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Fish biology

EFFECT OF FERMENTATION ON APPARENT TOTAL AND NUTRIENT DIGESTIBILITY OF SESAME (*SEASAMUM INDICUM*) SEED MEAL IN ROHU, *LABEO ROHITA* (HAMILTON) FINGERLINGS

WPŁYW FERMENTACJI NA STRAWNOŚĆ MĄCZKI Z NASION SEZAMU (*SEASAMUM INDICUM*) U PALCZAKÓW GRUBOWARGA INDYJSKIEGO, *LABEO ROCHITA* (HAMILTON)

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The apparent digestibility of both raw and treated (fermented) sesame (*Seasamum indicum*) seed meal by rohu, *Labeo rohita*, fingerlings was evaluated at 20, 30, and 40% levels of incorporation for each, using fishmeal based feed as the reference diet. The anti-nutritional factor, phytic acid, from raw sesame seed meal, could be reduced below detection limit by fermentation with lactic acid bacteria (*Lactobacillus acidophilus*). Fermentation of the oilseed meal resulted in reduction of tannin content from 2 to 1%. The dry matter digestibility from the fermented sesame seed meal was highest at 20% inclusion level. The protein digestibility at all levels of inclusion was high in comparison with that from the raw sesame seed meal diet. A similar trend was noticed with regard to fat digestibility. The results indicate that the digestibility of nutrients largely depend on the nature and level of incorporation of the ingredients. It further shows that sesame seed meal can be incorporated at a higher level (up to 40%) in the diet of rohu fingerlings, after suitable processing (fermentation).

INTRODUCTION

Plant oilseeds and their by-products usually constitute a major source of dietary protein within aquafeeds for warm water omnivorous/herbivorous fish species (Akiyama 1991; Lim and Dominy 1991). Some of the factors which limit incorporation of these ingredients at high levels in fish feeds are low protein content, amino-acid imbalance and presence of anti-nutritional factors (Wee 1991). Moreover, another problem of selecting these ingredients in aquafeeds is the lack of information on their digestibility. In feed formulation and manufacture, it is essential to have a knowledge of the digestibility of the main ingredients, as well as of the whole diet (De Silva and Anderson 1995). In India, very few studies have been conducted on the digestibility of locally available feed ingredients in carps (Jayaram and Shetty 1980; Nandeesha et al. 1991; Ray and Das 1994; Mukhopadhyay and Ray 1997).

Sesame seed meal is often used as a dietary ingredient in fish feed. Tacon (1993) suggested that maximum level of its inclusion is 35% in both omnivorous and herbivorous fish species. Hossain and Jauncey (1989) worked on protein, energy and amino acid digestibility of sesame seed meal in common carp, *Cyprinus carpio* L. They further worked on Bangladeshi variety of sesame oilseed meal in *Cyprinus carpio* L. and found that it can be included up to 25% in raw condition. Sesame usually contains anti-nutritional factor phytic acid which either forms complex with protein or binds with metal ions such as calcium and magnesium inhibiting the absorption of these important minerals (Gohl 1981). Hossain and Jauncey (1990) worked on detoxification of sesame oilseed meal and evaluated its nutritional value in *Cyprinus carpio* L. Hossain et al. (1992) substituted fish meat with sesame oilseed meal and tested it. in catfish, *Heteropneustes fossilis* and reported promising results.

It was the standard practice earlier to use 7 : 3 ratio of reference diet to ingredient to determine the digestibility of an ingredient (Cho et al. 1974). De Silva et al. (1990) however, have shown that the more desirable mix of the reference diet to ingredient would be 80–85% to 20–15%. In the present investigation an attempt has been made to evaluate the suitability of incorporation of raw and fermented sesame seed meal into the diets of *Labeo rohita* (Hamilton) fingerlings by determining the digestibility of coefficient of the ingredient. In addition, the digestibility coefficient of the diets was determined incorporating both types (raw and fermented) of sesame seed meal at different inclusion levels (20–40%) to ascertain the best level of incorporation of the ingredient.

MATERIAL AND METHODS

Experimental diets

Two types of deoiled sesame (*Seasamum indicum*) meals were used: raw and fermented as test ingredients. Prior to incorporation, a portion of the sesame seed meal was fermented by inoculating it with lactic acid (*Lactobacillus acidophilus*) bacteria. A reference diet was prepared according to Ray and Das (1994) using fish meal as the chief protein source. Six experimental diets (diets Dl to D6) were formulated using raw and fermented sesame seed meals. Finely powdered test ingredients (400 μm) were incorporated at 3 different levels for each namely 20, 30, and 40% by replacing equal proportions of all the ingredients from the reference diet. To each of the formulated diets, 1% chromic oxide was added as an external marker (Table 1). All the diets were in pelleted form using carboxymethyl cellulose as a binder.

Table 1

Ingredient composition (% dry weight) and proximate composition of the experimental diets (on dry matter basis)

Diets	Reference diet (RD)	Raw			Fermented		
		D1	D2	D3	D4	D5	D6
Ingredients							
Fish meal	40.0	32.0	28.0	24.0	32.0	28.0	24.0
Mustard oilcake	23.0	24.0	28.0	29.0	24.0	28.0	29.0
Rice bran	35.0	22.0	12.0	5.0	22.0	12.0	5.0
Sesame (raw)	—	20.0	30.0	40.0	—	—	—
Sesame (fermented)	—	––	—	—	20.0	30.0	40.0
Premix [1]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Chromic oxide	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Proximate composition (%)							
Dry matter	90.10	91.30	90.80	90.60	91.20	90.79	89.63
Crude protein	35.94	35.00	35.12	34.99	35.24	35.55	35.17
Lipid	6.72	6.71	8.98	9.40	7.01	7.09	7.11
Ash	15.39	13.63	13.60	12.20	12.59	11.42	10.64
Crude fibre	11.70	12.50	12.20	12.47	12.28	11.83	11.99
Nitrogen-free extract	20.35	23.46	20.90	21.54	24.08	24.90	24.72
Gross energy (kcal/g)	3.98	4.09	4.19	4.26	4.14	4.18	4.16
Tannin (%)	—	0.41	0.62	0.87	0.21	0.30	0.38
Phytic acid	—	0.34	0.51	0.68	—	—	—

[1] Vitamin and mineral mixture (Vitaminetes forte, Roche Products Ltd., Pt.M.M. Malviya Road Bombay 400034, India).

Experimental design

The experiment was conducted in a static indoor rearing system containing 90 dm^3 glass aquaria with continuous aeration. Rohu, *Labeo rohita* fingerlings were obtained from a local fish seed dealer and acclimated to the laboratory condition for 15 days and fed with a 1 : 1 mixture of rice bran and mustard oilcake. Fish fingerlings (mean weight 1.50 ±0.11 g) were randomly distributed at the rate of 10 fish per aquarium with two replicates for each treatment. Each experimental aquarium was fed with dechlorinated water from a deep tube well. The experimental fish were fed with the formulated diets twice daily at 0900 hours and 1500 hours at a fixed feeding rate of 5% wet body weight per day for the whole experimental period of 60 days duration. The quantity of feed given was readjusted every 10^{th} day after weighing the fish. The uneaten feed was collected after 1 h by siphoning after each feeding. The faecal samples were collected following the method outlined by Spyridakis et al. (1989). The faeces naturally released by the fish were easily detectable and removed from the water with a glass canula. The collection was performed everyday, 17 h after the last feeding. Faeces collected from replicate treatments were pooled, dried at 60°C in an oven, and stored for subsequent analysis. Pooled faecal samples for each treatment were analysed separately. At the termination of the experiment, all the fish were weighed individually and used for subsequent analysis. The water in each tank was changed every day throughout the experimental period in order to maintain the water quality. The ranges of the water quality parameters were: temperature, 26–30°C; pH, 6.3–7.3; dissolved oxygen, 4.9–7.7 $mg \cdot dm^{-3}$ and alkalinity, 189–1 90 $mg \cdot dm^{-3}$.

Chemical and statistical analyses:

Feed ingredients, experimental diets, faecal samples, and carcasses were analysed for proximate composition following the AOAC procedures (1990). Chromic oxide in the diets and faeces were estimated spectrophotometrically following the method of Bolin et al. (1952). The tannin content in the deoiled seed meals were determined using Folin-Denis reagent (Schanderi 1970), and the phytic acid was determined following the method outlined by Wheeler and Ferret (1971). Apparent total and nutrient digestibility values were calculated by employing the following formulae (De Silva and Anderson 1995):

(a) Apparent total digestibility of the reference (RD) and test diets:

(TD) (%)= 100 – 100 (% marker in diet / % marker in faeces)

(b) Apparent nutrient digestibility of the reference and test diets:

100 – 100 (% marker in diet / % marker in faeces × % nutrient in faeces / % nutrient in diet)

where, nutrient refers to any nutrient, such as protein or fat.

(c) Apparent dry matter digestibility of the ingredient:

100 / % test ingredients (Apparent total digestibility of TD – %RD / 100 × Apparent total digestibility of RD)

(d) Apparent nutrient digestibility of the ingredient:

100 / % test ingredient (Nutrient digestibility of TD – %RD / 100 – Nutrient digestibility of RD).

Statistical analyses of the data were made by analysis of variance (ANOVA) followed by Scheff's F-test for multiple comparisons (Das and Das 1993). Data were considered significant at the 0.01 level.

RESULTS

The proximate composition of sesame seed meal and other ingredients are presented in Table 2. The ingredient composition (% dry weight) and proximate composition of the experimental diets are presented in Table 1.The anti-nutritional factor, phytic acid from sesame seed meal, could be reduced below detection limit by fermentation with lactic acid bacteria (*Lactobacillus acidophilus*). Fermentation of the oilseed meal resulted in reduction of tannin content from 2 to 1%. The data regarding apparent dry matter (or total) and nutrient digestibility of the reference and test diets are presented in Table 3. The total digestibility of the reference and test diets was significantly lower ($P < 0.01$) than that for diets containing 20% raw and 40% treated sesame seed meal. The apparent protein digestibility was however, significantly ($P < 0.01$) higher at all the levels of incorporation containing fermented sesame oilseed meal, while diets containing raw sesame seed meal resulted poor protein digestibility. The apparent digestibility (AD) of protein was significantly ($P < 0.01$) higher than that of from the reference diet up to 40% levels of inclusion of treated (fermented) sesame seed meal. The apparent fat digestibility was significantly ($P < 0.01$) higher for the treated (fermented) sesame seed meal incorporated diet up to 40% level of incorporation as compared to that for the reference diet. At higher levels of inclusion of raw sesame seed meal a sharp decline in AD of fat was noticed. The AD of ash was significantly ($P < 0.01$) higher from diet D3, containing 40% raw sesame seed meal. The dry matter (or total) and nutrient digestibility from the test ingredient (both raw and fermented) at different levels of inclusion is presented in Table 4. The dry matter digestibility from the raw sesame seed meal at 20% inclusion level was recorded highest (89%). Lowest value (67.63%) of dry matter digestibility was obtained from fermented sesame seed meal at 30% level of inclusion which was not significantly different from that of the raw oilseed meal at 30% and 40% inclusion level. The protein from the fermented sesame seed meal was better digested at all levels of inclusion, the values ranging from 97.60 to 98.36%. A similar trend was noticed with regard to apparent fat digestibility. In general, the digestibility of ash from the fermented sesame oilseed meal was better digested at 20% inclusion level.

Table 2

Proximate composition of feed ingredients (% dry matter basis

Proximate composition	Fish meal	Mustard oilcake	Ricebran	Raw sesame meal	Fermented sesame meal
Moisture	2.96	14.00	4.45	2.83	2.82
Crude protein	58.50	35.93	13.00	24.41	25.16
Lipid	8.91	7.00	5.14	5.68	6.72
Ash	11.50	8.37	21.41	11.09	11.09
Crude fibre	3.93	5.53	25.50	21.62	21.60
Nitrogen free extract (NFE)	14.20	29.17	30.50	34.37	32.61
Gross energy (kcal/g)	4.19	4.11	3.52	4.21	4.21
Tannin	—	—	—	2.00	1.04
Phytic acid	—	—	—	1.71	—

Table 3

Apparent dry matter (or total) and nutrient digestibility (%) of raw and fermented test ingredients

Nutrient	D1	D2	D3	D4	D5	D6
Dry matter (or total)	89.00 ±0.16^c	68.69 ±0.20^a	71.10 ±0.14^a	74.60 ±0.11^b	67.63 ±0.13^a	74.10 ±0.09^b
Protein	68.70 ±0.11^a	71.06 ±0.10^a	72.57 ±0.13^a	97.60 ±0.09^b	98.36 ±0.17^b	98.15 ±0.08^b
Fat	89.45 ±0.21^b	77.09 ±0.20^a	74.90 ±0.21^a	95.40 ±0.19^c	93.91 ±0.20^c	93.25 ±0.18^c
Ash	41.15 ±0.16^a	51.08 ±0.11^c	52.88 ±0.09^c	58.70 ±0.15^d	49.22 ±0.20^c	46.88 ±0.13^b

Figures having the same superscript in the same row are not significant different ($P < 0.1$).

Table 4

Apparent dry matter (or total) and nutrient digestibility (%) of the reference and test diets

Nutrient	RD	D1	D2	D3	D4	D5	D6
Dry matter (or total)	70.32 ±0.16^a	74.05 ±0.11^c	69.85 ±0.12^a	70.63 ±0.20^a	71.17 ±0.19^a	69.53 ±0.16^a	71.83 ±0.09^b
Protein	88.97 ±0.11^d	84.91 ±0.17^a	83.61 ±0.09^a	82.41 ±0.12^a	90.69 ±0.20^c	91.81 ±0.19^c	92.64 ±0.16^d
Fat	89.42 ±0.21^b	89.42 ±0.19^b	85.74 ±0.11^a	83.61 ±0.09^a	90.61 ±0.13^c	90.79 ±0.17^c	90.95 ±0.19^c
Ash	46.11 ±0.18^a	45.11 ±0.21^a	47.61 ±0.23^b	48.81 ±0.22^c	48.62 ±0.16^c	47.05 ±0.19^b	46.41 ±0.20^a

Figures having the same superscript in the same row are not significant different ($P < 0.1$).

DISCUSSION

The replacement of fishmeal with alternate sources of protein have met with varied degree of success depending on the nature and composition of ingredients, inclusion level and method of processing (De Silva and Gunasekara 1991; Ray and Das 1992; Mukhopadhyay and Ray 1996, 1997, 1998, 1999). The present results are a clear evidence for the varied degree of digestibility on the nature, source and composition of ingredients and their level of inclusion. The apparent digestibility values for protein and lipid for the reference diet (88.97% and 89.42%, respectively) were highest in comparison to those of the raw sesame seed meal incorporated diets. This is in agreement with the values reported for carp and other species (Smith et al. 1980; Hossain and Jauncey 1989). The values of apparent digestibility of protein and lipid for the reference diet obtained during the present investigation were nearly equal to those obtained by Hossain and Jauncey (1989). The values of apparent digestibility of protein and lipid of the test diets containing raw sesame seed meal are comparable to those found by Hasan (1986) in common carp fry diets containing different proportions of raw mustard, linseed and sesame seed meals. Hossain and Jauncey (1989) found similar results in *Cyprinus capio* diets containing graded levels of raw sesame seed meal. During the present investigation, a progressive decline in apparent digestibility of protein and fat was noticed with increasing levels of incorporation of raw sesame seed meal. On the contrary, an increasing trend in AD of both protein and fat was noticed with increasing level of incorporation of fermented sesame seed meal, being highest with 40% inclusion level. Hossain and Jauncey (1989) also reported a progressive decline in apparent protein and fat digestibilities in *Cyprinus carpio* with increasing level of inclusion of sesame seed meal. De Silva et al. (1990) reported reduction in dry matter and protein digestibility with decreasing dietary protein content and increasing ash and fibre contents. In the present study, all the test diets were isonitrogenous (35% protein) and did not contain higher amounts of ash and crude fibre (ash: 10.64–13.63%; crude fibre: 11.83–12.50%).

It is generally recognised that digestibility data are useful only when ingredients do not contain gossypol-like substances, tannin, complex polysaccharides, antitrypsin, and other interfering substances which may influence the digestibility of various nutrients in the diet and give erroneous results (Lall 1991). In the present study diets containing raw sesame seed meal at 20, 30, and 40% levels of inclusion contained 0.41%, 0.62%, and 0.87% tannin and phytic acid may have been responsible for the observed declining trend in apparent digestibility of protein and fat with increasing level of inclusion of raw sesame seed meal. The diets containing fermented sesame seed meal contained 0.21%, 0.30%, and 0.38% tannin at 20, 30, and 40% inclusion levels, respectively. As stated earlier, the fermentation of the sesame seed meal resulted in complete elimination of phytic acid. Better digestibility of protein and lipid from the fermented sesame seed meal incorporated diets may be attributed to the absence of phytic acid in those diets. Teutino and Knorr (1985) also reported increased digestibility of protein and fat from the fermented samples of misco (rice + soya), wheat soybean tempeh and natto (soybean) in human. There is, however, no report on the digestibility of nutrients from fermented oilseed meal in fish.

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WPŁYW FERMENTACJI NA STRAWNOŚĆ MĄCZKI Z NASION SEZAMU (*SEASAMUM INDICUM*) U PALCZAKÓW GRUBOWARGA INDYJSKIEGO, *LABEO ROCHITA* (HAMILTON)

STRESZCZENIE

Pozorna strawność surowej oraz fermentowanej mączki z nasion sezamu (*Seasamum indicum*) u palczaków grubowarga indyjskiego, *Labeo rochita* była badana przy zawartości: 20, 30 i 40% każdej z nich, stosując jako dietę kontrolną paszę opartą na mączce rybnej. Fermentacja z udziałem bakterii kwasu mlekowego (*Lactobacillus acidophilus*) obniżała zawartość substancji antyżywieniowej – kwas fitynowy – z surowej mączki sezamowej do poziomu poniżej wykrywania. Fermentacja mączki sezamowej powodowała redukcję zawartości taniny z 2 do 1%. Strawność suchej masy fermentowanej mączki sezamowej była najwyższa przy 20% jej zawartości w paszy. Strawność białka przy wszystkich poziomach zawartości mączki była wysoka w porównaniu ze strawnością białka w paszy zawierającej surową mączkę sezamową. Podobną tendencję zaobserwowano w odniesieniu do strawności tłuszczu. Otrzymane wyniki sugerują, że strawność substancji odżywczych zależy głównie od ich rodzaju i zawartości w paszy. Stwierdzono ponadto, że mączka z nasion sezamu może być dodawana w większej ilości (do 40%) do paszy palczaków grubowarga indyjskiego, po jej uprzedniej fermentacji.

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