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Evaluation of some plant ingredients as dietary protein sources for common carp (*Cyprinus carpio* L.) fry

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Abstract

A laboratory growth trial was conducted to evaluate the suitability of various oilseed cakes and leucaena leaf meal as dietary protein sources for common carp fry (mean ± SE weight, 50 ± 1.6 mg). Eleven experimental diets were formulated containing different levels of mustard, sesame, linseed, copra and groundnut oil cakes and leucaena leaf meal to substitute for fish meal protein up to a maximum of 75% of the total protein content. Sesame oil cake was tested at three inclusion levels (25, 50 and 75% of total protein), whereas mustard (25 and 50%), linseed (25 and 50%) and groundnut (25 and 75%) oil cakes were each tested at two levels. Copra and leucaena were tested at one inclusion level (25% of total protein) only. The control diet was prepared with fish meal as the sole source of protein. All diets were isonitrogenous and contained about 40% protein. The experiment was conducted in a laboratory recirculation system with three replications for each treatment. The performances of the diets were evaluated on the basis of feed acceptability, survival, growth, feed conversion, protein utilisation, protein digestibility, body composition and histopathological changes. The results of the study showed that growth and performance were significantly affected by the type of plant protein as well as level of inclusion. There was no significant ($P > 0.05$) variation in the observed growth responses and feed conversion ratios among the control diet and the diets containing 25% linseed and 25% groundnut oil cakes. The diet containing 25% leucaena meal showed the poorest performance. Similar trends were also observed for protein efficiency ratio and apparent net protein utilisation. Apparent protein

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digestibilities were broadly similar (78–90%) for all diets except the diet containing 25% leucaena meal which showed the poorest value (63%). With the exception of the control and 25% leucaena diets, the carcass composition of experimental fish was relatively unaffected by different dietary treatments. Histopathological examination of liver revealed higher levels of intracellular lipid deposition in fish fed diet containing mustard cake. A large proportion of fish fed the 75% groundnut diet showed severe deformations of the body. Based on these results, the possibility of using plant ingredients as a partial replacement for fish meal in diets for the common carp fry is discussed. © 1997 Elsevier Science B.V.

Keywords: Fish nutrition; Common carp; *Cyprinus carpio*; Plant protein sources; Protein digestibility

1. Introduction

Various oilseed cakes and meals are produced in Bangladesh on a large scale as by-products of the edible oil industry. These include mustard, linseed, sesame, groundnut and copra. These oil cakes and meals are fairly rich in protein and are traditionally used as feeds for farm animals.

In recent years, intensification of carp fry production in Bangladesh has made it essential to develop suitable complete and supplemental diets for use in hatcheries and nursery ponds. Traditionally, fish meal has been the main source of dietary protein for fish fry. But the increasing cost of fish meal has severely restricted its use as a protein source for fry diets. Therefore, it is a matter of urgency that alternative protein sources for carp fry diets be found to support aquaculture development in the country.

The efficiency of various alternative protein sources as partial or complete dietary replacements for fish meal has been evaluated in fish diets, e.g. poultry by-product meal (Higgs et al., 1979), poultry offal meal (Hasan and Das, 1993), rapeseed meal (Higgs et al., 1979) and single cell protein (Atack et al., 1979). However, there is a paucity of information on the efficiency of mustard, linseed, sesame, groundnut and copra oil cakes or meals as alternative protein sources in feeds of carp fry. Hossain and Jauncey (1989) evaluated mustard, linseed and sesame oil cakes of Bangladeshi origin as dietary protein sources for the common carp fingerlings. However, there is no such information for common carp fry. Nevertheless, many of these ingredients have been used as dietary protein sources for other fish species, i.e. linseed (Hasan et al., 1989, 1991), mustard (Hasan et al., 1991), groundnut meal (Wu and Jan, 1977; Cruz and Laudencia, 1978; Jackson et al., 1982) and sesame (Hasan et al., 1991).

Leucaena leucocephala (ipil ipil), a leguminous plant, is grown in Bangladesh and used principally as cattle and poultry feed. It is fairly rich in protein and has been used as fish feed with variable success with different fish species (Jackson et al., 1982; Wee and Wang, 1987; Hasan et al., 1988, 1990, 1994; Santiago et al., 1988).

This study was undertaken to determine the nutritive value of mustard, linseed, groundnut, sesame, copra and leucaena meal fed to common carp fry in the form of compounded pelleted diets. The various plant protein sources were used at different dietary inclusion levels depending on their protein content. The aim of the investigation was to determine maximum inclusion levels for different plant protein sources and to identify the limitations to their use arising from palatability or apparent toxicity factors.

2. Materials and methods

The study was conducted in a laboratory recirculated water system, consisting of 36 8-l self-cleaning circular polypropylene tanks. Fry of mirror variety of common carp (*Cyprinus carpio*, Dinkesbuhl strain) (mean ± SE weight, 50 ± 1.6 mg) were used. Prior to the start of experiment, the fry were acclimatised to laboratory conditions for 3 days on ground commercial trout pellet (Edward Baker's Omega No. 3; protein content 49%). The experiment was carried out in the Tropical Aquarium Building of the Institute of Aquaculture, University of Stirling, UK. A water temperature of 27–29°C was maintained using thermostatic heaters (R. Aitchisens Ltd., Edinburgh) submerged in the header tank. A photoperiod of 12 h light:12 h dark was operated throughout the experimental period.

Twelve semi-purified isonitrogenous diets were formulated to evaluate mustard, sesame, linseed, copra, groundnut and leucaena meal as dietary protein sources for common carp fry. The control diet was prepared using herring meal as the source of protein. Mustard oil cake (Indian mustard, *Brassica juncea)* was brought directly from Bangladesh. Linseed and copra cake were obtained from Protein Supplements Ltd., Brakes Farm, Suffolk, UK and leucaena leaf (origin Malawi) and groundnut meal were obtained from the Tropical Development Research Institute (TDRI), London. Hexane extracted sesame meal was obtained locally from Stirling. Mustard, linseed and copra meal were expeller cakes whereas groundnut and sesame meal were solvent extracted.

The dietary protein sources and herring fish meal were analysed for proximate composition and amino acid content (Table 1) prior to the formulation of diets. All the diets were formulated to contain 40% protein (Ogino and Saito, 1970), 10–11% lipid (National Research Council, 1983), 6–8% crude fibre (Jauncey and Ross, 1982) and 25–30% digestible carbohydrate (Sen et al., 1978). Diets were also formulated to be isoenergetic. Wherever possible, the dietary plant protein was tested at three inclusion levels (25, 50 and 75% of the total protein). Both mustard and linseed meal were tested at two inclusion levels (25 and 50% of total protein). Sesame meal was tested at three inclusion levels (25, 50 and 75%), whereas groundnut meal was tested at two levels (25 and 75% of total protein). Leucaena and copra were tested at one inclusion level only (25% of total protein).

Each diet was formulated to contain 0.5% chromic oxide to study protein digestibility. The composition of the experimental diets are presented in Table 2. The proximate composition analysis and the calculated level of amino acids with the chemical score of each diet are presented in Tables 2 and 3, respectively. The diets were prepared by mixing the dry ingredients (ground to pass a 300 μm sieve), followed by the addition of oil and water until a stiff dough was obtained. The moist diet was then extruded through a mincer with a 2.0 mm die. The resultant pellets were then air dried at about 35°C using an electric fan convector heater. These pellets were ground by grinder and sieved to obtain particle sizes ranging between 300 and 500 μm as this size range was found to be optimum for carp fry weighing 50–100 mg (Hasan and Macintosh, 1992).

Feed ingredients, experimental diets and fish samples were analysed for their proximate composition according to the methods of the Association of Official Analytical Chemists (1970). The gross energy (GE) content of the diets was calculated using

Table 1
Proximate composition (% dry matter) and amino acid content (% crude protein) of dietary ingredients

Components	Ingredient						
	Fish meal	Mustard	Sesame	Linseed	Groundnut	Copra	Leucaena
Dry matter	93.61	92.53	95.34	90.52	88.05	90.04	92.39
Crude protein	74.95	34.71	50.72	38.08	59.01	24.60	30.22
Crude lipid	10.89	6.34	11.84	8.31	0.18	1.73	2.27
Ash	13.03	11.26	9.43	5.98	9.44	9.66	7.62
Crude fibre	1.18	13.40	7.44	10.05	12.06	13.95	21.92
NFE [a]	–	34.28	20.57	37.58	19.31	50.06	37.97
Amino acid content							
Arginine	7.79	5.85	10.24	7.45	11.31	9.19	4.53
Histidine	2.56	2.30	2.06	1.79	2.61	1.56	1.49
Isoleucine	4.05	3.25	2.60	3.10	3.24	2.80	6.53
Leucine	7.63	6.16	5.34	5.07	6.88	6.40	6.09
Lysine	7.84	4.25	2.25	3.22	3.49	2.45	4.82
Methionine	2.99	1.12	1.87	0.89	0.87	1.36	0.35
Phenylalanine	3.91	3.77	3.58	3.64	5.17	4.23	4.35
Threonine	4.64	3.51	2.68	2.99	2.82	2.78	3.70
Tryptophan [b]	1.08	1.19	1.61	1.41	0.96	0.96	–
Valine	5.87	3.96	3.37	3.76	3.86	4.70	4.03
Cystine	1.14	1.25	0.85	1.15	1.60	0.89	0.36
Tyrosine	3.34	2.50	2.82	2.06	3.93	3.03	3.47

[a] Nitrogen-free extracts.
[b] Tryptophan values were obtained from the National Research Council (1983) except that of mustard cake, which was obtained from Capper et al. (1982).

kilocaloric values of 5.5 g^{-1} protein, 9.1 g^{-1} lipid and 4.1 g^{-1} carbohydrate. The metabolisable energy (ME) content of the diets was calculated using kilocaloric values of 4.5 g^{-1} protein, 8.51 g^{-1} lipid and 3.49 g^{-1} carbohydrate (Jauncey, 1982b). The chromic oxide content of the experimental diets and faeces was determined by the acid digestion method of Furukawa and Tsukahara (1966).

All dietary protein sources were analysed for their amino acid content using an LKB 4151 Alpha plus amino acid analyser (LKB Biochrom Ltd., Cambridge). The amino acid

Notes to Table 2:
[a] Sodium carboxymethyl cellulose (high viscosity).
[b] Contained (kg^{-1} mix): thiamin, 2.5 g; riboflavin, 2.5 g; pyridoxine, 2.0 g; pantothenic acid, 5.0 g; inositol, 100.0 g; biotin, 0.3 g; folic acid, 0.75 g; para-aminobenzoic acid, 2.5 g; choline, 200 g; niacin, 10.0 g; cyanocobalamin, 0.005 g; retinol palmitate, 100000 IU; α-tocopherol acetate, 20.1 g; ascorbic acid, 50.0 g; menadione, 2.0 g; cholecalciferol, 500000 IU.
[c] Contained (g kg^{-1} mix): $MgSO_4 \cdot 2H_2O$, 127.5; KCl, 50.0; NaCl, 60.0; $CaHPO_4 \cdot 2H_2O$, 727.8; $FeSO_4 \cdot 7H_2O$, 25.0; $ZnSO_4 \cdot 7H_2O$, 5.5; $CuSO_4 \cdot 5H_2O$, 0.785; $MnSO_4 \cdot 4H_2O$, 2.54; $CoSO_4 \cdot 4H_2O$, 0.478; $Ca(IO_3)_2 \cdot 6H_2O$, 0.295; $CrCl_3 \cdot 6H_2O$, 0.128.
[d] Expressed as percent dry matter unless indicated otherwise.
[e] Calculated as crude fibre derived from test protein source and α-cellulose.
[f] Nitrogen-free extractive, calculated as NFE derived from test protein source, corn starch and dextrin.
[g] Protein to energy ratio in mg protein/kcal ME.

Table 2
Formulation (% dry weight) and chemical composition of the experimental diets

	Diet											
	1	2	3	4	5	6	7	8	9	10	11	12
Ingredient												
Mustard	–	28.81	57.62	–	–	–	–	–	–	–	–	–
Sesame	–	–	–	19.72	39.44	59.15	–	–	–	–	–	–
Linseed	–	–	–	–	–	–	26.26	52.52	–	–	–	–
Leucaena	–	–	–	–	–	–	–	–	33.09	–	–	–
Copra	–	–	–	–	–	–	–	–	–	40.65	–	–
Groundnut	–	–	–	–	–	–	–	–	–	–	16.95	50.84
Fish meal	53.37	40.03	26.68	40.03	26.68	13.34	40.03	26.68	40.03	40.03	40.03	13.34
Cod liver oil	1.50	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	4.00
Corn oil	3.50	3.00	3.00	3.00	2.50	2.00	3.00	3.00	4.00	4.00	4.00	4.50
Binder [a]	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Vitamin mix [b]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Mineral mix [c]	4.00	3.00	3.00	4.00	3.50	3.50	4.00	4.00	4.00	3.00	4.00	4.00
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
α-cellulose	6.00	2.00	–	4.50	3.00	2.00	3.00	1.00	–	–	4.00	–
Dextrin	16.50	10.00	2.50	13.75	11.00	8.50	10.00	4.00	8.00	4.00	15.00	12.00
Corn starch	11.13	7.16	1.20	9.00	7.88	5.51	7.71	2.80	4.88	2.32	10.02	7.32
Chemical composition [d]												
Crude protein	42.53	40.83	41.37	40.91	40.67	40.30	41.25	41.39	41.05	39.56	40.51	39.77
Crude lipid	10.76	10.95	10.39	11.72	12.31	13.06	10.84	11.87	11.56	11.69	10.78	9.89
Ash	10.70	11.96	13.02	11.01	11.36	10.85	10.34	10.27	11.70	12.75	10.68	10.61
Crude fibre [e]	6.63	6.33	8.03	6.44	6.25	6.56	6.11	6.59	7.72	6.14	6.51	6.29
NFE [f]	27.63	27.04	23.46	26.81	27.00	26.18	27.58	26.54	25.44	26.67	28.29	29.14
Chromic oxide	0.49	0.50	0.50	0.47	0.50	0.47	0.48	0.52	0.45	0.45	0.50	0.48
Gross energy (kcal g^{-1})	4.45	4.35	4.18	4.42	4.46	4.48	4.39	4.44	4.35	4.33	4.37	4.28
Metabolisable energy (ME) (kcal g^{-1})	3.79	3.71	3.56	3.77	3.82	3.84	3.74	3.80	3.72	3.71	3.73	3.65
PE ratio [g]	112.1	110.0	116.1	108.4	106.5	105.0	110.3	109.0	110.4	106.8	108.7	109.0

tryptophan could not be analysed owing to its destruction during acid hydrolysis. Therefore, tryptophan values for all ingredients were obtained from the National Research Council (1983) except that for mustard oil cake, which was obtained from Capper et al. (1982). The chemical score of the protein of each diet was calculated after

Table 3
Calculated level of amino acids in different experimental diets and amino acid requirement for carp in a 38.5% protein diet (Nose, 1979) (% crude protein)

Amino acid	Requirement for carp	Diet											
		1	2	3	4	5	6	7	8	9	10	11	12
Arginine	4.3	7.79	7.31	6.82	8.40	9.02	9.63	7.71	7.62	6.98	8.14	6.67	10.43
Histidine	2.1	2.56	2.50	2.43	2.44	2.31	2.19	2.37	2.18	2.29	2.31	2.57	2.60
Isoleucine	2.5	4.05	3.85	3.65	3.69	3.33	2.96	3.81	3.58	4.67	3.74	3.85	3.44
Leucine	3.3	7.63	7.26	6.90	7.06	6.49	5.91	6.99	6.35	7.25	7.32	7.44	7.07
Lysine	5.7	7.84	6.94	6.05	6.44	5.05	3.65	6.69	5.53	6.09	6.49	6.75	4.58
Methionine	2.1 [a]	2.99	3.52	2.06	2.71	2.43	2.15	2.47	1.94	2.33	2.58	2.47	1.40
Phenylalanine	3.4 [b]	3.91	3.88	3.84	3.53	3.75	3.66	3.84	3.78	4.02	3.99	4.23	4.86
Threonine	3.9	4.64	4.36	4.08	4.15	3.66	3.17	4.23	3.82	4.41	4.18	4.19	3.28
Tryptophan	0.8	1.08	1.11	1.14	1.22	1.35	1.48	1.16	1.25	0.81	1.05	1.05	0.99
Valine	3.6	5.87	5.39	4.92	5.25	4.62	4.00	5.34	4.82	5.41	5.58	5.37	4.36
Cystine	–	1.14	1.17	1.20	1.07	1.00	0.92	1.14	1.15	0.95	1.08	1.26	1.49
Tyrosine	–	3.34	3.13	2.92	3.21	3.08	2.95	3.02	2.70	3.37	3.26	3.49	3.78
Chemical score (%) [b]		115.0	111.8	98.1	106.4	88.6	64.0	108.5	92.4	101.3	107.2	107.4	66.7

[a] The values of methionine and phenylalanine are the requirements in the presence of 2% cystine and 1% tyrosine of the diet, respectively.
[b] Chemical score calculated based on the amino acid requirement of carp (Nose, 1979).

Jauncey and Ross (1982). The physico-chemical characteristics of the water in the experimental culture system were measured following the procedure recommended by the American Public Health Association, American Water Works Association and Water Pollution Control Federation (1980). Temperature, pH, dissolved oxygen, total ammonia and nitrite levels of the water were monitored at weekly intervals throughout the experimental period and had values of 27–29°C, 5.87–7.80, 6.20–7.5 mg l^{-1}, 0.08–0.84 mg l^{-1} ammonia-N and 0.007–0.28 mg l^{-1} NO_2-N, respectively.

At the end of the trial, fish samples from each diet were fixed in 10% buffered formalin and histopathological examinations of the gills, liver, muscle, kidney, pancreas and intestine were carried out to observe any changes between different dietary treatments.

Fish fry were randomly distributed between tanks at 40 fish per tank; three replicate tanks were used for each experimental diet. A number of fish representing about 10% of the experimental population was netted from the stock tank and weighed individually to the nearest 1.0 mg to obtain an average initial weight for the experimental population. All fish were fed four times a day at 3-h intervals from 09:00 to 18:00 h. Initially, fish were fed at 25% body weight day^{-1} for the first 3 weeks and then at 20% body weight day^{-1} for the remaining 2 weeks. Over the course of the experiment, which lasted for 5 weeks, all fish from each tank were weighed collectively at weekly intervals and feeding rates adjusted accordingly. On termination of the experiment, fishes were weighed individually and mean weight for each tank was calculated.

During the last week of the experiment, faeces were collected from each experimental tank. One hour after the feed was administered, any feed and faeces present in the tank were removed. Fresh faeces produced by the fish after this period and before the second

feed was given, were siphoned out. At least two siphonings were done from each tank during each collection period to minimise nutrient leaching from the faeces. The collected faeces were filtered onto filter paper and dried at 105°C for 12 h. The faecal samples from each replicate tank were pooled and used for the determination of protein digestibility. The protein content of faeces was determined by the micro-Kjeldahl method.

Specific growth rate (SGR), weight gain (%), feed conversion ratio (FCR), protein efficiency ratio (PER), apparent net protein utilisation (ANPU) and apparent protein digestibility (APD) were calculated after Castell and Tiews (1980). Comparison of treatment means was carried out by one-way analysis of variance (ANOVA), followed by testing for pairwise differences using Newman–Keul's multiple range test (SNK test) (Zar, 1974). For comparison of mortalities between treatments, mortality values were subjected to arcsin transformation (Zar, 1974) and the resultant data were subjected to analysis of variance.

3. Results

All fish became acclimatised to the experimental diets within 2–3 days of the start of feeding. However, the acceptability of all diets was not equal. The control diet and diets containing 25 and 50% of linseed and 25% groundnut meal were generally quite acceptable, and in general, diets with higher inclusion of plant protein (50–75% of total protein) showed poorer acceptability. Diets containing leucaena meal, even at the low inclusion level (25% of protein), showed the poorest acceptability. However, diets containing 25% of protein from mustard, sesame and copra meal were found to be moderately acceptable to fish fry.

Mortalities were recorded daily throughout the experimental period. Significantly different mortalities were observed between some of the dietary treatments during the experimental period. Total mortality data are presented in Table 4. Mortality ranged from 0.8% (diet 1, control) to 56.7% (diet 3, 50% mustard). The diet containing 25% leucaena meal (diet 9) also resulted in very high mortality (40%), second only to diet 3.

A large proportion of fish fed on diet 12 (75% protein from groundnut) showed severe deformations of the body. A small proportion of the fish fed diets 6 (75% sesame) and 9 (25% leucaena) also showed malformations. Histopathological examinations of the gills, liver, muscle, kidney, panaceas and intestine of fish reared under different dietary conditions revealed no significant changes except for diets containing mustard meal. Liver histology revealed a higher level of intracellular lipid deposition in fish fed diet containing 25% mustard meal (diet 2) compared with control fish (Figs. 1 and 2). Owing to loss of the sample, the histology of fish fed diet 3 (50% mustard) was not examined.

The growth responses of fry fed the different diets are presented in Table 4. The growth response of carp fry was significantly affected by the type of plant protein as well as by the level of inclusion. SGR ranged from 3.53 for diet 9 (25% leucaena) to 7.15 for diet 1 (control). Of the various plant protein sources tested, the diets containing both linseed and groundnut meals at 25% inclusion level resulted in comparable growth to that of the control. The SGRs for diets 1 (control), 7 (25% linseed) and 11 (25%

Table 4
Mortality, growth, feed conversion and protein utilisation of common carp fry (mean ± SE initial weight, 50 ± 1.6 mg)

	Diet												
	1	2	3	4	5	6	7	8	9	10	11	12	± SE [a]
Final weight (mg)	612a	400d	233f	466c	400d	216f	569b	323e	173g	326e	545b	263f	14.41
Mortality (%)	0.8e	14.2cd	67.7a	3.3e	8.3de	26.7c	0.8e	3.3e	40.0b	15.0cd	1.7e	20.0cd	2.68
SGR	7.15a	5.94c	4.40ef	6.38b	5.93c	4.17f	6.95a	5.32d	3.53g	5.35d	6.82a	4.74e	0.14
SGR as % of control	100	83.1	61.5	89.2	82.9	58.3	97.2	74.4	49.4	74.8	95.4	66.3	–
FCR	2.26a	2.92bc	6.61g	2.82abc	3.24c	5.77f	2.33ab	3.13c	8.13h	3.80d	2.63abc	4.52e	0.15
PER	1.06a	0.86c	0.37h	0.87c	0.86d	0.43g	1.04a	0.77d	0.30j	0.67e	0.94b	0.56f	0.01
ANPU (%)	13.71a	10.24cd	4.53h	10.77bc	9.22de	5.04g	13.06a	9.84cd	3.87h	8.31e	11.60b	6.31f	0.32
APD (%)	77.8	85.8	86.2	78.7	86.1	88.9	83.1	82.4	63.3	81.0	84.4	89.5	–

[a] Standard error of treatment means, calculated from the residual mean square in the analysis of variance.
Figures in the same row followed by the same letters are not significantly ($P > 0.05$) different.

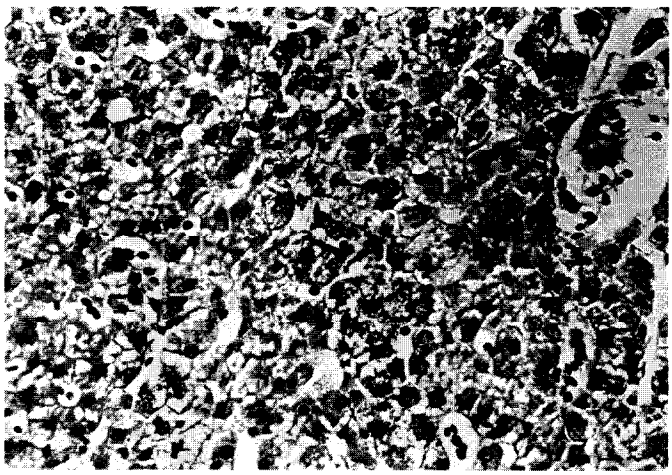

Fig. 1. Section of liver from carp fry fed the fish meal based control diet showing no visible intracellular lipid deposition (magnification × 400).

groundnut) were not significantly different from each other, but these were all significantly ($P < 0.05$) higher than the SGRs recorded for the other diets.

FCRs for the various diets tested are presented in Table 4. The control diet gave the best FCR (2.26), although this was not significantly different from the diets containing 25% linseed (2.33), 25% groundnut (2.63) and 25% sesame (2.82). Diets containing leucaena meal at the 25% level showed the poorest FCR (8.13). The level of protein utilisation of the different dietary protein sources was evaluated in terms of (a) protein efficiency ratio (PER) and (b) apparent net protein utilisation (ANPU%) and the values are presented in Table 4. The PER values of the control diet (1.06) and 25% linseed diet (1.04) were not significantly different, but both were significantly ($P < 0.05$) higher

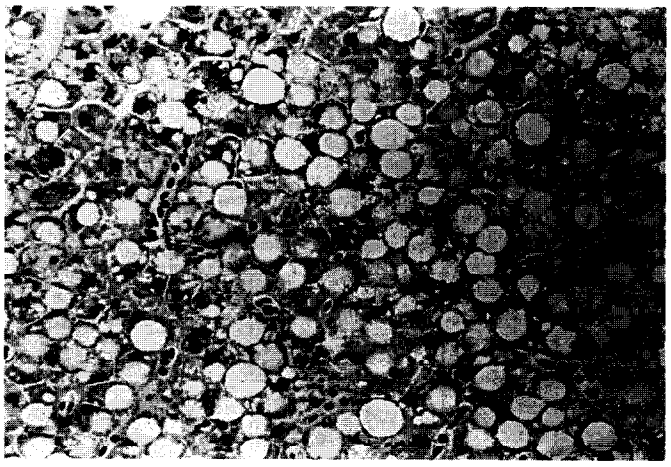

Fig. 2. Section of liver from carp fry fed diet 2 (25% mustard) showing severe intracellular lipid deposition (magnification × 400).

Table 5
Proximate carcass composition (% fresh weight) of fish samples at the start and end of the experiment

Components	Initial (all fish)	Final diet												
		1	2	3	4	5	6	7	8	9	10	11	12	± SE [a]
Moisture	82.7	8.4b	77.5d	77.9d	78.3d	77.9d	78.2d	79.6c	79.1c	82.2a	79.4c	79.0c	78.2	0.19
Crude protein	11.55	12.90a	12.35abc	12.23bc	12.44abc	12.26bc	11.84cd	12.45abc	12.54ab	11.59d	12.37abc	12.38abc	11.92bcd	0.13
Crude lipid	2.02	4.03g	7.52a	6.61c	6.31d	7.09b	6.72c	5.42f	5.52f	3.17h	5.55f	6.09e	7.14b	0.07
Ash	2.21	2.05	1.71	1.92	1.84	1.68	1.68	1.87	1.88	2.11	1.89	1.76	1.41	–

[a] Standard errors of treatment means calculated from the residual mean square in the analysis of variance.
Figures in the same row followed by the same letters are not significantly different ($P > 0.05$).

than for all the other diets. Diet 9 (25% leucaena) gave the poorest PER (0.30). Since the carcass protein contents of all the experimental fish groups were similar, the ANPU (%) values tended to reflect the PER values. The ANPU values ranged from 3.87% (diet 9) to 13.71% (control diet).

Apparent protein digestibility (APD) values are presented in Table 4. The control diet with fish meal as a protein source had fairly poor digestibility (77.8%). Apart from diet 9 (25% leucaena meal; APD 63.3%) and diet 4 (25% sesame meal; APD 78.7%), all the plant protein sources in combination with fish meal protein showed very good digestibility; the values ranged from 81.0 to 89.5%.

The proximate carcass composition of fish at the start and end of the trial are presented in Table 5. With the exception of fish fed diets 1 and 9, carcass composition was relatively unaffected by dietary treatment. Carcass moisture was highest ($P < 0.05$) for fish fed diet 9 (82.9%) followed by diet 1 (80.4%); the values for the other treatments ranged between 77.5 and 79.6%. The carcass lipid content was lowest ($P < 0.05$) for fish fed diet 9 (3.17%) followed by diet 1 (4.03%); the values for the other treatment groups ranged from 5.42 to 7.52%. The carcass crude protein and ash contents were similar for all dietary treatments and varied between 11.59 and 12.90% and 1.41–2.11%, respectively.

4. Discussion

The results of the present investigation indicate that linseed and groundnut meal at low inclusion levels (25% of protein) are good dietary protein sources for carp fry. Although the performance of sesame meal was not as good as linseed or groundnut, it proved to be a moderately good protein source for carp fry even at the 50% inclusion level. Leucaena and copra meal proved to be unsuitable even at low inclusion levels. Mustard meal was unsuitable at a high inclusion level (50% of protein), while at a low level, it did not produce growth comparable to linseed, groundnut or even sesame meal.

Several factors may have contributed to the observed variation in the growth responses of carp fry. These are:

1. variations in the acceptability of diets (Hasan and Macintosh, 1992; Hasan et al., 1989);
2. presence of various toxic and antinutritional factors such as glucosinolates in mustard oil cake (Tookey et al., 1980), phytic acid in sesame meal (Gohl, 1981), linamarin in linseed oil cake (Montgomery, 1980), mimosine in leucaena leaf meal (National Research Council, 1977; Liener, 1980) and aflatoxin in groundnut meal (Ashley, 1970);
3. imbalance of limiting essential amino acids in the diets containing higher levels of plant protein source (Jauncey and Ross, 1982);
4. variations in the digestibility of protein and energy (Jauncey, 1982a).

Despite none of the essential amino acids being limiting and with moderately good diet acceptability, diets containing mustard oil cake, even at 25%, showed poor growth and feed utilisation and high mortality. The above observations are in agreement with the findings of Capper et al. (1982) and Hossain and Jauncey (1989) for common carp fingerlings, and that of Hasan et al. (1991) for Indian major carp (*Labeo rohita*) fry.

The poor growth response and feed utilization of carp fry fed diets containing mustard meal were presumably due to the presence of toxic components in the mustard seed. Potentially the most toxic factors present in the mustard seed are glucosinolates, which upon hydrolysis by the enzyme thioglucosidase, usually present in the seed, yields highly toxic isothiocyanates (Tookey et al., 1980). Experimental evidence for the toxic action of isothiocyanates is well documented in rodents and poultry. Feeding rodents with rapeseed (*Brassica napus* and *Brassica campestris*) containing high levels of glucosinolates has been reported to cause depression of growth, marked enlargement of the thyroid, liver, adrenal and kidneys, delay in ovary development and histopathological changes in the pituitary (Tookey et al., 1980). An inclusion level of rapeseed meal as low as 5% of the diet has been shown to cause enlarged thyroid, depressed growth rate and perosis in poultry (McDonald et al., 1981).

The performance of carp fry fed 25% linseed meal protein was not significantly different from the control diet. With respect to growth, feed conversion, protein utilization and survival, the good performance of linseed meal may be attributed to its excellent acceptability. Although the linseed meal used in the present study was deficient in lysine, methionine and threonine, the prepared diet did not have any deficiency because of the amino acid profile contributed by the fish meal component. However, the significantly poorer growth response of carp fry at 50% linseed meal may be due to the presence of nutritional deficiency or growth inhibitory substances. Immature linseed contains a small amount of the cyanogenetic glucoside, linamarin, which in the presence of an associated enzyme, linase, liberates hydrogen cyanide (HCN) on hydrolysis. Unprocessed whole seeds, and linseed meal processed under low temperature, can be toxic to animals especially if wetted before being fed (Gohl, 1981; McDonald et al., 1981). Normal processing involving high temperature treatment, however, destroys linase and most of the linamarin, and the resultant meals are quite safe (Gohl, 1981; McDonald et al., 1981). The results of the present study support the finding of Hasan et al. (1989) for Asian catfish (*Clarias batrachus*), where no significant reduction in growth and feed conversion was observed when fish were fed diets containing 50% linseed oil cake.

The groundnut meal at low inclusion level (25% of protein) produced growth comparable to that of the control diet. However, when a higher inclusion (75% of protein; diet 12) was used, growth, feed conversion and protein utilisation were significantly reduced. The groundnut meal used in this study was deficient in three essential amino acids (lysine, methionine and threonine). Consequently, a higher level of groundnut resulted in amino acid deficiency in the diet, especially of methionine, giving a chemical score as low as 66.7% (Table 3). Similar depressed growth responses have been reported for *Oreochromis aureus* (Wu and Jan, 1977) and *Oreochromis mossambicus* (Jackson et al., 1982) when fed diets with high levels (50% or more) of groundnut meal.

Diets containing sesame meal at all three inclusion levels produced depressed growth and feed utilisation despite a good amino acid profile, and the absence of any known toxic factor (Liener, 1980). However, sesame seed has a high phytic acid content (Gohl, 1981) which has the capacity to bind di- and trivalent metal ions like phosphorus, calcium, magnesium, manganese, zinc and iron to form insoluble salts (Liener, 1977;

McDonald et al., 1981) and render them unavailable during digestion (Liener, 1977).

Animals with simple stomachs, such as fish, cannot utilise phytate-bound metal ions. Unless phytic acid is already saturated with metal ions naturally present in the plant and sufficient mineral supplementation is used, mineral deficiency may occur. The requirements for carp and other finfish for phosphorus, magnesium and zinc are well documented (Jauncey, 1982a).

Therefore, the presence of a high level of phytic acid in the sesame meal diets may have been responsible for the adverse growth and feed utilisation of carp fry. Sesame meal also has poor palatability, which might have reduced feed intake and resulted in poor performances. Essential amino acid deficiency might have been another factor contributing to the poor performance of carp fry. Diet 5 (50% sesame) had a deficiency of lysine, whereas diet 6 (75% sesame) was deficient in both lysine and threonine (Table 3). Moreover, during the processing of feedstuffs, the ϵ-amino group of the lysine may react with non-protein molecules present in the feedstuffs to form additional compounds that render lysine biologically unavailable, but chemically measurable (Cowey, 1979).

Leucaena leaf contains a toxic non-protein amino acid, mimosine, a lysine derivative (β-*N*(3-hydroxy-4-oxo-pyridine)α-amino propionic acid). It comprises about 3–5% of the dry weight of the protein of the leucaena leaf (National Research Council, 1977; Hasan et al., 1994). The poor performances of carp fry fed diets containing leucaena leaf are presumably due to the presence of this toxic amino acid in the leaf. In contrast to the widespread utilisation of leucaena in the rations of cattle and other terrestrial farm animals (National Research Council, 1977), feeding fish with unprocessed leucaena leaf has not proved successful.

Reduced performances when fed leucaena have been reported for Java tilapia (Jackson et al., 1982; Wee and Wang, 1987), Nile tilapia (Santiago et al., 1988), Indian major carps (Hasan et al., 1988, 1990, 1994). In contrast, improved growth performances in fish fed diets with leucaena as a dietary protein source have been reported for Java tilapia (Ghatnekar et al., 1983), Nile tilapia (Pantastico and Baldia, 1980) and Indian major carp (Ghatnekar et al., 1983).

Despite a high protein digestibility (81%) and a good amino acid profile (Table 3), the diet containing copra meal did not prove to be an acceptable protein source for carp fry. Copra meal is also not known to contain any toxic components (Jauncey and Ross, 1982). Published information regarding the efficiency of copra meal as a dietary protein source for fish is scarce. Cruz and Laudencia (1978) observed that complete or partial substitution of rice bran by copra meal did not improve the weight gain or feed utilisation for Nile tilapia. In contrast, Jackson et al. (1982) reported improved performances for Java tilapia with a diet containing 25% protein from copra meal.

The poor pelletability of copra meal may affect its suitability as a component in fish feed. Owing to the inclusion of a high proportion of copra meal in the test diet (40.65% of diet), the desired feed particle size could not be maintained while feeding. Feed particle size has been shown to be an important factor affecting the feed intake of carp fry (Hasan and Macintosh, 1992).

All the test protein sources, except leucaena leaf meal, in combination with fish meal protein showed very good crude protein digestibility. Generally, oilseed meal proteins have digestibilities of 80–95% for fish (Jauncey and Ross, 1982) and carp are reported

to be able to digest the proteins of plants as well as, or slightly better than monogastric mammals (National Research Council, 1983). APD values ranging between 68.3 and 72.9% have been reported for Indian major carps fed diets containing 20–25% protein from leucaena leaf meal (Hasan et al., 1988, 1990, 1994).

The carcass protein content of carp fry fed different diets did not show large variations, although some of the values were statistically different (Table 5). However, the carcass moisture and lipid contents showed large fluctuations. Carcass moisture and lipid contents of fish always tend to show greater fluctuations than other carcass components and they appear to be inversely related (Atack et al., 1979; Hasan et al., 1990; Hasan and Macintosh, 1993).

Microscopic examination of the liver of carp fry fed mustard oil cake showed a higher level of intracellular lipid deposition (fatty changes) in the liver. The vascular and fatty changes in the liver are presumably due to the presence of toxins in the feed which cause disturbances in fat metabolism. Intracellular lipid deposition in the liver of fish fed mustard oil cake have been reported for common carp fingerling (Hossain and Jauncey, 1989) and major carp fry (Hasan et al., 1991). Similarly, Higgs et al. (1979) for coho salmon and Oliver et al. (1971) for rats reported increased lipid deposition when the animals were fed diets containing rapeseed meal. The specific mode of toxic action of glucosinolates of mustard/rapeseed in fish is yet to be investigated. However, in the case of rats, it has been reported that the intestinal flora can hydrolyse glucosinolates to a variety of aglucon products and some of the nitrile aglucon products are liver and kidney toxins (Tookey et al., 1980).

Amongst several plant protein sources tested in this study as potential partial substitutes for fish meal, linseed followed by groundnut and sesame at 25–50% inclusion level gave the most promising results. Therefore, the use of linseed, groundnut and sesame meal, which are locally available and relatively inexpensive in many developing countries, as alternative protein sources for carp fry could reduce the feeding cost considerably.

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