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Original Contribution

DIETARY SAVORY EXTRACT (*SATUREJA HORTENSIS*) SUPPLEMENTATION OF RAINBOW TROUTS - INFLUENCE ON PRODUCTIVE TRAITS, BLOOD BIOCHEMISTRY AND FISH MEAT QUALITY

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ABSTRACT

PURPOSE: This study was designed to assess the effects of savory extract supplementation to fish feed on the mortality rate, growth parameters, economic performance, blood biochemistry, meat chemical composition and fatty acid content of rainbow trouts farmed in a recirculation system.

METHODS: Rainbow trouts (n=160) were assigned to two groups – control (C) and experimental (S.h). All fish were farmed in a recirculation system and were fed extruded feed. The fish of group S.h were supplemented via feed with 1% powdered savory extract, by lubrication of granules with 5 ml/100 g sunflower oil. The feed of the control fish (group C) was treated only with sunflower oil.

RESULTS AND CONCLUSIONS: The addition of savory extract to the fed of rainbow trouts had a beneficial effect on the main growth indicators and the economic performance of farmed fish. Final live weight and individual weight gain values were higher; also, FCR and ECR were better. Blood biochemical parameters and meat chemical composition were not influenced by dietary savory supplementation. Despite the statistically significantly higher SFA content and lower levels of UFA, PUFA and n-3 in trout meat after savory supplementation, the PUFA/SFA and n-6/n-3 ratios remained within the range recommended for human nutrition: between 0.4 and 5.

Key words: survival rate, weight gain, feed conversion ratio, economic efficiency, chemical composition, fatty acid profile

INTRODUCTION

In recent years, consumers have increasingly focused on ecologically clean and healthy foods. After the ban on utilization of nutritional antibiotics in livestock farming, alternatives have constantly been sought. With this regard, plants and plant extracts in particular seem promising because they are natural, comparatively inexpensive products, safe for fish, the environment and humans (1, 2). The influence of plant extracts on growth performance and meat quality of various fish species are still insufficiently studied. The interest in the application of various herbs as growth promoters in fish and as medicinal substances in aquaculture is increasing (3).

Plants from the *Satureja* genus have been used as spices in human nutrition since ancient times. The winter savory (*Satureja montana*) and the summer savory (*Satureja hortensis*) are encountered on the Balkan Peninsula. It has been reported that savory possesses antimicrobial, anticarcinogenic, antimutagenic, antigenotoxic, analgesic, spasmolytic, anti-inflammatory, antiparasitic, antiaggregation and antioxidant properties (4).

The effects of summer savory (*Satureja hortensis*) on productive and economic performance of fattening carps (*Cyprinus carpio*) were reported by (5), while (6) investigated intestinal microflora, immune response and blood biochemical parameters in juvenile carps. Data on fish meat quality after dietary supplementation with various plant

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extracts are few, and with regard to savory are absent.

This study was designed to assess the effects of savory extract supplementation to fish feed on the mortality rate, growth parameters, economic performance, blood biochemistry, meat chemical composition and fatty acid content of rainbow trouts farmed in a recirculation system.

MATERIAL AND METHODS

Ethical approval

The study was performed in line with international, national, and/or institutional guidelines for the care and use of animals (7, 8).

Experimental design

One hundred and sixty rainbow trouts were divided to two groups (two replications; 80 fish in group) – control (C) and experimental (S.h). The mean initial weight of trouts from the two groups was 13.43±0.33 g (C) and 13.53±0.55 g (S.h). Fish were farmed in a recirculation system and fed commercial extruded feed (2 mm size; Aqua garant UNI, –Austria). One percent savory extract powder (P.I.C.Co LTD) was fed to experimental fish after lubricating feed granules with sunflower oil (5 ml/100 g feed). The control group (C) received feed lubricated only with the vegetable oil **(Table 1)**. The feed amount was 3% of fish live weight and was offered 3 times per day. The duration of the experiment was 60 days.

Table 1. *Nutrient content of Oncorhynchus mykiss W. feed*

№	Item	Groups	
		C	S.h
1	Crude protein, %	45.00	45.00
2	Crude lipids, %	16.00	16.00
3	Crude fiber, %	2.40	2.40
4	Crude ash, %	8.00	8.00
5	Ca, %	1.60	1.60
6	P, %	1.20	1.20
7	Savory extract, %	-	1
8	ME, MJ/kg	18.50	18.50

*1 kg feed contains: vitamin A – 10000 IE; vitamin D_3 – 1500 IE; vitamin E – 200 mg; vitamin C - 150 mg
**1 kg feed contains: Fe – 62 mg; Mn – 26 mg; Cu – 5 mg; Zn – 103 mg; I – 2.6 mg; Se – 0.3 mg

Water analysis in the recirculation system

Temperature, dissolved oxygen, pH and electric conductivity of water were monitored every day (MultiLineP4 equipment, Xylem Analytics, Germany). The concentrations of ammonia (9), nitrates (10), nitrites (11), orthophosphates (12) were measured once per week.

Growth performance

Rainbow trouts were weighed individually in the beginning, in the middle and the end of the trial period for calculation of weight gain (g), mortality (%) and feed conversion ratio (FCR).

Economic performance

The economic results from savory extract supplementation were evaluated as described in a previous study of ours (13). The economic conversion ratio (ECR) was calculated by the formula (14):

ECR = Diet Cost x Feed Conversion Ratio (1)

Analysis of blood biochemical parameters

Blood samples were obtained through caudal vessels puncture in EDTA-anticoagulated vials for analysis of total protein (TP), albumin (ALB), glucose (GLU), urea, creatinine (CREA), calcium (Ca), inorganic phosphate (P), magnesium (Mg), transaminases (ASAT, ALAT), alkaline phosphatase (ALP), cholesterol (CHOL) and triglycerides (TG) (BS-120 Chemistry Analyzer, Mindray, China).

Chemical composition

After preparation of fish meat samples (15), their water content was determined by air drying (16). Crude protein (%) was determined by Kjeldahl's method, (Kjeltec 8400, FOSS, Sweden). Meat lipid content (%) was assayed according to the method of Soxhlet (Soxtec 2050, FOSS, Sweden). The ash content (%) was obtained after incineration at 550°C for 8 h (MLW, Germany).

Fatty acid composition

The fatty acid profile of meat lipids was assayed as previously described (13) on a gas chromatograph "Perkin Elmer" Clarus 500 with a flame ionization detector, and "Trace Gold T6-WAXMS GC Column" capillary column with column temperature – 130°C (1 min), with change 6.5°C/min to 170°C, with change

3.0°C/min to 215°C (12 min) 40.0/min to 230°C (1 min), detector temperature 280°C; injector temperature – 270°C, gas holder – hydrogen (H), split - 1:50.

Statistical analysis

Statistical analysis was performed with Student's t-test at the $p<0.05$ level (17).

RESULTS

Hydrochemical analysis

During the experiment, water parameters in the recirculation tanks were maintained within the optimum values for trouts **(Table 2)**. There were no differences in all studied parameters in recirculation system tanks in both groups. During the 60-day period, water temperature ranged between 12ºC and 15ºC, 13.50ºC on the average. Dissolved oxygen was over 9 $mg.l^{-1}$, and water pH in control (C) and experimental (S.h) groups –7.46±0.32 and 7.52±0.24, respectively. Water electric conductivity was 486 $\mu S.cm^{-1}$. The levels of ammonia, nitrates, nitrites and orthophosphates in the water were considerably lower than allowances (18) **(Table 2)**.

Table 2. *Parameters of recirculation tanks water throughout the experiment*

Parameter	n	C	S.h	Significance	Optimum values (18)
		$\bar{x}$±SD	$\bar{x}$±SD		
Temperature, ºC	60	13.50±1.25	13.50±1.42	NS	12.0-16.0
Dissolved oxygen, $mg.l^{-1}$	60	9.54±0.31	9.37±0.33	NS	> 9
pH	60	7.46±0.32	7.52±0.24	NS	6.0-9.0
Electric conductivity, $\mu S.cm^{-1}$	60	486±23.51	486±24.12	NS	-
Ammonia, $mg.l^{-1}$	8	0.61±0.28	0.66±0.22	NS	< 1.0
Nitrates, $mg.l^{-1}$	8	0.34±0.08	0.33±0.06	NS	< 2.0
Nitrites, $mg.l^{-1}$	8	0.005±0.001	0.006±0.001	NS	< 0.01
Orthophosphates, $mg.l^{-1}$	8	0.271±0.117	0.280±0.148	NS	<0.40

NS=Non-significant.

Determination of growth performance

In the beginning of the trial, the initial weight of trouts from groups C and S.h was 13.43±0.33 g and 13.53±0.55 g, respectively (P>0.05) **(Table 3)**. In the middle of the 60-day period, average individual weight of fish from group C was 23.73±0.85 g, i.e. 10.80% lower than that of group S.h, with significant differences (P<0.05) **(Table 3)**. The trend to higher average live weight of fish supplemented with savory via feed was preserved until the end of the experimental period, when the final live weight of S.h. fish was 3.32 % higher than that of controls (43.97±1.27 g, P>0.05) **(Table 3)**. Higher survival rate was established for control fish - 97.50% vs in fish supplemented with savory extract - 95.00% **(Table 3)**. The average individual weight gain of rainbow trouts from group C was 30.54±0.98 g, i.e. 4.45% lower than that of group S.h (P>0.05) **(Table 3)**. The feed conversion ratio (FCR) was better in the group fed 1% savory extract: 1.21±0.06, which was 4.96% lower than that of controls (P>0.05) **(Table 3)**.

Table 3. *Fish production parameters*

Parameter	n	C	n	S.h	Significance
		$\bar{x}$± SD		$\bar{x}$± SD	
Initial body weight, g	80	13.43±0.33	80	13.53±0.55	NS
Body weight in the middle of the trial, g	79	23.73±0.85	78	26.30±1.06	*
Final body weight, g	78	43.97±1.27	76	45.43±1.34	NS
Survival rate, %		97.50		95.00	
Average individual weight gain, g	78	30.54±0.98	76	31.90±1.03	NS
FCR	78	1.27±0.04	76	1.21±0.06	NS

Significant different *p<0.05, NS=Non-significant, FCR=Feed conversion ratio

Economic performance

The savory extract in the feed of group (S.h) added additional 65 €/t to production costs **(Table 4)**.

The economic conversion ratio of 1.77 was better in group (S.h.) compared to 1.78 for the control group **(Table 4)**.

Table 4. *Economic performance of fish feed supplementation with savory extract*

Item	C	S.h
Price, €/t feed (VAT excluded)	1400.00	1465.00
Price, €/kg feed (VAT excluded)	1.400	1.465
ECR	1.78	1.77*

*The lowest value shows the best ECR. ECR=Economic conversion ratio

Determination of blood biochemical parameters

In aquaculture, biochemical parameters in blood are used for monitoring fish health. In this experiment, there were no considerable differences between the control and experimental groups for all 13 studied blood biochemical parameters (P>0.05) **(Table 5)**.

Table 5. *Biochemical parameters of blood in farmed Oncorhynchus mykiss W.*

Parameters	n	C	S.h	Significance
		$\bar{x}$±SD	$\bar{x}$±SD	
GLU, mmol/l	6	4.87±0.42	5.39±0.63	NS
UREA, mmol/l	6	0.85±0.06	0.83±0.06	NS
CREA, μmol/l	6	16.17±1.58	14.67±1.61	NS
TP, g/l	6	40.43±1.31	40.65±2.33	NS
ALB, g/l	6	19.28±1.81	19.18±1.39	NS
ASAT, U/l	6	246.67±26.19	240.83±25.30	NS
ALAT, U/l	6	32.50±2.67	37.83±4.83	NS
ALP, U/l	6	590.67±90.39	573.50±21.72	NS
Ca, mmol/l	6	2.44±0.10	2.29±0.17	NS
P, mmol/l	6	5.85±0.51	5.81±0.78	NS
Mg, mmol/l	6	0.76±0.05	0.68±0.08	NS
TG , mmol/l	6	1.92±0.02	1.89±0.04	NS
CHOL, mmol/l	6	6.22±0.82	6.28±0.43	NS

NS=Non-significant.

Glu-glucose; Crea-creatinine; TP-total protein; Alb-albumin; ASAT-aspartate aminotransferase; ALAT-alanine transaminase; ALP-alkaline phosphatase; Ca-calcium; P-phosphorus; Mg-manganesium; TG-triglycerides; CHOL-cholesterol.

Chemical meat composition

The addition of 1% savory extract did not result in statistically significant differences in the chemical composition of rainbow trout meat (P>0.05). Water content of the meat of S.h group was 75.81±1.23% –, or 0.68% higher than that of the control fish. The respective dry matter content of S. h rainbow trouts was 24.19±1.23% - by 2.11% lower than that of non-supplemented fish **(Table 6)**. Meat protein content of fish supplemented with 1% savory extract was 1.49% higher than that of control trout: 18.58±0.26% **(Table 6)**. Fat and ash percentages of supplemented rainbow trouts were 3.74±0.44% and 1.59±0.16% respectively - lower than values in controls by 19.25% and 5.03% respectively **(Table 6)**.

Table 6. *Chemical analysis of rainbow trout fillets*

Parameters	n	C	S.h	Significance
		$\bar{x}$±SD	$\bar{x}$±SD	
Water, %	6	75.30±0.70	75.81±1.23	NS
Protein, %	6	18.58±0.26	18.86±0.66	NS
Lipids, %	6	4.46±0.82	3.74±0.44	NS
Dry matter, %	6	24.70±0.70	24.19±1.23	NS
Ash, %	6	1.67±0.04	1.59±0.16	NS

NS – non-significant.

Fatty acid composition

Meat fatty acid content of rainbow trouts farmed in recirculation system is presented in **Table 7**. The addition of 1% savory extract to the feed of trouts resulted in statistically significantly (P<0.05) higher myristic acid content (by 21.69%) compared to values in group C: 2.49±0.17%. No substantial differences were found for palmitic acid content of meat (P>0.05) between the groups. A considerably higher (P<0.01) stearic acid content – by 14.82%, was demonstrated in supplemented fish, than in controls, where this fatty acid content was 3.98±0.15%. Meat SFA in fish from group S.h was 25.79±0.95% –, or 5.28% higher compared to non-supplemented fish (P<0.05) **(Table 7)**.

The addition of 1% savory extract to the feed of rainbow trouts led to statistically significantly higher (P<0.01) palmitoleic acid content in the meat of the S.h group–, i.e. 23.97% than the control value of 2.92±0.12%. Meat oleic acid content of control and supplemented groups was 49.68±1.03% and 49.79±0.48%, respectively (P>0.05). Similarly, insignificant intergroup differences were established with respect to meat MUFA content of both groups.

Meat linoleic acid of control fish was 13.83±0.48%; this was by 3.11% lower compared to the level in group S.h (P>0.05). However, α-linolenic acid content of meat of control fish was 3.21±0.26% which was 14.02% lower than that of fish receiving savory extract (P<0.05). The dietary supplementation of rainbow trouts with 1% savory extract did not result in statistically significant differences (P>0.05) in meat contents of eicosadienoic, eicosatrienoic, eicosapentaenoic and docosapentaenoic acids. A substantial difference between the groups was noted with respect to docosahexaenoic acid, which was by 24.90% (P<0.001) lower in group S.h, vs the control fish percentage of 2.57±0.28%. The addition of 1% savory extract to the feed resulted in statistically significantly (P<0.05) lower meat PUFA and UFA content in supplemented fish from the S.h group: 7.48% and 1.80% respectively, as compared to contents in control group – 22.98±0.20% and 75.58±0.51%.

The amount of n-3 fatty acids in rainbow trout meat after dietary supplementation with 1% savory extract was 15.64% lower than the control percentage of 8.12±0.16%, with significant differences (P<0.001). The content of n-6 fatty acids in the meat of experimental fish was not influenced by savory extract in their feed (P>0.05).

Table 7. *Fatty acid analysis of rainbow trout fillets*

Parameters	**n**	**C**	**S.h**	**Significance**
		$\bar{x}$±SD	$\bar{x}$±SD	
C14:0 Myristic	6	2.49±0.17	3.03±0.50	*
C16:0 Palmitic	6	17.96±0.38	18.19±0.47	NS
C16:1 Palmitoleic	6	2.92±0.12	3.62±0.47	**
C18:0 Stearic	6	3.98±0.15	4.57±0.28	**
C18:1 Oleic	6	49.68±1.03	49.79±0.48	NS
C18:2 Linoleic	6	13.83±0.48	13.40±1.79	NS
C18:3n-3 α-linolenic	6	3.21±0.26	2.76±0.30	*
C20:2 Eicosadienoic	6	0.82±0.15	0.77±0.05	NS
C20:3 Eicosatrienoic	6	0.21±0.03	0.25±0.08	NS
C20:5 Eicosapentaenoic	6	1.71±0.30	1.72±0.44	NS
C22:5 Docosapentaenoic	6	0.64±0.12	0.45±0.19	NS
C22:6 Docosahexaenoic	6	2.57±0.28	1.93±0.16	***
SFA[1]	6	24.43±0.51	25.79±0.95	*
UFA[2]	6	75.58±0.51	74.22±0.95	*
MUFA[3]	6	52.60±0.91	53.41±0.95	NS
PUFA[4]	6	22.98±0.20	21.26±0.71	*
n-6[5]	6	14.86±0.36	14.42±1.91	NS
n-3[6]	6	8.12±0.16	6.85±0.20	***
PUFA/SFA	6	0.94±0.01	0.83±0.05	*
n-6/n-3	6	1.83±0.08	2.11±0.34	NS

***P≤0.001; ** P≤0.01; * P≤0.05; NS – non-significant.

[1]SFA – Saturated fatty acids; [2]UFA– Unsaturated fatty acids; [3]MUFA – Monounsaturated fatty acids; [4]PUFA – Polyunsaturated fatty acids; [5]n-6 – ΣC18:2;C20:2;C20:3;C20:4; [6]n-3 – ΣC18:3n-3;C20:5;C22:6

There were consistent intergroup differences (P<0.05) with regard to PUFA/SFA ratio in meat that was 11.70% lower in supplemented fish, compared to the control ratio of 0.94±0.01. The inclusion of the dietary herb supplement had no statistically significant effect (P>0.05) on n-6/n-3 fatty acids ratio.

DISCUSSION

During the experimental period, all water chemical parameters in the farming system were within the optimum ranges for rainbow trouts. Filtering system played an essential role for maintaining ammonia, nitrate, nitrite and orthophosphate concentrations below the maximum allowances as per (18) for trout farms - 1 $mg.l^{-1}$, 2 $mg.l^{-1}$, 0.01 $mg.l^{-1}$ and 0.40 $mg.l^{-1}$, respectively. There were no significant differences (P>0.05) between groups as the main water chemical parameters were concerned **(Table 2)**.

In the beginning of the trial, fish were uniform with regard to live weight. The control examination on day 30 showed statistically significantly higher live weight (P<0.05) in fish supplemented with 1% savory extract in feed – by 10.80% and this tendency was observed until the end of the experiment, but on day 60 the differences were no more significant (P>0.05) **(Table 3)**. These results confirmed findings (5) about higher live weight in carps supplemented with savory extract.

Regardless of the lower survival rate (by 2.50%) in rainbow trouts that received 1% savory extract **(Table 3)**, the detected mortality rate was within normal range for juvenile fish – up to 10%. The results from the present experiment were considerably worse than those of (5) in fattening carps supplemented with savory extract that demonstrated 100% survival rate. This is attributed to the different age category of fish used in our experiment.

The average weight gains of rainbow trouts from the S.h group was 4.45% higher than that group C (P>0.05) **(Table 3)** in line with data (5) for fattened carps.

The addition of 1% savory extract led to improved FCR – by 4.96% despite the lack of statistically significant differences (P>0.05) **(Table 3)**, as also reported in fattened carps (5). The better results for growth performance of the group supplemented with 1% savory extract could be attributed to the high content of biologically active substances in extruded feed, to the better flavor and digestibility of feed.

The economic conversion ratio in the savory-supplemented group **(Table 4)** reflected the slower growth and poorer FCR in the experimental group and supported the findings (5) for better ECR in fattened carps.

Blood biochemical parameters in fish are influenced by numerous factors (water quality, blood sampling technique, age, nutrition, sexual maturity and photoperiod) (19, 20). The savory extract added to the feed of rainbow trouts had no significant effect (P>0.05) on studied 13 blood biochemical parameters **(Table 5)**. In available literature, no data are available concerning the effects of dietary savory extract on fish blood biochemistry. Other studies found no substantial differences in blood biochemical indices of rainbow trouts fed *A. millefolium* and *T. officinale* extracts **(**21, 22), while (23) reported considerably elevated calcium in тхе blood of carps supplemented with *A. calamus*, and (24) – elevated blood TG and CHOL in the same species supplemented with *T. officinale*.

The proximate analysis of rainbow trout meat showed that parameters were not influenced by the addition of 1% savory extract to the feed, regardless of the tendency towards lower meat fat content in the S.h group **(Table 6)**. This aligns with the data reported by (25) in rainbow trouts after dietary supplementation with paprika, curcumin, garlic and thyme extracts, as well as in carps fed the same extracts (26). Considering that modern consumers prefer fish meat with lower lipid content, the observed tendency towards lower meat fat content after the addition of 1% savory extract seems promising.

The added savory extract to rainbow trout feed resulted in statistically significantly higher meat content of myristic (P<0.05) and stearic (P<0.01) acids and a trend to higher palmitic acid content – reason for considerably elevated total SFA content (P<0.05) **(Table 7)**. This contradicts the findings of (26) about lower concentrations of the same fatty acids in the meat of rainbow trouts fed diet containing paprika, curcumin, thyme, garlic and oregano. Due to increased blood cholesterol, myristic and palmitic fatty acids are especially important for human nutrition, whereas the role of stearic acid in this regard is neutral. Regardless of the statistically significantly higher meat SFA

content in S.h fish (25.79%), the percentage is normal for this fish species and far below the values of 28.28-29.03% reported by (27).

In this experiment, no significant differences were observed in total MUFA content, despite the substantially (P<0.01) higher palmitoleic acid content **(Table 7)**, a finding, in line with data (26) about the content of these fatty acids in rainbow trouts supplemented with paprika, curcumin, thyme, garlic and oregano.

Dietary supplementation of rainbow trouts with savory extract resulted in substantially lower (P<0.001) percentages of α-linolenic and docosahexaenoic acids in meat **(Table 7)**, as compared to the control group. Other findings (26) also showed lower meat α-linolenic acid in trouts that received feed with added thyme and curcumin, but not in fish supplemented with garlic, oregano and paprika. It should be noted that the cited study found no statistically significant differences with respect to docosahexaenoic acid contrary to our study. The percentages of the other long-chain fatty acids in trout meat did not differ significantly compared to untreated fish **(Table 7)**, in line with data from the supplementation of rainbow trouts' feed with paprika, curcumin, thyme, garlic and oregano (26). The addition of savory extract led to considerably lower content of PUFA (P<0.05), UFA (P<0.05) and n-3 (P<0.001) fatty acids in the meat of group S.h, but not in n-6 fatty acids **(Table 7)**. The latter results disagree with those reported by (26), noting a tendency towards higher levels of these fatty acid groups in trouts supplemented with paprika, curcumin, thyme, garlic and oregano.

PUFA/SFA ratio was statistically significantly lower (P<0.05) in rainbow trouts fed savory extract compared to controls, yet no relevant differences were found for n-6/n-3 ratio **(Table 7)**. Despite the less beneficial PUFA/SFA and n-6/n-3 ratios in the meat of group fed savory extract, their average values were 0.83 and 2.11 respectively, e.g. within the desired range of 0.4–5 (28).

The addition of savory extract to the fed of rainbow trouts had a beneficial effect on the main growth performance indicators and economic results of species' farming. Final live weight and average individual weight gain values were higher; FCR and ECR were also improved. Blood biochemical parameters and meat chemical composition were not affected by the dietary savory supplementation. Despite the statistically significantly higher SFA content and lower levels of UFA, PUFA and n-3 in rainbow trout meat after dietary savory supplementation, the PUFA/SFA and n-6/n-3 ratios remained within the range recommended for human nutrition: between 0.4 and 5.

CONCLUSION

The addition of savory extract to the feed of rainbow trouts had a beneficial effect on the main growth parameters and economic performance of the farmed species. Final live weight and average individual weight gain values were higher, and also FCR and ECR were better. Blood biochemical parameters and the chemical composition of meat were not influenced by the dietary savory supplementation. Despite the statistically significantly higher SFA content and lower levels of UFA, PUFA and n-3 in rainbow trout meat after feeding savory extract, the PUFA/SFA and n-6/n-3 ratios remained within the range recommended for human nutrition: between 0.4 and 5.

Conflict of Interest

The authors declare no conflict of interest.

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