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Partial and complete replacement of clover leaf (*Trifolium alexandrinum*) meal protein by cabbage leaf *(Brassica oleracea*) meal protein in Nile tilapia diets together with economic evaluation

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SUMMARY – Five diets were formulated including 0.0, 6.5, 13.5, 19.5 and 26% of cabbage leaf meal (CLM) instead of 0.0, 3.0, 6.0, 9.0 and 12% of Egyptian clover leaf meal (ECLM). Fish fed on the diet where 3% of ECLM was replaced by CLM showed the best body weight and specific growth rate compared with those obtained by fish fed on the diet containing ECLM. No significant differences were found in survival rates, food conversion ratio and protein efficiency ratio. Variable results were noticed in apparent net protein utilization of fish fed on the various diets. Fish fed on a diet containing 13.5% CLM had the highest carcass ash. No significant differences were found in hepatosomatic and blood parameters of fish fed on the experimental diets but these fish exhibited significant differences in terms of plasma glucose, pyruvic glutamic transaminase and plasma carotene levels. The cost per kg of feed was reduced by increasing cabbage leaf meal in diets. The best profit index was obtained by fish fed on the diets containing the highest level of cabbage leaf meal.

Keywords: Cabbage leaves, tilapia, performance parameters, nutrient utilization, physiological parameters, economic evaluation.

RESUME – *"Substitution partielle ou totale des protéines de farine de feuilles de trèfle (*Trifolium alexandrinum*) par des protéines de farine de feuilles de chou (*Brassica oleracea*) pour l'alimentation de tilapias du Nil, et évaluation économique". Cinq régimes ont été formulés où on a utilisé 0,0, 6,5, 13,5, 19,5 et 26% de farine de feuilles de chou (CLM) au lieu de 0,0, 3,0, 6,0, 9,0 et 12% de farine de feuilles de trèfle égyptien (ECLM). Les poissons recevant le régime où 3% d'ECLM étaient remplacés par CLM, ont eu le meilleur poids corporel et le meilleur taux de croissance spécifique en comparaison avec ce qu'obtenaient des poissons recevant le régime à ECLM. On n'a pas trouvé de différences significatives concernant le taux de survie, le taux de conversion alimentaire et le taux d'efficacité des protéines. Des résultats variables ont été notés pour l'utilisation apparente nette des protéines des poissons recevant les différents régimes. Les poissons recevant un régime contenant 13,5% de CLM présentaient de façon significative la plus forte teneur en cendres dans la carcasse. Aucune différence significative n'a été trouvée concernant les paramètres hépatosomatiques et sanguins des poissons recevant les régimes expérimentaux, mais ces poissons ont montré des différences significatives en termes de glucose dans le plasma, de transaminases glutamiques pyruviques et de niveaux de carotène dans le plasma. Le coût par kilo d'aliment a baissé en augmentant la farine de feuilles de chou dans l'aliment. Le meilleur indice de rentabilité a été obtenu par les poissons recevant le régime contenant le plus haut niveau de farine de feuilles de chou.*

Mots-clés : *Feuilles de chou, tilapia, paramètres de performances, utilisation des nutriments, paramètres physiologiques, évaluation économique.*

Introduction

The escalating fish production from the aquaculture sector, which is expected to increase, requires an increase in fish feed production in order to meet quantitative and qualitative requirements of fish species.

In Egypt in the year 2000, residues from vegetables amounted to 2.5 million tons and from fruits 1.5 million tons. Tekale and Joshi (1976) reported that cabbage by-product leaves and their yield of total nitrogen on a dry matter (kg/ha) basis were 3018 and 62, respectively. Cabbage is cultivated in several governorates in Egypt. The total surface area of cabbage cultivated in Egypt yields 496,000 tons. There are no reports on the use of cabbage leaf meal in fish nutrition, therefore, the present study was undertaken to establish the effects of partial and complete replacement of Egyptian clover leaf meal with cabbage leaf meal on performance, nutritional and physiological parameters in the Nile tilapia, *Oreochromis niloticus*, the best candidate fish species in the tropics for aquaculture.

Materials and methods

Cabbage leaf wastes were collected from a local market and dried in an oven at 60°C for 24 hours. The dried materials were ground using a 1 mm sieve. Leaves of Egyptian clover were treated similarly. Cabbage leaf meal (CLM) and Egyptian clover leaf meal (ECLM) were subjected to proximate analysis (AOAC, 1989) and the amino acid contents of each meal were determined using Beckman amino acid analyser Model 19 Cl. Five diets were formulated (Table 1), where 0.0, 6.5, 13.5, 19.5 and 26% of cabbage leaf meal (CLM) were used instead of 0.0, 3.0, 6.0, 9.0 and 12% of Egyptian clover leaf meal (ECLM). The substitution was based on crude protein, therefore, CLM substituted ECLM by 0.0, 24, 50, 73 and 100%, respectively. The chemical analysis of these diets is shown in Table 2. Each diet was randomly offered to duplicate aquaria and each aquarium was stocked with 20 fish of Nile tilapia with an average weight of 0.93 g. A fixed feeding regime of 5% of body weight was used for the first ten weeks and 3% for the 11-15 week. Fish were fed 3 times daily in equal proportions. Feeding was performed for six consecutive days with no food being given on the seventh day when fish were weighed. Fish were bulk weighed a tank at a time, except for the terminal weighing when fish were anaesthetized (Ross and Geddes, 1979), weighed and measured individually to allow calculations of the condition factor (weight x 100/length^3). An initial sample of fish, three per tank, was slaughtered prior to the start of the experiment and subjected to proximate analysis (AOAC, 1989). A final carcass sample of seven fish per tank was treated similarly.

Table 1. Composition (%) of the experimental diets fed to Nile tilapia containing various levels of cabbage leaf meal

Ingredient	Diets				
	D1	D2	D3	D4	D5
ECLM	12	9	6	3	0.0
CLM	0.0	6.5	13.5	19.5	26
Corn starch	14	10.5	6.5	3.5	0.0
Fish meal	30	30	30	30	30
Soybean meal	20	20	20	20	20
Meat and bone meal	12	12	12	12	12
Blood meal	4	4	4	4	4
Corn oil	5	5	5	5	5
Mineral mix.	1.0	1.0	1.0	1.0	1.0
Vitamin mix.	0.875	0.875	0.875	0.875	0.875
Ascorbic acid	0.125	0.125	0.125	0.125	0.125
Sodium alginate	1.0	1.0	1.0	1.0	1.0
Total	100	100	100	100	100

ECLM: Egyptian clover leaf meal.
CLM: Cabbage leaf meal.

Blood was collected, using heparinized syringes, from the experimental fish at the termination of the experiment for determination of haematocrit (Baker *et al*., 1966). Plasma total protein (Proteines-kit produced by bio Merieux, France) glucose (glucose enzymatique PAP-Kit produced by bio Merieux vitek Inc, USA). Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were determined using the method of Reitmen and Frankel (1957). Dietary and plasma carotene was also determined according to Hawks *et al*. (1965) and Alexander *et al*. (1985). Apparent net protein utilization was calculated from carcass analysis data. Analysis of variance and F test (Snedecor, 1966) and Duncan multiple range (Duncan, 1955) were used in evaluating the experimental results.

Table 2. Data of proximate analysis of experimental diets

Diets	Moisture	Crude protein	Ether extract	Ash	Crude fibre	NFE†	GE†† (KCal/100g)	P/E ratio†††
1	6.96	38.20	10.90	14.40	2.33	27.21	430.40	88.75
2	7.90	38.62	10.61	15.35	2.80	24.72	421.78	91.56
3	7.87	38.85	10.36	15.95	2.73	24.24	423.70	90.98
4	7.90	38.69	10.23	15.70	3.01	24.47	421.82	91.72
5	7.70	38.96	11.29	16.50	3.21	22.34	421.76	92.37

†Nitrogen free extract.
††Gross energy: (% protein x 5.5) + (% ether extract x 9.1) + (% carbohydrate x 4.1) (Jauncey and Ross, 1982).
†††Protein/energy ratio = mg protein/Kcal.

Results

Table 3 shows the proximate analysis and amino acid profile of CLM and ECLM. The crude protein content of CLM was inferior to that of ECLM but there were no much differences in ash and ether extract contents in both meals. Also CLM had a reasonable quantity of carotene (Table 3). The amino acid profile of both meals is presented in Table 4. The amino acid profile of ECLM is better than that of CLM. By the end of the experiment (16 weeks), fish fed the diet where 3% of ECLM was replaced by CLM, showed the best body weight and specific growth rate comparing with those obtained by fish fed the diet containing ECLM (Table 5). No significant differences were found in survival rates, food conversion ratio and protein efficiency ratio (Table 5). Variable results were noticed in apparent net protein utilization of fish fed the various diets (Table 5). Fish fed a diet containing 13.5% CLM had significantly the highest carcass ash and crude lipids but fish fed a diet containing 26% of this meal obtained the lowest values (Table 6).

Table 3. Chemical composition of Egyptian clover leaf meal (ECLM) and cabbage leaf meal (CLM)

Item	ECLM	CLM
Moisture %	6.96	7.35
Ash %	16.41	17.46
Ether Extract %	4.57	4.18
Crude Fibre %	7.20	10.80
Crude Protein %	32.7	14.69
True Protein %	30.84	13.50
TP/CP %	94.31	91.89
NFE %	32.16	45.52
GE (Kcal/100g)	382.8	349.74
Carotene (mg/kg)	296	42

No significant differences were found in hepatosomatic indices, haematocrit, plasma total protein, glutamic pyruvic transaminase and glutamic oxalotransaminase/glutamic pyruvic transaminase ratio of fish fed the experimental diets (Table 7), but these fish exhibited significant differences in terms of plasma glucose, glutamic oxaloacetic transaminase and plasma carotene levels (Table 7). The cost per kg of feed was decreased by increasing the amount of cabbage leaf meal in the diets (Table 8). The lowest incidence cost was observed in fish fed the highest incorporation level of cabbage leaf meal and the best profit index was obtained in fish fed the diets containing the highest level of cabbage leaf meal (Table 8).

Table 4. Amino acids composition of ECLM and CLM (g/100 g)

Amino acids profile	ECLM	CLM
1-Essential AA		
Arginine	1.74	0.48
Histidine	0.84	0.08
Isoleucine	1.24	0.30
Leucine	2.34	0.58
Lysine	1.72	0.32
Methionine	0.58	0.11
Phenyl alanine	1.86	0.26
Thereonine	1.66	0.89
Valine	2.20	0.64
2-Non essential AA		
Alanine	1.66	0.54
Aspartic acid	6.2	2.58
Cystine	0.24	0.06
Glutamic acid	0.44	1.58
Glycine	1.42	3.35
Proline	1.30	1.15
Serine	2.25	0.10
Tyrosine	1.34	0.23
Total	29.98	13.14

Table 5. Performance and nutritional parameters of Nile tilapia fry fed on diets containing different levels of cabbage leaf meal

Parameter	Diets					±SEM
	1	2	3	4	5	
Initial avg. B.W.	0.94	0.94	0.91	0.93	0.94	-
5 weeks						
Avg. BW (g)	2.2^{ab}	2.31^{a}	2.11^{b}	2.08^{b}	2.09^{b}	0.0431
SGR	2.42^{ab}	2.54^{a}	2.38^{ab}	2.28^{b}	2.27^{b}	0.4750
FCR	1.58^{ab}	1.46^{b}	1.45^{a}	1.73^{a}	1.66^{a}	0.0483
PER	1.64^{abc}	1.80^{ab}	1.82^{a}	1.54^{c}	1.59^{bc}	0.0601
10 weeks						
Avg. BW (g)	5.63	6.39	5.73	5.91	5.51	0.2820
SGR	2.55^{b}	2.73^{a}	2.61^{ab}	2.51^{b}	2.51^{b}	0.0454
FCR	1.42	1.35	1.34	1.50	1.44	0.0447
PER	1.83	1.96	1.95	1.76	1.83	0.0594
15 weeks						
Avg. BW (g)	14.9^{a}	15.25^{a}	12.55^{c}	13.40^{bc}	14.14^{ab}	0.3031
SGR	2.62^{ab}	2.64^{a}	2.49^{b}	2.53^{ab}	2.58^{ab}	0.0381
Survival rate	100	97.5	97.5	100	100	1.5811
CF	2.94^{b}	2.83^{b}	2.88^{b}	2.95^{b}	3.18^{a}	0.0577
FCR	1.52	1.71	1.74	1.49	1.50	0.0670
PER	1.70	1.55	1.50	1.79	1.78	0.0765
ANPU	27.56^{ab}	26.04^{bc}	23.32^{d}	28.65^{a}	24.77^{dc}	0.5418

SEM: Standard error of the means derived from the analysis of variance.
Only means with different superscript letters are significantly different (P<0.05).

Table 6. Results of body composition on a wet weight basis of fish fed on the experimental diets

Treatment	Wet weight (%)			
	Moisture	Ash	Crude lipids	Crude protein
Initial	80.40	5.41	3.80	10.50
Diet 1	74.06 ^{b}	5.13 ^{a}	5.03 ^{ab}	15.70
Diet 2	74.92 ^{ab}	4.95 ^{ab}	4.68 ^{b}	15.45
Diet 3	75.17 ^{ab}	4.65 ^{ab}	5.98 ^{a}	14.26
Diet 4	75.03 ^{ab}	4.88 ^{ab}	4.41 ^{b}	15.67
Diet 5	77.71 ^{a}	4.34 ^{b}	4.15 ^{b}	13.79
±SEM	0.8712	0.1667	0.3228	0.5999

SEM: Standard error of the means derived from the analysis of variance.
Only means with different superscript letters are significantly different (P<0.05).

Table 7. Physiological parameters of Nile tilapia fed on diets containing different levels of cabbage leaf meal

Parameter	Diets					±SEM
	1	2	3	4	5	
1-Organ						
HSI (%)	2.49	2.64	1.93	1.92	1.80	0.3317
GI (%)	4.72^{ab}	5.13^{ab}	5.71^{ab}	3.85^{b}	6.23^{a}	0.5034
GSI (%)	2.57^{ab}	6.17^{a}	4.76^{ab}	2.84^{ab}	1.56^{b}	1.2078
2-Blood						
HT	40.75^{a}	38.65^{a}	33.50^{b}	39.50^{a}	42.50^{a}	1.1355
PTP (g/dl)	4.21	4.38	4.47	4.23	3.83	0.2598
Gluc. (mg/dl)	82.21^{a}	88.19^{a}	57.40^{b}	66.84^{b}	67.43^{b}	3.5199
GOT (U/dl)	229^{b}	206.5^{b}	248^{a}	251^{a}	235^{b}	10.736
GPT (U/dl)	26	25.5	36	30	25.5	3.2939
GOT/GPT	8.85	8.64	8.89	8.51	9.21	1.2784
Carotene (mg/dl)	1.47^{b}	3.82^{a}	3.30^{a}	2.40^{ab}	2.55^{ab}	0.4025

HSI: Hepatosomatic index; GI: Gut index; GSI: Gonadosomatic index; HT: Haematocrit; PTP: Plasma total protein; Gluc.: Glucose; GOT: Glutamic oxaloacetic transaminase; GPT: Glutamic pyruvic transaminase; SEM: Standard error of the means derived from the analysis of variance.
Only means with different superscript letters are significantly different (P<0.05).

Table 8. Cost per kg of feed, incidence cost and profit index of fish fed the experimental diets

	Diets					±SEM
	1	2	3	4	5	
Cost/kg feed	2.52	2.46	2.38	2.32	2.25	-
Incidence cost	3.83 ^{ab}	4.22 ^{a}	4.15 ^{a}	3.46 ^{b}	3.37 ^{b}	0.1514
Profit index	1.83 ^{abc}	1.66 ^{c}	1.69 ^{bc}	2.03 ^{ab}	2.09 ^{a}	0.0895

SEM: Standard error of the means derived from the analysis of variance.
Only means with different superscript letters are significantly different (p<0.05).

Discussion

The problem faced by fish culturists around the world is that of finding economical sources of fish feed ingredients when demand and prices for many of the more commonly used products have raised. This problem is more serious in developing countries and a possible solution is to use unconventional ingredients, such as vegetable wastes and the present study is an attempt to incorporate cabbage leaf wastes in tilapia diets.

El-Hashimy (1971) reported that goedad *(Sonclus cleraccus*) and siris *(Cichorium intybus*) contain 16.9% and 16.1% crude protein, respectively, which agrees with that found in cabbage leaf meal (Table 3). Nile tilapia exhibited the best growth when they were fed a diet where 3% of ECLM was replaced by cabbage leaf meal (6.5%). The reduction in body weight was more related to:

(i) The amino pattern supplied by the vegetable leaf meal than to the total protein content of the diet since the diets contained equal amounts of protein. Increasing the replacement level of ECLM by CLM resulted in pronounced decrease in nine out of the ten essential amino acids, apart from a reduction in alanine, aspartic acid, serine and tyrosine. This can explain why 3% replacement of ECLM by 6.5% CLM is recommended. However, the odd results obtained by fish fed on diet 5 (full replacement of ECLM) cannot be explained on this basis because this diet had a poor amino acid profile and this high weight might be due to high growth rate of some fish individuals in this group.

(ii) Possible existing toxic substances in CLM. Glucosinolates are the main toxic effects of cruciferous vegetables. Yen and Wei (1993) reported that white cabbage contained 229 mg of glucosinolates per kg.

(iii) El-Hashimy (1971) reported that when 25% and 50% of protein content of casein were replaced by four leaf vegetables (radish, roquette, goedad and siris) the body weight of rats was reduced significantly when they were fed on diets at 50% replacement comparing with those fed on diets at 25% replacement, regardless of the vegetable source.

Carnivores have very short digestive tracts but herbivore tracts are relatively long and this is related to fish feeding habits. In this research study fish fed on a diet where full replacement of ECLM was achieved using CLM, obtained the highest gut index (Table 7) with increasing dietary crude fibre content (Table 2).

Plasma total protein (PTP) levels of fish fed on diets containing CLM seems to be affected by the dietary treatments in which fish were fed on the diets containing a complete replacement of ECLM by CLM exhibited the lowest values (Table 7). El-Hashimy (1971) reported the same results, which support the results of the present research. One of the most sensitive means of evaluating liver function is measuring appropriate serum parameters. GOT and GPT activities are localized primarily in the cytoplasmic compartment (Lemaire *et al*., 1991). Nile tilapia fed on diets D3, D4 and D5 where a gradual and full replacement of ECLM by CLM was used showed the highest level of plasma GOT (Table 7). Michael *et al*. (1987) reported that the elevation of GOT level may suggest cellulose destruction subsequent to pathological steatosis (accumulation of lipid globules and a reduction in glycogen reserves). Further histological studies are needed to investigate these suggestions.

Tangendjaja *et al*. (1984) have measured total carotene and total xanthophyll in the blood plasma of chicken that had been fed on different levels of leucaena leaf meal (0, 5, 10 and 20%). These authors found that plasma total xanthophyll was elevated proportionally as the level of leucaena in the diet increased, but carotene was not detected in the plasma although there were high levels in the feeds. In the present study low levels of carotene in plasma were detected (Table 7) even though ample carotene levels were found in the diets (Table 3). This may be explained on the basis that chicken and fish have little ability to utilize carotene existing in leucaena and cabbage.

In the present study, the cost reduction in diets supplemented with cabbage leaf meal may be due to the availability of this leaf waste free of charge. The same results have been reported by Yousif *et al*. (1994) when these authors included atriplex leaves in diets for Nile tilapia. Also, Hassan (1989) reported that the cost of the feed containing higher inclusion levels of soaked leucaena and water hyacinth leaf meal was lower than that of diets containing fishmeal.

In this study, the incidence cost of fish fed on the diets containing high levels of vegetable leaf waste meal is related to higher food conversion ratios in these fish. Pngilinan (1986) supported these findings and reported that when Nile tilapias were fed on a diet containing 25% leucaena they exhibited higher gain and the best food conversion ratios together with the best cost of producing a kilogram of fish.

Conclusion

From the results of the present investigation it can be concluded that 6.5% cabbage leaf meal can be incorporated into the diets for Nile tilapia.

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