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Original Article

The Effect of Linseed Oil and Kelp Meal in Diets on Fatty Acid Profile of Rabbitfish *Siganus Rivulatus*

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Abstract

Marine fish are an excellent source of dietary protein and omega-3 highly unsaturated fatty acids (n-3 HUFA). Various researchers claim that wild caught fish are healthier to consume than aquacultured fish. The assumption is that the diet of the wild fish is healthier and that translates to humans consuming the fish. The spinefoot rabbit fish is an herbivorous marine fish with a healthy fatty acid profile. Because diet can significantly influence the fatty acid profile of fish, we tested the effect of dietary non-marine lipids on rabbitfish proximate composition and fatty acid profile. Spinefoot rabbitfish juveniles were maintained in 50L glass tanks connected to a recirculating system. Fish were offered one of four dietary treatments with three replicate tanks per treatment. Three feeds were formulated to contain lipids with various fatty acid profiles. One diet was formulated with fish oil (Rich in EPA and DHA), one with linseed oil (Rich in alpha linolenic acid, ALA) and the third with linseed oil supplemented with DHA. Additionally, a commercial feed was tested as a reference and all results were compared to those of fish from another experiment that were reared using a high protein and lipid commercial diet. Results show that flesh of wild rabbitfish does not contain significantly more EPA and DHA than flesh of fish offered diets rich in ALA. Flesh of rabbitfish that consumed EPA and DHA in their diets contained significantly more of these two n-3 HUFA than flesh of wild fish. The present study demonstrates that consumption of aquacultured rabbitfish is as beneficial as, if not more beneficial than consumption of wild caught fish.

Key Words: n-3 HUFA, *Siganus rivulatus*, Rabbitfish.

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Introduction

Marine fish are known to be an excellent protein source as well as a good source of various minerals and vitamins necessary for good health. However, they differ from other

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foods in that they are also a source of essential omega-3 highly unsaturated fatty acids (n-3 HUFA). These n-3 HUFA are very important nutritional components that seem to mitigate many human diseases (Wijendran and Hayes, 2004 and Bourre, 2005). Societies with high fish intake such as the Inuit and the Japanese have considerably lower rates of acute myocardial infractions, other ischemic heart diseases (Bang and Dyerberg, 1980 and Blanchet, et al. 2000). Moreover, consumption of n-3 HUFA is inversely related to the risk of impaired cognitive function (Lie, 2004). Omega-3 HUFAs are also critical for normal neural and visual development in the human fetus (Innis and Elias, 2003). Recently, Sekikawa, et al. (2008) found that a decrease in atherosclerosis in humans is related to consumption of marine fish rich in n-3 HUFA and not to racial origin.

The important n-3 HUFAs, EPA (Eicosapentaenoic acid; 20:5 n-3) and DHA (Docosahexaenoic acid; 22:6 n-3) are produced mainly by marine phytoplankton (Liu and Lin, 2001) and transported through the food chain to be stored in secondary consumers such as marine fishes, which we then consume. Generally, consumers of marine organisms lack the ability to synthesize EPA and DHA although some can transform a limited amount of *A*-Linolenic acid (ALA) into longer chain HUFA. Some terrestrial and freshwater organisms also meet their EPA and DHA needs by elongating and desaturating other fatty acids (Lovell, 1998). For example, DHA can be formed in the human liver from the essential fatty acid ALA but only 1-4% of the ALA is actually converted to DHA (Sprecher, et al. 1996 and Innis and Elias, 2003). Consequently, humans need EPA and DHA in their diet and the most direct means to obtain these fatty acids is to consume foods rich in n-3 HUFAs such as marine fish. However, as various investigators have shown, aquacultured fish are not always as rich in n-3 HUFAs as wild caught fish. The presumed reason is a lack of EPA and DHA in the diet of the fish due to a decrease in the use of fish oil in fish feeds and to lack of elongation and desaturation abilities.

In the present work, we compared the effect of feeding rabbitfish a diet containing fish oil to that of feeding fish a diet containing linseed oil which is rich in ALA. The objective was to test whether rabbitfish, which naturally eat macro-algae which is not very rich in EPA and DHA, might be better at converting ALA into EPA and DHA. The filet composition of the experimental fish was also compared to that of wild fish in order to determine whether there is an advantage of consuming wild caught fish rather that farmed fish.

Materials and Methods

The present work was performed at the marine lab of the American University of Beirut, Lebanon. Juvenile spinefoot rabbitfish Siganus rivulatus were caught in traps off the Beirut coast and held in a quarantine tank for 10 days. Fish were then size sorted by hand to a relatively uniform size and stocked into a semi recirculation system consisting of twelve 50 L glass tanks connected to a biological filter and a physical filter. Oxygen concentration was maintained above 5.5 mg/l using a regenerative blower and submersible diffusers and temperature was held at 24^{o}C using a submerged heating element in the biofilter tank. Ammonia and nitrite nitrogen were maintained below 0.2 mg/L and pH ranged between 7.8 and 8.1.

Diets used in the present experiment were manufactured at the Auburn University Department of Fisheries and Aquaculture and are shown in (Table 1). A fourth commercial diet (Monodon Grower I-35 Premium, 35% crude protein, 7% crude fat, Zeigler Bros., Inc, Gardners, PA, USA) was used as a control. Twelve juvenile spinefoot rabbitfish (4.0±0.02g; Mean±SE) were stocked in each of twelve tanks and randomly assigned one of four dietary treatments with three replicates per treatment. Fish were offered feed at 4% body weight (BW) daily divided into a morning and an afternoon feeding event. Fish in each tank were group weighed weekly and ration adjusted accordingly. After the third weighing, we

noticed that growth was slower than usual so we increased the period between weightings. Consequently, every Monday the fish were offered feed until apparent satiation and then the amount of feed they consumed was increased by 10% and offered to the fish daily, for a week. When fish were weighed again, feeding at 4% BW was resumed for the following week. Seventy days after the start of the experiment, all fish were harvested, individually weighed and length measured and then fillet removed for further analysis. Proximate analysis was performed on samples of fish filets taken at the start of the experiment and on all filets collected at termination.

Filet from five fish in each tank were dried to constant weight at 98°C to estimate moisture content. Dried samples were then finely ground and stored at -20°C. Lipids were extracted from the samples according to the Continuous Solvent Extraction Method (Nielsen, 2003) with ethyl ether as the solvent and stored at -20°C for Fatty Acid analysis.

All samples were analyzed for protein content using a nitrogen analyzer (Thermo Finnigan/ EA1112 elemental analyzer) with aspartic acid as a calibration standard. Every tenth sample was duplicated for quality control and the machine was recalibrated after each 30 samples analyzed. Nitrogen values were multiplied by 6.25 to estimate protein content of samples. Fatty acid methyl esters were prepared on three of the five samples from each tank and on nine fish samples collected from another experiment (Hereafter labeled as treatment 5) that was performed simultaneously to the present work using fish from the same population but offered a trout feed (Golden Extruded, Chile; 50% crude protein, 20% lipid) as a diet. Fatty acid methyl ester profile was analyzed using a gas chromatograph (Shimadzu Scientific Inc., Model GC-2010) equipped with a flame ionization detector and Carbowax column (30m×0.53mm; Supelco). Finally, samples were combusted in a furnace at 550°C for two hours to estimate ash content.

Table 1: Ingredient composition of experimental diets (g per 100g dry weight) fed to spinefoot rabbitfish for 70 days. Diet 4 is a commercial shrimp feed (Monodon Grower I-35 Premium, Zeigler Bros., Inc, Gardners, PA, USA) and Diet 5 is a commercial trout feed (Golden Extruded, Chile):

	Diet 1	Diet 2	Diet 3	Diet 4
Fishmeal [a]	1.00	1.00	1.00	
Poultry by product meal [b]	16.00	16.00	16.90	
Soybean meal solvent extracted [c]	40.30	40.10	40.30	
Aqua Grow-schizochytrium-DHA	0.00	0.50	0.00	
AquaGrow ARA	0.00	0.13	0.00	
Menhaden Fish Oil [d]	5.02	0.00	0.00	
Soy oil	0.00	0.57	0.60	
Flax oil (linseed oil, Sigma)	0.00	4.18	4.40	
Wheat starch [e]	6.78	6.62	5.9	
Whole wheat [e]	20.00	20.00	20.00	
Trace Mineral premix [f]	0.50	0.50	0.50	
Vitamin premix w/o choline [g]	1.80	1.80	1.80	
Choline cloride	0.20	0.20	0.20	
Stay C 250mg/kg using 25%[h]	0.10	0.10	0.10	
CaP-dibasic [e]	2.60	2.60	2.60	
Lecethin (soy refined, USB)	0.50	0.50	0.50	
Cholesterol [e]	0.20	0.20	0.20	
Corn Gluten meal [e]	5.00	5.00	5.00	
Kelp meal	0.00	0.00	1.00	
Crude protein (%)	39.7	39.9	39.6	40.8
Crude lipids (%)	5.6	6.2	6.1	6.4
Ash (%)	9.3	8.9	8.8	13.4

[a] Omega Protein Inc., Hammond, LA, USA.

[b] Griffin Industries, Inc. Cold Springs, KY, USA.

[c] De-hulled solvent extracted soybean meal, Southern Sates Cooperative Inc., Richmond VA, USA.

[d] Omega Protein Inc., Reedville, Virginia, USA.

[e] United States Biochemical Corporation, Cleveland, Ohio, USA.

[f] g/100g premix: cobalt chloride 0.004, cupric sulfate pentahydrate 0.250, ferrous sulfate 4.0, magnesium sulfate heptahydrate 28.398, manganous sulfate monohydrate 0.650, potassium iodide 0.067, sodium selenite 0.010, zinc sulfate heptahydrate 13.193, filler 53.428.

[g] g/kg premix: thiamin HCl 0.5, riboflavin 3.0, pyrodoxine HCl 1.0, DL Ca-Pantothenate 5.0, nicotinic acid 5.0, biotin 0.05, folic acid 0.18, vitamin B12 0.002, choline chloride 100.0, inositol 5.0, menadione 2.0, vitamin A acetate (20,000 IU/g) 5.0, vitamin D3 (400,000 IU/g) 0.002, dl-alpha-tocopheryl acetate (250 IU/g) 8.0, Alpha-cellulose 865.266.

[h] 250 mg/kg active C supplied by Stay C®, (L-ascorbyl-2-polyphosphate 25% Active C), Roche Vitamins Inc., Parsippany, New Jersey, USA.

[i] ICN Biochemicals, Inc. Aurora, Ohio, USA.

All samples were analyzed for protein content using a nitrogen analyzer (Thermo Finnigan/ EA1112 elemental analyzer) with aspartic acid as a calibration standard. Every tenth sample was duplicated for quality control and the machine was recalibrated after each 30 samples analyzed. Nitrogen values were multiplied by 6.25 to estimate protein content of samples. Fatty acid methyl esters were prepared on three of the five samples from each tank and on nine fish samples collected from another experiment (Hereafter labeled as treatment 5) that was performed simultaneously to the present work using fish from the same population but offered a trout feed (Golden Extruded, Chile; 50% crude protein, 20% lipid) as a diet. Fatty acid methyl ester profile was analyzed using a gas chromatograph (Shimadzu Scientific Inc., Model GC-2010) equipped with a flame ionization detector and Carbowax column (30m×0.53mm; Supelco). Finally, samples were combusted in a furnace at 550^{o}C for two hours to estimate ash content.

Statistical analyses were performed using SPSS statistical software (V.12 for Windows, SPSS Inc., Chicago, IL, USA) and α= 0.05. The Fulton condition index (CI) of the fish was calculated as: CI= 100.W/L3, where W= fish weight (g) and L= total length (cm). Variables were compared among treatments using one-way ANOVA and Student-Newman-Keuls multiple-range test ($p < 0.05$).

Results

Survival was greater than 95% in the four treatments. Similarly, fish in the four treatments grew at the same rate (Table 2). The fact that some of the diets did not contain any EPA and DHA did not affect survival and growth over a period of 70 days. Feed conversion in all treatments was large (>3) and that is due to a slow growth rate. Results of a recent experiment in our lab (Ghanawi, et al. 2009) show that small fish in a population grow slower than the average growth rate for the population even when separated from larger fish. Consequently, in the present study we unknowingly selected for slow growing individuals with high feed conversion ratios. The CI of fish offered diet 3 was greater than CI of fish offered diets 1 and 2, and greater than CI of fish offered diet 4 but not significantly. A possible reason is that filet of fish offered diet 3 contained less lipids and more water than filet of fish offered other diets (Table 3), thus fish in treatment 3 were heavier per unit length than fish in other treatments. Such results imply that CI as an estimate of "Well being" is not always a reliable index.

The proximate composition of the fish filets in the present experiment did not vary significantly among treatments nor when compared to the fish captured from the wild. Even the fish in treatment 5, offered a high protein and lipid diet, had a body composition similar to fish in the other treatments (Table 3). Moisture, lipid content, protein content and ash in all treatments were similar to those reported by Saoud et al. (2007) in filets of rabbitfish collected from the wild during all seasons of the year. Consequently, any person using proximate composition of the flesh to compare nutritional benefits of consuming wild caught rabbitfish rather than aquacultured rabbitfish would find very few differences among them.

Table 2: Initial Wti, initial condition index CIi, percent survival, final Wtf, final condition index CIf and feed conversion ratio FCR of S. rivulatus offered various diets for 70 days:

Diets	Wt_i (g)	CI_i	% Survival	Wt_f (g)	WG (%)	CI_f	FCR
Diet 1	4.0 ^{a}	1.09	97.2 ^{a}	12.3 ^{a}	207.5 ^{a}	1.17 ^{a,b}	3.80 ^{a}
Diet 2	4.0 ^{a}	1.09	95.9 ^{a}	12.6 ^{a}	215.0 ^{a}	1.07 ^{a}	3.75 ^{a}
Diet 3	3.9 ^{a}	1.09	97.2 ^{a}	12.5 ^{a}	220.5 ^{a}	1.26 ^{b}	3.63 ^{a}
Diet 4	4.0 ^{a}	1.09	100.0 ^{a}	13.9 ^{a}	247.5 ^{a}	1.18 ^{a,b}	3.20 ^{a}
PSE	0.03	0.00	2.12	0.39	35.97	0.07	0.11

PSE= Pooled standard error.

Table 3: Proximate analysis of filets fish offered diets with various sources of fish oil for 70 days. There are no significant differences among treatments:

Diets	% Moisture	% Lipids	% Protein	% Ash
Wild	73.58	3.81	21.13	1.37
Diets 1	74.6	5.00	17.73	1.39
Diets 2	73.9	4.10	19.26	1.44
Diets 3	75.0	3.13	18.55	1.43
Diets 4	74.2	3.48	19.76	1.49
Diets 5	73.6	3.91	20.12	1.50
PSE	0.92	2.09	1.94	0.23

PSE= pooled standard error

As would be expected, the fatty acids in the diet had a strong effect on the fatty acid makeup of fish filets. Feeds that did not contain fish oil were devoid of pentadecanoic acid and consequently, so were the filets of fish that consumed those diets (Table 4). Moreover, the proportion of α-linolenic acid (ALA; 18:3 n-3) in the filet of fish also seems to be affected by the amount present in the diet. Linoleic acid (18:2 n-6) in the filet was also affected by the proportion present in the diet. Arachidonic acid (ARA), EPA and DHA were always found in fish flesh, even when absent, or below detectable levels, in the diet. Arachidonic acid in muscle of wild fish was greater than in muscle of fish in all cultured fish. These results are similar to those reported by Ogata et al. (2004) in other species of siganids. They postulate that the high proportion of ARA in the tissue is due to high levels of ARA in marine red and brown macro-algae, the natural food of the fish. The proportions of EPA and DHA in lipids of filets of fish that did not consume fish oil were less than in the filet of fish that consumed fish oil but were similar to the filets of wild fish. Similar results were observed by Lewis and Kohler (2008) working with sunshine bass offered graded levels of canola oil in their diet. However, if the sum totals of n-3 HUFAs are calculated, we find that fish which consumed diets with linseed oil have deposited more n-3 HUFA in their flesh than those that have consumed diets with fish oil. This is due to the abundance of ALA in linseed oil and its paucity in fish oil. Similarly, fish that consumed diets with linseed oil deposited in their flesh more n-6 HUFA than fish which consumed diets with fish oil. This is due to the fact that linseed oil contains much more linoleic acid (18:2 n-6) than fish oil. These results are similar to those of Suzuki et al. (1986) and Steffens (1997).

Scientists believe that the n-6:n-3 HUFA ratio is more important than total n-3 HUFA content of the diet (Yehuda, et al. 2002; Bourre, 2004 and Wijendran and Hayes, 2004). Although all diets investigated in the present work (Except diet 4) contained more n-3 HUFA than n-6 HUFA, fish flesh contained on average 50% more n-6 HUFA than n-3 HUFA, except for the flesh of fish in treatment 5 which contained twice as much n-3 than n-6 HUFA. The reason for a low n-6/n-3 ratio in treatment 5 can be attributed to the fact that diet 5 contained 20% lipids by weight (As opposed to 6% in other diets) and moreover, lipids of diet 5 contained 2.5 times more n-3 than n-6 HUFA per unit weight of lipid. In all treatments, the ratio of n-6/n-3 HUFA was 1.5 or less. These values correlate well with values reported by Saoud et al. (2007) in wild S. rivulatus, but not with reports of other investigators. Steffens (1997) cites various studies that report n-3 and n-6 proportions in fish lipids in which n-3s are more than n-6s but all fish tested are either zooplanktivorous or piscivorous. Similar results are reported by Regost et al. (2003) working with turbot Psetta maxima. However, rabbitfish is a macro-algaevore and thus does not benefit from the bioaccumulation of HUFAs as do organisms of a higher trophic level.

The proportions of EPA and DHA in the muscle lipids of fish appear to be affected by the amount of lipid in the diet of the fish as well as the source of the lipid. In all diets where lipids were of marine origin, EPA and DHA proportions of the muscle lipids were greater than when fish were offered diets with linseed

oil. However, when the amount of marine lipids in the diet was increased to approximately 20% (Diet 5) EPA and DHA increased a lot as a proportion of total flesh lipids although total lipids were not significantly different from those of fish in the other treatments. This suggests that EPA and DHA, essential FA, are well conserved in the body. Conservation of n-3 is reported in humans by Bourre (2004), crustaceans Dabramo (1997) and fish by Sargent et al. (2002).

Many consumers assume that wild caught fish are healthier to eat than aquacultured fish. Van Vliet and Katan (1990) evaluated n-3 and n-6 HUFA content in wild and aquacultured fish and reported that wild fish was better to consume than cultured fish. Similar findings were reported by George and Bhopal (1995). Even popular magazines sometimes claim that wild fish is better than farmed fish (Murphy, 2003). Such claims are not supported in the present work. The EPA and DHA content of wild fish filet in the present experiment was similar to filet of aquacultured fish that were offered diets devoid of fish oil. However, flesh of fish offered diets with fish oil contained significantly more EPA and DHA than flesh of wild fish. Such results do not corroborate findings by Van Vliet and Katan (1990) or those by George and Bhopal (1995). Since the lipid contents in the flesh of wild spinefoot rabbitfish and those in all treatments were similar to each other, our results show that consuming aquacultured fish is as healthy as, or healthier than consuming wild fish, depending on the diet of the fish. Rabbitfish that were offered feed with flax oil rather than fish oil contained similar amounts of EPA and DHA as wild caught con-specifics and more ALA in their flesh. Therefore, results of comparisons between wild caught and aquacultured fish vary with species, and what might be true for salmon does not necessarily apply to other species. The benefits of consuming wild caught fish as compared to aquacultured fish should be evaluated on a species by species basis. Moreover, in aquacultured fish, diet quality can be controlled. Hence, contaminants can be avoided and nutrients that might be beneficial to humans enhanced.

Hardy (2003) reminds readers that the amount of lipids in fish tissue multiplied by the proportion of n-3 HUFA results in a proper estimate of n-3 FA intake from fish consumption, as opposed to only evaluating the proportion of n-3 HUFA in the lipids. Wijendran and Hayes (2004) suggest that the total weight of fatty acid consumed is more important than the proportion of n-3 HUFA in the lipids. Furthermore, n-6/n-3 HUFA ratio is also important. Holub (2002) and Wijendran and Hayes (2004) recommend a n-6/n-3 ratio of 4:1 and 6:1, respectively. Such n-6/n-3 HUFA ratios were not found in flesh of aquacultured rabbitfish nor wild caught rabbitfish, and total content of flesh lipids did not vary among treatments. Accordingly, we suggest that fish consumption to be accompanied by the consumption of vegetable oils, high in n-6 HUFA.

As a conclusion, we believe that various fish species have various levels and kinds of fatty acids in their muscle, depending on ecological trophic level and on what they consume. The benefits of consuming wild caught fish vary with season and species (Saoud, et al. 2007), and the benefits of consuming aquacultured fish vary with species and diet. Blanket recommendations about the superiority of wild fish as compared to farm raised fish should be avoided and nutritional recommendations made on a case by case basis.

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Table 4: Fatty acid composition of flesh of wild fish at stocking, flesh of fish offered the various diets for 70 days and of the feeds offered to the fish. Values in the same row that do not have a similar superscript are significantly different from each other (α = 0.05):

	Diets offered to the fish.					
	Feed 1	**Feed 2**	**Feed 3**	**Feed 4**	**Feed 5**	
Pentadecanoic	5.3	0.0	0.0	6.6	5.7	
C15:1	0.5	0.0	0.0	0.5	0.6	
Palmitic	21.6	12.2	12.7	22.3	19.0	
Palmetoleic	8.9	1.9	1.9	8.5	5.7	
C17:0	0.0	0.0	0.0	0.0	0.0	
C17:1	0.0	0.0	0.0	0.0	0.0	
Stearic	5.4	4.6	5.2	5.6	4.5	
Oleic	21.2	26.0	28.3	17.5	21.4	
Linoleic	11.9	23.3	23.3	14.4	7.9	
C18:3ω6	0.0	0.0	0.0	0.0	0.0	
C20:1	0.0	0.0	0.0	2.5	3.9	
Linolenic	2.9	30.8	28.6	0.0	1.1	
C20:2	0.0	0.0	0.0	0.0	0.0	
C20:3ω6	0.0	0.0	0.0	0.8	0.0	
Arachidonic	0.5	0.3	0.0	0.0	0.8	
EPA	7.8	0.0	0.0	7.0	9.4	
DHA	7.8	0.8	0.0	7.2	10.9	
omega-3	18.5	31.6	28.6	14.3	21.3	
omega-6	12.4	23.6	23.3	15.2	8.7	
Ratio 6/3	0.7	0.7	0.8	1.1	0.4	
	Fish muscle tissue.					
	wild fish	**Trt. 1**	**Trt. 2**	**Trt. 3**	**Trt. 4**	**Trt. 5**	**PSE**
Pentadecanoic	3.5	3.8	0.0	0.0	4.1	4.9	
C15:1	0.7	0.0	0.0	0.0	0.2	0.6	
Palmitic	40.2	30.7	23.1	24.3	29.7	24.0	
Palmetoleic	6.3	9.8	5.6	6.1	10.4	8.3	
C17:0	0.8	0.0	0.0	0.0	0.0	0.0	
C17:1	0.6	0.0	0.0	0.0	0.1	0.0	
Stearic	6.7	5.5	5.1	5.0	5.3	4.6	
Oleic	21.4	26.3	28.2	29.4	23.3	25.7	
Linoleic	5.1	8.8	14.7	14.0	9.6	6.6	
C18:3ω6	0.4	1.2	2.3	2.1	1.1	0.0	
C20:1	0.3	0.5	0.0	0.0	1.1	3.0	
Linolenic	1.7	1.6	9.3	9.3	1.6	1.0	
C20:2	0.4	0.0	0.0	0.0	0.1	0.0	
C20:3ω6	0.4	0.7	0.8	0.9	0.7	0.0	
Arachidonic	1.7	0.7	0.9	0.7	0.6	0.6	
EPA	0.9^{a}	1.4^{b}	0.7^{a}	0.7^{a}	1.5^{b}	3.1^{c}	0.08
DHA	2.5^{a}	5.3^{b}	3.0^{a}	2.3^{a}	5.7^{b}	9.5^{c}	0.44
omega-3	5.1^{a}	8.4^{b}	13.0^{c}	12.3^{d}	8.7^{b}	13.6^{c}	0.16
omega-6	7.6^{a}	11.4^{bc}	18.7^{d}	17.6^{d}	12.1^{c}	7.1^{ab}	0.27
Ratio 6/3	1.5^{a}	1.4^{a}	1.4^{a}	1.4^{a}	1.4^{a}	0.5^{b}	0.03

PSE= pooled standard error.

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