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The effect of *Tenebrio molitor* larva meal instead of fish meal on growth performance and feed efficiency in juvenile Nile tilapia (*Oreochromis niloticus*) diets

Jüvenil Nil tilapia (*Oreochromis niloticus*) rasyonlarında balık unu yerine *Tenebrio molitor* larva ununun büyüme performansı ve yemden yararlanma üzerine etkisi

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ABSTRACT

The aim of this study was to evaluate the effects of feeding Nile tilapia juvenile (*Oreochromis niloticus*) with diets based on fish meal (FM) and *Tenebrio molitor* (TM) larvae meal on growth performance and feed efficiency. For this purpose, three diets were prepared: Control diet (FM); without *T. molitor* larvae meal, insect diet (TM); 100% of fish meal has been replaced by *T. molitor* larvae meal and mixture diet (FM/TM); 50% of FM has been replaced by *T. molitor* larvae meal. Each treatment was tested in three replications over a 46-day. In the experiment, 1-month-old Nile tilapia juveniles with an average weight of 0.12±0.001 g were used. At the end of the experiment, the best growth was in the FM group, followed by the TM group and FM/TM group, the differences being significant except for FM and TM groups ($P<0.05$). Weight gain was also in line with growth rates. The SGR was 5.35%, 5.27% and 5.21% in FM, TM and FM/TM groups, respectively, and The difference between FM and FM/TM groups was statistically significant ($P<0.05$). The best FCR was in the FM group which was similar to TM diet ($P<0.05$). The worst FCR was in the group fed FM/TM diet with a significant difference from the other treatments. The PER changed between 0.87±0.07 and 1.42±0.04 with significant differences among the treatments. The survival rate was 100% in all groups. The data obtained showed that TM has the potential to be used as a protein source in diets of juvenile tilapia.

Keywords: Nile tilapia juveniles, *Oreochromis niloticus*, Mealworm, Growth.

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ÖZET

Bu çalışmanın amacı, Nil tilapiya yavrularının (*Oreochromis niloticus*) balık unu (FM) ve *Tenebrio molitor* (TM) larva unu bazlı diyetlerle beslenmesinin büyüme performansı ve yem verimliliği üzerindeki etkilerini değerlendirmektir. Bu amaçla üç diyet hazırlanmıştır: Kontrol diyeti (FM); *T. molitor* larva unu içermeyen, böcek diyeti (TM); balık ununun %100'ü *T. molitor* larva unu ile değiştirilmiş ve karışım diyeti (FM/TM); FM'nin %50'si *T. molitor* larva unu ile değiştirilmiştir. Deneme 46 gün boyunca üç tekrarlı olarak test edilmiştir. Denemede, ortalama ağırlığı 0.12±0,001 g olan 1 aylık Nil tilapiya yavruları kullanılmıştır. Deneyin sonunda, en iyi büyüme FM grubunda gerçekleşmiş, bunu TM grubu ve FM/TM grubu izlemiş, FM ve TM grupları dışındaki farklar önemli bulunmuştur ($P<0.05$). Ağırlık artışı da büyüme oranlarıyla uyumluydu. SGR değerleri uygulamalar arasında karşılaştırılabilirdi ($P>0.05$). SGR, FM, TM ve FM/TM gruplarında sırasıyla %5.35, %5.27 ve %5.21, ve FM ile FM/TM grupları arasındaki fark istatistiksel olarak önemli bulunmuştur ($P<0,05$). En iyi FCR, TM diyetine benzer olan FM grubunda tespit edilmiştir ($P<0.05$). En kötü FCR, diğer uygulamalardan önemli bir farkla FM/TM diyetiyle beslenen grupta görülmüştür. PER 0.87±0.07 ile 1.42±0.04 arasında değişmiş ve uygulamalar arasında önemli farklılıklar görülmüştür. Tüm gruplarda yaşama oranı %100 olmuştur. Elde edilen veriler, TM'nin protein kaynağı olarak kullanılma potansiyeline sahip olduğunu göstermiştir.

Anahtar sözcükler: Nil tilapiya yavruları, *Oreochromis niloticus*, Unkurdu, Büyüme.

1. INTRODUCTION

Fish meal (FM) is the primary ingredient for juvenile fish, but is becoming more and more expensive. Alternative feed sources are required since faith in fishmeal has been questioned recently for social, economic, and environmental reasons (Tran *et al.,* 2015). Insects are promising in animal nutrition due to high nutritional, short intergenerational periods, low carbon emissions in their production, and easy and low production costs (Lange and Nakamura, 2023). Existing studies show that insects can convert 8-9% low-quality protein-containing food wastes into 44-61% good-quality insect protein in a period of 3-4 months under controlled conditions (Ramos-Elorduy, 1997).

Tenebrio molitor is an insect species that has the feature of being a model organism due to its features such as being an easily cultivated species in the laboratory environment, having low breeding and feeding costs, being able to be grown in large numbers in a unit area, short intergenerational time, and easy determination of its characteristics (Özsoy and Gündoğdu 2017). Therefore, *T. molitor* stands out as a promising species for mass production among insects (Ramos-Elorduy *et al.,* 2002; Ghaly and Alkoaik, 2009). *T. molitor* larvae have high protein content (37-47%) as well as high-fat content (Finke, 2002; Ramos-Elorduy *et al*., 2002; Ghaly and Alkoaik, 2009; Huang *et al*., 2011). The dried larvae of *T. molitor*, which is rich in unsaturated fatty acids, have a fat content of 46% (Siemianowska *et al.,* 2013; Ravzanaadii *et al.,* 2012). *T. molitor* meal have used as a protein source in diets of pet animals, broiler chick (Ramos-Elorduy *et al.,* 2002; Marco *et al.,* 2015) and fish (Gasco *et al.,* 2014; Ng *et al.,* 2001; Belforti *et al.,* 2016)

Juvenile fish, regardless of their feeding habit, require nutritionally high-quality diets based on FM under intensive farming conditions and are completely dependent on nutritionally complete diets (Tacon 1988). Insect proteins have become a valuable protein source juvenile fish (Odesanya *et al.,* 2011; Barroso *et al*., 2014; Henry *et al.,* 2015). Previous studies on tilapia fry have demonstrated that, when compared to FM-based control diets, meals from both housefly larvae (*Musca domestica*) and blowfly larvae (*Chrysomya megacephala*) can replace up to 100% of FM in practical diets without impairing fish performance. However, there is still room for the investigation of the dietary use of TM for juvenile Nile tilapia.

In recent years Ulva species have become important macroalgae investigated as a dietary component for a wide range of fish species. The inclusion of Ulva meal at low levels in the diet has resulted in improved growth, feed utilization, physiological activity, disease resistance, carcass quality and reduced stress response (Wassef *et al*., 2005; Valente *et al*., 2006). Furthermore, Ulva meal has been successfully used as a feed ingredient for tilapia and its addition to diets has shown that it can improve carcass composition (Güroy *et al*., 2007; Azaza *et al*., 2008). Ergün *et al*. (2009) reported that the addition of 5% of Ulva meal to diets improved growth performance, feed efficiency, nutrient utilization and body composition of *Oreochromis niloticus*. Therefore, in this study, a 3% *Ulva lactuca* meal was added to diets prepared with two different levels of TM (50% and 100%), and its effects on growth performance and feed efficiency of juvenile Nile tilapia were evaluated.

2. MATERIAL AND METHOD

2.1. Experimental Design and Feeding

The study was carried out at Sinop University Fisheries Faculty Research Center. In the experiment, 1-month-old Nile tilapia juveniles were used. The average weight of 0.12±0.001 g. The fish were distributed to nine square tanks (4 L) in groups of ten. Three diets were tested with three replications for 46 days. In order to keep the water in the tanks at a constant temperature, all aquariums were placed in a 100*50*40 L glass aquarium containing water, and the temperature of the water in the tanks and the aquarium 26ºC using a thermostatic heater. In addition, a water circulation motor was used to distribute the water in the glass aquarium evenly. The water quality parameters were monitored and recorded on a weekly basis.

Fish in all groups were hand fed twice a day (at 09:00 am and 15:00 pm) to apparent satiety under a natural light regime. Experimental tanks were daily siphoned and discharged water was renewed with fresh water afterwards.

2.2. Diet ingredients and formulation

T. molitor larvae obtained from a local producer for production purposes were first placed in a racked rearing system designed to be produced under laboratory conditions. *T. molitor* larvae placed in this rearing system were fed with potatoes or lettuce on a bed of whole wheat flour, cereal bran and rice bran (Figure 1).

Figure 1. A racked rearing system, adult insects and mealworms

After the larvae were transformed into insect form, they started to produce offspring regularly and were harvested before reaching the prepupa stage. *T. molitor* larvae produced for use in the study were frozen at -20°C. The *T. molitor* larvae were dried in an oven and homogenized by grinding just before the preparation of experimental diets.

All raw materials used in feed formulation were sieved and put into a mixer. After mixing homogeneously, water was added and mixing was continued. Since the fish were very small, no pelletizing was done. The feeds removed from the mixer were placed on separate trays and dried at 50ºC. The dried feeds were ground into powder and used as powder feed. Finally, they were stored in sealed feed boxes in the refrigerator. All feeds were used from the refrigerator during the experiment.

Three experimental diets were formulated in which fish meal was substituted with TM at different levels (Table 1):

- Control diet (FM): without TM
- Insect diet (TM): 100% of FM replaced by TM
- Mixture diet (FM/TM): 50% of FM replaced by TM

Other ingredients to be used in the feed formulation were obtained from a commercial feed company (SÜRSAN A.Ş.).

2.3. Chemical composition analysis of diets

The analyses of the chemical composition of feed ingredients and diets were performed according to the standard methods of AOAC (1995). The gross energy of the diets was estimated assuming 23.6 kJ/g protein, 39.5 kJ/g lipid and 17 kJ/g nitrogen-free extracts.

2.4. Calculations

Weight gain (WG, g) = (final body weight − initial body weight) (1)

Specific growth rate (SGR, %/day) = [(ln final body weight − ln initial body weight)/days] × 100. (2)

Protein efficiency ratio (PER) = wet weight gain/total protein given (3)

Feed conversion ratio (FCR) = feed intake/wet weight gain (4)

2.5. Statistical analysis

Anderson-Darling and Levene's tests were used for homogeneity of variances and equality of variance of groups, respectively. Square root transformations of percentage data, the significance of differences in growth and feed utilization variables among the treatments was tested using a one-way analysis of variance (ANOVA), followed by Tukey's method for multiple comparisons. Differences were considered significant when $P< 0.05$. Statistical analyses were performed using Minitab 17 software for Windows.

3. RESULTS

The inclusion levels of various ingredients in the experimental diets and their respective proximate composition are given in Table 1. Water temperature, dissolved oxygen, and pH varied slightly throughout the experiment and ranged from 26.2 to 28.1°C, 3.14 to 4.87 g/L, and 7.5 to 8.33, respectively.

At the end of the 46-day experimental period, juvenile tilapia showed the highest growth when fed FM diet, followed by TM and FM/TM diets. The growth differences were only significant between fish fed the control and FM/TM diets ($P<0.05$). Weight gain displayed a similar trend. The SGR was 5.35%, 5.27% and 5.21% in FM, TM and FM/TM groups, respectively. The difference between FM and FM/TM groups was statistically significant ($P<0.05$). The best FCR was in fish fed the FM diet, followed by those on TM diet without a statistically significance ($P>0.05$, Table 2).

Table 1. Formulation (g/kg) and proximate composition (%) of the experimental diets

	Experimental Diets		
	FM	TM	FM/TM
Nutrient (g kg⁻¹)			
Fish meal	180	-	110
Tenebrio molitor	-	280	170
Soybean meal	303	283	270
Wheat flour	300	230	243
Corn protein	150	150	150
Fish oil	50	10	10
Ulva lactuca meal	-	30	30
Vitamin premix(*)	3	3	3
Mineral premix(*)	3	3	3
Astaxanthin	1	1	1
Dicalcium phosphate	10	10	10
Proximate Composition (%)			
Moisture	3.46	2.87	3.79
Protein	42.85	41.05	42.99
Lipid	8.99	8.61	7.60
Ash	5.87	5.57	6.24
NFE[1]	38.83	41.90	39.37
Gross energy (kJg^{-1})[2]	20.26	20.21	19.84

[*] Vitamin-mineral premix (mg/kg premix): vitamin A, 210000 IU; Vitamin D_3, 35000 IU; vitamin E, 7000 mg; vitamin K_3, 322 mg; vitamin B_1, 588 mg; vitamin B_2, 252 mg; vitamin B_6, 294 mg; vitamin B_{12}, 826 mcg; niacin, 1400 mg; biotin, 7583 mcg; 182 mg folic acid, pantothenic acid, 1722 mg; inositol, 17220 mg; vitamin C, 933.31 mg; Ca, 1414mg.

[1]NFE=100-(%protein+ %lipid+ %ash+ %moisture)

[2]Gross energy is calculated according to 23.6 kJ g^{-1} protein, 39.5 kJ g^{-1} lipid and 17 kJ g^{-1} NFE

Table 2. Growth performance and feed efficiency of Nil tilapia juvenile fed the 3 experimental diets for 46 days

	Experimental Groups		
	FM	TM	FM/TM
IBW (g)	0.12±0.002[a]	0.12±0.001[a]	0.11±0.003[a]
FBW (g)	1.36±0.16[b]	1.32±0.16[b]	1.24±0.12[a]
WG (g)[1]	1.25±0.09[b]	1.21±0.09[b]	1.13±0.07[a]
SGR(%)[2]	5.35±0.19[a]	5.27±0.16[ab]	5.21±0.18[b]
FCR[3]	3.29±0.05[a]	3.48±0.23[a]	5.79±0.50[b]
PER (%)[4]	1.42±0.04[c]	1.21±0.10[b]	0.87±0.07[a]
Survival (%)	100[a]	100[a]	100[a]

Data are reported as mean ± standard errors of three replicates (3). Means with different superscript letter in a row are significantly different (p>0.05). IBW: Initial body weight, FBW: Final body weight. replicates.

The highest FCR was in the group fed FM/TM diet, being significantly different from the control.

The PER was calculated as 1.42±0.04 for the FM group, 1.21±0.10 for the TM group, and 0.87±0.07 for the FM/TM group and PER values were significantly different among the diets. The survival rate was 100% in all groups.

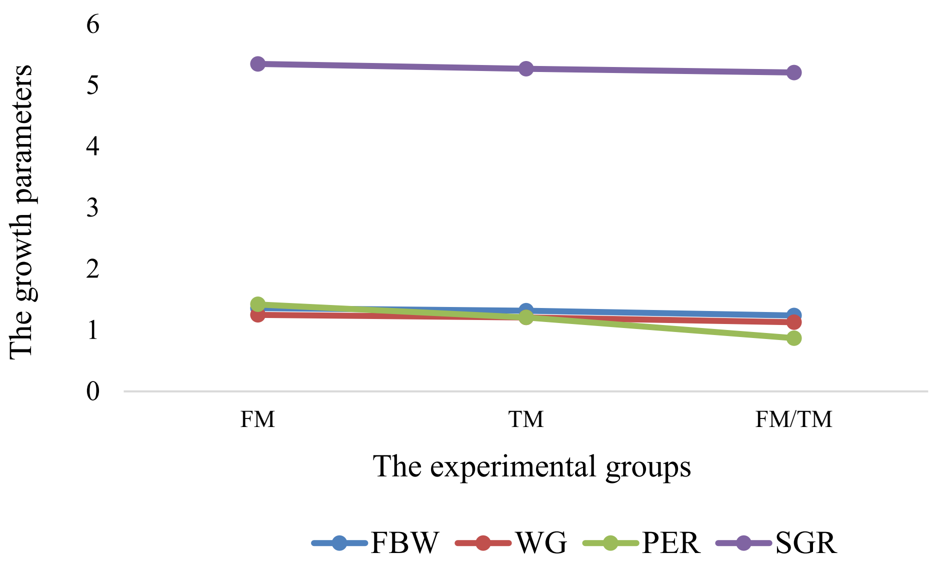

Figure 2. Distribution of specific growth rate (SGR), final body weight (FBW), weight gain (WG), and protein efficiency ratio (PER) values of Nil tilapia juveniles by groups

4. DISCUSSIONS

Today, feed formulation strategies take into account the nutritional quality of feed ingredients as well as cost, availability and sustainability. The TM produced for the purpose of replacement of dietary FM in the present study showed lower protein and higher lipid contents than FM. Dietary use of TM results in a reduction of dietary fish oil due to higher levels of TM.

In the current study, the highest growth, weight gain and protein efficiency rate were determined in the control group, followed by the TM and FM/TM groups in discending order. Gasco *et al.* (2014) reported that rainbow trout (*Oncorhynchus mykiss*) fed diets with up to 50% can be used without decreasing growth performance, thus saving fish meal protein. Gasco *et al.* (2016) reported that a full-fat Tenebrio molitor meal can be included up to 25% in the diets of European sea bass fry (*Dicentrarchus labrax*) without affecting their growth performance, while fish performance was negatively affected at a higher TM inclusion level (50%). Similarly, Ng *et al.* (2001) reported that African catfish (*Clarias gariepinus*) showed good growth performances when up to 35% TM meal was added to their diet. On the contrary, Roncarati *et al.* (2015) reported reduced growth when catfish (*Ameiurus melas*) fingerlings were fed a diet containing TM larval meal. In an experiment using the same amounts of TM (25% and 50%) in gilthead sea bream (*Sparus aurata*) diets, Piccolo *et al.* (2017) reported that dietary inclusion of 25% TM in the diet did not have any negative effects on final weight, specific growth rate, weight gain, protein efficiency ratio or feed conversion ratio of the fish, but at the inclusion level of (50%) a deteriorated nutrient digestibility was the case without detrimental effect on growth performance. Ng *et al.* (2001) reported that *T. molitor* larvae in both, live feed or meal form, were a good potential source of protein source and could replace up to 40% of FM component in practical diets for African catfish. Additionally, it was noted that catfish fed a diet containing a combination of mealworm and catfish pellet grew as well as or better than fish fed commercial catfish diet (Ng *et al.,* 2001). Interestingly, in the present study, the group fed with a 100% TM-supplemented diet showed a better growth rate than the group fed with a 50% TM-supplemented diet and had almost the same growth rate as the control group. This may be due to the *Ulva lactuca* added to the diet. However, although the same amount of *Ulva lactuca* was added to both groups, the TM group showed better growth. Further research on the use of *Ulva lactuca* in combination with TM

should be conducted to provide a definite explanation for this. As a matter of fact, Turan (2006) reported that the addition of red clover (*Trifolium pratense*) as a growth-promoting agent in tilapia (*Oreochromis aureus* Linnaeus) may improve feed utilization in fish, resulting in a higher growth rate. Also, according to Diab *et al*. (2008), Nile Tilapia fry fed diets enriched with medicinal plants showed faster growth than those fed a control diet. Medicinal plants have been used with similar success for common carp (*Cyprinus carpio*), guppy (*Poecilia reticulate*) and cichlid (*Cryptoheros nigrofasciatus*) (Yılmaz *et al*., 2006; Çek *et al*., 2007a,b).
Dietary protein plays an important role in determining fish growth rate. Accurate information on the protein requirements of fish is crucial for any aquaculture enterprise because of the high cost of protein components, which are generally required at high levels by most fish (NRC, 1993). Tubin *et al.* (2020) stated that it was possible to include up to 10% of TM in the diets of juvenile tilapia reared in biofloc systems without any loss growth performance, carcass composition, somatic and hematological indices. Sánchez-Muros *et al.* (2016) reported that the use of TM (TM has been partially or completely replaced by both SM and FM) instead of FM in tilapia diets, partial or complete replacement of SM with TM reduced feed intake in vitro and protein digestibility did not affect the amino acid composition of muscle. However, the inclusion of TM at both levels tested (25% and 50%) reduced growth by about 29% and also affected the fatty acid profile of the muscle. The results in the literature are partially in agreement with those obtained in this study, suggesting the need for further studies.

5. CONCLUSIONS

The study evaluated the effects of feeding Nile tilapia juveniles with FM and TM based diets on growth performance and feed efficiency. In conclusion, the main conclusion of this study is that TM can be used as a protein source in juvenile tilapia diets without a reduction in growth performance, thus saving fish meals and oil. But, more research is needed on the nutritional value of insects for fish. *T. molitor* larvae meal is an innovative raw material and its use as an alternative feed raw material to fish meal in fish diets seems promising. In addition, industrial-scale processes should be developed for the production of insect-based fish feeds, taking into account their impact on the environment, food safety and society, and their production should be promoted. In this way, both a new raw material will be brought to aquaculture and a new business area will be provided.

AUTHORSHIP CONTRIBUTION STATEMENT

Seval DERNEKBAŞI: Conceptualization, Methodology, Validation, Formal Analysis, Resources, Writing - Original Draft, Writing-Review and Editing, Data Curation, Software, Visualization, Supervision, Project administration. **İsmihan KARAYÜCEL:** Conceptualization, Methodology, Validation, Formal Analysis, Resources, Writing - Original Draft, Writing-Review and Editing, Data Curation, Software, Visualization, Supervision, Project administration.

CONFLICT OF INTERESTS

The author(s) declare that for this article they have no actual, potential or perceived conflict of interests.

ETHICS COMMITTEE PERMISSION

Authors declare that this study was conducted in accordance with ethics committee procedures of human or animal experiments.

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