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Research Article

Effects of Dietary Cold-Pressed Seed Oils on Growth, Coloration and Blood Parameters in Goldfish

Halime Pehlivanoğlu[1*], Mehmet Yardımcı[2], Çetin Yağcılar[3]

[1*] Tekirdağ Namık Kemal University, Faculty of Veterinary Medicine, Department of Food Hygiene & Technology, Tekirdağ, Turkey, (ORCID: 0000-0003-3138-9568), hpehlivanoglu@nku.edu.tr

[2] Tekirdağ Namık Kemal University, Faculty of Veterinary Medicine, Department of Biostatistics, Tekirdağ, Turkey, (ORCID 0000-0001-5650-437X), myardimci@nku.edu.tr

[3] Tekirdağ Namık Kemal University, Faculty of Veterinary Medicine, Department of Aquaculture, Tekirdağ, Turkey, (ORCID: 0000-0002-4683-820X), cyagcilar@nku.edu.tr

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Abstract

This research was conducted to examine the effects of dietary cold-pressed seed oils on growth, coloration and blood parameters in goldfish. A total of one-month-old 240 goldfish were used as the study material. The standard feed enriched with 5 different cold-pressed seed oils (fig, apricot, pomegranate, grape and plum) at a rate of 5% was given to the trial groups, while the control group was fed with the standard feed throughout the experiment. Groups were compared in terms of live weight, total length, specific growth rate (SGR), feed conversion rate (FCR), glucose, cholesterol and triglyceride values. Results showed that dietary seed oils improved the growth performance, feed utilization, coloration and decreased the blood glucose, cholesterol, and triglyceride levels. Seed oil-fed groups gained more weight compared to the control group ($p<0.05$). Remarkably fig and plum groups yielded higher SGR values ($p<0.05$). The glucose, cholesterol and triglyceride values in the fig group was found to be the lowest among all groups ($p<0.05$). Regarding the coloration, darker color shades were found in the pomegranate and fig groups. In conclusion, the use of dietary seed oils makes it possible to grow healthy and fast-growing fish in the desired color.

Keywords: Aquarium fish, Cholesterol, Cold-pressed oils, Coloration, Blood parameters.

Japon Balıklarında Büyüme, Renklenme ve Kan Parametreleri Üzerine Diyet Soğuk Pres Tohum Yağlarının Etkileri

Öz

Bu araştırma, soğuk sıkım meyve çekirdek yağlarının akvaryum balıklarında büyüme, renklenme ve kan parametreleri üzerindeki etkilerini incelemek için yapılmıştır. Çalışma materyali olarak 1 aylık toplam 240 Japon balığı kullanılmıştır. Deneme gruplarına çalışma boyunca beş farklı soğuk sıkım meyve çekirdek yağı (incir, kayısı, nar, üzüm ve erik) ile %5 oranında zenginleştirilmiş standart yem verilirken, kontrol grubuna deneme boyunca standart yem verilmiştir. Gruplar canlı ağırlık, toplam boy, spesifik büyüme oranı (SGR),yemden yararlanma oranı (FCR), glukoz, kolesterol ve trigliserit değerleri açısından karşılaştırılmıştır. Sonuçlar, meyve çekirdeği yağlarının büyüme performansını, yem kullanımını, renklenmeyi iyileştirdiğini ve kan şekeri, kolesterol ve trigliserit düzeylerini düşürdüğünü göstermiştir. Meyve çekirdek yağı ile beslenen gruplar, kontrol grubuna göre daha fazla ağırlık kazanmıştır ($p<0.05$). Dikkat çekici şekilde incir ve erik grupları daha yüksek SGR değerlerine sahiptir ($p<0.05$). İncir grubundaki glukoz, kolesterol ve trigliserit değerleri tüm gruplar arasında en düşük olarak bulunmuştur ($p<0.05$). Renklenme açısından ise nar ve incir gruplarında daha koyu renk tonlarına rastlanmıştır. Sonuç olarak, meyve çekirdek yağlarının kullanılması sağlıklı ve hızlı büyüyen balıkların istenilen renkte yetiştirilmesini mümkün kılmaktadır.

Anahtar Kelimeler: Akvaryum balıkları, Kolesterol, Soğuk sıkım yağlar, Renklenme, Kan parametreleri.

1. Introduction

The use of cold-pressed vegetable oils has increased significantly with the nutritional awareness in recent years. Cold-pressed oils are obtained by the mechanical method without heat treatment and they are functional in terms of nutritional and sensory aspects as they contain natural antioxidants such as essential fatty acids, phenolic substances and tocopherols important for human health and lipophilic, bioactive components such as sterols. They also have antihyperlipidemic, antioxidative, anticarcinogenic, anti-inflammatory, antimicrobial properties due to their unsaturated fatty acids, volatile compounds, phytosterol and vitamin E content (Arıcı et al., 2005).

The grape seed extract was reported to lower the triglyceride, total cholesterol, low-density lipoprotein-cholesterol and interleukin-6 values (Salmabadi et al., 2017) whereas apricot seed oil was reported to have positive effects on preventing the development of many chronic diseases by preventing free radicals from cell damage and increasing body resistance (Erdoğan & Kartal, 2011). Palm oil, peppermint and Amaranthus plants were found to be effective on growth, transformation into protein, and coloration as feed supplements in fish diets (Ahilan et al., 2008). Similarly, citrus peels and natural dyes obtained from seeds were found to have positive effects on color development in goldfish (Abbas et al., 2020; Dananjaya et al., 2019), while hot pepper oil was found to be effective in growth performance and blood parameters in trout (Vincenzo et al., 2018).

This research aimed to examine the effect of dietary cold-pressed seed oils on growth, coloration and blood parameters in goldfish.

2. Material and Method

2.1. Experimental Fish and Rearing Conditions

This research was conducted in the Fisheries and Diseases Laboratory of the Faculty of Veterinary Medicine at Tekirdağ Namık Kemal University. A female and two male ranchu goldfish were used as parents. The offspring obtained from these fish by milking method were fed with live food called artemia for seven days, then, they were fed with commercial powder feed for 20 days, and adapted to solid food. During the trial, biological filtration was applied by pipe filters separately in each experimental aquarium using SunSun brand aerator (19 m^3/h). Optimum growth conditions were achieved by applying a 30% water change in two-day intervals and keeping the nitrate level at an average of 10 ppm. The experiment was conducted in a completely randomized design with six treatments and two replications. A total of one-month-old 240 juvenile goldfish were randomly selected and stocked in 35*35*35 cm sized research aquariums. The fish were sorted into 6 groups and placed into 12 aquariums each consisting of 20 fish with two repetition groups. The trial material was transferred to 60*35*45 cm sized aquariums depending on the increased fish size. As routine measurements pH, salinity, Total Dissolved Solids (TDS), conductivity, aquarium temperature measurements were recorded with a waterproof ExStik® II pH/conductivity meter, EC500, "Extech" brand device, ambient temperature and humidity with a "Thermo HYGRO" device, and Oxygen measurements with a "JBL" brand test kit at weekly intervals (Table 1). There was no mortality associated with any of the treatments.

Table 1. Chemical and physical environmental conditions during the research

	n	Mean	Std. Dev.	Min	Max
pH	12	8,07	0,09	8,0	8,2
Salinity (ppm)	12	362,08	15,94	344,0	402,0
TDS (ppm)	12	581,67	20,69	548,0	626,0
Conductivity (µS)	12	714,50	46,35	601,0	807,0
Aquarium Temp (^{0}C)	12	26,18	1,27	24,7	28,7
Ambient Temp (^{0}C)	12	27,06	1,25	25,3	29,2
Ambient Humidity (% g/m^3)	12	61,83	4,43	54,0	69,0
Oxygen (ppm)	12	8,00	0,00	8,0	8,0

2.2. Preparation of Supplemented Diets

The standard feed enriched with 5 different cold-pressed seed oils (fig, apricot, pomegranate, grape and plum) at a rate of 5% was given to the trial groups (Table 2) while the control group was fed with the standard feed (fish meal, hydrolyzed fish meal, wheat flour, Cyrillic flour, wheat gluten, pea protein, fish oil, yeast and plant essential oils and organic acid, Ca, P, Na) throughout the experiment. The feed was prepared in an average of 20-day portions, each in an amount of 100 g and by the method of spraying cold-pressed seed oils into the ready-made feed using a 10-gauge injector, with a rate of 95 g standard feed and 5 g seed oil. Seed oils were purchased from Oneva-Neva Foods and Printing Materials Industry Trade Limited Company, Istanbul. The fatty acid composition of the cold-pressed seed oils used in the experiment was examined in the Thermo Scientific Trace 1300 Gas Chromatography device.

The food particle size given to fish was in the ranges of 300-500 microns during the first and second measurement periods and increased to 800-1200 microns parallel to the increase in the fish size for the 3, 4 and 5th measurement periods. Finally, 1 mm feed was used at the 6, 7 and 8th measurement periods according to the mouth opening degree of the fish. Feeding was done 3 times a day at a rate of 5%, based on the body weight (BW) of the fish (Table 3).

Table 2. Fatty acid composition (%) and color contents of seed oils

Fatty acid composition (%)	Groups				
	Apricot	Pomegranate	Grape	Plum	Fig
C16:0 Palmitic acid	4,10	4,30	7,50	5,20	6,0
C16:1 Palmitoleic acid	1,30	TE	0,22	0,56	ND
C18:0 Stearic acid	2,40	2,90	3,50	2,56	4,12
C18:1 Oleic acid	67,20	6,70	13,88	73,00	7,60
C18:2 Linoleic acid	23,70	6,20	71,20	15,88	34,33
C18:2 Linolenic acid	0,52	ND	0,88	0,45	45,38
C18:3 Conjugated (cis 9,11,13) Punicic acid	ND	75,90	ND	ND	ND
C20:0 Arachidic acid	0,14	0,35	0,29	0,38	TE
C20:1 Eicosanoic acid	0,26	0,47	0,30	0,43	TE
C22:0 Behenic acid	ND	0,29	0,46	ND	ND
C22:1 Erucic acid	ND	ND	0,22	ND	ND
C24:0 Lignoceric acid	ND	ND	0,38	ND	ND
ΣSFA	6,64	7,84	12,13	8,14	10,72
ΣMUFA	68,76	7,17	14,62	73,99	7,6
ΣPUFA	24,22	82,10	72,08	16,33	79,71
Redness	3,9	14	3,1	2,3	4,2
Yellowness	70	70	38	40	70

ND: Not Detected

Table 3. Fish feed formulation based on the control group

Serving Period	Feed size	Crude protein (%)	Crude fat (%)	Crude cellulose (%)	Crude ash (%)
1 (1st, 2nd measurements)	300-500 µ	55	15	0,5	12,3
2 (3, 4, 5th measurements)	800-1200 µ	60	10	1,5	11
3 (6, 7, 8th measurements)	1 mm	54	14	1,5	11

2.3. Measured parameters

2.3.1. Growth Performance

Live weight and total length measurements were taken at 20-day intervals. The fish was dried with a napkin to absorb the excess water in Petri dishes and body weights were measured (wet weight) under anesthesia with a Kern brand scale sensitive to 0.01 g. For anesthesia, fish were exposed to a concentration of clove oil at a dose of 1 ml/L. The clove oil was obtained from the "Kimbiotek Chemical Materials Industry and Trade Corporation" company and diluted in ethanol (99.8%) to 9 parts ethanol and 1-part clove oil (Yaşar et al., 2020). Total length was measured with a 20 cm ruler based on the distance between the tip of the nose and the tip of the tail, by straightening the tails of the fish in the petri dish just after the live weight measurement. Specific growth rate (SGR) and feed conversion rate (FCR) were calculated after collecting all the data.

SGR (% day-1) = ($\log_e$ Final weight – $\log_e$ Initial weight) / no. of days) * 100

FCR = Feed Given (dry weight in g) / Net weight gain (wet weight in g)

2.3.2. Hematological parameters

One of the reasons why goldfish were preferred in this study was that it was easier to take blood from goldfish compared to many other aquarium fish. In this respect, the blood samples were taken from the caudal peduncle of the 24 h fasted fish using insulin Syringes 30G 1/2 cc 1/2 inch 90/bx under anesthesia at the end of the study and glucose, cholesterol and triglyceride concentrations were recorded separately. As for the method followed in sampling, a drop of blood was placed onto a test strip (Accutrend® Plus; Roche Diagnostics, Vokietjia, Germany) for either cholesterol or triglyceride analysis. After the device is calibrated, the strips were inserted into the blood cholesterol and triglycride analyzer (Accutrend® Plus) and results were read 170-180 seconds. Glucose levels were measured with an IME-DC brand instrument with the same method and the results were read within 10-12 seconds.

2.3.3. Coloration

Being able to see the coloration more clearly was another advantage of choosing goldfish in our research. The red and yellow color contents of the seed oil samples were determined manually using a Lovibond Tintometer (PFXi 880 / L, Tintometer Ltd., Amesbury, UK) in a 1-inch cuvette according to AOCS Official Method Cc 13e-92. A goldfish color chart was used to evaluate the color changes (Anonymous, 2020) until the colors of the fish were formed to fit perfectly. In the color chart, the colors from dark to light were ranked as tangelo, orange, bright yellow, gold and mango. In color evaluation, the spread of the color from the back to the abdomen of the fish was considered better. When the colors were determined to be correct, 10 samples from each group were selected, photographed from the upper side of the right eye on the head and analyzed using the Just color picker 5.5 program on the computer (Table 9). In this program, each color is handled over the RGB codes and ratios of 255 in percentages. In this program, the color evaluation was made based on the average of 7 x 7 pixels. When the RGB codes are 255 255 255, the color is white; when 0 0 0, it is understood to be black. For example, if the RGB codes are 186, 73, 2 then the color values appear as 72,94% red, 28,63% green and 0,78% blue, respectively.

Saturation and lightness were the other criteria to define the colors. When the intensity of one of these three colors is higher than the others, the new tone that will emerge will be a shade closer to this primary color (like reddish, greenish or bluish). Also, if the intensity of the two primary colors is the same, then the

result will be a shade that is closer to the secondary colors (cyan, purple, or shades of yellow).

2.4. Statistical Analysis

IBM SPSS Statistics 25.0 package program has been used for statistical analysis. Live weight, total length, SGR, FCR, blood glucose, cholesterol and triglyceride concentration comparisons among the groups were made by the analysis of variance test, while comparisons between groups were performed by the Tukey test. Correlations between live weight and total lengths were determined by the Pearson correlation test.

2. Results and Discussion

3.1. Growth performance

A linear increase was observed in the body weight gain in all groups with a more evident rise after the fourth measurement period. Seed oil-fed groups gained more weight compared to the control group in all measurement periods following the initial measurement ($p< 0.05$). It was noteworthy that the weight gain differed significantly in the fig, pomegranate and plum groups in the final measurement period ($p<0.05$) compared to the other experimental groups (Table 4).

Table 4. Live weights (g) by the measurement periods

Groups	n	Measurements 1st (0-20 days) $\bar{x} \pm S_{\bar{x}}$	2nd (21-40 days) $\bar{x} \pm S_{\bar{x}}$	3rd (41-60 days) $\bar{x} \pm S_{\bar{x}}$	4th (61-80 days) $\bar{x} \pm S_{\bar{x}}$	5th (81-100 days) $\bar{x} \pm S_{\bar{x}}$	6th (101-120 days) $\bar{x} \pm S_{\bar{x}}$	7th (121-140 days) $\bar{x} \pm S_{\bar{x}}$	8th (141-160 days) $\bar{x} \pm S_{\bar{x}}$
Control	40	0,34±0,01	0,38±0,01^{a}	1,13±0,05^{a}	1,34±0,09^{a}	2,81±0,17^{a}	4,47±0,18^{a}	6,77±0,34^{a}	10,87±0,46^{a}
Pomegranate	40	0,34±0,01	0,45±0,02^{ab}	1,46±0,05^{b}	2,08±0,10^{b}	3,33±0,13^{ab}	5,27±0,20^{ab}	7,64±0,43^{ab}	13,89±0,47^{bc}
Grape	40	0,35±0,01	0,42±0,02^{ab}	1,39±0,05^{b}	1,93±0,08^{bc}	3,01±0,18^{ab}	4,76±0,24^{ab}	6,88±0,34^{a}	11,98±0,59^{ab}
Fig	40	0,33±0,01	0,49±0,03^{b}	1,48±0,03^{b}	2,26±0,09^{bc}	3,54±0,23^{b}	5,65±0,21^{b}	8,07±0,39^{ab}	14,29±0,54^{c}
Plum	40	0,33±0,01	0,48±0,02^{b}	1,40±0,04^{b}	2,01±0,07^{bc}	3,30±0,10^{ab}	5,34±0,24^{ab}	8,54±0,35^{b}	13,99±0,63^{bc}
Apricot	40	0,34±0,01	0,46±0,02^{b}	1,42±0,06^{b}	2,39±0,11^{c}	3,48±0,22^{ab}	5,00±0,28^{ab}	7,13±0,34^{ab}	12,29±0,40^{abc}
p		0,479	0,000	0,000	0,000	0,032	0,004	0,003	0,000

P: ANOVA; Different letters indicate significant difference among treatments by Tukey test ($p < 0.05$)

The lowest SGR (2.141) was observed in the control group, while fig and plum groups yielded higher values and differed significantly from the others ($p<0.05$) (Table 5). Feed conversion ratio (FCR) was variable among the groups but did not differ significantly ($p>0.05$).

Table 5. Specific growth rate (SGR) and feed conversion rate (FCR)

	Control	Pomegranate	Grape	Fig	Plum	Apricot
Mean initial weight (g)	0,345	0,335	0,355	0,329	0,325	0,335
Mean final weight (g)	10,871	13,888	11,979	14,289	13,992	12,288
Weight gain (g)	10,530	13,887	11,978	14,288	13,991	12,287
Growth ratio (g/d)	0,065	0,084	0,072	0,087	0,085	0,074
SGR (% BW/day)	2,141^{a}	2,324^{bc}	2,192^{ab}	2,351^{c}	2,344^{c}	2,258^{abc}
FCR	2,669	2,543	2,662	2,586	2,572	2,720

$p< 0.05$; P: ANOVA

No significant difference was observed between the groups in terms of total lengths except for some small differences at the 4th, 5th, and 6th measurement periods (Table 6). All groups had similar values in terms of initial and final total lengths.

Table 6. Total lengths (cm) by the measurement periods

Groups	n	Measurements 1st (0-20 days) $\bar{x} \pm S_{\bar{x}}$	2nd (21-40 days) $\bar{x} \pm S_{\bar{x}}$	3rd (41-60 days) $\bar{x} \pm S_{\bar{x}}$	4th (61-80 days) $\bar{x} \pm S_{\bar{x}}$	5th (81-100 days) $\bar{x} \pm S_{\bar{x}}$	6th (101-120 days) $\bar{x} \pm S_{\bar{x}}$	7th (121-140 days) $\bar{x} \pm S_{\bar{x}}$	8th (141-160 days) $\bar{x} \pm S_{\bar{x}}$
Control	40	2,36±0,04	2,42±0,04	2,92±0,08	3,32±0,09^{a}	3,93±0,11^{a}	4,43±0,11^{a}	5,64±0,16	6,02±0,16
Pomegranate	40	2,42±0,05	2,49±0,03	3,02±0,08	3,80±0,11^{b}	4,53±0,10^{b}	5,09±0,11^{b}	5,85±0,12	6,47±0,18
Grape	40	2,39±0,04	2,47±0,06	2,77±0,08	3,48±0,07^{ab}	4,30±0,08^{ab}	5,13±0,12^{b}	5,81±0,14	6,14±0,12
Fig	40	2,35±0,04	2,54±0,04	2,87±0,08	3,49±0,08^{ab}	4,61±0,15^{b}	5,22±0,13^{b}	6,16±0,14	6,51±0,12
Plum	40	2,35±0,03	2,49±0,04	2,96±0,05	3,54±0,06^{ab}	4,39±0,10^{b}	4,90±0,12^{b}	5,90±0,15	6,49±0,10
Apricot	40	2,35±0,02	2,51±0,04	3,07±0,06	3,69±0,12^{b}	4,57±0,11^{b}	4,83±0,10^{ab}	5,73±0,18	6,24±0,10
P		0,716	0,546	0,058	0,004	0,000	0,000	0,219	0,364

$p<0,05$; P: ANOVA; Different letters indicate significant difference among treatments by Tukey test ($p < 0.05$)

3.2. Correlations

Significant positive correlations were found between the body weight and total length values within all groups until the sixth measurement period ($p<0.01$). However, a serious drop was seen in the last three periods with some negative correlations (Table 7).

Table 7. Correlations between live weight and total lengths by the measurement periods

Groups	Measurements							
	1st	2nd	3rd	4th	5th	6th	7th	8th
	r	r	r	r	r	r	r	r
Control	,539**	,764**	,678**	,809**	,600**	,131	,255	-,146
P	,000	,000	,000	,000	,000	,419	,112	,370
Pomegranate	,874**	,873**	,441**	,221	,904**	,152	,212	,090
P	,000	,000	,004	,192	,000	,348	,189	,582
Grape	,988**	,765**	,373*	,371*	,582**	,631**	,583**	-,014
P	,000	,000	,018	,018	,000	,000	,000	,934
Fig	,951**	,504**	,985**	,628**	,887**	,356*	-,101	,292
P	,000	,001	,000	,000	,000	,024	,535	,067
Plum	,974**	,976**	,742**	,867**	,722**	,180	-,060	,024
P	,000	,000	,000	,000	,000	,267	,711	,882
Apricot	,978**	,788**	,940**	,586**	,852**	,310	,471*	-,063
P	,000	,000	,000	,000	,000	,051	,002	,701

***Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed)*

3.3. Hematological parameters

Significant changes were observed in glucose, cholesterol and triglyceride values ($p<0.05$) (Table 8). Blood glucose levels decreased in all groups whereas the value in the control group was quite high. In terms of cholesterol values, grape, fig, pomegranate, and apricot groups significantly differed from the plum and control groups ($p<0.05$). The lowest value for triglyceride concentration was in the fig group which was significantly differed from the control and apricot groups ($p<0.05$).

Table 8. Blood glucose, cholesterol and triglyceride concentrations

Parameters	Groups	n	$\bar{x} \pm S_{\bar{x}}$	P
Glucose	Control	20	101,85±6,56^{c}	0,000
	Apricot	20	67,25±4,91^{ab}	
	Pomegranate	20	76,90±6,46^{b}	
	Grape	20	74,90±6,31^{b}	
	Plum	20	66,40±6,10^{ab}	
	Fig	20	50,15±3,41^{a}	
Cholesterol	Control	14	250,29±8,12^{b}	0,000
	Apricot	14	168,07±9,72^{a}	
	Pomegranate	14	155,71±6,35^{a}	
	Grape	14	154,57±4,53^{a}	
	Plum	14	262,14±4,71^{b}	
	Fig	14	152,57±5,64^{a}	
Triglyceride	Control	14	136,21±16,16^{bc}	0,001
	Apricot	14	147,86±19,06^{c}	
	Pomegranate	14	110,07±9,33^{abc}	
	Grape	14	96,93±5,97^{ab}	
	Plum	14	91,29±4,59^{ab}	
	Fig	14	88,14±5,19^{a}	

p< 0.05; P: ANOVA

3.4. Coloration

The color values obtained with the Just color picker 5.5 program and complementary statistical values are given in Table 9.

Table 9. Final fish colors based on RGB codes

Color	Groups	n	Mean	SD	Min	Max
Red	Control	10	76,39	4,21	69,41	81,96
	Pomegranate	10	64,51	6,42	54,90	72,94
	Grape	10	71,88	3,77	65,88	76,86
	Fig	10	66,82	9,41	48,24	77,65
	Plum	10	72,12	8,69	58,43	89,80
	Apricot	10	67,69	5,00	58,04	76,08
Green	Control	10	65,69	7,67	51,76	75,29
	Pomegranate	10	29,92	6,58	23,92	41,96
	Grape	10	52,67	3,45	43,92	55,69
	Fig	10	36,70	7,94	25,10	47,84
	Plum	10	56,59	8,08	42,35	73,73
	Apricot	10	39,65	8,86	28,24	53,33
Blue	Control	10	48,27	11,99	30,98	65,88
	Pomegranate	10	2,31	0,77	0,78	3,53
	Grape	10	20,59	5,18	10,98	28,63
	Fig	10	4,98	1,36	3,53	7,84
	Plum	10	28,90	6,99	18,04	39,61
	Apricot	10	11,88	2,77	8,24	15,29
Saturation	Control	10	36,80	8,02	26,00	51,00
	Pomegranate	10	92,90	2,77	89,00	98,00
	Grape	10	56,40	7,03	47,00	71,00
	Fig	10	86,10	3,28	81,00	91,00
	Plum	10	49,50	9,01	39,00	71,00
	Apricot	10	70,40	5,56	63,00	79,00
Lightness	Control	10	62,30	7,42	50,00	74,00
	Pomegranate	10	33,50	3,06	29,00	37,00
	Grape	10	45,70	4,42	38,00	52,00
	Fig	10	35,80	4,94	26,00	42,00
	Plum	10	50,50	7,52	40,00	65,00
	Apricot	10	39,80	3,43	35,00	46,00

Coloration in fish was first observed at the 4th measurement (Fig 1) and 7 mango fish were recorded in the control group while 18 gold in apricot, 10 bright yellow in pomegranate, 22 gold in grape, 17 mango in plum, 13 gold were detected in the fig groups. At the 5th measurement, 24 bright yellow color fish were recorded in the control group while there were 19 bright yellow in apricot, 19 orange and bright yellow in the pomegranate, 29 bright yellow in grape, 29 gold and bright yellow fish in plum, and 21 bright yellow and orange in the fig group. At the 6th measurement, the colors were fully formed and neither the color nor the numbers changed in later periods. Therefore, final color shades and numbers in groups were as follows: 32 bright yellow fish in the control group, 32 orange and bright yellow in apricot, 36 tangelo and orange in pomegranate, 37 bright yellow in grape, 37 bright yellow in plum, and 31 orange fish in the fig group. Looking at the final color and intensity distribution based on the Just color picker 5.5 program on the computer, red, green and blue colors were seen to be more prominent in the control, plum and grape seed oil groups contrary to the saturation values. Saturation was higher in the pomegranate group followed by fig and apricot.

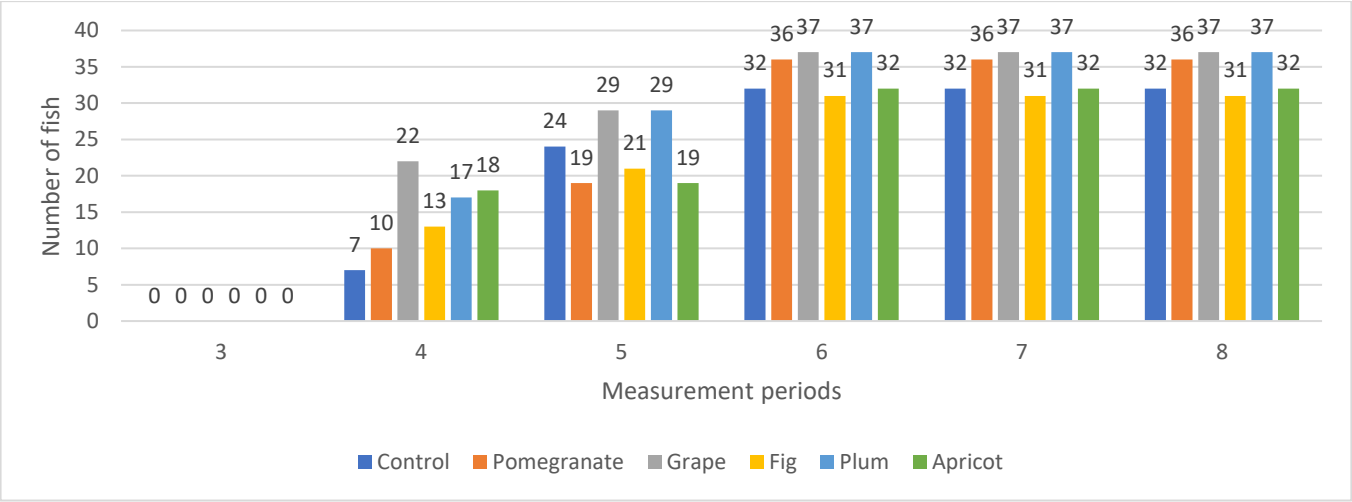

Figure 1. Coloration profile of the groups by periods

Positive effects of feeding with herbal products and active ingredients on growth are seen in many fish species such as goldfish, rockfish, tilapia fish, rainbow trout (Akoh & Min, 2008; Dananjaya et al., 2020; Gatlin et al., 2007; Gabriel et al., 2015; Heidarieh et al., 2013; Vieges & Contreras, 1994; Vincenzo et al., 2018). As one of those effective ingredients, essential fatty acids in which linoleic and linolenic fatty acids present in a balanced manner are recommended to be given externally for growth (Chou & Shiau, 1999; Aliyu-Paiko et al., 2010). Similar to the results of those papers, seed oils were found to have positive effects on fish growth in the current study. The weight gain values in the trial groups were higher than the control group due to the supplementation of cold-pressed seed oils to the diet because grape and fig seed oils are rich in linoleic and linolenic acid, while apricot and plum seed oils are rich in oleic acid. In this sense, the increase in weight in these groups could be attributed to the high amount of their essential fatty acid contents and other components (phenolic substances, vitamins and antioxidants).

The fact that the bodyweight gains were more pronounced with the preference of goldfish compared to other aquarium fish provided an advantage in terms of interpreting the research findings. Higher SGR values in all treatment groups particularly in fig and plum groups compared to the untreated control group indicates the positive effects of seed oils. Guoa et al. (2020) reported that the addition of different vegetable oils to feeds in juvenile golden pompano fish had a better effect on growth parameters and feed efficiency. They observed that a diet with vegetable oils had a significantly higher SGR compared to fish in the control group. However, they did not observe any statistical difference in feed conversion ratio (FCR). Abdel-Latifa et al. (2020) stated that a dose-dependent effect of thyme essential oil taken with diet in carp fish had a positive effect on growth performance (weight gain, SGR and weight gain) compared to the control group. On the other hand, they stated that FCR values among all groups were not significantly affected by the addition of dietary thyme oil for carp.

Regarding the total length measurements, all the groups became closer during the last two periods ($p>0.05$) whereas they were distinctly separated in terms of the bodyweight values ($p<0.05$). This was not a surprising result considering the morphological development of the fish because, after a certain age, there is no elongation in the body of the goldfish, conversely, there is just a transverse enlargement. The Kn value (Relative Condition Factor) was not calculated because the change in body structure due to growth is in the form of a transverse expansion and there is no regular increase in body length in goldfish. They do not have a fusiform body shape, but egg-shaped.

It is known that fatty acids in the diet affect blood parameters (Mensink et al., 2003). For this reason, most dietary recommendations propose limiting the intake of saturated fatty acids and trans fats and replacing them with unsaturated, especially polyunsaturated fatty acids (Anonymous, 2010; Schwab et al., 2014). The reason why the glucose values in all groups were lower than the control group in this study is thought to be due to the rich unsaturated fatty acid content of the seed oils. Beyond this, the glucose value in the fig group was found to be the lowest (Table 8) which could be attributed to the high ratio of linoleic and linolenic acid (total Omega 3) content.

On the other hand, except for the plum group, cholesterol values in the trial groups were lower than the control group, which can be interpreted as a positive effect of unsaturated fatty acids. In particular, fig, grape and pomegranate seed oils decreased the cholesterol levels in goldfish which can be explained by the fatty acid contents of these seed oils. Similar results are also consistent with previous findings. In studies supporting this view, using unsaturated and unprocessed products in the diet is recommended instead of saturated fatty acids due to their regulatory properties against cholesterol, triglyceride and glucose disorders (Siri-Tarino et al., 2010). For example, grape seed extract is reported to reduce the total cholesterol values in rats (Salmabadi et al., 2017; Doostan et al., 2017), in rabbits (Hassan et al., 2020), in broilers (Abu Hafsa & Ibrahim, 2018), and fish (Shakya, 2017). Similarly, a pomegranate peel extract supplemented diet is reported to decrease the blood cholesterol and triglyceride levels in Oreochromis niloticus (Sadeghipour et al., 2014) and dietary pomegranate seed oil is reported to decrease the blood cholesterol values of the rainbow trout (Acar et al., 2018). Moreover, 75.90% conjugated (cis 9,11,13). Additionally, punicic acid is specific to the pomegranate seed oil and not found in the other oils.

The intake of omega 3 and polyunsaturated fatty acids are reported to reduce the level of triglycerides (Weber & Raederstorff, 2000). In this research, triglyceride results in fig, plum, grape and pomegranate groups were lower than the control group whereas the apricot group was higher ($p<0.05$). The raw material source, process conditions (cold press), vitamin, phenolic substances, antioxidant values and the richness of the obtained oil in polyunsaturated fatty acids are thought to be effective on these results.

Inclusion of natural pigment in the diets is reported to improve skin pigmentation in Cyprinus carpio (Ninwichian et al., 2020), in goldfish (Dananjayaa et al., 2020) and the carotenoid level directly determines the commercial value of the fish (Gouveia & Rema 2005; Paripatanamont et al. 1999). It is also effective on fish growth, metabolism and reproductive functions (Miki, 1991). As shown in Fig. 1, darker coloration was observed in the pomegranate and fig groups. Coloration with different shades is thought to be caused by the carotenoid content because carotenoids are biological pigments and responsible for the skin color in fish. In the final skin color analysis by the just color picker 5.5 program on the computer, the region above the right eye was chosen as the coloration in the head region in goldfish is a more prominent region. This program takes into account the dominant color distributions and the saturation and lightness. A one-sided evaluation will be misleading when the results obtained are given separately in terms of color and saturation. While the pomegranate seed oil group has a high value in terms of saturation, the control group stands out in terms of dominant color. When the color and saturation data are taken together, it is seen visually why more distinct colors are perceived in some groups. Based on this, it can be concluded that saturation is more determinant in color perception.

The substances that give the orange color to the oils are the α and β carotene groups and xanthophylls while anthocyanins as polyphenolic pigments for the red color. The high red color of pomegranate seed oil indicates that it is rich in anthocyanin. It is known that polyphenols are also effective on heart health and diabetes. For this reason, it is thought that the effect of pomegranate seed oil on glucose, cholesterol and triglycerides is due to its anthocyanin content. These findings are thought to be a guide for researchers and breeders interested in the subject.

The dietary supplementation of different seed oils in the current study revealed a significant improvement in growth

performance, feed utilization, coloration, blood glucose, cholesterol, and triglyceride levels. Seed oil-fed groups gained more weight compared to the control group. Fig and plum groups yielded higher SGR values. The glucose, cholesterol and triglyceride values in the fig group were found to be the lowest among all groups. No significant change was observed in terms of the total lengths except for small differences. Regarding the coloration, darker color shades were found in the pomegranate and fig groups. Considering the positive effects of natural color substances on health, it is thought that they have also positive effects on blood values.

4. Conclusions and Recommendations

The dietary supplementation of different seed oils in the current study improved the growth performance, feed utilization, coloration and decreased the blood glucose, cholesterol, and triglyceride levels in goldfish. Since, health, rapid growth and desired skin color are the most important factors for both sustainability and commercial gains of aquarium fisheries, this research showed that the use of dietary seed oils makes it possible to grow healthy and fast-growing fish in the desired color.

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