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Evaluation of Sugar Beet Leave Extracts in Goldfish (*Carassius auratus*) Diets: Effects on Blood and Semen Parameters

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Research Article

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Abstract

Sugar beet (*Beta vulgaris* L.) is one of the common agricultural crops in the world. After harvesting of its roots, sugar beet leaves (SBL) become waste in the field. SBL could cause some inflammation problems in the digestive tract of cattle and decrease tasty of feed. This study was carried out to determine the effects of three different extracts from SBL on hematological and blood serum biochemical parameters, and semen quality of goldfish *Carassius auratus*. These extracts contained proteins, essential oils and carbohydrates from SBL were added to fish feed at ratios of 5 and 20 ‰. The serum total protein, glucose and albumin values increased while cholesterol and liver enzymes activities decreased in all SBL extract groups. Also, the highest hemoglobin values were found in the groups fed with the supplementations of the essential oils extract. Moreover, the lowest lipid peroxidation and the highest catalase activity levels of seminal plasma were found in these groups. The supplementations of the essential oils extract which contains phytol and vitamin E improved blood parameters and increased the motility parameters as well.

Keywords: Carasius auratus, sugar beet leaves, blood, semen.

Japon Balığı (*Carassius auratus*) Yemindeki Şeker Pancarı Yaprak Özütlerinin Kan ve Semen Parametrelerine Etkileri

Özet

Şeker pancarı (*Beta vulgaris* L.), dünyadaki en önemli tarım bitkilerinden biridir. Kök kısmının hasadından sonra, şeker pancarı yaprakları tarlada atıl hale gelmektedir. Bu yapraklar, büyükbaş hayvanlarda yem olarak kullanıldığında sindirim sistemi rahatsızlıklarına ve yem tadında bozulmaya neden olabilmektedir. Bu çalışma, şeker pancarı yapraklarından üç farklı şekilde elde edilmiş özütlerin, japon balığı *Carassius auratus* hematolojik ve kan serumu biyokimyasal parametrelerine, semen kalitesine etkilerini tayin etmeyi amaçlamıştır. Yapraklardaki proteinleri, esansiyel yağları ve karbonhidratları içeren bu özütler balık yemlerine ‰ 5 ve ‰ 20 oranlarında ilave edilmiştir. Yemlerine yaprak özütü eklenen tüm gruplarda serum toplam protein, glikoz ve albümin değerleri yükselmişken, kolesterol ve karaciğer enzimlerinin aktivite değerlerinde düşüşler gözlemlenmiştir. Ayrıca, en yüksek hemoglobin değerleri, yemine uçucu yağları içeren özüt ilave edilmiş grupta bulunmuştur. Bu gruplarda, en düşük lipid peroksidasyon ve en yüksek katalaz aktivitesi değerleri saptanmıştır. Fitol ve E vitamini açısından zengin olan esansiyel yağ özütünün, kan parametrelerini iyileştirmiş ve sperm kalite değerlerini artırdığı belirlenmiştir.

Anahtar kelimeler: Carasius auratus, şeker pancarı yaprağı, kan, semen.

INTRODUCTION

Aquaculture industry, as one of the fastest growing production industries, significantly provides animal protein source of human food, and this support is increasing from year to year. In the last decade, the global aquaculture produces around 80 million tons of fish per year, and its average growth rate is almost 10% per year (FAO, 2018). This huge production of the aquaculture industry creates a need for fish feed reflectively. Therefore, different ingredients which could offer the better both health conditions and growth rate have been studied.

For instance, some chemicals such as antibiotics, hormones have been considered as fish feed additives to increase fish production and reduce mortality caused by bacterial or fungal infections (Alderman and Hastings, 1998). Due to possible negative effects of these chemicals such as residue in fish tissues, accumulation in the aquatic ecosystem, herb and plant extracts or their essential oils evaluated as options for them (Rhodes et al., 2000; Acar et al., 2015). Moreover, in recent years, there has been an increasing amount of literature on the finding alternatives for proteins and lipids sources of fish feed from some terrestrial plant products instead of fishmeal and fishoil. Many common agricultural crops such as soybean, barley, canola, corn, pea, lupin, and wheat have been considered for inclusion in fish feed. However, no data was found on the utilization of sugar beet (*Beta vulgaris* L.) products in fish feed in reviewing the literature.

Sugar beet (SB) belonged to Chenopodiaceae family is cultivated in Europe, Asia, America, and Africa, provides around 16% of the total sugar production in the world. The main producers of SB are France, United States of America, Germany, Russian Federation, Ukraine, and Turkey (FAO, 2018). SB reaches about 2 feet high and is a succulent plant with large, fleshy, glossy leaves, angular stems and numerous leafy spikes of green flowers (Lange et al., 1999). Roots and leaves of SB are used in traditional medicine as a cure for liver and spleen diseases (Khare, 2007). Also, SB leaves are good sources of natural antioxidants such as betains, flavonoids, polyphenols, vitamin and folic acid (Lee et al., 2009).

After SB roots are harvested, a great part of its leaves become waste in the field even though they are restrictively used for animal feed (especially for cattle) or fertilizers. Usually, this by-product has not been valorized (Rabetafika et al., 2008) and no economic return. Moreover, like other plants, sugar beet leaves (SBL) could cause some inflammation problems in the digestive tract of cattle and decrease tasty of feed (Henry et al., 2015). To avoid any possible negative effects of SBL, proteins, essential oils and carbohydrates extractions of SBL (SBLE) were added to fish feed in this study. The aim of the current study was to determine the effects of dietary of three different SBLE on hematological and serum biochemical parameters, and semen quality of goldfish *Carassius auratus*, associating with the main components of the extracts.

MATERIAL and METHODS

Fish and Feed preparation

The study was approved by the local ethical committee of Bahri Dagdas International Agricultural Research Institute (ref. 2016/57-3822). 252 male goldfish (6.02±0.40 g) were obtained from at the Central Fisheries Research Institute, Antalya, Turkey. After an adaptation period of 14 days, the fish were transferred into 21 tanks (50 L) as 12 for each group in triplicate and then fed with experimental diets for 60 days. During the experiment, the average parameters of water were recorded once a week by a multimeter (YSI 556MPS, YSI Inc., Ohio, USA) and dissolved oxygen, temperature, and pH were measured as 7.5 ± 0.2 mg L^{-1}, 17 ± 1°C, and 7.9 ± 0.5 respectively. During experimental and adaptation periods, fish were offered the experimental diets to apparent satiation twice daily at 09:00 and 17:00. The fish were fed with the control diet during the adaptation period. The diet formulations are given in Table 1. The basal diet was maintained as the control diet therefore it was not supplemented with SBLE. The experimental diets were prepared using the basal diet supplemented with 5 and 20‰ SBLE obtained by three different methods. A laboratory food mixer was used to mix the dry ingredients for the diet preparation. The mixtures were primed with tap water to yield an applicable pulp. 1-mm pellets were fabricated using the wet ingredients, which were later dried at 40 °C in a drying cabinet, and stored at -20 °C until feeding.

Table 1. Composition of experimental feeds used in the feeding groups

	Control	P5	E5	C5	P20	E20	C20
Dietary ingredient (%)							
Fish meal	23.00	23.00	23.00	23.00	23.00	23.00	23.00
Soybean meal	37.00	37.00	37.00	37.00	37.00	37.00	37.00
Wheat meal	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Fish oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Mineral/vitamin premix[1.2]	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Starch	19.00	18.50	18.50	18.50	17.00	17.00	17.00
SBLE[3]	0	0.50	0.50	0.50	2.00	2.00	2.00
Total	100	100	100	100	100	100	100
Chemical analyses (Dry matter. %)							
Protein	35.17	35.59	35.22	35.18	35.47	35.17	35.63
Lipid	7.21	7.32	7.19	7.40	7.15	7.33	7.25
Ash	5.63	5.74	5.68	5.36	5.19	5.22	5.61

P5, feed with 5 ‰ protein extract; P20, feed with 20 ‰ protein extract; E5, feed with 5 ‰ essential oil extract; E20, feed with 20 ‰ essential oil extract; C5, feed with 5 ‰ carbohydrate extract; C20, feed with 20 ‰ carbohydrate extract.
[1]Vitamin Mix: Vit. A, 18000 IU; Vit. D3, 2500 IU; Vit. E, 250 mg/kg; Vit. K3, 12 mg/kg; Vit. B1, 25 mg; Vit. B2, 50 mg; Vit. B3, 270 mg; Vit. B6, 20 mg; Vit. B12, 0.06 mg; Vit. C, 200 mg; Folic acid, 10 mg; Calcium d-pantothenate, 50 mg; Biotin, 1 mg; Inositol, 120 mg; Choline chloride, 2000 mg.
[2]Mineral Mix: Fe, 75.3 mg; Cu, 12.2 mg; Mn, 206 mg; Zn, 85 mg; I, 3 mg; Se, 0.350 mg; Co, 1 mg
[3]SBLE; Sugar beet leaf extract

Extraction methods of sugar beet leaves and determination of their contents

The extraction methods were used for the extractions of proteins, carbohydrates and essential oils from SBL. To extract proteins (P extract), 100 g SBL were cut into pieces in a blender with 200 ml sodium phosphate buffer (100 mM, pH 7.5) containing 3 mM EDTA The extract was stirred for 2 h at 4°C, then centrifuged at 14.000 rpm for 10 min and the supernatant was obtained (Rabetafika et al., 2008). For the extraction of essential oils (E extract), 100 g SBL was homogenized in 150 ml ether, and then the extract was centrifuged (14.000 rpm, 10 min, at 4 °C). The clear supernatant was collected, and evaporated. The residue was resolved in 80% aqueous methanol, and centrifuged to obtain the supernatant (Kähkönen et al., 1999). For the extraction of carbohydrates (C extract), 100 g SBL powdered in liquid N_2 and extracted three times in 100 ml deionized water at 95 °C. The extract was centrifuged at 14.000 rpm for 10 min and the supernatants were taken (Sévenier et al., 1998).

The total protein in the protein extract was determined by Bradford assay (Bradford, 1976). The essential oil extract was analyzed by Shimadzu GCMS-QP 2010 Ultra (Kyoto, Japan) coupled with Rtx-5MS capillary column (30m; 0.25 mm; 0.25 µm). The column temperature was 40°C while ejection temperature adjusted to 250°C using with helium as the carrier gas. The sample injection was 1µL, and the analysis was taken 78 min at 100 kPa pressure. Main sugars (glucose, fructose, and saccharose) of the carbohydrates extract were analyzed by Shimadzu LC20A Prominence HPLC (Kyoto, Japan) equipped with Inertsil ODS-3 (5µm-25x4,6mm) column and a refractive index detector (RID). Acetonitrile /H2O mobile phase was 40/60 while its flow rate was 1.3 mL/min.

Blood sampling and analyses

At the end of the 60-day feeding trial, blood was taken from the fish (12.01±0.52 gr) as a total of 9 fish for each treatment (3 individuals from each replicate). After the fish were caught randomly from

the tanks, they were anesthetized with clove oil (50 μL L^{-1}) as soon as possible (Woody et al., 2002). After the anesthesia, immediately the anal region of the fish was cleaned with ethanol in order to prevent blood samples from contamination with mucous membrane. The blood was taken from the caudal vein of the fish by plastic syringes. A 1.5 ml volume of blood was collected from each fish. Hematological and serum biochemical analyzes were carried out by dividing blood samples into K3EDTA and cellular serum tubes. For serum analysis, the blood samples were centrifuged at 10500 g 5 min. The separated serum was stored at -80 °C until analyzed.

Red blood cells (RBC, 10^6 mm^3), hematocrit (Hct, %) and hemoglobin (Hb, g dL^{-1}) was determined by using the method of Blaxhall and Daisley (1973). RBC was counted with a Thoma hemocytometer with the usage of Dacie's diluting fluid. Hct was determined by using a capillary hematocrit tube. Hb concentration was determined with spectrophotometry (540 nm) by using the cyanmethemoglobin method. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated by using the following equations (Bain et al., 2006): MCV (μm^3) = [(Hct, %) × 10]/ (RBC, × 10^6 per mm^3), MCH (pg) = [(Hb, g/dL) × 10]/ (RBC, × 10^6 per mm^3), MCHC (%) = [(Hb, g/dL) × 100]/ (Hct, %).

Serum biochemical indices such as glucose (GLU), total protein (TP), albumin (ALB) triglyceride (TRI), cholesterol (COL), alkaline phosphatase (ALP), glutamic oxaloacetic transaminase (AST), and lactate dehydrogenase (LDH) were analyzed using bioanalytical test kits (Bioanalytic Diagnostic Industry, Co) and measured by a Shimadzu spectrophotometer (PG Instruments, UK).

Semen sampling and evaluation

After the blood sampling, the temperature of water in the tanks raised gradually to 22±0.3°C within a few days and the nine fish for each replicate were used for semen sampling. These fish received an intramuscular injection of human chorionic gonadotropin (Sigma-Aldrich, Schnelldorf, Germany) solved in Ringer's solution (150 mM NaCl, 3 mM KCl, 3.5 mM $MgCl_2$, 5 mM $CaCl_2$, 10 mM HEPES, pH 7.4) to induce spermiation at the ratio of 1 UI/g fish body weight (Goto-Kazeto et al., 2006). After overnight, the sperm samples were taken by abdominal massage with a micropipette avoiding any contamination. During semen collection, the anal fins of the fish were dried and the sample of each fish in a replicate was collected into the same Eppendorf tube. In this way, a pooled semen sample for each replicate which was sufficient to motility and seminal plasma analyses was constituted and stored on ice until ready for use.

Fresh spermatozoa samples were immediately counted hemocytometrically and motility of spermatozoa was recorded with a Leica DM750 (Leica Microsystems, Switzerland) microscope with a phase contrast attachment mounted a Leica MC190 HD camera. Duration of motility was determined by subjectively as the times until forward movement stopped and circular movement began. The evaluation of motility parameters was performed mainly according to Boryshpolets et al., (2013). The video recordings were saved in AVI format by using a microscope software platform (Leica Application Suite), and then processed using Virtual Dub software (http://virtualdub.org). The video recordings were evaluated by ImageJ which is open source software available with a specific CASA plug-in (Wilson-Leedy and Ingermann, 2007; Schneider et al., 2012). According to the video records, percentage of motility (%), curvilinear velocity (VCL, μm/s) in the 15^{th}-second record were determined with the CASA plug-in of ImageJ software. A spermatozoa activation solution (50 mM NaCl, 20 mM Tris, pH 8.5, Zadmajid et al., 2013) containing 0.5% bovine serum albumin (BSA) was used for triggering the motility, and a non-activating solution (95 mM NaCl, 48 mM KCl 1.7 mM $CaCl_2$, 25 mM $NaHCO_3$, pH 8.5, Ravinder et al., 1997), when needed. For seminal plasma analyses, the semen samples were centrifuged 10 minutes at 12000 rpm and 4°C two times. According to Bradford 1976, protein concentrations of seminal plasma samples were determined by the Bradford method, using calibration curve based on different BSA concentrations. Catalase activity (CAT) and lipid peroxidation level (LPO) in seminal plasma were measured spectrophotometrically. CAT was determined by hydrogen peroxide and ammonium molybdate ($(NH_4)_6Mo_7O_{24}$) complex at 405 nm (Goth, 1991) while LPO was deteced by thiobarbituric acid reactive substance assay that monitors malondialdehyde production (Buege and Aust, 1978).

Statistical analysis

Kolmogorov-Smirnov test and Levene's test were used to reveal normality and homogeneity of variance of each variable, respectively. A log transformation or *arcsin* transformation for the percentage data was performed on data. Mean values of blood and semen parameters were given with the standard error of the mean (SEM) for a parameter of all treatments and its *P* value to become more comprehensible. Each parameter was analyzed using ANOVA followed by Tukey's HSD test. Statistical analysis was performed using SPSS 17.0 software (SPSS Inc., Chicago, USA). $P < 0.05$ was set as statistical significance for all data.

RESULTS

The sugar beet extractions content

The main chemical constituents of three different SBLE which added to the feed were determined. The aerial parts of the E extract essential oils are given in Table 2. The major constituent of this extract was phytol. The other common essential oils are pentacosane, neophytadiene, hexatriacontane, diethyl phthalate, cycloheptasiloxane, tetradecamethyl, and vitamin E. Total protein in the P extract was found as 6.82 ± 0.92 mg g^{-1} wet weight. Glucose and fructose concentrations of the C extract were 2.19 ± 0.23 µg g^{-1} wet weight and 26.88 ± 2.42 µg g^{-1} wet weight, respectively while sucrose was not detected.

Table 2. Chemical composition of the aerial parts of sugar beet leaves essential oils (%)

Compounds	Composition %
Phytol	**56.28**
Phytol, trimethylsilyl ether	1.50
Phytene-2	1.80
Diethyl phthalate	3.02
Neophytadiene	**4.54**
Pentacosane	**5.43**
Hexatriacontane	**4.49**
Tetracontane	1.74
Eicosane	0.37
Nonacosane	0.35
Eicosamethylcyclodecasiloxane	0.37
Cycloheptasiloxane, tetradecamethyl-	2.70
Cyclooctasiloxane, hexadecamethyl-	1.76
Cyclononasiloxane, octadecamethyl-	1.39
Cyclohexasiloxane, dodecamethyl-	0.88
Silicone oil	0.38
Vitamin E	**2.66**
Tetracosamethylcyclododecasiloxane	2.33
Methyl linolenate	1.59
Ethyl linolenate	0.46
Squalene	1.09
2-Nonadecanone	0.53
Palmitic acid, trimethylsilyl ester	0.41
4,8,12,16-Tetramethylheptadecan-4-olid	0.30
Total	**96.36**

Effects of the sugar beet extractions on blood characteristics of goldfish

The fish hematology and their blood serum biochemical values are shown in Table 3. TP amounts were found to increase in the experimental groups compared to the control group. The highest value was observed in the P20 group ($P < 0.05$). Similarly, ALB results found in the highest amount in the group fed with the P20 extract supplementation and found statistically different from the control group ($P < 0.05$). GLU were lower in the control, the P5 and the E5 groups and found statistically different from the other groups ($P < 0.05$) at the end of 60 days feeding period. CHO levels showed a reducing trend in the E extract supplemented diet groups. The lowest CHO value obtained in the E20 group and found

statistically different from control (P < 0.05). TRIG values showed no significant differences between experimental groups (P > 0.05). AST activities were found lower in the E5, P20 and E20 groups and statistically different than the control group (P < 0.05). AST activities were tented to lower in all SBLE groups that the control however, there were statistically differences between the E extract supplemented diet groups and the control (P < 0.05). Similarly, LDH values were increasing in the E extract supplemented diet groups. Hematological blood parameters are similar in all experimental groups in terms of RBC. However, the lowest RBC value obtained in P20 group. The highest Hb values were determined in E5, E20 and C20 groups comparing to the control (P < 0.05). The significantly lowest Hct values were obtained in the P5 and P20 groups (P < 0.05). Other SBLE supplemented groups showed the similarities regarding to Hct. Also, the highest MCV, MCH), MCHC were calculated in the C20 group.

Table 3. Effect of dietary sugar beet extractions on the blood parameters of goldfish

Item	Control	P5	E5	C5	P20	E20	C20	SEM	P value
TP	6.57^{a}	6.78^{a}	7.69^{bc}	7.46^{b}	9.84^{d}	8.10^{c}	8.07^{c}	0.16	<0.01
GLU	51.26^{a}	53.73^{a}	58.54^{b}	68.62^{a}	62.21^{b}	67.07^{c}	74.47^{d}	1.27	<0.01
ALB	0.07^{a}	0.11^{b}	0.10^{b}	0.12^{b}	0.18^{c}	0.12^{b}	0.10^{b}	0.01	<0.01
CHO	179.68^{ab}	161.95^{abc}	140.97^{bc}	212.77^{a}	197.95^{a}	120.20^{c}	170.08^{abc}	6.18	<0.01
TRIG	76.15	90.75	130.19	101.22	107.27	90.79	91.46	4.88	0.09
AST	21.44^{a}	18.69^{ab}	15.85^{b}	16.07^{ab}	14.95^{b}	14.82^{b}	19.37^{ab}	0.57	<0.01
ALT	1.12^{abc}	1.24^{bc}	1.11^{abc}	1.30^{c}	0.81^{a}	1.04^{abc}	0.85^{ab}	0.04	<0.01
LDH	131.73^{ab}	138.35^{ab}	123.25^{ab}	169.84^{a}	130.39^{ab}	106.95^{ab}	134.47^{b}	4.89	0.03
RBC	2.21^{ab}	2.26^{ab}	2.39^{b}	2.34^{b}	2.05^{a}	2.32^{b}	2.28^{b}	0.02	<0.01
Hb	9.00^{abc}	8.50^{a}	10.44^{de}	9.68^{bcd}	8.83^{ab}	10.03^{cde}	10.80^{e}	0.15	<0.01
Hct	27.29^{ab}	17.20^{c}	27.75^{a}	26.28^{ab}	22.53^{e}	24.17^{de}	25.63^{bd}	0.55	<0.01
MCV	108.30^{a}	119.20^{b}	121.01^{b}	114.50^{c}	110.73^{ac}	119.97^{b}	136.87^{d}	1.39	<0.01
MCH	40.90^{a}	42.63^{ab}	44.34^{bc}	43.62^{b}	43.92^{b}	42.83^{b}	46.23^{c}	0.28	<0.01
MCHC	36.67^{a}	37.45^{a}	36.66^{a}	38.50^{abc}	40.46^{c}	39.28^{bc}	40.50^{c}	0.29	<0.01

P5, feed with 5 ‰ protein extract; P20, feed with 20 ‰ protein extract; E5, feed with 5 ‰ essential oil extract; E20, feed with 20 ‰ essential oil extract; C5, feed with 5 ‰ carbohydrate extract; C20, feed with 20 ‰ carbohydrate extract.
TP(g/L), total protein; GLU(mg/dL), glucose; ALB(g/dL), albumin; CHO(mg/dL), cholesterol; TRIG(mg/dL), triglycerides; AST(U/g protein), aspartate aminotransferase; ALT(U/g protein), alanine aminotransferase; LDH(U/g protein), lactate dehydrogenase; RBC($x10^{6}$/µL), red blood cells; Hb(g/dL), haemoglobin concentration; Hct (%), haematocrit; MCV(fL), mean corpuscular volume; MCH(pg/cell), mean corpuscular haemoglobin; MCHC(%), mean corpuscular haemoglobin concentration.
Data within each row of dietary groups with no common superscript differ significantly at P < 0.05. The standard error of the mean (SEM) for each parameter are given.

Effects of the sugar beet extractions on sperm characteristics of goldfish

Parameters of sperm motility and seminal plasma in different groups are presented in Table 4. In general, the motility increased in the treatment supplemented with SBLE, especially the E extract supplementations. Similarly, durations of sperm motility and sperm densities in the E20, E5, and C20 groups in descending order were higher than those in the control (P < 0.05). The fish fed diets with SBLE were found to have higher levels of protein concentrations their seminal plasma. Significant decreases in LPO levels and significant increases in CAT of the seminal plasma samples obtained from the E20 and C20 extract groups were measured (P < 0.05).

Table 4. Effect of dietary sugar beet extractions on the semen parameters of goldfish

	Control	**P5**	**E5**	**C5**	**P20**	**E20**	**C20**	SEM	P value
VCL 15th s	130.47^{a}	128.94^{a}	152.37^{b}	131.56^{a}	130.02^{a}	157.66^{c}	143.85^{d}	0.80	<0.01
% 30th s	59	56	61	57	59	66	62	0.92	0.06
Duration(s)	91^{a}	90 ^{a}	119^{b}	101^{c}	89^{a}	124^{d}	106^{c}	2.92	<0.01
Sperm density (x10^{9}/ml)	6.30^{a}	6.85^{a}	10.65^{b}	8.06^{c}	6.57^{a}	12.31^{d}	8.33^{c}	0.47	<0.01
Protein (g/l)	1.24^{a}	1.27^{a}	1.34^{a}	1.37^{a}	1.88^{b}	1.82^{b}	1.46^{a}	0.04	<0.01
LPO (nmol/mg protein)	0.37^{a}	0.39^{a}	0.36^{ab}	0.37^{a}	0.35^{ab}	0.25^{c}	0.30^{bc}	0.01	<0.01
CAT (U/mg protein)	9.76^{a}	9.25^{a}	11.13^{a}	10.57^{a}	11.75^{a}	16.74^{b}	16.07^{b}	0.49	<0.01

P5, feed with 5 ‰ protein extract; P20, feed with 20 ‰ protein extract; E5, feed with 5 ‰ essential oil extract; E20, feed with 20 ‰ essential oil extract; C5, feed with 5 ‰ carbohydrate extract; C20, feed with 20 ‰ carbohydrate extract.
VCL(µm/s) curvilinear velocity; LPO, lipid peroxidation level; CAT, catalase activity.
Data within each row of dietary groups with no common superscript differ significantly at $P < 0.05$. The standard error of the mean (SEM) for each parameter are given.

DISCUSSION

Plant extracts and their essential oils are frequently considered to have less side effects fish in comparison with chemical ones used in fish diets to improve health status of fish (Nya and Austin, 2009; Acar et al., 2015). Besides, increasing aquaculture production has been generated a rising demand of fish feed. Thus, alternative ingredients for it are attracting research interest. SB, as one of the common agricultural crops in the world, is needed to study its chemical and biological effects in animals. Furthermore, after harvesting of SB, a great majority of its leaves remains as a waste product while its roots have actual economic value. Also, each country has regional systems that link between harvesting of sugar beet from the field to sugar factories. These systems make plenty of SBL easily obtain without expense or at small expense. This study evaluated the effect of SBLE obtained by different methods on the blood hematological and serum biochemical profiles and sperm characteristics of goldfish.

Application of natural products in aquaculture should be continued to find suitable plant-derived feed additives to maximize growth and health status, sexual stimulation, hepatoprotective effects in fish (Citarasu et al., 2003). In the present study, the evident effect of SBLE used different levels in diets has been determined in serum biochemical profiles and hematological parameters of goldfish. Blood functions are related with particularly the transportation of nutrients and oxygen in the fish. Therefore, blood parameters are useful to determine fish health status response to dietary feed supplements (Lin et al., 2015). In the present study, serum TP values were found the higher in all groups fed with different level of SBLE than those in the control. Similarly, the increases in serum TP of hybrid grouper and crucian carp (*Carassius carassius*) caused by ginkgo leaves extract added to feed have been reported (Tan et al., 2018). The increase of serum TP levels in fish is related to a strong immune response (Wiegertjes et al., 1996). It may be explained by the fact that various immunostimulant originating from herbs do not induce to osmoregulatory dysfunction and any damage in tissues and blood vessels in fish. Moreover, serum GLU values of fish are affected by daily feeding (Kenari et al., 2011). In the present study, the serum GLU values were found highest in C5 and C20 groups. In this study, the high glucose and fructose concentrations in the C extracts were determined, and this could be the main reason for the high GLU values of these treatments. On the contrary, some plant products such as *Aloysia triphylla* and *Citrus sinensis* extracts in feed reduced GLU levels in fish (Acar et al., 2015; Gressler, 2014). Also, different fish species could give different response to saponins and flavonoids contents of used extracts in diets. ALB in fish blood performs the transportation of lipids and helps general metabolism of fish (Andreeva, 1999). This study showed that dietary SBLE increased serum ALB levels among the experimental groups compared to the control group. In the present study, serum CHO levels were found the lowest in the E20 group. Lowering effects of herbal extracts on CHO are well known and reported in Mozambique tilapia fed with the essential oils of ginger and orange peel (Immanuel et al., 2009; Acar et al., 2015). In the present study, phytol content of E extract was 56.28%. Rinchard et al. (2003) reported that the presence of phytosterols could be the main reason for the decrease of serum CHO levels. The

present study showed that dietary SBLE caused decreases in serum ALP, ALT and LDH values in the experimental groups compared to the control. The administrated SBLE in appropriate doses showed the positive effects of fish liver tissue if added diet with an appropriate extraction method. SBL have been used as phytotherapeutic in human for a long time due to their healing effects on liver (Ninfali and Angeino, 2013). Biochemical changes in hepatic enzymes activities are indicator for liver function (Abdel-Wahhab et al., 1999). Similar to our results, decreases in the liver enzymes activities were observed in rainbow trout (*Oncorhynchus mykiss*) fed with olive leaf extract (Baba et al., 2018). The haematological parameters such as RBC, Hb, Hct, MCV, MCH, and MCHC in blood indicate the health status of fish and their viscera when determining any abnormality occurring owing to the use of immunostimulants in diets (Tewary and Patra, 2011). There were no significant differences in RBC between treatment groups. Also, Hct values of treatment groups showed similar trend however, only P5 groups showed lower Hct values. Also, the dietary administration of SBLE did not cause any adverse effects on Hb content in all experimental groups. Moreover, the significantly higher Hb contents were obtained in the E groups fed with the E extracts supplemented feeds. A decrease in the amount of Hb reduces the transport capacity of oxygen in fish, even cause to reduce growth performance (Wells et al., 2005). Gabor et al. (2012) reported that anemia characterized by decreases Hct and RBC in common carp fed with 1% ginger powder for 93 days. This could be due to the prolonged time of this treatment. Therefore, it is necessary to adjust the dose of herbal supplement and the period of supply to avoid toxic effects in fish. It could be made an inference that any dietary SBLE do not cause anemia in the experimental fish for 60 days of feeding period.

Some factors such as photoperiod, feeding, stress, age, and diseases has been shown to affect the sperm quality in cultured fish (Rurangwa et al., 2004). Enrichment in nutrition of fish considerably improves the sperm quality and sperm production as well (Izquierdo et al., 2001). In this study, all measured semen parameters have been significantly affected by the feed with the addition of different SBLE. Sperm VCL values were improved by the feed added the E extracts. Also, the C20 extract had positive effects on sperm motility. Moreover, the lowest LPO level and the highest CAT were found in the E20 extract groups. The most remarkable difference was found in sperm density that the density values obtained from the E20 extract group were almost two times higher than those in the control. Tizkar et al. (2015) reported that dietary supplementations of astaxanthin and ß-carotene improved sperm motility and increased sperm density almost two times higher than the control. The SBLE caused increases in protein levels of seminal plasma. Additionally, LPO levels increased while CAT decreased in the seminal plasma of the fish fed with supplementation of the E20 extract compared to the seminal plasma of the fish in the control. Some substances like polyunsaturated fatty acids and vitamins like vitamin C and E in feed enhance reproductive performance of fish. Lipids in feed could change the composition of sperm membrane, which is a very critical factor for sperm quality (Labbé et al., 1995; Labbé and Maisse, 1996). Also, vitamin E has a role in the control of cell membrane permeability and stability (Lucy, 1972). Improvement on the sperm quality by dietary vitamin E has been shown in rainbow trout (Canyurt and Akhan, 2008). Vitamin E like Vitamin C could have a capacity to reduce lipid peroxidation, thus protects sperm cells (Dabrowski and Ciereszko, 1996). The E extract contains %2.66 of Vitamin E, this may be a reason for improvement in sperm quality. Moreover, phytol as a precursor for the production of tocopherol could be associated with this (Valentin and Qi, 2005). Herbal products are not always favorable for fish gametes. For instance, gossypol from cotton seeds prevented reproductive performance in female and reduced sperm motility whereas it had no effect on fish growth and mortality (Rurangwa et al., 2004).

The dietary supplementation of SBLE have positive effects on serum biochemical profiles, particularly the E extract which contains the essential oils improves semen quality parameters. This could be used especially for male broodstock nutrition, should test for female broodstock. Also, these extract could be a part of fish feeding as feed additives in further studies.

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