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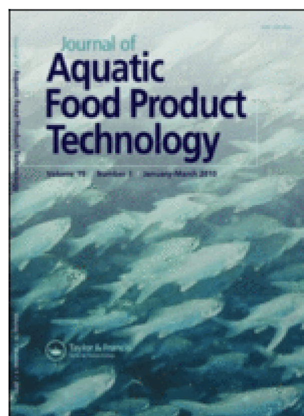

EFFECTS ON LIPID OXIDATION AND BIOACTIVE PROPERTIES OF RAINBOW TROUT FILLETS FED WITH BARLEY.

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Journal of Aquatic Food Product Technology
Pinedo-Gil et al., 2019-Ms Ref No. WAFT-2018-0102

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Valladolid, 2019

Dear Editor,

The authors would be grateful if you could consider the revised paper ***WAFT-2018-0102 "Effects on lipid oxidation and bioactive properties of rainbow trout fillets fed with barley". (by J. Pinedo-Gil, A. Tomás-Vidal, D. Rico, B. Tiwari, C. Álvarez García, , M. Jover-Cerdá, M.A. Sanz-Calvo and A.B. Martín-Diana)*** *for publication in Journal of Aquatic Food Product Technology.*

We wish to thank you and the Reviewers for comments and suggestions, which greatly improved the paper. Our answers to your comments and the changes on the manuscript according to your suggestions are detailed below. Changes in the revised manuscripts are blue-typed.

Comments Reviewer 1

Reviewer 1: Growth performance characteristics data could be included in the results to see if there is any relationship between fish performance, lipid oxidation and bioactive properties of barley used as dietary supplement.

Authors: According to the reviewer suggestion fish growth performance has been included in the results section (page 8 lines 168-172 and conclusion page 12 lines 271-275) in order to study the relationship between rainbow trout performance fed with barley and their fillets lipid oxidation and bioactive properties.

Results: Page 8, lines 168-172: Growth performance was not affected by the substitution of wheat from barley. Similar results were reported by Sealey *et al.* (2008) who studied the effect of three barley genotypes on growth performance of rainbow trout and did not observe significant differences on final weight regardless barley concentration used.

Conclusion: Page 12, lines 271-275: It can be observed that these effects may be due to the presence of certain bioactive components in barley and not related to the fish growth. More studies should be carried out to investigate which components in barley

are the responsible to inhibit lipid oxidation and improve antioxidant properties until a concentration of 8% of barley. And reason why higher barley concentrations showed a negative effect on the fish population.

Kind regards,
The authors.

EFFECTS ON LIPID OXIDATION AND BIOACTIVE PROPERTIES OF RAINBOW TROUT FILLETS FED WITH BARLEY.

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ABSTRACT

Barley concentrations ranging from 0% to 32% were incorporated into rainbow trout, *Oncorhynchus mykiss*, diets. The effect of barley concentration on lipid peroxidation and antioxidant activity were analysed on fish fillets. Results showed that the inclusion of barley on rainbow trout diets had an inhibitory effect on lipid oxidation probably associated with certain bioactive compounds reported on barley, which could interact scavenging and reducing metabolites involved in lipid oxidation. Concentrations up to 8% of barley produced an enhanced of fish fillets showing high antioxidant activity and higher levels of alpha-tocopherol.

Keywords: Barley, Rainbow trout, Lipid oxidation, antioxidant activity.

Practical Applications: Barley is a cereal not frequently used in aquaculture. The present study demonstrate that its use would be interesting due to its capacity to enhance quality, oxidative stability and the antioxidant activity of fish fillet. That makes rainbow trout fillet healthier and could promote its sale.

1. INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss*) is one of the major aquaculture fish species worldwide and is the second most consumed fish in Europe (FAO, 2013). During the last decade, the demand of rainbow trout has increased significantly for its high nutritional value, taste and aroma (Volpe *et al*. 2015, Shadman *et al*. 2017, Erbay *et al*. 2017). Trout is an important source of high-quality proteins, polyunsaturated fatty acids (PUFA´s), lipid soluble vitamins and micronutrients (Alparslan *et al*. 2014, Volpe *et al*.

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2015, Erbay *et al*. 2017), although can be rapidly oxidised leading to important sensory and nutritional quality losses (Pereira de Abreu *et al*. 2010, Yildiz *et al*. 2016, Erbay *et al*. 2017).

Incorporation of novel ingredients to subside the aquafeed cost is essential to balance product quality in order to control some negative aspects such as lipid oxidation (Pratoomyot *et al*. 2010, Valente *et al*. 2015, García-Romero *et al*. 2014, Pinedo-Gil *et al*. 2017). The use of natural antioxidants or ingredients preserve and reduce oxidation during and after fish processing. Therefore, organoleptic properties can be maintained, since lipid oxidation (hydroperoxide, peroxide value (PV) and their break down into other secondary compounds, most of them volatile products), is involved in the production of off-flavours (Razaei and Hosseini 2008).

Barley is an important source of β-glucans and other bioactive components such as phenolic compounds, which can control oxidative processes (Sandhu and Punia 2017). The use of barley on rainbow trout diets is not currently implemented at industrial scale, although few studies have investigated their incorporation on diet. It was observed that the incorporation of barley did not produce any negative effect on productive parameters (Sealey *et al*. 2008, Pinedo-Gil *et al*. 2017), however, more studies are required. For this reason, the objective of this study was the evaluation of oxidative parameters and bioactive properties of rainbow trout fed at different barley concentrations.

2. MATERIAL AND METHODS

2.1. Experimental design

Rainbow trout were provided by a commercial trout farm (IPEASA, Fuentidueña, Segovia, Spain). The average weight for each fish was 127 ± 2.62 g (least-square mean

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73 ± SEM). Fish were fed with five isoproteic (40% crude protein) and isolipidic diets 74 (18% crude fat), which contained different barley levels (0-31.9%, named 0B, 40B, 75 80B, 160B and 319B) (Pinedo-Gil *et al*. 2017). There were five feeding treatment 76 groups each in four replicates (n=4). Fish were randomly sampled every 28 days (0, 28, 77 56 and 84 days) and skinless fish fillets were prepared for the evaluation. Three fish per 78 replicate were evaluated for the different analysis (n=12). The skin was removed and 79 fillets were kept frozen until analysis. Prior to analyses, all fish were starved for 24 h 80 and anesthetized with MS222®; 200 mg L^{-1}. The duration of the trial was 84 days.

81 **2.2. Fatty acid profile (FAME)**

82 Fatty acid profile (FA) was determined in barley, diets and fish fillets by triplicate. 83 Blight and Dyer (B&D) method (Blight & Dyer 1959) was used for lipid extraction. 84 Lipid-containing chloroform phase was separated and after evaporated. The remaining 85 phase was dissolved in 1 mL of hexane and a methylated procedure carried out by 86 adding 100 µL of 0.5 M methanolic KOH and leaving the reaction for 10 min at room 87 temperature (RT). The upper layer was transferred to a 2 mL vial. Analysis of FA 88 methyl esters (FAME) were carried out on a gas chromatograph Agilent 7890A (Agilent 89 Technologies, PA, California, USA) and a flame ionization detector. For the analysis 90 the method was run on 50 ºC to 200 ºC during the first 7 min at a rate of 3 ºC min^{-1} and 91 held for 26 min. Injector and detector temperature were 250 ºC and 280 ºC, 92 respectively. After, 1 µL of the hexane extract was injected in split mode (ratio 25:1), 93 and FAMEs were identified by comparison of retention times with those of 37 FAMEs 94 standard mix (Supelco, Sigma-Aldrich, CO).

95 **2.3. Alpha-tocopherol content**

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96 Tocopherol content was determined according to the AOCS official method (1992) for
97 fish fillets samples, using Agilent 1200 series HPLC equipped with a diode array
98 detector. Two grams of the B&D extract was evaporated and resuspended in 2 mL of
99 hexane with 20 µL of tocopherol acetate as internal standard. An aliquot (10 µL) was
100 injected and a column (250 mm x 4.6mm 5 µm) (Teknokroma Anlítica S.A., Barcelona,
101 Spain) was used. Elution was performed using an isocratic mixture of hexane:2-
102 propanol (99.6:0.4; v:v) at a flow rate of 1.3 mL min^{-1}. Detection was set at 295 nm and
103 284 nm for tocopherol acetate. Results were expressed in µg tocopherol per gram of
104 fillet.

105 **2.4. Oxidative parameters**

106 **2.4.1 Peroxide value (PV)**

107 PV was measured on the fish fillet using B&D extract according to the International
108 IDF Standards method (1991). Results were expressed in meq of active oxygen per kg
109 of lipids.

110 **2.4.2. Conjugated hydroperoxides (dienes and trienes)**

111 Conjugated hydroperoxides (fish fillet B&D extract) were measured as described by
112 Undeland *et al*. (1998). Results were calculated as mmol of hydroperoxides per kg lipid.

113 **2.5. Antioxidant markers**

114 **2.5.1. Total Flavonoid determination (TFC)**

115 TFC was determined using the method described by Lin and Tang (2007) for barley and
116 diets. Aliquots of 0.1 g of sample were dissolved in 1 mL of 10% aluminium chloride
117 hexahydrate ($AlCl_3$), 0.1 mL of 1 M potassium acetate (CH_3COOK) and 2.8 mL of
118 deionized water. After incubation at room temperature (RT) for 40 minutes the reaction
119 was measured at 415 nm (Shimadzu PharmaSpec UV-1700. Milton Keynes, UK). The

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120 data were expressed as quercetin equivalent (QE) per 100 g of sample based on the
121 moisture content of lyophilized powder and "fresh sample".

122 **2.5.2. Extract preparation**

123 Barley, diets and fish fillet were used for extracts preparation to measure antioxidant
124 activity. One gram of blended sample was dissolved in 10 mL of 90% methanol. The
125 extraction was accelerated using a ceramic homogenizer in the test tubes and stirring for
126 30 s. Following samples were centrifuged at 1.635 x g for 10 min at 4 ºC and the
127 supernatants were collected, filtered and stored at -80 ºC. All the extracts were used for
128 the determination of total phenols and oxygen radical absorbance capacity (ORAC).

129 **2.5.3.Total phenols (TP)**

130 TP were measured using the Folin-Ciocalteu method (Slinkard and Singleton 1977) on
131 barley, diets and fish fillets. Results were expressed as mg of gallic acid per gram of
132 dried weight (dw) sample.

133 **2.5.4. Phenolic characterization using high-performance liquid chromatography**
134 **(HPLC)**

135 Phenolic characterization was determined on barley and diets. Five gramms of sample
136 were mixed with 45 mL of 80% ethanol (v/v) and after it was sonicated in a water bath
137 for 1 h. After centrifugation (5000 x g, 20 min., 10 ºC), the supernatant was removed
138 and the extraction was repeated twice. Supernatants were mixed and after evaporated at
139 40 ºC with nitrogen until complete dryness, reconstituted in 2 mL of 40% acetonitrile
140 and then it was filtered through 0.45 µm membrane for HPLC analysis (Bonoli *et al*.
141 2004, Zhao *et al*. 2006).

142 The phenolic compounds were separated and quantified using the method described by
143 Schieber *et al*. (2001) with modifications, briefly as follows. Water Alliance 2795

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144 Chromatography Separations Module (Waters Corp., Milford, USA) coupled to a
145 Waters 2996 PDA detector fixed at 280 nm of wavelength. Column employed was
146 Zorbax sb-c18 Agilent (4.6 x 150 nm) 5 microns. The mobile phases consisted in 0.5%
147 acetic acid (buffer A) and 20% (0.5% acetic acid):80% acetonitrile (buffer B). Initial
148 gradient started with 5% of buffer B for 1 minute, and then was increased up to a 55%
149 for 50 minutes; the column was cleaned for 5 minutes by pumping 95% of buffer B and
150 finally it was re-equilibrated for another 10 minutes. Calibration curves were
151 constructed using the following standards: gallic acid, chlorogenic acid, ferulic acid, p-
152 cumaric acids, synaptic acid, 3-coumaric acid, 4-coumaric acid, syringic acid, maleic
153 acid, transcinamic acid, vanilic acid, caffeic acid and 4-hydroxibenzoic acid at
154 concentration of 5, 10, 20, 40 and 80 µg mL^{-1}.

155 **2.5.5. Determination of the oxygen radical absorbance capacity (ORAC)**

156 The procedure was based on the method described by Ou *et al*. 2001. The determination
157 was measured on fish fillets. Results were expressed as µmol of Trolox Equivalent (TE)
158 per g of sample (dw).

159 **2.6. Statistical analysis**

160 Statistical analysis was performed using SAS version 9 (SAS Institute Inc., Cary, North
161 Caroline, USA) by a GLM procedure for the variance analysis (ANOVA) followed by a
162 t-Student test and considering significant differences between values with a P-value <
163 0.05.

164 **3. RESULTS AND DISCUSSION**

165 Proximate composition and β-glucan in barley, experimental diets and fillets were
166 determined in Pinedo-Gil *et al*. 2017. It was observed that all diets were isoproteic (40%
167 protein) and isolipidic (18% lipid), barley contained 5.2% β-glucan and its inclusion on

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experimental diets introduce this component to the diets (0 to 1.5% β-glucan). Growth performance was not affected by the substitution of wheat from barley. Similar results were reported by Sealey *et al*. (2008) who studied the effect of three barley genotypes on growth performance of rainbow trout and did not observe significant differences on final weight regardless barley concentration used. In fillet, the inclusion of barley increased significantly crude fat while protein was not affected.

3.1. Fatty acid profile

The replacement of wheat with barley in rainbow trout diets resulted in a marked decrease of linoleic acid (C18:2 n-6), linolenic acid (C18:3 n-3) and docosahexanoic acid (DHA, C22:6 n-3) in the experimental diets compared with the control (Table 1). However, results showed that although the fatty acid profile change with the concentration of barley, it had not a significant effect on fillets total SFA´s, MUFA´s or PUFA´s (Table 2). Significant differences ($P < 0.05$) were observed when individual FA´s were analysed: an increase on myristic acid (C14:0) and palmitic acid (C16:1) levels on fish fed with 319B diets. The fatty acid composition was in agreement with values for fresh rainbow trout fillets as reported by other authors (Ozden 2005, Volpe *et al*. 2015). It is well known that the fatty acid composition of fish fillets reflects the fatty acid composition of the diet (Turchini *et al*. 2009, Volpe *et al*. 2015) but is also modified by metabolic processes (Drew *et al*. 2007). Trout can elongate and desaturate C18:3 n-3 into the longer chain n-3 fatty acids (Tocher *et al*. 2001). Probably, for this reason, although fish fed with barley showed less PUFA content these differences were not significant.

3.2. Alpha-tocopherol content

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191 Alpha-tocopherol content was measured on fillets from fish fed at different barley
192 concentrations. Fish fed with diets 80B showed significantly higher α-tocopherol
193 content than control and fish from the rest of experimental diets (Figure 1). Alpha-
194 tocopherol has an important antioxidant activity and is well absorbed by rainbow trout
195 when included on their diets (Timm-Heinrich *et al*. 2013, Valente *et al*. 2015). In spite
196 of α-tocopherol was not determined on barley, the inclusion of 8% of barley on rainbow
197 trout diets improved fillet α-tocopherol content, and probably enhanced the antioxidant
198 properties of fillets of fish fed with 80B diets. Several studies have reported that some
199 vegetable ingredients contain some endogenous antioxidants, which are present in small
200 amounts and can enhance tocopherol antioxidant activity and this might explain the
201 stability of fish fed with 80B diets (Lauritzsen *et al*. 1999, Thiyam *et al*. 2006).

202 **3.3. Oxidative parameters**

203 **3.3.1. Peroxide value (PV) and conjugated hydroperoxides (dienes and trienes)**

204 No differences were observed regardless of the diet on the peroxide value (PV) (Table
205 6). However, when conjugated hydroperoxides (dienes and trienes) were evaluated, it
206 was observed that barley concentration only showed a significant effect ($P < 0.05$) on
207 trienes (Table 3). The highest value of trienes was observed in control fillets (7.61
208 mmol of hydroperoxides kg lipid^{-1}) and the lowest value in fish fillets at the highest
209 barley concentration (319B) (2.66 mmol of hydroperoxides Kg lipid^{-1}).

210 These results suggested that barley had a positive effect on the control of oxidative
211 process, since oxidative markers were lower in fillets came from fish fed with barley.
212 This could be related to the higher antioxidant activity of fish fed at higher barley
213 concentration and with the lower TBARS values as shown in Pinedo-Gil *et al*. 2017.

214 **3.4. Antioxidant markers**

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3.4.1. Total flavonoid content (TFC) and total phenolic content (TP)

Barley TFC values were of 0.02 µg QE g^{-1} (Figure 2). Differences in barley flavonoid content may be influenced by genotype, agronomic practices, climatic conditions, maturity at harvest, postharvest and handling and storage conditions (Erdman *et al.* 2007). Total flavonoid content was determined in the different experimental diets it was observed that the substitution of wheat for barley significantly decreased the TFC (Figure 2). Flavonoid content in diets comes from other compounds different from barley.

Phenolic acids are present in cereal grains and their content in cereals is usually lower than 1% of dry matter (Abidi *et al*. 2015). It has been reported that barley contains more total phenols than wheat (Ragaee *et al*. 2006, Fogarasi *et al*. 2015). The value obtained in the study was 1.17 mg of GAE g^{-1} (Figure 3). This result was in accordance with the values obtained by Zhao *et al*. (2006), who reported that the values of TP varied from 1.03 to 1.87 mg of GAE g^{-1}. However, TP can vary significantly among barley varieties (Abidi *et al*. 2015). Surprisingly, it was not expected that diets containing higher barley concentration showed significantly lower TP than control or diets with 4% and 8% of barley (Figure 3), since, as it was said before barley contains more total phenols than wheat. The reason of this result could be that phenolic compounds are heat labile (Sharma & Gujral 2011) and less resistant to the heat that can alter their nature (Sharma *et al*. 2012). The reduction in TP may be due to the decomposition of phenolic compounds due to the high extrusion temperature during the feed elaboration process.

According to these results when TP was determined on fish fillets, TP was higher in fillets of fish fed without barley than those fed with barley, regardless the concentration used (Table 5).

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239 **3.4.2. Individual phenolic compounds**

240 Individual phenolic compounds were determined in barley and experimental diets.
241 HPLC barley profile appears in Figure 4, and the contents of individual phenolic
242 compounds are summarized in Table 4. Eleven phenolic compounds were identified and
243 quantified in barley and the experimental diets. The results showed that maleic acid
244 (14.76 µg mL^{-1}) was the most abundant phenolic compound in barley, followed by 4-
245 hydroxybenzoic (5.34 µg mL^{-1}), 3-coumaric acid (2.87 µg mL^{-1}), caffeic acid (2.58 µg
246 mL^{-1}), vanillic acid (1.72 µg mL^{-1}), gallic acid (1.45 µg mL^{-1}), ferulic acid (1.43 µg mL^{-1}
247), 4-coumaric acid (1.40 µg mL^{-1}), syringic acid (0.97 µg mL^{-1}), chlorogenic acid (0.65
248 µg mL^{-1}) and transcinnamic acid (0.50 µg mL^{-1}). These results were not in agreement to
249 results reported by Naczk & Shahidi (2006) where ferulic acid and hydroxybenzoic acid
250 were the main phenolics. Probably differences can be associated to the variety of barley.
251 In this study naked barley was used and the lack of cover or peel can be the responsible
252 on the differences on the profile.

253 When the different phenolic compounds were determined in the experimental diets
254 results showed that the inclusion of barley increased 4-coumaric acid content and
255 decreased maleic acid content. This study showed no differences on the rest of the
256 phenolic compounds.

257 **3.4.3. Fillets antioxidant activity**

258 The antioxidant activity of fish fillets from fish fed with the different experimental diets
259 was evaluated through total phenol content (TP) and ORAC activity. Table 5 shows that
260 TP was higher in fillets of fish fed without barley than those fed with barley, regardless
261 the concentration used. However, antioxidant capacity measured using ORAC method,
262 showed that fillets from fish fed 8% barley concentration had the highest antioxidant

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263 activity. Probably, the absence of correlation between ORAC and TP could was due to
264 the presence of non-phenolics compounds with high antioxidant activity. It is important
265 to remember that barley is rich in β-glucans.

266 **CONCLUSION**

267 Results indicated that the inclusion of barley had an inhibitory effect on fish fillets lipid
268 oxidation probably associated with certain compounds present on barley, which could
269 act by scavenging and reducing lipid oxidation. Concentrations of 8% barley enhance
270 antioxidant properties, improving α-tocopherol content and ORAC values in fish fillets.
271 However, concentrations higher than 8% produced a negative effect on fish fillets. It
272 can be observed that these effects may be due to the presence of certain bioactive
273 components in barley and not related to the fish growth. More studies should be carried
274 out to investigate which components in barley are the responsible to inhibit lipid
275 oxidation and improve antioxidant properties until a concentration of 8% of barley. And
276 the reason why higher barley concentrations showed a negative effect on the fish
277 population.

278 **ACKNOWLEDGEMENT**

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1 **Table 1. Fatty acid profiles (FA) of barley and experimental diets.**

	Barley	DIETS[1]				
		0B	40B	80B	160B	319B
SFA						
C14:0	0.00	0.205	0.125	0.200	0.195	0.200
C16:0	0.36	2.22	2.28	2.29	2.30	2.38
C18:0	0.36	3.81	2.125	0.50	0.49	0.59
MUFA						
C16:1	0.00	0.23	0.24	0.23	0.24	0.22
C18:1 (n-9)	0.00	0.00	1.89	3.57	3.57	3.30
PUFA						
C18:2n6	0.84	5.61	5.49	5.03	5.01	3.82
C18:3n3	0.03	0.42	0.42	0.39	0.39	0.3
C20:5n3 (EPA)	0.00	0.27	0.27	0.11	0.21	0.09
C22:6n3 (DHA)	0.02	0.46	0.44	0.40	0.38	0.27

2 [1] Experimental diets: 0B (0% barley); 40B (4% barley); 80B (8% barley); 160B (16% barley);

3 319B (31.92% barley).

4 SFA (saturated fatty acid); MUFA (monounsaturated fatty acid); PUFA (polyunsaturated fatty

5 acid); DHA (docosahexaenoic acid, 22:6 n-3); EPA (Eicosapentaenoic acid, 20:5 n-3).

6 **Table 2. Fatty acid profile (FA) of rainbow trout fillets when fish were fed with**

7 **increasing barley levels (data are expressed as % of dry matter)** (n=12).

	DIETS[1]					SEM	Sign.
	0B	40B	80B	160B	319B		
SFA	20.24	20.74	21.27	17.29	19.94	1.47	N.S.
C14:0	1.57^{ab}	1.66^{abc}	1.70^{bc}	1.40^{a}	1.89^{c}	0.10	**
MUFA	33.22	33.95	35.52	43.49	37.70	3.58	N.S.
C16:1	2.37^{ab}	2.75^{b}	2.67^{b}	2.05^{a}	3.36^{c}	0.16	**
C18:1 (n-9)	27.27	27.61	29.08	38.05	30.40	3.86	N.S.
PUFA	46.54	45.31	43.21	39.22	42.36	2.56	N.S.
n-3	14.60	13.98	13.69	12.52	12.70	0.94	N.S.
n-6	31.39	30.97	29.09	24.79	29.18	1.63	N.S.
n-9	30.86	31.20	32.86	41.44	34.34	3.70	N.S.
n-3 / n-6	0.47	0.45	0.47	0.50	0.44	0.03	N.S.
ARA	0.75	0.68	0.68	0.61	1.43	0.36	N.S.
EPA	2.07	2.02	2.06	1.99	178	0.17	N.S.
DHA	8.88	8.28	7.95	7.25	7.37	0.60	N.S.
ARA / EPA	0.37	0.33	0.33	0.32	0.85	0.22	N.S.

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EPA / DHA	0.24	0.24	0.26	0.27	0.24	0.01	N.S.

[1] Experimental diets as shown in table 1.

SFA (saturated fatty acids); MUFA (monounsaturated fatty acids); PUFA (polyunsaturated fatty acids); ARA (arachidonic acid, 20:4 n-6); DHA (docosahexaenoic acid, 22:6 n-3); EPA (Eicosapentaenoic acid, 20:5 n-3).

Different superscript letters indicate significant differences ($P < 0.05$) between the experimental diets. ** indicates P-values < 0.0001.

Table 3. Effect of barley on the peroxide value (PV) and conjugated hydroperoxides (dienes and trienes) of rainbow trout fillets of fish fed at different barley concentrations (data are expressed as % of dry matter) (n=12).

	DIETS[1]						
	0B	**40B**	**80B**	**160B**	**319B**	**SEM**	**Sign.**
PV	13.26	12.87	11.83	11.77	7.62	3.40	N.S.
Dienes	18.65	15.70	18.00	17.46	13.71	1.35	N.S.
Trienes	7.61[b]	4.02[a]	4.46[a]	4.18[a]	2.66[a]	1.07	*

[1] Experimental diets as shown in table 1.

Different superscript letters indicate significant differences ($P < 0.05$) between the experimental diets. * indicates P-values >0.0001

Table 4. Contents in individual phenolic compounds ($\mu g\ mL^{-1}$) in barley and experimental diets.

	Barley	DIETS				
		0B	**40B**	**80B**	**160B**	**319B**
1 Gallic acid	1.45	69.97	18.65	89.28	40.78	59.06
2 Maleic acid	14.76	94.52	79.17	53.59	45.30	56.73
3 4-hydroxybenzoic acid	5.34	4.14	4.10	2.20	3.35	3.91
4 Chlorogenic acid	0.65	0.11	1.61	5.03	11.78	17.60
5 Vanillic acid	1.72	4.30	6.52	3.53	2.01	3.60
6 Caffeic acid	2.58	4.45	2.63	4.42	2.52	2.52
7 Syringic acid	0.97	0.23	0.89	0.41	2.65	7.68
8 4-coumaric acid	1.40	0.06	0.11	0.10	0.44	0.70

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9 Ferulic acid	1.43	1.02	0.68	0.65	0.07	0.01
10 3-coumaric acid	2.87	3.66	6.89	1.95	1.95	1.95
11 Transcinamic acid	0.50	5.78	8.11	1.04	11.58	12.93

22 • Experimental diets as shown in table 1.

23 **Table 5. Effect of barley on antioxidant properties of rainbow trout fillets of fish**
24 **fed at different barley concentrations (data are expressed as % of dry matter)**
25 (n=12).

	DIETS[1]						
	0B	**40B**	**80B**	**160B**	**319B**	**SEM**	**Sign.**
TP (µmol Trolox Eq. g-1)	224.39	194.20	214.91	216.54	188.63	15.45	N.S.
ORAC (µmol Trolox Eq. g^{-1})	18.43	18.93	20.07	16.70	17.04	1.39	N.S.

26 [1] Experimental diets as shown in table 1.

27 Total phenols (TP), ORAC (oxygen radical absorbance capacity).

28 Different superscript letters indicate significant differences ($P < 0.05$) between the experimental
29 diets.

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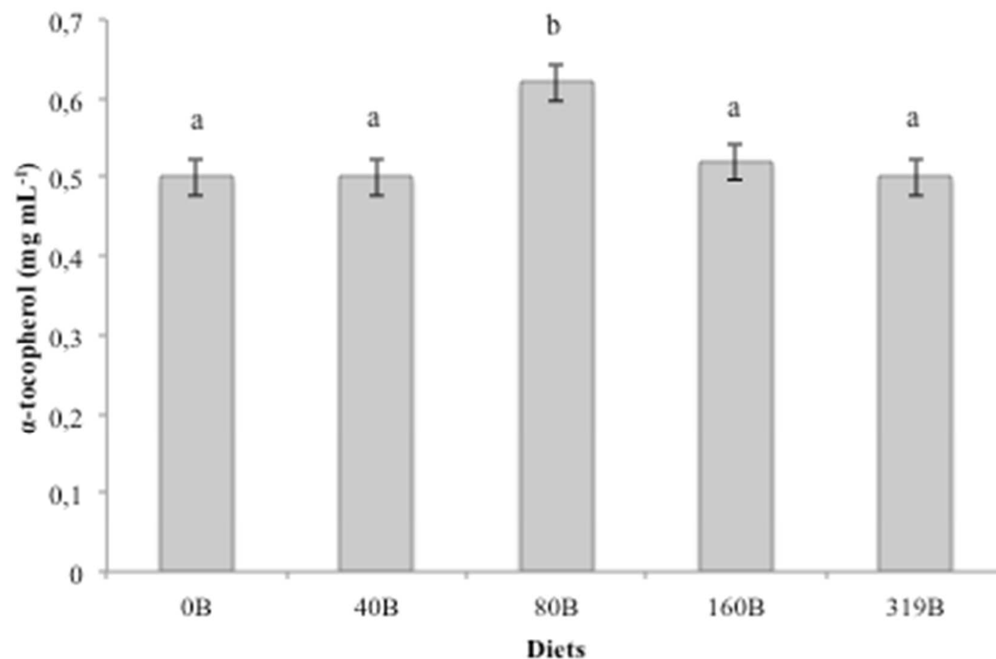
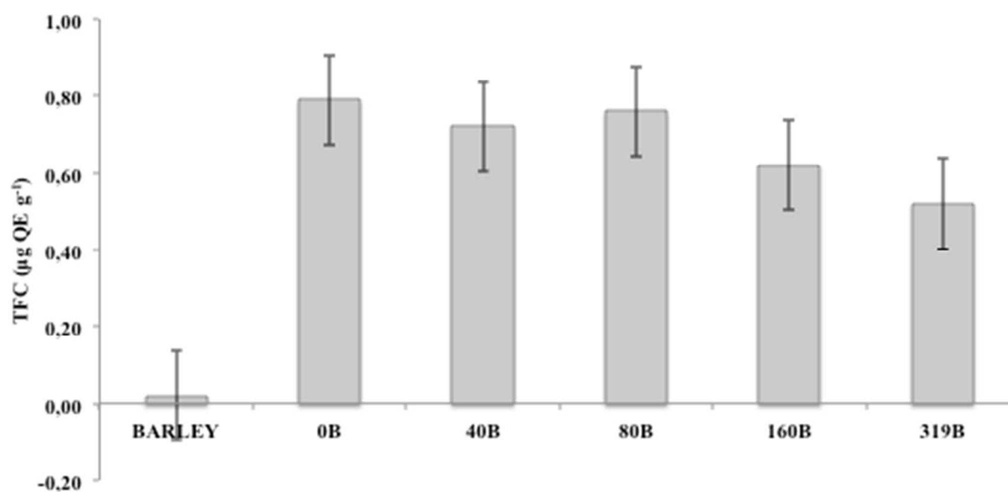


Figure 1. Fillet alpha-tocopherol content of fish fed different experimental diets (n=12). Different experimental diets: 0B (0% barley); 40B (4% barley); 80B (8% barley); 160B (16% barley); 319B (31.92% barley). Different small letters (a, b) correspond to significant differences (P < 0.05) between different samples.



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8 **Figure 2.** Barley and experimental diets total flavonoid contents (TFC). Different
9 experimental diets: 0B (0% barley); 40B (4% barley); 80B (8% barley); 160B (16%
10 barley); 319B (31.92% barley).

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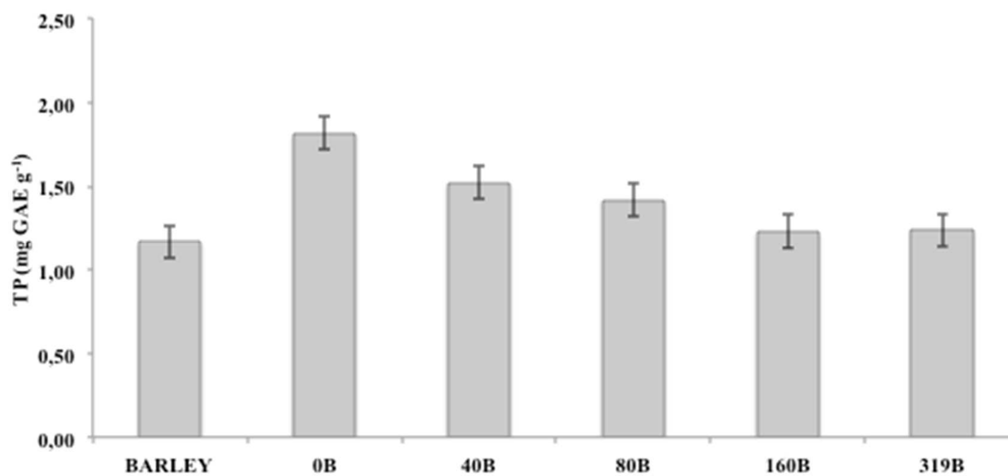

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13 **Figure 3.** Barley and experimental diets total phenolic contents (TP). Different
14 experimental diets: 0B (0% barley); 40B (4% barley); 80B (8% barley); 160B (16%
15 barley); 319B (31.92% barley).

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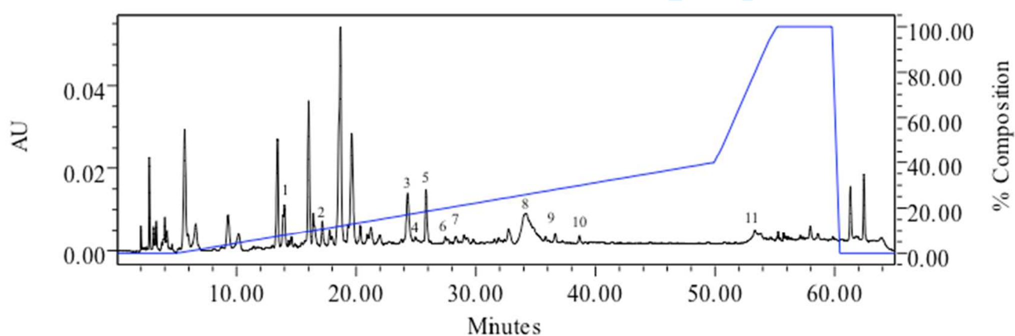

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18 **Figure 4.** HPLC chromatogram of phenolic compounds profile in barley extracts. 1.
19 Gallic acid; 2. Maleic acid; 3. 4-hydroxybenzoic acid; 4. Chlorogenic acid; 5. Vanillic

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4 20 acid; 6. Caffeic acid; 7. Syringic acid; 8. 4-coumaric acid; 9. Ferulic acid; 10. 3-
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