

FATTY ACIDS PROFILES OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS* WALBAUM, 1792), FED WITH ZEOLITE (CLINOPTILOLITE)

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ABSTRACT

In this trial, the lipid contents and fatty acid compositions (% of total fatty acids) of rainbow trout fed with four different ratios of clinoptilolite were studied. The fatty acid compositions of fish in groups ranged from 26.81% to 27.93 % saturated fatty acids, 25.35–31.435 % monounsaturated and 32.99–40.185 % polyunsaturated. Among them, those occurring in the highest proportions were oleic acid (C18:1n9, 19.85-22.27 %), palmitic acid (C16:0, 15.60-16.56 %), linoleic acid (C18:2n6, 11.43-18.88 %), cis-4,7,10,13,16,19-docosahexaenoic acid (C22:6n3, 13.36-15.52 %), stearic acid (C18:0, 4.25-4.75 %), palmitoleic acid (C16:1, 3.53-4.59 %), cis-5,8,11,14,17-eicosapentaenoic acid (C20:5 n3, 3.11-3.39 %), and myristic acid (C14:0, 2.56-2.85 %). The findings demonstrated that fatty acid compositions of the groups depend on feed, age, environmental conditions, and effects of feed additives like clinoptilolite. In this respect, it demonstrated that clinoptilolite can be added to fish feeds

Key words: Fatty Acids, *Oncorhynchus mykiss*, Clinoptilolite.

INTRODUCTION

Zeolites, one of the groups of the most important raw materials in present industry have attracted attention on especially environmental pollution and purification, and herbal and animal production (Danabas, 2009).

As a consequence of their properties, zeolite molecules called as “molecular sieves” are used generally in many fields as commercial absorbent and adsorbent (Alp, 2005; Leung, 2004; Aybal, 2001; Mumpton, 1999).

Clinoptilolite (CLNP), one of the types of zeolites or zeolites itself, are fundamentally used for four aims in aquaculture applications, at the present time. These are to provide pollution control in ponds; to remove N-compounds from water of hatcheries, fish transport and aquariums; to increase oxygen in aquarium and fish transport; and to increase growth parameter values of fish via adding into feed (Ravendra *et al.*, 2004; Kaiser *et al.*, 2006; Tore, 2006; Kanyilmaz, 2008).

Intensive aquaculture continues to expand, which requires high quality protein sources. Fish meal is major and increasingly expensive component of salmon and trout feeds, since it has high levels of digestible protein and energy, excellent amino acid and fatty acid profiles (Ozogul *et al.*, 2006). CLNP as feed additive can improve the effects of feed.

Flesh quality of fish is becoming an increasing concern in the aquaculture industry as total production increases (Johnston, 1999) by using different processes like feed additives. Flesh quality can be influenced by the biochemical composition of fish fillets (Hernández *et al.*, 2002). The biochemical composition may be affected by species, environmental factors, size, age, and diets

(Bandarra *et al.*, 1997; Yildiz *et al.*, 2006; Tang *et al.*, 2009).

The fish, especially freshwater fish as good as marine fish species, can be a source of essential fatty acids (Ozogul *et al.*, 2007). Compared to marine fish, freshwater fish contain high levels of C18 polyunsaturated fatty acids (PUFAs) and low levels of the C20:5 n3, eicosapentaenoic acid (EPA) and C22:6n3, docosahexaenoic acid (DHA) (Yildiz *et al.*, 2006). Freshwater fish are generally characterized by high levels of n6 PUFAs, especially C18:2n6, linoleic acid (LIA) and C20:4n6, arachidonic acid (ARA). Since freshwater fish contain lower proportions of long-chain n3 PUFAs than marine fish (Rahman *et al.*, 1995; Yildiz *et al.*, 2006), the ratio of total n3 to n6 fatty acids is much higher for marine fish than freshwater fish, varying from 5 to 10 or more (Ozogul *et al.*, 2007).

The significance of the long chain PUFAs has gained attention because of the prevention of human coronary artery disease (Conner, 2000; Mozaffarian *et al.*, 2005; Ward and Singh, 2005) and improvement of retina and brain development (Crawford, 1993; Su *et al.*, 2004), and also decreased incidence of breast cancer, rheumatoid arthritis, multiple sclerosis, psoriasis and inflammation (Kinsella, 1988). Marine lipids contain high level of PUFAs, especially EPA and DHA (Ackman, 1999). EPA and DHA are very beneficial to human health (Nestel *et al.*, 2002; Alasalvar *et al.*, 2002). EPA has been reported to be useful in the treatment of cancer and brain disorders (Fenton *et al.*, 2000). DHA is a major component of the brain, the eye retina, and heart muscles, also plays a vital role in the brain and the eye development (Ward and Singh, 2005). General recommendations for daily dietary intakes of DHA/EPA

are 0.5 g for infants and an average of 1 g/day for adults and patients with coronary heart disease (Kris-Etherton *et al.*, 2001; Ozogul and Ozogul, 2007).

Although Turkey has four seas and a great variety of fish species, the consumption of fish is small and the population should thus be encouraged to change their eating habits (Ozogul and Ozogul, 2007).

In this study, it is aimed to examine the effects of zeolite (CLNP), on lipid contents and fatty acids (FAs) profiles of rainbow trout (*Oncorhynchus mykiss*) which is in the first place (80.886 tone year⁻¹) among finfish culture in Turkey (TUIK, 2010).

MATERIALS AND METHODS

Experimental Site: The study was performed at Fisheries Faculty of Cukurova University (Adana, Turkey). In the trial, fingerlings of rainbow trout (*Oncorhynchus mykiss*) having 20.89±0.56 g average live weight (W) and 12.82±0.12 cm at average total length (L). They were cultured during 100 days and then the samples were taken after rearing period.

Channel type of concentrate ponds (4.75x1.0x0.75 m in size) and water from irrigation canal of The State Hydraulic Works were used in the Trial.

Zeolite (CLNP) added into the feed (100 µ in size) was assured from Enli Mining Corporation (Izmir, Turkey). In this study, trout feed (3 mm in size) which has min. 47 % crude protein (Abalioglu Company, Denizli, Turkey) were used in four groups; Control Group, 0 % CLNP feed⁻¹ (w/w); Group A, 1 % CLNP feed⁻¹ (w/w); Group B, 2 % CLNP feed⁻¹ (w/w); and Group C, 3 % CLNP feed⁻¹ (w/w) as triplicates. Feed was given to the fish at rate of 2 % average Ws of every group.

Sample preparation: At the beginning of the trial, 5 fingerlings were gutted, filleted and muscle tissue (edible muscle) was minced for analyses. After the Trial, 5 individuals of every group in cultured fish had same process.

FAME analyses: Lipid extraction followed the Bligh and Dyer (1959) method. Methyl esters were prepared by transmethylation using 2M KOH in methanol and n-heptane according to the method as described by Ichihara *et al.* (1996) with minor modification. Extracted lipids (10 mg) were dissolved in 2 ml heptane followed by 4 ml of 2 M methanolic KOH. The tube was then vortexed for 2 min at room temperature. After centrifugation at 4000 rpm for 10 min, the heptane layer was taken for gas chromatographic (GC) analyses.

Gas chromatographic condition: The FAs composition was analyzed by GC Clarus 500 with autosampler (Perkin Elmer, USA) equipped with a flame ionization detector

and a fused silica capillary SGE column (30 m x 0.32 mm, ID x 0.25 µm, BP20 0.25 UM, USA). The oven temperature was 140 °C, held 5 min, raised to 200 °C at a rate of 4°C min⁻¹ and to 220 °C at a rate of 1 °C min⁻¹, while the injector and the detector temperature were set at 220 °C and 280 °C, respectively. The sample size was 1 µl and the carrier gas was controlled at 16 psi. The split used was 1:100. FAs were identified by comparing the retention times of FAME with a standard 37 component FAME mixture (Supelco). Two replicate GC analyses were performed and the results were expressed in GC area % as a mean value and ± standard error of means.

Statistical Analyses: All statistical analyses (ANOVA) were performed using “SPSS 13.0” package program and comparison of means through DMRT at significance level 0.05.

RESULTS AND DISCUSSION

Table 1 shows the lipid contents of rainbow trout, water temperature, pH and dissolved O₂ values, for different groups.

The FAs compositions (% of total FAs) of rainbow trout fed with CLNP in Trial Groups, was given in Table 2.

In this study, lipid contents ranged from 2.15 % to 3.32 %; temperature, from 19.00°C to 19.28°C; pH, from 7.30 to 7.38; and dissolved O₂, from 9.86 mg l⁻¹ to 10.46 mg l⁻¹ (P>0.05).

The FAs compositions of fish in groups ranged from 26.81 % to 27.93 % saturated fatty acids (SFAs), 25.35–31.435 % monounsaturated (MUFAs) and 32.99–40.18 % PUFAs. Among them, those occurring in the highest proportions were C18:1n9, oleic acid (OLA, 19.85–22.27 %), C16:0, palmitic acid (PAA, 15.60–16.56 %), LIA (11.43–18.88 %), DHA (13.36–15.52 %), C18:0, stearic acid (STA, 4.25–4.75 %), C16:1, palmitoleic acid (PLA, 3.53–4.59 %), EPA (3.11–3.39 %), and C14:0, myristic acid (MYA, 2.56–2.85 %). These results show similarity with previous researches on FAs of rainbow trout (Saglik Aslan *et al.*, 2007) and some fresh water fish species (Ozogul and Ozogul, 2007).

It was also observed that the proportions of some FAs (C13:0, tridecanoic acid (TRA) in SFAs; OLA in MUFAs; and C18:3n6, γ-linolenic acid (LNA), ARA, and C22:2, cis 13,16 –docosadienoic acid (DDA) in PUFAs) changed significantly among groups and some others (C15:0, pentadecanoic acid (PEA), C17:0, heptadecanoic acid (HEA), and C22:0, behenic acid (BEA) in SFAs; PLA and C20:1, cis-11-eicosenoic acid (EIA) in MUFAs; and LIA and C20:3n6, cis-8,11,14-eicosatrienoic acid (ETA) in PUFAs), in age (P<0.05).

Table 1. According to Trial Groups, lipid contents of rainbow trout, water temperature, pH and dissolved O₂ values.

Parameters	Initial	Control Group	Group A	Group B	Group C
Lipid (%)	2,304±0,09 ^a	2,65±0,50 ^a	2,78±0,06 ^a	3,32±0,61 ^a	2,15±0,01 ^a
Temperature (°C)	-	19.09±0.13 ^a	19.28±0.12 ^a	19.00±0.06 ^a	19.27±0.10 ^a
pH	-	7.30±0.04 ^a	7.33±0.03 ^a	7.36±0.04 ^a	7.38±0.01 ^a
Dissolved O ₂ (mg l ⁻¹)	-	9.96±0.24 ^a	10.46±0.20 ^a	10.06±0.37 ^a	9.86±0.27 ^a

*The letters in the same line, show the differences in the results of statistical analyses.

Table 2. The fatty acids compositions (% of total fatty acids) of rainbow trout in trial groups (The letters in the same line show the differences in the results of statistical analyses)

Fatty Acids	Initial Time	Control Group	Group A	Group B	Group C	P Values
Saturated Fatty Acids						
C 12:0 (Lauric acid)	0.04±0.01 ^a	0.04±0.01 ^a	0.05±0.01 ^a	0.04±0.01 ^a	0.04±0.01 ^a	0.574
C 13:0 (Tridecanoic acid)	0.02±0.01 ^a	0.02±0.01 ^a	0.01±0.01 ^a	0.02±0.01 ^a	0.05±0.01 ^b	0.008
C 14:0 (Myristic acid)	2.56±0.03 ^a	2.77±0.10 ^a	2.85±0.07 ^a	2.84±0.13 ^a	2.72±0.04 ^a	0.210
C 15:0 (Pentadecanoic acid)	0.34±0.01 ^b	0.27±0.01 ^a	0.27±0.01 ^a	0.28±0.01 ^a	0.27±0.01 ^a	0.001
C 16:0 (Palmitic acid)	16.17±0.41 ^a	15.60±0.11 ^a	16.56±0.06 ^a	15.71±0.40 ^a	16.24±0.03 ^a	0.190
C 17:0 (Heptadecanoic acid)	0.37±0.03 ^b	0.28±0.01 ^a	0.26±0.01 ^a	0.28±0.02 ^a	0.28±0.01 ^a	0.013
C 18:0 (Stearic acid)	4.75±0.16 ^a	4.42±0.14 ^a	4.48±0.05 ^a	4.25±0.21 ^a	4.59±0.02 ^a	0.251
C 20:0 (Arachidic acid)	0.20±0.03 ^a	0.15±0.01 ^a	0.13±0.01 ^a	0.14±0.01 ^a	0.14±0.01 ^a	0.067
C 22:0 (Behenic acid)	1.08±0.10 ^a	2.14±0.11 ^b	1.97±0.02 ^b	2.06±0.02 ^b	2.02±0.07 ^b	0.001
C 23:0 (Tricosanoic acid)	0.07±0.03 ^a	0.04±0.01 ^a	0.04±0.01 ^a	0.04±0.01 ^a	0.04±0.01 ^a	0.486
C 24:0 (Lignoceric acid)	1.23±0.13 ^a	1.55±0.03 ^a	1.33±0.01 ^a	1.40±0.01 ^a	1.41±0.03 ^a	0.094
Total SFAs	26.81	27.25	27.93	27.03	27.77	
Monounsaturated Fatty Acids						
C 14:1 (Myristoleic acid)	0.02±0.01 ^a	0.03±0.01 ^a	0.03±0.01 ^a	0.03±0.01 ^a	0.03±0.02 ^a	0.558
C 16:1 (Palmitoleic acid)	3.53±0.11 ^a	4.30±0.20 ^b	4.49±0.10 ^b	4.40±0.14 ^b	4.07±0.17 ^b	0.028
C 17:1 (cis 10 -heptadecenoic acid)	0.24±0.01 ^a	0.23±0.01 ^a	0.23±0.01 ^a	0.24±0.01 ^a	0.23±0.01 ^a	0.739
C 18:1 <i>n</i> 9 (Oleic acid)	19.85±0.31 ^a	21.81±0.40 ^b	22.27±0.06 ^b	22.01±0.65 ^b	21.19±0.24 ^{ab}	0.033
C 20:1 (cis -11- eicosenoic acid)	0.92±0.74 ^a	3.63±0.16 ^b	3.41±0.02 ^b	3.48±0.02 ^b	3.42±0.03 ^b	0.010
C 24:1 (Nervonic acid)	0.80±0.01 ^a	0.86±0.09 ^a	1.01±0.01 ^a	0.96±0.17 ^a	1.06±0.09 ^a	0.393
Total MUFAs	25.35	30.845	31.435	31.095	29.98	
Polyunsaturated Fatty Acids						
C 18:2 <i>n</i> 6 (Linoleic acid)	18.88±1.05 ^b	12.06±0.29 ^a	11.53±0.31 ^a	12.41±0.53 ^a	11.43±0.14 ^a	0.001
C 18:3 <i>n</i> 6 (γ-linolenic acid)	0.30±0.01 ^a	0.29±0.01 ^a	0.42±0.01 ^b	0.41±0.02 ^b	0.29±0.01 ^a	0.001
C 18:3 <i>n</i> 3 (Linolenic acid)	2.01±0.13 ^a	1.94±0.01 ^a	1.86±0.05 ^a	1.99±0.10 ^a	1.86±0.01 ^a	0.540
C 20:2 (cis-11,14-eicosadienoic acid)	0.71±0.05 ^b	0.68±0.01 ^{ab}	0.60±0.02 ^a	0.63±0.02 ^{ab}	0.66±0.02 ^{ab}	0.101
C 20:3 <i>n</i> 6 (cis-8,11,14-eicosatrienoic acid)	0.37±0.01 ^b	0.24±0.01 ^a	0.22±0.01 ^a	0.23±0.01 ^a	0.24±0.01 ^a	0.001
C 20:3 <i>n</i> 3 (cis-11,14,17-eicosatrienoic acid)	0.85±0.26 ^a	0.58±0.01 ^a	0.55±0.01 ^a	0.54±0.05 ^a	0.60±0.01 ^a	0.414
C 20:4 <i>n</i> 6 (Arachidonic acid)	0.55±0.01 ^a	0.77±0.01 ^{bc}	0.69±0.01 ^b	0.79±0.05 ^c	0.78±0.01 ^{bc}	0.003
C 20:5 <i>n</i> 3 (cis-5,8,11,14,17-eicosapentaenoic acid)	3.11±0.21 ^a	3.36±0.04 ^a	3.19±0.01 ^a	3.35±0.02 ^a	3.39±0.02 ^a	0.301
C 22:2 (cis 13,16 -docosadienoic acid)	0.06±0.01 ^c	0.05±0.01 ^b	0.04±0.01 ^a	0.05±0.01 ^{ab}	0.05±0.01 ^b	0.011
C 22:6 <i>n</i> 3 (cis-4,7,10,13,16,19-docosahexaenoic acid)	13.36±0.08 ^a	14.55±0.45 ^{ab}	13.90±0.59 ^{ab}	13.84±0.89 ^{ab}	15.52±0.19 ^b	0.171
Total PUFAs	40.19	34.51	32.99	34.21	34.80	
PUFAs/SFAs	1.499	1.266	1.181	1.266	1.253	
Σ <i>n</i>6	20.090	13.350	12.855	13.825	12.730	
Σ <i>n</i>3	19.330	20.425	19.500	19.715	21.360	
<i>n</i>6/<i>n</i>3	1.039	0.654	0.659	0.701	0.596	
DHA/EPA	4.296	4.330	4.357	4.131	4.577	
Unidentified	7.66±1.05 ^a	7.40±0.07 ^a	7.65±0.19 ^a	7.67±0.09 ^a	7.47±0.17 ^a	

PEA, HEA, LIA and ETA levels decreased generally with increasing age, but, BEA, PLA and EIA levels increased. In addition to this; TRA, OLA, LNA, and ARA levels increased among groups when compared to the initial time; but DDA level decreased ($P<0.05$). Group C showed the highest level (0.05 %) in TRA ($P<0.05$). Levels of two groups (A and B) increased when compared to its own control and initial time, and the

highest levels obtained from Group A in OLA and LNA ($P<0.05$). The lowest levels of ARA and DDA obtained from Group A when compared to the Control Group ($P<0.05$). In this study, the lipid contents and percentage of FAs of rainbow trout were higher than that of the findings of Saglik Aslan *et al.* (2007). The findings demonstrated that FAs compositions of the groups depend on feed and effects of feed additives. However,

all Trial Groups had low levels of ARA (0.55–0.79 %) which may be advantageous to consumers for cardiovascular health due to the antagonistic effects to the health benefits of the *n3* FAs (Kinsella, 1988; Ozogul and Ozogul, 2007).

The proportions of FAs-*n3* (ranging from 19.330 to 21.360 %) were generally higher than those of FAs-*n6* (ranging from 12.730 to 20.090 %). The UK Department of Health recommends an ideal ratio of *n6/n3* of 4.0 at maximum (HMSO, 1994). Values higher than the maximum value are harmful to health and may promote cardiovascular diseases (Moreira *et al.*, 2001). In this study, the ratio of *n6/n3* was found to range from 0.596 to 1.039.

A minimum value of PUFAs/SFAs ratio is recommended as 0.45 (HMSO, 1994), which is lower than those (1.181–1.499 %) obtained from all Trial Groups treated with CLNP.

DHA/EPA ratios ranged from 0.72 to 6.89 in some fresh water fish species (Ozogul *et al.*, 2007) and it was 1.56 in rainbow trout (Sağlık Aslan *et al.*, 2007). In this study, the ratio of DHA/EPA in rainbow trout fed with CLNP was found to range from 4.131 to 4.557.

Conclusion: In this paper, the lipid contents and FAs compositions (% of total FAs) of rainbow trout fed with four different ratios of CLNP were studied and the results are discussed. According to the Trial results, the lipid contents and percentages of FAs of rainbow trout were higher than that of findings of Sağlık Aslan *et al.* (2007). The findings demonstrated that FAs compositions of the groups depend on feed, age, environmental conditions, and effects of feed additives like CLNP. It can be stated that cultured fish is a source of essential FAs for humans just like the wild fish. So that, it demonstrated that CLNP can be added to fish feeds.

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