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DIETARY UTILIZATION OF SELECTED VEGETABLE PROTEINS BY MULLET, *LIZA MACROLEPIS*

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ABSTRACT

Seven feeds having 40% fat free protein from each one of the sources viz.coconut kernel oil cake, sesame seed oil cake, ground-nut oil cake, horse-gram, field-bean, bengal-gram and casein fortified with phenylalanine, argenine and cystine were formulated. Casein was taken as the standard protein. Carbohydrate content was kept at not less than 35% while lipid (Cod liver oil) content was maintained at 5% vitamin mixture and mineral mixture (USP XIV) were at 2% level and Lambda varieties at 5% level was used as the binder. After feeding *Liza macrolepis* of 1.8 to 3.8 g (live weight with the experimental diets once a day for 15 days to satation, the performance of the diets towards eight conversion indices were ranked. The ranking of the vegetable proteins in the descending order of performance is as follows: coconut, bengal-gram, ground-nut, field-bean, sesame seed and horse-gram.

INTRODUCTION

Growth in living organisms, (the process of tissue addition) can be likened as the process of building up of protein. Thus for achieving higher rate of protein addition, the quality and quantity of protein available in the food and its protein source are vital. In the cultivable organisms studied, especially in fishes protein is also used as an energy source along with carbohydrate and fats. Earlier study conducted showed the dietary optimum protein level for *Liza macrolepis* as 40% (Kandasami *et al.*, 1987). Protein is not only expensive, and since being required for man and for every domesticated organism is becoming a dearer commodity. This amplifies the need for finding out non-conventional protein sources. Most of the fishes presently cultured being either carnivorous or omnivorous, the bulk of protein sources utilized in the preparation of fish feeds is of animal origin, like fish meal. The present study is an evaluation of six vegetable protein sources. Herein all the diets prepared are balanced having all the essential nutrients viz., lipids, vitamins and minerals at dietary optimal levels. An important factor which need to be seriously considered while using non conventional protein sources like proteins of vegetable origin is the presence of toxic or offensive chemicals present along with useful nutrients.

MATERIALS AND METHODS

As non-conventional protein sources, coconut (*Cocos nucifera*) kernel oil cake, sesame seed (*Sesamum indicum*) oil cake, groundnut (*Arachis hypoges*) oil cake, horse-gram (*Dolichos biflorus*), field-bean (*Dolichos lablab*) and bengal-gram (*Cicer arietinum*) available in the local market were chosen. The oil cakes were fresh and free of fungal growth and

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were freed from the residual oil by solvent extraction, in order to avoid the residual oils in any way influencing the results. The pulses were dried and powdered as whole seeds. The finely ground material was seived through a standard seive of BSS-60. The retained coarse portion was discarded after repeated grinding and seiving. Fat free casein (SISCO, Bombay - Batch No. 034 021) was used as the standard reference protein. Since casein is inadequate in certain amino acids viz., phenylalanine, argenine and cystine it was fortified with analar grade amino acids respectively at 1.2, 1.5, and 1.8 gramme per cent level. The fortified casein is termed herein as casein mix. The result of proximate composition analysis of the protein sources and the outline of formulation of seven diets were as given in the Table 1. In all the seven diets, cod-liver oil was used as lipid source at 5% W/W; vitamin mix (Table 2) and mineral mix as per formula USP XIV (SISCO) at 2% level each. In the vitamin mix vitamin C was added in excess, in view of loss due to leaching. Commercial carrageenan (Sigma - 4: 1 mixture of kappa and lambada varieties) at 5% level was used as the binder. Thus these essentials and the binder totally made about 14% of the diets. All the seven diets were having protein content around 40%. The sources chosen had protein content between 32-51%. Since by themselves 40% protein level could not be achieved, a high (90%) protein material viz., fat free casein mix was supplemented. Carbohydrate level was maintained at a minimum of 35% and in cases of deficiency was supplemented with D-glucose. Only in standard protein diet (diet 7) at 6.6 g % level $\propto$ - cellulose was added to make up to 100%. The meals being high in nonprotein nitrogen, nitrogen to protein ratio was also worked out (Table 1). First the binder was made into a pasty liquid with water and heated to 80°C. To this with constant stirring and heating other ingredients which were already well mixed with water was slowly poured in, heated to 80°C with continuous heating. Totally 3.5 times of water in weight of the ingredients was used. Afterwards the contents were cooked in a pressure cooker for 5 minutes, cooled, and kept in a refrigerator in covered stainless steel containers. The food was offered to fishes in wet form. Everytime only the required quantity was taken from the freezer, thawed to room temperature, weighed and offered to the fishes. After feeding, the remaining food out of thus taken out portion was not again returned to the food stock.

The young ones of mullet, *Liza macrolepis* of 1.8 to 3.8 g, collected from the Tuticorin Bay, Gulf of Mannar were used in the experiments. They were acclamatized for a period of 15 days before commencing the study. The duration of the study was 40 days. Triplicates were maintained for each diet group. Five fishes were taken in each of the 21 experimental tanks, each tank containing about 20 litres of sea water. Everyday the salinity was monitored using an automatic temperature compensated (15.5 - 37.7°C) hand refractometer having refractive index from 1.3330 - 1.3730 and salinity 0-160‰ (American Optical, Buffelo, NY - 1425, Model 10419) before water change and after change of water around 1000 hrs. Salinity was found to be 36 $\pm$ 1‰. The sea water used (from Tuticorin Bay) was sedimented and passed through a filter bed. (Nayar *et al.*, 1983). The water temperature too was monitored daily and it ranged from 29.5 to 32. 0° C. Gentle aeration was provided throughout.

Weighed quantity of food was offered soon after change of water, and after 4 hrs the left over was siphoned out on to a very fine bolting silk tied on to the mouth of a beaker, gently washed with distilled water and

TABLE 1. *Scheme for the formulation of diets*

	1 Coconut CNB	2 Sesame seed CMB	3 Ground-nut CMB	4 Horse-gram MB	5 Field-bean MB	6 Bengal-gram MB	7 Casein mix B
TEST PROTEIN MEALg	86	63	69	85.5	85.5	85.5	44.4
Carbohydrate content of meal%	47	26	28	48	59	57	0.0
Carbohydrate available from meal g	40.9	16.4	19.3	41.0	50.4	48.7	0.0
GLUCOSE supplemented g	0	18.6	15.7	0	0	0	35.0
Carbohydrate content in diet g%	40.9	35.0	35.0	41.0	50.4	48.7	35.0
Protein content of meal%	33	48	51	36	30	32	90
Protein available from meal g	28.4	30.2	35.2	30.8	25.6	37.4	40.0
CASEIN MIX supplemented g	14.0	11.1	5.5	11.0	16.0	14.0	0.0
Protein content in diet g%	39.6	38.3	40.0	39.6	40.2	38.1	40.0
N available from meal mg	5.7	6.9	6.3	6.1	4.5	5.8	6.4
N contributed %	71.6	79.4	87.8	77.7	63.5	72.0	100
N available from casein mix mg	2.2	1.8	0.9	1.8	2.5	2.2	-
N contributed %	28.4	20.6	12.2	22.3	36.5	28.0	-
Total N available in diet mg%	7.9	8.7	7.2	7.9	7.0	8.0	6.4
N to protein conversion factor	5.02	4.40	5.55	5.02	5.75	4.76	6.25

i. C-oil cake; M-meal; B-based diets; N-nitrogen.
ii Feed components have been given in capitals.

transferred to a pre - weighed and numbered petridish. Thus pooled, left overs from individual experimental tanks were oven dried at 55°C and weighed at the end of the study. In arriving at the quantity of dry food consumed, the quantity of thus dried leftover was deducted from the dry weight of the food offered. Faecal matter, which could be easily

TABLE 2. *Vitamin mixture*

Ingredients	gramme
Thiamin HCL (B1)	0.200
Riboflavin (B2)	0.400
Niacin (B5)	2.000
Pyriodoxin HCL (B6)	0.600
Cyanacobalamin (B12)	0.004
Calcium pantothenate	3.000
m- Inosital	20.00
Folic acid	0.040
Biotin (H)	0.020
Choline chloride	30.000
p-Aminobenzoic acid	0.500
Ascorbic acid (C)	100.000
ß-Carotene (A)	0.480
Calciferol (D)	0.060
α-Tocopherol (E)	1.000
Menadione (K)	0.200
α-Cellulose (filler)	91.496
Total	250.00

8.2 g of the above mixture will give 5g vitamins.

distinguished from the leftover, as tiny rods, were very carefully siphoned out on to a very fine bolting silk fastened over the mouth of a 250 ml glass beaker by a tight rubber band. Distilled water was gently poured from a dropper along the circumferance of the mouth of the beaker which made the faecal matter to concentrate in the centre and got washed of the salt over them. After washing in the said fashion, the faecal matter retained in the bolting silk was kept on a blotting paper for a while and then the cloth was up turned over a pre-weighed numbered pertridish and the faeces transferred into it with a gentle tap and a few drops of distilled water. Thus pooled faecal matter was then dried in a hot air oven at 55 - 60°C and weighed at the conclusion of the study.

The formulae used in the study were as follows :

1. Assimilation efficiency $= \frac{\text{Protein consumed - Protein in faeces}}{\text{Protein consumed}} \cdot 100$

2. Food consumption per day in percentage body wieght $= \frac{2C}{t(W_t + W_o)} .100$

3 Growth per day in percentage body weight $= \frac{2(W_t - W_o)}{t(W_t + W_o)} . 100$

4. Live weight gain $= \frac{W_t - W_o}{W_o} .100$

5. Protein efficiency ratio $= \frac{W_t - W_o}{\text{Protein consumed}}$

6. Feed conversion ratio (FCR) $= \frac{W_t - W_o}{C}$

7. Gross Conversion efficiency (% Nutrient retained k_1) $= \frac{\text{Protein gained}}{\text{Protein consumed}} .100$

8. Net conversion efficiency (K_2) $= \frac{\text{Protein gained}}{\text{Protein assimilated}} .100$

9. Specific growth rate (SGR) % per day (Tacon and Cowey, 1985) $= \frac{\text{In } W_t - \text{In } W_0}{t} .100$

Wo - Live weight of fishes at the beginning of experiment
Wt - Live weight of fishes at the end of experiment
t - duration of study ie., 40 days
C - total quantity of food consumed
ln - $\log_e$

Results and Discussion

The vegetable sources studied herein had varying amount of protein content on dry weight basis. Ground-nut oil cake was the richest (51%) in protein while field-bean was low (32%) in protein content. The low protein content was a major impediment in working out even for a 40% protein diet. A protein source need to have at least 75% protein on dry weight basis, to make by itself a dietary protein source for a dietary protein level of 40%. This fact can be well understood from the data in Table 1. Otherwise they can only be used as a supplementary source or used in combination with a high protein source as in the present study. Before the start of the study experimental fishes were fed with dry pelleted and moist dough diets of the same formulation and moist dough form of diet was found liked by fishes. Therefore in the present study the diets were given in wet dough form.

The initial moisture content of the fishes was 68%. At the end of the study the moisture content of the fishes reared with diets 1, 2, 3 & 6 were 68% while in those of 4, 5 & 7 it was 65, 69 and 67.5% respectively. The kjeldahl nitrogen content of the fishes remained 11.5mg% dry weight throughout. The survival rate was 100% except mortality to one fish in one of the triplicates in diet group 6 and one each in two of the tanks under diet 7. All these occurred towards the end most probably due to handling stress. The calculated values for the various indices were as given in Table 3.

For the coconut oil cake based diet, growth per day and specific growth rate were the highest, while ground-nut oil cake based diet gave the best weight gain. Bengal-gram based diet was best assimilated. The standard protein diet viz., casein mix diet gave best results towards assimilation, gross conversion, FCR, net conversion and protein efficiency. The ranking for the diets had been as given in Table 4. Thus towards each index the performance of the diets varied. This well evidences the need for studying various indices for the proper assessment of the diets. By taking average for the indices for each diet the final ranking could be arrived. Thus it was found that among the six commercial protein sources studied, coconut oil cake was the best and bengal-gram came close to it. These two sources fared better than standard protein diet. Among the six commercial protein sources horse-gram fared the least. The sesame seed diet stood better than horse-gram diet.

TABLE 3 . *Calculated values for the various indices*

		Coconut CMB	Sesame seed CMB	Ground-nut CMB	Horse-gram MB	Field-bean MB	Bengal-gram MB	Casein mix B
1. Assimilation efficiency %	1	72.1	57.4	70.9	63.7	77.5	78.6	78.6
	2	72.1	57.4	70.9	63.7	77.5	78.6	78.6
	3	72.1	57.4	70.9	63.7	77.5	78.6	78.6
	Mean	72.1	57.4	70.9	63.7	77.5	78.6	78.6
2. Consumption per day in % body weight	1	1.92	2.33	2.17	2.59	2.61	1.39	0.84
	2	2.34	1.91	2.52	2.28	2.30	1.84	0.87
	3	2.24	1.93	2.09	2.71	1.49	1.93	0.92
	Mean	2.20	2.06	2.26	2.53	2.13	1.72	0.88
3. Growth per day in % body weight	1	0.79	0.75	1.09	1.05	1.15	0.83	0.75
	2	1.31	0.91	0.97	0.86	0.83	1.00	0.80
	3	1.24	0.99	0.82	0.79	1.07	1.06	0.80
	Mean	1.11	0.88	0.96	0.90	1.02	0.96	0.78
4. Live weight gain %	1	38.0	34.6	70.4	53.1	60.1	35.7	35.6
	2	71.0	44.7	61.9	41.9	40.0	45.8	38.2
	3	65.8	49.5	52.6	37.6	54.5	49.0	38.0
	Mean	58.3	43.0	61.3	44.2	51.5	43.5	37.3
5. Protein efficiency ratio	1	1.05	0.83	1.25	1.02	1.09	1.58	2.23
	2	1.41	1.25	0.96	0.96	0.90	1.44	2.29
	3	1.39	1.35	0.98	0.73	1.78	1.44	2.16
	Mean	1.28	1.14	1.06	0.90	1.26	1.49	2.23
6. Feed conversion ratio	1	0.42	0.32	0.50	0.40	0.44	0.60	0.90
	2	0.56	0.48	0.38	0.38	0.36	0.55	0.64
	3	0.55	0.51	0.39	0.29	0.72	0.55	0.87
	Mean	0.51	0.44	0.42	0.36	0.51	0.57	0.80
7. Gross conversion efficiency	1	24.3	19.3	36.7	23.7	25.4	32.6	42.6
	2	32.8	29.1	27.7	22.2	20.8	30.2	44.1
	3	32.3	31.3	30.4	17.1	41.2	30.4	44.5
	Mean	29.8	26.6	31.6	21.0	29.1	31.1	43.7
8. Net conversion efficiency %	1	33.8	33.7	51.7	37.1	32.8	41.5	54.2
	2	45.5	50.7	40.4	34.9	26.8	38.4	56.1
	3	44.8	54.5	42.9	26.8	53.1	38.7	52.8
	Mean	41.4	46.3	45.0	32.9	37.6	39.5	54.4
9. Specific growth rate % per day	1	0.805	0.74	1.11	1.06	1.17	0.84	0.76
	2	1.34	0.92	0.98	0.87	0.84	1.02	0.81
	3	1.26	1.00	0.83	0.80	1.09	1.07	0.80
	Mean	1.13	0.88	0.97	0.91	1.03	0.97	0.79

TABLE 4 . *Ranking among the seven diets for the various indices*

Index	Coconut CMB	Sesame seed CMB	Ground-nut CMB	Horse-gram MB	Field-bean MB	Bengal-gram MB	Casein mix B
1. Assimilation	3	6	4	5	2	1	1
2. Growth per day	1	5	3	4	2	3	6
3. Specific growth rate	1	5	3	4	2	3	6
4. Weight gain	2	5	1	6	3	4	7
5. Gross conversion	4	6	2	7	5	3	1
6. Food conversion	3	4	5	6	3	2	1
7. Protein efficiency	3	5	6	7	4	2	1
8. Net conversion	4	2	3	7	6	5	1
Mean	2.6	4.7	3.4	6.1	3.4	2.9	3.0
Ranking	I	V	IV	VI	IV	II	III

Swaminathan (1974) had given amino acid profile for various protein sources and the values as given for the sources studied are presented in Table 5. In the table, for the purpose of comparision, essential amino acid composition for egg albumen is also presented. Coconut kernel though had a reasonably balanced composition, was slightly deficient in tryptophan and lysine, while rich in arginine. Probably this balanced nature made coconut oil cake based meal to fare better. The bengal-gram was just as good as coconut as protein source. Bengal-gram was slightly deficient in tryptophan, cystine and methionine. Both coconut and bengal-gram contain the same quantity of tryptophan. Casein mix contained slightly higher quantity of leucine and lysine. The deficiency for cystine, phenylalanine and arginine was made good by supplementation . Considering the overall performance, ground-nut oil cake and field-bean fared equally well. Ground-nut (Pea-nut) was deficient very much in methionine, less so in threonine and slightly in phenylalanine. Ground-nut, field -bean and coconut were high in arginine. Field-bean was lowest in methionine, cystine and tryptophan. It has slightly high amounts of leucine and lysine over egg albumen, but highest among the natural protein sources herein studied. Thus field-bean is very rich in the usual limiting amino acid lysine. In the present study sesame seed oil cake used was with the hard seed coat. The husk is rich in calcium oxalate and in tannine . This could probably be the reason for its low assimilation efficiency (57.4%). Sesame seed was very low in arginine and lysine but was a good source for methionine and tryptophan. Though its amino acid profile was not bad in

TABLE 5. *Amino acid composition (g/16gN) after Swaminathan,1974*

	Try.	Thr.	Iso.	Leu.	Lys.	Met.	Cys.	Phe.	Tyr.	Val.	Arg.	His.
Coconut kernel	0.8	3.2	4.5	6.7	3.8	1.8	1.3	4.3	2.5	5.3	12.1	1.7
Sesame seed	1.5	3.1	4.2	7.4	2.6	2.8	2.2	6.4	4.1	3.9	8.8	1.9
Ground-nut	1.1	2.7	4.1	6.1	3.6	0.9	1.5	3.1	3.6	5.0	10.7	2.4
Horse-gram	1.0	3.9	6.7	7.9	7.9	1.4	2.2	6.9	2.6	5.9	5.7	3.0
Field-bean	0.5	3.3	6.0	8.9	8.1	0.7	1.0	5.3	-	5.6	9.2	2.8
Bengal-gram	0.8	3.6	5.7	7.4	6.9	1.3	1.4	4.9	3.3	4.9	7.5	2.7
Casein mix	1.3	4.3	6.6	10.1	8.1	2.7	0.4 (1.8)	5.4 (1.2)	5.9	7.2	4.1 (1.5)	3.0
Egg albumen	1.4	4.9	6.0	8.7	6.7	3.6	2.4	6.5	3.0	8.3	5.9	2.5

The value given within brackets refer to supplementation at g% level in casein mix.
Expansion : Try.-Tryptophan; Thr.-Threonine; Iso.-Isoleucine; Leu.-Leucine; Lys.-Lysine; Cys.-Cystine; Met.-Methionine; Phe.-Phenylalanine; Val.-Valine; Arg.-Arginine; His.-Histidine

comparison with other, its low assimilation made it to fare low. This conclusion was made possible based on its high value for net conversion efficiency. Horse-gram ranked the lowest in the study. When compared with the rest, this was low in alanine and methionine but was a rich source for lysine.

From Tacon and Jackson (1985) it was understood that the protein sources studied could possibly harbour the following antinutritional factors. Since they did not say anything about coconut, the same is omitted from the following.

Protease inhibitor	: B,G,F, H
Phytohaemaglutinins	: G,F, H
Cyanogens	: B, F, S
Phytic acid	: B, G, S
Saponins	: G
Oestrogenic factor	: B, G
Flatulence factor	: B, G
Likelyhood to mycotoxin contamination	: B, G, S

B - Bengal- gram, S - Sesame seed, G- Ground-nut, H - Horse -gram and F - Field-bean.

The antinutritional factors can be profitably eliminated or controlled by controlled processing techniques. The following operations are recommended: removal of husks from sesame seed and legumes, solvent extraction (oestrogenic factor, gossypol and cyclopropenoic fatty acids), moist heat treatment with water extraction (enzyme inhibitors, anti-vitamin factors, haemaglutinins, cyanogens, lathyrogens, glucosinolates), alcohol extraction (flatulence factors and saponins) and autoclaving (phytic acid and limiting fungal growth) (Liener,1980).

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REFERENCES

KANDASAMI, D., R. PAULRAJ AND D.C. V. EASTERSON 1987. Effect of selected levels of dietary protein on the growth and feed efficiency of mullet, *Liza macrolepis* fry. *Indian J. Fish.*, **34** (3) : 306 - 311.

NAYAR, K.N., M.E. RAJAPANDIAN AND D.C.V. EASTERSON 1983. Purification of farm grown oysters. *Proc. Sym. Coastal Aquaculture*, MBAI, **2** : 505 - 508.

LIENER, I.E., 1980 *Toxic Constituents of Plant Foodstuffs*. II Edition, 502 pp. Academic Press, New York.

SWAMINATHAN, M. 1974. *Essentials of Food and Nutrition*. Vol 1. *Fundamental Aspects*. 576 pp. Genesh & Company, Madras. .

TACON, A.G. J., AND C. B. COWEY 1985. Protein and amino acid requirements. In : *Fish Energetics : New Perspectives*, Tytler, P. and P. Calow (Eds.), pp. 155 - 183. Croom Helm.

TACON, A.G. J. AND A.J. JACKSON, 1985. Utilization of conventional and unconventional protein sources in practical fish feeds. In : *Nutrition and Feeding in Fish*, C.B. Cowey, A.M. Mackie and J.G. Bell (Eds.), pp. 119 - 145. Academic Press, Lond.