The Israeli Journal of Aquaculture - Bamidgeh, IIC:63.2011.576, 6 pages

Use of Tuna Liver Meal as Partial Replacement of Fishmeal in Diets for Nile Tilapia Fry, *Oreochromis niloticus*

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(Received 4.5.10, Accepted 12.7.10)

Key words: *Oreochromis niloticus,* tuna liver meal, fishmeal replacement, growth, digestibility

Abstract

A 13-week feeding experiment was conducted to evaluate the use of tuna liver meal as a replacement for fishmeal in diets for Nile tilapia fry, *Oreochromis niloticus*. Six isonitrogenous (40%) and isoenergetic (18 kJ digestible energy/g) diets were formulated in which tuna liver meal replaced 0 (control), 10%, 20%, 30%, 40%, or 50% of the protein supplied by fishmeal. Triplicate groups of fish (2.29±0.07 g) were fed twice daily to apparent satiation. Results showed that final weight, weight gain, and specific growth rate of fish fed diets in which up to 30% of the protein was replaced by tuna liver meal did not significantly differ from fish fed the control diet. There were no significant differences in feed intake or protein efficiency ratio of fish fed diets containing up to 30% replacement. The apparent digestibility coefficients (ADC) of protein and energy significantly dropped with the increase in dietary tuna liver meal but there were no significant differences in the ADC of dry matter or lipid. There were no significant differences in protein or ash contents in the fish body, but dry matter and lipid contents significantly differed among treatments. Results indicate that up to 30% of fishmeal protein can be replaced by tuna liver meal in Nile tilapia fry diets without adverse effects on growth performance or feed efficiency.

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Introduction

One of the main protein sources in an aquaculture diet is fishmeal. However, the increasing demand, uncertain availability, and high price that accompany the expansion of aquaculture makes it necessary to search for alternative protein sources (Tacon and Metian, 2008). For this purpose, the efficiency of various alternative animal and plant protein sources as partial or complete replacement for dietary fishmeal has been evaluated in fish diets (El-Saidy and Gaber, 2003; Gaber, 2006; Yang et al., 2006; Zhang et al., 2006; Ahmad, 2008; Hu et al., 2008; Hernandez et al., 2009; Altan et al., 2010; Gümüş et al., 2009;2010)

Liver can be obtained from warm-blooded or large aquatic animals such as whales and bluefin tuna fish. Tuna liver meal is a by-product of the tuna fish rearing industry and cheaper (US$200/ton, local market price in 2008) than commercial fishmeal (US$1,051.17/ton, local market price in 2008) because of its reputation as a by-product. The bluefin tuna rearing industry has rapidly expanded in the Mediterranean. In 2007, Turkey cultured 918 tons of tuna fish while the entire Mediterranean are produced almost 29,500 tons (ICCAT, 2007). Therefore, because of its low price and nutritional quality, tuna liver meal may become an important animal protein that can be used to replace fishmeal in aquaculture diets. Fresh or frozen liver, minced together with other feed ingredients, is used as the first exogenous food for fish larvae. Thus, tuna liver meal can be considered for use in larvae diets.

Presently, little data exists on the use of tuna liver meal in aquaculture larvae diets. Our earlier study reported on its inclusion in diets for carp fry (Gümüş et al., 2009). There is a need to develop nutritious economical aquaculture feeds that do not rely on fishmeal as a major protein source. In the present study, we evaluated the effects of partial replacement of fishmeal by dietary tuna liver meal on the growth, body composition, and apparent digestibility of Nile tilapia fry.

Materials and Methods

Experimental diets. Six isonitrogenous (crude protein 40% wet wt), isolipidic (15% wet wt), and isoenergetic (18 kJ/g) diets were prepared in which 0 (control), 10%, 20%, 30%, 40%, or 50% of the fishmeal protein was replaced by tuna liver meal protein (Table 1). Tuna liver meal and other ingredients were from the same sources and the diets were prepared as in our study on carp fry (Gümüş et al., 2009). The experimental diets contained 0.5% chromic oxide as an inert marker. The ingredients were ground in a hammer mill and passed through a 0.5 mm mesh sieve, the dry ingredients were thoroughly mixed and blended with 100 ml/kg water to make a paste, and the resulting mixture was pelleted using a meat grinder with a 2-mm die. The pellets were dried in an oven at 70°C for 24 h, then crumbled to 0.8-1 mm diameter and stored in plastic bags -20°C until required.

Experimental conditions, fish, and feeding. The experiment was conducted in the Laboratory of the Fisheries Programme of the Ortaca Vocational School in Muğla, Turkey, from 15 October 2008 to 15 January 2009. Tilapia fry were obtained from the same laboratory and acclimatized in an indoor tank for two weeks. They were fed a control diet twice a day during acclimatization. At the beginning of the experiment, twenty fry with an average weight of 2.29±0.07 g were randomly distributed into each of 18 glass aquaria (65-l). Three aquaria were randomly assigned to each experimental diet and fry were fed manually to apparent satiation twice daily (9:00 and 16:00) for 13 weeks. The aquaria were supplied with chlorine-free tap water throughout the experimental period. The aquaria were cleaned daily and two-thirds of the water was replaced before feeding. Each aquarium was continuously aerated by air stones attached to a central compressor.

Water temperature was maintained with a 100-W automatic heater set at 24-26°C. Water temperature and dissolved oxygen were recorded daily using a Model WTW Oxi 330i multi-oxygen meter (WTW Wissenschaftlich-Weilheim, Germany). The photoperiod was maintained at 12 h light/12 h dark using a fluorescent lighting source. Dissolved oxygen was maintained 4.8-5.5 mg/l and pH at 7.8-8.4 pH (Stickney, 1979).

Table 1. Ingredients and proximate composition of the experimental diets (n = 3).

	Tuna liver meal replacement of fishmeal (%)					
	0 (Control)	*10*	*20*	*30*	*40*	*50*
Ingredient (%)						
Soybean meal	46.9	46.9	46.9	46.9	46.9	46.9
Fishmeal	17.14	15.42	13.71	11.99	10.28	8.57
Tuna liver meal	-	4.04	8.09	12.13	16.18	20.23
Corn meal	5.74	5.74	5.74	5.74	5.74	5.74
Corn gluten	7.23	7.23	7.23	7.23	7.23	7.23
Starch	10.27	10.12	10.06	9.93	9.90	9.83
Fish oil	11.12	8.95	6.7	4.52	2.24	-
L-methionine	0.5	0.48	0.46	0.44	0.42	0.4
Vitamin premix[1]	0.2	0.2	0.2	0.2	0.2	0.2
Mineral premix[1]	0.3	0.3	0.3	0.3	0.3	0.3
Iodized salt	0.1	0.1	0.1	0.1	0.1	0.1
Chromic oxide	0.5	0.5	0.5	0.5	0.5	0.5
Proximate composition (% wet wt)						
Moisture	5.62	4.89	5.16	5.03	5.68	4.97
Crude protein	40.03	40.01	40.36	39.96	40.03	39.92
Crude lipid	15.28	15.41	15.21	15.15	15.16	15.30
Ash	7.55	7.31	6.70	6.58	6.33	6.31
Crude fiber	2.27	2.28	2.30	2.31	2.33	2.35
NFE	29.25	30.10	30.27	30.97	30.47	31.15
Digestible energy (kJ/g diet)[2]	18.08	18.09	18.07	18.08	18.06	18.05

NFE = nitrogen-free extract
[1] Gümüş et al. (2009)
[2] Estimated using 20.9, 37.7, and 14.6 kJ/g for protein, fat, and carbohydrate, respectively (NRC, 1993)

Fecal collection and digestibility calculation. After one week acclimatization in the feeding trial, feces were carefully siphoned from each aquarium bottom on a daily basis. Uneaten feed was removed by siphon 1 h after feeding. Feces were siphoned through a fine (80 µm) mesh netting 3 h after feeding, and separately collected in individual jars. Pooled feces from each treatment were homogenized in individual jars and stored at -20°C until analysis. Fecal samples were dried in an oven at 70°C for 24 h, ground, and prepared for chemical analysis. Apparent digestibility co-efficients (ADC) were calculated using the following equations (Maynard and Loosli, 1969): ADC of dry matter (%) = 100 - [100(%Cr_2O_3 in feed/%Cr_2O_3 in feces)]; ADC of nutrients (%) = 100 - [100(%Cr_2O_3 in feed/%Cr_2O_3 in feces) x (%nutrient in feces/%nutrient in feed)].

Sample collection and chemical analysis. At the beginning of the experiment, 40 fry from the same stock and of similar body weight to the experimental fish were sampled and kept at -20°C for the chemical analysis. At the end of the 13 weeks, 15 fish were randomly removed from each aquarium, sacrificed, immediately frozen at -20°C, and held until analysis. Proximate compositions of diets, feces, and fish were analyzed after drying in an oven at 105°C for 24 h; crude protein was established (N x 6.25) by the Kjeldahl method after acid digestion; crude lipid by the soxhlet method after ethylether extraction; ash by combustion at 550°C in a muffle furnace for 24 h; crude cellulose after alkali and acid digestion, and nitrogen-free extract (NFE) as 100 - (moisture + protein + lipid + ash + fiber) according to the methods of AOAC (1995). Chromic oxide (Cr_2O_3) contents in the diets and feces were determined following the method of Furukawa and Tsukahara (1966).

Statistical analysis. Results are presented as means±SD of three replicates. All data were subjected to one-way analysis of variance (ANOVA) followed by the Duncan's multiple range test. SPSS 15.0 (SPSS Inc., Chicago, IL, USA) was used to perform statistical calculations. Differences were considered significant at $p<0.05$ (Steel et al., 1996).

Results

There were significant differences among treatments in final body weight, weight gain, and specific growth rate (Table 2). Generally, growth performance decreased as the replacement level increased. Diets significantly affected the feed intake, feed conversion ratio, and protein efficiency ratio. Feed intake and protein efficiency increased as the level of tuna liver meal rose to 30%, and then dropped. The poorest feed conversion

ratio (FCR), feed intake, and protein efficiency ratio (PER) were obtained in fish fed the 40% and 50% diets. There were no significant differences in whole body protein or ash contents but body lipid and dry matter were affected by dietary treatment. The ADC for dry matter and lipid were not affected by the level of tuna liver meal, but the ADC for protein and energy differed between treatments.

Table 2. Growth performance, body composition, and apparent digestibility coefficients of Nile tilapia fry fed six experimental diets for three months (means±SD; n=3).

	%TLM replacing FM in diets					
	Control	*10*	*20*	*30*	*40*	*50*
Initial wt (g)	2.215±0.06	2.360±0.18	2.240±0.07	2.325±0.02	2.290±0.05	2.285±0.07
Final wt (g)	18.64±1.11^{ab}	20.84±2.77^{a}	18.78±3.03^{ab}	20.57±1.18^{ab}	15.95±0.62^{bc}	12.81±0.02^{c}
Wt gain (g)	16.42±1.05^{a}	18.48±2.96^{a}	16.54±3.10^{a}	18.25±1.15^{a}	13.66±0.56^{b}	10.53±0.09^{b}
SGR (%/d)[1]	3.041±0.04^{a}	3.092±0.10^{a}	2.979±0.15^{a}	3.176±0.05^{a}	2.769±0.01^{b}	2.463±0.05^{c}
Feed intake (g/fish)	18.40±2.2^{a}	18.66±1.98^{a}	18.52±2.50^{a}	18.61±0.44^{a}	15.45±0.67^{b}	16.00±0.49^{b}
FCR[2]	1.127±0.13^{a}	1.011±0.04^{a}	1.122±0.27^{a}	1.019±0.08^{a}	1.131±0.04^{a}	1.519±0.03^{b}
PER[3]	1.82±0.28^{ab}	2.10±0.13^{ab}	2.16±0.00^{a}	1.95±0.16^{ab}	1.76±0.05^{b}	1.31±0.02^{c}
Survival (%)	96.67±1.67	98.33±.1.67	98.33±.1.67	100.00±0.00	98.33±.1.67	100.00±0.00
Final body composition (%;dry matter basis)						
Dry matter	26.11±0.50^{ab}	25.17±0.47^{b}	24.80±0.15^{b}	26.38±1.03^{ab}	25.39±1.06^{ab}	27.12±0.74^{a}
Protein	75.57±0.57	76.97±1.17	77.68±2.72	74.79±4.79	77.54±2.09	73.26±2.68
Lipid	16.20±0.63^{ab}	14.86±0.21^{b}	14.23±0.59^{b}	18.19±1.77^{ab}	15.33±3.43^{ab}	19.93±2.53^{a}
Ash	8.219±0.06	8.315±0.75	8.199±1.95	6.277±0.17	7.444±1.61	7.036±0.01
Apparent digestibility coefficient (ADC, %)						
Dry matter	70.67±0.68	71.00±0.87	70.46±0.70	71.34±0.80	72.77±2.52	70.87±0.58
Protein	83.08±0.38^{b}	85.50±0.69^{a}	82.32±0.81^{b}	84.06±0.03^{ab}	83.80±1.49^{ab}	83.40±0.28^{b}
Lipid	86.28±0.23	87.02±1.84	88.86±0.14	88.10±1.23	87.08±0.38	86.19±1.97
Energy	81.36±0.36^{ab}	81.58±1.41^{a}	81.40±0.25^{ab}	82.33±0.72^{a}	80.78±1.84^{ab}	78.56±1.23^{b}

Values in a row with different superscripts significantly differ (p<0.05).
[1] Specific growth rate = (Ln final wt - Ln initial wt/time in days) x 100
[2] Feed conversion ratio = feed intake/wt gain
[3] Protein efficiency ratio = wt gain/protein intake

Discussion

Growth performance and feed utilization were affected by the fishmeal replacement level. Final body weight, weight gain, and SGR of fish fed tuna liver meal up to a replacement level of 30% were higher than those of fish fed the control diet, but dropped beyond this level. The lowest growth was obtained in fish fed the 50% tuna liver meal diet. These results are comparable to those of Gümüş et al. (2009) who used the same diets for carp fry, *Cyprinus carpio*, and other studies on cyprinid larvae in which liver meal resulted in improved growth and survival (Charlon and Bergot, 1984; Szlaminska et al., 1990). Similar results were obtained in studies on post-larvae of the freshwater prawn, *Macrobrachium rosenbergii* (Garces and Heinen, 1993; Molina-Vozzo et al., 1995). Fish growth tends to be reduced when a high proportion or all of the fishmeal is substituted (Yang et al., 2006; Ahmad, 2008; Hu et al., 2008; Gümüş et al., 2009, 2010).

Feed intake, FCR, and PER were also influenced by dietary treatment. Feed intake and PER slightly improved up to 30% replacement without significant differences, then decreased significantly to 50%. FCR improved up to 40% replacement. The reduced utilization variables in fish fed diets with more than 30% replacement can be attributed to reduced palatability or attractiveness of the diet, causing reduced feed intake. These results are in agreement with Gümüş et al. (2009) who reported that partial replacement of fishmeal with tuna liver meal affected feed utilization of carp fry. Feed utilization variables of other fish species tend to be similar when a high proportion or all of the fishmeal is substituted (Adebayo et al., 2004; Gaber, 2006; Yang et al., 2006; Ahmad, 2008; Gümüş et al., 2010).

Partial replacement of fishmeal with tuna liver meal did not influence the whole-body crude protein or ash contents, suggesting that the tuna liver meal protein was efficiently digested and assimilated. Similarly, there were no effects of dietary mixture on the whole-body protein content in carp (Gümüş et al., 2009) or Nile tilapia fry (El-Saidy and Gaber, 2003; Gaber, 2006; Ahmad, 2008; Gümüş et al., 2010). Whole-body dry matter and lipid contents increased in fish fed diets containing high amounts of tuna liver meal. However, whole-body lipid decreased in carp fed diets containing high tuna liver meal (Gümüş et al., 2009) and there were no significant differences in whole body lipid and dry matter content in gibel carp (Yang et al., 2006) or Nile tilapia (Gümüş et al., 2010) fed diets containing different levels of fishmeal replacement.

The ADC of dry matter and lipid were slightly but insignificantly decreased by the high addition of tuna liver meal in the diets. Protein and energy ADC were similar to findings of Wee and Shu (1989), Ahmad (2008), and Gümüş et al. (2009).

In conclusion, tuna liver meal can be a substitute for up to 30% of the fishmeal protein in diets for Nile tilapia fry without negative effects on growth performance, feed utilization efficiency, or digestibility. Further research should investigate the possibility of including higher levels of tuna liver meal in various fish diets after applying different processing techniques for the tuna liver meal.

Acknowledgements

This study was financially supported by the Research Funds from the Akdeniz University.

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