

Effects of Dietary Tuna By-Products on Feed Intake and Utilization of Rainbow Trout *Oncorhynchus mykiss*

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Abstract: A 12 week experiment was conducted to investigate the effect of feeding rainbow trout *Oncorhynchus mykiss* with diets containing different levels of tuna by-products (tuna fish meal and tuna fish oil) on feed intake, growth performance, nutrient utilization and body composition in seawater condition. Fish were fed 5 experimental diets (42% crude protein and 12% crude fat), commercial diet (Com) with 42% protein and 16% crude fat, anchovy meal diet (Anc) and the diets contained different levels of tuna by-products (54.6, 45.5 and 36.4%) to replace 75, 60 and 50% of dietary protein, respectively. Specific Growth Rate (SGR) and Feed Conversion Ratio (FCR) were significantly influenced by replacement of fish meal by tuna by-products ($p < 0.05$). The poorest performance in terms of SGR and FCR were recorded in Tuna groups irrespective of dietary inclusion. The hepatosomatic index, crude protein content and crude ash in whole body were not influenced ($p > 0.05$); however the condition factor, moisture and lipid in whole body were significantly affected by dietary treatment ($p < 0.05$). Within the conditions of this experiment, the results showed that tuna by-products with the present quality are not qualified protein or energy source for rainbow trout at high inclusion levels. However, further studies are required to investigate the processing techniques and storage conditions of these products to resolve the palatability problem.

Key words: Salmonids, feed formulation, fish meal, dietary lipids, nutrient utilization, growth

INTRODUCTION

Feed constitutes approximately 50-70% of the production cost in commercial fish production and Fish Meal (FM), mainly derived from stocks of small pelagic fish, is the basic ingredient for most fish diets because of its high protein content with balanced amino acid profile, a good source of Essential Fatty Acids (EFA), minerals and vitamins (Hardy 1996; Hardy and Tacon, 2002). However, market price of FM, has risen significantly for three decades, due to decrease in supply of stocks and increase in demand for aquaculture (Hardy and Tacon, 2002). Therefore, many attempts have been in progress investigating alternative animal/plant protein sources such as pulses, oilseeds, grains, rendered animal meals, seafood processing discards and fisheries by-catch to substitute FM in fish feeds (Stickney *et al.*, 1996). The use of such ingredients in the diets of some carnivorous species has decreased the amount of FM by approximately 35% (Fornshell, 2002).

Fish processing waste and fisheries by-catch exceed the global landings of FM production and presented as

alternative feedstuffs for aquaculture feeds (Hardy *et al.*, 2005), even though they have significant variations in proximate compositions.

Tuna by-products (tuna fish meal and tuna fish oil) could also be potential ingredients for fish feeds in Turkey where approximately 40% of 70.000 metric tonnes (wet weight) of processed skipjack tuna (*Katsuwonus pelamis*) is discarded and turned into inexpensive fish meal with high level of ash and fish oil.

This preliminary study was designed to investigate the effects of produced tuna by-products on feed intake, growth performance, nutrient utilization and body composition of rainbow trout, which is one of the leading fish species of Turkish aquaculture.

MATERIALS AND METHODS

Experimental layout: Rainbow trout (*O. mykiss*) were obtained from a private trout farm (Çanakkale, Turkey) and transferred to the Net Cage Unit of the Faculty of Fisheries, Onsekiz Mart University (Çanakkale, Turkey). Prior to the experiment, the fish were fed by hand to

apparent satiation with a commercial trout feed (Biomar; crude protein: 45%, crude lipid: 18%) and acclimated until the beginning of the feeding trial. Total 960 fish of mean weight 155.0 ± 0.2 g were randomly distributed into experimental net cages in duplicate (diameter: 2 m and depth 2.5 m).

During the experiment, the dead fish were removed, weighed and recorded daily to correct the calculations by the end of the experiment. The feeding trial was conducted for 12 weeks. The experimental diets were fed close to apparent satiation by hand twice. Water temperature, dissolved oxygen, pH and salinity during the experimental period, were varied between 6.8 and 16.3°C, 8.5 and 9.8 mg L⁻¹, 7.3 and 8.8, 22.9 and 27.5%, respectively. Fish individually weighed at the start and the end of feeding trial and bulk-weighed fortnightly.

Experimental diets: Proximate composition of fish meal sources is displayed in Table 1. Formulation and nutrient composition of the experimental diets are presented in Table 2. All the 4 diets were deemed to prepare to be isonitrogenous and isoenergetic. Anchovy diet (Anc) was mainly composed of anchovy meal, whereas anchovy meal was substituted by tuna fish meal in Tuna 50, 60 and 70 at the inclusion levels of 54.6, 45.5 or 36.4% to provide 50, 60 or 70% of total dietary protein from tuna fish meal, respectively. Anchovy fish oil was incorporated in Anc diet, whereas tuna fish oil was used in tuna diets. Commercial (Com) diet formulation and ingredients were not revealed by the supplier due to commercial reasons. All dietary ingredients were blended thoroughly, moistened and cold pelleted with a laboratory feed mixture, then pellets were dried at 40°C for 16 h and stored at -20°C until delivery.

Sample collection and analyses: At the beginning of the feeding trial, 10 fish were sampled and frozen at -25°C for subsequent proximate analysis of whole body. At the end of the feeding trial, all fish starved for 48 h to ensure that there is no feed in digestive system. Three fish from each cage were killed and stored -25°C for subsequent whole-body proximate analysis. Determination of dry matter, crude ash, crude protein and crude lipid of experimental diets, commercial diet and whole fish body were done by 24 h drying at 105°C, 12 h combustion at 525°C, Kjeldahl method (N×6.25) and petroleum ether extraction, respectively. Nitrogen-Free Extract (NFE) was calculated by subtraction of the sum of crude ash, crude protein and crude lipid from 100. The energy content of the diets was estimated using the conversion factors of 23.7, 39.5 and 17.2 kJ g⁻¹ for protein, lipid and carbohydrate, respectively (Brett and Groves, 1979).

Table 1: Proximate composition of fish meal sources

| Proximate composition (%) | Tuna by-product meal | Anchovy fish meal |
|---------------------------|----------------------|-------------------|
| Moisture | 6.6 | 8.2 |
| Protein | 61.3 | 66.2 |
| Lipid | 13.6 | 12.0 |
| Ash | 19.4 | 15.3 |

Table 2: Formulation and proximate analyses of experimental diets fed to rainbow trout reared in netcages

| Ingredients | Diets | | | | |
|--|-------|---------|---------|---------|-------|
| | Anc | Tuna 50 | Tuna 60 | Tuna 75 | Corn* |
| Anchovy fish meal ¹ | 44.12 | - | - | - | - |
| Tuna by-product meal ² | - | 36.40 | 45.46 | 54.60 | - |
| Corn gluten ³ | 9.00 | 19.00 | 14.00 | 9.00 | - |
| Full-fat soybean meal ³ | 9.00 | 19.00 | 14.00 | 9.00 | - |
| Sunflower meal ³ | 5.00 | 5.00 | 5.00 | 5.00 | - |
| Wheat meal ⁴ | 19.28 | 7.70 | 7.94 | 8.80 | - |
| Anchovy fish oil ¹ | 8.60 | - | - | - | - |
| Tuna by-product oil ² | - | 8.60 | 8.60 | 8.60 | - |
| Vitamin/mineral premix (1:1) ⁵ | 2.00 | 2.00 | 2.00 | 2.00 | - |
| Binder ⁶ | 1.00 | 1.00 | 1.00 | 1.00 | - |
| Calsid ⁶ | 2.00 | 2.00 | 2.00 | 2.00 | - |
| Proximate composition (Dry matter%) | | | | | |
| Protein | 43.50 | 42.40 | 42.50 | 42.20 | 42.0 |
| Lipid | 14.20 | 12.60 | 12.40 | 12.10 | 16.0 |
| Ash | 7.50 | 9.70 | 11.60 | 13.60 | 7.6 |
| NFE | 34.80 | 35.30 | 33.60 | 32.10 | 34.4 |
| Energy (kJ g ⁻¹) | 21.90 | 21.10 | 20.80 | 20.30 | 22.2 |
| P:E (mg kJ ⁻¹) | 19.90 | 20.10 | 20.50 | 20.80 | 18.9 |

¹Anchovy fish meal and oil; Can Kardesler Fish Meal Co., Samsun, Turkey;

²Tuna by-product meal and oil. Dardanel Onentas Incorporated, Canakkale, Turkey; ³Abide Feed Mill Company, Canakkale, Turkey; ⁴Kepez Un, Canakkale, Turkey; ⁵Per g vitamin mixture: vitamin A: 342 IU; vitamin D₃: 329 IU; vitamin E: 0.0274 IU; vitamin K₃: 5.48 mg; vitamin B₁: 2.05 mg; vitamin B₂: 3.42 mg; vitamin B₃: 20.5 mg; vitamin B₅: 5.48 mg; vitamin B₆: 2.05 mg; vitamin B₁₂: 2.74 mg; vitamin C: 24.0 mg, Kartal Chemical Incorporated, Kocaeli, Turkey; Per g mineral mixture: biotin: 0.411 mg; folic acid: 0.685 mg; Zn: 12.3 mg; Mn: 4.80 mg; Cu: 1.64 mg; I: 0.274 mg; Se: 0.0274 mg; Ca: 125 mg; K: 189 mg, Kartal Chemical Incorporated; Kocaeli, Turkey; ⁶Kartal Chemical Incorporated, Kocaeli, Turkey

Statistical analysis: The collected data were subjected to one-way ANOVA and Duncan's multiple range test (p<0.05) using the statistical software package Statgraphics 7.0 (Manugistics Incorporated, Rockville, MD, USA) (Zar, 2001).

RESULTS

The results of overall performance, feed intake and nutrient utilisation of fish are presented in Table 3. Survival rates of rainbow trout ranged between 78.7% and 94.3% and did not differ significantly among the dietary treatments except the commercial group (p<0.05). The unexpected mortality was recorded in Com group because of attacks by cormorants during the first 2 weeks of the trial. The trout fed Anc or Com diets accepted the experimental diets and fed actively for the duration of the experiment, however, fish fed tuna diets showed suppressed feed intake ranged from 1.1-1.3 g feed 100 g body weight⁻¹.

Table 3: Growth parameters of rainbow trout fed experimental diets reared in netcages

| Growth parameters | Initial | Anc | Tuna 50 | Tuna 60 | Tuna 75 | Com | *±SEM |
|---|---------|--------------------|--------------------|--------------------|--------------------|--------------------|-------|
| Survival (%) | - | 87.8 ^{ab} | 93.3 ^b | 94.3 ^b | 84.7 ^{ab} | 78.3 ^a | 2.6 |
| Mean initial weight (g) | - | 155.0 | 156.0 | 156.0 | 156.0 | 155.0 | 0.2 |
| Mean final weight (g) | - | 502.0 ^b | 280.0 ^a | 296.0 ^a | 304.0 ^a | 547.0 ^b | 12.2 |
| SGR (% day ⁻¹) ¹ | - | 1.3 ^b | 0.6 ^a | 0.7 ^a | 0.7 ^a | 1.4 ^b | 0.1 |
| FCR ² | - | 1.3 ^{ab} | 1.6 ^{ab} | 1.7 ^{ab} | 1.9 ^c | 0.9 ^a | 0.3 |
| Feed intake (g fish ⁻¹) | - | 2.1 ^b | 1.1 ^a | 1.3 ^a | 1.3 ^a | 1.8 ^b | 0.1 |
| PER ³ | - | 1.7 | 1.3 | 1.3 | 1.5 | 2.1 | 0.4 |
| NPU (% ⁴) | - | 58.5 | 56.5 | 45.9 | 63.4 | 60.8 | 8.6 |
| CF ⁵ | 1.4 | 1.5 ^b | 1.1 ^a | 1.1 ^a | 1.1 ^a | 1.5 ^b | 0.8 |
| HSI (% ⁶) | 1.5 | 1.6 | 1.5 | 1.3 | 1.3 | 1.3 | 0.2 |
| DO (% ⁷) | 90.0 | 88.0 | 89.2 | 90.1 | 90.1 | 86.6 | 3.2 |

Values within the same row with different letters are significantly different (p<0.05); *Values Represent Mean±S.E.; ¹SGR: Specific Growth Rate (% day⁻¹) = 100×((ln final fish weight)-(ln initial fish weight))/experimental days; ²FCR: Feed Conversion Ratio = feed intake (g)/weight gain (g); ³PER: Protein Efficiency Ratio = weight gain (g)/dietary protein consumption (g); ⁴NPU: Net Protein Utilisation (%) = ((final body protein (g) -initial body protein (g))/dietary protein consumption (g))×100; ⁵CF: Condition Factor = (fish weight (g)/(fish length)³ (cm))×100; ⁶HSI: Hepatosomatic Index (%) = (liver weight (g)/fish weight (g))×100; ⁷DO: Dress-Out (%) = ((fish weight (g) -gut weight (g))/fish weight (g))×100

Table 4: Composition of whole body of rainbow trout reared in netcages

| Proximate composition (%) | Initial | Anc | Tuna 50 | Tuna 60 | Tuna 75 | Com | ±SEM |
|---------------------------|---------|-------------------|-------------------|-------------------|-------------------|-------------------|------|
| Moisture | 71.0 | 65.9 ^b | 72.1 ^b | 71.1 ^b | 70.8 ^b | 56.4 ^a | 3.2 |
| Protein | 21.3 | 16.6 | 16.9 | 16.2 | 17.5 | 21.6 | 3.2 |
| Lipid | 3.9 | 12.9 ^b | 7.6 ^a | 7.6 ^a | 7.3 ^a | 18.0 ^c | 2.7 |
| Ash | 2.2 | 2.2 | 2.4 | 2.2 | 2.8 | 1.8 | 1.1 |

Significant differences (p<0.05) in growth performance and feed utilisation were observed among the experimental groups. Irrespective of inclusion level, trout fed tuna by-product diets displayed the poorest growth and feed conversion, whereas the groups fed either Anc or Com diets almost tripled their weight during the feeding trial. Similarly, PER of trout were higher in Com and Anc groups than other treatments. ANPU of both groups fed Tuna 50 and 75 diets were high and comparable with groups fed Anc diet. Condition Factor (CF) of both Anc and Com groups ranked highest with 1.5, whereas Tuna groups scored 1.1, irrespective of inclusion level. HSI ranged from 1.3 (Com) to 1.6 (Anc). Dress out of Tuna 50, 60 or 75 groups were similar to Anc and higher than Com group, but this is not proven statistically.

Table 4 presents the proximate composition of whole body of experimental fish at the end of the feeding trial. The crude protein and ash contents of whole-body ranged from 16.2-21.6 and 1.8-2.8%, respectively and were not affected by experimental diets (p>0.05). On the other hand, body lipid levels of Anc and Com groups were significantly higher than tuna groups irrespective of substitution level of tuna by products (p<0.05).

DISCUSSION

The results showed that tuna fish meal and tuna fish oil with the present quality are not qualified protein or energy source for rainbow trout at high inclusion levels within the conditions of this experiment, since the lower palatability of tuna by-products influenced the feed

acceptance in tuna groups. Anc group consumed almost double amount of feed compared to tuna groups during the 12 week feeding trial. Variations in feed intake of rainbow trout reflected the growth performance and feed utilization. Anc and Com groups displayed significantly superior SGR compared to tuna groups irrespective of substitution level, although, tuna groups doubled their body weight at the end of the trial. Growth performance and feed utilisation results of trout fed on Anc and Com diets corresponded well with same species studied (Satoh *et al.*, 2003; Morris *et al.*, 2005), except for trout fed tuna by-products. Previous studies have been demonstrated that growth performance was influenced in different species by parameters such as processing temperature, raw material and freshness of dietary fish meal (Moksness *et al.*, 1995; Aksnes and Mundheim, 1997). Hernandez *et al.* (2004) did not report any palatability problem with dietary wet tuna viscera fed shrimp (*Litopenaeus vannamei*) probably because of the co-extrusion of the tuna meal with corn meal.

The most efficient feed conversion was observed in Com group, followed by Anc, Tuna 50, 60 and 75 groups, respectively. The difference in FCR between Com and Anc fish could be due to the fact that Anc diet was cold extruded under laboratory condition whereas Com diet was extruded by using heat and steam which clearly effects the gelatinization rate of starch in the diet. Trout consumed diets Com and Anc produced better PER results than that of groups delivered diets of Tuna by-products. These suggests that imbalances or deficiencies of nutrients could be present in the tuna diets. However, nutrient retention parameter somehow does not support

this since Tuna 75 and Com groups displayed superior NPU compared to other groups although, this could not be proven statistically. As a whole, low nutrient utilization of tuna groups may suggest an amino imbalance so that essential aminoacids were not simultaneously present for tissue building (Hardy *et al.*, 2005).

Hepatosomatic index of the experimental fish were similar that of reported by Samuelsen *et al.* (2001) and Caballero *et al.* (2002). Variations in feed intake among the experimental groups also effected the body composition of trout. Trout fed on Anc and Com diets showed significantly higher body lipid level than tuna groups which had similar levels of body lipid ($p>0.05$). This parameter was highest in Com group ($p<0.05$) probably due to the higher rate of dietary lipid level in Com diet. It is well demonstrated that body lipid is affected by dietary lipid level and body lipid is conversely related to body moisture (Jobling, 2001).

CONCLUSION

In conclusion, Tuna fish meal and tuna fish oil might still have potential as feed ingredients in trout diets with a lower inclusion levels than the present study. However, some challenges exist because of the variation in its biological availability depending on quality and profile of amino acids, fatty acids and other nutrients. Heat treatment, extrusion process (Hernandez *et al.*, 2004), conservation methods of fragile nutrients such as lipids may be alternative ways for converting Tuna industry waste into a high quality aquafeed ingredient. Also the palatability of these products can be improved by attractant supplementation and also rehabilitating storage conditions of tuna fish meal and oil.

ACKNOWLEDGEMENT

The authors are indebted to Dardanel Ltd. (Turkey) for providing tuna by-products. Betül Guroy and Soner Bilen also are acknowledged for their help during the feeding trial. This research was funded by Council of Scientific Research Projects (2002/17) of Çanakkale OnsekizMart University and was submitted as MSc thesis by Emrah Deveciler.

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