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NOVEL PROTEIN SOURCES FOR FISH: 3- NUTRITIONAL EVALUATION OF BEET (*BETA VULGARIS*) LEAVES CONCENTRATE IN DIETS OF RED TILAPIA

A.K. Soliman, Manal M.A. El-Razik , M.A. Abaza and T.A.Taha

Animal and Fish Production Department, Faculty of Agriculture, Alexandria University,Egypt

SUMMARY

Beet leaf concentrate meal (BLCM) was prepared in our laboratory. BLCM contains an adequate level of crude protein (31.5%) and very low crude fiber level (1.70%) however, its amino acid levels especially essential amino acids were inferior to those of soybean meal with the exception of methionine. Dried beet leaves concentrate was used to replace 0, 25, 50, 75 and 100% of soybean meal of the basal diet. Fish fed the diets containing different levels of beet leaves concentrate performed equally in terms of growth performance and nutritional parameters whereas no significant differences in body weight, specific growth rate, condition factor, survival rate, food conversion ratio, protein efficiency ratio, apparent net protein utilization and carcass body composition (moisture , crude lipids, and ash) were found but fish diet 3 where 50% of soybean meal was replaced exhibited the best growth response, nutritional parameters and carcass crude protein. No significant differences were noticed in plasma total protein, plasma albumin and plasma total globulins of red tilapia fed the experimental diets. It could be concluded from the results of the present study that beet leaf concentrate meal could replace safely 50% of soybean meal in tilapia diets.

Keywords: Beet leaves concentrate, nutrient utilization, soybean meal, red tilapia

INTRODUCTION

The escalating fish production from aquaculture sector, which is expected to increase has necessitated an increase in fish feeds production in order to meet fish species demand on quantitative and Qualitative requirements. Fish meal and soybean meals are the major protein sources in diets of many fish species and increasing prices, fluctuation in quality and unavailability of both ingredients have forced the nutritionists to look for alternative protein sources to be incorporated in fish feeds (Shiau *et al.*,1990; Hughes, 1991; Tidwell *et al.*, 1993; Gallagher, 1994; Kaushik *et al.*, 1995; Robaina *et al.*, 1995; Boomyaratpalin *et al.*, 1998; Refstie *et al.*, 1998; Storebakken and Refstie,2000; Soliman 2000 a, b). Another approach is using leaf protein concentrate from different protein sources as a possible alternative protein sources in diets of some fish species (Lu *et al.*,1977;Ogino *et al.*, 1978; Alvera-Novoa *et al.*,1990; Johansson,1991; Soliman, 2000c).

Ben-Gera and Kramer (1975) reported that the percentage of residues were 25% for vegetables and 40% for fruit .In Egypt, vegetables and fruit production were 7.7 and 2.71 million tons and the residue were 2.3 and 1.08 million tons. Abou Akkada and Nour (1985) reported that in year 2000, the residue is expected to be 2.5 million tons from vegetables and 1.5 million tons from fruit. Tekale and Joshi (1976) reported that the by-product leaves and their yield of total nitrogen on dry matter (kg/ ha) basis for beet were 1668 and 43 respectively. Therefore, the present study was undertaken to establish the effects of partial and complete replacement of soybean meal with beet leaf concentrate meal on performance, nutritional and physiological parameters of red tilapia one of the most salinity tolerant fish species in the tropics.

MATERIALS AND METHODS

Experimental system and animals

Ten glass aquaria with dimensions of 70 x 30 x 40 cm were used. Each aquarium was filled with 75 liters of dechlorinated tap water. During the experimental period (15 weeks) 12 liters of water aquarium were removed daily and equal amounts of water were added. Each aquarium was supplied with an automatic heater to maintain water temperature at 28 ± 1°C, air pump and stone to provide continuos aeration to water (dissolved oxygen was 6.8-8.2 ppm). Also, each aquarium was supplied with a power filter to filter the faeces and fine matter from the water. Water pH was in the range of

7.2-7.5 during the experiment. Fry of red tilapia (*Oreochromis niloticusxO.mossambicus*) was obtained from the Maruit Fish Farm Company located in Alexandria. Fish were fed the control diet (diet 1,Table 1) for one month as a conditioning period before starting the experiment.

Table 1. Composition of the experimental diets and proximate analysis

Item / Substitution level(%)	Diets 0.0	25	50	75	100
Ingredients(%)	1	2	3	4	5
Soybean meal	20.00	15.00	10.00	5.00	0.00
Beet leaf concentrate meal	0.00	7.12	14.24	21.36	28.48
Corn starch	13.00	10.88	8.76	6.64	4.52
Fish meal	30.00	30.00	30.00	30.00	30.00
Meat &bone meal	15.00	15.00	15.00	15.00	15.00
Yellow corn meal	14.00	14.00	14.00	14.00	14.00
Corn oil	5.00	5.00	5.00	5.00	5.00
Sodium alginate	1.00	1.00	1.00	1.00	1.00
Mineral mix[1]	1.00	1.00	1.00	1.00	1.00
Vitamin mix[2]	0.875	0.875	0.875	0.875	0.875
Ascorbic acid	0.125	0.125	0.125	0.125	0.125
Proximate analysis (%)					
Moisture	6.97	6.98	5.69	6.09	5.44
Ash	12.73	12.98	13.46	14.04	14.05
Crude protein	37.40	37.53	36.61	37.20	36.89
Crude lipids	12.36	12.42	12.14	12.36	12.30
Crude fiber	1.24	0.95	0.89	0.82	0.72
Nitrogen free extract	29.30	29.14	31.21	29.49	30.00
Gross energy[3](Kcal/100g)	438.361	438.91	439.79	437.98	437.82
Protein: energy (mg:Kcal)	85.33	85.51	83.24	84.94	84.26

1- Soliman *et al.*(1994)

2- Each 100 g contain: Vit A 960,000 IU; Vit D_3 160,000 IU; Vit E 0.89 g; Vit K 0.16 g; Vit B_1 80 mg; Vit B_2 0.32 g; Vit B_6 0.12 g; Vit B_{12} 0.8 mg; Pantothenic acid 0.89; Niacin 1.6 g; Folic acid 80 mg; Biotin 4 mg; Choline chloride 40 g; the rest is a carrier

3-Gross energy (see Jauncey and Ross, 1982)

Preparation of beet leaf concentrate meal and feed ingredients analysis

Beet leaves were collected from local market. Beet leaves were disintegrated using a food grinder (Oster, Oster cooperation, USA) and subsequently pressed in a presser , a squeezy type(Passaverdura, Italy). The expressed green juice was dried in oven at 60o C for 24 hrs. The dried material was ground using a Retsch mill where it passes through a 1.0 mm sieve resulting in producing beet leaf concentrate meal (BLCM). BLCM and feed ingredients were subjected to proximate analysis (AOAC, 1989). Amino acids contents of BLCM and soybean meal (SBM) were determined according to the method described by Duranti and Cerelli (1979) using Beckman amino acid analyzer Model 119 CL.

Diets and Feeding Regime

Five diets were formulated (Table 1) where 0, 25, 50, 75 and 100% of soybean meal were substituted by beet leaf concentrate meal. Diet preparation and storage have been previously described (Soliman, 1985). Chemical composition of the experimental diets is shown in Table 1. Each diet was fed to duplicate randomly assigned aquaria for 15 weeks. Each aquarium was stocked with 15 fish (aveage. weight 2.45-2.54 g). A fixed feeding regime of 5% of the body weight per day (dry food/whole fish) was employed for the first ten weeks and 3% of the body weight from 11-15 week. Fish fed 3 times daily in equal portions. Fish were fed for six consecutive days, weighed on the seventh and feeding rates for the following week adjusted accordingly.

Experimental Methodology

Fish were bulk weighed, aquarium at a time, in water without anesthesia except for the terminal weighing when fish were anaesthetized (Ross and Geddes, 1979) and weighed and measured to allow calculation of condition factor (Weight x 100/standard length^3). An Initial sample of fish, 3 per aquarium were killed and subjected to proximate analysis and a final sample of 7 fish per aquarium

was treated similarly (AOAC, 1989). Blood was collected using heparinized syringes from the caudal vein of the experimental fish at the termination of the experiment. Blood was centrifuged at 3000 rpm for 5 minutes to allow separation of plasma which was subjected to determination of plasma total protein (Armstrong and Carr, 1964) and plasma albumin (Doumas *et al.*, 1977). Apparent net protein utilization was calculated from carcass analysis data by method of Nose (1962). For evaluation of the results of the present study, analysis of variance (Snedecor, 1966) and Duncan's multiple range test (Duncan, 1955) were employed.

RESULTS

Proximate analysis of BLM, BLCM and BRM (Table 2) showed that processing of beet leaves to produce BLCM resulted in an appreciable increase in crude protein of BLCM(31.50%) compared with 21.81 in BLM and producing BRM with a good nutritive value(Table 2) and this meal could be used in feeding ruminate animals. The amino acid profile especially essential amino acids was inferior to that of soybean meal with the exception of methionine (Table 3). Growth response, nutritional parameters and carcass composition at the start and end of experiment of fish fed the experimental diets are presented in Tables 4 and 5. Fish fed the diets containing different levels of beet leaf concentrate meal performed equally in terms of performance and nutritional parameters whereas no significant differences were found in body weight, specific growth rate, condition factor, survival rate, food conversion ratio, protein efficiency ratio, apparent net protein utilization and carcass body composition (moisture, crude lipids, and ash) but fish diet 3 where 50% of soybean meal was substituted by BLCM exhibited the best growth response, nutritional parameters and carcass crude protein(Tables 4 and 5). No significant differences were noticed in plasma total protein, plasma albumin and plasma total globulins of red tilapia fed the experimental diets (Table 4).

Table 2. Proximate analysis (%)of beet leaf meal(BLM), beet leaf concentrate meal(BLCM), residue meal(RM) and Soybean meal (SBM)

	Moisture	Ash	Crude protein	Crude lipids	Crude fiber	NFE
BLM	9.30	21.18	21.81	4.59	5.78	36.34
MLCM	12.72	26.84	31.50	1.70	0.89	26.35
RM	10.33	19.89	18.39	2.92	11.76	36.71
SBM	10.84	6.25	44.80	1.21	4.26	32.66

Table 3. Amino acid composition (%) of beet leaf concentrate meal and soybean meal (g /100g)

Amino acids profile	Beet leaf concentrate meal	Soybean meal
Alanine	1.39	1.85
Arginine	0.77	3.02
Asparatic acid	1.85	3.11
Cystine	0.11	0.52
Glutamic acid	3.29	4.14
Glycine	10.19	11.73
Histidine	0.49	1.18
Isoleucine	1.44	2.38
Leucine	1.37	2.56
Lysine	0.75	1.57
Methionine	0.37	0.39
Phenylalanine	0.92	1.48
Proline	1.94	1.36
Serine	0.88	1.30
Threonine	1.03	1.34
Tyrosine	0.65	1.11
Valine	1.36	1.79

Table 4. Effect of partial and complete replacement of soybean meal Protein with beet leaf concentrate meal protein on performance , nutritional and physiological parameters[1] of red tilapia

Item	Diets					
	1	2	3	4	5	±SE M[2]
Ave.initial wt.g	2.52	2.46	2.45	2.54	2.49	
Final ave.wt.g	14.92	15.89	16.13	14.78	14.65	0.473
Condition factor	3.22	3.28	3.23	3.13	3.14	0.056
SGR[3](%d-1)	1.69	1.76	1.78	1.67	1.68	0.047
FCR[4]	2.31	2.23	2.18	2.30	2.24	0.078
PER[5]	1.15	1.20	1.26	1.19	1.20	0.039
ANPU[6],%	17.27	18.81	21.47	18.27	17.13	0.479
Survival rate,%	100.00	96.66	100.00	100.00	96.66	2.109
PTP[7] (gdl-1)	4.77	4.23	4.46	4.45	4.66	0.549
PA[8] (g dl-1)	1.03	0.87	1.61	0.98	1.17	0.278
PTG[9] (g dl-1)	3.74	3.36	2.85	3.47	3.49	0.699

1-Values in the same row with a common superscript are not significantly different (P>0.05).
2-Standard error of the means derived from analysis of variance.
3-Specific growth rate 4-Food conversion ratio 5-Protein efficiency ratio
6-Apparent net protein utilization 7-Plasma total protein
8- Plasma albumin 9- Plasma total globulins

Table 5. Body Composition data on wet weight basis of fish fed the experimental diets

	Wet weight (%)			
	Total moisture	Ash	Crude lipids	Crude protein
Initial	79.10	4.08	7.09	9.73
1	73.41	4.77	7.49	14.43b
2	72.39	5.04	7.64	14.93b
3	71.42	4.83	7.42	16.32a
4	72.90	5.31	7.09	14.70b
5	73.27	5.62	6.84	14.24b
± SEM[1]	0.865	0.313	0.281	0.345

a, b, c and d: Only means with different superscript letters are significantly different (P<0.05)
1-Standard error of the means derived from the analysis of variance

DISCUSSION

The advantages from producing leaf concentrate meals over other protein sources such as fish meal, soybean meal and meat meal are low production cost(due to use plant by-products) and availability but the disadvantage of these meals are their organoleptic protperties and the inferior quality of protein(Johansson *et al.*(1991). Goel *et al.*(1979) conducted a partial amino acids analysis of cauliflower(*Brassica oleraca* Var.Bortrytis)leaf protein concentrate . These authors found that arginine, cystine ,histidine, lysine and methionine levels were 1.0, 0.11, 0.44,1.03 and 0.37% respectively whereas values of these amino acids in beet leaf concentrate meal were 0.77, 0.11, 0.49, 0.75 and 0.37% respectively . The results reported above support findings of Johansson *et al.*(1991) who stated that leaf nutrient concentrate was deficient in lysine and sulpher-containing amino acids. Even though the amino acid profile of BLCM was inferior to SBM ,the growth response (final body weight and specific growth rate) of fish fed diets 2 and 3 (25 and 50% replacement) were higher than that of fish fed the control diet but above these replacement levels ie 75 and 100% substitution (diets 4 and 5) growth decreased .This could be due a complementary effects between soybean and beet leaf concentrate meals . The first make a compensation in the amino acids deficient in BLCM whereas the BLCM make a compensation for the vitamins and minerals deficient in SBM. Hasan *et al.*(1997) reported that in common carp(*Cyprinus carpio*)diets when linseed and peanut meals (both meals deficient in lysine ,methionine and threonine) replacing 25% of fish meal protein in the control diet for the first and 25 and 50% of fish meal in the control diet for the second , growth performance of fish fed these diets was comparable to that of fish fed the control diet and the authors attributed this to the amino acid profile contributed by the fish meal component. Also ,these authors reported that at higher inclusion level(75%) growth performance deteriorated because the contribution was not enough to support fish growth. Tekale and Joshi (1976) reported that vegetables and of course leaf protein

concentrates are the principle sources of vitamins and minerals. Also increasing levels of beet leaf concentrate meal may cause hypervitaminosis which can cause reduction in fish growth observed in fish fed diets containing higher levels of beet leaf concentrate meal. No significant differences were detected in carcass moisture, ash and crude lipids but the differences were significant in carcass crude protein where red tilapia fed a diet replacing 50% of soybean meal protein obtained the highest carcass crude protein and the same results were reported more recently by Soliman (2000c) for red tilapia fed diets where a partial replacement of fish meal protein with protein of water hyacinth protein concentrate meal

CONCLUSION

It could be concluded from the results of the present study that beet leaf concentrate meal could replace safely 50% of soybean meal in tilapia diets.

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