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Digestibility of Three Feed Ingredients by *Catla catla* (Hamilton, 1822)

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Abstract

Digestibility of dry matter and nutrients by *Catla catla* was investigated from three feed ingredients-Azolla, soybean and silkworm pupa incorporated at levels ranging from 0-40% incorporated into a diet consisting equal amounts of rice bran and groundnut oil cake. Total dry matter digestibility (DMD) and protein digestibility of Azolla diets were comparable with the control diet up to 20% incorporation ($p>0.05$), reducing there after ($p<0.05$). On the other hand, fat digestibility increased with the level of Azolla incorporation ($p<0.05$). The digestibility of nitrogen-free extract (NFE) was higher ($p<0.05$) at 10 and 20% levels. DMD and protein digestibility of soybean diets were higher ($p<0.05$) at 40% incorporation, and that of fat and NFE were higher ($p<0.05$) at both 30 and 40% levels. Protein digestibility of silkworm pupa diets showed no difference ($p>0.05$) with that of the control, while fat and NFE digestibility were higher above 20 and 30% pupa incorporation, respectively. The results of this study would be useful in formulating diets for catla using the ingredients tested.

Keywords: Digestibility; *Catla catla*; Azolla; Soybean; Silkworm pupa

Introduction

Alternative protein sources and inclusion levels need to be optimized in aqua feeds to make aquaculture production efficient and cost-effective. Best aqua feeds are not defined by nutritional composition, but the degree to which a fish can digest, absorb and assimilate the nutrients. Therefore, digestibility determinations of various feed ingredients are very important to develop cost-effective diet formulations, evaluate ingredient quality, and limit the excretion of nutrients into the environment, which may cause environmental problems [1,2]. Digestibility is the quantification of the digestive processes. It gives a relative measure of the extent to which ingested food and its nutrient components are digested and absorbed by the animal. Apparent digestibility coefficients (ADCs) vary between fish species and feed stuffs. Digestibility of individual ingredients in the compounded diet is considered as one of the important factors affecting the growth of fish [3] and hence, it has been recommended to evaluate the digestibility of each ingredient before its incorporation in the diet.

Azolla pinnata is one of the aquatic plants found in lakes, paddy fields, freshwater ponds, rivers and irrigation channels round the year [4]. It is easy to cultivate and has higher productivity and nutritive value. The use of azolla as a fish feed ingredient is well documented [5-7]. Azolla is rich in protein, total protein ranging from 25 to 30%. Other constituents in Azolla are minerals, chlorophyll, carotinoids, amino acids, and vitamins.

Soybean meal is a prominent ingredient used in prepared diets for fish because of its high protein content and excellent amino acid profile,easy availability, reasonable price and lower fecal nutrient output [8]. Partial substitution of fish meal with soybean meal or its products in feeds at quantities of up to 50-60% has been in practice for many years [9,10]. While aquaculture production continues to expand worldwide to meet the growing demand for fish, the use of soybean products will play an even more important role in providing high-quality protein for various fish species.

Sericulture has become an important cottage industry in countries such as Brazil, China, France, India, Italy, Japan, Korea, and Russia.Spent silkworm pupae,the waste material from silkworm industry, is often discarded in the open environment or used as fertilizer. Due to its high protein content, silkworm pupae meal has been found suitable as a livestock feed, notably for monogastric species and also for ruminants [11]. Studies with Indian major carps and common carp have shown that it

is more suitable than mustard oil cake and rice bran as a feed ingredient [12]. Studies with common carp [13], catla and rohu [14] and mahseer [15] have shown that it could be included up to 50% and 30% levels in carp and mahseer diets respectively.

Catla (*Catla catla*) is an economically important South Asian freshwater fish. Its higher growth rate and compatibility with other major carps, surface feeding habit, and consumer preference have increased its popularity in carp polyculture systems among fish farmers in India, Bangladesh, Myanmar, Laos, Pakistan and Thailand. The present study was conducted to estimate the digestibility of dry matter and nutrients from three non-conventional ingredients – Azolla, silkworm pupa and soybean meal by catla.

Materials and Methods

Table 1: Ingredient proportion (%) and proximate composition (%) of experimental feeds.

Ingredients	Control	10% Azolla/Soya/ SWP	20% Azolla/Soya/ SWP	30% Azolla/Soya/ SWP	40% Azolla/Soya/ SWP
Groundnut cake	45	40	35	30	25
Rice bran	45	40	35	30	25
Ragi (Finger Millet)	9	9	9	9	9
Azolla/Soya/SWP	0	10	20	30	40
Vitamin & mineral mix	1	1	1	1	1
Proximate composition					
Azolla feeds					
Moisture	5.79±0.08	5.60±0.07	4.87±0.10	4.53±0.06	4.60±0.06
Crude protein	26.23±0.31	25.85±0.43	25.98±0.25	24.96±0.32	24.85±0.68
Fat	7.76±0.43	7.46±0.07	7.12±0.10	6.66±0.06	6.23±0.06
Ash	6.99±0.02	8.51±0.07	10.23±0.02	12.12±0.14	13.55±0.11
Crude fiber	7.81±0.02	7.88±0.30	6.24±1.11	6.41±0.31	6.69±0.85
NFE	45.42±1.51	44.70±0.69	45.56±0.89	45.32±1.83	44.08±2.21
Gross energy (kJ/g)	17.07	16.82	16.72	16.53	16.03
Soybean feeds					
Moisture	6.64±0.04	6.09±0.07	5.15±0.04	5.99±0.06	5.08±0.06
Crude protein	27.61±2.16	29.44±0.03	30.81±0.02	31.11±0.05	33.68±0.04
Fat	6.78±0.34	6.82±0.05	5.68±0.36	4.13±0.30	4.07±0.87
Ash	6.75±0.04	7.16±0.02	7.46±0.02	7.61±0.07	7.92±0.02
Crude fiber	7.61±0.17	7.10±0.04	6.85±0.08	5.65±0.16	5.74±0.13
NFE	44.62±2.08	43.40±0.12	44.05±1.42	45.51±1.04	43.51±1.77
Gross energy (kJ/g)	16.55	16.77	16.75	16.46	16.68
Silkworm pupa feeds					
Moisture	6.67±0.02	6.42±0.02	6.22±0.01	5.95±0.01	5.53±0.02
Crude protein	26.74±0.17	29.27±0.60	32.97±0.74	34.91±0.60	35.65±1.02
Fat	6.92±0.36	9.02±0.23	10.28±0.38	12.47±0.97	15.45±1.06
Ash	6.65±0.04	6.20±0.02	5.81±0.05	5.52±0.03	5.38±0.06
Crude fiber	6.83±0.31	8.66±1.48	6.29±0.38	6.35±0.04	6.61±0.56
NFE	46.18±0.51	40.42±1.69	38.43±0.89	34.81±0.83	31.38±2.31
Gross energy (kJ/g)	16.68	17.08	18.06	18.73	19.46

The basal diet consisted of groundnut cake, rice bran, finger millet (binder) and vitamin- mineral mixture (Table 1). Four experimental diets were formulated with each ingredient, replacing groundnut cake and rice bran of the basal diet at 10, 20, 30 and 40% levels. Whole plants of *A. pinnata* were harvested from fish culture ponds, sun dried for 2-3 days, packed in polythene bags and powdered at requisite quantities before feed preparation. Soybean meal, silkworm pupae, groundnut oil cake, rice bran and finger millet were procured locally. Groundnut oil-cake, soybean meal, silkworm pupae and finger millet were dried and powdered. All the ingredients were sieved through a fine meshed screen (0.5mm). The required quantity of the

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ingredients was mixed with hot water to make a dough and then pressed through a hand pelletiser to get uniform sized pellets (2mm). The pellets were sun dried and packed in polythene bags till further use.

Digestibility of the test ingredients was estimated *in vivo* through a short-term trial [16] conducted in 50L indoor plastic tubs at the Regional Research Centre, Bengaluru. Ten fingerlings each of catla (av. wt. 10.11±1.57g) were maintained in a set of thirty separate aerated aquaria and acclimated to laboratory conditions for a week. Every morning at 10.00hr., each diet was fed to fish in duplicate tanks. The fish were allowed to feed for 6 hours. The pelleted feed was siphoned out at the end of the feeding period. On the following day, fecal matter was collected from each tank by filtering the water with a fine meshed nylon cloth (15µm), dried, pooled and stored for proximate analysis. About 50% of water from each aquarium was replaced with fresh water every day after fecal matter collection. This feeding and fecal matter collection trial was conducted for a period of 60 days. Proximate composition of pelleted feed and fecal matter was analysed [17]. Energy content of the feed ingredients and diets was calculated using values of 22.6kJg^{-1} for protein, 38.9kJg^{-1} for lipid and 17.2kJg^{-1} for carbohydrate as NFE. Dry matter and nutrient digestibility were determined according Maynard & Loosli [18]. Crude fibre was used as the reference marker [3,19].

Data on digestibility was compared employing one-way analysis of variance. Pair-wise comparison of treatment means was done by Duncan's multiple range test (p=0.05) [20], when a parameter was significant.

Results and Discussion

The crude protein content of Azolla incorporated diets ranged from 24.85 to 26.23%, while fat and NFE contents varied from 6.23 to 7.76% and 44.08 to 45.56% respectively. The values of these nutrients in soybean incorporated feed were in the range of 27.61-33.68%, 4.07-6.82% and 43.40-45.51% respectively. The crude protein content of silk worm pupae incorporated diets ranged from 26.74 to 35.65%, while fat and NFE contents varied from 6.92 to 15.45% and 31.38 to 46.18% respectively. The differences in the major nutrient contents between the diets are attributable to the proximate composition of the ingredients tested.

Table 2: Digestibility (%, mean±SD) of dry matter, protein and fat by *Catla catla* fed experimental feeds.

Feeds	Total DMD	Protein Digestibility	Fat Digestibility	NFE Digestibility
Azolla				
Control	60.20±2.20^{cd}	68.26±1.91^{c}	82.79±1.47^{a}	79.31±1.90^{a}
10%	62.48±0.18^{d}	75.45±4.03^{cd}	87.08±0.29^{b}	86.66±0.72^{c}
20%	55.61±0.64^{c}	65.44±0.65^{c}	89.80±0.08^{c}	83.74±1.25^{bc}
30%	49.34±2.04^{b}	55.64±2.58^{b}	95.29±0.57^{d}	82.14±0.48^{ab}
40%	36.21±3.33^{a}	44.91±1.92^{a}	96.83±0.70^{d}	81.14±1.16^{ab}
Soy bean				
Control	60.61±2.29^{a}	69.21±2.65^{a}	80.93±0.52^{a}	76.36±1.88^{a}
10%	60.25±1.76^{a}	68.76±1.74^{a}	81.16±0.17^{a}	76.68±1.15^{a}
20%	60.08±1.10^{a}	71.37±2.59^{a}	82.70±1.84^{a}	77.10±0.87^{a}
30%	63.25±0.98^{a}	72.25±0.64^{a}	86.43±0.80^{b}	80.68±0.81^{b}
40%	68.71±0.14^{b}	84.96±0.15^{b}	88.32±0.08^{b}	84.83±0.19^{c}
Silkworm pupa				
Control	61.28±3.26^{b}	68.57±3.41^{a}	85.20±1.35^{a}	78.61±0.19^{a}
10%	58.61±0.40^{ab}	69.04±0.33^{a}	84.18±0.22^{a}	77.37±0.06^{a}
20%	58.43±0.52^{ab}	70.87±0.45^{a}	88.00±0.39^{b}	79.56±0.47^{a}
30%	59.00±1.77^{ab}	69.64±1.29^{a}	94.66±0.87^{c}	85.73±0.54^{b}
40%	55.31±2.63^{a}	67.17±1.41^{a}	93.72±0.30^{c}	85.40±0.63^{b}

DMD and the major nutrient digestibility values recorded are given in Table 2. DMD and protein digestibility (PD) of Azolla diets were comparable with the control diet up to 20% incorporation ($p>0.05$), which showed a reduction thereafter ($p<0.05$). On the other hand, fat digestibility increased with the level of Azolla incorporation ($p<0.05$). The digestibility of NFE was higher ($p<0.05$) at 10 and 20% levels. DMD and PD of soybean diets were higher ($p<0.05$) at 40% incorporation, and that of fat and NFE were higher ($p<0.05$) at both 30 and 40% levels. Protein digestibility of silkworm pupa diets showed no difference ($p>0.05$) with that of the control, while fat and NFE digestibility were higher above 20 and 30% pupa incorporation, respectively.

Azolla pinnata, has been considered a potential source of nutrients which is directly used as a food component by a number of herbivorous fish [21,22]. In the present study,

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incorporation of azolla up to 20% did not have any adverse effect on DMD and protein digestibility, both decreasing ($p<0.05$) at higher inclusion levels. Asadujjaman & Hosain [23] reported poor growth of catla fed fresh Azolla as compared to those fed control diet consisting of rice bran, wheat bran and mustard cake (30:30:40). According to Datta [5], Azolla can be incorporated up to 25% in the diet of another Indian major carp *L. rohita*. Inclusion of *A. pinnata* at 10-25% replacing fish meal in the diet of rohu has yielded positive growth due to the presence of ω-6 fatty acids [24]. Azolla inclusion at 40% enhanced growth in *L. rohita* fingerlings [25]. The differences in the utilization of Azolla by the two major carps can be attributed to their feeding habits. Catla is omnivorous, mainly subsisting on vegetable matter, algae and microcrustaceans [25-27]; on the other hand, rohu is herbivorous, mainly consuming phytoplankton and submerged vegetation [28-30]. Addition of more than 25% Azollain fish diet has led to reduced food utilisation, poor growth performance and altered muscle composition [31]. *A. pinnata* protein is reported to be limiting in tryptophan and slightly deficient in threonine [32], increasing the demand for the deficient amino acids with increased concentration of Azolla in the diet. Catla requires 4.5% and 1.03% of dietary protein as threonine and tryptophan, respectively [33].

The DMD and protein digestibility of soybean diets were higher ($P<0.05$) at 40% incorporation and that of fat and NFE were higher ($p<0.05$) at both 30 and 40% levels. Negative effects on PD in soybean feeding, attributable to trypsin inhibitors and phytic acid, have been reported by Francis et al. [34]. However, Venou et al. [35] observed that soy inclusion did not have any effect on nutrient digestibility. Heat treatment destroys a large number of anti-nutritional factors contained in plant derived ingredients and improves their utilization by fish [34]. Since hot water was used during the preparation of feeds in the present study, similar deactivation of anti-nutrients is expected, mitigating their adverse effects on digestibility.

The digestibility of the protein fraction of soybean products has been reported to be more than 90% for species such as common carp [36], channel catfish [37], tilapia [38] and silver perch [39]. However, Hossain et al. [40] reported apparent PD value of 84.06% with rohu. The highest PD recorded in the present study was 84.96%. This value is comparable to the value of 85% reported for channel catfish [41], 84.67% for tilapia [42], 86.8% for carp [43], but higher than the values of 69.5% in jalawat [44], 76.08% in *Anabas testudineus* [45] and 80% in rainbow trout [46]. Eid & Matty [47] and Atack et al. [48] reported PD values of 83.2%. and 83.7% respectively for soybean meal in common carp. Highest lipid digestibility value observed in catla was 88.32%. Lipid digestibility values of 81 and 90% were reported earlier for channel catfish [49] and hybrid tilapia [38]. The present results suggest that soybean meal at the tested levels of incorporation does not affect lipid digestibility.

Protein digestibility of silkworm pupa diets showed no difference ($P>0.05$) with that of the control, while fat and NFE digestibility were higher above 20 and 30% pupa incorporation, respectively. Complete replacement of dietary protein (30%) by silkworm pupa resulted in significantly better growth performance of catla fingerlings than the control diet [50]. Studies with catla and rohu [14] have shown the best growth at 30% pupa inclusion, it being the highest level tried. Silkworm pupa meal could be used up to 50% in the diet of common carp without adversely affecting growth and flesh quality [13]. The highest protein digestibility value recorded for pupa diets with catla was 70.87%. For rohu, Hossain et al. [40] and Jayaram & Shetty [14] reported fairly high APD values of 85.21% and 91.89% with silkworm pupae. Higher APD values were also recorded in rainbow trout (85%, Hastings, 1969) and tilapia (85.74%,) [42]. In contrast, Kim [43] observed low APD (63.9%) in common carp. Fat digestibility in common carp increased with increasing level of pupa meal [51]. In the present study also, higher fat digestibility was observed with pupa incorporated diets. Incorporation of pupa reduced dietary starch levels due to which an increase in NFE digestibility was recorded in catla, as observed in other species [52].

The digestibility of fat by fish is known to be quite variable and depends on a number of factors, including the source [53]. In the present study, higher fat digestibility was observed with the test diets, indicating that the crude fat from all the ingredients was well digested by catla. This is particularly evident in the case of Azolla and soybean, where, in spite of a reduction in dietary fat content with their incorporation, there was an improvement in fat digestibility. Dietary fat at higher levels (>10.28%) had a negative effect on PDingilthead bream [52]. In contrast, in catla, silkworm pupa diets having fat above 10% recorded higher ($p<0.05$) fat digestibility. Lipid supplementation studies have shown levels of 5-12% to be optimal for carp diets [54-56]. Formulated diets having 9.99, 13.26 and 16.91% fat with 3,6 and 9% pupa oil supplementation, respectively, had higher fat digestibility in common carp compared to control diet having 7.35% fat [57].

Carbohydrate digestibility in fish depends on the type, inclusion level and the processing treatments applied to it as reported for carp [58], yellow tail, *Seriola quinqueradiata* [59], rainbow trout, *Salmo gairdneri* [60] and cod, *Gadus morhua* [61]. The digestibility of NFE in the present study ranged between 77.37 and 86.66%. Herbivores and omnivores fish are known to utilize carbohydrate better [42]. Catla, being an omnivore, is expected to better utilize carbohydrate from feed ingredients. Dietary fat level is reported to have a negative effect on starch digestibility in sea bream [52]. In the present study, the increase in dietary fat levels due to pupa incorporation, however, did not have a negative effect on NFE digestibility in catla [62,63].

Conclusion

The study reveals the usefulness of the tested ingredients for inclusion in catla diets. Azolla can be profitably used up to 20%, while due to the higher protein and fat digestibility, soybean

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and silkworm pupae can be used at 40% and 30%, respectively. Further, there is scope for trying still higher levels of soybean in the diet of catla since the highest level tried has shown the best results.

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