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SUMMARY – In order to reduce reliance on fishmeal in fish feed formulations, several alternative protein sources or supplements were tested in Egypt. Feeding trials on Nile and blue tilapias, *Oreochromis niloticus* and *O. aureus,* gilthead seabream, *Sparus aurata,* European seabass, *Dicentrarchus labrax,* and mullet, *Mugil cephalus,* were conducted. The main target was the efficient utilization of waste animal and plant resources, such as trash fish and macroalgae, for fish feed production. Acid and fermented fish silage, a mixture of fish silage and soybean meal, green and red macroalgae *Ulva* and *Pterocladia* meals were all investigated. For each ingredient, a series of experiments were carried out to assess their nutritional properties, amino and fatty acid profiles as well as optimum dietary inclusion level to produce growth performance and feed utilization efficiency equivalent to those of fish meal.

Key words: Fish silage, *Ulva, Pterocladia*, *Oreochromis niloticus, O. aureus, Sparus aurata, Dicentrarchus labrax.*

***RESUME* –** *"Sources alternatives de protéines pour l'aliment des poissons en Egypte". Afin de réduire la dépendance de la farine de poisson en ce qui concerne la formulation des aliments pour aquaculture, plusieurs sources alternatives de protéines ou suppléments ont été testés en Egypte. Des tests d'alimentation ont été menés sur le tilapia du Nil et le tilapia bleu,* Oreochromis niloticus *et* O. aureus*, sur la daurade royale* Sparus aurata*, sur le bar européen* Dicentrarchus labrax*, et sur le mullet* Mugil cephalus*. L'objectif principal était l'utilisation efficace de sous-produits animaux et de ressources végétales, telles que les poissons de rebut et les macroalgues, pour la production d'aliment poisson. L'ensilage acide et fermenté de poisson, un mélange d'ensilage de poisson et de farine de soja, la farine de macroalgues vertes et rouges* Ulva *et* Pterocladia*, ont fait l'objet de recherches. Pour chaque ingrédient, une série d'expériences ont été menées pour en évaluer les propriétés nutritionnelles, les profils en acides aminés et acides gras ainsi que le niveau optimum d'incorporation dans le régime pour produire des performances de croissance et une efficacité d'utilisation de l'aliment équivalents à ceux de la farine de poisson.*

Mots-clés : *Ensilage de poisson,* Ulva, Pterocladia, Oreochromis niloticus, O. aureus, Sparus aurata, Dicentrarchus labrax.

Introduction

Egyptian aquaculture has developed rapidly in recent years, accounted for 47% of total fish produced in year 2000, and the potential for further diversification and expansion is excellent. In the mean time, domestic aquafeed industry is still under-developed and supplies for reliable fish feed at economically viable prices are greatly in need. At present, there are few feed mills manufacturing species-specific pelleted diets for cultured finfish and shrimp. High quality fish meals supply the major portion of protein in commercial rations of fish in semi-intensive and intensive culture systems. Therefore, the great demand for fish meal, and consequently, their escalating prices may represent a future limitation in the growth of Egyptian aquaculture. Clearly, ideally and less expensive feed ingredients are being sought and use made of the substantial discards, which are currently wasted in fishery or fish farming, will be necessary. Several approaches employed in Egypt, as well as in other regions, for the partial or total replacement of fish meal and the results reported have been promising. A wide variety of animal and plant foodstuffs were nutritionally evaluated for fish, however, the selection is based on their local availability, cost, nutritive value, and the ultimate market value of the farmed fish. Among the alternative protein sources or supplements that hold a particular promise for finfish cultured on the commercial scale in Egypt are: fish silage, marine macroalgae, processed soybean meal and yeast (single cell protein).

The present paper summarizes the major results of experiments and efforts directed towards the development of practical fish feeds from locally available ingredients and to lower, as far as possible, their fish meal content (Wassef *et al*., 1988, Wassef, 1990 and 1991, Gobran, 2000, Wassef *et al*., 2001a,b, 2002 and 2003 and Sakr, 2004). The major targets of these experiments were: (i) efficient utilization of waste animal and plant protein resources for fish feed production; (ii) determination of the nutritional properties of suitable ingredients readily available to a potential fish feed industry; and (iii) development and testing fish feed formulations based upon these novel protein sources for the main cultured fish species, namely tilapias (*Oreochromis niloticus and O.aureus)*, mullet (*Mugil cephalus),* gilthead seabream (*Sparus aurata)* and european seabass *(Dicentrarchus labrax)*.

Feed availabilty in Egypt

For a long time, extensive fish farming was the type practiced in Egypt, where only chemical and/or organic fertilizers were applied for promoting the natural productivity of ponds. Agricultural by-products such as wheat bran and rice bran were used for supplementation in some farms. As the technology of fish farming has developed, aquaculture started to exert some significant demand on fish feed. At present, there are twelve feed mills that produced about 68 500 tonnes of specialized feeds in year 2001. Most of feeds are produced for self-sufficiency to support the needs of Governmental fish farms, but some quantities are available for sale to private sector. Because of the cost, such mills produce fish feeds of 18-32% protein of sinking type pellets, however, higher protein floating feeds could be produced upon request. High quality fish meal provide the major component in the commercial fish feeds and may constitute up to 60% of the total diet for marine species, with higher levels being used in starter and fingerling rations. Generally, a good range of raw materials is available for fish manufacture in Egypt. However, price and competition from the human food and animal feed industries limits the choice. High quality feed materials are in short supply and are expensive. Apart from fish meal (imported and indigenous), the main available protein sources are: soybean meal (hexane-extracted), cottonseed meal (expeller), meat meal, poultry offal meal and feather meal. Other possibilities for new feed materials may be the wide spread marine macroalgae or fresh water weed hyacinth (El Sayed and Tacon, 1997). On local basis there is a scope for their incorporation into fish feeds particularly for tilapia and mullets. Tables 1 and 2 show the proximate composition of the tested feed ingredients, namely: acid fish silage (AFS), fermented fish silage (FFS), soybean meal (SBM), a mixture of FFS and SBM (MIX), green macroalga *Ulva* meal (UM) and red macroalga *Pterocladia* meal (PM) compared to fish meal (FM) from different sources and their amino acid profiles respectively.

Table 1. Composition (% dry matter) of tested proteins sources or supplements for fish feeds

Ingredient	Protein	Lipid	Ash	Moisture	NFE†	Fiber	DE††
AFS†††	72.90	13.12	12.76	73.28	1.22	-	164
AFS	73.40	17.1	8.3	-	1.20	-	178
AFS	63.00	22.10	9.68	75.00	-	-	177
FFS††††	56.67	12.7	20.04	0.98	-	-	135
SBM_G†††††	44.80	20.60	5.40	5.50	29.2	-	161
SBM_B	44.00	1.80	8.00	8.94	37.26	-	103
SBM_D	44.00	4.00	6.53	11.00	38.17	7.30	110
UM††††††	17.44	2.50	32.85	3.69	41.47	5.47	64
PM†††††††	22.61	2.18	37.30	3.05	28.29	9.62	35
FM_I††††††††	72.05	10.94	7.00	5.00	8.98	1.02	160
FM_D	61.00	8.95	20.72	6.20	9.73	-	136
FM_D	61.00	5.00	16.6	5.00	16.70	0.70	127

†Nitrogen free extract, by difference.
††Digestible energy (MJ/Kg).
†††Acid fish silage.
††††Fermented fish silage.
†††††Soybean meal (G: germinated; B: boilled fullfat; D: defatted).
††††††*Ulva* meal.
†††††††*Pterocladia* meal.
††††††††Fish meal (D: domestic product; I: imported Manhaden).

Table 2. Amino acid (g/100g protein) profiles of tested protein sources or supplement as compared to fish meal (FM)

Amino acid (AA)	AFS	FFS	SBM	MIX	UM	PM	FM
Indispensable (IAA)							
ARG	3.62	2.86	5.59	6.20	5.85	4.46	5.88
HIS	2.36	1.33	4.30	2.48	2.80	2.70	2.48
ILE	2.66	1.87	3.64	3.27	3.47	4.53	4.41
LEU	4.43	3.73	6.09	0.51	5.21	5.92	5.71
LYS	5.27	3.95	4.49	5.44	5.62	6.90	4.42
MET	1.81	1.35	1.25	2.22	4.40	3.26	2.50
PHE	2.36	2.30	4.30	3.06	4.45	4.78	3.87
THR	2.60	1.41	2.97	3.74	3.94	4.23	3.76
VAL	3.01	2.41	3.86	3.94	7.46	6.69	4.75
TRP	0.63	0.36		0.72			0.80
Total IAA	28.75	21.57	36.94	31.58	43.20	43.47	38.58
Dispensable (DAA)							
ASP	5.97		15.20		11.54	10.59	2.04
SER	2.62		4.15		4.48	4.08	0.66
GLU	8.81		13.03		9.35	10.22	3.30
GLY	3.50		3.14		5.53	7.49	4.13
ALA	3.74		3.54		7.19	7.23	1.47
TYR	2.04		4.03		3.31	3.65	1.47
PRO	2.60		4.46		5.15	4.64	
CYS	0.73		1.13		1.27	1.51	0.97
Total (DAA)	30.01		48.68		47.82	49.41	12.57
Total AA	58.76		85.62		91.02	92.88	51.15

AFS: acid fish silage; FFS: fermented fish silage; SBM: boiled full fat soy meal; MIX: mixture of FFS and SBM; UM: *Ulva* meal; PM: *Pterocladia* meal; FM: fish meal.

Fish silage (acidified, AFS and fermented, FFS)

Ensilage of fish, as a method of preservation, is not a new technique but still applicable nowadays (Vidotti *et al.,* 2002). It may be one way to convert waste fish into usable by-product for incorporation into fish (or animal) feeds (Austreng and Asgard, 1986). In Egypt, 94,000 tones of trash fish, unfit for human consumption, was available in 2000, representing 20% of fishery production plus 5% unsalable farm crop. Fish silage is a liquid product manufactured by mincing preferably whole fish (or processing waste) and mixing with an acid (acid preserved silage) or by lactic acid bacterial fermentation (fermented fish silage). The resulting silage was relatively stable at ambient storage (16-30^{o}C) for at least 3 months (dependant on the composition of raw fish used) with no marked changes in its nutritive quality (Wassef, 1990). Fish silage is generally a product of high biological value presenting practically the same composition as the original raw material (Tacon, 1993), easy to produce and involves simple artisanal technology, which is adaptable on farm level in Egypt. Liquid silage, characterized by a strong fish odor and yellowish color, can be further sun dried or directly mixed with the soybean ingredient of the diet before incorporation into compounded feeds.

A. Feeding trial with acid fish silage (AFS) in diets for gilthead bream *Sparus aurata*

A preliminary feeding trial was initiated to test two unconventional protein sources, namely AFS and dry germinated soyameal (DGS) in diets for gilthead bream fry. Three balanced diets (I,II,III) were formulated (Table 3) on the basic idea of FM replacement either partly, by SBM (diet I) or DGS (diet III) or completely by a mixture of both AFS and SBM (diet II). Gilthead seabream fry (mean 3.1 cm length and 0.65 g weight) were kept into nine 230 l seawater glass aquaria at a rate of 40 fry per aquarium at tree replicates for each treatment. Fish were fed test diets to apparent satiety for 41 days at ambient temperature 18-27°C. Weight gain was highest (10.5 g/fish) for fish fed diet II (AFS and SBM), followed by diet III (9.45 g) then diet I (7.7 g). Growth rate was comparable with that previously recorded in earthen ponds without any supplementary feeding or fertilization (Eisawy and Wassef, 1984). These preliminary observations indicated that a mixture of AFS and SBM (defatted) is a potential FM-replacer for *S.aurata* fry (Wassef, 1991). However, further experiments are required for longer feeding periods to elucidate a precise effect on feed utilization efficiency.

Table 3. Ingredients and composition (%DM) of test diets fed to *Sparus aurata* fry

Ingredient	Diet I	Diet II	Diet III
	(SBM/FM)	(SBM/AFS)	(DGS/FM)
Fish meal (FM)†	24.2	-	24.2
Soybean meal (SBM)††	66.5	45.0	-
Fish silage (AFS)†††	-	42.9	-
Dry germinated soyameal (DGS)††††	-	-	66.7
Cod liver oil	2.7	-	2.7
Soybean oil	6.6	5.6	-
Wheat starch	-	4.7	-
Calcium carbonate	-	1.8	-
Cellulose	-	-	6.4
Calculated Proximate analyses (%DM)			
Crude protein	45.0	45.0	45.0
Ether extract	11.0	10.0	14.8
Metabolic energy (MJ/Kg DM)	12.5	12.0	14.1
Calcium	1.6	1.01	1.5
Phosphorus	1.1	0.73	1.1

†Local FM [(65% P (protein), 4% L (lipid)].
††Defatted SBM (44% P, 1% L).
†††Mixture (73.4% P, 17.1% L).
††††DGS (43.9% P, 16.7% L).

B. Feeding trials with AFS in diets for Nile and blue tilapia (*O.niloticus and O.aureus)*

The major objective was to test acid fish silage (AFS) as the main protein source in diets for Nile and blue tilapia fry and fingerlings.

A 30% crude protein control diet (CTR, 1), containing fishmeal (FM) and defatted soybean meal (SBM) as the protein ingredients, was formulated to fulfill nutritional requirements of the species (Santiago and Lovell, 1988). Liquid AFS was firstly blended with the SBM portion of the diet. In a similar way, three experimental diets (2, 3 and 4) were prepared, by total substitution of the FM portion by AFS, at dietary protein levels 25, 30 and 35% respectively. Formulation and composition of test diets is given in Tables 4 and 5. Before the experimental period, fish were fed with an acclimation diet to satiation for two weeks. Thereafter, test diets were fed as moist pellets to *O. niloticus* and *O. aureus* fry (1±0.75 g) and fingerlings (5.5±2 g) stocked into 32 glass aquaria (120 l each), in triplicate groups for each treatment, to apparent satiation. Diets were provided as 2-3 meals per day, six days a week for 17 weeks at ambient temperature 24-28°C (mean 26°C). Growth rate was estimated at biweekly intervals. Supplemental aeration was provided by a blower system and water quality parameters in the experimental system were measured (APHA, 1995) at biweekly intervals prior to removal of fishes for weighing (dissolved oxygen ranged from 6.6-8.8 mg/l, nitrites 0.01-0.07 mg/l and pH 7-7.3). Proximate composition of fish and diets (% dry matter) were determined according to the standard methodology of AOAC (1995). Diets-cost analysis was also estimated (Gobran, 2000) Statistical ANOVA and Duncan multiple range test were applied to compare treatment means for significant differences ($P<0.05$).

Table 4. Ingredients and proximate composition of acid fish silage (AFS) experimental diets fed to Nile and blue tilapias *(O. niloticus* and *O.aureus)* for 17 weeks

Ingredients (g / 100g)	Experimental diets (%protein)			
	CTR1(30%)	2 (25%)	3(30%)	4 (35%)
Fish meal (FM)[†]	15.0	–	–	–
Acid fish silage (AFS)	–	5.0	15.0	25.0
Soybean meal (hexan-extracted)	34.8	34.8	34.8	33.8
Wheat bran	35.0	35.0	35.0	26.0
Yellow corn	15.0	25.0	15.0	25.0
Vitamin and mineral premix[††]	0.2	0.2	0.2	0.2
Proximate composition (%DM)				
Dry matter	90.07	85.97	79.57	73.08
Crude protein (CP)	29.98	24.86	30.28	35.06
Lipid (L)	3.76	4.50	6.32	8.22
Fiber	6.83	6.94	6.72	5.66
Ash	5.61	3.71	4.56	5.41
Nitrogen free extract (NFE)[†††]	53.82	60.64	54.03	48.85
Gross energy (GE, Kcal/Kg)	2049.2	1769.2	2127.1	2455.9
Protein / energy ratio (mg /Kcal GE)	146.3	140.5	142.4	142.8

[†]Local FM (61% protein and 5% lipid).
[††]NRC (1993).
[†††]Estimated by difference.

Table 5. Amino acid composition of acid fish silage (AFS)-experimental diets

Amino acid (AA) (g/100g protein)	Experimental diets (% protein)			
	CTR1 (30%)	2 (25%)	3 (30%)	4 (35%)
Indispensable AA				
Arginine	0.69	0.28		0.84
Histidine	0.32	0.06	0.39	0.28
Isoleucine	0.27	0.09	0.52	0.25
Leucine	0.45	0.20	0.82	0.46
Lysine	0.06	0.28	1.29	0.44
Methionine	0.16	0.02	0.44	0.02
Phenylalanine	0.24	0.23	0.79	0.31
Threonine	1.61	0.64	2.05	1.68
Valine	0.47	0.19	1.04	0.41
Total IAA	4.27	1.99	8.23	4.69
Dispensable AA				
Aspartic acid	0.93	0.36	1.05	0.99
Serine	0.22	0.09	0.29	0.22
Glutamic acid	2.26	0.96	3.44	2.48
Glycine	0.72	0.29	0.79	0.79
Alanine	0.28	0.11	0.15	0.29
Tyrosine	0.27	0.07	0.69	0.24
Proline	1.02	0.38	0.91	1.06
Cystine	0.07	0.24	0.46	0.63
Total DAA	5.77	2.50	7.78	6.70
Total AA	10.04	4.49	16.01	11.39

Results of this experiment have shown that under our feeding protocol, feed intake (FI, g/fish/d) or palatability were unaffected significantly by AFS dietary level (Table 6). Variations of daily weight gain, (DWG, g/fish/d) were found insignificant among all treatments. Among the tested levels of AFS-based diets, diet 3 (30% protein) supported weight gain and growth rate higher or similar to those in CTR1 group without significant difference (P<0.05). Fish fed diet 3 produced significantly higher percentage weight gain (PWG, 834%) and specific growth rate (SGR, 2.97) as compared to CTR1 group (428% and 2.33 respectively) or other tested diets. Daily weight gain (DWG) insignificantly varied among treatments. In the mean time, diet 3 resulted in best feed conversion ratio (FCR, 2.97) and protein efficiency ratio (PER, 1.44) compared to CTR1 diet (4.66 and 0.88 respectively) or other tested diets (Table 6).

Table 6. Growth performance and feed utilization indices for *O. niloticus* and *O. aureus* fed acid silage diets for 17 weeks†

Diet	% CP	IW (g)	FW†† (g)	TWG†† (g/fish)	PWG†† (%)	DWG (g/d)	SGR (%)	DFI (g/fish/d)	FCR	PER
O. niloticus fry										
CTR 1	30	1.24	6.55	5.31[a]	428.2[a]	0.07	2.33	0.37	4.66	0.88
Diet 2	25	1.75	5.67	3.92[a]	224.1[b]	0.04	1.45	0.29	5.30	0.72
Diet 3	30	0.31	2.89	2.58[ab]	832.3[a]	0.03	2.97	0.10	2.97	1.44
Diet 4	35	0.34	2.43	2.09[b]	614.7[a]	0.02	1.94	0.09	4.80	0.80
O. niloticus fingerlings										
CTR 1	30	7.21	62.18[a]	54.97[a]	762.4[a]	0.49	2.02	2.02	5.87	1.08
Diet 2	25	7.20	45.0[abc]	37.80[b]	524.8[b]	0.33	1.66	1.57	5.92	0.95
Diet 3	30	6.73	73.55[b]	66.82[a]	992.9[a]	0.58	2.21	2.20	4.63	1.19
Diet 4	35	6.54	66.65[c]	60.11[a]	919.1[a]	0.52	2.20	1.88	5.09	0.91
O. aureus fry										
CTR 1	30	1.20	4.77	3.57[a]	297.5[a]	0.05	2.38	0.26	8.01	1.20
Diet 2	25	1.27	4.20	2.93[a]	230.7[b]	0.04	1.86	0.22	6.59	1.05
Diet 3	30	1.51	5.22	3.71[a]	245.7[a]	0.04	1.62	0.26	8.81	0.73
Diet 4	35	1.48	4.83	3.35[a]	226.4[b]	0.04	1.79	0.24	9.92	0.67
O. aureus fingerlings										
CTR 1	30	2.12	57.35	55.23[a]	2605.2[a]	0.48	3.14	1.66	3.61	2.68
Diet 2	25	3.39	43.10	39.66[b]	1170[b]	0.34	2.35	1.41	4.27	1.85
Diet 3	30	3.44	61.65	58.21[a]	1692.2	0.51	2.72	1.98	4.09	1.86
Diet 4	35	4.12	58.55	54.43[a]	1321.1	0.47	2.45	1.79	3.94	1.29

†IW: initial weight; FW: final weight; TWG: total weight gain; PWG: %weight gain; DWG: daily weight gain; SGR: specific growth rate; DFI: daily feed intake; FCR: feed conversion ratio; PER: protein efficiency ratio.

††Values in the same column with different superscript, for each group, are significantly (P<0.05) different.

In addition, economical evaluation proved that the least cost diet was diet 2 (0.67 LE), the least AFS level, followed by Diet 3 (0.79 LE), while CTR1 diet was the most expensive among all (1 LE). Nevertheless, diet 3 was recorded best incidence cost per kg fish gain (2.66 and 2.76 LE for *O.niloticus* and *O.aureus* respectively) among all tested levels or compared to CTR1 diet (3 LE) (Gobran, 2000). Data in Table 6 showed also that fingerling groups recorded higher growth performance and feed utilization indices than fry groups indicating better AFS utilization for bigger fish sizes. Moreover, fish composition was insignificantly affected by dietary acid silage level (Tables 7 and 8).

Results of this experiment indicated that Nile and blue tilapia can utilize efficiently acid fish silage protein up to 30% dietary level to produce growth performance equivalent, or even better, to that of FM diet, with no marked alteration in their nutrient composition.

Table 7. Proximate composition of *O.niloticus* and *O. aureus* at start and end of feeding trial

Diets	Mean (% dry weight) ± SD†			
	Moisture	Protein	Lipids	Ash
O. niloticus fry				
Initial	85.20±0.3	65.63±0.2	17.40±0.1	11.08±0.2
Final				
CTR1	74.36±1.0	50.63±0.5	26.80±0.6	14.49±0.2
Diet 2 (25%)	75.05±0.7	48.13±0.4	28.10±1.6	14.42±0.2
Diet 3 (30%)	75.38±0.2	51.25±0.2	23.80±0.5	14.12±0.7
Diet 4 (35%)	75.68±0.5	48.13±0.5	24.02±0.6	12.69±0.9
O.aureus fry				
Initial	82.52±0.9	64.45±0.9	12.65±1.0	12.05±0.8
Final				
CTR1	73.59±0.8	50.63±0.3	30.50±1.1	11.94±0.2
Diet 2 (25%)	74.88±1.3	52.50±0.6	26.30±1.0	14.99±0.7
Diet 3 (30%)	74.94±0.5	48.76±0.4	26.10±0.1	14.11±0.7
Diet 4 (35%)	75.26±0.5	49.07±0.5	22.40±0.1	15.62±0.3
O.niloticus fingerlings				
Initial	78.04±1.2	55.00±0.8	17.60±1.0	12.80±0.8
Final				
CTR1	79.53±1.0	71.17±1.3^{a}	10.63±1.0a	10.77±0.9^{a}
Diet 2 (25%)	78.80±1.1	70.63±1.2^{b}	13.64±1.1a	9.76±0.8^{a}
Diet 3 (30%)	78.70±1.4	66.57±0.7^{abc}	15.89±1.4bc	9.49±0.6^{b}
Diet 4 (35%)	78.70±1.3	72.59±0.7^{c}	12.59±0.7c	12.54±0.3^{ab}
O.aureus fingerlings				
Initial	80.52±1.5	56.62±0.9	17.60±1.5	13.39±0.7
Final				
CTR1	78.23±1.0	72.15±1.7	13.51±1.0	10.31±0.4
Diet 2 (25%)	78.37±0.8	68.97±1.3	13.15±0.8	10.74±0.5
Diet 3 (30%)	79.93±1.2	70.09±1.4	14.04±1.2	10.84±0.7
Diet 4 (35%)	78.95±1.2	70.13±1.1	15.56±1.2	11.61±0.5

Values in the same column, for each group, with different superscript are significantly ($P<0.05$) different.
†SD = Standard deviation.

Many authors stated that fish silage is the best alternative of fish meal (Austreng and Asgard, 1986, Lapie and Benitez, 1992, Fagbenro, 1994 and Vidotti et al., 2002). Blending AFS with a binder meal, such as SBM ingredient of the diet, inhibits further autolysis and therefore greatly improve its nutritional quality (Fagbenro and Jauncey, 1994). Heat treatment (to 85^{o}C), is another alternative practice suggested following the initial stages of liquefaction to prevent further break down or hydrolyze the protein structures in the slurry and stabilize the mixture, but will adds cost (Gobran, 2000). In Egypt, successful sun drying may offer a solution for the high water content of silage.

C. Feeding trials with fermented fish silage (FFS) in practical diets for Nile tilapia *O. niloticus*

The aim was to use dried FFS as a replacement for FM in practical diets for fry, fingerling and growout *O. niloticus.*

A basal CTR diet (28% P) was formulated with FM as the major protein source and fulfilled the nutritional requirements of the species; then FM was replaced by 25, 50, 75 and 100% by either FFS or a mixture of FFS and SBM (1:1, w/w) to prepare eight experimental diets. Diets had almost equal protein content, metabolizable energy (140 Kcal/100g) and protein/energy ratio (144.7). *O. niloticus* fry (1 g), fingerlings (11 g) and growout (26 g) kept in fiberglass tanks (120 l each) or cement ponds, in triplicate groups for each treatment, were fed test diets to near satiation, at 2-3 meals per day for 18 weeks at ambient temperature (20-30^{o}C).

Table 8. Amino acid composition (g/100 g protein) of *O. niloticus* and *O. aureus* fingerlings fed acid fish silage-test diets for 17 weeks

Amino acid (AA)	*O. niloticus*				*O. aureus*			
	CTR1	2(25%)	3(30%)	4(35%)	CTR1	2(25%)	3(30%)	4(35%)
Indispensable AA								
Arginine	8.18	5.69	6.80	6.64	5.46	5.59	6.58	9.09
Histidine	2.52	2.39	3.02	2.28	2.03	2.22	2.18	2.53
Isoleucine	2.32	2.0	2.93	2.6	1.99	2.27	2.56	2.01
Leucine	3.68	3.29	4.20	3.99	3.12	3.19	4.21	3.54
Lysine	3.58	3.34	5.15	3.90	4.15	3.24	6.24	6.79
Methionine	1.59	0.58	3.54	2.65	1.72	1.53	2.85	0.29
Phenylalanine	2.42	2.02	2.69	3.16	2.21	2.08	3.48	1.87
Threonine	9.25	7.85	9.87	9.29	7.40	7.59	8.56	9.52
Valine	3.34	3.34	4.39	4.09	2.98	3.38	4.26	3.01
Total IAA	36.90	31.07	42.59	38.65	31.06	31.09	40.92	38.65
Dispensible AA								
Aspartic acid	5.86	4.89	7.13	7.39	5.24	5.18	6.24	7.03
Serine	1.16	1.18	1.37	1.25	1.04	1.11	1.35	1.19
Glutamic acid	12.78	10.48	14.36	15.51	11.83	11.33	14.27	14.73
Glycine	4.31	3.76	4.91	4.67	3.70	3.84	4.89	5.21
Alanine	2.08	1.83	1.94	1.99	1.67	1.76	2.23	2.25
Tyrosine	3.05	3.01	3.87	3.29	2.39	2.82	3.34	2.34
Proline	6.34	5.55	6.52	5.89	4.79	5.59	6.82	6.36
Cystine	2.23	1.32	1.98	1.58	1.76	1.13	2.37	4.49
Total DAA	37.81	32.02	42.08	41.57	32.42	32.76	41.51	43.60
Total AA	74.71	63.09	84.67	80.22	63.48	63.85	82.43	82.25

Results for nutritive value of FFS or MIX (Table 1) and amino acid profiles (Table 2) indicated the suitability of both ingredients for inclusion in Nile tilapia diets. Dried FFS contained comparatively lower total indispensable amino acid (IAA) content (22%) than MIX (32%) however, little variations occurred between IAA profile of MIX and local FM. Results indicated that feed consumption (FI) of test diets was unaffected significantly by dietary FM level (Tables 9 and 10). Generally, fish fed MIX diets yielded, at most, better growth rate and protein utilization as compared to FFS- diets. Growth performance as well as feed utilization indices emphasized that Nile tilapia utilized test diets efficiently up to 75% FM- replacement level for MIX or 50% for FFS, to attain growth performance comparable to that of CTR diet, however, complete FM substitution resulted in depressed growth and relatively poor feed utilization among all treatments. Furthermore, relatively higher growth performance and feed utilization indices were recorded for growout than for fingerling or fry, which signified their better utilization of FFS-based diets.

In the mean time, no marked change in carcass or liver nutrient composition (protein, lipid, ash, moisture and liver glycogen) was noticed up to 75% FM replacement level, except for the complete substitution level (100% of FM) which produced relatively lower protein and higher lipid contents compared to CTR group.

Table 9. Growth performance and feed utilization indices for *O. niloticus* fry fed FFS- or MIX-diets

	FFS-Diets (% FM replacement)					MIX-Diets (% FM replacement)				
	0	25	50	75	100	0	25	50	75	100
Indices†	CTR_F	F1	F2	F3	F4	CTR_M	M1	M2	M3	M4
IW (g)	1.30	1.30	1.30	1.30	1.30	0.96	0.96	0.96	0.96	0.96
FW (g)	18.8^a	15.58^b	15.0^b	13.0^c	10.33^d	21.0^a	18.49^b	16.38^c	13.65^d	11.13^e
TWG (g)	17.5^a	14.28^b	13.7^b	11.7^c	9.03^d	20.04^a	17.53^b	15.42^c	12.69^d	10.17^e
PWG	1346^a	1098^b	1054^b	900^c	695^d	2088^a	1826^b	1606^c	1322^d	1059^e
DWG	0.13	0.11	0.10	0.09	0.07	0.15	0.13	0.11	0.09	0.08
SGR	2.41	2.24	2.20	2.04	1.75	2.69	2.57	2.45	2.10	1.92
K (%)	1.87	1.81	1.88	1.82	1.87	1.86	1.86	1.83	1.78	1.71
DFI	0.22	0.19	0.18	0.16	0.14	0.23	0.22	0.20	0.15	0.14
FCR	1.52	1.66	1.71	1.79	2.12	1.45	1.88	2.0	1.81	2.38
PER	2.79	2.57	2.55	2.38	2.13	3.36	3.30	3.19	2.94	2.78
PPV	8.80^a	8.36^a	8.10^a	7.74^a	6.47^b	9.28^a	8.60^a	8.22^a	8.80^a	7.59^b

†IW: initial weight; FW: final weight; TWG: total weight gain; PWG: % weight gain; DWG: daily weight gain (g/fish/d); SGR: specific growth rate (%); K: condition factor; DFI: daily feed intake (g/fish/d); FCR: feed conversion ratio; PER: protein efficiency ratio; PPV: protein productive value.
Means in the same row with the same letter, for each tested material, are insignificantly (P<0.05) differ.

Table 10. Growth performance and feed utilization indices for *O. niloticus* fingerling fed FFS- or MIX-diets

	FFS-Diets (% FM replacement)					MIX-Diets (% FM replacement)				
	0	25	50	75	100	0	25	50	75	100
Indices	CTR_F	F1	F2	F3	F4	CTR_M	M1	M2	M3	M4
IW (g)	12.75	12.75	12.75	12.75	12.75	10.35	10.35	10.35	10.35	10.35
FW (g)	77.0^a	78.95^b	75.0^c	66.1^d	47.78^e	66.75^a	63.86^b	60.78^c	52.77	40.38^e
TWG (g)	64.25^a	66.2^b	62.25^c	53.34^d	35.03^e	56.05^a	53.51^b	50.43^c	42.42^d	30.03^e
PWG	589.9^a	546.2^b	489.6^c	400.7^d	292.7^e	530.0^a	523.7^b	467.2^c	354.4^d	265.6^e
DWG	0.48	0.49	0.46	0.40	0.26	0.42	0.40	0.37	0.31	0.22
SGR	1.21	1.30	1.27	1.17	0.91	1.61	1.58	1.52	1.37	1.18
K (%)	1.9	1.87	1.81	1.79	1.75	1.98	1.89	1.82	1.77	1.74
DFI	0.94	1.00	0.99	0.89	0.72	0.86	0.83	0.80	0.74	0.66
FCR	2.08	2.04	2.04	2.52	2.83	2.10	2.28	2.56	2.43	3.52
PER	1.75	1.78	1.75	1.44	1.31	1.81	1.80	1.78	1.64	1.37
PPV	1.96	1.90	1.80	1.69	1.78	1.92	1.91	1.85	1.71	1.27

The good protein utilization of test diets may be attributed to the fact that diets were nutritionally balanced, particularly IAAs content, and rapidly consumed by fish, as visually observed. Besides, both FFS and MIX ingredient contained predigested proteins, however higher inclusion levels reduced their nutritional quality.The superior performance of CTR fish group, in some treatments, was referred to the fact that the nutritional characteristics of FM-protein approximated almost exactly to the nutritional requirements of cultured finfish (Tacon, 1993).

These experiments concluded that both dried FFS if used singly or in combination with SBM (MIX) can be used efficiently as FM replacers, at the recommended levels of 50 or 75% respectively, in Nile tilapia practical diets (28% P) (Wassef *et al.,* 2003).

Macroalgae *Ulva lactuca (Chlorophyta) and Pterocladia capillacea (Phodophyta)*

Among the wide spread seaweed along the Egyptian Mediterranean coasts all the year around, are the green macroalga *Ulva lactuca* and the red *Pterocladia capillacea.* Their nutrient composition (Table 1), amino acid profiles (Table 2) and fatty acid profiles (Wassef *et al.,* 2002) indicated suitable ingredients for fish feeds. Besides, previous *Ulva* meal feeding trial with mullet (*Mugil cephalus)* was successful and resulted in higher growth performance, feed intake and protein utilization efficiency (Wassef *et al.,* 2001a,b). Therefore, two experiments were performed aimed to investigate the effect of feeding seabream *Sparus aurata* and seabass *Dicentrarchus labrax* fry *Pterocladia* or *Ulva* meals (PM and UM respectively) as a protein supplement.

In these experiments, six 50% crude protein experimental diets were developed, for each species, to contain 5, 10 and 15 % of either PM or UM (designated as P_5, P_{10}, P_{15} and U_5, U_{10}, U_{15} respectively) and evaluated as an additive, versus the 50% CP fishmeal control diet (CTR). Fish were fed to apparent satiation three to four times daily for eight weeks at an average ambient temperature of 27^{o}C. Growth performance, feed utilization indices as well as amount of food consumed (feed intake), survival rates and fish nutrient composition were all measured at the end of feeding trial.

Nutritional properties of *Pterocladia* and *Ulva* meals (PM and UM)

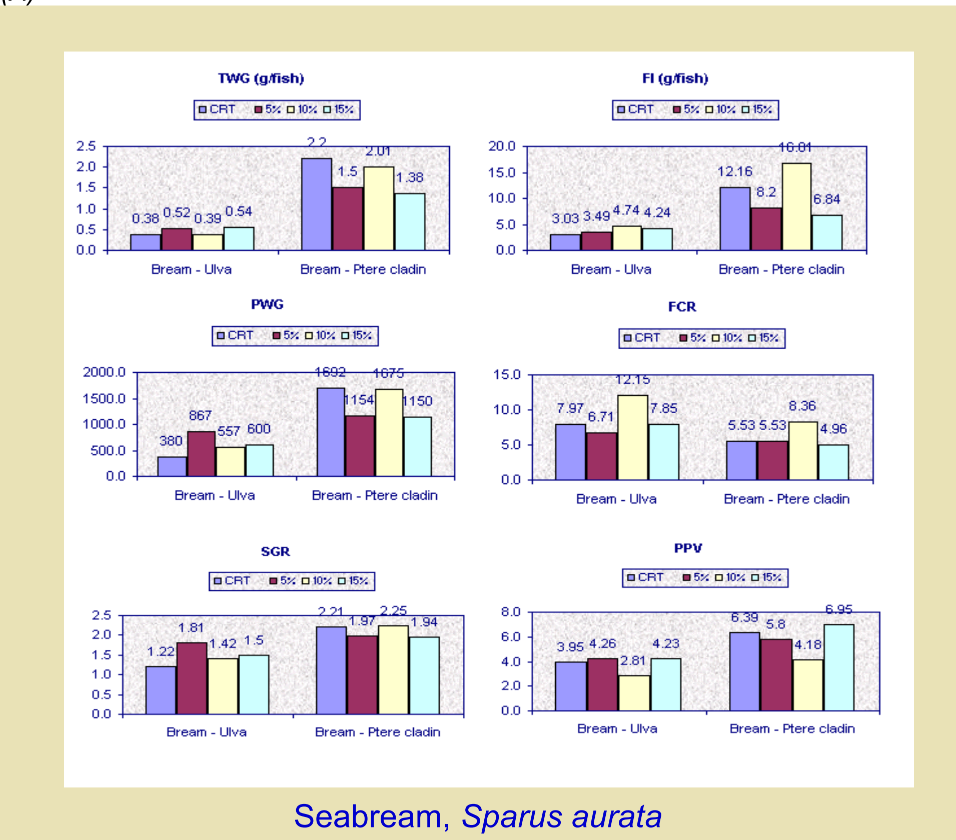
Red alga, *Pterocladia capillacea,* have relatively higher protein (23% of dry matter), ash (37%) and fiber (10%) contents, but lower carbohydrates (28%) and digestible energy (185 kcal/g of dry matter), than green alga, *Ulva lactuca* (Table 1). Both algae meals (PM and UM*)* have almost equal lipids (2-2.5%), indispensable and dispensable amino acids (IAA and DAA), as well as relative abundance of individual AA (Table 2). PM lysine content was slightly higher (7%), but methionine was lower (3%) relative to UM (6% and 4% respectively). Moreover, PM contains higher amounts of polyunsaturated fatty acids (PUFA) (11% versus 5%) and omega-*6* FA (10% vs 1%), but lower omega-*3* FA (1% vs 4%) compared with UM. Oleic (18:1) and palmitic (16:0) acids are the most dominant FAs in both meals (55 and 21% for 18:1 and 31 and 29% for 16:0 in PM and UM respectively). It was considered in diet formulation that PM and UM test diets contained almost equal indispensable amino acids (IAA), dispensable AA (DAA) and PUFA levels to that of CTR diet (43.6%, 47% and 20.6% respectively).

Results for gilthead seabream, Sparus aurata

Results of feeding *Sparus aurata* fry PM and UP test diets, under aquaria conditions, showed that dietary supplementation with PM at 10% level (diet P_{10}) has significantly increased food consumption (food intake, 16.0 vs 12.2 g/fish/d for CTR), protein intake (8.6 vs 6.1 g/fish for CTR) and survival rate (85.5% vs 70% for CTR) compared with the control group (Fig. 1). Fish fed this diet also produced significantly higher percentage weight gain (PWG, 1675%) and daily weight gain (DWG, 36 mg/fish/d) among all tested levels. Although total weight gain (TWG, 2 g) and specific growth rate (SGR, 2.3%) were the highest for fish fed P_{10} diet, variations were tested to be insignificant ($P<0.05$). In the meantime S. aurata fry can utilize PM diets efficiently up to the highest inclusion level (15%). Protein efficiency ratio (PER) was unaffected significantly among all treatments, but best feed conversion ratio (FCR, 4.96%) and protein productive value (PPV, 6.95) were insignificantly different from those of CTR group (Fig 1A).

On the other hand, dietary UM supplementation at 5% level (diet U_5) produced significantly higher PWG (867%), DWG (9 mg/fish/d) and survival rate (SR, 76.5%) compared to CTR group (380%, 3 mg/fish/d and 65 % respectively). This increase in PWG or DWG is approximately 2.5-3 folds that of CTR group. Similarly, feeding fish U_5 diet had improved feed intake (3.5 g/fish) and protein intake (1.9 g/fish) relative to CTR group (3 and 1.5 g/fish respectively). In the meantime, this diet produced better FCR (6.7) and highest PPV (4.3) among all treatments, whereas PER was unaffected significantly (Fig. 1A).

(A)



(B)

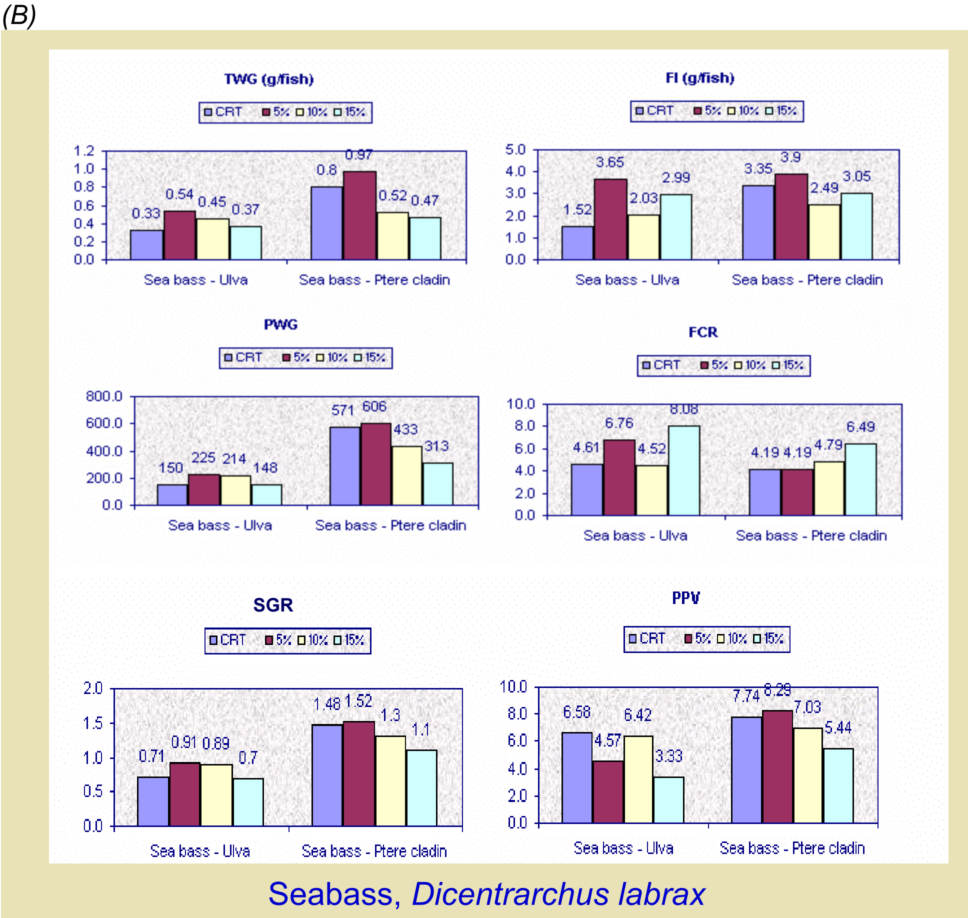


Fig. 1. Growth performance and feed utilization indices for seabream (A) and seabass (B) fed UM- or PM-supplemented diets for eight weeks.

Despite feeding seabream PM or UM tested diets had improved their major nutrients composition, no marked change in fish composition was detected among treatments, except for the slightly higher moisture content for fish fed PM diets (72%) relative to CTR group (70%) (Fig. 2A). Final IAA content of fish was almost equal when fed P_5 and P_{10} (45%) or U_5 and U_{10} (44%) diets, unexpectedly higher than CTR group (43%), whereas higher dietary algae meal level (P_{15} or U_{15}) produced slightly lower IAA levels in experimental fish (44% and 41% each in turn). In the meantime, highest PUFA and total FAs contents were observed for fish fed P_{10} diet (20 and 94% respectively) compared to CTR group (10 and 92% each in turn). Similarly, among the tested UM supplementation levels, fish fed the U_5 diet recorded the highest PUFA content (9.4%) comparable to that of CTR group (9.6%), whereas total fatty acid content in all treatments were almost equal (91%) (Wassef et *al*., 2002).

Results for seabass, Dicentrarchus labrax

Supplementation of bass fry diets with 5% PM or UM*,* under laboratory conditions, had resulted in significantly improved PWG (606% and 225% respectively), DWG (17 and 10 mg/fish/d), feed intake (FI, 3.9 and 3.7 g/fish/d), survival rate (SR, 73% and 85 %) and PPV (8.3) relative to CTR group. In the mean time TWG, SGR, FCR and PER were unchanged significantly among treatments indicating best utilization for the 5% PM or UM supplemented diets among all tested levels (Fig. 1B).

Concomitant with the observed increase in weight gain, feed intake and survival rate, these diets (P_5 and U_5) yielded better fish nutrient composition at the end of feeding trial. Significant decrease in moisture content (73% and 76% respectively) relative to those of CTR fish (75% and 87% respectively) and slight increase in lipid for the PM diet only (6.6% vs 4.4 % for CTR). Protein and ash contents were unaffected among treatments (Fig. 2B).

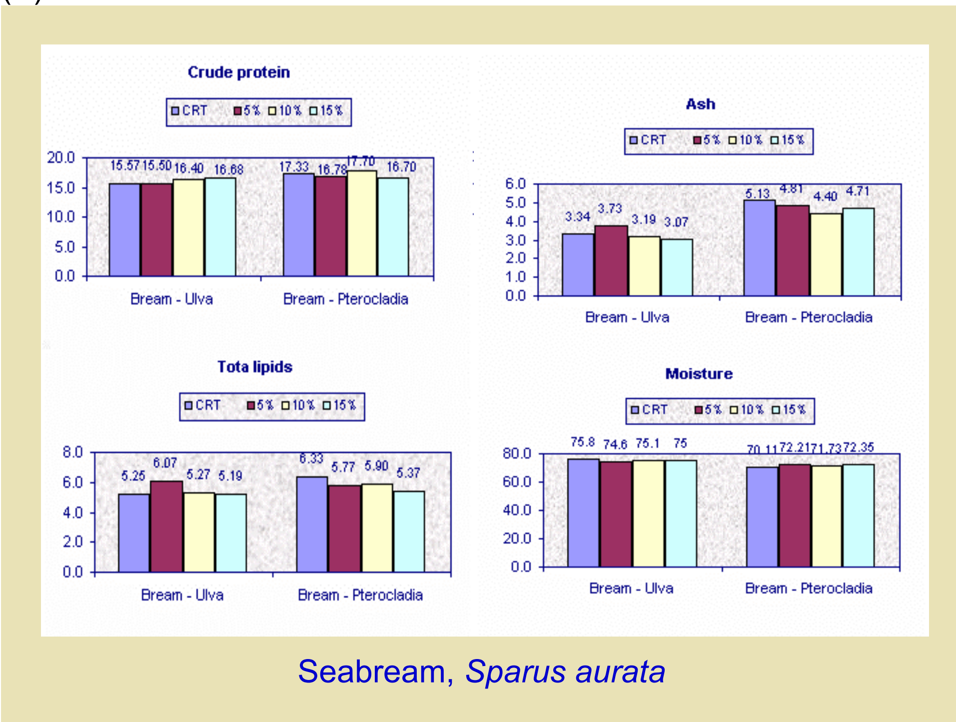
Furthermore, results of a 5- minutes air exposure test for experimental fish at the end of feeding trial indicated remarkably improved survival rate of fish fed the P_{10} and U_5 diets (Table 11).

Table 11. Effect of feeding dietary *Ulva* or *Pterocladia* meal supplement on survival rate of bream and bass after 5 min air-exposure test

Test diet	% Survival rate (SR)	
	Bream	Bass
Ulva meal		
Control	20	20
5%	40	20
10%	10	10
15%	30	30
Pterocladia meal		
Control	95.9	55
5%	95	65
10%	100	66
15%	95.5	55

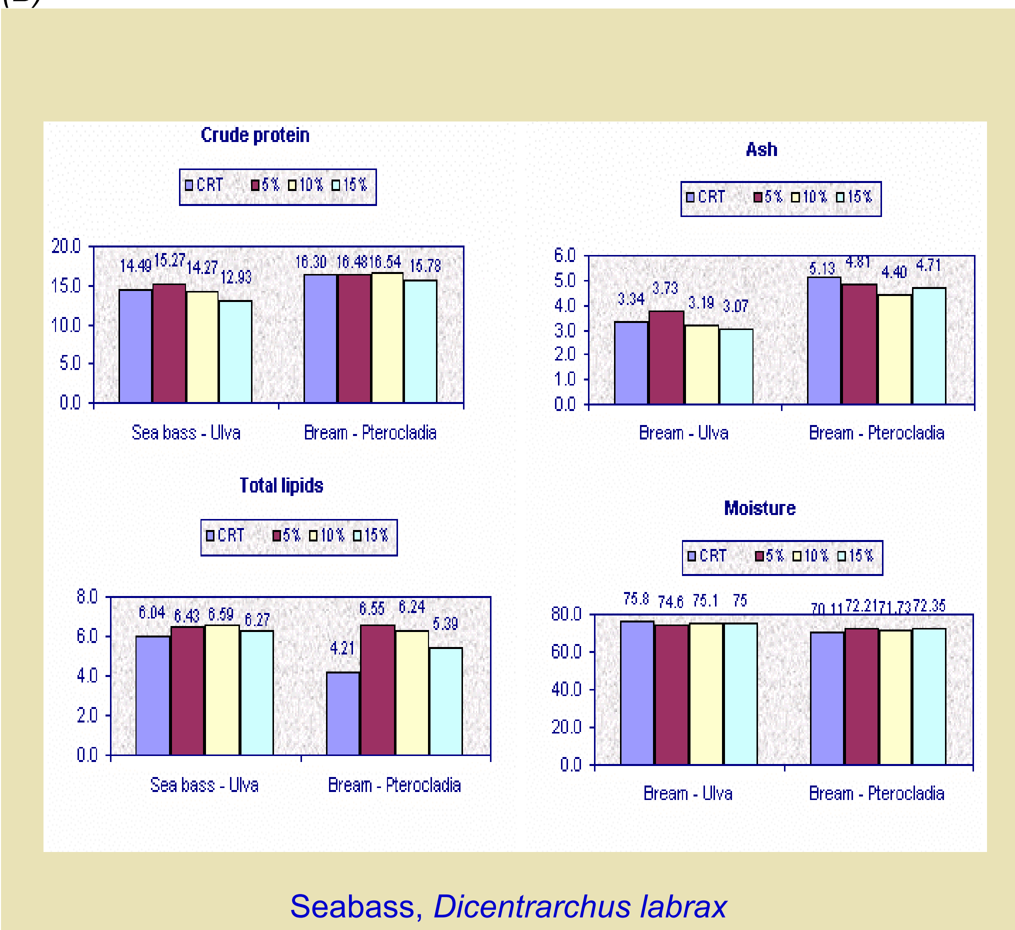
Earlier feeding trials with macroalgae in Egypt, have taken place on grey mullet (*Mugil cephalus*) and emphasized that 20% UM supplemented diets resulted in enhancement of growth performance, feed utilization and muscle quality (Wassef *et al.,* 2001a,b). Numerous reports on the dietary benefits of using *Ulva* sp. in fish feeds are summarized by Mustafa and Nakagawa (1995) in general and Mustafa *et al*., (1995) for a closely relative species red seabream, while Kissil *et al.* (1992) found no improvement in growth rate for gilthead bream *S. aurata* growout fed 8% UM supplemented diet. Unfortunately no previous records on feeding seabass macroalgae supplement is available. It may also be worthy mentioning that our experiment on feeding fish red alga *Pterocladia* is considered the first study for seabream (Wassef *et al*., 2002a) and seabass (Sakr, 2004).

(A)



Seabream, *Sparus aurata*

(B)



Seabass, *Dicentrarchus labrax*

Fig. 2. Proximate composition (%DM) of seabream (A) and seabass (B) fed UM or PM Supplemented diets for eight weeks.

Final conclusions

(i) Properly prepared fish silage, acid or fermented, has a good amino acids profile and proved suitable protein ingredient in tilapia diets.

(ii) Fish silage / soybean meal blend, in a 30% CP diet, provided the total dietary protein required for best performance (equivalent to FM) of Nile and blue tilapias.

(iii) Dry fermented fish silage if used singly or mixed with soybean meal, satisfactorily replace 50% or 75% respectively of dietary FM in Nile tilapia diets.

(iv) Addition of macroalgae meal (5% *Ulva or 10% Pterocladia)* in gilthead seabream and (5% PM or UM) in seabass fry diets has improved growth rate, nutrient composition and survival rates.

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