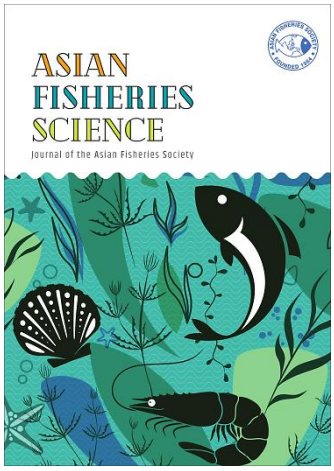

Nutrient Digestibility and Digestive Enzyme Activity in Fringe Lipped Carp, *Labeo fimbriatus* (Bloch, 1795), Fed Diets Containing Cottonseed Meal

GANGADHAR BARLAYA*, ANANADA KUMAR B.S., GANESH HEGDE, HEMAPRASANTH KANNUR
Central Institute of Freshwater Aquaculture, RRC, Hesarghatta Lake PO, Bangalore 560089, Karnataka, India

*E-mail: gbarlaya@yahoo.co.in | Received: 03/05/2021; Accepted: 17/09/2021

E-ISSN: 2073-3720
https://doi.org/10.33997/j.afs.2021.34.3.007

Abstract

Advanced fingerlings of *Labeo fimbriatus* (Bloch, 1795) were reared in aerated indoor plastic tanks to elucidate the dry matter and nutrient digestibility of cottonseed meal (CSM) in the feed. The fish were fed isocaloric formulated diets containing CSM replacing the main ingredients – groundnut cake and rice bran of the control diet at 10, 20, 30 and 40 % levels. Total dry matter digestibility and major nutrient digestibility were estimated using acid insoluble ash as the marker. In general, the digestibility of dry matter, protein, fat, and nitrogen-free extract was higher in CSM incorporated diets than the control diet. While the dry matter and nitrogen-free extract digestibility values were highest at 40 % CSM incorporation level, those for protein and fat digestibility were highest in 30 % and 10 % levels, respectively, showing an apparent reduction thereafter. The incorporation of CSM had a stimulatory effect on most of the intestinal digestive enzymes tested. Fish fed 20 % CSM diet recorded the highest activity of total protease and carboxypeptidases while those fed 30 % CSM diet had highest trypsin and amylase activities; all showing reducing trend consequently. In the case of hepatopancreas, the reducing trend in the activities of protease, chymotrypsin, carboxypeptidase A, and lipase observed with increased incorporation levels of CSM was significant ($P < 0.05$) and the reduction in the activities of trypsin, amylase and cellulase in CSM fed fish was not significant ($P > 0.05$). The study indicates the possibility of incorporation of CSM in the diet of *L. fimbriatus* up to 40 % level without affecting nutrient digestibility.

Keywords: feed ingredient, minor carp, alternate protein source, medium carp, fish feed

Introduction

Cottonseed meal (CSM) is a by-product of oil extraction and is a relatively cheap source of protein. It has been used in animal feeds since the early part of the 20th century (Cheng and Hardy, 2002). Cottonseed meal has been reported to be very palatable to fish (Gohl, 1981). However, the presence of high fibre content, anti-nutritional factors like gossypol and protease inhibitors and low availability of essential amino acids lysine, methionine and cysteine limits its use in formulated fish diets (NRC, 2011; Heuzé et al., 2013). India is among the top five cottonseed meal producing countries; USA, Brazil, China and Pakistan are the others (FAO, 2012). Because of the process by which oil is extracted, CSM yields a predominately bound form of gossypol compared to the whole cottonseed. After removing the hull, the cottonseed is moistened, flaked, cooked, passed through an expander, extracted and then de-solventised and toasted before being ground into a meal (Jones and King, 1996). This processing method binds much of the gossypol leaving only 0.1–0.2 % as free gossypol, which is the anti-nutritional factor in the ingredient. Further, the negative effects of CSM due to gossypol can be overcome by supplementation of diets with limiting amino acids (Li and Robinson, 1998; Fagbenro, 1999).

Cottonseed meal has been evaluated as a fishmeal alternative for several fish species (Cheng and Hardy, 2002; Rincharda et al., 2003; Cai et al., 2011) and the recommended dietary level is 15 %–50 % based on feeding habit and life stage. Channel catfish (*Ictalurus punctatus* (Rafinesque, 1818)), Nile tilapia (*Oreochromis*

niloticus (Linnaeus, 1758)) and Siberian sturgeon (*Acipenser baerii* Brand, 1869) can adapt to 40–50 % dietary CSM content if they are supplemented with amino acids (El-Saidy and Gaber, 2004; Li and Robinson, 2006; Wu et al., 2010). Whereas rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)), coho salmon (*Oncorhynchus kisutch* (Walbaum, 1792)), and parrotfish (*Oplegnathus fasciatus* (Temminck and Schlegel, 1844)) can tolerate only 12–22 % dietary CSM (Fowler, 1980; Lee et al., 2002; Lim and Lee, 2009). With lysine supplementation, up to about 50 % of fishmeal (FM) or soybean meal (SBM) can be replaced by CSM without negative impacts on the growth performance of tilapia *Oreochromis* sp. (Mbahinzireki et al., 2001) and channel catfish (Robinson and Li, 2008).

The fringed lipped carp (*Labeo fimbriatus* (Bloch, 1795)) is a minor carp and a potential candidate for species diversification due to its moderate growth and high consumer preference (Basavaraju et al., 1995). The conventional feed of carps is a 1:1 mixture of groundnut cake and rice bran. The cost of a kilogram of groundnut cake varies from INR30–50 (USD0.40–0.67) depending on quality, while rice bran ranges from INR20–25 (USD0.27–0.33). As CSM is available at around INR20–30 (USD0.27–0.40) per kilogram, its possible utilisation as an ingredient for fish feed production is likely to reduce the feed cost considerably. The present study was conducted to evaluate the effect of incorporating CSM in the conventional diet of carps at varying levels on the digestibility of dry matter and major nutrients of different diets. The activity of major digestive enzymes was also estimated in the gut of fish fed varying levels of CSM, to validate the results.

Materials and Methods

Experimental diets

The basal diet (control) was formulated with groundnut oilcake and rice bran (Table 1). Finger millet (*Eleusine coracana* (L.) Gaertn.) was used at 9 % as the binder for preparing the pelleted feed. Vitamin and mineral mixture pre-fortified with amino acids methionine and lysine compensate for the limiting amino acids in CSM was incorporated at 2 %. Four experimental diets were formulated replacing groundnut oilcake and rice bran in the basal diet with CSM at 10 (CSM 10 %), 20 (CSM 20 %), 30 (CSM 30 %) and 40 % (CSM 40 %) levels as per Umesh et al. (1994). The feed was prepared as follows. Groundnut oilcake, CSM and finger millet were dried and powdered. All the ingredients were sieved through a fine meshed screen (0.5 mm). The required quantity of the ingredients was mixed with hot water to make dough, which was pressed through a hand pelletiser (Kalsi hand-operated machine, Model No. 10, India) to get uniform sized pellets (2 mm). The pellets were sun-dried and packed in air-tight bags till use.

Experimental fish

All applicable institutional guidelines for the care and use of animals were followed by the authors. Digestibility of the test diets was evaluated *in vivo* through a feeding experiment conducted in 50 L indoor, aerated plastic tanks. A total of 150 advanced fingerlings of *L. fimbriatus* with body weight ranging from 7.39–10.88 g were stocked at 10 fish per tank in such a way that the total biomass per tank is almost uniform. The fish were initially acclimated for 10 days with the control diet. Triplicate tanks were maintained for each treatment, and control and test diets were provided once daily at 5 % of body weight, every morning at 10:00. Fish were allowed to feed for 6 h, and at the end of the feeding period, the unconsumed pellets were siphoned out completely. On the following day, faecal matter collected at 09:00 a.m. by filtering through a 15 µm meshed nylon cloth was dried and stored for proximate analysis. About 50 % of water from each tank was replaced with freshwater every day following faecal matter collection. Feeding and faecal matter collections were done for a period of 45 days.

Table 1. Ingredient proportion (%) of experimental diets for evaluation of the effect of incorporating cottonseed meal (CSM) in the diet of *Labeo fimbriatus*.

Ingredients	Control	CSM 10 %	CSM 20 %	CSM 30 %	CSM 40 %
Groundnut cake	45	40	35	30	25
Rice bran	45	40	35	30	25
Finger millet	8	8	8	8	8
Cottonseed meal	0	10	20	30	40
Vitamin and mineral mixture*	2	2	2	2	2

*Each kg has calcium - 25.5 %, phosphorus - 12.75 %, magnesium - 6000 mg, sulphur - 0.72 %, sodium - 5.9 mg, potassium - 100 mg, copper - 1200 mg, cobalt - 150 mg, zinc - 9600 mg, iron - 1500 mg, iodine - 325 mg, selenium - 10 mg, manganese - 1500 mg, vitamin A - 700000 IU, vitamin D3 - 70000 IU, vitamin E - 250 mg, nicotinamide - 1000 mg, DL-methionine - 1929 mg, L-lysine - 4400 mg, *Lactobacillus* sp. 1.5×10^{11} CFU, *Saccharomyces cerevisiae* - 30000 million CFU.

Pelleted feed and faecal matter were subjected to proximate analysis (AOAC, 1995). The gross energy content of the diets was calculated following Mayes (1990). Acid insoluble ash was used as the reference marker (Goddard and McLean, 2001; Li et al., 2008; Bob-Manuel, 2013). Dry matter and nutrient digestibility were calculated according to Maynard and Loosli (1972). While the water quality parameters - dissolved oxygen and total alkalinity analysed titrimetrically following APHA (2005) standard procedures, pH and temperature were recorded using probes (Orion 2 Star pH meter, Thermo Electron Corporation, USA), on fortnightly intervals.

Analysis of digestive enzyme activity

At the end of the experiment, the intestine and hepatopancreas of six fish from each treatment group were dissected and individually macerated with ice cold distilled water (intestine/ hepatopancreas weight: volume of distilled water = 1:4), and centrifuged in a refrigerated centrifuge at 16,000 rpm for 20 min. The extract was decanted, and the pellets were re-suspended in equal volume of cold distilled water and centrifuged as before. The washing procedure was repeated, and all the extracts collected as supernatant were pooled. The crude enzyme extract thus obtained was divided into 1 mL aliquots and stored at -20 °C. All extraction procedures were carried out at 4 °C. Protein in the crude enzyme extracts was estimated according to Lowry et al. (1951) using bovine serum albumin as standard. The activity of proteases viz., total protease (Kunitz, 1947), trypsin and chymotrypsin (Erlanger et al., 1961) and carboxypeptidases (Appel, 1974) was estimated. Amylase and cellulase were assayed by hydrolysing starch and carboxymethyl cellulose, respectively, followed by the estimation of reducing sugars (Nelson, 1944). Para-nitro phenyl acetate was used as the substrate for the assessment of lipase activity (Licia et al., 2006).

Statistical analysis

Data on digestibility and digestive enzyme activity were compared employing one-way ANOVA. Further, pair-wise comparison of treatment means was done by Duncan's multiple range test ($P < 0.05$) (Duncan, 1955).

Results

The average water temperature ranged from 24.70 to 25.60 °C. Water showed alkaline conditions with average pH ranging from 7.34 to 8.10 and total alkalinity from 323.01 to 338.78 mg.L^{-1}. Dissolved oxygen levels ranged between 5.67 and 7.86 mg.L^{-1} (Table 2).

The proximate composition parameters of the experimental diets are given in Table 3. Higher levels of CSM incorporation resulted in significantly ($P < 0.05$) lower moisture, fat, and nitrogen-free extract (NFE) and higher crude protein levels in the diets. Ash and gross energy levels remained similar ($P > 0.05$).

In general, the digestibility of dry matter (DM), protein, fat and NFE was higher in CSM incorporated diets compared to the control diet (Table 4).

Table 2. Water quality parameters recorded in the experimental tanks during the study period.

Water quality	Control	CSM 10 %	CSM 20 %	CSM 30 %	CSM 40 %
Temperature (°C)	25.10 ± 0.32	24.90 ± 0.45	25.60 ± 0.23	24.70 ± 0.01	25.30 ± 0.14
pH	7.59 ± 0.31	8.10 ± 0.02	7.34 ± 0.03	8.00 ± 0.02	7.42 ± 0.16
Dissolved oxygen (mg.L^{-1})	5.86 ± 1.11	6.87 ± 1.09	7.16 ± 1.12	7.86 ± 0.78	5.67 ± 0.49
Total alkalinity (mg.$CaCO_3$ L^{-1})	323.01 ± 8.31	327.87 ± 7.61	338.78 ± 6.81	330.16 ± 5.26	331.49 ± 3.18

Values expressed as mean ± SD. n = 4.

Table 3. Proximate composition (%) of experimental diets.

Parameter	Control	CSM 10 %	CSM 20 %	CSM 30 %	CSM 40 %
Moisture	8.34 ± 0.11^{a}	8.15 ± 0.01^{ab}	7.68 ± 0.36^{ab}	7.65 ± 0.03^{ab}	7.48 ± 0.44^{b}
Crude protein	25.41±1.28^{a}	26.74 ± 0.85^{a}	27.79 ± 0.46^{ab}	29.57 ± 0.15^{b}	31.20 ± 0.02^{b}
Fat	7.85 ± 0.17^{a}	8.07 ± 0.27^{a}	7.83 ± 0.46^{a}	6.08 ± 0.85^{b}	5.76 ± 0.06^{b}
Ash	8.19 ± 0.03^{a}	8.23 ± 0.02^{a}	8.24 ± 0.03^{a}	7.99 ± 0.03^{a}	7.55 ± 0.57^{a}
Crude fibre	12.71 ± 0.02^{a}	13.59 ± 0.20^{b}	13.80 ± 0.12^{b}	13.35 ± 0.34^{b}	13.43 ± 0.29^{b}
Nitrogen-free extract	37.51 ± 1.27^{a}	35.22 ± 1.10^{ab}	34.66 ± 0.20^{ab}	35.36 ± 0.69^{ab}	34.58 ± 0.50^{b}
Gross energy (kJ.g^{-1})	15.25 ± 0.08^{a}	15.24 ± 0.05^{a}	15.27 ± 0.15^{a}	15.16 ± 0.24^{a}	15.24 ± 0.06^{a}

Figures in the same row with same superscript do not differ significantly ($P > 0.05$). Values expressed as mean ± SD. n = 3.

Table 4. Apparent digestibility coefficient (%) of dry matter and major nutrients of the cottonseed meal (CSM) incorporated diets.

Diets	Dry matter	Protein	Fat	Nitrogen-free extract
Control	71.21 ± 1.60^{a}	83.01 ± 0.03^{a}	91.60 ± 0.26^{ab}	88.15 ± 0.80^{a}
CSM 10 %	74.12 ± 1.95^{ab}	85.37 ± 1.49^{ab}	93.15 ± 0.45^{c}	90.23 ± 0.64^{b}
CSM 20 %	75.17 ± 1.04^{bc}	86.03 ± 0.78^{b}	92.79 ± 0.79^{c}	90.53 ± 0.27^{bc}
CSM 30 %	76.22 ± 0.45^{bc}	86.69 ± 0.28^{b}	92.42 ± 1.40^{bc}	90.84 ± 0.15^{bc}
CSM 40 %	78.07 ± 2.54^{c}	86.15 ± 1.73^{b}	89.68 ± 0.80^{a}	91.55 ± 0.93^{c}

Figures in the same column with same superscript do not differ significantly ($P > 0.05$). Values expressed as mean ± SD. n = 3.

While the value for DM and NFE digestibility was the highest at 40 % CSM incorporation level, that for protein and fat digestibility were the highest in 30 % and 10 % levels, respectively, showing an apparent reduction thereafter. The incorporation of CSM had a stimulatory effect on most of the intestinal digestive enzymes tested (Fig. 1).

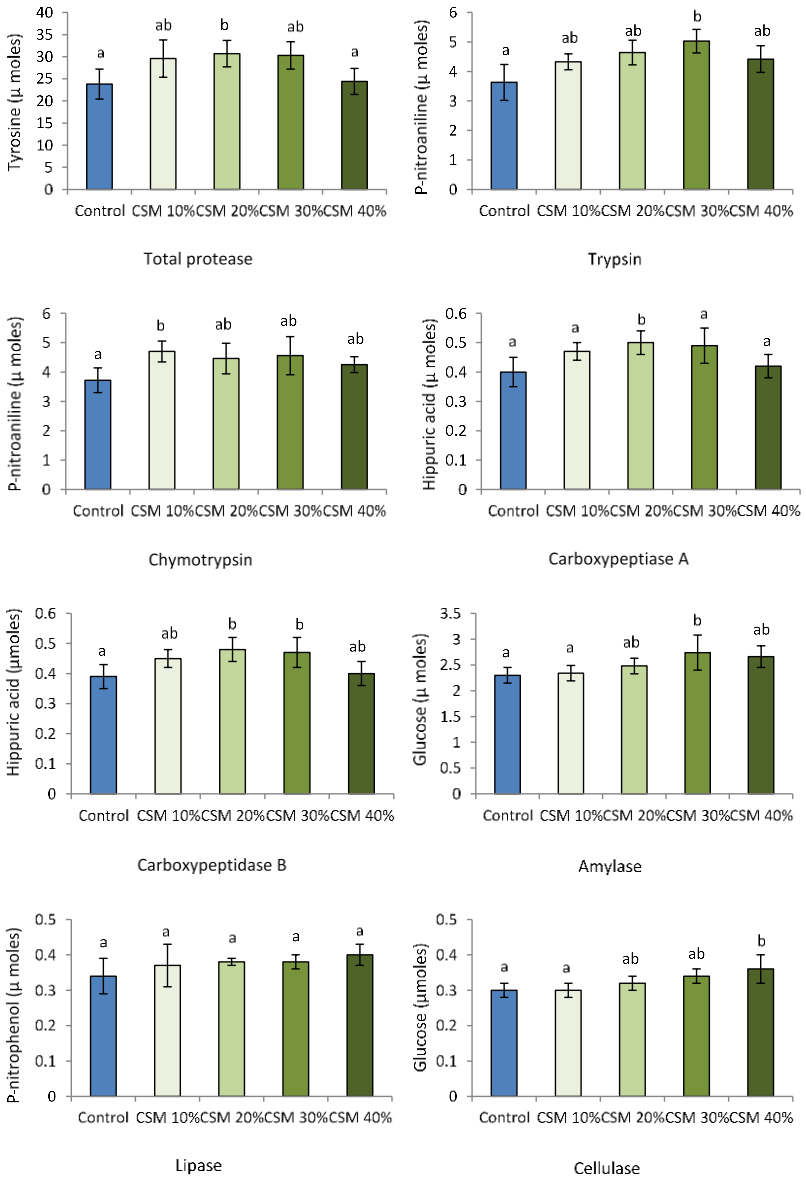

Fig. 1. Activity of digestive enzymes (µ moles of product liberated.h^{-1}. mg tissue protein^{-1} at 25 °C) in the intestine of *Labeo fimbriatus* fed experimental diets. Different alphabets on bars in the same graph indicate statistical difference among treatments ($P < 0.05$). Values expressed as mean ± SD. n = 3.

Fish fed 20 % CSM diet recorded the highest activity of total protease and carboxypeptidases while those fed 30 % CSM diet had the highest trypsin and amylase activities; all showing reducing trend consequently. The activity of chymotrypsin, lipase and cellulase did not differ significantly ($P > 0.05$) among treatments. In the case of hepatopancreas, the reducing trend in the activities of total protease, chymotrypsin, carboxypeptidase A, and lipase observed with increased incorporation levels of CSM was significant ($P < 0.05$) and the reduction in the activities of trypsin, amylase and cellulase in CSM fed fish was not significant ($P > 0.05$)(Fig. 2).

Discussion

The major water quality parameters analysed during the study period were in the acceptable range (Jena et al., 2011) and did not show significant ($P > 0.05$) difference between the treatments indicating that the difference in digestibility between groups is attributable to experimental diets.

The nutritive value of food depends on the capacity of the animal to digest and absorb the nutrients and the efficiency of digestion of the ingested food largely determines the growth performance of the animal (Deganp and Yehuda, 1999). Deganp and Yehuda (1999) reported a digestibility value of 79 % for CSM in tilapia. In the present study, the apparent digestibility of DM in CSM incorporated diets ranged from 74.12 to 78.07 %, while protein from 85.37 to 86.69 %, fat from 89.68 to 93.15 % and NFE from 90.23 to 91.55 %. Cheng and Hardy (2002) also recorded the apparent digestibility

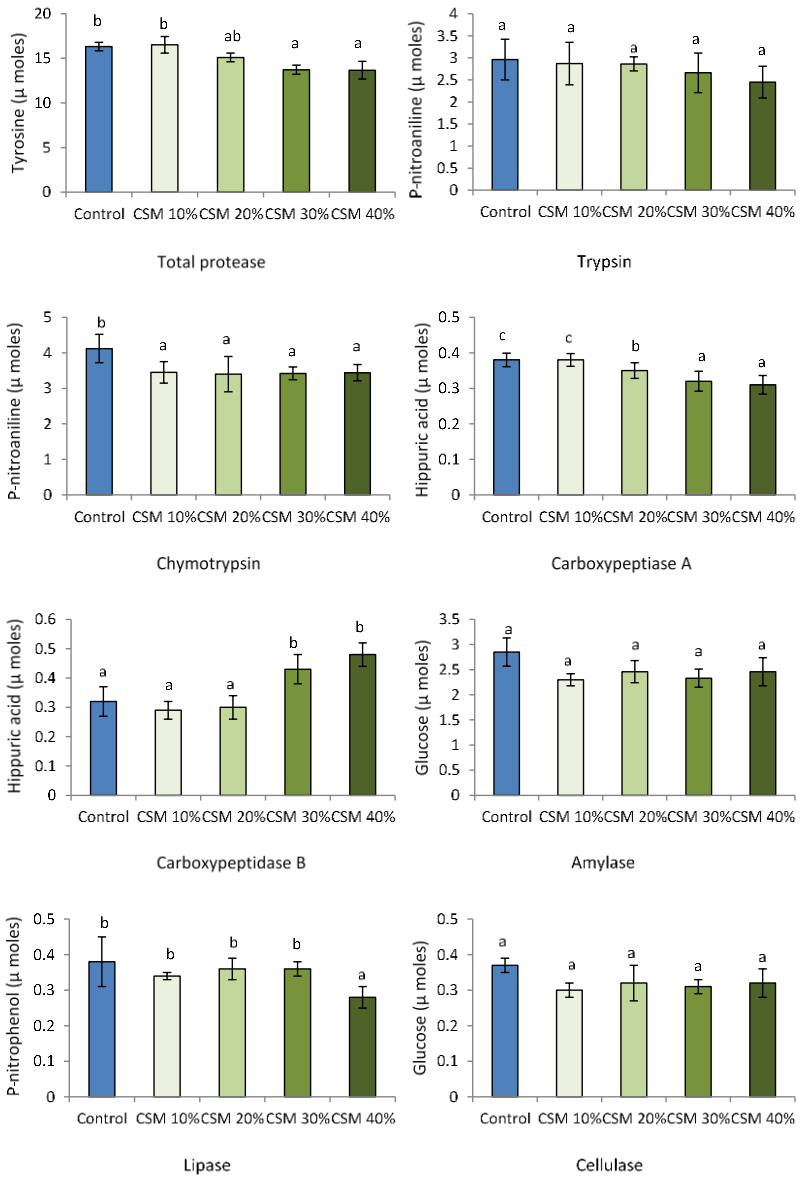

Fig. 2. Activity of digestive enzymes (µ moles of product liberated.h^{-1}. mg tissue protein^{-1} at 25 °C) in the hepatopancreas of *Labeo fimbriatus* fed experimental diets. Different alphabets on bars in the same graph indicate statistical difference among treatments ($P < 0.05$). Values expressed as mean ± SD. n = 4.

of DM ranging from 50.8–75.5 %, crude protein ranging from 81.6–87.9 % and fat ranging from 60.2–78.6 % in the four CSM groups tested in rainbow trout, *O. mykiss*. Several studies have reported the apparent digestibility values for CSM in fish. The values were: 81.6–87.9% in rainbow trout (*O. mykiss*) (Cheng and Hardy, 2002), 76.35–84.5 % in red drum (*Sciaenops ocellatus* (Linnaeus, 1766)) (Gaylord and Gatlin, 1996; McGoogan and Reigh, 1996), 78.6–81.8 % in Nile tilapia (*O. niloticus*) (Guimarães et al., 2008), 78.0–81.0 % in Korean rockfish (*Sebastes schlegeli* (Hilgendorf, 1880)) (Lee, 2002), 83.7–87.6 % in Siberian sturgeon (*A. baerii*) (Liu et al., 2009), 83.8 % in hybrid striped bass (*Morone saxatilis*) (Walbaum, 1792) ♀ × *Morone schrysops* (Rafinesque, 1820) ♂ (Sullivan and Reigh, 1995), 75.4–80.0 % in gilthead sea bream (*Sparus auratus* (Linnaeus, 1758)) (Nengas et al., 1995) and 93.3–97.2 % in Florida pompano (*Trachinotus carolinus* (Linnaeus, 1766)) (Cook et al., 2016). The differences in apparent digestibility coefficients (ADC) between the species can be attributed to species-specific differences and ingredients in the feed other than CSM. While Cheng and Hardy (2002) used pure ingredients like casein, gelatine, dextrin, carboxymethyl cellulose (CMC) and cellulose for their diets in addition to vitamins and mineral supplements, Gaylord and Gatlin (1996) used a mixture of fishmeal (FM), dextrin, CMC, cellulose and fish oil. Others like McGoogan and Reigh (1996), Guimarães et al. (2008), Sullivan and Reigh (1995) used FM, soybean meal (SBM) and wheat middling with carbohydrate sources like corn/broken rice/dextrin.

Mbahinzireki et al. (2001) observed a decrease in the digestibility of crude protein with an increase in the total CSM level in the diet of tilapia. He opined that CSM at higher levels makes the diets unpalatable and suppress the appetite of the fish. But this is not always true since the results of present study and the study by Cook et al. (2016) with Florida pompano does not support this. While the values for DM and NFE digestibility were the highest at 40 % CSM incorporation level, protein and fat digestibility were the highest in 30 % and 10 % levels, respectively, showing an apparent reduction thereafter. El Saidy (1999) also reported that increasing levels of CSM beyond 30 % level results in decreased digestibility coefficient of protein and fat. Liu et al. (2016) reported that feed utilisation of silver carp (*Hypophthalmichthys molitrix* (Valenciennes, 1844)) fed diets containing CSM up to 37 % were not affected after 6-week feeding trial.

The incorporation of CSM had a stimulatory effect on most of the intestinal digestive enzymes tested in *L. fimbriatus*. Similar to our findings, Liu et al. (2016) reported an increasing trend in intestinal trypsin activity ($P > 0.05$) and no effect of dietary CSM level on chymotrypsin activity in grass carp (*Ctenopharyngodon idella* (Valenciennes, 1844)), fed diets containing CSM up to 61 %. The structure of the mid-intestinal tissues and the ultra-structure of the enterocyte microvilli were normal when dietary CSM level was <37 %. Based on the quadratic regression analysis of the thermal growth co-efficient, the maximum incorporation level for juvenile grass carp arrived at 35 % (Liu et al., 2018).

In the case of hepatopancreatic enzymes of *L. fimbriatus*, the reducing trend in the activities of total protease, chymotrypsin, carboxypeptidase A, and lipase observed with increased incorporation levels of CSM was significant ($P < 0.05$) and the reduction in the activities of trypsin, amylase and cellulase was not significant ($P > 0.05$). As per studies by Liu et al. (2016), damage to hepatopancreas function was apparent when the dietary CSM level was raised to 48 %. Though histopathological studies were not conducted, the reduced activity of hepatopancreatic digestive enzymes in *L. fimbriatus* may be attributed to similar structural changes caused to the hepatopancreas by CSM.

Conclusion

In the present study, the dry matter and nitrogen-free extract digestibility values were highest at 40 % CSM incorporation level and protein digestibility was highest in 30 % level of incorporation. The incorporation of CSM had a stimulatory effect on majority of the intestinal digestive enzymes tested. From the results of the present study, it is concluded that incorporation of CSM in the diet of *L. fimbriatus* up to 40 % level does not affect nutrient digestibility. This can result in considerable savings on feed cost. However, further studies to evaluate the growth of fish fed CSM incorporated diets need to be undertaken before recommending utilisation of this unconventional source of protein in fish feed.

Acknowledgements

The authors are grateful to the Director, ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar for providing infrastructure facility for conducting this experiment.

Conflict of interest: The authors declare that they have no conflict of interest.

References

AOAC. 1995. Official methods of analysis. 16th Edition. Association of Official Analytical Chemists, Washington DC, USA, pp. 32–42.

APHA. 2005. Standard methods for the examination of water and wastewater. American Public Health Association, Washington DC. 1268 pp.

Appel, W. 1974. Peptidases. In: Methods of enzymatic analysis, vol. 2, Bergmeyer, H.U. (Ed.), Academic Press, New York, pp. 949–954.

Basavaraju, Y., Devaraj, K.V., Ayyar, S.P. 1995. Comparative growth of reciprocal carp hybrids between *Catla catla* and *Labeo fimbriatus*. Aquaculture 129:187–191. https://doi.org/10.1016/0044-8486(94)00246-K

Bian, F., Zhou, H., He, G., Wang, C., Peng, H., Pu, X., Mai, K. 2017. Effects of replacing fishmeal with different cottonseed meals on growth,

feed utilization, haematological indexes, intestinal and liver morphology of juvenile turbot (*Scophthalmus maximus* L.). Aquaculture Nutrition 23:1429–1439. https://doi.org/10.1111/anu.12518

Bob-Manuel, F.G. 2013. Methods used in digestibility evaluation of fish diets: a review and challenges. Continental Journal of Fisheries and Aquatic Sciences 7:25–37.

Cai, C., Li, E., Ye, Y., Krogdahl, A., Jiang, G., Wang, Y., Chen, L. 2011. Effect of dietary graded levels of cottonseed meal and gossypol on growth performance, body composition and health aspects of allogynogenetic silver crucian carp, *Carassius auratus gibelio* ♀ × *Cyprinus carpio* ♂. Aquaculture Nutrition 17:353–360. https://doi.org/10.1111/j.1365-2095.2010.00801.x

Cheng, Z.J., Hardy, R.W. 2002. Apparent digestibility coefficients and nutritional value of cottonseed meal for rainbow trout (*Oncorhynchus mykiss*). Aquaculture 212:361–372. https://doi.org/10.1016/S0044-8486(02)00260-0

Cook, R.L., Zhou, Y., Rhodes, M. A., Davis, D.A. 2016. Evaluation of various cottonseed products on the growth and digestibility performance in Florida pompano *Trachinotus carolinus*. Aquaculture 453:10–18. https://doi.org/10.1016/j.aquaculture.2015.11.029

Deganp, G., Yehuda, Y. 1999. Digestibility of protein sources in feed for *Oreochromis aureus* × *O. nilotica*. Indian Journal of Fisheries 46:33–39.

Duncan, D.B. 1955. Multiple range and multiple F-tests. Biometrics 11:1–42. https://doi.org/10.2307/3001478

El Saidy, D.M., Gaber, M.M. 2004. Use of cottonseed meal supplemented with iron for detoxification of gossypol as a total replacement of fish meal in Nile tilapia, *Oreochromis niloticus* (L.) diets. Aquaculture Research 35:859–865. https://doi.org/10.1111/j.1365-2109.2004.01077.x

El-Saidy, D.M. 1999. Evaluation of cottonseed meal as partial and complete replacement of fish meal in practical diets of Nile tilapia (*Oreochromis niloticus*) fingerlings. Egyptian Journal of Aquatic Biology and Fisheries 3:441–457. https://doi.org/10.21608/ejabf.1999.3459

Erlanger, B.F., Kokowsky, M., Cohen, W. 1961. The preparation and properties of two new chromogenic substrates for trypsin. Archives of Biochemistry and Biophysics 95:271–278. https://doi.org/10.1016/0003-9861(61)90145-X

Fagbenro, O.A. 1999. Equi-protein replacement of soybean meal with winged bean meals in diets for the African clariid catfish, *Clarias gariepinus*(Burchell). Journal of Aquaculture in the Tropics 14:93–99.

FAO. 2012. The state of food and agriculture. Food and Agriculture Organization of the United Nations, Rome, Italy. 182 pp.

Fowler, L.G. 1980. Substitution of soybean and cottonseed products for fish meal in diets fed to chinook and coho salmon. The Progressive Fish Culturist 42:92–97. https://doi.org/10.1577/1548-8659(1980)42[87:SOSACP]2.0.CO;2

Gaylord, T.G., Gatlin, D.M. 1996. Determination of digestibility coefficients of various feed stuffs for red drum (*Sciaenops ocellatus*). Aquaculture 139:303–314. https://doi.org/10.1016/0044-8486(95)01175-7

Goddard, J.S., McLean, E. 2001. Acid-insoluble ash as an inert reference material for digestibility studies in tilapia (*Oreochromis niloticus*). Aquaculture 194:93–98. https://doi.org/10.1016/S0044-8486(00)00499-3

Gohl, B. 1981. Tropical feeds. FAO Animal Production and Health Series No. 12. FAO, Rome. 529 pp.

Guimarães, I., Pezzato, L., Barros, M. 2008. Amino acid availability and protein digestibility of several protein sources for Nile tilapia, *Oreochromis niloticus*. Aquaculture Nutrition 14:396 –404. https://doi.org/10.1111/j.1365-2095.2007.00540.x

Heuzé, V., Tran, G., Bastianelli, D., Hassoun, P., Lebas, F. 2013. Cottonseed meal. Feedipedia, a programme by INRAE, CIRAD, AFZ and FAO. 14:21. https://www.feedipedia.org/node/550

Jena, J.K., Das, P.C., Mitra, G., Patro, B., Mohanta, D., Mishra, B. 2011. Evaluation of growth performance of *Labeo fimbriatus*(Bloch), *Labeo gonius* (Hamilton) and *Puntius gonionotus* (Bleeker) in polyculture with *Labeo rohita* (Hamilton) during fingerlings rearing at varied densities. Aquaculture 319:493–496. https://doi.org/10.1016/j.aquaculture.2011. 07.028

Jones, L.A., King, C.C. 1996. Cottonseed oil. In: Baileys industrial oil and fat products. Volume 2, Edible oil and fat products: Oils and oilseeds. Hui, Y.H. (Ed.), John Wiley, New York, pp. 159–240.

Kunitz, M. 1947. Crystalline soybean trypsin inhibitor. II. General properties. Journal of General Physiology 30:291–310. https://doi.org/10.1085/jgp.30.4.291

Lee, K.J., Dabrowski, K., Blom, J.H., Bai, S.C., Stromberg, P.C. 2002. A mixture of cottonseed meal, soybean meal and animal by-product mixture as a fishmeal substitute: growth and tissue gossypol enantiomer in juvenile rainbow trout (*Oncorhynchus mykiss*). Journal of Animal Physiology and Animal Nutrition 86:201–213. https://doi.org/10.1046/j.1439-0396.2002.00375.x

Lee, S.M. 2002. Apparent digestibility coefficients of various feed ingredients for juvenile and grower rockfish (*Sebastes schlegeli*). Aquaculture 207:79–95. https://doi.org/10.1016/S0044-8486(01)00751-7

Li, M.H., Robinson, E.H. 1998. Effects of supplemental lysine and methionine in low protein diets on weight gain and body composition of young channel catfish, *Ictalurus punctatus*. Aquaculture 163:297–307. https://doi.org/10.1016/S0044-8486(98)00239-7

Li, M.H., Robinson, E.H. 2006. Use of cottonseed meal in aquatic animal diets: a review. North American Journal of Aquaculture 68:14–22. https://doi.org/10.1577/A05-028.1

Li, P., Webb, K.A., Gatlin, D.M. 2008. Evaluation of acid-insoluble ash as an indicator for digestibility determination with red drum, *Sciaenops ocellatus* and hybrid striped bass, *Morone chrysops* × *M. saxatilis*. Journal of the World Aquaculture Society 39:120–125. https://doi.org/10.1111/j.1749-7345.2007.00144.x

Licia, M.P., Mario, M.R., Guillermo, R.C. 2006. Catalytic properties of lipase extracts from *Aspergillus niger*. Food Technology and Biotechnology 44:247–252.

Lim, S.J., Lee, K.J. 2009. Partial replacement of fish meal by cottonseed meal and soybean meal with iron and phytase supplementation for parrot fish *Oplegnathus fasciatus*. Aquaculture 290:283–289. https://doi.org/10.1016/j.aquaculture.2009.02.018

Liu, H., Wu, X., Zhao, W., Xue, M., Guo, L., Zheng, Y., Yu, Y. 2009. Nutrients apparent digestibility coefficients of selected protein sources for juvenile Siberian sturgeon (*Acipenser baerii* Brandt), compared by two chromic oxide analyses methods. Aquaculture Nutrition 15:650–656. https://doi.org/10.1111/j.1365-2095.2008.00634.x

Liu, H., Yan, Q., Han, D., Jin, J., Zhu, X., Yang, Y., Xie, S. 2019. Effect of dietary inclusion of cottonseed meal on growth performance and physiological and immune responses in juvenile grass carp, *Ctenopharyngodon idellus*. Aquaculture Nutrition 25:414–426. https://doi.org/10.1111/anu.12867

Liu, H., Yan, Q., Han, D., Jin, J., Zhu, X., Yang, Y., Xie, S. 2016. Effect of dietary cottonseed meal on growth performance, physiological response, and gossypol accumulation in pre-adult grass carp, *Ctenopharyngodon idellus*. Chinese Journal of Oceanology and Limnology 34:992–1003. https://doi.org/10.1007/s00343-016-4115-4

Lowry, O.H., Rosebrough, N.J. Farr, A.L., Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. Journal of Biological

Chemistry 193:265–275. https://doi.org/10.1016/S0021-9258(19)52451-6

Mayes, P.A. 1990. Nutrition. In: Harper's biochemistry, 22nd Edition, Murray, R.K., Granner, D.K., Mayes, P.A., Rodwell, V.W. (Eds.), Prentice Hall International Inc., USA, pp. 571–579.

Maynard, L.A., Loosli, J.K. 1972. Animal nutrition. 6th Edition. McGraw-Hill, New York, USA. 613 pp.

Mbahinzireki, G.B., Dabrowski, K., Lee, K.J., El-Saidy, D., Wisner, E.R. 2001. Growth, feed utilisation and body composition of tilapia (*Oreochromis* sp.) fed with cottonseed meal-based diets in a recirculating system. Aquaculture Nutrition 7:189–200. https://doi.org/10.1046/ j.1365-2095.2001.00172.x

McGoogan, B.B., Reigh, R.C. 1996. Apparent digestibility of selected ingredients in red drum (*Sciaenops ocellatus*) diets. Aquaculture 141:233–244. https://doi.org/10.1016/0044-8486(95)01217-6

National Research Council (NRC) 2011. Nutrient requirements of fish and shrimp. National Academies Press, Washington, DC. 63 pp.

Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. Journal of Biological Chemistry 153:375–380. https://doi.org/10.1016/S0021-9258(18)71980-7

Nengas, I., Alexis, M., Davies, S., Petichakis, G. 1995. Investigation to determine digestibility coefficients of various raw materials in diets for gilthead sea bream, *Sparus auratus* L. Aquaculture Research 26:185–194. https://doi.org/10.1111/j.1365-2109.1995.tb00901.x

Rinchard, J., Lee, K.J., Czesny, S., Ciereszko, A., Dabrowski, K. 2003. Effect of feeding cottonseed meal-containing diets to broodstock rainbow trout and their impact on the growth of their progenies. Aquaculture 227:77–87. https://doi.org/10.1016/S0044-8486(03)00496-4

Robinson, E.H., Li, M.H. 2008. Replacement of soybean meal in channel catfish, *Ictalurus punctatus*, diets with cottonseed meal and distiller's dried grains with solubles. Journal of the World Aquaculture Society 39:521–527. https://doi.org/10.1111/j.1749-7345.2008.00190.x

Serrano, J.A., Nematipour, G.R., Gatlin, D.M. 1992. Dietary protein requirement of the red drum (*Sciaenops ocellatus*) and relative use of dietary carbohydrate and lipid. Aquaculture 101:283–291. https://doi.org/10.1016/0044-8486(92)90031-F

Smith, R.R., Winfree, R.A., Rumsey, G.W., Allred, A., Peterson, M. 1995. Apparent digestion coefficients and metabolizable energy of feed ingredients for rainbow trout *Oncorhynchus mykiss*. Journal of the World Aquaculture Society 26:432–437. https://doi.org/10.1111/j.1749-7345.1995.tb00839.x

Sullivan, J.A., Reigh, R.C. 1995. Apparent digestibility of selected feed stuffs in diets for hybrid striped bass (*Morones axatilis* ♀ × *M. chrysops* ♂). Aquaculture 138:313–322. https://doi.org/10.1016/0044-8486(95)01071-8

Umesh, N.R., Dathathri, K., Nandeesha, M.C., Gangadhar, B., Varghese, T.J. 1994. Digestibility of dry matter and protein and *Spirulina platensis* by common carp, *Cyprinus carpio*, with a note on time of faeces collection in digestibility estimations. In: Fish nutrition research in Asia, proceedings of the fifth Asian fish nutrition workshop, De Silva, S.S. (Ed.), Asian Fisheries Society Special Publication 9. Asian Fisheries Society, Manila, Philippines, pp. 81–84.

Wang, X.F., Li, X.Q., Leng, X. J., Shan, L.L., Zhao, J.X., Wang, Y.T. 2014. Effects of dietary cottonseed meal level on the growth, hematological indices, liver and gonad histology of juvenile common carp (*Cyprinus carpio*). Aquaculture 428–429:79–87. https://doi.org/10.1016/j.aquaculture.2014.02.040

Wu, X. F., Xue, M., Guo, L.Y., Wu, L.X., Zheng, Y.H. 2010. Effects of substitution of solvent-extracted cottonseed meal for part fishmeal on growth, body composition and serum biochemical indices of juvenile Siberian Sturgeon (*Acipenser baerii* Brandt). Chinese Journal of Animal Nutrition 22:117–124.