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ARAŞTIRMA MAKALESİ RESEARCH PAPER

The Effect of Whey on the Immune Parameters of Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract: The aim of this study was to investigate the effect of whey on the immune system of cultured rainbow trout (*Oncorhynchus mykiss*). It was targeted to use whey, which is a by-product of the dairy industry, in the aquaculture industry as a promoter of fish immune system and as a source of fish feed protein. Rainbow trout individuals with an average weight of 70.28 ± 1.50 g were placed in 6 tanks with a stock density of 10 kg/m^3. While fish meal (52%) was used as a protein source in the control group feeds, in the experimental group feeds whey powder (48%) was used. The fish were fed these pellet feeds that were isocaloric (21 kj/g) and isonitrogenous (41% protein) for 3 weeks. Blood samples were collected once a week and while hematocrit and hemoglobin values were determined using a fully automated hematology analyzer, standard hemocytometry methods were used in erythrocyte and leukocyte counts. In addition, free radical production and complement 3 concentrations were determined quantitatively. According to the one-way ANOVA analysis, the control group and the experimental group were statistically similar (p> 0,05). However, it was found that C3 complement concentration, which was measured equally at the beginning in the control and experimental groups, showed a statistically significant increase (p <0.05) in the experimental group compared to the control group in the second week.

Keywords: Diet, rainbow trout, whey, immune system, C3 complement.

Peynir Altı Suyunun (PAS) Gökkuşağı Alabalığı (*Oncorhynchus mykiss*) Bağışıklık Parametreleri Üzerine Etkisi

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Öz: Bu çalışmanın amacı kültürü yapılan gökkuşağı alabalıklarının (*Oncorhynchus mykiss*) bağışıklık sistemi üzerine peynir altı suyunun (PAS) etkisinin incelenmesidir. Süt ve süt ürünleri endüstrisi atığı olan PAS'ın akuakültür endüstrisinde balık bağışıklık sistemi destekleyicisi ve balık yemi protein kaynağı olarak kullanılması hedeflenmiştir. Ortalama ağırlığı 70,28 ± 1,50 g olan gökkuşağı alabalığı bireyleri 10 kg/m^3 stok yoğunluğunda olacak şekilde 6 adet tanka yerleştirilmiştir. Kontrol grubu yemlerinde protein kaynağı olarak balık unu kullanılırken deney grubu yemlerinde ise balık unu yerine PAS kullanılmıştır. Balıklar izokalorik (21 kj/g) ve izonitrojenik (%41 protein) olarak hazırlanan bu pelet yemlerle 3 hafta boyunca beslenmiştir. Haftada birer kez olmak üzere balıklardan kan örnekleri alınmış; hematokrit ve hemoglobin değerleri tam otomatik hematoloji analizatörü ile belirlenmiş, eritrosit ve lökosit sayıları ise standart hemositometri yöntemleri kullanılarak yapılmıştır. Bunlara ek olarak oksidatif radikal üretimi ve kompleman 3 konsantrasyonu da kantitatif olarak belirlenmiştir. Sonuçlar tek yönlü varyans analizi (one-way ANOVA) ile değerlendirilmiştir. Sonuç olarak kontrol grubu ile deney grubu arasında istatistiki açıdan herhangi bir farklılık olmadığı görülmüştür (p>0,05). Bununla birlikte, kontrol ve deney grubunda başlangıçta eşit olarak ölçülen C3 kompleman konsantrasyonunun, deney grubunda ikinci haftada kontrol grubuna göre istatistiksel açıdan önemli (p<0,05) bir artış gösterdiği tespit edilmiştir.

Anahtar kelimeler: Diyet, gökkuşağı alabalığı, peynir altı suyu (PAS), bağışıklık sistemi, C3 kompleman.

INTRODUCTION

The rapid growth of aquaculture in recent years has been accompanied by increased demand for aquaculture feeds (Gatlin et al., 2007). Approximately 50-60% of the operating cost is feed expenses and the price of the fish meal used as the main protein source in fish feeds increases in proportion to the increasing aquaculture production (Rana et al., 2009). It is predicted that the current state of fish meal production will not meet the future needs of the developing aquaculture sector. For this reason, aquaculture feed industry has tried to replace fish meal and fish oil in feeds with sustainably produced vegetable raw materials, however, such protein sources may lack some indispensable amino acids such as methionine or lack components such as taurine, hydroxyproline and nucleotides (Bañuelos-Vargas et al., 2014).

To date, many studies have been conducted to replace fish meal such as soybean flour, corn gluten, wheat gluten and canola flour as vegetable protein, and it has been reported that the alternative vegetable protein sources adequately meet the amount of essential amino acids needed by rainbow trout (Bell and et al., 2001; Rosenlund et al., 2001; Caballero et al., 2002; Yang et al., 2011; Kriton et al., 2018; Yıldız et al., 2018). Diseases that lead to economic losses, the decreased number of effective antibiotics that can be used, and failures in treatment have directed the aquaculture feed industry to using food additives that can activate the fish's immune system (WHO, 2006).

Whey is a natural ingredient derived from fresh milk containing high quality proteins, lactose, bioactive ingredients, minerals and vitamins and is used in human and animal nutrition as a food additive that can activate the immune system (EWPA, 2020). Whey does not contain anti-nutritional factors, it can be easily given with other foods and it is easy to digest. Whey has been reported to have a probiotic effect associated with bioactive functions such as supporting tissue repair, destroying pathogens, and eliminating toxins (Clare et al., 2003). It has been observed that humoral immune responses and neutrophil levels have been increased in animals fed diets supplemented with whey. It has also been reported that whey proteins modulate immune functions and protect glutamine stores in the muscle by increasing the production of glutathione (GSH) in various tissues, an important part of the body's antioxidant protection system, which regulates immune functions. Therefore, combining whey proteins with diet is important because of providing strong immunity and health-protecting effect (Wong et al., 1997; Wong et al., 1998; Amer et al., 2019). While the mice fed with whey-added feeds did not change the cellular immune response functions, there was a significant increase in natural leukocyte cellular response and lymphoid cellular response compared to the control group (Rutherfurd-Markwicket et al., 2005). Wong and Watson (1995) reported that the effect of whey on the immune system of mice increased cellular immune response in the 5th-8th week of feeding in their study.

The effect of whey on some blood parameters of male athletes was investigated by Eslami et al. (2010) and there was no difference in the blood parameters (such as WBC, RBC, HGB, HCT) of the athletes fed with 6.6 g whey every day for a month compared to the control group. Rusu et al. (2009) showed that different concentrations of whey on human neutrophils have no direct effect on chemotaxis, phagocytosis, oxidative metabolism and degranulation. Szczurek et al. (2013) investigated the effect of whey added to the breeding chicken feed on some blood parameters, it was reported that whey had no effect on blood leukocytes, hematocrit and hemoglobin, while increasing the number of erythrocytes. Abou-El-Atta et al. (2019), fed Nile tilapias (*Oreochromis niloticus*) with probiotic *Lactobacillus plantarum* and feeds with different ratios of whey added (1, 2 and 3 g/kg feed) and after 60 days, they examined the effects of fish on growth, antioxidant activity, lysozyme activity, NBT and some blood parameters. As a result of the study, it was found that feeding with high whey doses (2 and 3 g/kg feed) significantly increased the antioxidant capacity of fish, NBT, lysozyme and red blood cell count. In addition, the addition of 2 and 3 g/kg whey doses with *L. plantarum* has been reported to show synergistic effects and increase their resistance to *Aeromonas sobria*. Similarly, another study with Nile tilapias (*O. niloticus*) was investigated by adding whey in various ratios to fish feed and its effects on growth and some immune parameters (Amer et al., 2019). It has been reported that adding 27.7% PAS to feed instead of fish meal increases C3 complement, lysozyme and phagocytic activity without any pathological disorder in fish tissues.

In this study, some immune parameters of rainbow trout fed with pellet feeds prepared with whey, which is a valuable by-product due to its nutraceutical and pharmaceutical properties, were determined and the effects of whey on the immunity were investigated.

MATERIAL AND METHODS

This study was carried out in Çanakkale Onsekiz Mart University, Faculty of Marine Sciences and Technology. The rainbow trout with an average weight of 70.28 ± 1.50 g were stocked in 6 tanks (3 control, 3 experimental groups) each with 140 liters, with a stock density of 10 kg/m^3. During the adaptation and the experiment, the water temperature was recorded as 15.2 ±

0.3°C, pH 7.85 ± 0.08, and oxygen as 7.56 ± 0.16 mg/L. During the adaptation, there were no signs of disease in the fish and before the experiment, the fish were examined for bacteria and parasites.

The experimental and control groups were carried out triplicates and the experiment was carried out by providing 15% water change daily in a recirculated system with heating and cooling system. In the control group, anchovy fish meal (72% protein / 8% fat) at the rate of 52% was used as a source of protein in feeds. In the experimental group, Hardline Hipro Iso Whey concentrate (96% protein / 0.5% fat), a commercial product, was added to the feeds at the rate of 38%. The ration of the feed used in this study is given in Table 1. Pellet feeds were prepared in La Monferrina - P3 brand feed machine at Çanakkale Onsekiz Mart University, Faculty of Marine Sciences and Technology and feed analyzes were done according to AOAC (1998).

Table 1. Feed ration used in the experiment (% dry matter).

	Control Diet (%)	Experimental Diet (%)
Fish meal	52	0
WHEY	0	38
Soybean meal	0	0
Wheat flour	29	39
Wheat gluten	0	0
Tryptophan	0	0,2
Arginine	0	0,2
Mineral premix[a]	2	2
Protector	0.001	0.001
Vitamin premix[a]	2	2
Starch	3.499	4.599
Fish oil	11.500	14
Total	100	100
Analyzed proximate diet composition (% DM)		
Crude protein (%)	40.67	41.22
Lipid (%)	16.21	14.93
Ash (%)	6.34	0.52
NFE[b] (%)	27.32	35.60
Gross energy (kjg $^{-1}$)	20.65	21.68

[a]Premix of vitamins and minerals according to NRC (1993) recommendations for fish.
[b]NFE: nitrogen-free extract calculated by difference

Blood samples from the fish in the experimental groups were collected on the 7th, 14th and 21st days. Five fish from each tank (15 fish per group) were used for the sampling. To do this, after the fish were caught randomly from the test tanks, they were treated with 2-Phenoxyethanol at 0.2 ml concentration (Summerfelt and Smith, 1990; Çetinkaya and Şahin, 2005). Blood samples were taken into K_3EDTA and gel serum tubes. From hematological analyzes, hematocrit and hemoglobin values were determined using Mindray / BC 3000 Plus fully automated blood count device, which was calibrated for fish (Yılmaz and Ergün, 2018; Yılmaz, 2018). In addition, erythrocyte and leukocyte counts were made from the blood samples by hemocytometry (Buckley et al., 1976; Hofmann and Lomel, 1984). For serum analysis, the blood taken into gel tubes was centrifuged at 5000 g for 10 minutes and the obtained serum samples were stored at -80°C until the analysis.

Oxidative radical production, nitro blue tetrazolium (NBT) activity was quantitatively determined according to the method reported by Siwicki et al (1996). In the quantitative determination of complement 3 concentration in fish serum, CUSABIO - Fish complement 3 (C3) ELISA Kit (Cat. No: CSB-E09727s) was used according to the manufacturer's instructions. To do this, briefly: 50 µl Standard solutions (in duplicate) and serum samples supplied with the kit were added to each well, then 50 µl HRP-conjugate and then 50 µl antibody were added. After washing and adding Substrate (A and B), the optical densities of the wells treated with stop solution after incubation at 37°C for 15 minutes in a dark environment, were measured at 450nm wavelength with a microplate reader.

One-way analysis of variance (one-way ANOVA) was applied using the SPSS 21 software (IBM) and evaluated at a statistically significant level.

RESULTS AND DISCUSSION

Whey, which contains high quality proteins, bioactive components, minerals, as well as amino acids and vitamins that play a role in carbohydrate metabolism, constitutes 70-90% of the total milk volume. In cases where whey cannot be utilized, it must be removed from the environment by treatment. Because its high organic load, direct discharging to the nature leads to environmental pollution and the removal from the production facility is very expensive. This reason has led researchers to focus on the utilization of this waste product. The use of this product in human and animal health is rapidly increasing in both European Union and other countries (González-Siso, 1996; De Wit, 1998; Macwan et al., 2016). Although fish meal constitutes the main animal protein of fish food, because of low fish stocks and high cost of fish meal, novel protein sources are needed. In Turkey, there is a study conducted to investigate the effect of whey protein on various biochemical parameters in the tissues of rainbow trout by Özcan et al. (2021). They feed rainbow trout with fed diets containing four doses of whey protein for 42 days and found that the lipid levels of fish fed diets with 1.5% whey protein was essentially higher than that of fish fed the control diet. They suggested that whey could be an alternative protein sources.

In this study, blood and immune parameters between the control group and the experimental group were analyzed statistically with samples taken over a 3-week period. The red blood cell count, leukocyte cell count, NBT activity, hemoglobin value and hematocrit value of the control and experimental groups were statistically similar ($p>0.05$). The mean values/counts of erythrocyte, leukocyte, NBT activity, hemoglobin, hematocrit, C3 complement and weekly standard deviations are shown in Figure 1.

A statistically significant ($p<0.05$) increase was observed in the C3 complement concentration, which was measured equally in the control and experimental group at the beginning, compared to the control group in the second week (Figure 2). When the findings obtained in this study evaluated, it was determined that whey had no effect on blood parameters. This result is opposed to the results of Amer et al. (2019) and Abou-El-Atta et al. (2019), but similar to Rusu et al. (2009) and Wong and Watson (1995).

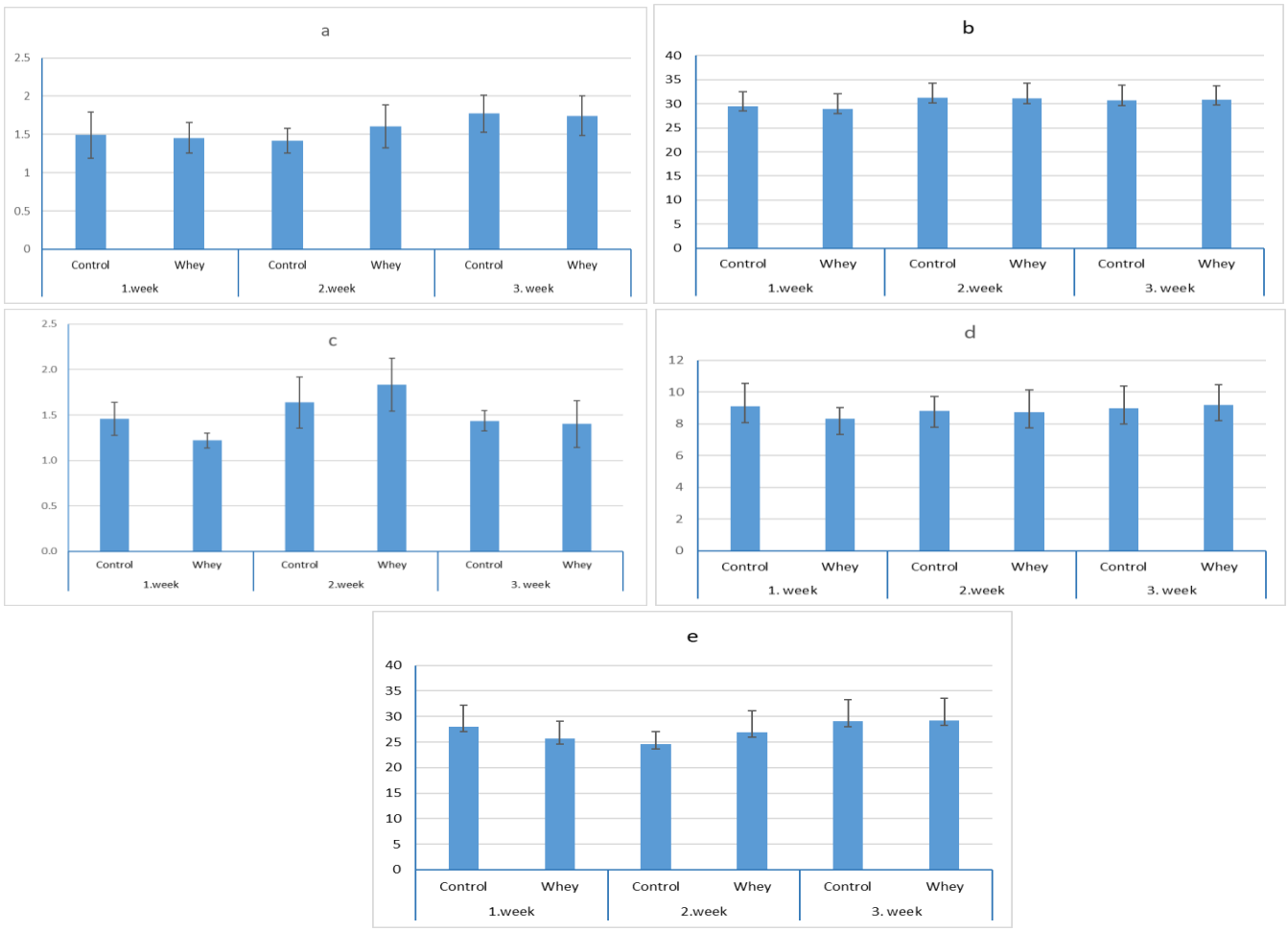

Figure 1. The mean values of (a) erythrocyte count (10^6/mm^3), (b) leukocyte count (10^3/mm^3), (c) NBT activity (mg/ml), (d) hemoglobin (g/100 ml), (e) hematocrit (%) (N = 15).

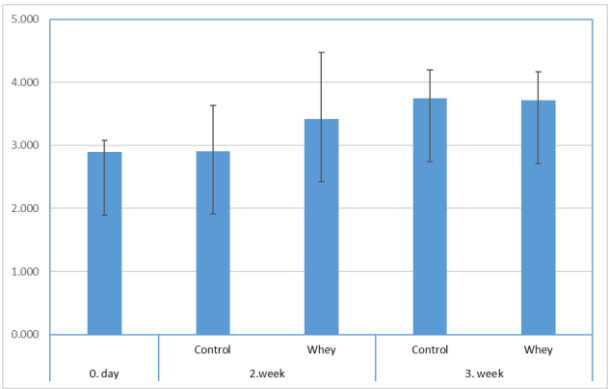

Figure 2. The mean values of C3 complement concentrations (µg/ml).

The use of whey which can help reduce the use of feed additives imported from abroad and used extensively in aquaculture, was investigated in this study. As a result, the use of whey in fish did not have any negative effects on the fish during the research and in terms of the parameters examined. In the future studies, investigating the effects of whey on the immune system by feeding with different ratios and for longer periods of time will provide more accurate results regarding this protein source.

ETHICAL APPROVAL

This study was carried out the approval of Istanbul University Animal Experiments Local Ethics Committee (Decision no: 2015/21).

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