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Effect of Feeding Different Levels of Alfalfa Meal on the Growth Performance and Body Composition of Nile Tilapia (*Oreochromis niloticus)* Fingerlings

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Abstract

The study was conducted to evaluate the growth performance and body composition of *Oreochromis niloticus* fingerlings (average initial body weight 12.38 ± 0.39g) fed isonitrogenous and isoenergetic diets containing different levels (0, 5, 10, 15 and 20 %) of alfalfa meal, for a period of 67 days. The inclusion of alfalfa meal over 5% in the diets reduced ($P < 0.05$) growth performance and nutrient utilization in fish as compared to control diet (without alfalfa meal). The decrease in growth performance was linearly correlated with level of alfalfa meal in the diet. No significant ($P < 0.05$) differences were, however, observed in specific growth rate and body condition factor of fish fed either 10 or 15% of alfalfa meal. The diet containing 20% of alfalfa meal showed poorest ($P < 0.05$) growth performance and nutrient utilization. Inclusion of alfalfa meal over 5% in diets significantly ($P < 0.05$) reduced feed intake in fish compared to the control diet. Diets containing 15 or 20% alfalfa meal however, did not show any significant ($P > 0.05$) difference in feed consumption of fish. The efficiency of feed utilization decreased linearly when the level of alfalfa meal in diets increased over 5%. No significant ($P < 0.05$) differences were, however, observed in the feed conversion ratio (FCR) of diets containing 10 or 15% of alfalfa meal. Similar results were observed for protein efficiency ratio (PER). Net protein

retention (NPR) and apparent net energy retention (ANER) values however, differed significantly ($P < 0.05$) at all inclusion levels. Body moisture content of fish increased whereas fat and gross energy contents decreased when the level of alfalfa meal in diets was either 15 or 20%. No significant differences were however observed in the body moisture, fat and gross energy contents of fish fed either 15 or 20% of alfalfa meal. The level of alfalfa meal in fish diets did not affect body crude protein and ash contents. The results of the present study indicated that up to 5% level of alfalfa meal could be used in the diets for *Oreochromis niloticus*.

Introduction

The search for alternate feed resources in aqua feeds is increasing and has gained increasing significant as traditional ingredients are becoming costly or less available (Alexis 1985; El-Sayed 1999; Naylor et el. 2000). Because of its high protein content and balanced amino acid profile, fish meal is regarded as the best natural feed ingredient for aqua feeds. Possibilities for substituting fish meal with alternate plant protein sources in diets of herbivorous and omnivorous fishes are much higher than for carnivorous fishes. The inclusion of plant protein sources in aqua feeds however, depends on a number of factors such as protein quality, nutritional constraints, type and processing conditions and economic feasibility (Tacon 1997; El-Sayed 1999). Tilapia is the most widely cultured fish in tropical and subtropical regions and can be grown year round, with some variability in fish yields depending upon seasonal differences related to the area (Green et al. 1990). It is marked as the 3rd largest group of farmed finfish species in the world and its global production has increased more than 3 fold since 1984 (FAO 1997).

Soybean meal, cotton seed meal and other oilseed meal byproducts have shown promising results as fish meal substitutes in tilapia diets (Sadiku and Jauncey 1985; Shiau et al. 1990; El-Sayed 1999). Excellent growth of *O. niloticus* and tilapia hybrids (*O. niloticus* x *O. aureus*) was observed when they were fed all plant diets containing corn by-products and soybean meal as protein sources (Wu et al. 1996; Twibell and Brown 1998). Aquatic plants, single cell proteins, grain legumes, rye grass and leaf protein concentrates have also been tried in tilapia diets but with variable results (Naegel 1997; Essa, 1997; El-Sayed 1999). Olvera-Novoa et al. (1990) reported that the growth performance of *O. mossambicus* fingerling fed purified alfalfa leaf protein concentrate (69% CP) as a replacement of 35% of fish meal protein in a 40% crude protein diet, was better than that obtained with fish meal based diet. Jia et al. (1990) reported that inclusion of alfalfa in practical diets of Chinese blunt snout bream (*Megalobrama amblycephala*) resulted in better palatability, increased feed intake and improvement of fish flesh. Yousif et al. (1994) however did not recommend the use of dehydrated alfalfa in the diets of tilapia (*O. mossambicus*). Because of its high biomass production and good nutritional quality as animal feed, alfalfa is regarded as the “Queen of Forages”. It is an excellent source of minerals and vitamins and contains 15-22% crude protein. There is, however, a paucity of information on the use of alfalfa meal in aqua feeds. The present study was therefore conducted to evaluate the use alfalfa meal in practical diets for *Oreochromis niloticus*.

Materials and Methods

Oreochromis niloticus fingerlings with an average initial weight of 12.38 " 0.39g were collected from the fish hatchery of King Abdulaziz City for Science and Technology (KACST) Deerab, Riyadh. The fish were acclimatized to the experimental conditions for a period of two weeks before the start of actual experiment. During this period they were kept on the same standard diet as fed previously at the hatchery. Thirty randomly captured fishes (divided into three replicates of 10 fish in each) were killed immediately and after recording their body weight and length were stored at -30EC for subsequently determining initial body composition. One hundred and fifty fish were then randomly divided into 5 different groups with 3 replicates containing 10 fish in each replicate. The fish were kept in glass tanks (100 x 42.5 x 50.0 cm) containing dechlorinated and well-aerated tap water and fitted with waste filtration facility. The water temperature was maintained at 28 " 1EC with the help of a thermostatically controlled heating system. Compressed air was used to maintain the oxygen supply. Regular fortnightly monitoring of water quality parameters was carried out. Values recorded were, pH (7.1 - 8.0), dissolved oxygen (5.6 - 6.7 $mg.l^{-1}$), ammonia nitrogen (0.12 – 0.20 $mg.l^{-1}$), nitrite nitrogen (0.33 – 0.58 $mg.l^{-1}$) and alkalinity as $CaCO_3$ (235 – 350 $mg.l^{-1}$).

Five isonitrogenous and isoenergetic diets containing different levels of alfalfa meal (0, 5, 10, 15 and 20%) were prepared using a pellet press with 2 mm die (Table 1). The diets were dried at 60EC and stored at -18EC throughout the experimental period. The proximate chemical composition of the ingredients and the finished diets was determined by the methods of AOAC (1990) and is given in Tables 2 and 3. The gross energy content of the diets was calculated on the basis of their protein, fat and carbohydrate (nitrogen free extract) contents using the equivalents of 23.64, 39.54, and 17.15 $MJ.kg^{-1}$ respectively (Kleiber, 1975). Each diet was randomly allotted to 3 replicates in a completely randomized design. The diets were offered *ad-libitum* twice daily to satiation of fish for a period of 67 days. Daily feed intake and fortnightly weight gains were recorded. To quantify the exact amount of dry matter feed intake, any feed refused was immediately siphoned out, dried and weighed. The experiment was conducted under artificial light with a light and dark cycle of 12:12 hours. At the end of the experimental period all fish were killed and their body weight and length were recorded. To determine whole body composition, fish were cut into pieces, minced through a meat grinder, homogenized and immediately frozen at -30EC for further analysis. The proximate chemical composition was determined according to the methods of AOAC (1990). The gross energy (GE) content of fish was calculated from the fat and protein contents using the equivalents of 39.54 $MJ.kg^{-1}$ crude fat and 23.64 $MJ.kg^{-1}$ crude protein (Kleiber, 1975). Feed conversion ratio (FCR), specific growth rate (SGR), protein efficiency ratio (PER), net protein retention (NPR), apparent net energy retention (ANER) and body condition factor were calculated as already described by Ali and Al-Asgah (2001). The data so collected was subjected to statistical analysis using one-way analysis of variance and means were compared by Fisher's LSD test according to Snedecor and Cochran (1989).

Results

Data on growth performance of *Oreochromis niloticus* fed different levels of alfalfa meal is presented in Table 4. Significant ($P < 0.05$) differences were observed in body weight gain of fish fed different levels of alfalfa meal. Inclu-

Table 1. Composition of the experimental diets (%)

Ingredients	Diets				
	A	B	C	D	E
Fish meal	35.00	35.00	35.00	34.00	33.00
Soybean meal	20.00	20.00	20.00	20.00	20.00
Maize grain	25.00	25.00	25.00	23.00	19.00
Wheat bran	12.00	7.00	2.00	-	-
Alfalfa meal	-	5.00	10.00	15.00	20.00
Corn oil	2.00	2.00	2.00	2.00	2.00
Cod liver oil	2.00	2.00	2.00	2.00	2.00
Gelatin	1.00	1.00	1.00	1.00	1.00
Mineral mixture	2.00	2.00	2.00	2.00	2.00
Vitamin mixture	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00

1. Per Kg of the premix contains: $CaHPO_4$, 530g; K_2HPO_4, 80g; Na_2HPO_4, 90g; $MgCl_2.6H_2O$, 100g; KCl, 67.5g; K_2SO_4, 80g; NaCl, 30g; KI, 0.05g; $ZnSO_4.7H_2O$, 2.5g; SeO_2, 0.03g; $CuSO_4.5H_2O$, 0.15g; $FeSO_4.7H_2O$, 18g; $(NH_4)_6Mo_7O_{24}.4H_2O$, 0.01g; $MnSO_4.H_2O$, 0.5g; NaF, 1.2g; $CoCl_2.6H_2O$, 0.01g.
2. Per Kg of the premix contains: Vitamin A, 400,000 I.U.; D_3, 200,000 I.U.; E, 5000 I.U.; K_3, 1g; B_1, 1g; B_2, 1.5g; B_6, 1g; Pantothenic acid, 5g; Niacin, 3g; Folic acid, 0.5g; B_{12}, 2mg; Biotin, 100mg; Vitamin C, 20g.

Table 2. Proximate chemical composition of the experimental diets (on %dry matter basis)

Parameters	Diets				
	A	B	C	D	E
Dry matter (%)	92.21	92.39	92.59	92.48	92.97
Crude protein	35.73	36.17	36.24	36.35	36.05
Crude fibre	3.68	4.52	5.33	6.01	7.07
Total fat	8.97	8.58	8.32	8.46	8.47
Ash	10.65	10.75	11.15	10.92	11.14
Nitrogen free extract (NFE)	40.97	39.98	38.76	38.26	37.27
Gross energy ($MJ.kg^{-1}$)	19.01	19.09	18.65	18.49	18.25
Lysine	2.75	2.76	2.76	2.78	2.79
Methionine	0.98	0.97	0.97	0.97	0.97

Table 3. Chemical composition of the ingredients used for the diets preparation (on % dry matter basis)

Ingredients	Parameters							
	DM	CP	CF	Fat	Ash	NFE	Lysine	Methionine
Fish meal	91.40	70.24	1.09	5.47	16.10	7.10	5.55	2.13
Soybean meal	90.00	52.78	4.33	1.11	6.50	35.28	3.29	0.74
Maize grain	88.50	9.61	2.49	4.29	1.50	82.11	0.29	0.20
Wheat bran	89.00	17.64	10.26	3.37	6.90	61.83	0.69	0.26
Alfalfa meal	92.50	18.38	25.71	2.70	10.60	42.61	0.79	0.26
Gelatin	90.00	97.40	0.00	0.10	-	-	3.95	0.81

sion of alfalfa meal over 5% in diets reduced ($P < 0.05$) growth performance and nutrient utilization in fish as compared to the control diet (without alfalfa meal). The decrease in growth performance was linearly correlated with dietary alfalfa meal level. No significant ($P < 0.05$) differences were however observed in specific growth rate and body condition factor of fish fed either 10 or 15% of alfalfa meal. The diet containing 20% alfalfa meal showed the poorest ($P < 0.05$) growth performance and nutrient utilization. Inclusion of alfalfa meal over 5% in diets significantly ($P < 0.05$) reduced the feed intake in fish as compared to the control diet. Diets containing 15 or 20% of alfalfa meal, however, did not show any significant ($P > 0.05$) difference in feed consumption. The efficiency of feed utilization decreased linearly when the level of alfalfa meal in the diets increased over 5%. No significant ($P < 0.05$) differences were, however, observed in the feed conversion ratio (FCR) of diets containing either 10 or 15% of alfalfa meal. Similar results were observed for protein efficiency ratio (PER). Net protein retention (NPR) and apparent net energy retention (ANER) values, however, differed significantly ($P < 0.05$) at all inclusion levels. The body moisture content of fish increased whereas the fat and gross energy contents decreased when the level of alfalfa meal in diets was either 15 or 20%. No significant ($P < 0.05$) differences were however observed in the body moisture, fat and gross energy contents of fish fed either 15 or 20% of alfalfa meal. The level of alfalfa meal in fish diets did not affect ($P > 0.05$) the body crude protein and ash contents (Table 5).

Discussion

The results of the present study indicate that inclusion of alfalfa meal over 5% in diets reduced ($P < 0.05$) growth performance and nutrient utilization in fish as compared to the control diet (without alfalfa meal). The decrease

Table 4. Growth performance of *Oreochromis niloticus* fed different levels of Alfalfa meal

Parameters	Diets					
	A	B	C	D	E	S.E.
Initial weight ($g.fish^{-1}$)	12.56	12.16	12.36	12.35	12.49	$\pm$ 0.41^{NS}
Final weight ($g.fish^{-1}$)	45.47^{a}	44.20^{a}	38.74^{b}	36.76^{c}	34.14^{d}	$\pm$ 1.52
Total weight gain ($g.fish^{-1}$)	32.91^{a}	32.04^{a}	26.38^{b}	24.41^{c}	21.65^{d}	$\pm$ 1.08
Specific growth rate (SGR) %	1.92^{a}	1.93^{a}	1.71^{b}	1.63^{b}	1.50^{c}	$\pm$ 0.11
Condition factor (k)	2.97^{a}	2.95^{a}	2.81^{bc}	2.71^{c}	2.58^{d}	$\pm$ 0.13
Total feed dry matter consumed ($g.fish^{-1}$)	37.75^{a}	37.98^{a}	34.88^{b}	33.59^{c}	32.71^{c}	$\pm$ 1.29
Feed conversion ratio (FCR)	1.15^{c}	1.19^{c}	1.32^{b}	1.38^{b}	1.51^{a}	$\pm$ 0.11
Protein efficiency ratio (PER)	2.44^{a}	2.33^{a}	2.09^{b}	2.00^{b}	1.84^{c}	$\pm$ 0.15
Net protein retention (NPR) %	36.10^{a}	35.30^{a}	31.80^{b}	29.81^{c}	27.74^{d}	$\pm$ 1.29
Apparent net energy retention (ANER) %	23.54^{a}	23.59^{a}	21.20^{b}	19.48^{c}	18.09^{d}	$\pm$ 1.04

SE = Pooled standard error
NS = non significant
a, b, c, d = Different superscripts in the same row mean significant differences between the values at 5%.

in growth performance and nutrient utilization was linearly correlated with the level of alfalfa meal in diets. Diets containing either 10 or 15% of alfalfa meal however did not show any significant ($P < 0.05$) differences between them. Diets used in the present study were isonitrogenous and isoenergetic. The chemical composition of the experimental diets indicated that with increasing level of alfalfa meal, the crude fibre content of the diets increased. The level of lysine and methionine in all the diets was the same and met the essential amino acid requirements of *Oreochromis niloticus* (NRC 1993). The decrease in growth performance and poor nutrient utilization of fish with the increasing level of alfalfa meal in the diets may be attributed to a number of factors such as higher levels of crude fibre, protease inhibitors and anti-nutritional factors.

The possible nutritional and physiological actions of the tannin content of alfalfa meal (Liener 1989) may also play a role in growth depression and nutrient utilization as little is known about the effect of tannins on fish (Becker and Makkar 1999). Although the diets did not show any essential amino acids deficiency, the presence of protease inhibitors and other anti-nutritional factors in alfalfa meal might be responsible for poor protein digestibility and subsequent absorption of amino acids. Elevated levels of crude fibre in the diet exert a negative effect on the nutrient digestibility (Kirchgessener 1986) and may lead to growth depression in fish (Dioundick and Stom 1990; Al-Asgah and Ali 1996). Olvera-Novoa et al. (1990) reported that weight gain, specific growth rate, feed intake and nitrogen deposition in *Oreochromis mossambicus* were best at low levels (15-20%) of alfalfa protein inclusion. Purified (cytoplastic) leaf protein concentrate showed better performance than crude (chloroplastic) leaf protein concentrate. Yousif et al (1994) reported that growth rate and nutritional parameters decreased with increasing levels of dehydrated alfalfa in the diets of tilapia (*O. aureus*). They did not recommend the use of dehydrated alfalfa in the diets of tilapia. Our results showed that diet containing 5% level of alfalfa meal produced comparable results to the control diet but depressed the growth at higher inclusion levels.

Table 5. Body composition of *Oreochromis niloticus* fed different levels of Alfalfa meal (on % wet basis)

Parameters	Diets					
	A	B	C	D	E	S.E.
Moisture	74.91^{b}	75.24^{b}	75.31^{b}	76.17^{a}	76.23^{a}	± 0.81
Crude protein	14.42	14.69	14.67	14.43	14.51	$\pm 0.27^{NS}$
Total fat	3.99^{a}	4.14^{a}	3.89^{a}	3.53^{b}	3.38^{b}	± 0.13
Ash	4.50	4.52	4.70	4.53	4.66	$\pm 0.18^{NS}$
Gross energy ($MJ.kg^{-1}$)	4.98^{a}	5.11^{a}	5.00^{a}	4.81^{b}	4.76^{b}	± 0.15

1 = Composition of fish slaughtered at the beginning of the experiment (moisture, 76.40%; crude protein, 13.45%; fat, 3.45%; ash 6.05% and gross energy, 4.53 $MJ.kg^{-1}$).
SE = Pooled standard error
NS = non significant
a, b, c, d = Different superscripts in the same row mean significant differences between the values at 5%.

Jia et al. (1990) reported that inclusion of alfalfa in practical diets of Chinese blunt snout bream (*Megalobrama amblycephala*) resulted in better palatability, increased feed intake and improvement of fish flesh. At 20–40% inclusion levels, in which 15-20% of fish meal and soybean meal were substituted, growth, feed conversion ratio, and protein utilization were comparable to a control diet. The better utilization of alfalfa meal by Chinese blunt snout bream as compared to Tilapia may be because of species differences. Tilapia has relatively long digestive tract that allows the establishment of polysaccharide decomposing microflora of fairly high efficiency that may positively influence the digestion of some apparently indigestible feed ingredients by this fish (Sakata et al. 1980; Sugita et al. 1982; Viola and Arieli 1983). The inclusion of alfalfa meal over 5% in the diets not only reduced the feed intake but also the efficiency of nutrient utilization. The poor growth performance and nutrient utilization at 20% level of alfalfa meal in tilapia diets may be explained in this context.

Both endogenous and exogenous factors operate simultaneously to affect the efficiency of transfer of nutrients from feed to fish (Burtle 1990; Shearer 1994). The results of the present study indicate that body moisture content of fish increased whereas the fat and gross energy contents decreased when the level of alfalfa meal in diets was either 15 or 20%. The carcass lipid is directly related to dietary energy intake (Sargent 1989). Although all the diets were isonitrogenous and isoenergetic, the lower feed intake at higher levels of alfalfa meal in the diets might have been responsible for lower energy intake by fish. The increase in dietary crude fibre content may also be another factor. The results of the present study are in line with the findings of Anderson et al. (1984); Shiau et al. (1988); Al-Asgah and Ali (1996) but do not agree with those of Leary and Lovell (1975) and Davies (1985) who reported that the level of crude fibre in the diet had no effect on the chemical composition of channel catfish (*Ictalurus punctatus*) and rainbow trout (*Oncorhynchus mykiss*) respectively. No significant ($P < 0.05$) differences were observed in the body moisture, fat and gross energy contents of fish fed either 15 or 20% of alfalfa meal. The level of alfalfa meal in fish diets did not affect ($P > 0.05$) the body crude protein and ash contents. Olvera-Novoa et al. (1990) also reported only minor effects of alfalfa leaf protein concentrate on the carcass composition of fish.

The results of the present study indicated that up to 5% level of alfalfa meal could be used in the diets for *Oreochromis niloticus* without any performance loss. Higher levels may be tried depending upon the cultural management practices and economic viability. However many aspects of digestion, nutrient utilization and mechanism of action of anti-nutritional factors still remain to be studied, to allow the formulation of effective economical aqua feeds based on alternate plant protein sources.

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