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ORIGINAL ARTICLE

Effect of dietary *Ulva* and *Spirulina* on weight loss and body composition of rainbow trout, *Oncorhynchus mykiss* (Walbaum), during a starvation period

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Summary

A study was conducted to determine the effect of dietary algal supplementation on weight loss and proximate composition in rainbow trout, *Oncorhynchus mykiss*, during starvation. An algae-free control diet (C) and four experimental diets, including varying levels of raw *Ulva* meal (5% = U5; 10% = U10) and *Spirulina* meal (5% = S5; 10% = S10) were formulated. Fish were fed to satiation for 12 weeks and then subjected to a 3-week starvation period. Body weight, viscerosomatic index (VSI, %), hepatosomatic index (HSI, %), dress-out (DO, %) and chemical composition of carcass were analysed at the end of each starvation week. The accumulated weight loss of the fish fed dietary algae was almost 50% less than the control group ($p < 0.05$) after 2 and 3 weeks starvation. No significant differences in weight loss were observed among the fish fed different algal diets during the starvation period. Total body protein and ash contents remained constant in all groups throughout the starvation period. Fish in all treatments lost a significant level of total lipids and changes of liver fatty acid profiles were also observed. Results indicate that low level inclusion of algae in aquafeeds may have economical advantages in terms of reducing weight loss in fish when subjected to a short-term fasting period, that sometimes is necessary after a heavy rainfall or when fish stocks are held back to meet market demand.

Introduction

Macro and micro algae have been used as dietary supplements to enhance health and nutritional performance of a range of farmed fish species. Improvements of growth, feed utilization, lipid metabolism, physiological activity, stress response, disease resistance and carcass quality have been reported with dietary algal inclusion levels of 1–5% (Mustafa and Nakagawa, 1995; Nakagawa and Montgomery, 2007).

Among the main areas of dietary algal research for finfish is the potential for protein replacement by micro algal species (Nandeesha et al., 1998; Olivera-Novoa et al., 1998; Palmegiano et al., 2008). *Spirulina* is one of the most commonly used micro algae in aquafeeds; it is a rich source of protein, vitamins, essential amino acids, minerals, essential fatty acids and antioxidant pigments such as carotenoids (Nakagawa and Montgomery, 2007). The effects of *Spirulina* on fish growth, feed intake, pigmentation and nutrient utilization have been investigated for

several fish species, including tilapia, *Oreochromis niloticus* (Takeuchi et al., 2002) carp, *Cyprinus carpio* (Nandeesha et al., 1998), red sea bream, *Pagrus major* (Mustafa et al., 1997), white sturgeon, *Acipenser transmontanus* (Palmegiano et al., 2008) and rainbow trout, *Oncorhynchus mykiss* (Matty and Smith, 1978).

Compared to micro algae, macro algae are relatively poor sources of protein, however, they may be considered as feed additives for growth promotion, lipid mobilization and improvement of flesh quality (Nakagawa et al., 1987; Wassef et al., 2001). *Ulva* species are important macro algae which have been used as a dietary ingredient for a wide range of fish species and reported benefits include improved growth performance, diet utilization, immune response and fish quality (Satoh et al., 1987; Güroy et al., 2007; Azaza et al., 2008; Ergün et al., 2009). *Ulva* spp. are also a good source of vitamins and minerals; they are especially rich in ascorbic acid (Ortiz et al., 2006; García et al., 2007). Ascorbic acid induces lipolysis which decreases tissue lipid and may alter body composition and nutrient deposition in fish (Miyasaki et al., 1995; Ji et al., 2003a).

Rainbow trout is a fast growing species which have become highly economically important both globally and more specifically in Europe where production totalled 297 738 tones in 2007 (FAO, 2009). Modern trout farming practices are geared towards optimizing feed conversion; the use of high energy, high lipid diets, with reduced protein levels have become common practice. Whilst economically successfully, these practices are not always acceptable to the consumer. It is widely accepted that wild fish contain lower lipid levels than intensively farmed fish (Haard, 1992; Rasmussen, 2001). Higher lipid levels can be unappealing to consumers and are often problematic in freezing and smoking practices. However, these problems can be overcome by altering production strategies to manipulate lipid deposits. Such strategies include reverting to low lipid diets, reduced rations and starvation. Starvation is common practice in reared trout prior to slaughter in order to empty the gastrointestinal tract and prevent fish spoilage. Starvation of salmonids is also practiced in order to hold back stocks to regulate supply in accordance with consumer demand and to reduce excess lipid levels. Starvation periods may also be conducted in trout feeding strategies during periods of heavy downpour when turbidity and poor water qualities lead to wasted feed. There is limited information regarding the effects of dietary algal supplementation on rainbow trout lipid metabolism. The aim of the present study was to evaluate the effects of *Ulva rigida* and *Spirulina platensis*, on rainbow trout weight loss and carcass composition during starvation.

Materials and methods

Diets

Ulva rigida was freshly collected from the near-shore waters of the Dardanelles, Canakkale-Turkey. Algal samples were thoroughly washed with sea water, dried at 40 °C for 48 h, and fine-milled with a laboratory blender to produce raw *Ulva* meal. *Spirulina platensis* was obtained as a dried powder from the Phytoplankton Production Unit of Canakkale Onsekiz Mart University, Faculty of Fisheries. An algae-free control diet (C) and four experimental diets, including varying levels of raw *Ulva* meal (5% = U5; 10% = U10) and *Spirulina* (5% = S5; 10% = S10), were formulated. The proximate composition of dietary ingredients is given in Table 1. Formulation and chemical composition of the experimental diets is displayed in Table 2. Experimental diets were prepared as explained by Güroy et al. (2007).

Juvenile rainbow trout *O. mykiss* were transported from a local fish farm (Keskinler Trout Farm, Bayramic, Canakkale, Turkey) and acclimated to laboratory conditions at the Fish Nutrition and Aquarium Unit of Canakkale Onsekiz Mart University, Faculty of Fisheries for 4 weeks whilst fed a commercial diet (Bagci Feed Company, crude protein: 50%, crude lipid: 18%). Three hundred fish (~7 g) were randomly allocated into 150 l circular tanks (20 fish per tank) within a recirculation freshwater system. Fish were fed experimental diets to satiation three times a day for 12 weeks. Each treatment was conducted in triplicate. During the experimental period temperature was maintained at 16 ± 1.5 °C and dissolved oxygen levels at >80% saturation. Water flow rate was approximately 12 l/min per tank. After

Table 1 Chemical composition of dietary ingredients (%)

	Protein (N × 6.25)	Lipid	Ash	NFE*
Fish meal	64.07	6.90	18.61	10.42
Wheat meal	12.09	1.96	3.37	82.58
Soybean meal	45.99	1.39	7.28	45.34
Spirulina meal	60.00	7.00	10.00	23.00
Ulva meal	16.00	0.50	24.00	59.50

*NFE (Nitrogen-free extracts) = dry matter – (crude protein + crude lipid +ash).

Table 2 Formulation and composition of the experimental diets

Ingredients	C	U5	U10	S5	S10
Fish meal[1]	50.0	50.0	50.0	45.0	40.0
Ulva[2]	0.0	5.0	10.0	0.0	0.0
Spirulina[3]	0.0	0.0	0.0	5.0	10.0
Soybean meal[4]	24.5	24.5	24.5	24.5	24.5
Wheat meal[5]	14.0	9.0	4.0	14.0	14.0
Fish oil[6]	10.0	10.0	10.0	10.0	10.0
Vitamin premix[7]	0.9	0.9	0.9	0.9	0.9
Mineral premix[8]	0.6	0.6	0.6	0.6	0.6
Chemical analyses (dry matter %)					
Protein (N × 6.25)	45.0	45.2	45.2	44.8	44.6
Lipid	15.1	15.0	15.0	15.1	15.1
Ash	9.1	11.6	11.4	8.5	9.1
Nitrogen free extract[9]	30.8	28.1	28.4	31.6	31.2

[1]Anchovy fish meal. Can Kardesler Fish Meal, Samsun, Turkey.
[2]Dardanelles.
[3]Canakklae Onsekiz Mart University, Phytoplankton Production Unit, Turkey.
[4]Abide Feed Mill Company, Canakkale, Turkey.
[5]Kepez Un, Canakkale, Turkey.
[6]Anchovy fish oil. Can Kardesler Fish Meal, Samsun, Turkey.
[7]Per g mixture: vitamin A: 342 IU; vitamin D_3: 329 IU; vitamin E: 0.0274 IU; vitamin K_3: 5.48 mg; vitamin B_1: 2.05 mg; vitamin B_2: 3.42 mg; vitamin B_3: 20.5 mg; vitamin B_5: 5.48 mg; vitamin B_6: 2.05 mg; vitamin B_{12}: 2.74 mg; vitamin C: 24.0 mg, Kartal Chemical, Kocaeli, Turkey.
[8]Per g mixture: biotin: 0.411 mg; folic acid: 0.685 mg; Zn: 12.3 mg; Mn: 4.80 mg; Cu: 1.64 mg; I: 0.274 mg; Se: 0.0274 mg; Ca: 125 mg; K: 189 mg, Kartal Chemical, Kocaeli, Turkey.
[9]Nitrogen-free extracts (NFE) = dry matter − (crude protein + crude lipid + ash).

12 weeks, all tanks were batch weighed and then subjected to a 3-week starvation period. During the starvation period, fish from each tank were batch weighed at the end of each week and six fish per treatment were sampled for carcass composition and biological indexes.

Chemical analysis

All analyses were performed in triplicate. Analyses of crude protein, moisture and ash in feed ingredients, diets and the whole body of fish were performed according to standard procedures (AOAC, 2000). Total lipids in the diets and carcass of fish were extracted according to the procedure of Folch et al. (1957) with chloroform/methanol (2:1 v/v). The fatty acids in the total lipid were esterified into methyl esters by saponification with 0.5 N methanolic NaOH and transesterified with 14% boron trifluoride–methanol (AOAC (Association of Official Analytical Chemists), 2000). Fatty Acid Methyl Esters (FAME) were analysed using a flame ionization gas chromatograph (Shimadzu GC-2014) equipped with an Omegawax 250 capillary column (30 m L × 0.25 mm internal diameter), a Flame Ionization Detector (FID) and a split injection system with nitrogen carrier gas. Injector port and detector temperatures were maintained at 250 °C and 260 °C, respectively. The column temperature program was held at 140 °C for 5 min and then elevated at a rate of 3 °C/min to 200 °C. Total run time was 60 min per sample. Fatty acids were identified by comparing their retention times of the standard fatty acid standards (Sigma-Aldrich, St Louis, MO, USA).

Biological measurements

The somatic indexes were calculated as follows:

Condition Factor (CF, %) =fish weight (g)/(fish length cm)^3 × 100; Dress-out (DO, %) = (fish weight (g) − gut weight (g))/fish weight (g) × 100; Hepatosomatic index (HSI, %) = liver weight (g)/fish weight (g) × 100; Viscerosomatic index (VSI, %) = viscera weight [excluding liver weight] (g)/fish weight (g) × 100.

The liver weight was not included in the VSI, which is not the usually formulae, in order to determine if other visceral chemical composition were affected irrespective of the liver contribution to the VSI.

Statistical analysis

Data were subjected to a one-way analysis of variance (ANOVA) and Tukey's HSD multiple range test using Statgraphics 7.0 version for Windows (Manugistics Incorporated, Rockville, MD, USA). The results were treated statistically significant at the $p < 0.05$ level.

Results

The percentage of body weight loss of rainbow trout during the starvation period is illustrated in Fig. 1. The accumulated weight loss of the control group was significantly greater than that of the algal fed groups after 2 and 3 weeks of fasting, whilst no significant differences of weight loss were observed between the groups of fish fed on the different algal diets. No pathological signs were observed during the starvation period and no mortalities occurred.

The effects of starvation period on carcass composition and biological indexes are shown in Table 3. Total body protein and ash remained constant within the groups irrespective of starvation week. However,

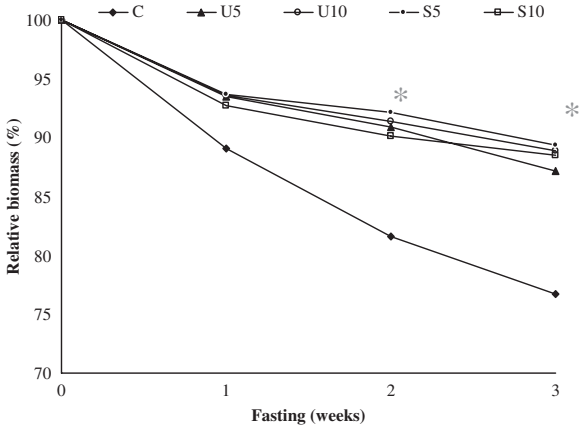


Fig. 1 Accumulated weight loss (%) of rainbow trout during starvation after 12 weeks feeding on experimental diets. C = Control (71.7 g); U5 = 5% *Ulva* meal (70.4 g); U10 = 10% *Ulva* meal (78.4 g); S5 = 5% *Spirulina* (79.1 g) and S10 = 10% *Spirulina* (78.9 g). The numbers given in brackets after each experimental group sign in the latter sentence are mean final body weights after 12 weeks feeding on experimental diets, prior to starvation period. *Significant difference between the control group and dietary algal supplemented groups (no significant differences between different algal groups).

compared to pre-starvation levels, all groups lost a significant level of total lipids after the 3-week fasting period. During the same period, no significant differences in carcass moisture were observed. The condition factors, hepatosomatic and viscerosomatic indexes in all treatments decreased significantly ($p < 0.05$) after 3 weeks of fasting.

The effects of starvation period on liver fatty acid composition is presented in Table 4. Myristic acid (14:0) levels were observed to decrease significantly in the liver of the control group with starvation but was myristic acid levels remained constant in the algal groups. Starvation resulted in a reduction of arachidic acid (20:0), oleic acid (18:1n-9) and α-Linolenic acid (18:3n-3) in the livers of all groups. The level of oleic acid decreased in all treatments but the rate of the decrease was significantly higher ($p < 0.05$) in the control group than the algal groups. Eicosenoic acid (20:1n-9) remained constant in the control group, but eicosenoic acid significantly decreased ($p < 0.05$) in the algal groups. The total levels of monounsaturated fatty acids (MUFA) decreased significantly ($p < 0.05$) in all groups after starvation. Linoleic acid (18:2n-6) did not change in the all treatments with fasting, but the linoleic acid level of groups S5 and S10 was significantly higher ($p < 0.05$) than the control group at the end of the 12-week feeding period. The level of docosahexaenoic acid (22:6n-3 – DHA), docosapentatenoic acid (22:5n-3 – DPA) and eicosatrienoic acid (20:3n-3) increased in all treatments after fasting. It should be noted however, DHA levels of groups U10, S5 and S10 was significantly higher than in the control group at the end of the 12-week feeding experiment. All treatments lost a significant level of eicosapentaenoic acid (20:5n-3 – EPA) after the 3-week starvation period. The levels of polyunsaturated and n-3 fatty acids were higher in fish after fasting. Dietary inclusion of macroalgae and microalgae meals resulted in a significant increase of PUFAs in liver at the end of the feeding period.

Discussion

At the end of the 3-week starvation period, the control group displayed approximately double the weight loss of algal fed groups. To our knowledge, there is no data regarding the effects of dietary algae on rainbow trout weight loss during starvation. However, reduced weight loss has been reported in black sea bream *Acanthopagrus schlegeli* fed macro algae prior to starvation (Nakagawa et al., 1987; Nakagawa, 1997). Nakagawa et al. (1987) observed that during a 49-day wintering period, black sea bream previously fed a control diet lost 27.9% of their initial body weight, whilst a group fed 5% dietary *Ulva* meal suffered only a 12.5% loss. In another black sea bream study, dietary supplementation of 5% *Ulva* meal, prior to a 3-month starvation period resulted in reduced body weight loss from 15.2% to 1.6% (Nakagawa, 1997). Nakagawa (2004) supplemented waste *Porphyra* to an artificial diet at either 3% or 5% for red sea bream *Pagrus major* and reported that *Porphyra* meal supplementation improved growth performance, feed utilization and enhanced triglyceride accumulation. Starvation for 10 days at the end of the feeding trial resulted in reduced body weight loss. It was suggested that preferential lipid mobilization to energy suppressed muscle protein catabolism and thus reduced weight loss. Reduced weight loss in fish fed other microalgae such as *Chlorella*, prior to starvation has also been reported (Nakagawa et al., 1984; Nakagawa, 1985).

In a recent study a reduction of flesh lipid levels was observed in *Sparus macrocephalus* after a fasting period for 28 days which demonstrated that fasting could be used as a technique to reduce lipid content in fish (Zhang et al., 2007). However, Zhang and colleagues did not incorporate algal supplementation in their diet, so it was interesting to see that besides the reduction in muscle lipid observed from day 3 of starvation, the protein content of fish muscle also decreased after 14 days of starvation (Nakagawa,

Table 3 Body proximate composition and biological indexes of rainbow trout during 3 weeks of fasting

Week	Body proximate composition (% wet basis)					Biological index (%)		
/diet	Moisture	Protein	Lipid	Ash	CF	HSI	VSI*	Dress-Out
C								
0	66.3 ± 1.12	17.9 ± 0.06	13.5 ± 0.35^{b}	2.3 ± 0.03	1.78 ± 0.05^{c}	1.79 ± 0.11^{b}	13.82 ± 0.04^{c}	80.26 ± 0.46^{a}
1	66.5 ± 1.66	17.5 ± 0.09	13.0 ± 0.22^{b}	3.0 ± 0.04	1.51 ± 0.06^{bc}	1.14 ± 0.07^{a}	13.85 ± 0.03^{c}	81.98 ± 0.62^{a}
2	69.0 ± 1.36	16.2 ± 0.15	12.6 ± 0.47^{ab}	2.2 ± 0.06	1.43 ± 0.09^{ab}	1.06 ± 0.09^{a}	11.57 ± 0.04^{b}	84.09 ± 0.98^{ab}
3	69.7 ± 1.89	16.5 ± 0.26	11.0 ± 0.66^{a}	2.8 ± 0.03	1.19 ± 0.05^{a}	0.98 ± 0.13^{a}	9.90 ± 0.05^{a}	86.00 ± 1.83^{b}
U5								
0	67.6 ± 1.02	17.8 ± 0.03	12.4 ± 0.41^{b}	2.2 ± 0.01	1.79 ± 0.09^{b}	1.55 ± 0.10^{b}	14.44 ± 0.16^{c}	80.19 ± 0.55^{a}
1	67.0 ± 1.86	17.7 ± 0.16	12.6 ± 0.55^{b}	2.7 ± 0.03	1.41 ± 0.08^{a}	1.33 ± 0.09^{b}	12.01 ± 0.04^{c}	82.60 ± 0.68^{ab}
2	67.5 ± 1.24	17.9 ± 0.28	11.3 ± 0.36^{ab}	3.3 ± 0.07	1.36 ± 0.06^{a}	1.21 ± 0.05^{ab}	11.21 ± 0.11^{b}	84.54 ± 0.77^{ab}
3	72.7 ± 1.55	16.2 ± 0.32	8.4 ± 0.74^{a}	2.7 ± 0.01	1.32 ± 0.04^{a}	1.14 ± 0.06^{a}	10.43 ± 0.10^{a}	86.87 ± 1.38^{b}
U10								
0	66.8 ± 1.35	17.4 ± 0.08	13.4 ± 0.59^{b}	2.4 ± 0.04	1.81 ± 0.02^{b}	1.57 ± 0.08^{b}	16.04 ± 0.37^{c}	78.80 ± 0.40^{a}
1	68.5 ± 1.13	16.1 ± 0.11	12.5 ± 0.89^{ab}	2.9 ± 0.02	1.46 ± 0.06^{ab}	1.52 ± 0.13^{b}	12.02 ± 0.23^{b}	83.49 ± 0.73^{b}
2	66.4 ± 1.08	17.4 ± 0.23	13.9 ± 0.48^{b}	2.3 ± 0.05	1.32 ± 0.05^{a}	1.39 ± 0.07^{ab}	10.18 ± 0.22^{a}	85.78 ± 0.88^{bc}
3	70.7 ± 1.50	17.0 ± 0.37	9.4 ± 0.50^{a}	2.9 ± 0.02	1.20±0.06^{a}	1.07 ± 0.07^{a}	9.38 ± 0.11^{a}	86.60 ± 1.05^{c}
S5								
0	67.0 ± 1.98	17.3 ± 0.27	13.1 ± 0.39^{b}	2.6 ± 0.07	1.77 ± 0.09^{b}	1.71 ± 0.06^{b}	14.87 ± 0.09^{c}	80.10 ± 0.63^{a}
1	69.1 ± 1.22	16.1 ± 0.36	12.4 ± 0.49^{b}	2.4 ± 0.02	1.53 ± 0.12^{ab}	1.43 ± 0.04^{ab}	12.49 ± 0.11^{b}	82.89 ± 0.59^{ab}
2	69.0 ± 1.34	16.8 ± 0.96	11.6 ± 0.55^{ab}	2.6 ± 0.05	1.41 ± 0.03^{ab}	1.35 ± 0.02^{ab}	9.06 ± 0.21^{a}	86.02 ± 1.06^{b}
3	68.8 ± 1.12	18.3 ± 0.55	10.2 ± 0.95^{a}	2.7 ± 0.05	1.18 ± 0.09^{a}	1.22 ± 0.03^{a}	8.06 ± 0.17^{a}	87.45 ± 1.89^{b}
S10								
0	69.2 ± 1.05	16.7 ± 0.14	11.8 ± 0.22^{b}	2.3 ± 0.05	1.68 ± 0.08^{b}	1.44 ± 0.06^{b}	15.20 ± 0.23^{c}	80.66 ± 0.45^{a}
1	69.0 ± 1.58	17.4 ± 0.09	11.1 ± 0.81^{ab}	2.5 ± 0.06	1.52 ± 0.07^{ab}	1.42 ± 0.09^{b}	12.29 ± 0.14^{b}	84.64 ± 0.67^{ab}
2	69.1 ± 1.29	17.5 ± 0.23	10.8 ± 0.69^{ab}	2.6 ± 0.04	1.43 ± 0.10^{ab}	1.16 ± 0.05^{a}	11.41 ± 0.08^{a}	83.60 ± 0.46^{ab}
3	71.0 ± 1.89	17.2 ± 0.38	9.0 ± 0.93^{a}	2.8 ± 0.04	1.15 ± 0.06^{a}	1.12 ± 0.08^{a}	10.25 ± 0.13^{a}	85.26 ± 1.04^{b}

Different superscripts in the same column denotes a significant difference ($p < 0.05$) between the starvation weeks within the same experimental groups. Values expressed as mean ± SE ($n = 6$).

*Viscerosomatic index calculations excluded liver.

2004). In the present trial however, it is likely that much of the energy requirement of fish during starvation was utilized from fat reserves, particularly from liver and visceral stores as indicated by the observed reductions of HSI and VSI. Relative to initial lipid levels, *Ulva* fed fish displayed the highest levels of lipid loss at the end of the starvation period (a reduction of over 30% of total body lipids). The lower condition factor and an increased dress-out from an initial level of about 80–86% after the fasting period may lead to increased profitability and more aesthetically pleasing fish product. *Ulva* species are rich in vitamin C and it is hypothesized that this suppresses lipid peroxidation and improves lipid metabolism (García-Casal et al., 2007) which may result in the changes of fish carcass composition and nutrient retention (Miyasaki et al., 1995; Nakagawa, 1997; Nakagawa et al., 2000; Ji et al., 2003a).

In the current study, dietary inclusion of *Spirulina* had similar effects to *Ulva* meal with regards to total body weight loss over the starvation period (accumulated weight loss was approximately 50% less than the control group). Watanabe et al. (1990) reported that a 5% dietary inclusion of *Spirulina* decreased muscle lipid and enhanced product quality of striped jack *Pseudocaranx dentex*. Mustafa et al. (1997) reported that dietary *Spirulina* significantly elevated ascorbate levels in blood serum and the hepatopancreas in red sea bream. The authors suggested that *Spirulina* plays a protective role against degradation of dietary ascorbic acid. Dietary supplementation of 2.5% *Spirulina* in conjunction with vitamin C enhanced body weight gain, feed efficiency and digestibility which indicates a synergistic effect of *Spirulina* and Vitamin C on red sea bream.

In the present study, liver MUFA levels decreased after the 3-week fasting period which is suggestive that MUFA were the major energy sources for fasting rainbow trout, similar to that observed in black sea bream (Ji et al., 2003b). In the current trial, clear differences in the liver were in regards to n-3 fatty acids, with an increase in DHA and a reduction in EPA during starvation. These effects are also in agreement with previous findings in black sea bream

Table 4 Fatty acid composition of the liver at the end of feeding phase and after 3 weeks of fasting

	C		U5		U10		S5		S10	
	Feeding	Fasting	Feeding	Fasting	Feeding	Fasting	Feeding	Fasting	Feeding	Fasting
14:00	6.64	2.59*	7.82	5.56†	7.65	5.68†	5.02	3.51†	5.61	4.58†
15:00	1.03	0.58	1.12	0.79	1.12	0.57	0.79	0.58	0.85	1.15
16:00	25.15	26.00	25.70	28.19	25.79	27.39	22.54	24.78	22.15	28.92
17:00	0.90	0.63	0.90	0.79	0.91	0.78	0.69	0.71	0.77	0.83
18:00	8.07	7.50	7.03	7.23	7.05	8.60	5.26†	8.25	4.95†	6.82
20:00	0.86	0.20*	0.72	0.32*	0.72	0.25*	0.60	0.37*	0.57	0.23*
∑Saturated‡	43.56	37.85	43.87	43.14	43.87	43.49	35.40†	38.47	35.46†	42.93
18:1n-9	22.11	10.92*	22.66	17.10*†	22.75	13.12*†	23.38	17.17*†	21.87	15.53*†
18:1n-7	2.96	2.57	3.97	2.95	3.84	2.64	3.17	3.25	2.88	2.57
20:1n-9	3.82	3.33	2.62	1.35*†	2.62	0.92*†	2.19	1.51*†	1.89	1.11*†
∑Monounsaturated§	39.31	30.00*	36.05	26.61*	34.03	23.23*	38.79	28.66*	38.21	25.42*
18:2n-6	2.20	2.53	3.30	3.11	3.07	2.61	4.88†	3.31	5.21†	3.17
18:3n-6	0.28	0.19	0.28	0.24	0.27	0.26	0.19	0.26	0.49	0.14
20:4n-6	0.07	0.11	0.07	0.42	0.07	0.16	0.17	0.14	0.17	0.30
18:3n-3	0.63	0.35*	0.95	0.45*	0.90	0.53*	1.05	0.56*	1.08	0.53*
20:3n-3	0.17	3.05*	0.16	2.10*	0.17	3.07*	0.64	2.55*	0.69	2.12*
20:5n-3	3.43	0.22*	3.90	0.59*	4.31	0.61*	3.42	0.51*	3.57	0.57*
22:5n-3	0.18	1.07*	0.20	1.28*	0.21	1.35*	0.20	1.18*	0.53	1.22*
22:6n-3	10.04	21.75*	10.92	17.74*	11.95†	21.65*	14.01†	21.31*	12.25†	19.25*
∑Polyunsaturated¶	17.11	29.58*	20.03†	28.50*	21.20†	31.58*	24.99†	30.91*	24.41†	30.49*
∑n-6	2.60	3.02	3.71	3.93	3.48	3.19	5.48	4.11	6.07	4.09
∑n-3	14.52	26.56*	16.32	24.57*	17.72	28.39*	19.51	26.79*	18.34	26.40*

*Significant difference between feeding phase value and the value after 3 weeks of fasting ($p < 0.05$).

†Significant difference between the value of the algal fed group and the value of control group ($p < 0.05$).

‡ Includes: 21:00, 22:00, 23:00, 24:00.

§Includes: 14:1, 15:1, C16:1, C17:1, 22:1n-9.

¶Includes: 18:4n-3, 20:3n-6.

(Ji et al., 2003b). In the present trial, macro and micro algae meal incorporated into rainbow trout diets enhances the level of PUFAs at liver both feeding and starvation periods. Dantagnan et al. (2009) observed that the inclusion of 3–6% macroalgae meal (*Macrocystis pyrifera*) in diets fed to rainbow trout diets elevated the flesh levels of PUFAs, especially EPA and DHA, after 124 days feeding. These findings are similar to those observed in the present study with regards to liver PUFA levels.

The present study suggests that low level dietary algal supplementation may be helpful for rainbow trout farmers whom occasionally subject their fish to short periods of starvation. This can occur after heavy rainfall when sedimentation increases rearing water turbidity or when fish stocks are held back to meet consumer demands. The addition of dietary *Ulva* and *Spirulina* can reduce weight loss by enhancing mobilization of lipid reserves in rainbow trout as was evident by reductions of HSI and VSI. Future work should be undertaken to ascertain optimum inclusion levels and to evaluate the potential synergistic effects of combing *Spirulina* and *Ulva* meal in aquafeeds.

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