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Effect of dietary supplementation of copepod meal in goldfish *Carassius auratus* (Linnaeus, 1758) on growth performance, fatty acid profile and colouration

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ABSTRACT

This study assessed the use of copepod meal (*Arctodiaptomus salinus)* as a carotene source for colour enhancement in goldfish *Carassius auratus* (Linnaeus, 1758). The effect of copepod meal on growth and fatty acid profile of goldfish was also evaluated. Copepod meal with 4,338 μg g^{-1} of total carotene and 1,655.78 μg g^{-1} of β-carotene per gram dry weight was added to the diet containing 0 (control), 50, 150 and 300 μg g^{-1} of β-carotene. Four diets with similar protein (35.17% CP) and energy (3745 kcal g^{-1}) levels were prepared. The fish were fed with diets twice a day for 60 days. The results showed that there was no statistically significant difference between body weight, total length, condition factor and survival rates of the groups fed on different diets. Body coloration measured in terms of $L^*a^*b^*$ values increased with the increase in level of copepod meal in the diet. In all experimental groups, copepod meal incorporated diets had a significant effect on the fish flesh fatty acid profile ($p<0.05$), especially in terms of ecosopentaenoic acid (EPA) and docohehexoenoic acid (DHA) compared to those of the control group. However, a positive trend was observed in terms of all parameters in the group fed with the diet containing 300 μg g^{-1} of β- carotene.

Keywords: β-carotene, Colour, Fatty acids, Live feeds, Ornamental fish, Survival rate

Introduction

The calanoid copepod *Arctodiaptomus salinus* is a species completely adapted to planktonic life. It is a cosmopolitan species distributed in the aquatic systems of Eurasia and North Africa (Anufriieva and Shadrin, 2014; Shadrin and Anufriieva, 2018). It is also well adapted to harsh environmental conditions of the salty lakes in the Caucasian and Siberian regions (Tolomeev *et al.,* 2010). *A. salinus* is a predominant species of the Yarıslı Lake in the Burdur Province, Turkey (Demirhindi, 1972). The lake Yarıslı is a closed basin that has an altitude of 913 m from mean sea level, surface water spread area of approximately 15 km^2 and a maximum depth of 2 m. It is a hyposaline and highly alkaline lake. The population density of *A. salinus* in Lake Yarıslı has been reported as 3,74,266 individuals per m^3 (Anon., 2013).

Copepods are used as a source of protein in fish feeds because of their high nutritional content (protein 24-84 and lipid 8-12% of dry matter) (Stottrup, 2003). It is well known that copepods contain various carotenoids and are an important source of natural carotenoids (Pasarin and Rovinaru, 2018; da Costa and Miranda-Filho, 2019). Stottrup (2003) reported that only astaxanthin and its esters as a carotenoid derivative can be found @ 1133 μg g^{-1} wet weight of copepods.

Synthetic and natural carotenoid sources are used for colour enhancement of aquacultured species. Synthetic astaxanthin and cantaxanthin are the most used carotenoid sources in fish feeds. Current market trends are towards natural carotene sources considering the high cost of synthetic pigment materials (Duru, 2014). Several studies show that the most suitable natural carotenoid sources for use in fish feeds are blue green algae (*e.g. Spirulina*), fungi, copepods and water fleas (Hekimoglu, 2005; Duru, 2014). As in other animals, fishes are unable to perform "*de novo* synthesis" of carotenoids and therefore rely on diet to meet the need of carotenoids (Duru, 2014). Carotenoids taken up by different tissues and organs (skin, scales, fin, operculum, liver, bile, egg, blood and adipose tissue) can accumulate in different amounts. However, their rates of acumulation vary with factors such as age, size, sexual maturity and gender of the fish. Carotenoids accumulated in muscles towards the breeding time are transferred to the ovaries in females and especially to the skin in males (Yanar and Tekelioglu, 1999; Yesilayer *et al.,* 2008), suggesting a function in reproduction (Torrissen, 1984).

Fish meal with high nutritional value and flavour is preferred as the main source of protein and lipid in the preparation of fish feeds. However, difficulties experienced in fish meal production and high cost of raw material have led research to find cheaper alternative protein and lipid sources that can replace fish meal completely or partially (Ajiboye *et al.,* 2011; Tacon and Metian, 2015). In this study, the effect of copepod (*A. salinus)* meal on the growth performance, colour and fatty acid profile of goldfish *Carassius auratus* (Linnaeus, 1758) was investigated with partial substitution of commercial fish meal.

Materials and methods

Fish

Goldfish *C. auratus* used for the experiments were obtained from a private ornamental fish producer. The fish were fed with control diet for 15 days to adapt to the experimental conditions. After the 15th day, goldfish with a total length of 4.585±0.085 cm and an average body weight of 1.175±0.075 g were randomly assigned to 8 aquaria at a stocking density of 15 individuals per group.

Copepod supply and meal preparation

A. salinus was collected (1.5 kg) from Yarıslı Lake using 500 µm pore size plankton net from a dominant population in January 2015, when intense copepod reproduction occurred. The collected samples were selected, washed with freshwater and, after squeezing out excess water (Fig. 1a), transported to the laboratory in cold chain and kept at -20°C. In order to prepare copepod meal, copepod stored at -20ºC was thawed at 4ºC and dried in oven for 24 h at 50°C and then stored at -20ºC in deepfreeze until use (Fig. 1b).

Experimental diets

Feed ingredients used as the basic protein source in experimental diets were supplied from Korkutelim Yem Gıda Sanayi Ticaret A.S. Company, Turkey. Nutritional content of feed ingredients is given in Table 1.

The experimental diets were prepared in the Akdeniz University Fisheries Faculty Research Laboratory. Feed ingredients and copepod meal used for experimental diets were ground in Sinbo SCM-2906 coffee grinder to the desired particle size. The ground ingredients were then passed through a sieve with 595 µm mesh.

(a) (b)

Fig. 1. (a) Live copepod (*A. salinus*) samples collected from the lake Yarıslı. (b) Dried copepod (*A. salinus*) meal

Table 1. Chemical composition of copepod (*A. salinus*) and fishmeal (g 100 g^{-1}, wet wt.)

Parameter	Copepod meal	Fish meal
Dry matter	93.14±0.13	89.87±0.04
Moisture	6.86±0.13	10.13±0.04
Crude protein	38.57±0.57	65.98±0.66
Crude fat	31.84±0.39	11.70±0.53
Crude ash	9.44±0.39	12.15±0.38

Values are average of three analyses (±SD).

Control diet having 45% crude protein, 10% crude lipid and 3744.60 kcal g^{-1} digestible energy (DE) was prepared with fish meal and soybean meal according to the nutritional requirements for goldfish specified by NRC (1993). Experimental diets (Diet-50, Diet-150 and Diet-300) were prepared by adding copepod meal to have β-carotene content of 50, 150 and 300 µg g^{-1}, respectively, replacing corresponding quantity of commercial fish meal in the control diet (Diet-0).

In order to prepare the experimental diets, each feed ingredient was weighed using Scaltec SPB 42 digital scales with an accuracy of 0.001 g. All weighted ingredients were thoroughly blended for uniform distribution before adding oil and water (up to 40%). The mixture was passed through a meat grinder with a 2 mm sieve. After feeds were dried at room temperature in a closed room for one day, they were dried in an oven at 40°C for about a day. Dried feeds were broken in to suitable dimensions so that the juvenile fishes are able to ingest, placed in plastic bags and stored at -20ºC until use. Details of diet formulation and nutritional content of experimental diets are given in Table 2.

Experimental design

The experiments were carried out in a special aquarium fish production facility (Gurler Pet Shop, Antalya) between 10 August 2015 and 24 October 2015, in 8 aquara of 30x30x40 cm during a 60-day feeding period. After the 15-day adaptation period, a total of 120 goldfish were equally assigned to 8 aquaria. The length, weight and colour measurements of the fish were performed at the start of the experiment. The experiment was planned in 2 repetitions according to the random parcel trial pattern. Natural daylight was used during the experiment. A central air motor (size S-400B airpump) was used to aerate the aquarium water and the air was evenly distributed to the experimental aquaria with air stones.

The fish were manually fed for 60 days, 3 times a day (07:00, 14:00 and 19:00 hrs) until saturated (Davies *et al.,* 1990). The feed consumption of groups was determined in 15-day periods.

Table 2. Formulation and proximate composition of the experimental diets (%) prepared according to β-carotene level of copepod (*A. salinus*, μg g^{-1})

Ingredients	Experimental groups			
	Diet-0 (Control)	Diet-50	Diet-150	Diet-300
Fish meal	44.0	43.3	42.0	40.0
Copepod meal	0.0	1.162	3.478	6.926
Soybean meal	32.0	32.0	32.0	32.0
Wheat meal	10.0	10.0	10.0	10.0
Dextrin	3.900	3.738	3.322	2.674
Fish oil	4.5	4.2	3.6	2.8
Vit mix.[1]	2.0	2.0	2.0	2.0
Mineral mix.[2]	2.0	2.0	2.0	2.0
NaCl[3]	0.1	0.1	0.1	0.1
$CaHPO_4.2H_2O$[4]	0.5	0.5	0.5	0.5
CMC[5]	1.0	1.0	1.0	1.0
Total	100	100	100	100
Proximate composition (%, wet wt.)				
Crude protein	35.32±0.29	34.79±0.11	35.33±0.18	35.26±0.14
Crude lipid	12.26±1.55	11.09±1.12	11.02±0.48	10.95±1.36
Crude ash	11.71±0.12	11.24±0.27	11.36±0.17	11.36±0.25
Moisture	6.38±0.35	6.98±0.02	7.18±0.25	6.49±0.37
Dry matter	93.64±0.00	93.04±0.02	92.84±0.27	93.52±0.10
NFE[6]	34.35±0.79	35.92±1.49	35.13±0.57	35.95±1.34
DE (kcal g^{-1})[7]	3747	3745	3743	3743

[1]Vitamin mix. (per kg contains; Vit-A: 4 000 000 IU, Vit-D3: 600 000 UI, Vit-E: 40 000 mg, Vit-K3: 2 400 mg, Vit-B1: 5 000 mg, Vit-B2 : 8 000 mg, Vit-B6: 4 000 mg, Vit-B12: 12 mg, Vit-C: 40 000 mg, Niacin: 50 000 mg, Folic acid: 1 400 mg, Calcium D-Pantothenate: 8 000 mg, D-Biotin: 50 mg, Inositol: 40 000 mg); [2]Mineral mix (per kg contains; manganese 60 000 mg, iron 10,000 mg, zinc 75 000 mg, copper 5 000 mg, cobalt 1 000 mg, iodine 2 500 mg, selenium 100 mg and magnesium 65 000 mg); [3]Sodium chloride; [4]Calcium hydrogen phosphate; [5]Carboxy-methyl cellulose; [6] Nitrogen free extract; [7] Digestible energy (DE) was calculated using 4.9 kcal g^{-1} for digestible energy protein, 9.01 kcal g^{-1} for fat and 3.49 kcal g^{-1} for carbohydrate (Chiou and Ogino, 1975; NRC, 1993). Values are an average of three analyses (±SD).

Weight and length measurements

The fish were weighed individually using a Scaltec SPB 42 digital scale with an accuracy of 0.001 g and the total length measurements were taken using a 1 mm division ruler, every 15 days. Clove oil (20%, v:v) was used to anesthetise the fish during measurement and weighing processes (Kanyilmaz *et al.,* 2007).

Colour measurement

L*a*b* values were used for colour measurements (Anon., 2008). The colour of the fish in each group was measured individually using the Konica Minolta CR400/410 Portable Colour Meter (Chrometer) to determine the L*a*b* values.

Water quality

The optimal water temperature in the aquaria was adjusted to ambient conditions. The average values of water quality parameters recorded are given in Table 3.

Chemical analysis

On termination of the feeding experiments, 3 fish were randomly removed from each aquarium and stored

Table 3. Avarage values of water quality parameters of experimental aquaria

Parameter	Experimental groups			
	Diet-0 (Control)	Diet-50	Diet-150	Diet-300
Light intensity (lux)	119.50±58.50	105.00±25.00	112.00±2.00	93.00±13.00
Temperature (°C)	19.60±0.10	19.50±0.10	19.50±0.10	19.50±0.10
Conductivity (μS cm^{-1})	755.00±5.00	752.50±2.50	752.50±2.50	752.00±3.00
Salinity (g l^{-1})	0.40±0.00	0.40±0.00	0.40±0.00	0.40±0.00
pH	7.88±0.01	7.89±0.01	7.89±0.01	7.90±0.02
Disolved oxygen (mg l^{-1})	8.39±0.01	8.46±0.07	8.46±0.06	8.48±0.08
Turbidity (NTU)	0.85±0.14	0.93±0.21	0.93±0.16	0.87±0.03

at -20°C for chemical analysis. The feed ingredients, copepod, experimental diets and the final fish flesh were analysed for chemical composition, fatty acids and total β-carotene.

Moisture and dry matter analyses were performed by weighing the feed ingredients, fish flesh and diet samples in a desiccator after drying them at 110°C until they reached a constant weight. Protein analysis was determined by burning a certain amount of nitrogen-containing sample according to the Kjeldahl method with sulfuric acid, converting all nitrogen $(NH_4)_2SO_4$, basifying the solution with sodium hydroxide, and titrating the released NH_3 in the boric acid solution by the amount of hydrochloric acid. Crude lipid analysis was carried out by the ether extract method employing soxhlet apparatus and the crude ash content was determined by burning the total inorganic substance at 550ºC (AOAC, 1995). Nitrogen-free extract (NFE) of feeds were calculated using the formula, NFE (%) = 100 - (%Moisture + %Protein + %Fat + %Ash) (Morales *et al*., 1994).

Carotene content and fatty acid analysis of copepod meal, lipids of experimental diets and fish flesh were performed after derivatising for GC-MS (AGILENT 5975 C AGILENT 7890A GC) (Bardakcı and Secilmis, 2006). Derivatisation of lipids in the copepod sample, experimental diets and fish flesh was carried out using 1.5 m methanolic HCl (Bligh and Dyer, 1959). For the analysis of fatty acids, HP-88 (100*0.250*0.20 µm) column was used. The column starting temperature was set at 60ºC and after 1 min it was increased to 175°C with an increase of 13°C per min and then to 215°C with 4ºC increments and maintained at this temperature for 35 min. Injector and detector temperatures were set at 250ºC (Bardakcı and Secilmis, 2006).

Statistical analysis

The experiment data were analysed using the SPSS 15.00 package. Levene's test was used to test if data were homogeneously distributed. After applying variance homogeneity tests to all data, variance analysis (ANOVA) was performed and differences between group averages were determined by Duncan's multiple comparison test ($p<0.05$). Results are given as mean±standard deviation (Mean±SD).

Results and discussion

Growth performance

Initial and final growth parameters of goldfish fed with experimental diets incorporating copepod (*A. salinus)* meal replacing appropriate quantity of fish meal to have 0, 50, 150 and 300 µg g^{-1} β-carotene, to the control diet are given in Table 4.

Differences in weight change between the experimental groups was statistically insignificant ($p>0.05$), but the live weight tended to increase with increase in copepod meal incorporation. Best growth was observed in the group fed with diet containing 300 µg g^{-1} β-carotene compared to the control group. Yanar and Tekelioglu (1999) found that the difference in body weight gain between groups was statistically insignificant in goldfish fed with diets with the addition of 0, 200, 400, 600, 800, 1000 µg g^{-1} of cantaxanthin. A similar result was obtained in a study on goldfish fed with feed containing 25, 50 and 75 mg kg^{-1} of *Spirulina platensis* powder (Duru, 2014). The results of the present study are consistent with their findings. The change between length values of the experimental groups was statistically insignificant ($p>0.05$). Although there was an irregular change in the length values between groups, the control group and Diet-300 group containing 300 µg g^{-1} β-carotene had higher total and fork length values than others, which is consistent with the results of Yanar and Tekelioglu (1999).

There were no statistically significant differences between the groups in terms of the condition factor values at the end of the experiment ($p>0.05$). Duru (2014) had also reported that there was no significant difference in condition factors between goldfish feeds supplemented with 25, 50 and 75 mg kg^{-1} of *S. platensis* powder. Survival rates of groups containing control, 50, 150 and 300 µ g^{-1} β-carotene were also not statistically different.

Table 4. Growth performances of fish fed with experimental diets

Parameters	Experimental groups[1]			
	Diet-0 (Control)	Diet-50	Diet-150	Diet-300
Initial body weight (g)	1.44±0.19	1.49±0.37	1.41±0.01	1.45±0.21
Final weight (g)	3.67±0.42	3.39±0.37	3.34±0.30	3.81±0.58
Initial body length (cm)	4.65±0.19	4.64±0.25	4.74±0.18	4.98±0.01
Final body length (cm)	5.63±0.16	5.49±0.02	5.61±0.02	5.90±0.16
Condition factor (CF)[2]	2.23±0.08	2.15±0.21	1.98±0.15	1.81±0.11
Survival rate (%SR)[3]	83.34±16.67	86.67±13.34	83.34±16.67	83.34±16.67

[1]The differences between the averages in the same row are statistically insignificant ($p>0.05$). [2]Condition factor (CF, g cm^{-3}) = W / L^3 x 100, W: Fish weight (g), L: Fish length (cm); [3]Survival rate (% SR) = (A / B) x 100, A: Number of fish at final period, B: Number of fish at initial period.

Colour parameters

In the present study, there was a statistically significant difference between the groups in terms of L*a*b* values (colour measurement parameters) ($p<0.05$) (Table 5). In the experimental group whose average baseline L* values were 41.03±1.91, the best a* and L* values (15.05±3.78 and 48.93±2.49, respectively) were observed in the group fed with Diet-300; on the other hand, b* value was highest and statistically similar to that of the group fed with Diet-150. The increase in the amount of copepod meal used in the feed led to increase in L*a*b* values. Using carophyll-red colourant giving 10% red colour, Yanar and Tekelioglu (1999) found that 1000-dose group produced the best result in terms of red colouration. In a colouration study on goldfish fed with *S. platensis* powder supplemented feed, Duru (2014) observed highest colouration in 75 mg kg^{-1} diet group.

At the end of the experiment, the lowest and highest survival rates of the experimental groups were 83.34 and 86.67%, respectively, which suggested no statistical difference between the groups. In a study on effect of carotenoid sources on colouration in goldfish, Rema and Gouveia (2005) also reported that differences in survival rates were statistically insignificant.

β-Carotene and fatty acid profile of copepod meal, experimental diets and fish flesh

β-carotene and fatty acid profile of copepod meal, experimental diets and fish flesh are given in Tables 6, 7 and 8. β-carotene content of the copepod was 1655.78 μg g^{-1}. It is well known that copepods inhabiting high altitude shallow lakes accumulate a copious amount of carotenoids in response to ultraviolet radiation stress (Rautio and Tartarotti, 2010; Schneider *et al.,* 2012). It is also known that the carotene content of copepods is higher than β-carotene content of *Artemia* and rotifers (Stottrup, 2003). The β-carotene content of *A. salinus* was reflected in the experimental diets, which caused the carotene content to increase due to the increase in level of copepod meal incorporation in the trial diets. β-carotene content of fish flesh in the experimental groups was found similar to that of β-carotene content of the experimental diets. Similar increases were observed in the fish flesh of the experimental groups. When compared to the control group, β-carotene content in fish flesh increased with increase in level of copepod meal in the experimental diets.

The saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) contents were found to be 35.83, 5.43 and 26.91%, respectively. The most important saturated fatty acids were heptadecanoic acid (C17: 0), palmitic acid (C16: 0) and stearic acid (C18: 0), respectively. The most important monounsaturated fatty acid was oleic acid (C18: 1). When the composition of PUFA was examined, the most important polyunsaturated fatty acids were eicosapentaenoic acid (EPA, C22: 5n-3), docosahexaenoic acid (DHA, C22: 6n-3), linoleic acid (C18: 2n-6) and arachidonic acid (C22: 4n-6), respectively. *A. salinus* was found to be rich in EPA and DHA, the most important components of PUFA.

Eryalcın (2018), found significant ($p<0.05$) differences in fatty acid contents of rotifer and *Artemia franciscana* enriched with six enrichers such as w-3 Olio, n-3 Top Rich, red pepper, Culture Selco, microalgae mixture (*Dunaliella salina* + *Chlorella vulgaris*) and Emulsion T. The enrichment process with n-3 Top Rich led to an increase in the amount of n-3 HUFA retention, whereas

Table 5. Initial and final colour L*a*b* values of fish fed with experimental diets

Parameter		Experimental groups			
		Diet-0 (Control)	Diet-50	Diet-150	Diet-300
L*	Initial value	41.37±4.27[b]	40.60±2.32[c]	39.80±0.43[d]	42.35±0.62[a]
	Final value	48.07±1.00[b]	48.01±1.07[b]	48.51±2.58[a]	48.74±2.53[a]
a*	Initial value	9.67±0.61[c]	11.62±1.27[b]	12.21±1.04[a]	12.14±2.05[a]
	Final value	11.33±1.02[d]	13.20±1.73[c]	14.37±2.37[b]	15.05±3.78[a]
b*	Initial value	32.36±0.12[d]	34.05±1.04[c]	34.64±2.72[b]	35.31±3.52[a]
	Final value	32.61±0.29[c]	33.58±1.19[b]	35.94±1.94[a]	35.18±3.04[a]

* [a-d]Values in the same row with different superscripts are significantly different ($p<0.05$).

Table 6. β-carotene composition of copepod meal (μg g^{-1}, dry wt.), experimental diets (μg g^{-1}, dry wt.) and fish flesh (μg g^{-1}, wet wt.)

Parameters		Experimental groups			
		Diet-0 (Control)	Diet-50	Diet-150	Diet-300
Copepod meal	1655.78	-	-	-	-
Experimental diets	-	2475.93	3609.88	5299.96	6723.01
Fish flesh	-	2902.77	2410.84	2689.22	5712.44

Values are an average of two analyses (±SD).

Table 7. Fatty acid composition of copepod (*A. salinus*) and experimental diets (%, dry wt.)

Fatty acids	Copepod *(A. salinus)*	Experimental groups			
		Diet-0 (Control)	Diet-50	Diet-150	Diet-300
C13:0	0.07	0.01	0.01	0.01	0.01
C14:0	5.88	5.44	5.33	6.05	7.29
C15:0	2.13	0.91	1.05	0.98	0.16
C16:0	15.46	24.43	25.57	34.35	33.60
C17:0	0.49	0.31	0.31	0.28	0.20
C18:0	10.84	18.93	18.15	11.47	10.66
C20:0	0.17	0.25	0.25	0.18	0.08
C24:0	0.79	0.82	0.88	0.74	1.01
∑SFA[1]	35.83	50.79	51.55	54.15	53.01
C14:1	0.84	0.28	0.35	0.30	0.03
C15:1	0.37	0.14	0.16	0.13	0.06
C16:1	0.56	6.60	7.97	6.94	8.50
C18:1n-9	2.79	6.43	5.69	8.83	9.75
C20:1	0.87	1.26	1.28	1.52	0.92
∑MUFA[2]	5.43	14.7	15.45	17.72	19.26
C18:2n-6	3.49	8.40	8.37	6.28	2.86
C18:3n-3	0.81	2.95	4.15	1.23	0.49
C20:4n-6	3.96	0.94	1.15	0.48	1.15
C22:2n-6	0.53	0.35	0.37	0.34	0.30
C20:5n-3	9.06	6.18	7.14	8.88	8.92
C22:6n-3	9.06	7.33	8.73	9.34	11.29
∑PUFA[3]	26.91	26.15	29.91	26.55	25.01

Fatty acids composition values are mean (±SD) of two analyses.
Total saturated fatty acids are: C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0 and C24:0. [2] Total monounsaturated fatty acids are: C14:1, C15:1, C16:1, C18:1n-9 and C20:1n-9. [3] Total polyunsaturated fatty acids are: C18:2n-6, C18:3n-3, C20:4n-6, C22:2n-6, C20:5n-3 and C22:6n-3.

Table 8. Fatty acid composition of fish flesh fed with experimental diets (%, dry wt.)

Fatty acids	Experimental groups			
	Diet-0 (Control)	Diet-50	Diet-150	Diet-300
C13:0	0.08±0.03^{a}	0.03±0.02^{b}	0.03±0.02^{b}	0.05±0.04^{b}
C14:0	0.48±0.00^{a}	0.41±0.10^{c}	0.35±0.14^{d}	0.44±0.15^{b}
C15:0	0.28±0.04^{b}	0.31±0.17^{a}	0.19±0.04^{d}	0.22±0.06^{c}
C16:0	21.24±4.25^{a}	14.65±0.70^{c}	14.53±1.99^{d}	17.31±3.71^{b}
C17:0	0.32±0.13^{a}	0.18±0.12^{b}	0.18±0.13^{b}	0.08±0.00^{c}
C18:0	15.42±3.19^{a}	12.76±1.35^{c}	12.25±3.10^{d}	12.85±2.19^{b}
C20:0	0.25±0.13^{a}	0.01±0.00^{d}	0.11±0.08^{b}	0.05±0.04^{c}
C24:0	1.23±0.10^{a}	1.04±0.10^{c}	1.07±0.17^{b}	1.23±0.22^{a}
∑SFA[1]	39.30±0.07^{a}	29.39±0.05^{c}	28.71±0.07^{d}	32.23±0.05^{b}
C14:1	0.43±0.35^{a}	0.04±0.01^{b}	0.05±0.01^{b}	0.04±0.01^{b}
C15:1	0.05±0.03	0.07±0.02	0.05±0.01	0.07±0.01
C16:1	2.81±1.00^{a}	1.56±0.05^{b}	1.43±1.28^{c}	0.98±0.52^{d}
C18:1n-9	25.52±13.10^{d}	43.72±0.69^{a}	41.09±12.15^{b}	32.48±11.27^{c}
C20:1	1.39±0.12^{a}	1.21±0.10^{c}	1.23±0.2^{b}	1.12±0.20^{d}
∑MUFA[2]	30.20±0.07^{d}	46.60±0.05^{a}	43.85±0.07^{b}	34.6±0.05^{c}
C18:2n-6	3.31±1.45^{b}	3.17±0.47^{c}	2.93±0.25^{d}	3.61±0.75^{a}
C18:3n-3	1.14±0.49^{a}	0.57±0.02^{c}	0.49±0.05^{d}	0.74±0.25^{b}
C20:4n-6	1.86±0.15^{b}	1.29±0.13^{d}	1.95±0.27^{a}	1.65±0.50^{c}
C22:2n-6	0.37±0.05^{a}	0.31±0.02^{ab}	0.30±0.02^{b}	0.37±0.07^{a}
C20:5n-3	3.03±0.64^{b}	2.69±0.23^{c}	2.45±0.87^{d}	3.69±0.77^{a}
C22:6n-3	13.54±1.81^{b}	10.43±0.00^{d}	12.10±3.20^{c}	14.78±3.49^{a}
∑PUFA[3]	23.25±0.05^{b}	18.46±0.07^{d}	20.22±0.07^{c}	24.84±0.05^{a}

* $^{a-d}$Values in the same row with different superscripts are significantly different ($p<0.05$). Fatty acids composition values are mean (±SD) of two analyses. [1]Total saturated fatty acids are: C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0 and C24:0. [2] Total monounsaturated fatty acids are: C14:1, C15:1, C16:1, C18:1n-9 and C20:1n-9.[3] Total polyunsaturated fatty acids are: C18:2n-6, C18:3n-3, C20:4n-6, C20:5n-3, C22:2n-6 and C22:6n-3.

A. franciscana nauplii enriched red pepper showed the highest HUFA accumulation.

With respect to the increase in the copepod meal level added to the experimental diets, SFA, MUFA and PUFA ratios were 50.79-54.15, 14.70-19.26 and 25.01-29.91%, respectively. Due to the decrease in fish meal and the increase in copepod meal in experimental diets, the SFA and MUFA values increased while the PUFA ratios decreased. Due to the increase in the rate of palmitic acid in saturated fatty acids having a high content in copepod meal, especially saturated fatty acids, it also caused an increase in SFA in experimental diets. The low MUFA value of *A. salinus* meal also affected that of the experimental diets. The EPA, DHA and linoleic acid values of the copepod meal led to a decrease in the PUFA values of the experimental diets.

A decrease in the SFA content of the fish flesh was observed depending on the reduction in the fish meal and the increase in the copepod meal in the experimental diets. The MUFA and PUFA contents of the fish fed on diets supplemented with copepod meal were higher than those of the control group. The MUFA content consists mainly of oleic acid. However, PUFA increased with decrease in fish meal and the increase in copepod meal in the experimental diets. The most important PUFA fatty acids were DHA, EPA and linoleic acid. The high content of DHA and EPA in copepod meal is reflected in the fish flesh, suggesting that the fatty acid content of the fish flesh is derived greatly from the fatty acid content of the fish feed.

Koca and Beyhan (2014) reported that red pepper produced the best results in terms of the EPA and DHA of fatty acid composition of *A. salina* fed with Oligo ω3, red pepper and *Nannochloropsis oculata*. The highest n3 HUFA and PUFA contents were also reported to be found in groups enriched with Oligo ω 3.

Live feed including DHA:EPA ratio of 2 prevented pigmentation disorder in flatfish larvae (Reitan *et al.,* 1994) and the recommended ratio of DHA:EPA for normal growth of marine larvae is >1.0 (Takeuchi *et al.,* 1998; Seoka *et al.,* 2007). Optimum DHA:EPA ratio of at least 1.2 has been reported for the development of seabream larvae (Rodriguez *et al.,* 1997). *A. salinus* in the present study was found to be rich in EPA and DHA, but rather low in monounsaturated fatty acids, especially compared to the fatty acid values in earlier studies on different copepod and rotifer species.

In the present study, compared to the control group, growth performance increased and improved in goldfish fed with experimental diets prepared with the addition of increasing levels of copepod meal. Similar effects on improvement in $L^*a^*b^*$ values and colouration were also observed in goldfish fed with copepod meal incorporated diets.

The results of fatty acid analysis of *A. salinus* revealed that PUFA contains especially 18: 2n-6, 22: 5n-3 and 22: 6n-3 fatty acids and similar trends were also observed in experimental diets. The fatty acid composition of feeds directly affects the fatty acid content of fish flesh (Borquez *et al.,* 2011). The results of the present study indicated that the addition of copepod meal instead of fish meal to experimental diets changed the fatty acid composition of fish flesh. The increase in PUFA content in the experimental diets was reflected in the fish texture and especially PUFA content of the fish flesh.

Overall, copepod (*A. salinus*) meal was found to be valuable raw material for fish nutrition due to its high carotene, EPA and DHA fatty acid contents. Further studies in different fish species are required to reveal optimum utilisation of copepod in fish feed formulations.

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References

Ajiboye, O. O., Yakubu, A. F., Adams, T. E., Olaji, E. D. and Nwogu, N. A. 2011. A review of the use of copepods in marine fish larviculture. *Rev. Fish Biol. Fish.,* 21: 225-246. DOI:10.1007/s11160-010-9169-3.

Anon. 2008. *CIE L*a*b* color scale. HunterLab.* https://support.hunterlab.com/hc/en-us/articles/203996325-CIE-L-a-b-Color-Scale-an07-96a (Accessed 07 June 2017).

Anon. 2013. *Yarıslı Lake Wetland Sub-basin Biodiversity Research Project.* T. R. Ministry of Forestry and Water Affairs, General Directorate of Nature Conservation and National Parks, Sensitive Areas Department, Wetlands Branch, Ankara. https://kutuphane.tarimorman.gov.tr/vufind/Record/20435/Description (Accessed 07 June 2017).

Anufriieva, E. V. and Shadrin, N. V. 2014. Factors determining the average body size of geographically separated *Arctodiaptomus salinus* (Daday, 1885) populations. *Zool. Res.*, 35(2): 336-341. DOI:10.11813/j.issn.0254-5853.2014.2.132.

AOAC 1995. *Official methods of analysis*, 16th edn. AOAC International, Association of the Official Analytical Chemists, Arlington, Virginia, USA.

Bardakcı, B. and Seçilmis, H. 2006. Investigations of the chemical content of rose oil in Isparta region by GC-MS and FTIR Spectroscopy techniques. *SDU Faculty of Arts and Sciences Journal of Science (EDJournal),* 1: 64-69 (In Turkish).

Bligh, E. G. and Dyer, W.J . 1959. A rapid method for total lipid extraction and purification. *Can. J. Biochem. Physiol.*, 37: 911-917. DOI: 10.1139/o59-099.

Borquez, A. S., Hernandez, A. J., Dantagnan, P., Saez, P. and Serrano, E. 2011. Incorporation of whole lupin, *Lupinus albus,* seed meal in commercial extruded diets for rainbow trout, *Oncorhynchus mykiss*: Effect on growth performance, nutrient digestibility and muscle fatty acid composition. *J. World Aquac. Soc.,* 42(2): 209-221.

Chiou, C. Y. and Ogino, C. H. 1975. Digestibility of starch in carp. *Bull. Japan Soc. Sci. Fish.,* 41(4): 465-466.

da Costa, D. P. and Miranda-Filho, K. C. 2019. The use of carotenoid pigments as food additives for aquatic organisms and their functional roles. *Rev. Aquac.*, 12: 1567-1578. DOI: 10.1111/raq.12398.

Davies, S. J., Connell, S. and Bateson, R. 1990. Potential of rapeseed meal as alternative protein source in complete diets for tilapia (*Oreocromis mossambicus*). *Aquaculture*, 87: 145-154.

Demirhindi, U. 1972. The preliminary planktonic investigations in the coastal lagoons and several brackishwater lakes of Turkey. *Istanbul University Faculty of Science Magazine*, 37(3-4): 205-232 (In Turkish).

Duru, M. 2014. *Effects of Spirulina platensis added to different amounts of feed on coloring and growth performance of goldfish (Carassius auratus)*. Masters Thesis, Mersin University, Graduate School of Natural and Applied Sciences, Mersin, Turkey, 68 pp.

Eryalçın, K. M. 2018. Effects of different commercial feeds and enrichments on biochemical composition and fatty acid profile of rotifer (*Brachionus plicatilis*, Muller 1786) and *Artemia franciscana*. *Turk. J. Fish. Aquat. Sci.*, 18: 81-90. DOI:10.1016/j.seares.2013.04.014.

Hekimoglu, M.A. 2005. A study on growing and coloring of gold fish in colored tanks (*Cyprinus auratus*, 1778). *E. U. J. Fish. Aquat. Sci.,* 22(1): 137-141.

Kanyılmaz, M., Sevgili, H., Ercen, Z. and Yılayaz, A. 2007. Use of clove oil as fish anesthetic. *Turk. J. Aquat. Life*, 3-5(5-8): 671-680 (In Turkish).

Koca, S. and Beyhan, T. 2014. The effects of some commercial enhancers on the fatty acid composition of *Artemia* sp. *Suleyman Demirel University Egirdir Aquatic Products Faculty Journal*, 10(1): 25-32. DOI: 10.22392/egirdir.246368 (In Turkish).

Morales, M. T., Aparicio, R. and Rios, J. J. 1994. Dynamic headspace gas chromatographic method for determining volatiles in virgin olive oil. *J. Chromatogr. A*, 668: 455-462. https://doi.org/10.1016/0021-9673(94)80139-8.

NRC 1993. *Nutrient requirements of fish*. National Research Council, National Academy Press, Washington, DC, USA.

Pasarin, D. and Rovinaru, C. 2018. Sources of carotenoids and their uses as animal feed additives - A review. *Scientific Papers, Series D. Anim. Sci.,* 61(2): 75-85.

Rautio, M. and Tartarotti, B. 2010. UV radiation and freshwater zooplankton: Damage, protection and recovery. *Freshw. Rev.,* 3: 105-131. DOI:10.1608/FRJ-3.2.157.

Rema, P. and Gouveia, L. 2005. Effect of various sources of carotenoids on survial and growth of goldfish larvae and juvenils. *J. Anim. Vet. Adv.*, 4(7): 654-658.

Rodriguez, C., Perez, J. A., Diaz, M., Izquierdo, M. S., Fernsndez-Palacios, H. and Lorenzo, A. 1997. Influence of the EPA:DHA ratio in rotifers on gilthead seabream (*Sparus aurata*) larval development. *Aquaculture*, 150(1): 77-89.

Schneider, T., Herzig, A., Koinig, K. A. and Sommaruga, R. 2012. Copepods in turbid shallow soda lakes accumulate unexpected high levels of carotenoids. *PLoS One,* 7: e43063. DOI:10.1371/journal.pone.0043063.

Seoka, M., Kurata, M. and Kumai, H. 2007. Effect of docosahexaenoic acid enrichment in artemia on growth of pacific bluefin tuna (*Thunnus orientalis*) larvae. *Aquaculture*, 270(1): 193-199.

Shadrin, N. V. and Anufriieva, E. V. 2018. Integral indicators of variability of *Arctodiaptomus salinus* (Daday, 1885) (Copepoda, Diaptomidae) and their possible use in assessing the population state. *Inland Water Biol.,* 11: 456-464. DOI:10.1134/S1995082918030173.

Stottrup, J. G. 2003. Production and nutritional value of copepods. In: J. G. Støttrup and L. A. Mcevoy (Eds.), *Live feeds in marine aquaculture*. Blackwell Publishing, Oxford, UK, p. 145- 205.

Takeuchi, T., Dedi, J., Haga, Y., Seikai, T. and Watanabe, T. 1998. Effect of vitamin A compounds on bone deformity in larval japanese flounder (*Paralichthys olivaceus*). *Aquaculture,* 169(3): 155-165.

Tacon, A. G. J. and Metian, M. 2015. Feed matters: Satisfying the feed demand of aquaculture. *Rev. Fish. Sci. Aquac.,* 23(1): 1-10. DOI:10.1080/23308249.2014.987209.

Tolomeev, A. P., Sushchik, N. N., Gulati, R. D., Makhutova, O. N., Kalacheva, G. S. and Zotina, T. A. 2010. Feeding spectra of *Arctodiaptomus salinus* (Calanoida, Copepoda) using fatty acid trophic markers in seston food in two salt lakes in South Siberia (Khakasia, Russia). *Aquat. Ecol.*, 44: 513-530. DOI 10.1007/s10452-010-9331-y.

Torrissen, O. J. 1984. Pigmentation of salmonide-effect carotenoids in eggs and start feeding diet on survival and growth rate, *Aquaculture,* 43: 185-193. https://doi.org/10.1016/0044-8486(84)90021-8.

Yanar, M. and Tekelioglu, N. 1999. The effect of fish size on pigmentation in goldfish (*Carassius auratus*). *Turk. J. Biol.,* 23: 101-106 (in Turkish).

Yesilayer, N., Dogan, G. and Erdem, M. 2008. The use of natural carotenoid sources in fish feed. *J. Fisheries Sciences.com,* 2(2): 241-251.

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