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Improvement of nutritive value of grass pea (*Lathyrus sativus*) seed meal in the formulated diets for rohu, *Labeo rohita* (Hamilton) fingerlings after fermentation with a fish gut bacterium

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Abstract

Eight isonitrogenous (35% crude protein approximately) and isocaloric (4.0 kcalg^{-1} approximately) diets were formulated incorporating raw and fermented grass pea (*Lathyrus sativus*) seed meal at 10%, 20%, 30% and 40% levels by weight into a fish meal based diet and fed to rohu, *Labeo rohita*, fingerlings for 80 days and fish performance was studied. A particular bacterial strain (*Bacillus* sp.) isolated from the intestine of adult common carp (*Cyprinus carpio*) reared in the wild having significant amylolytic, cellulolytic, lipolytic and proteolytic activities were used for fermentation of seed meal for 15 days at 37 °C. Fermentation of grass pea seed meal was effective in significantly reducing the crude fibre content and anti-nutritional factors, such as tannins, phytic acid and the neurotoxin, β-ODAP and enhancing the available free amino acids and fatty acids. In terms of growth response, feed conversion ratio and protein efficiency ratio, 30% fermented grass pea seed meal incorporated diet resulted in significantly ($P < 0.05$) better performance of rohu fingerlings. In general, growth and feed utilization efficiencies of fish fed diets containing fermented seed meal were superior to those fed diets containing raw seed meal. The apparent protein digestibility (APD) values decreased with increasing levels of raw seed meal in the diets. The APD for raw seed meal was lower at all levels of inclusion in comparison to those for the fermented seed meals. The highest deposition of carcass protein was recorded in fish fed the diet containing 40% fermented seed meal. The results indicated that fermented grass pea seed meal can be incorporated in carp diets up to 30% level compared to 10% level of raw seed meal.

Keywords: *Lathyrus sativus*; Anti-nutrients; Fish intestinal bacteria; Fermentation; Diets; Growth performance; *Labeo rohita* fingerlings

1. Introduction

Grass pea (*Lathyrus sativus*) is one of the important food legumes in countries like Bangladesh, India and Ethiopia. It is a low production cost legume adapted to harsh and low rainfall environments having considerable potential as a good quality, cheap protein source (Tadelle et al., 2003). This plant is resistant to drought and low quality of soil. It is cultivated commonly for the seeds, consumed mainly by humans. They are rich in protein, about 20%–32% (Castell et al., 1994; Grela and Günther, 1995). However, the seeds of legumes such as *Lathyrus* sp. contain a variety of anti-nutritional substances, which hinder free nutritional utilization in monogastric animals (Hanbury et al., 2000) and humans (Grela and Winiarska, 1998). The most frequently occurring anti-nutritional substances in this legume are tannins, protease and amylase inhibitors, lectins, saponins, alkaloids, non-starch polysaccharides, vicine and convicine, phytates and lathyrogens (Lambein et al., 1993; Riepe et al., 1995). The seeds of *Lathyrus sativus*

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contains an acidic neurotoxic amino acid, 3-*N*-oxalyl-L-2,3-diaminopropionic acid or β-ODAP (Grela et al., 2001). Fermentation of legumes is a potentially important processing method that can be expected to improve the nutritive value and decrease certain anti-nutritional factors, like phytates, protease inhibitors and flatulence factors (Yigzaw et al., 2001). Feed accounts for the major part of the production cost in aquaculture industry. Fishmeal is considered as an essential ingredient in feeds for carnivorous fish species and to a lesser extent in the feeds for omnivorous fish and fresh water prawn. However, the cost of fishmeal has soared so high in recent times that it is becoming uneconomical to use them in fish feeds. There is need, therefore, to look for locally available and cheap sources of feed ingredients. One possible source of cheap protein is grass pea. Although there are reports on the use of grass pea in broiler (Tadelle et al., 2003) and pig (Castell et al., 1994) diets, its use as an ingredient in fish feeds has hitherto not been examined. Considering the constant increase of grass pea production and its low cost coupled with the shortage and high cost of fish meal justifies investigation into possible use of grass pea as an alternative plant protein source for carps in India. The use of fermented grass pea seed meal as a partial substitute for fish meal in carp diets is expected to bring down the cost of fish production. In the present study, grass pea seed meal was fermented by an enzyme producing bacterium, *Bacillus* sp., isolated from the intestine of common carp, *C. carpio* and an experiment was designed to evaluate the nutritive value of fermented seed meal in the formulated diets for the Indian major carp, rohu, *L. rohita* (Hamilton) fingerlings.

2. Methods

2.1. Isolation and characterization of fish intestinal bacteria

The bacterial strain used for fermentation of *Lathyrus* seed meal was isolated from the intestine of common carp, *C. carpio* on sterilized Tryptone soya agar plates. The bacterial isolate was screened for the production of extracellular amylase (Bernfeld, 1955), cellulase (Denison and Koehn, 1977), protease (Walter, 1984) and lipase (Colowik and Kaplan, 1955) in specific media. The strain was identified and characterized by carrying out the tests described in the Manual of Microbiological Methods Society of American Bacteriologists (1957).

2.2. Preparation of bacterial seed culture

The selected bacterium was grown in shake bottles in 4% tryptone soya broth (Hi-media, India) for seed culture. After 24 h of growth at 37 °C, an average viable count was about 10^7 cells/ml of broth. This was used as bacterial seed for *Lathyrus* seed meal fermentation.

2.3. Fermentation of Lathyrus seed meal

The dry seeds of *L. sativus* were finely ground and passed through a fine meshed sieve to ensure homogeneity. A portion of sieved *Lathyrus* seed meal was moistened with 50% w/v liquid basal medium containing ($g\,l^{-1}$): KH_2PO_4, 4; Na_2HPO_4, 4; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2$, 0.001; $FeSO_4 \cdot 7H_2O$, 0.004 and autoclaved for sterilization. The sterilized seed meal was fermented with *Bacillus* culture at the rate of 10^8 bacterial cells/g of dried seed meal for 15 days at 37 ± 2 °C in an incubator.

2.4. Diet preparation

Two sets of experimental diets were formulated using either raw (diets D1–D4) or fermented (diets D5–D8) *Lathyrus* seed meal at 10%, 20%, 30% or 40% levels by weight. A diet containing fish meal as the main protein source was used as the reference diet (RD). To each of the formulated diet, 1% chromic oxide was added as an external digestibility marker. All the diets were prepared in pelleted form using 0.5% carboxymethylcellulose as a binder. The pellets were sun dried for a few days and crumbled prior to feeding.

2.5. Experimental design

The feeding trial was conducted in flow-through 90 l circular fibre-glass tanks. Rohu fingerlings, obtained from a local fish dealer, were acclimatized to the laboratory conditions for 15 days and fed with a mixture of rice bran and mustard oil cake. The fingerlings (mean weight 7.6 ± 0.12 g) were randomly distributed at the rate of 15 fish per tank. There were three replicates for each experimental diet. Each experimental tank was supplied with unchlorinated water from a deep tube well with continuous aeration. All the fish were fed once daily at a fixed feeding rate of 3% body weight per day for 80 days. The quantity of feed given was readjusted every 15th day after weighing the fish. To determine the feed consumption, any leftover feed was collected 6 h after each feeding and weighed after oven drying. The digestibility study was conducted separately in static aquaria. The faecal samples were collected everyday in the morning by siphoning 17 h after removal of the uneaten feed following the ''immediate pipetting'' method outlined by Spyridakis et al. (1989), from three replicates of each dietary treatment. The faeces naturally released by the fish could be easily detected and were immediately removed from the water with a glass canula. At the termination of the 80 day experiment the fish were weighed and analyzed for carcass composition.