fishmeal by VTBP reduced feed and production cost. So it can be concluded that the according to the present findings, tanning by-product has significant effect on the growth performance of Nile tilapia and can be used as fishmeal replacement an as alternative cheaper protein source for fishes without compromising the growth.

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