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Nutritive value of diets containing dried lactic acid fermented fish silage and soybean meal for juvenile *Oreochromis niloticus* and *Clarias gariepinus*

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Abstract

Dry diets containing varying levels of dried fermented fish silage and soybean meal blend (FS: SBM) as replacement for 25,50 or 75 % of fish meal protein were fed to juvenile *Oreochromis niloticus* (8.2±0.1 g) and *Clarias gariepinus* (10.8±0.3 g) at 5 % body weight per day for 70 days. Diets containing co-dried FS: SBM supported weight gains and growth rates similar to those in the control treatment without significant differences ($p > 0.05$). Feed conversion, protein efficiency ratio and protein productive values followed a similar trend with growth performance values. Apparent protein digestibility decreased in both fish species with increasing dietary level of co-dried FS: SBM. Carcass composition of experimental fish were not affected by the diets and morphological defects were not observed. Results indicate that co-dried FS: SBM can partially replace fish meal protein as well as establish the potential of utilizing waste/undersized tilapias as a protein feedstuff in dry aquaculture diets.

Keywords: Nutrition, tilapia, catfish, lactic acid fermentation, fish silage, diets.

*Valeur nutritive d'aliments à base d'un mélange co-séché d'ensilage biologique de poisson et de tourteau de soja pour des juvéniles d'*Oreochromis niloticus *et de* Clarias gariepinus.

Résumé

Des aliments secs renfermant des taux croissants d'ensilage microbiologique de poisson et de tourteau de soja co-séchés en substitution à 25, 50 et 75 % de protéines de farine de poisson ont été distribués à des juvéniles d'*Oreochromis niloticus* (8,2±0,1 g) et de *Clarias gariepinus* (10,8±0,3 g) à raison de 5 % du poids vif pendant 70 jours. Les régimes renfermant le mélange co-séché ensilage/tourteau de soja entraînent des gains de poids et des taux de croissance similaires à ceux du traitement témoin sans différence significative. Les indices de consommation, les coefficients d'efficacité protéique et la rétention protéique suivent une tendance similaire à celle des performances de croissance. La digestibilité apparente des protéines diminue chez les deux espèces de poissons lorsque le niveau d'incorporation d'ensilage co-séché augmente. La composition corporelle des poissons expérimentaux n'est pas affectée par les différents régimes alimentaires et aucun défaut de morphologie n'a été observé. Les résultats indiquent qu'un mélange d'ensilage de poisson co-séché avec du tourteau de soja peut remplacer partiellement les protéines de farine de poisson. Il s'agit là d'une possibilité de valoriser les tilapias de faible taille, habituellement écartés, en tant que matière première pour les aliments composés utilisables en pisciculture.

Mots-clés : Nutrition, tilapia, poisson-chat, ensilage microbiologique, aliments composés.

INTRODUCTION

Fish meal remains the most expensive ingredient in aquaculture feeds and fish farmers now seek its replacement with less expensive alternative protein feedstuffs. One of such alternatives is fish silage prepared from whole fish, fishery wastes/by-products or fish farm mortalities (Lo *et al.*, 1993). Fish silage is prepared either by mineral and/or organic acid preservation (acid silage) or by anaerobic microbial fermentation (fermented silage). The latter is preferred in developing countries because it is cheaper to produce, involves simple artisanal technology which is adaptable at cottage level and possesses good storage properties (Dong *et al.*, 1993). As fish silage is liquid, it is bulky and difficult to transport, stir or store; hence it has to be dried mixed with other dry ingredients or filler material. Several methods of removing or reducing the water content of fish silages include sun drying, kiln drying, spray drying, vacuum evaporation, drum drying or co-drying (Disney *et al.*, 1978; Jayawardena *et al.*, 1980). Co-drying is a process whereby dry products are added to the wet silage to adsorb the solubilized proteins and some of the moisture.

Co-dried silages have been evaluated in pig and poultry diets (Tibbetts *et al.*, 1981; Ologhobo and Balogun, 1988; Ologhobo *et al.*, 1988), and as protein feedstuff in diets fed to marine fishes - salmon and trout (Hardy *et al.*, 1983; 1984; Lie *et al.*, 1988) and freshwater fishes - common carp and tilapias (Djajasewaka and Djajadiredja, 1980; Phromkunthong and Chetanon, 1987). The results from these studies indicated that co-dried fish silage based diets yielded lower weight gains compared with fish meal based diets, which were explained by the indigestibility of the added filler materials or increased susceptibility to Maillard type reactions between the free amino acids in the silage and the carbohydrate in the dry additives, thus reducing the nutritional quality of the feed, as well as the presence of relatively high levels of free amino acids whose absorption may not have been synchronized with protein synthesis or energy absorption.

However, information on the evaluation of co-dried fermented silages in fish diets is limited and has not been directly compared to fish meal in fish feeding trials (Lopez, 1990). Further to earlier studies on the preparation, preservation and properties of fermented tilapia silage (Fagbenro and Jauncey, 1993 *a*, *b*, 1994), this study evaluates co-dried fermented tilapia silage and soybean meal blend (FS: SBM) as protein source (partially replacing fish meal) in dry diets for juveniles of all-male Nile tilapia *O. niloticus* and the African clariid catfish *C. gariepinus*. The effects on growth performance, feed conversion, protein utilization and digestibility, and carcass composition of experimental fish were investigated.

Table 1. – Proximate and essential amino acid composition of herring fish meal and co-dried FS: SBM.

Proximate composition (g/100 g)	Herring fish meal	Co-dried FS: SBM
Dry matter	91.27	94.39
Crude protein	74.85	56.08
Crude lipid	10.42	5.61
Ash	11.54	9.06
Amino acid content (g/100 g protein)		
Arginine	7.48	6.87
Histidine	2.54	2.75
Isoleucine	3.47	3.63
Leucine	6.66	6.57
Lysine	6.54	6.04
Methionine	2.55	2.47
Phenylalanine	3.32	3.40
Threonine	4.23	4.15
Tryptophan	1.07	0.80
Valine	4.14	4.38

MATERIALS AND METHODS

Experimental diets

Fermented silage was prepared from minced ungutted mixed-sex tilapias (< 100 g) mixed with 5 % sugar beet molasses and 2 % *Lactobacillus plantarum* (NCIMB 11974, NCIMB Ltd., Aberdeen Scotland) starter culture and incubated at 30 °C for 7 days. No antioxidant was added to the substrates because of the low lipid content of tilapias but to prevent mould growth, the surface of the silage and inner walls of the fermentation bin were sprayed with 1 % potassium sorbate solution. Soybean meal was blended with the wet tilapia silage (1 : 1, w/w) and oven-dried (45 °C for 48 h). The co-dried FS: SBM was milled and analyzed for proximate and amino acid composition *(table* 1). Four dry semi-purified diets were formulated separately for *O. niloticus* (30 % protein) and *C. gariepinus* (40 % protein) (*table* 2) and contained increasing levels of the co-dried FS: SBM as replacement for fish meal at 0 % (control, FM), 25 % (low silage, LS), 50 % (medium silage, MS) and 75 % (high silage, HS). Levels of essential amino acids in fish meal and co-dried FS: SBM were considered in formulating the diets. All diets were stored at −20 °C in polyethylene bags until fed. Triplicate determinations of pH and water stability (expressed as percentage loss of dry matter, % LDM) were made per diet as previously described (Fagbenro and Jauncey, 1993 *c*).

Growth trials

Growth trials were conducted in 1 m^2 (400-l) rectangular fibreglass tanks in a recirculating system with water flow adjusted to 3 l/min and temperature maintained at 27.5 ± 1.1 °C. Supplemental aeration was provided by a low-pressure blower system and

Table 2. – Formulation (g/100 g dry weight) of experimental diets for *O. niloticus* and *C. gariepinus*.

	Tilapia diets				Catfish diets			
Diet code	T-FM	T-LS	T-MS	T-HS	C-FM	C-LS	C-MS	C-HS
% fish meal remplacement	0	25	50	75	0	25	50	75
Herring fish meal	40	30	20	10	53.5	40	27	13.5
Co-dried FS: SBM	0	13.4	26.75	40.13	0	17.85	35.7	53.5
Corn starch	35	32.5	30	27.5	25	22.5	20	17.5
Cellulose flour	13	12.1	11.25	10.37	9.5	7.65	5.3	3.5
Cod liver oil	3	3	3	3	3	3	3	3
Corn oil	3	3	3	3	3	3	3	3
Carboxymethyl cellulose (binder)	2	2	2	2	2	2	2	2
Mineral mix[1]	2	2	2	2	2	2	2	2
Vitamin mix[1]	1	1	1	1	1	1	1	1
Chromic III oxide (marker)	1	1	1	1	1	1	1	1

([1]) according to Jauncey and Ross (1982) and Haylor (1992) for tilapia and catfish, respectively.

Table 3. – Proximate composition, energy content and properties of experimental diets (all values represent mean of three replicates).

	T-FM	T-LS	T-MS	T-HS	C-FM	C-LS	C-MS	C-HS
Moisture	7.64	7.83	7.78	7.81	7.49	7.60	7.62	7.65
Crude protein	30.97	31.25	31.20	31.34	40.91	41.17	40.83	40.69
Crude lipid	7.40	7.47	7.52	7.48	8.48	8.52	8.56	8.60
Crude fibre	3.50	3.56	3.58	3.60	3.52	3.57	3.58	3.61
Ash	9.01	9.18	9.45	9.50	10.47	10.42	10.63	10.66
Gross energy (kcal/g DM)	4.35	4.39	4.36	4.38	4.55	4.62	4.58	4.55
pH	6.3	6.3	6.5	6.4	6.3	6.5	6.5	6.4
Water stability (% LDM)	5.4	5.3	5.5	5.4	5.5	5.2	5.3	5.5

dissolved oxygen ranged from 6.8 to 8 mg/l. Before the experimental period, juvenile all-male *O. niloticus* and *C. gariepinus* were fed with an acclimation diet to satiation for 7 days. The fish were then sorted into groups of 30 *O. niloticus* (8.2±0.1 g) or *C. gariepinus* (10.8±0.3 g). Triplicate random groups of fish were fed 5% of their body weight per day, divided into two equal feedings at 09.00-9.30 h and 16.30-17 h, for 70 days. This amount was close to maximum daily ration for both species according to the observed levels of dietary intake during acclimation. Ten fish from each tank were anaesthetized and individually weighed biweekly, and the amount of diet fed was adjusted accordingly. In each growth trial, six fish at stocking and two per tank at the end were sacrificed, homogenized and stored at −20°C in polyethylene bags for subsequent proximate analysis. Mortality was monitored daily and recorded; and water quality (pH, dissolved oxygen, total ammonia and total nitrite concentration) in the experimental systems was monitored at biweekly intervals prior to removal of fishes for weighing. Diet performance was evaluated as follows:

– average daily weight gain,

$$ADG\ (g/day) = W_t - W_0/t$$

– specific growth rate,

$$SGR\ (\%/day) = 10^2 \times (\ln W_t - \ln W_0)/t$$

where W_t is weight of fish at time t, W_0 is weight of fish at time 0, and t is the culture period in days.

– feed gain ratio,

$$FGR = \text{dry feed fed/wet weight gain}$$

– protein efficiency ratio,

$$PER = \text{wet weight gain/protein fed}$$

– protein productive value

$$PPV = 10^2 \times (\text{protein gain/protein fed}).$$

Digestibility trials

Digestibility trials were conducted in 60-l cylindro-conical polypropylene tanks. At the bottom of each tank was a settling column to which was attached a control valve. Ten tilapia or catfish were stocked per tank (three tanks per treatment), acclimated for 7 days and fed with the experimental diets at 5% body weight per day, twice daily for 30 days. Faeces were collected at the bottom of a settling column in 150 ml conical flasks and pooled for each treatment. Pooled faeces were oven-dried to constant weight at 105°C. The chromic oxide (Cr_2O_3) content of diets and faeces was determined using Furukawa and Tsukahara (1966) methods. Proximate composition analysis of faeces were conducted according to AOAC (1990) methods. Apparent digestibility coefficient (ADC) for protein was calculated according to Austreng and Refstie (1979):

$$ADC = 10^2 \times (a - b)/a$$

where: a = protein in feed/Cr_2O_3 in feed; b = protein in faeces/Cr_2O_3 in faeces.

Analytical procedures

Moisture, crude protein (% N × 6.25), crude lipid, crude fibre and ash contents of protein feedstuffs, diets and faeces were determined in triplicate according to AOAC (1990) methods as follows: moisture was determined by oven-drying at 105 °C for 24 h; lipid by extracting the residue with 40-60 °C petroleum ether for 8 h; fibre as loss on ignition of dried lipid-free residues after digestion with 1.25 % H_2SO_4 and 1.25 % NaOH; ash by ignition at 550 °C to constant weight; total nitrogen by micro-Kjeldahl method using a TECATOR-KJELTEC system 1 003 unit. Amino acid composition was determined in acid hydrolysate (6 mol/lHCI under reflux for 24 h at 110 °C) of fish samples, protein feedstuffs and diets using an LKB 4151 ALPHA-PLUS (LKB Biochrom Ltd., Cambridge) automatic amino acid analyzer. Tryptophan was determined by colorimetry (Fischl, 1960) after hydrolysis in 4.2 mol/l NaOH. Moisture-free samples were milled and assayed for gross energy by combustion in an adiabatic bomb calorimeter (GALLENKAMP Ltd., U.K.)

Statistical analysis

Statistical comparisons of growth performance and protein utilization values were made by using one-way analysis of variance (ANOVA) for multiple comparison tests (Zar, 1984).

RESULTS

The pH and water stability of all diets were similar (*table* 3). Little variations occurred between moisture, protein, lipid and fibre contents of tilapia silage diets. Fish became accustomed to the diets within the first week. Low mortality (< 10 %) occurred during the acclimation period and was replaced with fish of similar size. No mortality occurred throughout the duration of either tilapia or catfish growth trial.

The summary of growth responses (*table* 4) shows that the mean final fish weights were not significantly different ($p > 0.05$). Differences in ADG and SGR values were also not significant ($p > 0.05$). FGR values less than 2 were obtained in all treatments, similarly with no significant differences ($p > 0.05$) between them. Results of protein utilization, expressed as PER and PPV values, are also presented in *table* 4. PER and PPV values obtained from both *O. niloticus* and *C. gariepinus* in all treatments were not significantly different ($p > 0.05$). $ADC_{protein}$ values (*table* 4) were >80 % for both experimental fishes in the co-dried silage treatments and decreased as the dietary level of co-dried FS: SBM increased. $ADC_{protein}$ in the control treatments were higher than those in co-dried silage treatments. The carcass composition of both *O. niloticus* and *C. gariepinus* at the beginning and end of the growth trials (*table* 5) shows that fish fed with both the control and co-dried silage diets had higher carcass protein and lipid contents than the initial carcass. The differences in final composition of fish fed with the co-dried silage diets were, however, not significant ($p > 0.05$).

DISCUSSION

Experimental diets

The near neutral pH of co-dried silage based-diets does not pose any problem to either tilapia or catfish because much lower pH occurs in their stomachs, pH 2 in *O. niloticus* (Jauncey and Ross, 1982) and pH 4 in *C. gariepinus* (Haylor, 1992). Water stability of all diets was suitable for fast and competitive feeders such as *O. niloticus* and *C. gariepinus* as it ensured optimum feed uptake before disintegration of pellets. High fish survival was attributable to conductive water quality conditions in the experimental systems.

Table 4. – Summary of growth and protein utilization by *O. niloticus* and *C. gariepinus* fed co-dried silage diets for 70 days.

Diet treatments	Initial weight (g)	Final weight (g)	ADG (g/day)	SGR (%/day)	FGR	PER	PPV (g)	$ADC_{protein}$ (%)
O. niloticus								
T-FM	8.19 ± 0.12	52.65 ± 2.27	0.63 ± 0.03	2.66 ± 0.04	1.59 ± 0.04	2.02 ± 0.04	29.48 ± 0.62	85.5
T-LS	8.19 ± 0.16	52.25 ± 2.04	0.63 ± 0.03	2.65 ± 0.03	1.60 ± 0.02	2.00 ± 0.03	29.65 ± 0.33	84.3
T-MS	8.19 ± 0.10	52.16 ± 1.24	0.63 ± 0.02	2.64 ± 0.02	1.60 ± 0.02	2.01 ± 0.02	29.45 ± 0.27	81.2
T-HS	8.20 ± 0.11	52.15 ± 1.61	0.63 ± 0.02	2.64 ± 0.05	1.59 ± 0.01	2.01 ± 0.02	29.73 ± 0.23	80.6
C. gariepinus								
C-FM	10.83 ± 0.37	65.95 ± 2.13	0.79 ± 0.03	2.58 ± 0.02	1.61 ± 0.03	1.52 ± 0.03	24.84 ± 0.33	87.0
C-LS	10.83 ± 0.37	66.03 ± 2.02	0.79 ± 0.02	2.58 ± 0.00	1.60 ± 0.02	1.52 ± 0.02	24.87 ± 0.23	85.3
C-MS	10.82 ± 0.32	65.86 ± 2.11	0.79 ± 0.02	2.58 ± 0.02	1.60 ± 0.02	1.53 ± 0.02	24.94 ± 0.37	84.1
C-HS	10.83 ± 0.32	65.90 ± 1.91	0.79 ± 0.02	2.58 ± 0.00	1.60 ± 0.02	1.53 ± 0.01	24.92 ± 0.22	81.9

Table 5. – Carcass composition of *O. niloticus* and *C. gariepinus* fed co-dried silage diets for 70 days.

Diet treatments	Moisture	Crude protein	Crude lipid	Ash
O. niloticus				
Initial	79.65	12.17	4.06	3.92
T-FM (control)	76.80	14.19	5.21	3.56
T-LS	76.57	14.40	5.25	3.53
T-MS	76.62	14.26	5.34	3.60
T-HS	76.45	14.38	5.38	3.59
C. gariepinus				
Initial	78.60	13.47	5.11	2.69
C-FM (control)	75.61	15.85	5.93	2.48
C-LS	75.48	15.92	6.00	2.46
C-MS	75.46	15.88	6.06	2.49
C-HS	75.49	15.80	6.13	2.47

Growth response and feed conversion

Growth responses by fish in all co-dried silage treatments compared favourably with the control treatment (*table* 4) which suggests that co-dried FS: SBM can replace fish meal as dietary protein in dry diets for *O. niloticus* and *C. gariepinus*. This agrees with reports that high levels of fish meal replacements by acid or fermented silage was accepted and gave good growth responses in *O. niloticus* (Phromkunthong and Chetanon, 1987) and *C. batrachus* and *C. macrocephalus* (Wee *et al.*, 1986; Edwards *et al.*, 1987). Lower growth responses by fish in the co-dried silage treatments (*table* 4) were probably caused by reduced palatability of diets or loss of appetite (due to the exclusion of fish meal during formulation), hence resulting in reduced feed intake.

Poor performance of various fishes fed with silage-based diets have been ascribed to acidity of diets, high proportion of free amino acids and hydrolysed proteins (Bromley and Smart, 1981; Jackson *et al.*, 1984; Hardy *et al.*, 1984; Wood *et al.*, 1985; Goncalves *et al.*, 1989; Stone *et al.*, 1989). Acidity reduces diet acceptance and affects protease activity in fish guts (Rungruangsak and Utne, 1981). Alternatively high level of free amino acids may act as an appetite depressant. Wilson *et al.* (1984) attributed reduced performance of channel catfish to a marginal presence of histidine, isoleucine and total aromatic amino acids. Hardy *et al.* (1984) and Lall (1991) suggested that liquefaction of silage should be restricted if higher levels of dried fish silage were to be used in fish diets. In this study, pH of all diets were near neutral (*table* 3) and fermentation was restricted to 7 days which ensured limited proteolysis (Fagbenro and Jauncey, 1993 *a*, *b*).

Protein utilization and digestibility

The lack of significant difference in growth performance (MWG, ADG, SGR, FGR) and protein utilization (PER, PPV) values by experimental fish fed co-dried silage diets, compared with fish fed fish meal diets (*table* 4) indicates that co-dried FS: SBM can replace some fish meal protein in fish diets without affecting fish growth performance, feed conversion or protein utilization. Loss of appetite coupled with the slightly lower $ADC_{protein}$ of co-dried silage diets may have produced tendency towards poorer performance of fish. Considering that all diets met the amino acid requirements of both fish species, stipulated by Santiago and Lovell (1988) and Haylor (1992), lower $ADC_{protein}$ of the co-dried silage diets could have been caused by factors other than amino acid imbalance; probably by the presence of alcohol-soluble carbohydrates in soybean meal (Arnesen *et al.*, 1989). As soybean has lower digestibility and metabolizable energy values than fish meal (NRC, 1983), the lower digestibility (*table* 5) is not surprising.

Nevertheless, $ADC_{protein}$ was higher than those of co-dried acid silages used as dietary protein for *O. niloticus* (Hernandez, 1983; Adejumo, 1987) or moist fermented tilapia silage for Asian catfishes, *C. batrachus* and *C. macrocephalus* (Wee *et al.*, 1986; Edwards *et al.*, 1987), which have $ADC_{protein}$ in the range of 75-80% and 65-76%, respectively. Lower $ADC_{protein}$ values reported in salmonids fed co-dried acid silages (Hardy *et al.*, 1983; 1984) were attributed to high levels of non-protein nitrogen (free amino acids, peptides) resulting from proteolysis which interfered with protein absorption. Such loss of dietary protein is detrimental to efficient protein utilization. It is unlikely that this was the case in this study because the degradation of protein in fermented tilapia silage is minimal, particularly if fermentation is terminated after 7 days (Fagbenro and Jauncey, 1993 *a*).

Carcass composition

Increasing level of carcass lipid in fish fed with diets containing increasing levels of co-dried FS: SBM may be due to the occurrence of reductions in their moisture contents. Wee *et al.* (1986) and Edwards *et al.* (1987) noted that *C. batrachus* and *C. macrocephalus* hardly increased fat deposition in their muscular tissue when fed with moist fermented tilapia silage diets. No morphological deformities were observed in both *O. niloticus* and *C. gariepinus* fed with any of the co-dried silage diets, and particularly no back deformities (usually associated with tryptophan deficiency in fish silages), contrary to the observation in *C. batrachus* (Wee *et al.*, 1986).

Acid-ensiled fish and fish by-products co-dried with protein feedstuffs, used as protein feedstuff in moist or dry aquaculture diets, were reported to have nutritional value comparable with that of commercial fish meal (Stone *et al.*, 1984; Lall, 1991) but to our knowledge there have been no studies evaluating the suitability of dried fermented silage as dietary protein source in dry aquaculture feeds. In this study, co-dried fermented tilapia silage and soybean meal blend was suitable as protein source in dry diets for *O. niloticus* (omnivore) and *C. gariepinus* (carnivore). Results show that a well-balanced dry feed based on co-dried fermented silage is as efficient in supporting growth as a dry fish meal-based diet, and that it represents an alternative to fish meal in utilizing waste/trash fish, undersized or low-value fish, as protein feedstuff for warmwater aquaculture species as suggested by Foltz *et al.* (1982).

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