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# Effects of dietary yeast autolysate on performance, some blood parameters and lysozyme activity in rainbow trout (*Oncorhynchus mykiss*)*

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**Summary:** The aim of this study was to determine the effects of dietary yeast autolysate (*Saccharomyces cerevisiae*) supplementation on performance characteristics, some blood parameters and lysozyme activity in rainbow trouts. A total of 180 rainbow trouts (*Oncorhynchus mykiss*) weighing approximately 35 g were used in this experiment. They were divided into one control group and two treatment groups each containing 60 fish. Each group was divided into three subgroups each containing 20 fish. The diets of the first and second treatment groups were supplemented with 0.1% and 0.2% yeast autolysates, respectively. The experimental period lasted for eight weeks. Fish, fed diets with yeast autolysate exhibited higher body weights and specific growth rates than those fed with control diet after eight weeks of experimental period. Feed conversion ratio was improved by dietary yeast autolysate. There were no significant differences among the groups on length, condition factor, serum lysozyme activity, serum total protein and serum cholesterol at the end of the experiment.

Serum triglyceride levels decreased with yeast autolysate. Liver lysozyme level was higher in fish fed with diets including yeast autolysate than that of fed with control diet ($P<0.05$). It was concluded that dietary yeast autolysate supplementation can be effective in rainbow trout feeding due to the improvement in performance, specific growth rate and liver lysozyme level. Yeast autolysate can be used at 0.1% because no differences between the doses of yeast autolysate were seen in the performance and other parameters in the feeding of rainbow trout.

Keywords: Blood parameters, lysozyme, performance, rainbow trout, yeast autolysate.

## Gökkuşağı alabalığı (*Oncorhynchus mykiss*) rasyonlarına maya otolizatı ilavesinin performans, bazı kan parametreleri ve lizozim aktivitesi üzerine etkisi

**Özet:** Bu araştırma, gökkuşağı alabalıkları rasyonlarına farklı düzeylerde maya otolizatı ilavesinin performans, bazı kan parametreleri ve lizozim aktivitesi üzerine etkilerini belirlemek amacıyla yürütüldü. Araştırmada hayvan materyali olarak ortalama ağırlıkları 35 g olan 180 adet gökkuşağı alabalığı (*Oncorhynchus mykiss*) kullanıldı. Deneme, her biri 60 adet hayvandan oluşan bir kontrol ve iki deneme olmak üzere toplam üç grup halinde yürütüldü. Her grup 20 balıktan oluşan üç alt gruba ayrıldı. Birinci ve ikinci deneme grupları rasyonlarına sırasıyla %0.1 ve %0.2 düzeylerinde maya otolizatı (*Saccharomyces cerevisiae*) ilave edildi. Deneme sekiz hafta sürdürüldü. Deneme sonunda yemlerine maya otolizatı ilave edilen balıklarda canlı ağırlık ve spesifik büyüme hızı kontrol grubuna gore daha yüksek bulundu. Rasyonlarda maya otolizatı kullanımı yem dönüşüm oranını olumlu yönde etkiledi. Alabalık rasyonlarına maya otolizatı ilavesi deneme sonunda boy, kondisyon faktörü, serum lizozim aktivitesi, toplam protein ve kolesterol değerleri arasında farklılık yaratmadı, serum trigliserit düzeyi maya otolizatı ilavesi ile azaldı. Rasyonlarına maya otolizatı ilave edilmiş balıklara ait karaciğer lizozim düzeyleri kontrol grubundan yüksek bulundu ($P<0.05$). Sonuç olarak, Gökkuşağı alabalık rasyonlarına maya otolizatı ilavesi performans, spesifik büyüme hızı ve karaciğer lizozim düzeyinde artış sağladığından etkili bir katkı maddesi olabileceği kanısına varıldı. Ayrıca maya otolizatının %0.1 ve %0.2 düzeyinde kullanılması arasında performans ve diğer parametreler bakımından bir farklılık gözlenmediğinden, Gökkuşağı alabalık rasyonlarında maya otolizatı %0.1 düzeyinde kullanılabilir.

Anahtar sözcükler: Gökkuşağı alabalığı, kan parametreleri, lizozim, maya otolizatı, performans.

## Introduction

The culturing of rainbow trout has become more popular in the world because of its fast growth, nutritive quality and easy culturing system. Rainbow trout, *Oncorhynchus mykiss,* is one of the most important fish species for freshwater aquaculture in Turkey and have been cultured in sea cages in the Black Sea since early 1990s (2).

* This study was summarized from PhD thesis of the first author.

Baker's yeast, *Saccharomyces cerevisiae*, is used for the baking industry and it contains various immunostimulating compounds such as β-glucans, nucleic acids as well as mannan oligosaccharides (MOS) and it has the capability to enhance growth and immune responses (1, 11, 12, 16). Yeasts are also the best sources of protein, amino acid and vitamin B. Yeast and yeast products have been used increasingly in animal diets as feed additives, natural growth promoters, after the ban on the use of antibiotic growth promoters in the EU and Turkey. Within yeast products yeast autolysates consist of ruptured or lysed cells and contain both intracellular and cell wall fractions (17). Studies in aquaculture have shown favourable responses to dietary yeast products supplementation. Li and Gatlin (12) concluded that dietary supplementation of 2% GroBiotic-A (partially autolyzed brewers yeast, dairy ingredient components and dried fermentation products) indicated significant protection against mycobacterial infection and dietary supplementation of partially autolyzed brewers yeast may enhance growth performance and chronic infections of mycobacteria. Hisano et al. (10) showed that 0.02% yeast and yeast derivatives improved the growth performance. Dietary usage of *Saccharomyces cerevisiae* has improved the growth performance and feed efficiency in tilapia cultivation (11). Mohammad et al. (13) also observed that supplementation of 0.1% *Saccharomyces cerevisiae* enhanced the growth performance and feed utilization of *Oreochromis niloticus* fingerlings. Barnes et al. (4) reported that rainbow trout weights were significantly heavier in the groups fed yeast culture containing diets than the control group. Barnes et al. (4) also concluded that the inclusion of yeast culture (*Saccharomyces cerevisiae*) provided significant increases in trout survival and growth through the first four weeks of feeding, with continued benefits for the next four weeks. Researches about dietary yeast autolysate in rainbow trout are limited. Therefore in the present study the effects of dietary yeast autolysate (*Saccharomyces cerevisiae*) supplementation on the performance characteristics, some blood parameters and lysozyme activity in rainbow trout (*Oncorhynchus mykiss*) were evaluated.

## Materials and Methods

*Materials:* A total of 180 rainbow trouts (*Oncorhynchus mykiss*) having average weight of 35 g were used in this study. Fish were obtained from two local aquaculture farms in Oltu and Tortum districts in Erzurum. They were randomly allocated into one control group and two treatment groups each containing 60 fish. Each group was divided into three replicates as subgroups, comprising 20 fish each. Fish were put in nine fiberglass cages (1mx1mx1m) having the hole diameter of 4 mm, at the Department of Aquaculture, Faculty of Agriculture, Atatürk University. Water flow rate in the cages was about 1 l/min. Temperature, pH and dissolved oxygen values of the cage water were about 8-10ºC, 7.6 and 7.7 mg/l, respectively. The feeding trial lasted for eight weeks. Feed was provided in pellet form. Basal diets were supplemented with yeast autolysate derived from bakers yeast, *Saccharomyces cerevisiae* (InteWall, NCYC R 625, Integro Food and Feed Manufacturing Company, İstanbul, Turkey) at the level of 0.1 and 0.2% for the diets of the first and second treatment groups, respectively. Yeast autolysate had 93.78% dry matter, 42.90% crude protein, 0.75% ether extract, 0.55% crude fibre and 5.83% crude ash. Basal diet was formulated to contain about 4370 kcal/kg digestible energy and 47% crude protein. The ingredients and composition of basal diet were given in Table 1.

Table 1. Ingredients and nutrient composition of basal diet.
Tablo 1. Bazal rasyonun içeriği ve besin madde bileşimi.

| Ingredients | % | Analyzed composition, | % |
|---|---|---|---|
| Fish meal | 51 | Dry matter | 92.81 |
| Soybean meal | 12 | Crude protein | 47.21 |
| Fullfat soya | 8 | Ether extract | 18.65 |
| Corn gluten meal | 2 | Crude fibre | 1.80 |
| Wheat gluten | 2 | Crude ash | 10.92 |
| Wheat | 8 | | |
| Fish oil | 13 | | |
| Soy lecithine | 2 | | |
| Vitamin and mineral premixes[a] | 2 | | |

[a:] Each 2 kg vitamin and mineral premixes contain 12.000.000 IU vitamin A, 200.000 mg vitamin E, 150.000 mg niacine, 10.000 mg folic acid, 200 mg vitamin B12, 200.000 mg vitamin C, 1.000 mg biotin, 20.000 mg manganese, 30.000 mg zinc, 5.000 mg copper and 2.000 mg cobalt

[a]: Her 2 kg vitamin ve mineral karması 12.000.000 IU vitamin A, 200.000 mg vitamin E, 150.000 mg niasin, 10.000 mg folik asit, 200 mg vitamin B12, 200.000 mg vitamin C, 1.000 mg biotin, 20.000 mg mangan, 30.000 mg çinko, 5.000 mg bakır ve 2.000 mg kobalt içermektedir.

*Traits measured:* Nutrient composition of the diets were determined according to the AOAC (3). Fish were hand-fed daily at 2% of initial body weight in two equal meals a day. Feeding levels were at or above satiation for all of the groups based on observed residual feed. Feed consumption of groups was monitored every meal time in a day. Fish were weighed individually for calculating body weight gains every two weeks of the trial. Feed conversion ratio was calculated as kg feed per kg body weight gain. Fish were observed daily to calculate survival rate.

At the end of the trial body length and body weight of six fish in every group (two from each replicate) were measured. To calculate condition factor, total length and fork length were used with the following formula (18):

Condition factor = (Body weight*100)/(Length)$^3$, where body weight is in g and length is in cm.

Spesific growth rate (SGR) was calculated with the following formula (18):

SGR = (($\log_{10}$W2- $\log_{10}$W1)/D)*100, where W1 is the initial weight (g), W2 is the final weight (g), D is the duration of experiment (day).

At the end of the experiment nine fish from each group (three from each replicate) were weighed and slaughtered. Their heads and livers were separated from body.

Blood samples were taken from Vena caudalis before slaughtering. After 3 hours blood samples were centrifuged at 3500 g for 8 min. Serum was collected and stored at -20ºC for determination of total protein, triglyceride and cholesterol by an autoanalyser (Vitros 350 autoanalyser) using their accompanying commercial kits (Chemistry Products, Ortho-Clinical Diagnostics, Johnson and Johnson Company, New York, NY, USA). Lysozyme activity was determined in the samples of liver and serum with a commercial kit (thebinding side, Lot No: GT073.3) by immune-diffusion technique.

*Statistical analyses:* Statistical analyses were done using SPSS programme (SPSS Inc., Chicago, IL, USA). Data for condition factor, body length, lysozyme activity and blood parameters were analysed as a completely randomized block design, with three dietary treatments and nine samples using One-way ANOVA procedure to examine the differences among groups. The effects of graded levels of dietary yeast autolysate on these variables were analysed using polynomial contrasts. The significance of mean differences between groups were tested by Tukey. Level of significance was taken as $P<0.05$ (5). Due to limited replicates of performance data, statistical analyses for performance were not made.

## Results

The changes in body weight, feed intake and feed conversion ratio by dietary yeast autolysate supplementation were shown in Figure 1. At the end of the 8th week, body weights of the treatment groups fed diets containing 0.1 and 0.2% yeast autolysate were higher than that of control group. Feed conversion was improved with yeast autolysate supplementation as shown in Figure 1c. Dietary yeast autolysate supplementation had no effects on condition factors, total length and fork length (Table 2). Specific growth rate was improved with dietary yeast autolysate as shown in Figure 2. During eight weeks of the experiment, one fish from the first treatment group and one fish from the second treatment group had died.

The effects of yeast autolysate supplementation on lysozyme activity and blood serum parameters are shown in Table 3. Dietary yeast autolysate supplementation increased liver lysozyme activity ($P<0.001$). No differences were observed in serum lysozyme activity, serum protein and serum cholesterol levels among the groups. Serum triglyceride level was decreased ($P<0.05$) in the group fed diet containing 0.2% yeast autolysate.

Table 2. Effects of different levels of dietary yeast autolysate on condition factor and body length of rainbow trout.
Tablo 2. Rasyona farklı düzeylerde maya otolizatı ilavesinin gökkuşağı alabalıklarında kondisyon faktörü ve vücut uzunluğu üzerine etkisi.

| | Treatments | | | | Statistics | |
|---|---|---|---|---|---|---|
| | 0 | 0.1 | 0.2 | Pooled SEM | Linear | Quadratic |
| Condition factor$_{fork}$ | 1.56 | 1.65 | 1.59 | 0.02 | 0.627 | 0.138 |
| Condition factor$_{total}$ | 1.09 | 1.16 | 1.10 | 0.02 | 0.677 | 0.042 |
| Total length, cm | 18.70 | 18.72 | 19.66 | 0.24 | 0.112 | 0.372 |
| Fork length, cm | 16.58 | 16.67 | 17.41 | 0.20 | 0.087 | 0.426 |

n=9

No significant differences among groups.
Gruplar arasında önemli farklılık bulunamamıştır.

Table 3. Effects of different levels of dietary yeast autolysate on lysozyme activity and some blood parameters of rainbow trouts.
Tablo 3. Rasyona farklı düzeylerde maya otolizatı ilavesinin gökkuşağı alabalıklarında lizozim aktivitesi ve bazı kan parametreleri üzerine etkisi.

| | Treatments | | | | Statistics | |
|---|---|---|---|---|---|---|
| | 0 | 0.1 | 0.2 | Pooled SEM | Linear | Quadratic |
| Liver lysozyme (mg/dl) | 24.30$^b$ | 29.90$^a$ | 28.77$^a$ | 0.67 | 0.001 | 0.004 |
| Serum lysozyme (mg/dl) | 1.24 | 1.27 | 1.27 | 0.01 | 0.158 | 0.440 |
| Serum total protein (g/dl) | 4.87 | 4.98 | 4.93 | 0.07 | 0.765 | 0.637 |
| Serum triglyceride (mg/dl) | 489.22$^a$ | 467.39$^{ab}$ | 430,17$^b$ | 8.95 | 0.005 | 0.649 |
| Serum cholesterol (mg/dl) | 363.33 | 363.67 | 366.83 | 5.96 | 0.820 | 0.915 |

n=9

a,b: Means within a row followed by the different superscripts differ significantly ($p<0.05$).
a,b: Aynı sırada farklı harf taşıyan ortalama değerler arasındaki fark istatistiksel bakımdan önemlidir ($P<0.05$).

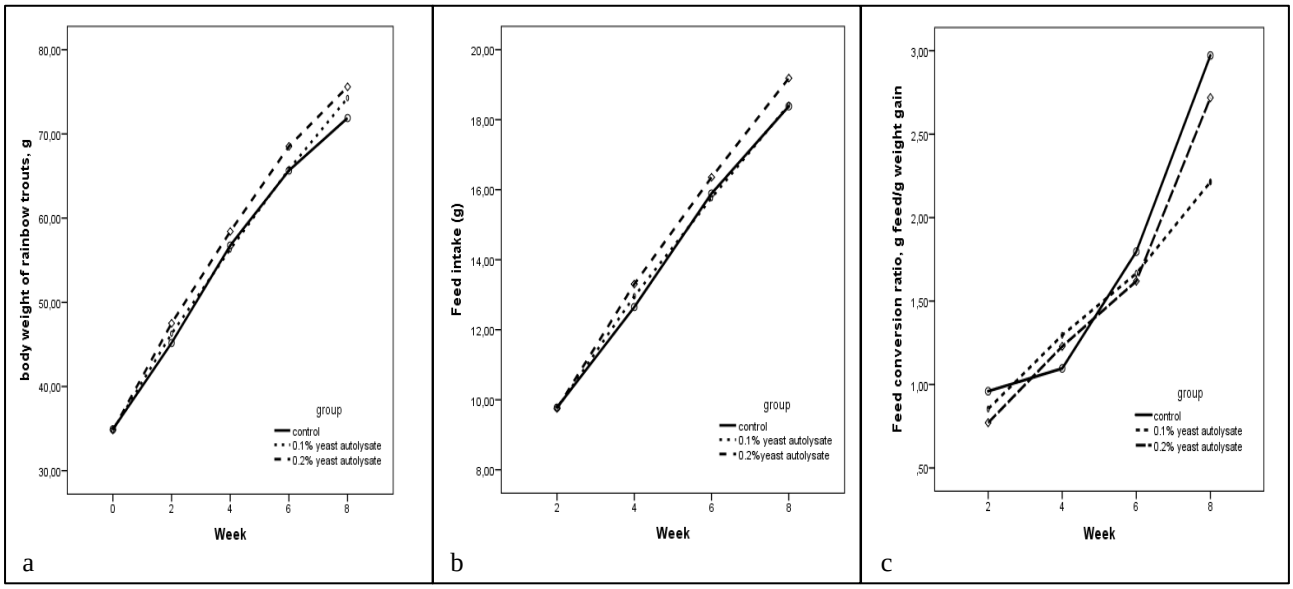


Figure 1. Changes in body weight (a), feed intake (b), and feed conversion ratio (c) of rainbow trouts by dietary yeast autolysate supplementation during experimental period.
Grafik 1. Deneme süresince rasyonlara maya otolizatı ilavesinin gökkuşağı alabalıklarında canlı ağırlık (a), yem tüketimi (b) ve yem dönüşüm oranındaki (c) değişimler.

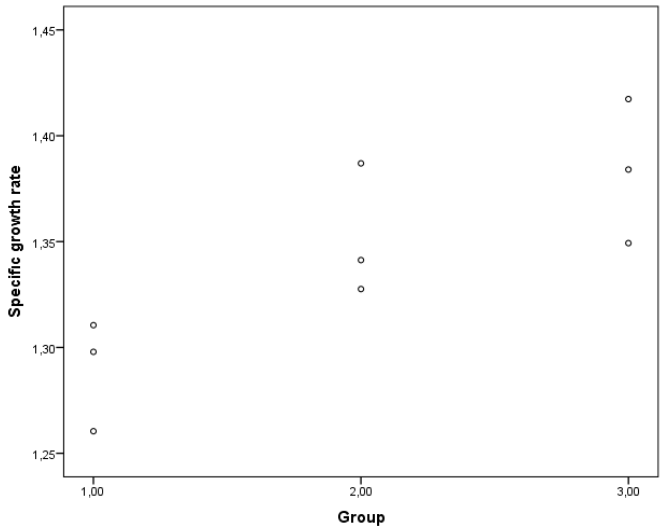


Figure 2. Scatterplot for specific growth rate of rainbow trouts according to the dietary yeast autolysate supplementation.
Grafik 2. Gökkuşağı alabalıklarında rasyona maya otolizatı ilavesinin spesifik büyüme hızı için saçılım grafiği.

## Discussion and Conclusion

Dietary yeast autolysate supplementation during eight weeks of experimental period improved final body weight, feed conversion and specific growth rate as shown in Figure 1a, 1c and Figure 2. Similar results were obtained when yeast and yeast products were added to fish diets for Nile tilapia (1, 11), Israeli carp (15), rainbow trouts (4) and hybrid striped bass (12). Feed intake of the group fed 0.2% yeast autolysate was higher than that of control group as shown in Figure 1b. Improvement in fish growth and feed utilization with yeast and yeast products supplementation may possibly be due to increased nutrient digestibility (1, 11). Eleraky et al. (7) indicated that *Cyprinus carpio* frys fed prebiotics (having MOS and β-glucans) had significantly improved body weight, weight gain, specific growth rate, feed intake and feed conversion. Li and Gatlin (12) reported that dietary supplementation of inactivated or autolyzed brewers yeast could serve as a growth enhancer under certain conditions such as chronic infection. Hisano et al. (9) indicated that 0.2% of MOS supplementation provided better growth responses of juvenile *M. amazonicum*. MOS in yeast cell wall are known to improve digestion and gut health in animals by binding and blocking glycoprotein receptors

on pathogens to make them pass through the gut instead of colonising and invading the host (8, 14). MOS also have a function as a prebiotic, promote the growth of beneficial bacteria and depress the growth of *E.coli* in the gut (19). Similar to the present study, Mohammad et al. (13) indicated that there was a linear relationship between dietary yeast level and growth performance in *Oreochromis niloticus* fingerlings.

During 8 weeks of the study, mortality from the treatment groups was not related to the diet. There were no differences among the groups in survival rate. Similar to the present study Hisano et al. (9) indicated that survival rate was not significantly affected by the supplementation of yeast and yeast derivatives. However, Eleraky et al. (7) reported that *Cyprinus carpio* frys fed prebiotics (having MOS and β-glucans) had significantly improved survival rate. Barnes et al. (4) concluded that yeast culture supplementation may also have improved survival rate by providing nutritional benefits, enhancing fish health by immunomodulation or some combination of these actions in conjunction with palatability.

Dietary yeast autolysate supplementation did not significantly affect the condition factors, total length and fork length of fish. However quadratic increase (P=0.042) was seen in total condition factor. Similar to the present study, Hisano et al. (9) observed that final length was not significantly affected by the supplementation of yeast and yeast derivatives. However Eleraky et al. (7) indicated that *Cyprinus carpio* frys fed 0.25% of prebiotics (having MOS and β-glucans) had significantly increased final body length and condition factor.

Dietary yeast autolysate supplementation increased liver lysozyme activity (P<0.05). Serum trigyceride level was decreased (P<0.05) with 0.2% yeast autolysate supplementation. Linear reduction in serum triglyceride (P=0.005) and linear (P=0.001) and quadratic (P=0.004) increase in liver lysozyme were obtained with increasing the dose of yeast autolysate. No differences were observed in serum lysozyme activity, serum protein and serum cholesterol levels among the groups. However, Eleraky et al. (7) observed that *Cyprinus carpio* frys fed prebiotics (having MOS and β-glucans) had significantly increased serum lysozyme level and this effect as an immunostimulant may be attributed to its glucan content which enhanced phagocytic activity of macrophages (7). In contrast with the present experiment serum protein concentration was also increased with dietary prebiotics supplementation at 0.15% (7). Denji et al. (6) reported that dietary MOS supplementation did not affect serum cholesterol, triglyceride and total protein levels. The contradictory results may be attributed to different fish species, different diets and different properties of the yeast products.

It was concluded that dietary supplementation of yeast autolysate can be an effective feed additive in rainbow trout feeding due to the improvement in performance, specific growth rate and liver lysozyme level.

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