Responses of *Labeo rohita* to Dietary *Lathyrus sativus* Seeds

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ABSTRACT : Lathyrus sativus, locally known as Khesari, is a leguminous pulse crop grown in many parts of the world for food (used by poor people) and animal feed/fodder. Its seeds are rich in protein and energy but contains anti-nutritional factors prominent among which is β-N-Oxalylamino-L-Alanine (BOAA), a neurotoxin causing lathyrism in humans due to prolong consumption. Keeping in view the chemical characteristics of this toxin and literary facts on *L. sativus* feeding in terrestrial animals, it was hypothesized that aquatic species may better utilise this ingredient in mixed extruded diets. Diets were prepared with varying levels (0, 10, 18, 26 and 34%) of *L. sativus* seeds and fed for 60 days to study growth, body composition and digestibility of nutrients. Final body weight, specific growth rate and feed and protein conversion ratio did not differ ($p>0.05$) between treatments. Crude protein digestibility was reduced ($p<0.01$) beyond 26% inclusion level of *L. sativus*. Final carcass composition with regard to protein, lipid and ash did not differ ($p>0.05$) among the treatments. Comparable craniosomatic, viscerosomatic, renosomatic and hepatic indices and no mortality implies no apparent adverse effect on the vital organs and fish health. It was concluded that *L. sativus* can be a promising feed ingredient that can be used up to 34% or possibly higher level in fingerlings diet. To our knowledge, it appears to be the first report of its kind under laboratory conditions. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 1 : 127-130)***

Key Words : *Labeo rohita*, Dietary *Lathyrus sativus*, BOAA, Growth, Body Composition

INTRODUCTION

In aquaculture, feed plays a very important role as it accounts for about 40 to 60% of the total recurring costs depending on the intensity of the operation. Any reduction in the feed costs through diet development is therefore crucial to the development of the industry. Protein supplements of animal and plant sources are used in aquafeed. Due to expensive nature and uneven supply of animal proteins, mixing of vegetable protein sources satisfies the need. Vegetable proteins otherwise also are more important in the diet of herbivorous and omnivorous fishes particularly in carps. Groundnut cakes, soybean cake, rice and wheat bran are commonly incorporated in poultry and other livestock rations besides fish. Many other protein sources are available but some are not in use for one or other reason such as the presence of anti nutritional factors. Economic and production efficiency in aquaculture can be increased by incorporation of locally available agro-industrial by-products at the disposal of farmers.

Lathyrus sativus (chickling vetch or chick pea) locally known as "Khesari " or "Lakhodi" is one of the pulse crop belonging to the family leguminoceae, which is cultivated despite its ban on cultivation in some states of India (Dwivedi, 1992). Reason attributed to cultivation of this pulse which is used as a food and green manure is due to its drought and disease resistant and high yielding nature. It is grown in Maharashtra (Bhandara, Chandrapur, Gadchiroli and some parts of Nagpur districts), Madhya Pradesh (third largest pulse crop in this state), Uttar Pradesh, Bihar, and also some other states of India. Worldwide cultivation trends indicate that it is grown in Europe (France, Italy, Spain, Poland), Africa (Ethiopia and Algeria), Asia (Bangladesh, Pakistan, China), Australia, Latin America and many other countries. Khesari seeds are good source of protein (28 to 32%) and energy (2.700 kcal ME/g) and are superior to other pulses (Dhiman et al., 1983). Ingesting the seeds for 3-6 months can cause neurolathyrism, a syndrome characterised by muscular rigidity, weakness and paralysis of the leg muscles.

Experiments in ruminants (Cerci and Ozer, 1993), poultry (Panda et al., 1972; Latif et al., 1975; Choudhary and Davis, 1988; 1989; Rotter et al., 1990), pigs (Castell et al., 1994) and other animals (Tiwari et al., 1975) suggest that *L. sativus* has limitations for long-term use in terrestrial species. Although other legumes are tried (see review Petterson, 2000), but there is a lack of information pertaining to use of *L. sativus* in aquafeed (see review Hanbury et al., 2000). Cheeke and Shull (1985) reported the main toxic principle as β-N-oxalyl-amino-L-alanine (BOAA, $C_5H_8N_2O_5$, mol. wt 176.1) which causes nuerolathyrism. The concentration of BOAA in seed varies from 0.1 to 2.5 %. Literary facts reveal that neurotoxin (BOAA) is fully removed by soaking, washing and drying (CFTRI, 1992-93). Looking to the advantages of natural (aquatic) habitat (wherein feed soaks) and method of feed preparation (extrusion involves steaming which may deactivate and or destroy toxin) and typical metabolism of fishes, the hypothesis was tested if such *L. sativus* seeds can be used in aquafeed.

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Table 1. Physico-chemical parameters of ambient water

Dietary groups	Temperature	pH	DO (ppm)	CO_2 (ppm)	Alkalinity (ppm)	Hardness (ppm)
I (Control)	23-26	8.22	7.4	Nil	150	226
II	23-24	8.24	7.8	Nil	154	226
III	22-24	8.26	7.3	Nil	156	242
IV	23-25	8.22	7.5	Nil	152	228
V	23-26	8.39	7.6	Nil	156	222

Table 2. Gross and chemical composition of the experimental diets

Gross composition	Dietary groups				
	I (Control)	II	III	IV	V
Lathyrus sativus seeds	0	10	18	26	34
Fish meal	15	15	15	15	15
Acetes	5	5	5	5	5
Soybean meal	21	18	15	13	12
Wheat bran	20	19	15	14	12
Groundnut cake	20	16	15	12	8
Wheat floor	12.6	10.6	10.6	8.6	7.6
Sunflower oil	1.5	1.5	1.5	1.5	1.5
Cod liver oil	1.5	1.5	1.5	1.5	1.5
Chromic oxide	1.0	1.0	1.0	1.0	1.0
Vitamin*+mineral Premix**	2.0	2.0	2.0	2.0	2.0
Betaine	0.4	0.4	0.4	0.4	0.4
Chemical composition (%DM basis)					
Dry matter	93.25	93.01	92.84	93.66	94.72
Organic matter	89.29	89.74	89.74	90.00	89.63
Crude protein	35.65	35.48	34.89	33.72	32.67
Lipid	9.27	10.70	10.98	11.38	11.67
Gross energy (kcal/g)	4.709	4.792	4.807	4.821	4.805
Digestible energy, DE*** (kcal/g)	4.035	4.115	4.139	4.169	4.169

* Composition (per kg): Vitmin A 2,000,000 IU, Vit D_3 400,000 IU, Vit B_{12} 800 mg, Vit E 300 IU, Vit K 400 mg, Ca pantothenate 1 g, Nicotinamide 4 g, Vit B_{12} 4.2 g, Choline chloride 60 g, Vit C was an additional supplementation in the form of Celin tablets, Glaxo Co India at 300 mg^{-1} kg^{-1} diet.

** Composition (per kg): Calcium 750 g, Mangenese 27.5 g, Iodine 1 g, Iron 7.5 g, Zinc 15 g, Copper 2 g, Cobalt 0.45 g.

*** DE (kcal/100 g)=Protein%×4+lipid%×9−CHO%×4.

MATERIALS AND METHODS

Experimental setup

The experiment was designed to explore the use *Lathyrus sativus* in the diet of *Labeo rohita* fingerlings (wt. 1.52±0.04 to 1.62±0.06 g) over a period of two months. The fingerling of *L. rohita* used in the present experiment were procured from the Khopoli fish seed farm district in the Raigad (Maharashtra state). The fingerlings were acclimatised in a rectangular fibre tank (6×3 ft) before the experiment and fed with pelleted feed containing 35% protein. The experiment was set up in 5 distinct experimental groups having 4 replicates each in 20 uniform size plastic tubs (50 L capacity). Ten fingerlings of uniform size were stocked in each tub. A uniform volume of 40 L chlorine free borewell water was maintained in each tub through out the experimental period. Round the clock aeration was provided to all the tubs with a 2 HP air blower. The aeration pipe of each tub was provided with air stone and 1/8 inch diameter plastic regulator to adjust the air pressure uniformly in all tubs. Experimental tubs were cleaned manually by siphoning the water along with faecal matter and left over feed on alternate days and 50% water was replaced by fresh water. During experimental period. Water quality viz. water temperature, pH, dissolved oxygen, free carbon dioxide, total alkalinity, total ammonia and total hardness were recorded as per APHA (1985) on alternate day and given in Table 1.

Feed ingredients, diet preparation and feeding

Feed ingredients were procured from local markets, except *Lathyrus sativus* was procured from Bhandara district (M.S.). Dried anchovy fish was used for formulating rations. All the dried ingredients were finely ground with a pulverizer to pass through 60 mesh size nylon netting. All the ingredients were thoroughly mixed (Table 2) except vitamins and required amount of water (20%) was added to make dough. Later on vitamins were added. Pellets were prepared by using extruder (BTPL twin screw extruder. Calcutta) with 2 mm die size. Finally pellets were dried and kept in airtight pack and stored till further use. Feeding rate was adjusted based on daily observation. Feed was given at 5% of total biomass by accessing the body weight at fortnight interval till the end of the experiment. Daily ration

Table 3. Response of *Labeo rohita* fed varying levels of *Lathyrus sativus* seeds

Attribute	Percent of *Lathyrus sativus* seeds in diet					Significance
	0	10	18	26	34	
Initial body weight (g)	1.62±0.06	1.55±0.05	1.55±0.09	1.58±0.08	1.52±0.04	NS
Final body wt (g)	3.72±0.10	2.85±0.80	3.44±0.08	3.60±0.10	3.77±0.16	NS
Specific growth rate (SGR)	1.38±0.06	1.39±0.07	1.38±0.06	1.38±0.08	1.52±0.08	NS
Feed conversion ratio (FCR)	2.55±0.11	2.66±0.16	2.75±0.65	2.70±0.14	2.36±0.19	NS
Feed efficiency (FE)	0.39±0.02	0.38±0.02	0.36±0.01	0.38±0.02	0.42±0.03	NS
Protein efficiency ratio (PER)	1.11±0.04	1.08±0.06	1.03±0.03	1.11±0.06	1.27±0.10	NS
Final body composition of fish						
Dry matter	23.15^{a}±0.82	24.00^{ab}±1.80	27.37±0.63	26.78^{bc}±0.80	29.05^{c}±0.38	*
Crude Protein	58.55±2.06	56.60±1.36	56.19±1.22	56.80±1.43	59.99±0.39	NS
Lipid	24.80±2.22	26.82±5.40	28.70±1.27	30.60±1.50	29.40±0.82	NS
Ash	7.52±1.67	10.13±0.32	12.46±1.58	12.89±2.14	9.62±0.55	NS
DM digestibility (%)	52.41±6.28	51.86±8.02	55.74±3.31	47.88±2.05	44.38±3.37	NS
CP digestibility (%)	84.04^{bc}±2.75	81.29^{b}±2.83	85.17^{c}±1.27	73.65^{a}±0.95	75.92^{a}±1.05	**
Indices						
Hepatosomatic index (%)	1.21±0.13	1.19±0.09	1.13±0.10	1.11±0.10	0.83±0.04	NS
Craniosomatic index (%)	1.14±0.122	1.22±0.12	1.44±0.10	1.36±0.04	1.22±0.05	NS
Viscerosomatic index (%)	4.30±0.54	5.13±0.62	5.06±0.18	4.90±0.25	4.20±0.36	NS
Renosomatic index (%)	0.63±0.07	0.58±0.09	0.76±0.09	0.69±0.07	0.53±0.06	NS

$^{a, b, c}$ Means bearing different superscript letters with in a row differ significantly (* $p<0.05$, ** $p<0.01$).

SGR % (SGR)= (Log_e Final body weight- Log_e Initial body weight)×100/Total duration.

FCR=Feed given (dry weight)/wet gain (wet weight). FE=Net weight gain (wet weight)/feed given (dry weight). PER=weight gain (g)/protein intake.

was divided in two parts and two third of total ration was given in the morning at 9 am and rest one third at 5 pm. The animals were kept starved over night before taking body weight measurements. Digestibility trial was conducted after a month feeding during which quantitative collection of total faeces was done. As the voluntary feed intake was closely monitored, the left-over was rarely encountered, which was separated from faeces and taken into account for digestibility calculation purposes.

Biochemical analysis

Proximate composition was done as per AOAC (1980). Crude protein content was determined by micro-Kjeldahl's method, whereas fiber and ether extract content was determined using Fibertech system (Tecator Model M 1047) and Soxtech system (Tecator Model ST2, 1045), respectively. Moisture and ash was estimated using hot air oven and muffle furnace, respectively. Gross energy was calculated using the standard values of energy for protein, carbohydrates and fats (Maynard et al., 1979).

Statistical analysis

Data were subjected to one way analysis of variance and differences between groups were tested as per methods of Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Response of *Labeo rohita* fed diets with varying levels of *Lathyrus sativus* seeds are presented in Table 3. The initial and final body weights of the experimental groups were comparable with control group. Similarly, specific growth rate were not significantly different ($p>0.05$) in all the groups indicating no growth retardation of fishes even at higher inclusion level of 34% *L. sativus* in the diet.

Dry matter digestibility was also not affected due to inclusion of different level of *L. sativus* seeds in the feed. However, CP digestibility was significantly ($p<0.01$) lowered beyond 26% of inclusion. This might be due to toxic principle β-N-Oxalylamino-L-Alanine (BOAA) in the *L. sativus* as reported by Panda et al. (1972). The feed and protein conversion efficiency were not affected ($p>0.05$) among experimental groups even if lower protein digestibility at higher inclusion rates (>26%) of *L sativus* seeds. This indicates better acceptability of extruded feeds and that the protein requirement of those groups was satisfied by higher inclusion of *L. sativus*. *L. sativus* is not only good source of protein but also carbohydrates, which are gelatinised during extrusion process and it is established that gelatinisation improves quality and acceptability of diets (Biliaderis, 1991; Mohapatra et al., 2003). Moreover, it has been found that pea proteins are better utilised by turbot and rainbow trout (Burel et al., 2000) as well as carps (Garg et al., 2002). Our results are in agreement with these reports.

Final body composition in terms of CP, lipid and ash were also not significantly different ($p>0.05$) among groups, except dry matter (Table 3). Feeding of *L. sativus* to fingerlings of *L. rohita* was not found to be fatal as no mortality was recorded during the experimental period.

Craniosomatic, viscerosomatic, renosomatic and hepatic indices were similar in all the groups indicating no significant changes in vital organs due to feeding of *L. sativus*. This implies that activity of BOAA in the pulse was either inactivated or destroyed during extrusion or soaking while the feed was in water. Panda et al. (1972) reported reduced growth and leg paralysis in chicks offered feed with 25 to 50% *L. sativus* for 6 weeks. Response of *L. rohita* to higher levels of *L sativus* implies that fish have higher tolerance level for BOAA. The oral LD_{50} of feed was reported to be 20,115, 14,710, 11,450 and 8,333 ppm at 24, 48, 72 and 96 h, respectively (Barse et al., 2002), which was higher than that of terrestrial species (Mehta et al., 1983; Chaudhary and Davis, 1989)

From the above results, it can be concluded that *L. sativus* can be efficiently used at 26% level in the diet of *L. rohita* fingerlings. However, no adverse effect was noted at maximum 34% level of *L. sativus* in their diet when fed for two months. Using higher levels, further research needs to be undertaken to see if prolong feeding beyond two months cause any adverse effect in different species during different life stages.

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