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EFFECT OF DIFFERENT PROCESSING TECHNIQUES ON THE NUTRITIVE VALUE OF GRASS PEA, *LATHYRUS SATIVUS* L., SEED MEAL IN COMPOUND DIETS FOR INDIAN MAJOR CARP ROHU, *LABEO ROHITA* (HAMILTON), FINGERLINGS

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ABSTRACT. Six isonitrogenous (35% crude protein approximately) and isocaloric (16.73 kJ g^{-1} approximately) diets incorporating processed grass pea, *Lathyrus sativus* L., seed meal at a 30% level by weight into a fish meal – based control diet were fed to rohu, *Labeo rohita* (Hamilton) fingerlings (average initial body weight 3.18 ± 0.11 g) in triplicate treatments at the rate of 3% of body weight daily for 80 days, and fish performance was studied. Four processing methods, namely fermentation, extrusion, autoclaving, and germination, were employed prior to the incorporation of the grass pea seed meal into the diets. The fermentation of grass pea seed meal was effective in significantly reducing the anti-nutritional factors, tannins, phytic acid, and the neurotoxin β-ODAP (β-oxalyl-diaminopropionic acid). The extrusion of grass pea seed was effective in significantly reducing tannins, trypsin inhibitor, and the neurotoxin β-ODAP. Autoclaving the grass pea seed meal resulted in the reduction of tannins but was not effective in reducing other anti-nutritional factors. The level of trypsin inhibitor was reduced to non-detectable limits in germinated grass pea seeds. The tannin content was also reduced considerably in the germinated grass pea seed meal. In terms of growth response, feed conversion ratio, and protein efficiency ratio, 30% fermented, extruded, and germinated grass pea seed meal incorporated diets resulted in significantly ($P < 0.05$) the best performance of rohu fingerlings. The apparent protein digestibility (APD) values obtained with processed grass pea seed meal were significantly higher as compared to those with raw seed meal incorporated diets ($P < 0.05$). The accumulation of carcass protein was comparatively higher in the groups of fish reared on diets containing 30% autoclaved, germinated, and extruded grass pea seed meal. The results of this study indicate that processing grass pea seed meal is effective in improving the nutritive value of *L. sativus* seed meal and that the processed grass pea seed meal can be incorporated into rohu diets up to a 30% level without any adverse effect.

Key words: GRASS PEA, ANTI-NUTRITIONAL FACTORS, PROCESSING, DIETS, ROHU, *LABEO ROHITA*, FINGERLINGS

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INTRODUCTION

The intensive cultivation of grass pea, *Lathyrus sativus* L., one of the important food legumes, occurs in India, Bangladesh, 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). It is cultivated commonly for the seeds for human consumption. 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, convicine, phytates, and lathyrogens (Lambein et al. 1993, Riepe et al. 1995). The seeds of *L. sativus* also contain an acidic neurotoxic amino acid, 3-*N*-oxalyl-L-2,3-diaminopropionic acid or β-ODAP (Grela et al. 2001). Therefore, the anti-nutritional factors in the seeds require inactivation by different processing methods such as autoclaving, extrusion, fermentation, and germination prior to inclusion in fish feeds. Feed accounts for the major part of the production cost in aquaculture. Fish meal is considered as an essential ingredient in feeds for carnivorous fish species, and to a lesser extent in the feeds for omnivorous fish and freshwater shrimp. However, the cost of fish meal has soared so high recently that it is becoming uneconomical to use them in fish feeds. There is a need, therefore, to look for locally available, cheap sources of feed ingredients. One possible source of cheap protein is the grass pea. The constant increase of grass pea production and its low cost coupled with the shortage and high cost of fish meal justifies investigating the possible use of grass pea as an alternative plant protein source for carps in India. In view of the aforementioned, the present study was designed to evaluate raw, autoclaved, fermented, extruded, and germinated grass pea seed meals as partial replacement for fish meal in practical diets for the Indian major carp, rohu, *Labeo rohita* (Hamilton), fingerlings based on its effects on growth, protein utilization, and carcass chemical composition.

MATERIALS AND METHODS

PROCESSING OF GRASS PEA

The grass pea seed required for the trial was obtained from the local market. The material was subdivided into lots that were processed as follows:

EXTRUSION

The extrusion of finely ground grass pea was performed in a Twin-Screw Extruder (Basic Technology Pvt. Ltd., India). The extruder was operated at 400 rpm and the temperature was set at 130°C. Moisture content of the sample was increased to 12% by adding water.

FERMENTATION

Finely ground seeds of grass pea were passed through a fine meshed sieve to ensure homogeneity. Grass pea seed meal was fermented by an enzyme producing the bacterium, *Bacillus* sp., isolated from the intestine of common carp, *Cyprinus carpio* L. (Bairagi et al. 2002a). The selected bacterium was grown in shake bottles in 4% tryptone soya broth (Hi-Media, India) for seed culture. After 24 h growth at 37°C, the average viable count was about 10^7 cells ml^{-1} of broth. This was used as bacterial seed for seed meal fermentation. A portion of sieved grass pea seed meal was moistened with 50% w/v liquid basal medium containing (g l^{-1}): KH_2PO_4, 4; Na_2HPO_4, 4; $MgSO_4.7H_2O$, 0.2; $CaCl_2$, 0.0001; $FeSO_4.7\ H_2O$, 0.004 and autoclaved for sterilization. The sterilized seed meal was fermented with *Bacillus* culture at the rate of 10^8 bacterial cells $g^{-1}$of dried seed meal for 15 days at 37 ± 2°C in an incubator.

GERMINATION

The dry seeds of grass pea were sowed in wet soil and allowed to germinate for three days. The germinated seedlings were oven dried, finely ground and passed through a fine meshed sieve to ensure homogeneity.

AUTOCLAVING

A part of the raw seed sample was autoclaved for 30 min at 121°C after soaking in water for 20 h. The autoclaved seeds were dried, finely ground and passed through a fine meshed sieve to ensure homogeneity.

EXPERIMENTAL DIETS

The proximate composition of feed ingredients used in the present experiment is presented in Table 1. Five sets of approximately isonitrogenous (35% crude protein) and isocaloric (16.73 kJ g^{-1}) experimental diets were formulated using raw (diet D1), fermented (D2), autoclaved (D3), germinated (D4), and extruded (D5) grass pea seed meal at 30% levels by weight (Table 2). A diet containing fish meal as the main protein source was used as the control diet (CD). To each of the formulated diets, 1% chromic oxide was added as a 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.

TABLE 1

Proximate composition (mean ± SE; n = 3) of feed ingredients (% dry matter basis)

Nutrients	Fish meal	Mustard oil cake	Rice bran	Raw *Lathyrus sativus* seed meal	Fermented *Lathyrus* seed meal	Autoclaved *Lathyrus* seed meal	Germinated *Lathyrus* seed meal	Extruded *Lathyrus* seed meal
Moisture	2.26 ± 0.07	14.00 ± 0.49	4.45 ± 0.16	6.50 ± 0.23	7.50 ± 0.25	9.50 ± 0.47	8.50 ± 0.42	6.50 ± 0.32
Dry matter	97.04 ± 3.4	86.00 ± 3.04	95.55 ± 3.38	93.50 ± 3.31	92.50 ± 3.25	90.50 ± 4.52	91.50 ± 4.57	93.5 0 ± 3.31
Crude Protein	58.50 ± 2.07	35.93 ± 1.27	13.00 ± 0.46	21.45 ± 0.76	23.45 ± 0.83	21.45 ± 1.07	28.0 ± 1.4	21.45 ± 0.76
Crude lipid	8.91± 0.32	7.00 ± 0.25	5.14 ± 0.18	3.50 ± 0.12	3.0 ± 0.11	3.50 ± 0.17	3.90 ± 0.19	3.50 ± 0.12
Ash	11.50 ± 0.40	8.37 ± 0.29	21.41 ± 0.76	3.50 ± 0.12	3.0 ± 0.11	3.50 ± 0.17	3.60 ± 0.18	3.50 ± 0.12
Crude fiber	3.93 ± 0.14	5.53 ± 0.20	25.50 ± 0.91	7.50 ± 0.27	5.0 ± 0.17	7.50 ± 0.37	7.70 ± 0.38	7.50 ± 0.27
Nitrogen-free extract	14.20 ± 0.5	29.17 ± 1.03	30.50 ± 1.08	57.55 ± 2.04	58.05 ± 2.06	54.55 ± 2.72	51.70 ± 2.58	57.55± 2.04
Gross energy (kJ g^{-1})	20.29 ± 0.17	17.19 ± 1.05	14.72 ± 0.12	17.44 ± 0.15	17.36 ± 0.15	16.98 ± 0.2	17.74 ± 0.21	17.44 ± 0.15

EXPERIMENTAL DESIGN

The feeding trial was conducted in glass aquaria containing 90 l of water in each. Rohu fingerlings, obtained from a local fish seed dealer, were acclimatized to laboratory conditions for 15 days and fed with a mixture of rice bran and mustard oil cake. The fingerlings (mean body weight 3.18 ± 0.11 g) were randomly distributed at a rate of 15 fish $tank^{-1}$. 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 twice a day at 08.00 and 12.00 at a feeding rate of 3% of the total body weight day^{-1}. The feeds were delivered on the surface of the

TABLE 2

Ingredient composition (% dry weight) and proximate composition (on % dry matter basis; n=3) of experimental diets

		Diets with 30% processed *Lathyrus* seed meal				
Ingredients	Control diet	D1	D2	D3	D4	D5
Fish meal	40	34	34	34	34	34
Lathyrus seed meal	-	30	30	30	30	30
Mustard oil cake	24	29	29	29	29	29
Rice bran	34	5	5	5	5	5
Vitamin and mineral premix[1]	1	1	1	1	1	1
Chromic oxide	1	1	1	1	1	1
Proximate composition (%)						
Dry matter	94.5	91.5	91.5	92.0	91.05	93.0
Crude protein	35.94	35.03	35.93	35.04	35.98	35.06
Crude lipid	9.0	5.0	4.0	5.0	5.2	3.0
Ash	17.0	20.5	14.5	20.4	20.2	13.05
Crude fiber	10.5	9.5	5.8	9.5	9.7	9.5
NFE[2]	22.06	21.47	31.27	22.06	19.97	32.39
Gross energy (kJ g^{-1})	17.27	15.56	16.44	15.64	15.64	16.65
Tannins (%)	-	0.39	0.07	0.09	0.21	0.1
Phytic acid (%)	-	1.95	0.23	1.93	1.87	0.19
Trypsin inhibitor (TIU g^{-1})	-	3416.4	3414.6	3360.6	N.D.	N.D.
β-ODAP (%)	-	0.38	0.29	0.36	0.38	0.20

N.D. – not detectable, [1]Vitamin and mineral mixture (Vitaminetes Forte, Roche Products Ltd., 24/28, Pt. M. M. Malviya Road, Mumbai 400034, India), [2]Nitrogen-free extract

water. The feeding trial continued for a period of 80 days. The quantity of feed given was readjusted every 15^{th} day on bulk weighing the fish. To determine the feed consumption, any leftover feed was collected 6 h after each feeding and weighed after oven drying. The fecal 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 feces 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.

The water quality parameters from each tank were monitored each week throughout the experimental period following the methods outlined by the American Public

Health Association (1985). The ranges of water quality parameters were temperature, 29-32°C, pH 7-7.8, dissolved oxygen 4.6-5.5 mg l^{-1}, and alkalinity 154-165 mg l^{-1}.

CHEMICAL ANALYSIS AND DATA COLLECTION

Proximate composition of feed ingredients, experimental diets, fecal samples, and fish carcasses were analyzed according to the standard methods of the Association of Official Analytical Chemists (Helrich 1990) as follows: moisture was determined by oven drying at 105°C for 24 h; crude protein (Nitrogen × 6.25) by micro Kjeldahl digestion and distillation after acid digestion using a Kjeltec 1026 Distilling unit together with a Tecator Digestion System (Tecator, Sweden); lipid was determined by extracting the residue with 40-60°C petroleum ether for 7-8 h in a Soxhlet apparatus; crude fiber was determined as loss on ignition of dried lipid-free residues after digestion with 1.25% H_2SO_4 and 1.25% NaOH using a Fibertec System 2021 (Foss Tecator, Sweden), and ash was determined by ignition at 550°C in a muffle furnace to a constant weight. Nitrogen-free extract (NFE) was computed by taking the sum of values for crude protein, crude lipid, crude fiber, and moisture and subtracting this from 100 (Maynard et al. 1979). The proximate analyses of the carcasses were done before initiation and after termination of the experiment following the aforementioned procedures. Chromic oxide in the diets and fecal samples was estimated following the method of Bolin et al. (1952). Tannin content in the raw and processed grass pea seed meals was determined using Folin-Denis reagent (Schanderi 1970). The content of neurotoxin β-ODAP in grass pea seed meal was determined with the spectrophotometric method of analysis developed by Rao (1978). Trypsin inhibitor was quantified following the method of Kakade et al. (1974). Trypsin inhibitor activity was expressed either as trypsin inhibitor unit (TIU) mg $protein^{-1}$ (in the case of grass pea seed meal) or g $sample^{-1}$ (in the case of formulated feeds). Phytic acid content was determined according to Wheeler and Ferrel (1971).

Average live weight gain (%), specific growth rate (SGR, % day^{-1}), feed conversion ratio (FCR), and protein efficiency ratio (PER) were calculated using standard formulae (Steffens 1989). The apparent protein digestibility (APD) was calculated according to De Silva and Anderson (1995), using the formula: APD (%) = 100 – 100 × (% Cr_2O_3 in diet/% Cr_2O_3 in feces) × (% protein in feces/% protein in diet).

STATISTICAL ANALYSIS

Statistical analysis of the data was performed by analysis of variance (ANOVA) using Microsoft software Statistica. Mean differences between treatments were tested for significance at $P < 0.05$ and comparisons were made with Duncan's multiple range test (Duncan 1955).

RESULTS

The level of tannin in the seed meal was reduced by all the processing techniques employed (Table 3). The fermentation of grass pea seed meal reduced the phytic acid content from 6.52 to 0.97% (85.1% reduction). However, the phytic acid content remained almost unaffected in extruded, autoclaved, and germinated grass pea seed meals. Extrusion and germination of the seed meal reduced the trypsin inhibitor level to undetectable limits, whereas fermentation and autoclaving did not affect it. Extrusion and fermentation of grass pea seed meal resulted in the reduction of β-ODAP contents (46.09 and 24.2% reduction, respectively). The β-ODAP content was not affected by germinating or autoclaving the seed meal. The average final weight of the fish increased from the initial value in all dietary treatments. Rohu fingerlings fed diet D4 containing 30% germinated *Lathyrus* seed meal had the highest weight gain, which was

TABLE 3

Effect of different processing methods on anti-nutritional factors encountered in *Lathyrus* seed meal (mean ± SE; n = 3). Values in parentheses indicate percentage reduction of anti-nutritional factors

Anti-nutritional factors	Raw *Lathyrus* seed meal	Fermented *Lathyrus* seed meal	Autoclaved *Lathyrus* seed meal	Germinated *Lathyrus* seed meal	Extruded *Lathyrus* seed meal
Tannin (%)	1.3 ± 0.05	0.25 ± 0.008 (80.7)	0.32 ± 0.02 (75.3)	0.69 ± 0.03 (46.9)	0.25 ± 0.01 (80.76)
Phytic acid (%)	6.52 ± 0.23	0.97 ± 0.043 (85.1)	6.42 ± 0.32 (1.5)	6.23 ± 0.31 (4.4)	6.48 ± 0.32 (0.61)
Trypsin inhibitor (TIUmg protein^{-1})	31.42 ± 0.93	Unaffected	Unaffected	N.D.	N.D.
β-ODAP* (%)	1.28 ± 0.05	0.97 ± 0.05 (24.2)	1.21 ± 0.06 (5.4)	1.27 ± 0.06 (0.78)	0.69 ± 0.03 (46.09)

*N.D. – not detectable, * β-oxalyl-diamino propionic acid*

significantly higher ($P < 0.05$) than those fed other experimental diets (Table 4). The highest attained fish body weight, average percentage live weight gain, and SGR were recorded in the group of fish reared on diet D4 (30% germinated grass pea seed meal incorporation). Live weight gain (%) and the SGR varied significantly ($P < 0.05$). PER was highest in fish fed diet D4 containing 30% germinated grass pea seed meal. FCR was best for fish fed diet D4 (30% germinated grass pea seed meal) and worst for diet D1 containing 30% raw grass pea seed meal. Apparent protein digestibility (APD) was highest for diet D2 (containing 30% fermented grass pea seed meal). There was no significant ($P < 0.05$) difference in APD values among the other diets.

TABLE 4

Growth performance, feed utilization efficiency and apparent protein digestibility in *Labeo rohita* fingerlings fed experimental diets for 80 days (mean ± SE; n = 3)

		Lathyrus seed meal diets				
Parameters	Control diet	D1	D2	D3	D4	D5
Initial body weight (g)	3.18 ± 0.1^{a}	3.13 ± 0.11^{a}	3.18 ± 0.11^{a}	3.19 ± 0.11^{a}	3.09 ± 0.11^{a}	3.15 ± 0.11^{a}
Final body weight (g)	5.68 ± 0.21^{a}	5.27 ± 0.18^{b}	6.00 ± 0.21^{a}	5.34 ± 0.19^{b}	6.00 ± 0.21^{a}	5.93 ± 0.21^{a}
Weight gain (%)	78.62 ± 2.78^{b}	68.37 ± 2.42^{c}	88.68 ± 3.14^{a}	67.40 ± 2.39^{c}	94.17 ± 3.34^{a}	88.25 ± 3.13^{a}
Feed intake (g 100 g fish^{-1} day^{-1})*	0.90	1.04	0.93	0.74	0.84	1.12
SGR (% day^{-1})	0.74 ± 0.03^{b}	0.65 ± 0.02^{c}	0.80 ± 0.03^{a}	0.64 ± 0.02^{c}	0.83 ± 0.03^{a}	0.79 ± 0.03^{a}
FCR	1.62 ± 0.05^{b}	2.04 ± 0.07^{a}	1.58 ± 0.06^{b}	1.48 ± 0.05^{b}	1.38 ± 0.05^{c}	1.90 ± 0.07^{a}
PER	1.98 ± 0.07^{a}	1.37 ± 0.05^{b}	1.37 ± 0.05^{b}	1.89 ± 0.06^{a}	2.02 ± 0.07^{a}	1.46 ± 0.05^{b}
APD (%)	85.04 ± 3.02^{a}	70.25 ± 2.81^{c}	84.62 ± 3.00^{a}	83.24 ± 2.95^{a}	80.27 ± 2.84^{b}	83.26 ± 2.95^{a}

Values in the same row with the same superscripts are not significantly different ($P < 0.05$)
**Statistical analyses were not possible as determinations were performed on pooled samples*

The deposition of protein and lipid in the carcasses of experimental fish increased over the initial value in all dietary treatments (Table 5). The highest accumulation of carcass protein was recorded in the group of fish reared on diet D3 (autoclaved) and D5 (extruded). The highest tissue lipid accumulation occurred in fish fed diet D4 (containing 30% germinated grass pea seed meal). The ash content of fish carcasses was highest ($P < 0.05$) in the fish fed diet D2 (fermented).

TABLE 5

Proximate carcass composition (% wet weight) of the experimental fish at the start and end of the 80 day feeding experiment (mean ± SE; n = 3)

Carcass composition	Initial	Control	D1	D2	D3	D4	D5
Moisture	81.5 ± 2.88	78.2 ± 2.77^{a}	79.6 ± 2.82^{a}	74.25 ± 2.63^{a}	71.9 ± 2.55^{a}	75.26 ± 2.69^{a}	79.8 ± 2.84^{a}
Crude Protein	8.01± 0.40	9.87 ± 0.35^{b}	8.75 ± 0.31^{c}	8.13 ± 0.29^{c}	12.0 ± 0.42^{a}	10.87 ± 0.38^{a}	11.5 ± 0.41^{a}
Crude Lipid	1.5 ± 0.06	3.92 ± 0.14^{b}	2.84 ± 0.09^{c}	1.5 ± 0.05^{d}	1.56 ± 0.06^{d}	5.06 ± 0.17^{a}	3.99 ± 0.14^{b}
Ash	2.42 ± 0.09	2.98 ± 0.11^{c}	3.23 ± 0.11^{c}	6.36 ± 0.23^{a}	3.17 ± 0.11^{c}	3.85 ± 0.13^{b}	2.44 ± 0.09^{d}

Values in the same row with the same superscripts are not significantly different (P < 0.05)

DISCUSSION

The results of this study corroborate the suitability of processed grass pea seed meal as an alternate protein source in formulated diets for rohu fingerlings. It is evident from this investigation that processed grass pea seed meal could be incorporated at a 30% level in the diet of rohu fingerlings without any adverse effect on growth performance.

Tannins, phytates, lathyrogens, and trypsin inhibitors are some of the frequently occurring anti-nutritional substances in legumes. Dietary tannins interfere with protein and dry matter digestibility by inhibiting protease and also forming indigestible complexes with dietary protein (Krogdahl 1989). There are reports of tannin toxicity in sheep and cattle (Makkar and Becker 1994) and growth retardation and inhibition of digestive enzymes in fish (Hossain and Jauncey 1989, Mukhopadhyay and Ray 1996, 1999a, b, Bairagi et al. 2002b, Maitra and Ray 2003, Ramachandran et al. 2005). Phytic acid acts as a chelator, forming protein and mineral phytic acid complexes, the net result being reduced protein and mineral bioavailability (Spinelli et al. 1983, Hossain and Jauncey 1989). The seeds of grass pea contain a neurotoxin, β-ODAP, which causes paralysis of the lower or hind limbs in humans and animals and general weakness in skeletal muscles (Grela et al. 2001). In the present investigation, the tannin content was comparatively reduced by different processing methods. Fermentation and extrusion of the grass pea resulted in the reduction of tannin content by 80.7%. The tannin content was reduced by 75.3% in the case of autoclaved grass pea seed meal and by 46.9% in the case of germinated grass pea seed meal. High extrusion temperatures can affect the molecular structure of condensed tannins and polyphenols. This chemi-

cal modification may alter tannin solubility or chemical reactivity (Barroga et al. 1985). The fermentation of grass pea seed meal resulted in the reduction of phytic acid content by 85.1%, whereas it remained unaffected by autoclaving, extrusion, and germination. The trypsin inhibitor content in the raw grass pea seed meal was 31.42 TIU mg protein^{-1}, whereas in the case of extruded and germinated grass pea seed meals, the trypsin inhibitor was reduced to non-detectable limits. Marzo et al. (2002) also demonstrated that trypsin inhibitory activity in kidney beans could be reduced by extrusion processing. Many anti-nutritional factors, such as protease inhibitors are heat labile and are thus denatured in this manner (Krogdahl et al. 1994). The β-ODAP content was reduced by 24.2% by means of fermentation and by 46.09% by means of extrusion. In the case of autoclaved grass pea seed meal, the β-ODAP was reduced to 5.47%. The germination of grass pea seed did not affect the β-ODAP content. While studying the effects of different methods of treatment on β-ODAP content in grass pea, Tadelle et al. (2003) reported that cooking at 90°C for 20 minutes was effective in reducing its level by 63.64%. During extrusion processing in the present study, the temperature was set at 130°C, which resulted in a 46.09% reduction of β-ODAP.

The growth performance and feed utilization efficiencies in rohu fingerlings fed extruded, germinated, and fermented grass pea seed meal incorporated diets were significantly better than those fed diets containing raw grass pea seed meal at similar levels of incorporation. Fish fed diets containing 30% extruded, fermented, and germinated grass pea had better weight gain and SGR than the group of fish reared on a control diet containing fishmeal as the protein source. Ramachandran et al. (2005) observed better growth response in rohu fingerlings fed diets containing 30% grass pea seed meal fermented with *Bacillus* sp. Leaf meals of *Lemna* and *Leucaena* fermented with the same bacterial strain were successfully used to replace fish meal in diets for rohu fingerlings up to a 30% level (Bairagi et al. 2002b, 2004). The growth performance of the fish on the fermented grass pea diet could also be attributed to the presence of high concentrations of L-homoarginine in the seeds. Since homoarginine could be a substrate for arginase (Beruter et al. 1978, Petyala and Rao 1999), it is possible that the resulting lysine, being an essential amino acid, could contribute to the growth of the fish on a grass pea diet. It is evident that the reduced growth rate of fish fed raw grass pea meal diets is due to the effects of anti-nutritional factors and high fiber content. Extruded grass pea seed meal was used to replace fish meal in diets for rohu

fingerlings up to a 40% level without any adverse effect on growth performance (Ramachandran and Ray 2004).

The apparent protein digestibility (APD) values were highest in the group of fish fed 30% extruded grass pea seed meal and 30% fermented grass pea seed meal incorporated diets and lowest in the case of raw seed meal incorporated diets. Similar trends have been reported with higher levels of inclusion of raw mustard, *Sinapis* sp. (Hossain and Jauncey 1989), linseed, *Linum* sp. (Hasan et al. 1991), sesame, *Sesamum* sp. seed (Mukhopadhyay and Ray 1999a), copra meal (Mukhopadhyay and Ray 1999b) and leaf meals (Ray and Das 1994, Bairagi et al. 2002b, 2004) in carp diets. The presence of anti-nutritional factors may influence the digestibility of various nutrients in the diet and give erroneous results (Lall 1991).

The proximate composition of the carcasses of the experimental fish at the termination of the feeding trial had significantly increased protein and fat in comparison to the initial value in all the dietary treatments. These results conform to the reports of others, where similar trends were noted with higher levels of inclusion of fermented sesame seed and leaf meals in carp diets (Mukhopadhyay and Ray 1999a, Bairagi et al. 2002b, 2004).

The results of the present experiment demonstrate that the methods of extrusion and germination were effective in the reduction of trypsin inhibitor to non-detectable levels and fermentation was effective in the reduction of phytic acid. However, maximum growth was recorded in the group of fish fed on germinated and fermented grass pea seed meals. Hence, it can be concluded that both germination and fermentation are effective processing techniques to reduce the toxicity of raw grass pea seeds for incorporation into the formulated diets and to obtain better fish growth. However, it is too early to recommend to the industry to use processed grass pea seed meal in the formulation of aquafeeds. The costs involved in pre-incorporation treatments of the seed meal have yet to be justified. Further research is necessary in this direction to evaluate the efficacy of different pre-treatment strategies and the economic benefits of utilizing these products in aquafeeds.

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STRESZCZENIE

WPŁYW PRZETWARZANIA ROZMAITYMI TECHNIKAMI MĄCZKI Z NASION *LATHYRUS SATIVUS* L. NA WARTOŚĆ ŻYWIENIOWĄ W PASZY DLA NARYBKU *LABEO ROHITA* (HAMILTON)

Celem badań było określenie tempa wzrostu oraz składu chemicznego ciała narybku *Labeo rohita* (Hamilton) żywionego paszą z dodatkiem nasion groszku zwyczajnego, *Lathyrus sativus* L., które przetwarzano różnorodnymi technikami produkcji mieszanek paszowych. Średnia masa początkowa ryb wynosiła 3,18 ± 0,11 g, a czas trwania podchowu 80 dni. Fermentacja nasion okazała się efektywną metodą obniżenia zawartości kwasu fitynowego, tanin oraz neurotoksyny (β-ODAP). Ekstruzja nasion groszku zwyczajnego doprowadziła do znaczącego obniżenia poziomu tanin, inhibitorów trypsyny oraz β-ODAP. Przetwarzanie nasion poprzez autoklawowanie doprowadziło do istotnego obniżenia poziomu tanin, ale nie wpłynęło na spadek zawartości innych elementów antyżywieniowych. Natomiast pasza przygotowana z nasion groszku przetworzonego w wyniku procesu kiełkowania cechowała się obniżoną zawartością inhibitorów trypsyny i tanin. Średnia masa ciała narybku *L. rohita* na zakończenie eksperymentu w zależności od stosowanej paszy wynosiła od 5,27 do 6,0 g, a najszybszym tempem wzrostu wyróżniały się ryby karmione paszą z dodatkiem nasion *L. sativus* poddanych kiełkowaniu lub fermentacji.