Evaluation of silkworm pupae and tannery waste as dietary protein sources for Thai koi, *Anabas testudineus* (Bloch)

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Abstract

Fish meal (FM) was partially replaced by silkworm pupae meal (SWPM) and tannery waste meal (TWM) as dietary protein sources in diets for Thai koi, *Anabas testudineus* (Bloch). Four different diets were prepared using SWPM and TWM replacing half the fish meal as the sole source of protein. Growth performance was observed for 10 weeks using these four different diets on the basis of feed utilization and digestibility. Growth responses in terms of weight gain (%) and feed utilization (specific growth rate, feed conversion ratio, protein efficiency ratio and apparent protein digestibility) in fish fed diet containing 20% FM, 15% SWPM and 5% TWM (Diet 3) was found to be similar to that of control diet containing 100% FM (Diet 1); both were significantly ($p < 0.05$) higher than those of diet 2 (20% FM, 10% SWPM and 10% TWM) and Diet 4 (20% FM, 5% SWPM and 15% TWM). Diet 3 was found to be the most economical among the four diets and cost the least to produce a unit of biomass. The survival of fish fed Diet 1 (Control) and Diet 3 was higher (83-88%) than those fed Diet 2 and Diet 4. Biosyntheses of crude protein and crude lipids in fish fed diets 1 and 3 were significantly greater than in the fish fed the other two diets and initial fry. There was no difference ($p < 0.05$) between treatments or the control in relation to bioaccumulation of minerals (ash). It was found that the diet containing low levels of TWM (5%) and a moderate amount of SWPM (15%), replacing half the Fish Meal gave best growth performances for Thai koi, *A. testudineus*. Crude protein, crude lipids, ash and nitrogen free extract had a significant ($p < 0.01$) combined effect on percent weight gain ($R = 0.895$), specific growth rate ($R = 0.920$), feed conversion ratio ($R = 0.908$) and protein efficiency ratio ($R = 0.86\ 9$), contributing 80.10%, 84.64%, 42.45% and 75.52% to the total, respectively. This study showed that tannery waste, before adding chromium oxide (Cr2O7), may be used as a dietary ingredient to replace fish protein to a limited extent, reducing the cost of fish diets as well as the pollution of the environment from tannery waste disposal.

Keywords: Silkworm

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Introduction

'Thai koi', *Anabas testudineus* (Bloch), was introduced into Bangladesh around 1996 due to its high growth rate (Shoma, 2005). The growth rate of 'Thai koi' is higher than the climbing perch 'native koi', *Anabas testudineus,* and attains a marketable size within three months. The 'native koi' is one of the most preferred farmed fish in Bangladesh due to its good taste, high acceptability among consumers, tolerance of low pH and dissolved oxygen, and its resistance to disease (Singh and Samuel, 1981; Habib and Hasan, 1994). There is some research information available on the native climbing perch mainly with regard to parasitic, bacterial and fungal diseases (Chandra and Yasmin, 2003; De and Mandal, 2003; Sahoo *et al*., 2000 and Santhakumar *et al*., 2000). Due to the scarcity of the native koi, however, market price of Thai koi has been increasing day by day, and there is a need to cultivate them using artificial feed.

The protein requirement of the of fry of 'native koi', *A. testudineus* is high and around 40% (Habib and Hasan, 1994; Mahmood *et al*., 2004) and farmers are faced with the problem of obtaining low cost protein rich local ingredients. There is some information available on the food habits (Singh and Samuel, 1981), reproductive biology (Banerji and Prasad, 1974; Chanchal *et al*., 1978; Misra, 1994), haematological and enzymatic changes of *A. testudineus* (Das *et al*., 2005). But there is no research work conducted, however, to evaluate the performance of low cost indigenous ingredients of feed for this recently introduced fish. The available information on the optimum level of protein and lipid requirements of native climbing perch, *A. testudineus* fry is still very scanty except for the reports on its nutritional requirements reported by Patra and Ray, 1988; Ray and Patra, 1989; Patra, 1993; Habib and Hasan, 1994. The information on nutritional requirements of major dietary components such as protein and lipid is a prerequisite for formulation of least cost and balanced diet for fish (Pathak, 1978; Page and Andrews, 1973; Daniels and Robinson, 1986; De Silva *et al*., 1989). Fish meal has been used as a sole protein source in artificial feed for fish. But due to high cost of fish meal and its limited availability, it is essential to develop alternate protein rich sources to prepare artificial feed for fish from locally available ingredients.

Silkworm pupae (Habib *et al*., 1994) and tannery waste are cheap and available indigenous ingredients, rich in protein, fat, vitamins and minerals, that may be used as an alternative to fish meal in feeds for Thai koi, *A. testudineus*. Keeping the protein requirement of 'native koi' *Anabas testudineus* as a standard (Habib and Hasan, 1994), an experiment was conducted to evaluate protein requirement of 'Thai koi', *A. testudineus* using silkworm pupae and tannery waste meal as local ingredients.

Materials and Methods

The experiment was conducted in the wet laboratory of the Department of Aquaculture, Bangladesh Agricultural University, Mymensingh, Bangladesh over a period of 10 weeks. The fry were collected from a nearby hatchery and conditioned to acclimatize with the laboratory environment and the control diet for 10 days. The fish were raised in thirty liter rectangular glass aquaria containing 20 liters of water. Details regarding the experimental methods are shown in Table 1 below:

Table 1. Experimental Methods.

Experimental fish	Thai Koi, *Anabas testudineus* (Bloch)
Age & Weight	21 days, 1.54 g per fry
Duration	70 days with aeration
Number of fish per aquarium	16
Temperature	28°C (Thermostatic heater was used in November & December)
pH & Dissolved oxygen	Measured every day
Change of water	Every day
Adjustment of ration	After weekly weighing of the fish
Treatments (Diet with 40% protein)	4
Feeding frequency & time	Twice daily(morning & evening)
Treatment 1 (Control)	100% Fish meal (FM)
Treatment 2	29% FM, 10% Silkworm pupae meal (SWPM) & 10% Tannery waste meal (TWM)
Treatment 3	20% FM, 15% SWPM & 5% TWM
Treatment 4	20% FM, 5% SWPM & 5% TWM
Proximate composition	Ingredients, Diets, Fish (Initial & Final)
Economic analysis	Cost benefit analysis on the basis of diets
Statistical analyses	One way ANOVA & Multiple correlation

Fish meal (Grade 1) was purchased from the market, silkworm pupae were collected from a nearby sericulture farm, Mymensingh, Bangladesh and tannery waste - collected before adding chromic oxide - from a Tannery of Hazaribagh, Dhaka, Bangladesh. Fish meal was dried in an oven at 35°C overnight. Silkworm pupae and tannery waste were first sun dried for three days and then oven dried at 35°C for 2 days. After drying, all the ingredients were sieved using a mesh size of 500 ìm. The proximate composition of all these ingredients were analyzed following standard methods Horwitz (1984) (Table 2). The diets were prepared according to Habib and Hasan (1994). Four different diets including the control were formulated to contain 40% protein to evaluate the growth efficiency of Thai koi, *Anabas testudineus* (Bloch) fed diets prepared with fish meal (FM), silkworm pupae meal (SWPM) and tannery waste meal (TWM) as dietary protein sources (Table 3). The control diet was prepared using fish meal as the sole source of protein while in the experimental diets, around half of the fish meal was replaced with the other two local ingredients (Table 3).

The mixed feeds and the fish at the end of the study were analyzed for proximate composition following Horwitz (1984). Specific growth rate ($\log_e$ of mean final body weight – $\log_e$ of mean initial body weight) / time x 100, feed conversion ratio (dry food intake / mean live weight gain), protein efficiency ratio (mean live weight gain / protein intake), apparent protein digestibility {100-100 (% chromic oxide in feed / % chromic oxide in faeces x % nutrient in faeces / % nutrient in feed)} and survival rates{(mean fish fry survived / mean fish fry died) x100} were determined. An economic analysis was performed to estimate the cost of feed to raise a unit biomass of fish by considering the cost of ingredients only (Habib *et al.*, 1994). Comparison of treatment means for the different growth parameters of fish of was done by one-way ANOVA. The combined effects of proximate composition of feed such as crude protein, crude lipids, ash and nitrogen free extract (NFE) on the growth parameters of fish were analyzed by multiple regression coefficient analysis. All the statistical analyses were carried out using a statistical package (Microstat) following Zar (1984).

Table 2. Proximate composition (% on dry weight basis) of dietary ingredients.

Constituents	Fish meal	Silkworm pupae	Tannery waste
Moisture	8.65	8.40	8.50
Crude Protein (CP)	64.90	70.30	77.00
Crude Lipids (CL)	12.80	15.40	5.85
Ash	13.60	3.35	7.20

Table 3. Formulation and composition (%) of diets using different ingredients such as fish meal (FM), silkworm pupae meal (SWPM) and tannery waste meal (TWM).

	T1 (Diet 1)	T2 (Diet 2)	T3 (Diet 3)	T4 (Diet 4)
FM	65	31.67	31.67	31.67
SWPM	-	22.73	34.09	11.36
TWM	-	17.24	8.62	25.86
Wheat flour	10	10	10	10
Cod liver oil	1.50	1.50	1.50	1.50
Vitamin/mineral	2.0	2.0	2.0	2.0
Binder	12.50	6.86	7.12	12.31
Alpha-cellulose	7.0	6.0	3.0	3.30
Chromic oxide	5.0	5.0	5.0	5.0
Cost/ Kg(TK)	32	32	30	32
Moisture	7.31	7.10	7.77	7.43
Crude Protein (CP)	39.26	39.84	39.04	39.55
Crude Lipids (CL)	10.43	10.95	11.38	10.24
Ash	11.00	11.78	12.00	12.22
Crude Fibre	5.40	4.60	4.80	4.65
NFE[a]	26.60	25.73	25.01	25.91
Gross energy	4.17	4.17	4.18	4.09
ME[c]	3.66	3.67	3.68	3.60

[a]NFE (Nitrogen free extract) = 100- (Moisture + CP + CL + Ash + Crude fibre). [b]Gross energy of diets was calculated using caloric values of 5.7/g protein, 9/g lipid and 4/g carbohydrate. [c]Metabolizable energy of diets was calculated using caloric values of 4.5/g protein, 8.5/g lipid and 3.4/g carbohydrate.

Results and Discussion

The acceptability of the experimental diets to the fry, estimated by observing the feed consumed within 15 minutes, ranged from 60 to 80%. Consumption rates were highest for the control (treatment 1) diet and diet three (20% FM, 15% SWPM and 5% TWM) with 75 to 80% of the diet being eaten within this time. The fry showed delayed interest to eat feed in treatment 4 (Diet 4) whilst 60-70% feed was eaten in the case of Diet 2. The water quality parameters such as temperature (27-28°C), pH (6.8-8.5) and dissolved oxygen (3.5-6.5 ppm) were found to be in the optimum range for good water quality (see Table 4).

Table 4. The range of physico-chemical values for water during the experiment.

	T1 (Diet 1)	**T2 (Diet 2)**	**T3 (Diet 3)**	**T4 (Diet 4)**
Temp. (°C)	27.80-28.20	27.90-28.10	27.90-28.10	28.10-28.20
pH	6.90-7.15	7.00-7.10	7.10-7.15	7.10-7.20
Dissolved O_2 (mg/L)	4.80-5.40	4.90-5.10	5.00-5.30	4.20-4.40

Diets were evaluated on the basis of feed utilization and digestibility. The growth rates and feed utilization by the Thai koi fry on the four treatment diets in terms of weight gain, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), apparent protein digestibility (APD), survivability (%) and cost per kg of diet weight gain (in US$) are shown in Table 5.

Weekly weight gain of fry shows that fish fed diets 1 and 3 was similar and higher than that of fish fed diets 2 and 4 (Fig. 1). There were only numerical differences but no significant differences ($p > 0.05$) among the initial weight of fry of four treatments (Table 5). The weight gain and feed utilization on the diet containing 20% fish meal, 15% SWP meal and 5% TW meal (Diet 3) was similar to that on the control diet (Diet 1). In the case of Diet 2 (20% fish meal, 10% SWP meal and 10% TW meal) and Diet 4 (20% fish meal, 5% SWP meal and 15% TW meal), however, these parameters were significantly different ($P<0.05$) from the control. The variations in SGR, FCR and PER of fish in respect to diets are shown in Fig. 2. Diet 3 (US$ 0.69) was found to be the most economical and cost the least to produce a unit biomass of fish (Table 5). The survival rate of fish fed Diet 1 (control) and Diet 3 was higher (83-88%) than those fed Diets 2 and 4.

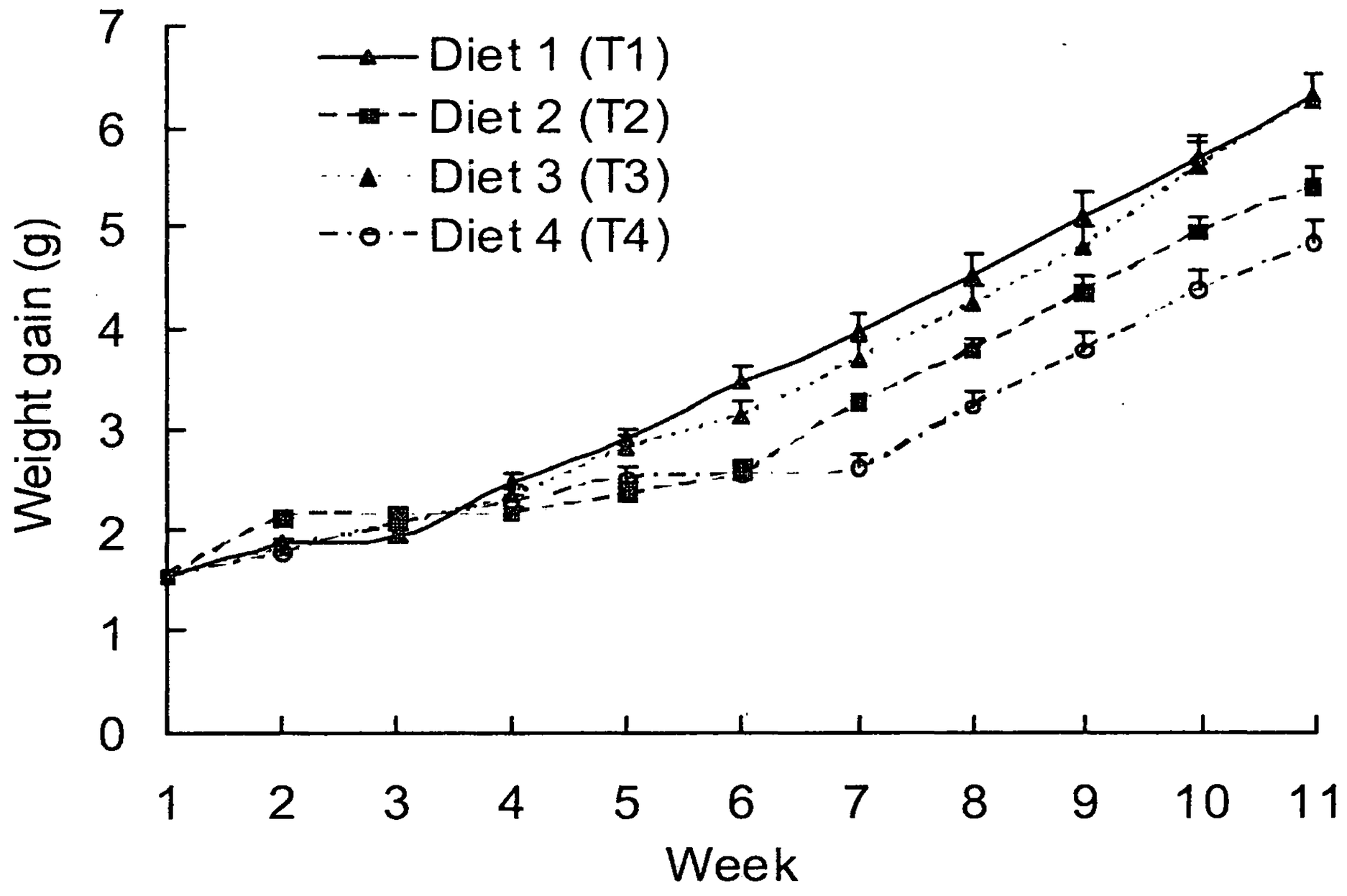

Fig. 1. Weekly growth of Thai koi, *Anabas testudineus* fed four different diets.

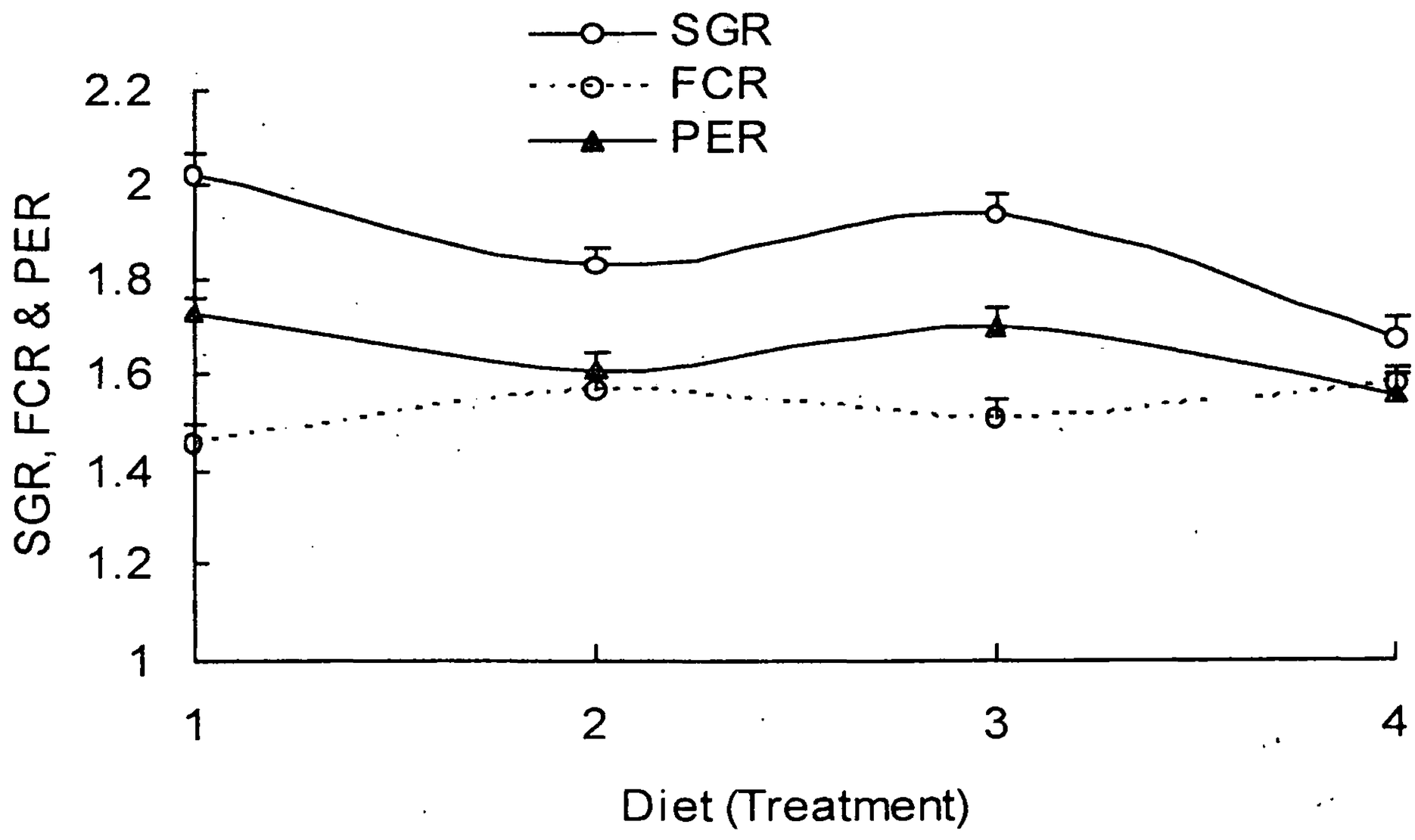

Fig. 2. Specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) of Thai koi, *Anabas testudineus* fed four different diets.

Table 5. Growth, weight gain, food utilization, digestibility and mortality of Thai koi (*Anabas testudineus*) fed four different diets.

	T1 (Diet 1)	**T2 (Diet 2)**	**T3 (Diet 3)**	**T4 (Diet 4)**
Initial wt. (g)	1.53	1.54	1.55	1.54
Final wt. (g)	6.30	5.52	6.97	4.96
Weight gain (%)	301.28a	260.32b	288.72a	223.04c
SGR	2.02a	1.83b	1.94a	1.68c
FCR	1.45a	1.57b	1.51a	1.58b
PER	1.75a	1.61b	1.70a	1.58b
APD	81.25a	73.45b	80.40a	69.15b
Survivality (%)	83	60	83	60
Cost of diet per kg Weight gain (in US$)	0.68	0.73	0.69	0.72

SGR = Specific growth rate, FCR = Food Conversion Ratio, PER = Protein Efficiency Ratio. Means with common superscripts do not differ significantly ($p < 0.05$). 1.0 US$ = Tk. 68.50.

The proximate composition of the fish is presented in Table 6. The moisture content of the fish at the beginning was significantly higher ($p < 0.05$) than at the end in the case of all four diets. On diets 1 and 3 crude protein and crude lipids were biosynthesized in greater amounts than on other two diets ($p < 0.05$) or by the fry. Bioaccumulation of minerals (ash) in the fish fed the different diets was similar, but all were higher than that of the initial fry ($p > 0.05$). The combined effects of proximate composition (crude protein, crude lipids, ash and nitrogen free extract) of diets had highly significant ($p < 0.01$) correlation with the growth parameters of fish such as weight gain ($R = 0.895$), SGR ($R = 0.920$), FCR ($R = 0.908$) and PER ($R = 0.869$) which contributed 80.10%, 84.64%, 82.45% and 75.52% of total contribution, respectively (Table 7).

Table 6. Average moisture, crude protein, crude lipids and ash (% fresh weight basis) content of fish fed the four experimental diets.

	Moisture	**Crude protein**	**Crude lipids**	**Ash**
Initial	76.40a	16.55d	2.20c	3.80a
Control (Diet 1)	75.20b	17.60a	3.50a	2.98b
1 (Diet 2)	75.22b	17.40b	3.35b	2.96b
1 (Diet 3)	75.16b	17.62a	3.52a	2.94b
1 (Diet 4)	75.19b	17.25c	3.25b	2.96b

Means with common superscripts do not differ significantly ($p < 0.05$).

Table 7. Multiple correlation coefficient (R), MR^2, F-values of crude protein, crude lipids, ash and nitrogen free extract (NFE) of diets with weight gain (%), specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER) of Thai koi (*Anabas testudineus*).

Growth parameters	**R**	**MR^2**	**F-values, estimated equation**
Weight gain (%)	0.895*	80.10	$12.45, 33.25 + 55.35X_1 + 40.14X_2 + 10.12X_3 + 4.20X_4$
SGR	0.920*	84.64	$19.30, 66.80 + 88.20X_1 + 75.28X_2 + 31.24X_3 + 10.10X_4$
FCR	0.908*	82.45	$15.34, 44.22 + 69.55X_1 + 58.21X_2 + 25.28X_3 + 5.59X_4$
PER	0.869*	75.52	$12.60, 28.15 + 34.76X_1 + 43.25X_2 + 16.25X_3 - 3.59X_4$

*$F_{0.01(31,5)} = 4.00$

The results from the experiment showed that the feed containing low levels of tannery waste meal (5%) and moderate levels of silkworm pupae meal (15%) replacing around half the fish meal gave best results with Thai koi, *Anabas testudineus* (Bloch). The weight gain and growth responses such as SGR, FCR, PER and APD of Thai koi fed diet 1 (Control) and diet 3 were significantly ($p < 0.05$) higher than those on the other two diets. It was observed that the growth responses of fish were diminished with increasing levels of tannery waste meal (above 5%). Apparent protein digestibility (%) of the diets by the fish was directly related to the amount of tannery waste. Crude protein and crude lipid content of the fish fed diets 1 and 3 were also increased, as the amount of tannery waste meal decreased. But biosynthesis of crude lipids of fish body was not increased in the same manner as for crude protein. The combined effect of proximate composition of feed on growth parameters such as weight gain (%), SGR and FCR of fish was high but the protein efficiency ratio was not as satisfactory as the other parameters. Temperature, pH and dissolved oxygen of water of aquaria were within the optimum ranges which showed that the quality of water for fish culture was satisfactory. There is no recorded information on growth performances of Thai koi in the country. Habib *et al.*, (2001) recorded that a diet containing fish meal and silkworm pupae meal was highly palatable to fish fry (*Clarias batrachus*) which agrees with the present findings. Mahmood *et al.*, (2004) who reared native climbing perch, *A. testudineus,* on live food recorded good growth when fed on tubicid worms. Patra (1993) used a pelleted diet with 35% protein to feed native koi but there was no information regarding the ingredients used. Akhteruzzaman (1988) used a supplemental diet prepared with a mixture of rice bran, mustard oil cake and fish meal at a ratio of 3:1:1 to feed native climbing perch, *A. testudineus* but did evaluate the individual ingredients separately.

Conclusion

Tannery waste before adding chromium oxide may be used as a dietary ingredient for fish in aquaculture to replace 5% fish protein. It can reduce the cost of the diet and increase the production of fish and ultimately the income of the fish farmer. Utilization of tannery waste in this manner will also reduce the environmental pollution due to this material to some extent.

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