



Growth Performance of Advanced Fry of Grass Carp *Ctenopharyngodon idella* (Valenciennes) Fed with Cocoa Pod Husk Powder Incorporated Diet

K. B. Shabitha, Aneykutty Joseph* and Gijo Ittoop¹

Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology, Cochin - 682 016, India

Abstract

An experiment was conducted to find out the efficacy of cocoa pod husk incorporated feed in the diet of grass carp as protein substitute. Seven isonitrogenous diets were prepared with proportion of cocoa pod husk powder varying from 0 to 30%, replacing fish meal. The diets were fed to advanced fry of grass carp in three replicates. Growth performance was assessed by using indicators such as net weight gain, weight increment per day, percentage weight gain, specific growth rate, feed conversion ratio, protein efficiency ratio and apparent digestibility coefficient. Net weight gain, weight increment per day and protein efficiency ratio were higher for diet containing 25% cocoa pod husk powder. Percentage weight gain and specific growth rate were higher for diet containing 15% and 25% cocoa pod husk powder. The results revealed that the inclusion of cocoa pod husk powder upto 25% could promote the growth of advanced fry of grass carp better than the control diet with 40% fish meal.

Keywords: Cocoa pod husk powder, growth performance, *Ctenopharyngodon idella*

Received 20 July 2011; Revised 07 June 2012; Accepted 09 September 2012

¹Present Address: Government Regional Fisheries Vocational Higher Secondary School, Thevara, Cochin - 682 013, India

* E-mail: aneykuttyj@yahoo.co.in

Introduction

Feed and labour are the major cost incurring components of aquaculture practices. Reducing feed

costs is considered to be crucial for ensuring long term sustainability of aquaculture industry. One way to reduce feed costs is by the use of low cost ingredients such as plant protein sources (Hertramp & Piedad-Pascual, 2000; Booth et al., 2001; Khan et al., 2003; Cremer et al., 2003; Muzinic et al., 2004). A variety of ingredients ranging from oil seed meals, pulses, leaf meals and agricultural by-products have been shown to be capable of supporting fish growth (El-Saidy & Gaber, 2003; De Silva, 2006). This is especially true in the case of purely herbivorous fish like grass carp (*Ctenopharyngodon idella*) which makes use of carbohydrate for its growth and as main source of energy. Studies conducted by Law (1986) have revealed that in the diet of grass carp, fish meal could be replaced with Napier and carpet grass meals with best results.

Cocoa (*Theobroma cacao*) is an economic cash crop in tropical African, Asian and Latin American countries. Cocoa pods constitute about 75% of the weight of whole cocoa fruit and are waste products after cocoa processing. The cocoa pod husk, with crude protein in the range of 5.69 – 9.69% (Nambuthiri & Shivshankar, 1985), has been evaluated as a component in poultry feed, pig rations (Branckaert et al., 1973) and for fish (Lim & Dominy, 1992; Falaye et al., 1999). It was found to be desirable, being relatively inexpensive and abundantly available throughout the year. Therefore, in the present study, an attempt has been made to find out the feasibility of using cocoa pod husk powder for replacing fish meal in the diet of advanced fry of grass carp (*C. idella*), which is an important species used in aquaculture, particularly in the tropics. The species was chosen as it is herbivorous and has better ability to digest and utilize plant components in the diet for growth and energy requirements.

Materials and Methods

The ingredients used for the preparation of the test diets were cocoa pod husk powder, fish meal, ground nut oil cake, wheat bran, tapioca flour and vitamin and mineral mixture. In order to determine the apparent digestibility coefficient of protein in the diet, chromic oxide was included at 1% level in all the experimental diets. Fresh cocoa pods were peeled and dried in a hot air oven at 100°C for 10 h. All the ingredients, except vitamin were powdered and made into a dough. It was steam cooked for 30 min and was cooled to room temperature. Vitamin powder was then added and again mixed thoroughly. Seven isonitrogenous diets ($29.83 \pm 0.36\%$ crude protein content) were prepared with a control diet containing 40% fish meal without cocoa pod husk powder. In all the other experimental diets, fish meal was progressively replaced by cocoa pod husk powder. Pelletising was done in a hand operated extruder to obtain pellets of about 3 mm diameter. Pelletised feeds were dried in a hot air oven at 60°C to a moisture content of less than 10%. The dried pellets were crumbled, cooled to room temperature and stored in airtight containers for further use.

All the seven diets were analyzed for crude protein, fat, fibre, moisture, ash and total carbohydrate using standard methods (AOAC, 1990). Crude carbohydrate was estimated by the method of Maynard & Loosli (1969) by subtracting the sum of protein, lipid, moisture, ash and fiber from 100.

The experiment was conducted in an indoor rearing system comprising of 21 tanks of 100 l capacity. The tanks were filled with 80 l of fresh clean water and mild aeration was provided. Healthy advanced fry of grass carp of uniform size (3 ± 0.24 cm) were obtained from a local fish farm. They were stocked at a rate of 4 numbers per tank area (0.2 m²) after acclimatization. Each diet was fed to fish in three tanks. Thus for 7 diets, 21 tanks were maintained. The fish were fed respective diets once daily. For digestibility assessment, faecal matter was collected using a glass canula from the second day onwards and dried to constant weight at 60 °C in an oven and stored in air tight containers for subsequent analysis (Ramachandran & Ray, 2007). The tanks were cleaned and 75% of water was exchanged daily. Water quality parameters such as temperature, pH, dissolved oxygen and ammonia were monitored as per AOAC (1994) throughout the study. The

experiment was carried out for a period of 50 days. The length and weight of the fish were recorded at fortnightly intervals. Mortality was recorded daily.

Parameters like net weight gain (NWG), weight increment per day (g day^{-1}), percentage weight gain (PWG), specific growth rate (SGR) and feed conversion ratio (FCR) and protein efficiency ratio (PER) were computed using appropriate formulae as given below,

NWG = Mean final body weight - mean initial body weight

WI (Weight increment) day^{-1} = Net weight gain / Number of days of experiment

PWG = Mean weight gain $\times 100$ / Mean initial weight

SGR = $(\text{Log}_e W_2 - \text{Log}_e W_1) \times 100 / (T_2 - T_1)$

Where,

W_2 = Weight of fish at time T_2 in days

W_1 = Weight of fish at time T_1 in days

Log_e = Natural log to base e

FCR = Weight of dry feed fed (g) / Live weight gain of fish (g)

PER = Gain in weight of fish (g) / Protein consumed (g)

Apparent digestibility coefficient (ADC) of protein was calculated according to Zhao et al. (2006) by using the formula,

$$\text{ADC} = [100 - (100 \times \% \text{Cr}_2\text{O}_3 \text{ in diet} \times \% \text{protein in diet})] / (\% \text{Cr}_2\text{O}_3 \text{ in faeces} \times \% \text{protein in faeces})$$

The experiment was carried out by using completely randomized design and data obtained was analysed using analysis of variance technique at 1% level of significance. Comparison of means was done using Tukey's Honesty significant difference test (Snedecor & Cochran, 1991).

Results and Discussion

Proximate analysis of feed ingredients is given in Table 1. The proportion of the ingredient of the experimental diets are given in Table 2. Proximate composition of the six formulated diets and the control diet is given in Table 3.

During the course of the experiment, there was no mortality of experimental fishes. The mean net weight gain, percentage weight gain, specific growth

Table 1. Proximate composition of feed ingredients used in the formulation of feed

Ingredients	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Fibre (%)	Carbohydrate (%)
Cocoa pod husk powder	8.7	0.41	7.48	11.1	31.48	40.83
Fish meal	9.4	5.78	58.41	19.2	3.1	4.11
Ground nut oil cake	6.1	6.78	41.18	2.35	14.2	29.39
Wheat bran	9.8	2.85	14.32	3.52	13	56.51
Tapioca flour	10.89	2.09	2.85	1.25	2.6	80.32

Table 2. Ingredient proportion of the test diet

Treatment/ingredient	Control	T1	T2	T3	T4	T5	T6
Cocoa pod husk powder	—	5	10	15	20	25	30
Fish meal	40	35	30	25	20	15	10
Wheat bran	20	20	15	15	12	5	2
Ground nut oil cake	15	20	25	30	38	45	51
Tapioca flour	20	15	15	10	5	5	2
Vitamin premix ¹	2	2	2	2	2	2	2
Mineral premix ²	2	2	2	2	2	2	2
Cr ₂ O ₃	1	1	1	1	1	1	1
Total	100	100	100	100	100	100	100

1. One kg of vitamin premix: Vitamin A- 400 000 I.U.; D3- 200 000 I.U.; E- 5000 I.U.; K3- 1g; B1- 1 g; B2- 1.5 g; B6- 1 g; Pantothenic acid- 5 g; Niacin- 3 g; Folic acid- 0.5 g; B12- 2 mg; Biotin- 100 mg; Vitamin C- 20 g.

2. One kg of mineral premix: CaHPO₄- 530 g; K₂HPO₄- 80 g; Na₂HPO₄- 90 g; MgCl₂.6H₂O- 100 g; KCl- 67.5 g; K₂SO₄- 80 g; NaCl- 30 g; KI- 0.05 g; ZnSO₄.7H₂O- 2.5 g; SeO₂- 0.03 g; CuSO₄.5H₂O- 0.15 g; FeSO₄.7H₂O- 18 g; (NH₄)₆Mo₇O₂₄.4H₂O- 0.01 g; MnSO₄.H₂O- 0.5 g; NaF- 1.2 g; CoCl₂.6H₂O- 0.01 g.

Table 3. Proximate composition of the formulated diets

Diets	Moisture (%)	Fat (%)	Protein (%)	Ash (%)	Fibre (%)	Carbohydrate (%)
Control	4	3.8	30.67	15	6.58	39.95
T1 (5% Cocoa pod husk powder)	3.5	3.6	30.38	13	8.01	41.51
T2 (10% Cocoa pod husk powder)	3	3.5	30.12	14	10.1	39.28
T3 (15% Cocoa pod husk powder)	4.5	4.12	30.09	13.5	11.81	35.98
T4 (20% Cocoa pod husk powder)	3	3.45	30.02	12	13.76	37.77
T5 (25% Cocoa pod husk powder)	3.5	3.61	30.06	13.25	16.1	33.48
T6 (30% Cocoa pod husk powder)	3	3.42	30.01	16	17.2	30.37

Table 4. Growth performance of grass carp fry feed with the experimental diet

Treat Ments	Initial weight (g)	Final weight (g)	NWG (g)	In/day (g)	PWG	SGR	FCR	PER	ADC
T1	1.03 ±0.04	4.68± 0.80	3.65±0.13 ^a	0.07±0.00 ^a	354.06±27.44 ^a	3.00±0.07 ^a	7.78±1.02 ^a	0.12±0.00 ^a	0.90
T2	1.07 ± 0.2	4.69± 0.12	3.62±0.11 ^a	0.07±0.00 ^a	351.45±96.12 ^a	2.71±0.16 ^a	8.33±1.63 ^a	0.12±0.00 ^a	0.91
T3	0.80 ±0.03	4.69± 0.06	3.86±0.04 ^b	0.08±0.00 ^a	469.79±18.51 ^b	2.70±0.11 ^a	7.33±0.70 ^a	0.13±0.00 ^a	0.91
T4	1.20 ±0.09	5.20±7 0.10	4.06±0.06 ^b	0.08±0.00 ^a	336.51±32.27 ^a	2.96±0.17 ^a	8.21±0.72 ^a	0.14±0.00 ^a	0.91
T5	1.02 ±0.04	4.73± 0.03	3.71±0.07 ^a	0.07±0.00 ^a	366.06±24.61 ^a	3.03±0.07 ^a	8.08±0.16 ^a	0.12±0.00 ^a	0.94
T6	1.01 ±0.14	3.40± 0.05	2.39±0.11 ^c	0.05±0.00 ^b	242.09±46.70 ^c	2.21±0.10 ^b	12.56±2.19 ^b	0.08±0.00 ^b	0.93
Control	0.92 ±0.05	4.34± 0.06	3.42±0.02 ^d	0.07±0.00 ^a	370.88±21.20 ^a	2.77±0.15 ^a	8.29±0.83 ^a	0.11±0.00 ^a	0.91

Values which are differently superscribed in each column are statistically different

rate, food conversion ratio and protein efficiency ratio showed significant difference between the treatments ($p<0.05$) as given in Table 4. Net wet gain in the treatment T_4 gave significantly high value ($p<0.05$) compared to all the other treatments. Treatment T_6 showed significantly low weight gain ($p<0.05$). Percentage weight gain was highest ($p<0.05$) for treatment T_3 . Here also T_6 gave a significantly low value ($p<0.05$). Specific growth rate showed no significant difference between the treatments and the control except for T_6 in which the value was significantly low ($p<0.05$). There was no significant difference in feed conversion ratio between the treatments and control, except for T_6 in which the value was significantly high. Protein efficiency ratio was significantly different ($p<0.05$) in all treatments except T_6 . Apparent digestibility coefficient was 0.9 for all the treatments. The result revealed that cocoa pod husk powder could be incorporated up to 25% replacing fish meal in the diet of grass carp without affecting its growth, compared to conventional diet with 30% fish meal. But inclusion at the rate of 20% gave better growth compared to control.

In the present study, fish meal and ground nut oil cake served as the protein sources and wheat bran, tapioca flour and cocoa pod husk as energy supplements. Although protein is used as an energy source for fish, a number of studies have pointed out the importance of using less expensive energy, in the form of lipid and carbohydrate, in order to save protein (Nandeesh et al., 1991; De Silva & Anderson, 1995; Booth et al., 2001; Khan et al., 2003;

Cremer et al., 2003; Muzinic et al., 2004). Carbohydrates not only supply the necessary energy but also have protein sparing effect in fish diet (Lovell, 1989). Work on the use of cocoa pod meal in fish feeds has been limited to a few species viz., *Clarias isheriensis* and *Oreochromis niloticus* where 10% inclusion level gave best growth performance (Fargbenro, 1992; Falaye et al., 1999). The tolerance of fish to higher level of cocoa pod husk powder may be attributed to the macrophytic feeding habits of the grass carp (Jhingran, 1992).

The growth depression observed at 30% inclusion level may be because of the toxicity of theobromine in cocoa which has been proved to be fatal for other domesticated animals and birds (Tarka, 1982; Haines & Echeverria, 1955). Theobromine is an antinutritional factor which would reduce the activity of digestive enzymes, thereby reducing the digestibility of feed stuffs (Krogdahl, 1989). Studies conducted by Adamafio et al. (2011) indicated that fermentation of cocoa pod husk powder could reduce the theobromine content in it to a level safe for animal consumption. Therefore, further studies with fermented cocoa pod husk powder in fish diets may be undertaken for higher inclusion levels of this cheap ingredient.

In conclusion, results obtained from this investigation showed that, up to 25% cocoa pod husk powder can be incorporated in to the diets of advanced fry of grass carp, *C. idella* without any compromise in the growth performance and deleterious effects on health of the fish.

Acknowledgements

The authors wish to express their gratitude to the Head and staff of Department of Marine Biology, School of Marine Sciences, Cochin University of Science and Technology, Cochin for the facilities provided and also for the help rendered for the above study. The first author acknowledges the receipt of University merit scholarship of CUSAT during the study period.

References

- AOAC (1990) Methods of Analysis. 15thedn., 72 p, Association of Official Analytical Chemists, Arlington
- AOAC (1994) Official Analytical Methods of the AOAC. 685 p, AOAC, Washington, D.C. USA
- Adamafio, N. A., Ayombil, F. and Tano-Debrah, K. (2011) Microbial detheobromination of cocoa (*Theobroma cacao*) pod husk. Asian J. Biochem. 6: 200-207
- Booth, M. A., Allan, G. L., Frances, J. and Parkinson, S. (2001) Replacement of fish meal in diets for Australian silver perch, *Bidyanus bidyanus* iv, Effects of dehulling and protein concentration on digestibility of green legumes. Aquaculture, 196: 67-85
- Branckaert, R., Vallerand, F. and Vincent, J. D. (1973) Cocoa pod meal for pig feeding. The café Cocoa.17: 313-320
- Cremer, M., Jian Z. Y. and Ehnua, Z. (2003) Performance of grass carp and tilapia with soy based aquafeeds in China. International Aquafeed, 6: 24-30
- De Silva, S. S. (2006) Reducing feed costs in aquaculture: Is the use of mixed feeding schedules the answer for Semi-intensive practices. Aqua. Asia Mag. 10: 7-12
- De Silva S. S. and Anderson, T. A. (1995) Fish Nutrition in Aquaculture. 319 p, Chapman & Hall Aquaculture Series, London
- EL-Saidy D. M. S. and Gaber M. M. A. (2003) Replacement of fish meal with a mixture of different plant protein sources in juvenile Nile tilapia (*Oreochromis niloticus* (L.) diets. Aquacult. Res. 34: 1119-1127
- Fargbenro O. A. (1992) Utilisation of cocoa pod husk in low cost diets by the Clariid cat fish, *Clarias isheriensis sydenham*. Aqua. Fish Mgt. 23: 175-182
- Falaye, A. E., Jauncey, K. and Tewe, O. O. (1999) The growth performance of tilapia (*Oreochromis niloticus*) fingerlings fed varying levels of cocoa husk diets. J. Aqua trop. 14: 1-10
- Haines, C. E. and Echeverria, A. J. (1955) Cocoa pods as substitutes in tropical dairy rations. Foreign Agriculture 19: 99-101
- Hertramp, J. W. and Piedad - Pascual, F. (2000) Handbook on Ingredients for Aquaculture Feeds, 573 p, Kluwer Academic Publishers, The Netherlands
- Jhingran, V. G. (1992) Fish and Fisheries of India 3rd edn., 750 p, Hindustan Publication Corporation of India Ltd
- Khan, A. M., Jafri, A. K., Chadha, N. K. and Usmani, N. (2003) Growth and body composition of rohu (*Labeo rohita*) fed diets containing oilseed meals: partial or total replacement of fish meal with soybean meal. Aquaculture Nutri. 9: 391-396
- Krogdahl, A. (1989) Alternative protein sources from plants contain antinutrients affecting digestion in salmonids. pp253-261, Proc. 3rd Int. Symposium on Feeding and Nutrition in Fish, Toba (Japan), 28 August to 1st September, 1989
- Law, A. T. (1986) Digestibility of low-cost ingredients in pelleted feed by grass carp (*Ctenopharyngodon idella* C.et V.). Aquaculture 51: 97-103
- Lim, C. and Dominy, W. (1992) Substitution of full-fat soybean meal for commercial soy bean meal in the diets of shrimp, *Penaeus vannamei*. J. Appl. Aquacult. 1: 35-45
- Lovell, R.T. (1989) Nutrition and Feeding of Fish, 260 p, A. VI. Van Nostrand Rein rold Co, New York.
- Maynard, L. A. and Loosli, J. K. (1969) Animal Nutrition. 6th edn., McGraw-Hill, New York, USA
- Muzinic, L.A., Thompson, K.R., Morris, A., Webster, C.D., Rouse, D.B. and Manomaitis, L. (2004) Partial and total replacement of fish meal with soybean meal and brewer's grains with yeast in practical diets for Australian red claw crayfish *Cherax quadricarinatus*. Aquaculture, 230: 359-376
- Nambuthiri, E. S. and Shivshankar, S. (1985) Cocoa waste and utilization. Ind. Cocoa, Arecanut & Spices. J. 8: 78-80
- Nandeesh, M. C, Srikanth, G. K., Keshavnath P. and Das, S. K. (1991) Protein and fat digestibility of five feed ingredient by an Indian major carp, *Catla catla* (Hamilton). In: Fish Nutrition Research in Asia. Proceedings of the Fourth Asian Fish Nutrition Workshop (De Silva S.S. Ed), pp 75- 81, Asian Fisheries Society, Manila, Philippines.
- Ramachandran, S. and Ray, A.K. (2007) Nutritional evaluation of fermented black gram (*Phaseolus mungo*) seed meal in compound diets for rohu, *Labeo rohita* (Hamilton), fingerlings. J. Appl. Ichthyol. 23: 74-79
- Snedecor, G. W. and Cochran, W. G. (1991) Statistical Methods. 8th edn., Iowa State University Press, Ames.Iowa, USA
- Tarka, S. M. (1982) The toxicology of cocoa and methylxanthines: A review of the literature. Crit. Rev. Toxicol. pp 275-312,
- Zhao, M., Xie, S., Zhu, X., Yang, Y., Gan, L. and Song, L. (2006) Effect of inclusion of blue-green algae meal on growth and accumulation of microcystins in gibel carp (*Carassius auratus gibelio*). J. Appl. Ichthyol. 22: 72-78