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Menba Kastamonu Üniversitesi Su Ürünleri Fakültesi Dergisi 2024; 10 (3): 119-128 Araştırma Makalesi/Research Article

Effect of Spirulina Suplementation to Canola Meal-Containing Goldfish (*Carassius auratus*) Diets on Growth Performance, Nutrient Digestibility and Digestive Enzyme Activity

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Abstract

The aim of this study was to determine the effects of spirulina added to diets containing canola meal in juvenile goldfish (*Carrasius auratus*) on growth, feed utilization, nutrient digestibility and intestinal digestive enzyme activity. In the experiment planned as 4 groups and 3 replications, fish with an average weight of 2.3 ± 0.9 g were placed in 12 glass aquaria with a capacity of 60 liters (60x30x35) with 20 fish per aquarium. Diets were prepared to contain 30% canola meal. 10%, 20% and 30% spirulina was added to the diets except the control diet, respectively. Fish were fed twice a day until satiated for 7 weeks. At the end of the experiment, it was determined that diets containing spirulina significantly ($p<0.05$) increased the growth of fish. The best FCR value was obtained from the group containing 30% spirulina. It was determined that nutrient digestibility (dry matter, protein, lipid) increased significantly ($p<0.05$) in groups fed diets containing spirulina compared to the control group and similarly, the activity of the digestive enzyme lipase enzyme in the intestines increased ($p<0.05$). While an increase in protease enzyme activity was detected, it was determined that there was no significant difference between the groups ($p>0.05$). The results showed that adding spirulina to diets containing 30% canola meal increased the nutrient digestibility that decreased with the presence of canola meal and the digestive enzyme activity, thus increasing fish growth. When the groups added spirulina together with canola meal were compared among themselves, it was shown that adding 30% spirulina was more effective on these parameters.

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INTRODUCTION

Ornamental fish farming is a sector that supports the development of the aquaculture sector, provides billions of dollars of employment opportunities to developing countries and constitutes the livelihood of many people (Wei and Wee, 2014; Thomas et al., 2023). Among ornamental fish, goldfish (*Carassius auratus*) is preferred for hobby purposes and used as a model species in scientific studies (Blanco and Unniappan, 2022). Goldfish, which eat various plant and insect species, are omnivorous fish and balanced diets can be created by adding other raw materials to flake and pellet feeds to be used in aquaculture (Brown et al., 2019).

Determining the utilization rates of vegetable protein sources in fish feeds to reduce feed cost has been the focus of researchers for many years. Canola meal, which is a by-product of the oil industry, is one of the alternative vegetable protein sources. However, canola meal contains antinutritional factors (ANF) such as glucosinolate, phenolic compounds, tannin, phytic acid (Lim et al., 2008). When ANFs in plant sources are taken into the fish body with diets, they limit the utilization of nutrients in the feed by the fish and thus negatively affect the growth and development of the fish (Francis et al., 2001; Kokou and Fountoulaki, 2018). Although these compounds have a negative effect on growth and development, they are also known to have strong antioxidant properties (Wang et al., 2018; Dossou et al., 2018a). Studies have investigated the effects of canola on growth and feed evaluation parameters, immune system, antioxidant defense system, digestion rate, digestive enzymes, liver and intestinal histopathology of fish (Erdogan and Ölmez, 2009; Kurtoglu et al., 2019; Hernandez et al., 2020). Although it varies from species to species, it has been determined that it can be used in various ratios instead of fish meal (Erdogan and Ölmez, 2009; Cheng et al., 2010; Yigit et al., 2012; Yigit et al., 2013; Yigit, 2018; Kurtoglu et al., 2019; Hernandez et al., 2020; Mohammadia et al., 2020). The increase in the amount of canola meal in diets causes difficult digestion by fish. The difficulty in digestion is associated with its high content of cellulose (Yigit, 2018). Although decreases in digestion rate are more pronounced in carnivorous species, they have been detected in almost all fish species (Cheng et al., 2010; Yigit et al., 2012; Kurtoglu et al., 2019; Hernandez et al., 2020). In addition, tannins, which are phenolic compounds contained in canola (Cheng et al., 2010; Mohammadia et al., 2020), inhibit important digestive enzymes such as amylase and trypsin (Cheng et al., 2010; Dossou et al., 2018b).

Spirulina is one of the plant protein sources added to fish feeds. Spirulina is an extremely valuable source due to its amino acid and fatty acid composition as well as the minerals, vitamins and carotenoids it contains (Grosshagauer et al., 2020; Han et al., 2021). Studies with various fish species have reported that spirulina is a suitable vegetable protein source for fish (Alagawany et al., 2021). Spirulina addition to fish diets increases feed intake (Abdurrahman, 2014) and growth (El-Sheekh et al., 2014; Velasques et al., 2016; Erdogan, 2019; Liu et al., 2019; Mohammadiazarm et al., 2021; AlMulhim et al., 2023), blood profile, pigmentation (Sun et al., 2012; Teimouri et al., 2013; Ansarifard et al., 2018; Erdogan, 2019; Kargın and Dikbas, 2020; Sehgal et al., 2022; Güroy et al., 2022), immune system (Abdel Tawwab and Ahmed, 2009; Yeganeh et al., 2015; Adel et al., 2016; Güroy et al., 2022) and improves reproductive performance (Güroy et al., 2012; Khanzadeh et al., 2016). The positive effect of spirulina, especially on growth and feed utilization, has been explained by its good digestibility by fish (Abdel Tawwab and Ahmed, 2009; El-Sheekh et al., 2014; Cao et al., 2018). Based on this point, spirulina was added to *Carasius auratus* diets containing canola meal in this study. The effects of the combination of canola meal and spirulina in the diet on growth, feed utilization, apparent digestibility and digestive enzymes were investigated.

MATERIAL and METHOD

Test Diets

Formulation and nutrient composition of 4 diets were formulated as given in Table 1. All diets were prepared to contain 30% Canola meal. Spirulina was added to the diets except the control diet at 10%, 20% and 30%, respectively. While preparing the diets, dry ingredients were mixed first. Then Chromium oxide was added and mixing continued. Oil and then distilled water were added to the mixture to obtain a homogeneous dough. The dough was passed through a mincing machine and given the form of pellets. The pellets were dried at 60 °C and then cooled. The cooled pellets were stored at -20 °C until used in the experiment.

Table 1. Diet ingredients and proximate composition

Ingredients	Control	CM+SP10	CM+SP20	CM+SP30
Fish meal[1]	35	25	17	8
Canola meal[2]	30	30	30	30
Spirulina[3]	0	10	20	30
Wheat starch[4]	25.5	24.5	21.5	19.5
Fish oil[5]	5	6	7	8
Vitamin premix[6]	2	2	2	2
Mineral premix[6]	2	2	2	2
Chromium oxide[7]	0.5	0.5	0.5	0.5
Proximate composition (%)				
Dry matter	93.9	94.2	94.5	93.9

Crude protein	35.6	35.4	36.2	35.9
Crude lipid	10.5	10.2	10.5	10.4
Ash	1.3	1.3	1.2	1.3
Nitrogen-free extract[8]	46.5	47.3	46.6	46.30
Gross energy[9] (kj g^{-1})	20.45	20.42	20.61	20.45

[1]Sürsan, Türkiye; crude protein, 72.3%; crude lipid, 10.2%.
[2] Vamos Tarım, Türkiye; crude protein, 38.3%; crude lipid, 4.5%.
[3] Naturiga, Türkiye; crude protein, 65.2%; crude lipid, 1.3%
[4]İnci, Türkiye
[5]Sürsan, Türkiye
[6] Sürsan, Türkiye
[7] Sigma-Aldrich, USA
[8] Nitrogen-free extract = Dry matter − (protein + lipid + ash).
[9] Gross energy is calculated according to 23.6 kj g^{-1} protein, 39.5 kj g^{-1} lipid and 17 kj g^{-1} NFE.

Experimental design

Approval was obtained from Ordu University Animal Experiments Local Ethics Committee (date-decision no: 06/11/2023-05/23) for the study. The experiment was conducted in Fatsa Marine Sciences Faculty Aquarium Fish Laboratory. Juvenil goldfish (*Carrasius auratus*) were obtained from a commercial enterprise. The fish were adapted to laboratory conditions for 2 weeks. The fish were fed with the Control diet during the adaptation period. 240 goldfish with an average weight of 2.3 ±0.9 g were used in the experiment. In the experiment planned as 4 groups and 3 replications, the fish were placed in 12 glass aquariums with a capacity of 60 liters (60x30x35) with 20 fish in each aquarium. The aquariums were continuously aerated with an air motor. 30% of the aquarium water was changed with chlorine-free and aerated water every day. The fish were fed twice a day (9:00-16:00) for 7 weeks until they were full. During the trial, water temperature was determined as 24.3±1.5 °C, pH 7.82±0.35 and oxygen 6.98±1.6 mg L-1. A 12-hour light: 12-hour dark photoperiod was applied in the aquariums.

Sampling

Before the sampling at the beginning and end of the trial, the fish were fasted for 24 hours. At the end of 24 hours, the fish were sampled after applying an overdose of anesthetic (Clove oil, 100 mg/L). A total of 20 fish were taken at the beginning of the trial and 8 fish from each tank at the end of the trial to determine proximate analysis, hepatosomatic index, viscerosomatic index, conditional factor and digestive enzyme activity. The samples were frozen at -20 °C. Intestinal samples to be used in the measurement of digestive enzyme activity were sent to tissue homogenization on ice.

Feces were collected from the 3rd week of the trial to determine the digestibility of the feeds. Until the end of the experiment, feces were collected regularly every day by siphoning. Immediately after feeding, the aquariums were cleaned and feces were collected 30 minutes later. The collected feces were placed in sampling containers and stored at -20 °C until the time of analysis.

Analysis

Diets, feces samples and dry matter, crude protein, ash content in fish meat were determined according to AOAC (1995) and lipid content was determined according to the Bligh and Dyer (1959) method. The contents of chromic oxide in diets and feces were determined as described by Furukawa & Tsukahara (1966). Intestines were homogenized on ice with buffered saline solution and centrifuged at 10,000 g for 15 minutes at 4 °C. The protease activities were measured with the method described by Sotoudeh and Esmaeili, (2022), Amylase (AMS) activity was assayed by the method introduced in Yılmaz et al., (2018), Lipase activity was performed as described in Ravardshiri et al., (2021). The protein content of the intestine supernatants was evaluated following the method of Bradford (1976).

Growth Performance

Growth, feed evaluation and digestibility coefficient were calculated using the following formulas.

Specific growth rate (SGR, %) = (Ln (Final weight) − Ln (Initial weight)/days) × 100

Feed conversion ratio (FCR) = total feed intake (g)/weight gain (g)

Protein efficiency ratio (PER) = (final body weight (g) - initial body weight (g))/Protein intake

Hepatosomatik index (HSI, %) = (liver weight (g)/body weight (g)) x 100

Survival rate (SR, %) = (Final amount of fish/initial number of fish) x 100

Condition factor (CF) = (g/cm^3) = 100 x (body weight)/(body length)^3

Apparent digestibility coefficients (ADC, %) = 100-{100 x (% Cr_2O_3 in diet/% Cr_2O_3 in faeces) x (% nutrient in faeces/% nutrient in diet)}

Statistical analysis

Differences in mean values between groups were analyzed using SPSS 11.5 (SPSS, Chicago, IL, USA) software based on one-way ANOVA and Tukey test. Significant differences were determined at a level of $p < 0.05$.

RESULTS

Growth, feed and protein utilization, CF and HSI values are given in Table 2. Final weight and SGR values increased significantly ($p<0.05$) in the groups containing spirulina with canola meal compared to the Control group. The highest FI value was determined in the CM+SP20 and CM+SP30 groups. The best FCR value was determined in the CM+SP30 group. 10

% and 20% spirulina addition did not affect FCR. No significant effect of spirulina added to the diets on PER, CF and HSI was determined ($p>0.05$).

Table 2. Growth and feed utilization of fish fed with experimental diets

	Experimental Diets				
	Control	**CM+SP10**	**CM+SP20**	**CM+SP30**	***P value***
Initial weight	2.33±0.05	2.34±0.12	2.32±0.14	2.33±0.10	-
Final weight	5.24±0.34^{c}	5.89±0.17^{b}	6.14±0.15^{ab}	6.50±0.22^{a}	0.000
SGR	1.65±0.08^{c}	1.88±0.07^{bc}	1.99±0.09^{ab}	2.10±0.16^{a}	0.004
FI	5.36±0.62^{a}	6.40±0.32^{ab}	6.79±0.21^{b}	6.75±0.54^{b}	0.007
FCR	1.84±0.04^{a}	1.80±0.04^{a}	1.78±0.02^{a}	1.61±0.00^{b}	0.000
PER	1.53±0.03^{a}	1.57±0.03^{a}	1.56±0.02^{a}	1.73±0.01^{a}	0.000
Survival	100	100	100	100	-
CF	2.43±0.12^{a}	2.38±0.23^{a}	2.41±0.17^{a}	2.42±0.08^{a}	0.062
HSI	2.84±0.31^{a}	2.95±0.32^{a}	2.91±0.23^{a}	3.01±0.43^{a}	0.076

Values are mean ± SD (n=3). Values having different superscripts in the same line are significant different ($p<0.05$).

Table 3 shows the proximate composition of the muscle tissue of *Carasius auratus*. No significant effect of spirulina addition to the diets on the dry matter, crude protein and ash values in the muscle tissue was detected ($p>0.05$). Crude lipid content increased with spirulina addition to the diets, but a significant ($p<0.05$) difference was found between the Control group and the CM+SP20 and CM+SP30 groups.

Table 3. Proximate composition of fish muscle

		Experimental Diets				
	Initial	**Control**	**CM+SP10**	**CM+SP20**	**CM+SP30**	***P value***
Dry Matter	25.23	24.92±0.35^{a}	25.40±0.31^{a}	25.23±0.24^{a}	25.42±0.40^{a}	0.125
Crude Protein	16.34	16.19±0.31^{a}	15.79±0.29^{a}	15.83±0.19^{a}	16.03±0.27^{a}	0.094
Crude Lipid	4.97	5.54±0.41^{b}	6.02±0.26^{ab}	6.15±0.20^{a}	6.12±0.29^{a}	0.027
Ash	3.99	3.18±0.08^{a}	3.59±0.05^{a}	3.21±0.10^{a}	3.12±0.09^{a}	0.132

Values are mean ± SD (n=3). Values having different superscripts in the same line are significant different ($p<0.05$).

Dry Matter, Protein and Lipid ADC are given in Table 4. While the lowest Dry Matter ADC was detected in the Control group, Dry Matter ADC increased in parallel with the increase in the amount of spirulina in the diet ($p<0.05$). Protein digestibility also showed a similar trend to dry matter digestibility. The highest protein ADC was detected in the CM+SP20 and CM+SP30 groups. Lipid ADC value also showed a significant increase ($p<0.05$) with the increase in the amount of spirulina in the diet to 20% and 30%, while the difference between the Control group and the CM+SP10 group was found to be insignificant ($p>0.05$).

Table 4. Apparent digestibility coefficients (ADC) of dry matter, protein and lipid of *Carassius auratus* fed experimental diets.

ADC (%)	Experimental Diets				
	Control	**CM+SP10**	**CM+SP20**	**CM+SP30**	***P value***
Dry matter	67.34±0.50^{d}	68.62±0.35^{c}	70.43±0.31^{b}	73.45±0.20^{a}	0.000
Protein	85.98±0.12^{c}	87.87±0.26^{b}	90.27±0.12^{a}	90.83±0.47^{a}	0.000
Lipid	83.33±0.29^{c}	83.45±0.64^{bc}	85.26±0.66^{ab}	86.65±1.26^{a}	0.002

Values are mean ± SD (n=3). Values having different superscripts in the same line are significant different ($p<0.05$).

The results obtained regarding digestive enzyme activities are given in Table 5. No significant difference ($p>0.05$) was detected between the groups in terms of protease and amylase activity. Although lipase activity was statistically similar ($p>0.05$) in the groups containing spirulina, the lipase activity detected in the Control group was found to be significantly ($p<0.05$) lower than that detected in the CM+SP20 and CM+SP30 groups.

Table 5. Protease, lipase and amylase activities (u/mg protein) in *Carasius auratus* fed experimental diets.

	Experimental Diets				
	Control	**CM+SP10**	**CM+SP20**	**CM+SP30**	***P value***
Proteaz	4.64±0.25^{a}	4.71±0.26^{a}	5.27±0.32^{a}	5.42±0.70^{a}	0.222
Lipaz	1.13±0.04^{b}	1.17±0.03^{ab}	1.19±0.01^{a}	1.19±0.02^{a}	0.017
Amilaz	11.44±0.64^{a}	10.77±1.58^{a}	11.69±1.07^{a}	11.34±0.59^{a}	0.737

Values are mean ± SD (n=3). Values having different superscripts in the same line are significant different ($p<0.05$).

DISCUSSION

In this experiment to determine the effect of rapeseed and spirulina added to *Carassius auratus* diets, the lowest growth rate was obtained in the control group without spirulina and it was found that the growth rate increased as the amount of spirulina added to the rapeseed meal diets increased. In previous studies, it was determined that canola meal added to the diets suppressed growth, but it can be added to the diets in certain amounts, although it varies from species to species. For example, Mohammadia et al., (2020) reported that up to 25% can be added to the diets of young Nile tilapia (*Oreochromis niloticus*), an omnivorous species. In studies conducted with carnivorous fish species, Shafaeipour et al., (2008) added up to 30% in *Onchorhynchus mykiss* diets, Kurtoglu et al., (2019) added 30% of fish meal in Gilthead Seabream (*Sparus aurata*) feeds, Yigit et al., (2012) reported that 8% canola meal can be added to juvenile rainbow trout diets. Studies have found that growth is suppressed as the amount of canola meal in the diets increases. Growth suppression has been associated with the presence of antinutritional factors (fiber, glucosinolates, phytic acid, etc.) in canola meal. It has been reported that glucosinolates, which are among these antinutritional factors, suppress thyroid hormones that affect growth (Burel et al., 2000) and cellulose, tannin and phytic acid inhibit nutrient digestibility (Cheng et al., 2010; Yigit et al., 2012; Dossou et al., 2018b; Hernandez et al., 2020; Mohammadia et al., 2020). Also, Nagel et al., (2012) reported that as the amount of plant protein added to diets increases, the palatability of the feed deteriorates and feed consumption decreases accordingly, and the decreased feed consumption suppresses growth. In this study, it was determined that growth was increased by adding spirulina to diets containing canola meal. Similarly, in studies conducted on fish species with different feeding habits, spirulina supplementation was found to improve growth and feed utilisation (Dernekbasi et al., 2010; Teimouri et al., 2013; Adel et al., 2016).

In this study, it was determined that the amount of feed consumed and Dry Matter, Protein and Lipid ADC increased with the addition of spirulina to the diet. Similar results were obtained in studies with omnivorous fish species such as Nile tilapia

(*Oreochromis niloticus*) and *Oreochromis niloticus* x *Oreochromis mossambicus* (Abdel Tawwab and Ahmed, 2009; El-Sheekh et al., 2014). Roohani et al., (2019) Caspian brown trout (*Salmo trutta caspius*) juveniles had feed intake (appetite enhancer) and nutrient digestibility enhancer properties, and this (increase in feed intake and digestibility) led to an increase in growth and feed utilization.

Spirulina contains essential amino acids, vitamins, minerals, phenols, polyphenols and carotenoids, which stimulate the growth of fish, have an immunostimulant effect and are indirectly a nutrient source for beneficial microorganisms in the digestive system (GIT) (Soni et al., 2017). Although not investigated in this study, studies with different fish species have reported that spirulina (rainbow trout) can increase feed efficiency by increasing bacterial colonization in the intestine (Teimouri et al., 2013), improve intestinal flora, break down indigestible feed components and provide more nutrients (James et al., 2006). Man et al., (2020) reported that *S. platensis* can act as a potential trigger that increases the number of probiotic microorganisms in the fish intestine.

In the current study, it was determined that the addition of spirulina to diets containing canola increased lipase enzyme activity and, although no statistical difference was detected, it also improved protease enzyme activity. Mohammadiazarm et al., (2021) Oscar fish (*Astronotus ocellatus*), Ansarifard et al., (2018) Koi (*Cyprinus carpio*), Adel et al., (2016) sturgeon (*Huso huso*) reported that addition of spirulina to their diets increased growth and feed utilization and that this increase obtained in growth and feed utilization parameters was associated with the increase in digestive enzyme activity of spirulina. On the contrary, Cao et al., (2018) reported that feeding gibel carp (*Carassis auratus gibelio* var. CAS III) with spirulina-added diets did not cause an increase in digestive enzymes (lipase, protease and amylase) and that the increase detected in growth performance may be a result of increased digestibility of nutrients and better absorption of digested nutrients. Indeed, Al-Deriny et al., (2020) reported that dietary *S. platensis* improved the FCR of Nile tilapia, and this was explained by the algae improving intestinal morphometry indices and increasing intestinal absorption capacity.

Liu et al., (2019) yellow catfish (*Pelteobagrus fulvidraco*), Teimouri et al., (2013) rainbow trouth (*Onchorhyncus mykiss*), Roohani et al., (2019) Caspian brown trout (*Salmo trutta caspius*) juveniles, Cao et al., (2018) juvenile gibel carp (*Carassis auratus gibelio* var. CAS III), AlMulhim et al., (2023) Nile Tilapia, *Oreochromis niloticus* reported that the lipid content of the fish decreased as the amount of spirulina in diets increased. The reason for the decrease in lipid content was explained by the hypolipidic activity of polyphenols found in spirulina (Kim et al., 2013; Liu et al., 2019). Contrary to this finding found in both omnivorous and carnivorous fish species, in this study, higher lipid levels were detected in the groups containing 20% and 30% spirulina compared to the control group, and the lipid level in fish meat was affected by the amount of spirulina in the diet. The detection of low lipid levels in the control group can be explained by the low lipase enzyme activity (decreased lipid digestibility) detected in this group. The low lipid digestibility detected in the control group can also be attributed to the ANF content such as non-starch polysaccharides, glucosinolate and phytate (Dossou et al., 2018b).

In conclusion, spirulina added to *Carasius auratus* diets containing canola meal increased nutrient digestibility and digestive enzyme activity, resulting in improved growth and feed utilization. Under current experimental conditions, optimal growth could be achieved by adding 30% spirulina to diets containing 30% canola meal.

Compliance with Ethical Standards

a) Author Statement

F. B. H.: Conceptualization, Methodology, Formal analysis, Investigation, Writing – Original Draft, Visualization.

E. Y.: Conceptualization, Methodology, Visualization, Investigation.

b) Conflict of interest

The authors declare that they have no actual, potential, or perceived conflict of interest for this article.

c) Ethics committee approval: This study was conducted following the ethical protocol (06.11.2023/ 05 /23 Number) of Ordu University Animal Experiments Control Council.

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