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RESEARCH ARTICLE

Effects of Two Seaweeds (*Ulva lactuca* and *Enteromorpha linza*) as a Feed Additive in Diets on Growth Performance, Feed Utilization, and Body Composition of Rainbow Trout *(Oncorhynchus mykiss)*

Önder YILDIRIM * ✎ Sebahattin ERGÜN ** Seval YAMAN *** Ali TÜRKER *

* Department of Aquaculture, Faculty of Fisheries, Muğla University, 48000, Kötekli, Muğla - TURKEY
** Department of Aquaculture, Faculty of Fisheries, Çanakkale Onsekiz Mart University, 17100, Çanakkale - TURKEY
*** Department of Aquaculture, Faculty of Fisheries, Sinop University, 57000, Sinop - TURKEY

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Summary

In the present study, it was aimed to determine the effects of diet containing two seaweed species, *Ulva lactuca* and *Enteromorpha linza*, on the growth performance, feed utilization and body composition of rainbow trout. Two experimental diets were formulated with the usage of 10% *U. lactuca* meal and 10% *E. linza* meal in feed and control group had no seaweed ingredients. Each experiment was triplicate and each group had fourteen fish specimens with an average weight of 32.96±0.29 g. Fish were hand fed three times per day for 60 days. Significant differences were determined in weight gain, specific growth rate, relative growth rate and feed utilization between experimental and control groups (P<0.05). Fish fed with the diet containing 10% *E. linza* meal had the poorest feed utilization. The survival rate ranged from 96% to 98% in all groups during trial period. Apparent net protein retention, protein efficiency rate, daily dry feed intake and total feed intake were significantly lower in fish groups which fed with the diet containing *U. lactuca* and *E. linza* than control group (P<0.05). The final levels of crude protein, crude lipid and crude ash were in higher rates in the body composition all the groups compared when compared to the initial level (P<0.05). The results of the experiment revealed that a diet with *U. lactuca* and *E. linza* inclusion at 10% levels resulted in a poorer growth and feed utilization for rainbow trout when compared to those of control group. Hence, more defined experiments seem to be necessary in order to determine the optimum dietary inclusion level of these seaweeds in rainbow trout diets.

Keywords: *Rainbow trout (Oncorhynchus mykiss), Seaweeds, Ulva lactuca, Enteromorpha linza, Feeding, Growth and feed utilization*

Rasyonlarda Yem Katkı Maddesi Olarak İki Deniz Yosununun *(Ulva lactuca, Enteremorpha linza)* Gökkuşağı Alabalığının *(Oncorhynchus mykiss)* Büyüme Performansı, Yem Değerlendirme ve Vücut Kompozisyonu Üzerine Etkileri

Özet

Bu çalışmada, iki deniz yosunu *(Ulva lactuca, Enteremorpha linza)* içeren rasyonun gökkuşağı alabalığının büyüme performansı, yem değerlendirmesi ve vücut kompozisyonu üzerine etkisinin belirlenmesi amaçlanmıştır. İki deneme rasyonu % 10 *U. lactuca* ve %10 *E. linza* unu içerecek düzeyde formülüze edilmiş, kontrol grubuna ise hiçbir deniz yosunu ilave edilmemiştir. Her bir deneme grubu üç tekerrürden oluşmuş, her bir tekerrürde ortalama ağırlığı 32.96±0.29 g olan 14 balık yer almıştır. Balıklar günde üç kez elle yemlenmiş ve çalışma 60 gün sürmüştür. Deniz yosunu içermeyen Kontol grubu ile *U. lactuca* ve *E. linza* unu ihtiva eden gruplar arasında ağırlık artışı, spesifik büyüme oranı, nispi büyüme oranı ve yemden yararlanma yönünden farklılık istatistiki olarak önemli bulunmuştur (P<0.05). % 10 *E. linza* unu içeren grupta, yem değerlendirmenin en düşük olduğu tespit edilmiştir (P<0.05). Yaşama oranı tüm deneme gruplarında % 96'dan %98'e değişim göstermiştir. Görünür net protein alımı, protein etkinlik oranı, günlük yem ve toplam yem alımı *U. lactuca* ve *E. linza* içerikli rasyonlarda kontrol grubuna nazaran nispeten daha düşük bulunmuştur (P<0.05). Deneme sonunda, balıketinde yapılan ham protein, ham yağ ve ham kül oranı deneme başıyla karşılaştırıldığında tüm deneme gruplarında daha yüksek bulunmuştur (P<0.05). Bu çalışma sonuçları, gökkuşağı alabalığı rasyonlarında %10 seviyesinde *U. lactuca* ve *E. linza* kullanmanın kontrol grubuna göre, balıkların daha düşük bir büyüme ve yem değerlendirmesine neden olduğunu ortaya çıkarmıştır. Bu yüzden, alabalık rasyonlarında bu deniz yosunlarının, optimum kullanımı için daha fazla araştırma yapmaya gerek vardır.

Anahtar sözcükler: *Gökkuşağı alabalığı (Oncorhynchus mykiss), Deniz yosunları, Ulva lactuca, Enteromorpha linza, Besleme, Büyüme ve yem değerlendirme*

✎ İletişim (Correspondence)
☎ +90 252 2111891
✉ onderyildirim@mu.edu.tr & onyil@hotmail.com

INTRODUCTION

Aquafeed is the most expenditure in intensive aquaculture operations. Cheaper alternative ingredients receive increasing priority in feed formulating for the development of low-cost fish feed. Aquafeed industry worries about the picture that the major ingredients which fish meal, fish oil soon will be scarce; so much effort is put into the research for substitute feedstuff in developed and developing countries.

The importance of algae as a potential substitute protein source for cultured fish feeding has been documented in recent years [1,2]. The annual global aquaculture production of marine algae was $14.5x10^6$ tonnes (including brown, green and red seaweeds and miscellaneous aquatic plants) in 2007 [3].

Seaweeds are receiving increasingly consideration for their high protein value, essential amino acid content, vitamins and trace metals in fish feeding [4-15].

Ulva spp. is one of the most generally as a feed ingredient in fish diets [2,16]. Enteromorpha spp. is rich in minerals, primarily Ca and P, and contains acceptable essential amino acids and increases bile production for digestion of fatty acids [17]. Even though, valuable effects of both *Ulva* spp. and *Enteromorpha* spp. are well known, there is no information about the influence of these two macroalgae on the growth, feed utilization in rainbow trout. The green algae *Ulva lactuca (U. lactuca)* and *Enteromorpha linza (E. linza)* (Chlorophyta) are widely available coast of the Black Sea and the Sinop, in Turkey. The main purpose of this experiment was to evaluate the two seaweeds, *U. lactuca* and *E. linza*, as feed ingredients on the growth performance, feed utilization, and body composition of rainbow trout *(Oncorhynchus mykiss)*.

MATERIAL and METHODS

Experimental fish

Triplicate tanks of rainbow trout, obtained from a commercial local fish farm, were fed one of three experimental diets in lasting 60 days at research unity of the Fisheries of Faculty, Sinop University, Sinop in Turkey. The fish were acclimated in the experimental tanks one-month prior to the start of study. 126 fish were randomly distributed into 300 L cylindrical fiberglass tanks with 14 fish each (mean weight 32.96±0.29g). The tanks were supplied with freshwater. During the experiment, water was constantly and equally replaced by continuous flow at a rate of 3 L min^{-1}. Water temperature, dissolved oxygen and pH were maintained 13-17°C, 5-7 mgL- and at pH 6.4-8.1, respectively. Fish were weighed and recorded every 2 weeks from the beginning of the study. Before weighing, fish were starved for one day.

Experimental diet and feeding regime

The *U. lactuca* and *E. linza* were freshly collected from the near-shore waters in Sinop, Turkey. The seaweeds were cleaned, washed and dried before being added in the laboratory and stranger materials were cleaned off. The seaweeds were aired for 1 or 2 days darkness. They were dried in a drying cabinet at 45°C for 48 h and milled with a laboratory blender. Diet ingredients included fish meal, wheat gluten, soybean meal, *U. lactuca* meal, *E. linza* meal, fish oil, vitamin and mineral premix. A control diet was used without inclusion of any seaweed. After mixing of grounded ingredients of each diet, warm water was added and the feed pressed into pellets of 3 mm holes in a food grinder. Two experimental diets, comprising varying levels of *U. lactuca* meal (10%) and *E. linza* (10%) were formulated. The diets were isonitrogenous and isolipid on a crude protein (45%) and lipid (11%) basis. The proximate compositions of the experimental diets are reported in *Table 1*. The nutrient composition and amino acid profiles of feedstuff (fishmeal, soy meal, wheat meal, *U. lactuca* and *E. linza* meal) are given in *Table 2*. The fish in the all groups were fed 2% of their body weight per day under a natural light regime. Each diet was fed to the groups to apparent satiation 3 times a day (08:30, 12:00 and 16:30). The tanks were siphoned daily.

Chemical analysis

All experimental diets and fish samples were analyzed for chemical composition according to Association of official analytical Chemist (AOAC) [18]. Dry matter was determined by oven-drying at 105°C for 24h until constant weight; ash was determined by burning in a muffle furnace at 550°C for 4h; crude protein and total lipid content were determined by Kjeldahl method, by extraction with di-ethyl ether by Soxhlet method, respectively. Before starting the experiment, 9 fish from the initial batch were sacrificed by lowering the body temperature in a freezer, stored in polyethylene bags, and frozen (-20°C) for subsequent analysis of body composition. At the end of the feeding trial, three fish from each tank (nine fish per treatment) were randomly sampled, sacrificed, and stored for analysis in the above manner. Prior to analyses, samples were prepared by homogenizing the whole fish body in a blender. All analyses were performed in triplicate.

Table 1. *Ingredients and nutrient composition of diets used in the experiment*

Tablo 1. *Denemede kullanılan rasyonların besin madde içerikleri ve kimyasal analizleri*

Ingredients		**Diets**		
		Control	***U. lactuca***	***E. linza***
Fish meal		45.5	45	45
Soybean meal		25	25	25
Wheat meal		19	10	10
Fish oil		9.5	9	9
U. lactuca		-	10	-
E. linza		-	-	10
Vit.Min. Premix		1	1	1
Proximate analysis of diets (dry basis)				
Dry matter (%)		88.83	90.76	87.55
Crude protein (%)		45.49	44.36	43.15
Crude lipid (%)		11.20	10.77	10.60
Crude ash (%)		8.48	10.40	10.05
NFE1		34.83	34.47	36.20
Gross energy (kJg^{-1}diet)[2]		20.71	20.22	20.18
Digestible energy (kJg^{-1}diet)[2]		15.04	14.67	14.59
EAA (g 100g^{-1} DM)[3]	**Trout**[4]			
Arg	2.0	4.51	5.02	4.74
His	0.7	1.85	2.09	2.13
Ile	0.8	3.49	3.76	3.71
Leu	1.4	5.57	5.97	5.87
Lys	1.8	5.27	5.76	5.49
Met	1.45	1.70	2.11	1.95
Phe	1.86	3.29	3.66	3.58
Thr	0.8	3.30	3.64	3.61
Tyr	0.2	0.86	0.85	0.85
Val	1.3	3.74	4.41	4.06

1 *NFE, nitrogen free extract calculated: 100% - % (protein + lipid + fibre + ash)*
2 *Calculated using gross and digestible energy values of 23.01, 38.05 and 17.15 kJg^{-1}; 16.84, 33.47 and 10.46 kJg^{-1} for protein, fat and carbohydrate, respectively* [27].
3 *Essential amino acid contents calculated from data in Table 2.*
4 *As a percent of diet essential amino acid requirements of rainbow trout to Hardy* [28].
5 *Met+cystine*
6 *Phe+tyrosine*

Statistical analysis

Statistical analysis included one-way analysis of variance (ANOVA) and Tukey's multiple significant difference tests using the software program (Minitab 13 for windows). Differences were regarded as significant when P<0.05 level.

Table 2. *Proximate analysis of fishmeal, soy meal, wheat meal Ulva and Enteromorpha meal (as % dry matter)*

Tablo 2. *Soya unu, buğday unu, Ulva ve Enteromorpha unlarının % kuru madde cinsinden kimyasal analizleri*

Proximate analysis	**FM** [1]	**Soybean meal** [1]	**Wheat meal** [1]	**UM** [2]	**EM** [3]
Dry matter	92	89	88	96.31	93.3
Protein	70.7	45	14.3	17.44	14.1
Lipid	5.3	1.2	1.7	2.5	2.2
Fibre	-	6.1	1.1	5.47	33.1
NFE	7.1	41.6	81.9	41.74	18
Ash	16.9	6.1	1	32.85	32.64
EAA (g 100g^{-1} DM)					
Arg	5.9	6.94	0.45	5.85	3.09
His	2.5	2.64	0.27	2.8	3.14
Ile	4.7	5.01	0.50	3.47	2.91
Leu	7.7	7.54	0.94	5.21	4.27
Lys	8.0	6.28	0.31	5.62	2.85
Met	2.9	1.38	0.20	4.40	2.83
Phe	4.2	5.03	0.64	4.45	3.72
Thr	4.4	4.92	0.35	3.94	3.69
Tyr	1.2	1.18	0.12	-	-
Val	5.4	4.72	0.53	7.46	4.00

[1] *Data on proximate composition and amino acid contents of fishmeal are from Hertrampf and Pascual* [29]; [2] *Data on proximate composition and amino acid contents of Ulva are from Wassef et al.*[10]; [3] *Data on proximate composition and amino acid contents of Enteromorpha are from Aguilera-Morales et al.*[17].

RESULT

Over the 60 days feeding period, data in *Table 3* show that all fish fed *U. lactuca* and *E. linza* diets resulted in different growth to the control diet There was significantly in growth performance among fish fed the different diets (P<0.05). But, Specific growth rate (SGR) and Relative growth rate (RGR) were similar to *U. lactuca* and *E. linza* diets (P>0.05). Survival rate in fish fed all the experimental diets was high and ranged from 96 to 98%.

The effects of algae as a feed additive on feed utilization are shown in *Table 4*. Total feed intake, daily dry feed intake, protein efficiency rate and apparent net protein retention were markedly higher fish fed the control diet than for fish fed the *U. lactuca* and *E. linza* diets. But there were no significant differences between fish fed feeds the *U. lactuca* and *E. linza* contents, except for daily protein intake feed conversion rate, protein retention.

At the end of the 60 days trial, proximate composition of rainbow trout fed the experimental diets is offered in *Table 5*. Final crude protein content of the fish at the end of all treatments raised significantly (P<0.05) compared to the initial measurements; however, there were no significant differences (P>0.05) in the final crude protein content among the treatments. The lipid content of the fish fed *U. lactuca* and *E. linza* diets was lower significantly (P<0.05) than of control diet. Ash content was similar control and *U. lactuca* diets except for *E. linza*.

Table 3. *Growth performance and survival of rainbow trout fed the experimental diets*

Tablo 3. *Deneme rasyonlarıyla beslenen gökkuşağı alabalıklarında büyüme performansı ve yaşama oranı*

Parameters	Control	*U. lactuca*	*E. linza*
Initial wet weight (g)	32.66±7.53	33.14±6.11	33.08±6.03
Weight gain (g)	62.79±2.45a	46.18±1.55b	41.19±0.58c
Relative growth rate (%) [1]	192.34±10.45a	139.39±6.12b	124.54±1.6b
Specific growth rate (%/day) [2]	1.79±0.06a	1.45±0.04b	1.35±0.01b
Survival (%)	98	97	96

** Values (mean±SD) with different superscripts in the same row are significantly different at the 5% level*
[1] Relative growth rate (%) = (Final wet weight - Initial wet weight / Initial wet weight) x 100
[2] Specific growth rate (%) = [(ln final wet weight - ln Initial wet weight) / days] x 100

Table 4. *Feed intake and feed conversion rate in experimental groups*

Tablo 4. *Deneme gruplarının yem alımı ve yem değerlendirme oranları*

Feed utilization	Control	*U. lactuca*	*E. linza*
Total feed intake (g)	1318.94±51.67a	1101.86±40.17b	1039.92±14.15b
Daily dry feed intake(g/fish)	1.39±0.05a	1.17±0.04b	1.1±0.01b
Daily protein ntake (g/fish)	0.63±0.02a	0.52±0.02b	0.47±0.06c
Feed conversionrate [1]	1.5±0.01a	1.7±0.01b	1.8±0.01c
Protein efficiency rate [2]	1.65±0.01a	1.49±0.06b	1.45±0.01b
Protein retention (g) [3]	10.41±0.43a	7.55±0.43b	6.50±0.23c
ANPR (%) [4]	24.31±0.67a	21.08±0.67b	19.22±0.91b

Values (mean±SD) with different superscripts in the same row are significantly different at the 5% level
[1] Feed conversion rate=Total feed intake (g)/wet weight gain (g)
[2] Protein efficiency rate =Wet weight gain in g / protein intake
[3] Protein retention= Final body protein (g) - initial body protein (g)
[4] Apparent net protein retention (%) = [(Final weight in g x Final body protein in %) - (Initial weight in g x Initial body protein in %) / protein intake in g] x 100

Table 5. *Whole body composition (% fresh weight basis) at beginning and end of experiment*

Tablo 5. *Deneme başı ve deneme sonunda yaş madde cinsinden tüm vücut kompozisyonu*

Groups	Moisture (%)	Crude Protein (%)	Crude lipid (%)	Crude ash (%)
Initial	70.90	13.6	11.1	1.4
Control	69.1a	15.60a	13.30a	1.66a
U. lactuca	70.90b	15.20a	12.50b	1.53ab
E. linza	69.90ab	14.80a	12.10b	1.45b

Values in a column with different superscripts are significantly different at the 5% level

DISCUSSION

This present experiment reports the first use *U. lactuca* and *E. linza* in rainbow trout diets. In this study, growth performance of fish tended to reduce with using *U. lactuca* and *E. linza* meal. The inclusion of vegetable origin protein in diets for fish has been related with decline feed intake and decreased growth performance [11,19,20]. This is in agreement with the results noted by Azaza [13], Güroy et al.[15], Valente et al.[11] and Wassef et al.[10]. They found that the inclusion of different seaweeds (*Cystoseira barbata, Ulva lactuca, Ulva rigida* and *Gracilaria cornea*) at a level of 10% poorer growth and feed utilization compared to fish fed a control diet. A carnivorous fish like trout would rather animal origin ingredients than plant feedstuff. The growth retardation was attributed to the effects of various antinutrients (saponins, tannins, phytic acid) which are published to happen in the numerous plant -derived sources [21]. Moreover, they can reduce the palatability of diets. Azaza et al.[13] reported that 10% *Ulva rigida* meal including of antinutrients to exemplify saponins (1.13%), tannins (0.16%) and phytic acid (0.47%). Saponins could diminish tastiness of a diet by their bitterness and interference with the absorption of dietary lipids, bile salts [22,23]. So, these compounds with antinutritional characteristic might suppress growth performance and feed utilization. In other case, the most plant ingredients contain a certain amount fiber [24,25] likewise the seaweeds, so they may have poor effects on both their nutritional value and palatability. In the present study, *U. lactuca* and *E. linza* diets fed groups were affected depression in growth performance when compared to group fed the non-algal diet. *U. lactuca* and *Enteromorpha* spp. meal contain fiber significantly [12,17], thus might reduce their value in aquafeeds, especially for trout diets.

The trend growth performance was similar to that noticed for feed utilization. Feed conversion rate (FCR) and Protein efficiency rate (PER) remarks of this study demonstrated that the inclusion of 10% of *U. lactuca* and *E. linza* meal recorded poorer nutrient and energy utilization compared to the control diet. Similar results were arrived at different fish species fed algae meal varies (for 10% including *Ulva lactuca, Ulva rigida* and *Gracilaria cornea*) supplemented diets such as European sea bass *(Dicentrarchus labrax)* [11], gilthead sea bream *(Sparus aurata)* [10], Nile tilapias *(Oreochromis niloticus)* [13]. In contrast, Nakagawa et al.[5] revealed that the best FCR's in red sea bream (Pagrus major) was observed with a diets containing different amount of *Ascophyllum* (5-10%). Furthermore, Güroy et al.[15] and Kala et al.[26] indicated that better FCR in Nile tilapia and red sea bream at 5%-%10 of dietary *Cystoseira barbata* and *Porphyna purpurea* supplementation. Davies et al.[19] observed poor feed utilization, when they fed grey mullet with the red seaweed *P. purpurea* at level tested (16% and 33%). Omnivorous fish like the tilapia can utilize effectively algae sources. On the other hand, a carnivorous fish like trout would rather animal origin ingredients than vegetable feedstuff. Feed ingredients of rations may have affected the feed utilization.

After feeding trial, there were little differences in moisture, crude protein and crude ash among the *U. lactuca, E. linza* and control groups, however crude lipid displayed higher value in free-algal group. The final level of crude protein, crude lipid, and crude ash remarkable was obtained superior rates in the body composition all treatment compared to initial. These results were similar to earlier studies of seabream [10], Nile tilapia [13] and red sea bream [26] at inclusion level 10% seaweeds. It might be that the body composition was enhanced by feeding regularly diets in the compared to initial.

The results of the this experiment showed that feeding experimental diets containing 10% level *U. lactuca* and *E. linza* as a feedstuff for rainbow trout resulted in poor growth and feed utilization. But, further investigations are needed to determine the optimum dietary inclusion level of these seaweeds in trout diets.

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