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1   **Enhancement of quality of rainbow trout (*Oncorhynchus mykiss*) flesh incorporing**

2   **barley on diet without negative effect on rearing parameters**

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4   Julia Pinedo-Gil[1,2*], Ana Tomás-Vidal[2], Ana María Larrán-García[1], Cristina Tomás-

5   Almenar[1], Miguel Jover-Cerdá[2], Miguel Ángel Sanz-Calvo[1], Ana Belén Martín-Diana[1]

6   [1] Subdirection of Research and Technology. Agro-Technological Institute of Castilla y

7   León. Consejería de Agricultura y Ganadería. Finca de Zamadueñas, Ctra. Burgos km.

8   119, 47171, Valladolid, Spain.

9   [2] Research Group of Aquaculture and Biodiversity, Institute of Animal Science and

10   Technology, Polytechnic University of Valencia, Camino de Vera, 14. 46071-Valencia,

11   Spain.

12   Correspondig author: pingilju@itacyl.es, Julia.pinedo.gil@gmail.com*

13   Phone number: +34921412716

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21   **ABSTRACT**

22   Barley concentrations ranging from 0% to 32% (0B, 40B, 80B, 160B and 319B) were

23   incorporated into rainbow trout, *Oncorhynchus mykiss* (Walbaum) diets. The

24   experiment started with an initial average fish weight of 127.72 ± 5.65 g and finished

25   when they reached commercial weight (final weight between 312-330 g) after 84 days.

26   The inclusion of barley in the diets did not show a significant effect on growth and

27   biometric parameters, fat and carbohydrate digestibilities, however, protein digestibility

28   decreased significantly with the incorporation of barley on diets. Glucose levels

29   increased significantly with barley concentration in the diet and lactate and cortisol

30   levels were also significantly affected after a stress period regardless of the diet. Meat

31   quality was influenced as well by barley concentration. Lower water activity values and

32   an enhancement in textural and colour properties, were observed in fish fed with the diet

33   containing the highest barley concentration. Trout fed feed with higher concentrations

34   of barley (160B) showed lower lipid oxidation levels than those fed with lower

35   concentrations (control and 40B). The sensory panel found that fish fed with diets

36   higher than 8% in barley content (80B), exhibited a brighter red colour in gills and a

37   better texture, also meat colour became redder with a higher barley inclusion (160B and

38   319B), being all these sensory parameters correlated with fish freshness. Thus, results

39   indicate that barley can be substituted for wheat fraction without any detrimental effect

40   on production efficiency and enhancing fish quality.

41   *Keywords: Barley, β-glucans, growth parameters, meat quality, trout diet.*

42   **1. INTRODUCTION**

43   In the course of just a few decades fish farming has evolved into a highly productive

44   and efficient industry in animal protein production for human consumption (Caballero

45   *et al*., 2002). Rainbow trout, *Oncorhynchus mykiss* (Walbaum) is one of the most

46   important freshwater cultured fish worldwide. European rainbow trout production

47   represents 21% (176.983 metric Tons in 2012; APROMAR, 2014) of the world

48   production and Spain holds 10% of this production (14.009 metric Tons in 2015,

49   MAGRAMA). Aquaculture requires nutrition optimization in order to raise fish with

50   food production purposes efficiently (Hixson 2014).

51   Incorporation of novel ingredients need to balance economic and product quality

52   aspects (Pratoomyot *et al*., 2010; Valente *et al*., 2015) without compromising sensory

53   attributes and consumer acceptance. Cereals are usually incorporated in extruded diets

54   of rainbow trout as a carbohydrate and starch source. Wheat is the cereal traditionally

55   used as a carbohydrate source in commercial trout diet (Sealey *et al*., 2008, Gaylord *et*

56   *al*., 2009), however, barley has not been used widely as an ingredient in aquaculture

57   feed, although a few studies showed that its incorporation into fish feed did not have

58   any detrimental effect on growth parameters (Sealey *et al*., 2008). Probably one of the

59   reasons of the scarce use of barley is due to the presence of anti-nutritive components in

60   its composition, such as phytic acid (Cheng and Hardy 2003). The presence of phytic

61   acid limits the absorption of some minerals in diets such as phosphorus, zinc and

62   calcium caused by the formation of insoluble salts (Cheng and Hardy 2003, Overturf *et*

63   *al*., 2003, Gaylord *et al*., 2009, Kumar *et al*., 2012). However, in order to decrease the

64   presence of phytates, new varieties low in phytic acid levels have been developed

65   (Overturf *et al*., 2003, Gaylord *et al*., 2009). Another limiting factor for the use of

66   barley is the low protein content compared to that found in other different sources

67   (wheat, soy, corn, etc).

68   However, barley presents many advantages due to its β-glucan content (Sealey *et al*.,

69   2008; Meena *et al*., 2013). β-glucans in nature are in the cell walls of several plants

70   such as barley, oats, rye and wheat at concentrations of about 7%, 5%, 2% and < 1%

71   respectively. However depending the variety of barley β-glucan content can range from

72   4 to 11% (Gatlin *et al*. 2007). The acceptance of β-glucans as a functional, bioactive

73   ingredient has increased their popularity (Lazaridou and Biliaderis 2007) and potential

74   due to their immunostimulant effect. Different studies have been carried out to evaluate

75   the beneficial effects of β-glucans on the growth and survival rates (Hai and Fotedar

76   2009; Lin *et al*., 2011), disease resistance and protection against pathogens (Dalmo and

77   Bøgwald 2008; Lokesh *et al*., 2012), and immune system enhancement (Gu *et al*., 2011)

78   in a wide range of aquaculture species (Sealey *et al*., 2008; Meena *et al*., 2013). In

79   particular, several studies on trout have reported the growth enhancement when adding

80   β-glucans to fish feed (Heidarieh *et al*., 2012; Ghaedi *et al*., 2015). Jeney *et al*. (1997)

81   observed that low doses of β-glucans (0.1%) in the feed may prevent stress caused by

82   transport.

83   The purpose of the present work has been to study the effect of the inclusion of barley,

84   as an alternative ingredient in rainbow trout, *Oncorhynchus mykiss* (Walbaum) diets and

85   evaluate the impact on growth performance, apparent digestibility, response to stress

86   and final fish meat quality parameters.

87   **1. MATERIAL AND METHODS**

88   1.1.Production system

89   The trial was conducted in 20 cylindrical fiberglass tanks (500 L) within a freshwater

90   recirculation system (RAS). Throughout the experiment temperature remained constant

91   at 13.58 ± 1.06 ºC and so were dissolved oxygen levels, kept between values of $9.18 \pm$

92   1.35 mg L^{-1}. All tanks were equipped with aeration and an oxygen probe. Water pH was

93   8.03 ± 0.07 and ammonia and nitrites concentration in water were $0.16 \pm 0,23$ and $0,15$

94    $\pm$ 0.11 mg L^{-1} respectively. Water flow was 12.2 $\pm$ 0.5 L h^{-1}. The photoperiod consisted

95   on 12 hours light and 12 hour dark intervals and all tanks had identical lightning

96   conditions.

97   1.2. Fish and experimental design

98   A total of 500 rainbow trout from a commercial trout farm (IPEASA, Fuentidueña,

99   Segovia, Spain) were used. Fish were randomly allocated in 20 tanks, 25 fish in each

100   tank (initial stocking density 6.7 $\pm$ 0.4 kg m^{-3}). Prior to the feeding trial, all fish were

101   acclimated to the indoor rearing conditions for 2 weeks and fish were fed once a day

102   (8:00) to apparent satiation using exclusively a control diet. The study lasted 84 days.

103   Rearing parameters (growth (final weight, biomass increment, survival and SGR),

104   nutritional parameters (FI and FCR) and biometric indexes (CF, VSI and HSI) and meat

105   quality (proximate composition, water activity, colour, texture and sensory analysis)

106   were evaluated approximately every 28 days. All fish were starved for 24 h and

107   anesthetized with (MS222®; 200 mg L^{-1}) prior to taking weight and length

108   measurements. Fish were randomly sampled from each tank to determine rearing and

109   meat quality parameters during the growth period (0, 28, 56 and 84 days). At day 44,

110   fish were controlled stressed by decreasing the concentration of oxygen from 8 to 4 mg

111   L^{-1}. The concentration of oxygen was decreased by lowering water level to a volume of

112   50 L and removing the aeration. When the levels of dissolved oxygen in water reached

113   4 mg L^{-1} it is started to count 10 minutes in these conditions, reaching levels of < 2 mg

114   L^{-1}. Biochemical parameters in blood plasma (glucose, lactate and cortisol levels) were

115   determined.

116   1.3. Diets and feeding

117   Five isoproteic (40% crude protein) and isolipidic diets (18% crude lipid) were

118   developed containing different barley levels (0B (0% barley, 0% β-glucans); 40B (4%

119   barley, 0.14% β-glucans); 80B (8% barley, 0.22% β-glucans); 160B (16% barley,

120   0.53% β-glucans); 319B (31.92% barley, 1.5% β-glucans). Control diet (0B) has been

121   prepared with the same ingredients as experimental diets but without barley. This diet

122   was not a commercial diet. There were five feeding treatment groups each in four

123   replicates (n=4).

124   The barley which was used corresponded to an H13 genetically modified and bred

125   variety of Merlin and VOLGA varieties, harvested in the 2012/2013 period and

126   commercially known as GALIS. This barley is bare with a β-glucan content of 5.2%.

127   The formulation and composition of the diets are shown in Table 1. Diets were prepared

128   by an extrusion process using a semi-industrial twin-screw extruder (CLEXTRAL BC-

129   45. St. Etienne, France). Raw material was processed at a speed of 100 rpm, at 110ºC

130   and a pressure of 40-50 atmospheres.

131   Fish were fed twice a day (8:00 am and 15:00), 6 days per week to apparent satiation

132   level during the whole experimental period. Pellets were distributed manually to allow

133   all fish to eat. The uneaten pellets were collected to determine feed intake (FI).

134   1.4.Apparent digestibility coefficients

135   Simultaneously to the feeding trial, digestibility studies were conducted. After fish were

136   fed for a second time, tanks were completely cleaned and faeces were collected in a

137   settling column (Cho *et al*. 1982), which was emptied in the following morning at 8:00

138   hours. Wet fecal content was then collected and dried at 60ºC for 48 hours prior to

139   analysis (crude protein (CP), crude fat (CF), carbohydrates (CHO) and acid-insoluble

140   ashes (AIA)). Over the whole experimental period, samples of feces were collected

141   from each tank (n=4).

142   The ***apparent digestibility coefficients (ADCs)*** of protein, fat and carbohydrates in the

143   diets tested were calculated according to the following formula:

144    $$ADC\,(\%) = 100 \times \left[100 - \left(\frac{marker\ in\ diet}{marker\ in\ faeces} \times \frac{PN\ in\ faeces}{PN\ in\ diet} \right) \right]$$

145   Where *PN* is the percentage of nutrient.

146   1.5.Biochemical parameters in blood plasma

147   To determine the stress response (hypoxia conditions, < 4 mg L^{-1} per 10 min) 3 fish per

148   tank (n=3) were alternatively captured before stress conditions (basal levels), during

149   stress condition and after one and two weeks, to measure their ability to recover basal

150   levels.

151   Blood samples were withdrawn from the caudal vein using 1 ml syringes (BD

152   Plastipak) with ethylenediaminetetracetic acid (EDTA) as anticoagulant, 0.5 ml were

153   centrifuged (Hettich Zentrifugen, Universal 320 R, Germany) at 5000 rpm for 20 min at

154   4ºC and the plasma was extracted to measure cortisol, glucose and lactate levels.

155   Samples were stored at -80ºC till analysis.

156   ***Concentration of plasma cortisol*** was determined using the method described by

157   Thomas (1992), using a Enzyme-Linked Immunosorbent Assay (DEMEDITEC

158   CORTISOL ELISA® Ref. DE1887). Briefly, aliquots (20 µl) from plasma that were

159   dispensed into appropriate wells and incubated with 200 µl of enzyme conjugate

160   solution for 60 min at room temperature. After incubation the wells were rinsed 3 times

161   with wash solution (400 µl per well) and incubate with 100 µl substrate solution for 15

162   min at room temperature. The enzymatic reaction was stopped by adding 100 µl of stop

163   solution and the absorbance was measured at 450 nm with an absorbance microplate

164   reader (Bibby Scientific Limited, Jenway 7315, United Kindom).

165   ***Concentration of glucose and lactate*** were measured by an enzymatic colorimetric

166   assay, in particular by GOD-POD (SPINREACT® Ref. 1001191) and LOD-POD

167   (SPINREACT® Ref. 1001330) method respectively (Kaplan and Pesce 1984). Briefly,

168   aliquots (5 µl) from plasma samples were mixed with 500 µl of reactive and incubated

169   for 10 min for glucose determination and 5 min for lactate determination at 37ºC in

170   dark. The absorbance was determined at 490 nm in a 96-well microplate reader (Bibby

171   Scientific Limited, Jenway 7315, United Kindom).

172   1.6.Quality markers of fish meat

173   1.6.1. Proximate composition analysis

174   Proximate analyses (moisture, crude protein, crude fat and ash, % of dry weight) were

175   evaluated from ingredients, diets and feces obtained from the digestibility trial and from

176   fish flesh (n=4 for flesh, one fish per tank). Analyses were determined according to

177   AOAC (1990) procedures: Dry matter (60ºC to constant weight), ash (incinerated at

178   550ºC to constant weight), crude protein (N $\times$ 6.25 and nitrogen was analyzed by

179   Dumas principle, TruSpec CN; Leco Corporation, St. Joseph, MI, USA) and crude lipid

180   content using the Soxhlet extraction method. AIA was used as an indicator for the ADC,

181   and was analyzed according to the method described by Atkinson *et al*. (1984) with

182   some modifications. Briefly, 5 g of sample were ashed for 5 hours at 550ºC to ensure

183   complete combustion of the organic material in the sample. The resulting ash was

184   boiled till dryness in 75 mL of HCl (2N) and boiled in other 75 mL HCl during 15 min.

185   Samples were filtered hot through ashless filter paper and washed in boiling destilled

186   water till neutralized the samples. Finally as Atkinson *et al*. (1984) method, samples

187   were ashed for 5 hours at 550ºC.

188   β-glucan content was measured in barley, control and all experimental diets. β-glucan

189   content on barley and different diets were evaluated using McCleary method

190   (Megazyme mixed-linkage beta-glucan assay procedure K.BGLU04/06). Briefly, 0.5 g

191   of sample were mixed with 1 mL ethanol (50% v/v) and 5 mL of sodium phosphate

192   buffer (20 mM, pH 6.5). It was incubated in a water bath during 5 minutes. It was

193   cooled at 40ºC and mixed with 0.2 mL of liquenase (10 U) during 1 h at 40ºC. After this

194   time, the mixture was centrifuged at 100 xg during 10 minutes. 0.1 mL of supernatant is

195   transferred and mixed with 0.1 mL of sodium acetate buffer (50 mM pH 4) and 0.1 mL

196   of β-glucosidase (0.2 U). The mixture was incubated during 15 minutes at 40ºC for the

197   determination of β-glucan. The absorbance was determined at 510 nm in a 96-well

198   microplate reader (Bibby Scientific Limited, Jenway 7315, United Kindom).

199   1.6.2.   Water activity (a_w)

200   Water activity (a_w) was instrumentally measured using an Aqualab 4TE (Decagon

201   Devices inc., Pullman, WA, USA). Measurements were taken directly from the muscle.

202   Six measurements were made in each flesh at three different locations (front, central

203   and tail). The study was evaluated in four independent fish flesh (n=4).

204   1.6.3.   Colour

205   CIELAB parameters (lightness (L*), redness (A*), yellowness (B*)) were evaluated

206   using a portable colorimeter (Minolta CM-2002, Osaka, Japan). Hue and Chroma were

207   calculated using the formulas.

208    $Chroma = (a^{*2} + b^{*2})^{1/2}$

209    $$Hue = arctan\left(\frac{b^*}{a^*}\right)$$

210   Measurements were taken directly over the muscle. Six measurements were evaluated

211   randomly over skinless fish meat. The study was evaluated in four independent fish

212   flesh (n=4).

213   1.6.4. Texture analysis

214   Texture was determined using a texture analyzer TA-XT2i (ANAME, Stable Micro

215   System, Vienna Court, Lammas Road, Godalming, Surrey, UK). A texture profile

216   analysis (TPA) was carried out using a penetration probe of 4 mm diameter at speed of

217   1 mm s^{-1} with a 5 mm distance and the instrument was equipped with a 25 kg load cell.

218   The time delay between cycles was 5 seconds. Previous to analysis, samples were

219   peeled manually and texture was analyzed in the front, middle and tail parts. Fish flesh

220   were evaluated in the same position (with the muscle fibers perpendicular) to the test

221   probe. The study was evaluated in four independent fish flesh per treatment (n=4).

222   Curves were evaluated and the following parameters were determined: **hardness** (g)

223   (maximum force required to compress the sample), **cohesiveness** (capacity of the

224   sample to deform before rupture (A_2 / A_1, where A_1 is the total energy required for the

225   first compression and A_2 is the total energy required for the second compression)),

226   **elasticity** (mm) (capacity of the sample to recover its original shape after the

227   deformation force ends) and **gumminess** (g) (strength to disintegrate a sample to a

228   constant state of swallowing (hardness $\times$ cohesiveness)).

229   1.6.5. Thiobarbituric Acid Reactive Substances (TBARS)

230   TBA as an indicator of lipid oxidation was evaluated using the methodology described

231   by Vyncke (1975). Briefly, 10 g of samples were mixed with 30 mL of 7.5% TCA. The

232   mix was homogenized and centrifuged for 5 min at 4 ºC and 4000 rpm, then filtered

233   with Whatman nº 1 filters (Prat Dumas, France). 5 mL of the filtrate were mixed with 5

234   mL 0.02 M TBA, incubated at 90ºC in a water bath during 40 min and then read in

235   spectrophotometer (Fluostar® Omega, BMG labtech, The microplate reader company,

236   Germany) at 530 nm. One fish per tank was analysed during the entire experiment (n=4)

237   and results were expressed as µmol malondialdehyde (MDA) per kilogram of fresh

238   muscle.

239   1.7.Sensory analysis

240   All sensory analysis were performed according to ISO standards (ISO 2001, 2008) in a

241   sensory room compliant with ISO 2007 by a panel of 8 people (4 male and 4 female

242   aged between 25 and 50) with previous experience in sensory analysis of food products.

243   Nonetheless, in order to familiarise the panel with the sensory assessment of fish

244   products and optimise the tables used for sensory evaluation, the panel were trained in

245   the main characteristics we wished to study.

246   Sensory analysis comprised fresh whole fish and fish meat samples (n=4). Whole fish

247   were evaluated using the quality index method (QIM) and fish flesh were analysed

248   using a quality descriptive method (QDM). Panellists were trained to perform both

249   analysis. QIM was assessed following the guideline of QIM Eurofish (Martinsdóttir *et*

250   *al*., 2001). Freshness was evaluated by giving demerit points according to certain

251   aspects of general appearance (skin, stiffness, odour, gills pots colour and odour, belly,

252   and eyes brightness and shape). The trained judges gave a score ranging from 0-3 for

253   each attribute. The maximum score of 3 corresponded to the worst quality signs and

254   lower values were given when quality attributes were very good (0-1).

255   For the QDM, panellists were trained to discriminate colour, texture, odour and

256   acceptability of fish meat. A continuous non-structured scale (1-10) was used for

257   evaluation. The left side of the scale corresponded to the lowest intensity (value 1:

258   white, soft, fresh odour and acceptable sample) whereas the right side corresponded to

259   the highest intensity (value 10: dark, hard, rancid odour and non-acceptable sample).

260   Panellists evaluated one fish per treatment every 28 days during the whole experiment.

261   5 samples, in pairs of whole fish and flesh of each treatment, were individually

262   presented in porcelain dishes to each panellist. Samples were coded with random

263   numbers and maintained at room temperature during evaluation.

264   1.8.Statistical analyses

265   Statistical analysis was performed using SAS version 9 (SAS Institute Inc., Cary, North

266   Carolina, USA) by a GLM procedure for the variance analysis (ANOVA). In the rearing

267   parameters, the initial weight (Wi) was included as covariable. ~~to analysis growth~~

268   ~~parameters until day 28, and for the rest of the moments the final weight form the~~

269   ~~previous moment was considered as initial weight for the next moment and included as~~

270   ~~covariable to analysis growth parameters.~~

271   For the rearing parameters and digestibility diet was included as the only fixed factor.

272   For biochemical parameters, in order to evaluate the differences between diets at a

273   certain moment and at different moments within a same diet, the fixed factors of diet

274   and time and their interaction were included in the model. ~~For digestibility diet was~~

275   ~~included as the only factor.~~ For proximal composition, colour, sensorial analysis,

276   TBARs, water activity (a_w) and texture in the GLM model only the diet was included as

277   fixed effect, ~~the moment of sampling and the interaction between diet and time were~~

278   ~~included as fixed effects.~~ In addition, for a_w and texture the section where these

279   parameters were measured (front, middle or tail of the fish flesh) was also included as a

280   fixed effect.

281   When the ANOVA revealed a significant effect, values were compared using the t-

282   Student test and were considered to be significant at p<0.05. When the interaction was

283   proven to be significant the data correspond to the double interaction and are presented

284   as least-squares means (LSM) ± the standard error of the mean (SEM).

285   1.9.Ethical statement

286   The rainbow trout study complied with the European Union Council Directive

287   2010/63/UE, which provides the minimum standards for animal protection, and was

288   also in accordance with the Spanish national legislation (Spanish Royal Decree

289   53/2013) based on animal protection in experimentation and other scientific practices

290   and approved by the Animal Ethics Committee of Agro-Technological Institute of

291   Castilla y León (Spain).

292   Fish in tanks were checked on a daily basis. Every four weeks, fish were weighed

293   individually and their health status was assessed by observation, after sedation with

294   MS222 dissolved in water (MS222®; 200 mg L^{-1}) to minimize animal suffering.

295   Animals were euthanized by an excess of MS222 (300 mg L^{-1}) or with ice (when quality

296   samples were taken) and then dissected.

297   **2. RESULTS**

298   2.1.Rearing parameters: growth and biometric analysis

299   The experiment started with an initial average fish weight of 127.72 ± 5.65 g and

300   finished when fish reached commercial weight (range 312-330 g). Every 28-days fish

301   were weighed and length measured to determine the growth and biometric indexes

302   (Table 2). The study did not show significant differences in any of the parameters

303   studied.

304   2.2. Apparent digestibility coefficients (ADC)

305   The results showed that ***protein digestibility*** of fish fed with the control and 40B diets

306   were significantly higher (98.28%) ($p<0.05$) than that of fish fed with higher barley

307   concentrations. The ***ADC of fat*** and ***carbohydrate*** in experimental diets was not

308   significantly affected by diet alone (Table 3).

309   2.3.Biochemical parameters

310   Higher concentrations of barley in the diet showed higher concentration of glucose in

311   blood plasma in the moment of stress. But not significant effects were observed in

312   lactate and cortisol levels (Figure 1). When the stress response results were analyzed a

313   significant increase ($p<0.05$) of all parameters (glucose, lactate and cortisol) were

314   observed under stress, recovering basal levels of cortisol and lactate in 7 days, while

315   hyperglycemia persisted 7 days more (Figure 1). An interactive effect was only

316   observed in glucose levels in the different experimental diets. Changes in glucose levels

317   have been significantly ($p<0.05$) affected by both, the inclusion of barley and the effect

318   of stress.

319   2.4.Quality markers of fish meat

320   2.4.1.   Proximate composition

321   Results showed that barley increased significantly ($p<0.05$) crude fat and ash content on

322   meat proximate composition (Table 4) while moisture and crude protein were not

323   affected. At the end of the experimental growth, crude fat content of fish fed 160B diets

324   increased significantly (p<0.05), while ash content decreased significantly with the

325   concentration of barley.

326   2.4.2. Water activity (a_w)

327   A_w was significantly ($p<0.05$) affected by the different diets (Figure 2). Lower a_w values

328   were observed in fish fed with diets high in barley (319B), at the end of the

329   experimental growth period and in the front and middle parts of the fillet (results not

330   shown).

331   2.4.3. Colour

332   No significant differences were observed despite barley concentration in meat of fish

333   fed the different diets (Table 5).

334   2.4.4. Texture

335   The results obtained in the present study showed that barley concentration in the diet

336   have a significant effect on gumminess of fish fillet (Table 6 and Figure 3). Compared

337   to control diet (0B) increasing barley levels on diet decreased significantly gumminess

338   of fillets. Hardness, cohesiveness and elasticity was not significantly affected by the

339   diet. Different sections of the fish flesh were also studied (front, middle and tail).

340   Results showed the tail region to be the stiffest part of the flesh (results not shown).

341   2.4.5. Thiobarbituric Acid Reactive Substances (TBARS)

342   The inclusion of barley in the diet had an inhibitory effect. Trout fed with diets higher in

343   barley concentration had a lower level of TBARS in meat than those obtained from

344   trouts fed with lower barley concentrations diets (Figure 4).

345   2.5. Sensory analysis

346   Results from QIM showed that barley concentration significantly affected (p<0.05) gill

347   colour (Figure 5). Gills became pale on fish fed with 40B diets but diets higher in barley

348   concentrations caused gills to become redder, so barley with a β-glucan content of

349   0.22% or more, enhance fish freshness by making gills appear redder. ~~Eye brightness~~

350   ~~increased significantly with the inclusion of barley in the diets, but with fish growth~~

351   ~~those differences disappeared, showing barley concentration no effect. When QIM~~

352   ~~parameters were evaluated along the experimental growth period, significant differences~~

353   ~~(p<0.05) were observed in almost all parameters. Fish fed with control diets (0B) lost~~

354   ~~eyes brightness during the experimental growth. However a significant increase of that~~

355   ~~brightness was observed when fish reached their commercial weight. Those fish, also,~~

356   ~~showed a redder colour on their gills, which was related to fish growth. Fish fed with~~

357   ~~40B diets lost eyes brightness and their characteristic red colour on their gills during the~~

358   ~~experimental growth. Gills odour was also significantly affected by growth, acquiring~~

359   ~~fish a more rancid odour while reaching their commercial weight. Fish fed with 80B~~

360   ~~diets lost their skin brightness during the experimental period, but when they reached~~

361   ~~their commercial weight, a significant increase of brightness appeared on their skin.~~

362   ~~Those fish, also suffered from an impact on eye brightness along the experimental~~

363   ~~growth period and gills became redder. Fish fed with 160B diets lost their skin~~

364   ~~brightness, got a more rancid odour and duller eyes during the experimental growth~~

365   ~~period, however, their stiffness increased significantly with fish growth. And in fish fed~~

366   ~~319B diets, the stiffness and eye brightness increased significantly with the growth of~~

367   ~~the fish, on da 28 (T1) fish were softer and had duller eyes than on day 84 (T3).~~

368   On the other hand, QDM was evaluated in fish flesh. Experimental diets showed a

369   significant (p<0.05) effect on meat colour (Figure 6). Fish colour was redder in those

370   fish fed with diets higher in barley concentrations up to a β-glucan concentration of

371 0.53%. ~~Fish fed with diets higher in barley concentrations showed no differences with~~

372 ~~those fed with the control diet. However when fish reached commercial weight, fish fed~~

373 ~~with 319B were significantly redder than fish fed with control diets~~. Texture was also

374 affected by diets, fish fed with 80B showed a higher hardness than those fish fed with

375 diets higher in barley concentrations (Figure 7). ~~During the experimental growth period~~

376 ~~significant (p<0.05) differences were only observed in meat colour. The redness of meat~~

377 ~~increased with fish size as fish reached their commercial weight.~~ When acceptability

378 was analyzed, no significant differences were observed regardless barley concentration,

379 so fish samples were considered to be acceptable for human consumption.

380 **3. DISCUSSION**

381 Currently there is increased research interest on the use of new ingredients in the

382 aquaculture industry. The present study was mainly focused on evaluating the effect of

383 increasing levels of barley, as an ingredient rich in β-glucans, on rainbow trout diets.

384 The findings concerning growth performance and digestibility obtained in the present

385 study have demonstrated the potential use of barley in commercial extruded diets of

386 rainbow trout. In the present study, substituting wheat portion for barley did not

387 substantially altered growth of rainbow trout suggesting that both cereals were equally

388 used even though barley contains more dietary fiber than wheat did. However, barley, in

389 contrast to wheat, enhances fish meat quality and is rich in β-glucans. Similar results

390 obtained Sealey *et al*. (2008), who studied the effect of 3 barley genotypes on growth

391 performance of rainbow trout and did not observed significant differences on weight

392 gain regardless barley concentration and so β-glucan concentration. The fact that growth

393 has not been disadvantaged could also been explained that the phytic acid content of

394 this barley variety has not a negative effect on the growth of rainbow trout, as it has also

395 been reported by Overturf *et al*., 2003 and Gaylord *et al*., 2009. Despite barley has more

396   dietary fibre than wheat it was not observed a greater feed intake (FI). It is common that

397   when fibre levels are very high, digestive transit is faster and FI increase, possibly as a

398   result that this higher fibre level is not harmful for rainbow trout. Results did not show

399   significant differences on survival rate. Probably one of the reasons of this could be that

400   experimental diets studied in the present study were very well balanced nutritionally.

401   However, it has been reported a significant increase on survival rates with the

402   incorporation of β-glucan in other fish species: croaker (*Pseodosciena crocea*) (Ai *et*

403   *al*., 2007), Pacific white prawns (*Penaeus monodon*) (Chang *et al*., 2003) and juvenile

404   western king prawns (*Penaeus latisulcatus kishinouye*) (Hai and Fotedar 2009). In the

405   case of Chang *et al*. and Ai *et al*. they tested immunity, so the survival rate is related to

406   resistance of fish to a disease factor.

407   In the present work has been observed that diets with barley and wheat were properly

408   digested by rainbow trout, since all apparent digestibility coefficients are high,

409   independently barley concentration on the diet. It is true that protein digestibility

410   coefficient was slightly lower in trouts fed with diets containing higher barley levels,

411   but considering the high percentage of this coefficient it is not possible to conclude a

412   negative effect of this ingredient in the overall digestibility of diets. The ability of

413   salmonids to digest fibre is rather limited due to the low α-amylase activity, and large

414   amounts of undigested starch in the intestinal content also reduce digestibility of other

415   macronutrients (Skrede *et al*. 2002, Stone 2003, Krogdahl *et al*. 2005, Couto *et al*.

416   2016). The concentration of undigested carbohydrate in the gut has been related to

417   reduction in fat digestibility in rainbow trout (Storebakken *et al*. 1998, Morken *et al*.

418   2011). In the present study the ADC of fat in experimental diets was not significantly

419   affected by diet alone, fat digestibility was higher than previously found in other

420   carnivorous fish species studies: rainbow trout (*Oncorhynchus mykiss*) (Storebakken *et*

421   *al*, 1998), atlantic salmon (*Salmo salar*) (Skrede *et al*., 2002), gilthead seabream

422   (Sparus aurata) (Couto *et al*., 2016). The ADC for protein and fat were higher than 80%,

423   values in agreement with the results reported by Cheng and Hardy (2002, 2003) who

424   reported ADC for protein and fat in barley were also higher than 80% for rainbow trout.

425   Starch digestibility diminished with increasing levels of wheat and barley in the diets, in

426   accordance with previously reported data (Grisdale-Helland and Helland 1997, Skrede

427   *et al*. 2002). Skrede *et al*. (2002) performed a study with lactic acid fermentation of both

428   barley and wheat, reporting a higher starch digestibility in the case of barley. Results

429   which are comparable to those obtained in the present study, indicating that barley

430   would be an interesting ingredient in extruded diets for rainbow trout.

431   For rainbow trout, it has been shown that high glucose values follow feeding with high

432   levels of available carbohydrates (Walton 1986, Krogdahl *et al*. 2004). When the stress

433   response results were analyzed a significant increase of all parameters (glucose, lactate

434   and cortisol) were observed under stress, recovering basal levels of cortisol and lactate

435   in 7 days, while hyperglycemia persisted 7 days more. Rainbow trout, as a carnivorous

436   fish, has limited capability to digest fibre (Skrede *et al*. 2002, Stone 2003, Krogdahl *et*

437   *al*. 2005, Couto *et al*. 2016), which will explain why plasma glucose levels increased

438   significantly with the inclusion of barley in the diet. During any type of stress, cortisol

439   levels can reach up to more than 100 ng ml^{-1} and later drop to 10-20 ng ml^{-1}, their basal

440   level (Flores-Quintana 2002). Changes in cortisol levels during hypoxia produced a

441   hyperglycemia due to glucogenolysis and gluconeogenesis pathways (Hemre *et al*.

442   2002). Changes in cortisol and glucose plasma levels occurred at different kinetics

443   (Mommsen *et al*. 1999), that is why the hyperglycemia persisted for 14 days while basal

444   cortisol levels were reached in 7 days. Lactate is produced by glucose from anaerobic

445   glycolysis, and as glucose, it incremented significantly at the time stress occurred, but

446   recovered basal levels in 7 days. Hemre (1992) reported in the case of Atlantic cod, that

447   even 96 h after transport stress, sustained hyperglycemia was detected only in fish

448   adapted to high dietary starch levels, while adaptation to a low starch diet resulted in a

449   lower glucose peak coupled with a shorter recovery period to establish basal levels. This

450   adaptation also influenced muscle and liver ability to regulate plasma glucose levels

451   after peaking, assuming that the space for glycogen storage can be modified by an

452   adaptation diet, in agreement with studies on glucose space in halibut (*Hippoglosus*

453   *hippoglossus*) (García-Riera and Hemre, 1996) and Atlantic salmon (*Salmo salar*)

454   (Hemre and Krogdahl, 1996).

455   Proximate composition values were similar to those reported by other authors (Yildiz *et*

456   *al*. 2004, Popelka *et al*. 2014). Substituting barley for wheat portion did not

457   substantially affect proximate composition of rainbow trout flesh, results that are in

458   accordance with ones reported by Sealey *et al*. (2008). Lower a_w values were observed

459   in fish fed with diets high in barley (319B), at the end of the experimental growth period

460   and in the front and middle parts of the flesh. These decrease values of a_w would help to

461   reduce lipid oxidation process and microbial growth.

462   The appearance of food products is of major importance to consumers, both from the

463   acceptability and preference point of view. The colour of rainbow trout is generally

464   considered as one of the most relevant quality parameters. Therefore, colour plays a

465   decisive characteristic during quality evaluation of the product at the point of sale (Ortiz

466   *et al*., 2013). No significant differences were observed despite barley concentration in

467   the different diets. These results differed from the studies obtained in the sensory

468   analysis. In the QDM analysis it was observed that when fish reached commercial

469   weight, fish fed with 319B diets were significantly redder than fish fed with control

470   diets. The redness of fish flesh increased with fish size as fish reached their commercial

471   weight, meat became redder and brighter.

472   Fish muscle texture is based on many intrinsic biological factors such as collagen or fat

473   content. Some autolytic enzymes and microbiological effects could be induced in

474   degradation, which made muscles less elastic and softer, were activated after fish death

475   (Asghari *et al*. 2014, Xu *et al*. 2015). Casas *et al*. (2006) reported cohesiveness as a

476   parameter to measure muscle elasticity since it describes the ability of the muscle to

477   recover from deformation and its resistance to subsequent deformation. If cohesiveness

478   is < 1, the deformation suffered by the first compression is partly irrecoverable. In the

479   present samples, the deformation along the experimental growth period was < 1, and

480   significantly lower at the beginning of the experiment than at the end. Different sections

481   of the fish flesh were also studied (front, middle and tail). Results showed the tail region

482   to be the stiffest part of the flesh also in accordance with the results obtained by Casas

483   *et al*. (2006).

484   Lipid oxidation of fish meat was measured through TBARs indicators. Lakshmanan

485   (2000) proposed a range of 1-2 mg malonealdehyde per kg of sample as the limit of

486   acceptability, when TBAR index is above this value it affects to the fish. At the end of

487   the experimental period TBARs index was between the range proposed by

488   Lankshmanan (2000) and fish fed with 80B and diets higher in barley concentration

489   reached those TBAR index levels. It should be noted that diet 160B maintained such

490   levels during the whole experiment. This decrease on the TBAR index was correlated

491   with the lower water activity of fish fed with diets higher in barley concentrations at the

492   end of the experimental growth period, which probably reduced microbial and

493   enzymatic activity and probably with a positive effect of different compounds of barley

494   which act as endogenous antioxidants.

495   Barley is a cereal with certain bioactive components, not only β-glucans, but also

496   phenolic acids, polyphenols and non polar compounds such as tocols that can enhance

497   growth and quality parameters, however, with the obtained data we cannot claim those

498   improvements to be associated to the combined effect of all of these components or just

499   to one of them, and so further studies should be done to evaluate the cause of those

500   beneficial effects on rainbow trout. β-glucans are potential immunostimulant

501   components, thus some immunological studies should be carried out to explain their

502   efficiency in growth and quality parameters.

503   **4. CONCLUSIONS**

504   Results indicated that wheat can be substituted by barley without any significant

505   detrimental effect on rearing parameters and with a positive enhancing effect on fish

506   quality, lower water activity values, as well as an enhancement in textural and colour

507   properties, were observed in fish fed with the diet containing the highest barley

508   concentration. Trout fed feed with higher concentrations of barley showed lower lipid

509   oxidation levels than those fed with lower concentrations. The sensory panel found that

510   fish fed with diets higher than 8% in barley content, exhibited a brighter red colour in

511   gills and a better texture, also fillet colour became redder with a higher barley inclusion,

512   being all these sensory parameters correlated with fish freshness. Considering the total

513   of the results obtained and taking into account that the product quality (fish flesh) is a

514   balance between rearing parameters (fish health) and quality of fish (fish flesh), is

515   considered that barley concentrations of 31.9 g kg^{-1} is a suitable concentration to

516   achieve this balance.

522   **REFERENCES**

523   Ai Q., Mai K., Zhang L., Tan B., Zhang W., Xu W. & Li H. (2007). Effects of dietary

524   β-1,3- glucan on innate immune response on large yellow croaker, *Pseudosciaena*

525   *crocea*. Fish Shellfish Immun 22, 394-402.

526

527   A.O.A.C., Association of Official Analytical Chemists (1990). Official Methods of

528   Analysis, 15th ed. Association of Official Analytical Chemists, Arlington, VA, USA.

529   1298 pp.

530

531   APROMAR 2014. La acuicultura en España 2013. Report by the Spanish Association

532   of marine Aquaculture (APROMAR) and the Spanish Association of Freshwater

533   Aquaculture (ESCUA). Available at: http://www.apromar.es/content/la-acuicultura-en-

534   españa-2014

535

536   Asghari M., Shabanpour B. & Pakravan S. (2014). Evaluation of some qualitative

537   variations in frozen fillets of beluga (Huso huso) fed by different carbohydrate to lipid

538   ratios. J Food Sci Tech 51 (3): 430-439.

539

540   Atkinson J. L., Hilton J. W. & Slinger S. J. (1984). Evaluation of acid-insoluble ash as

541   an indicator of feed digestibility in rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat.

542   Sci. 41: 1384-1386.

543

544   Caballero M. J., Obach A., Rosenlund G., Montero D., Gisvold M. & Izquierdo M. S.

545   (2002). Impact of different dietary lipid sources on growth, lipid digestibility, tissue

546   fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*.

547   Aquaculture 214, 253-271.

548

549   Casas C., Martinez O., Guillen M. D. Pin C. & Salmeron J. (2006). Textural properties

550   of raw Atlantic salmon (*Salmo salar*) at three points along the fillet, determined by

551   different methods. Food control 17, 511-515.

552

553   Chang C-F., Su M-S., Chen H-Y. & Liao I-C. (2003). Dietary β-1,3-glucan effectively

554   improves immunity and survival of Penaeus monodon challenged with white spot

555   syndrome virus. Fish Shellfish Immun 15 297-310.

556

557   Cheng Z. J. & Hardy R. W. (2002). Effect of microbial phytase on apparent nutrient

558   digestibility of barley, canola meal, wheat and wheat middlings, measured in vivo using

559   rainbow trout (*Oncorhynchus mykiss*). Aquacult Nutr 8, 271-277.

560

561   Cheng Z. J. & Hardy R. W. (2003). Effects of extrusion processing of feed ingredients

562   on apparent digestibility coefficients of nutrients for rainbow trout (*Oncorhynchus*

563   *mykiss*). Aquacult Nutr 9, 77-83.

564

565   Cho C. Y., Slinger S. J. & Bayley H. S. (1982). Bioenergetics of salmonid fishes:

566   energy intake, expenditure and productivity. Comp Biochem Physiol 73B, 25-41.

567

568   Couto A., Peres H., Oliva-Teles A. & Enes P. (2016). Screening of nutrient

569   digestibility, glycaemic response and gust morphology alterations in gilthead seabream

570   (*Sparus aurata*) fed whole cereal meals. Aquaculture 450, 31-37.

571

572   Dalmo R. A. & Bøgwald J. (2008). B-glucans as conductors of immune symplhonies.

573   Review. Fish Shellfish Immun 25, 384-396.

574

575   Flores-Quintana C. (2002). Respuestas neuroendocrinas al estrés en peces teleósteos.

576   Rev. ictiol. 10 (1/2): 57-78.

577

578   García-Riera M.P. & Hemre G-I. (1996). Effect of adaptation to three different levels of

579   dietary carbohydrates on the incorporation of ^{14}C-glucose in several organs of Atlantic

580   halibut (*Hippoglosus hippoglossus*). Aquac Res 27, 565-571.

581

582   Gatlin D.M., Barrows F., Brown P., Dabrowski K., Gaylord T.G., Hardy R.W., Herman

583   E., Hu G., Krogdahl Å., Nelson R., Overturf K., Rust M., Sealey W., Skonberg D.,

584   Souza E.J., Stone D., Wilson R. & Wurtele E. (2007). Expanding the utilization of

585   sustainable plant products in aquafeeds: a review. Aquac Res 38, 551-579.

586

587   Gaylord T. G., Barrows F. T., Rawles S. D., Liu K., Bregitzer P., Hang A., Obert D. E.

588   & Morris C. (2009). Apparent digestibility of nutrients and energy in extruded diets

589   from cultivars of barley and wheat selected for nutritional quality in rainbow trout

590   *Oncorhynchus mykiss*. Aquac Nutr 15, 306-312.

591

592   Ghaedi G., Keyvanshokooh S., Azarm H. M. & Akhlaghi M. (2015). Effects of dietary

593   β-glucan on maternal immunity and fry quality of rainbow trout (*Oncorhynchus*

594   *mykiss*). Aquaculture 441, 78-83.

595

596   Grisdale-Helland B. & Helland S.J. (1997). Replacemente of protein by fat and

597   carbohydrate in diets for Atlantic salmon (Salmo salar) at the end of the freshwater

598   stage. Aquaculture 152, 167-180.

599

600   Gu M., Ma H., Mai K., Zhang W., Bai N. & Wang X. (2011). Effects of dietary β-

601   glucan, mannan oligosaccharide and their combinations on growth performance,

602   immunity and resistance against *Vibrio splendidus* of sea cucumber, *Apostichopus*

603   *japonicus*, Fish Shellfish Immun 31, 303-309.

604

605   Hai N. V. & Fotedar R. (2009). Comparison of the effects of the prebiotics (Bio-Mos®

606   and β-1,3-D-glucan) and the customized probiotics (*Pseudomonas synxantha* and *P.*

607   *aeruginosa*) on the culture of juvenile western king prawns (*Penaeus latisulcatus*

608   *kishinouye*, 1896). Aquaculture 289, 310-316.

609

610   Heidarieh M., Mivaghefi A. R., Akbari A., Sheikhzadeh N., Kamyabi-Moghaddam Z.,

611   Askari H. & Shahbazfar A. A. (2012). Evaluation of Hilyses[TM], fermented

612   *Saccharomyces cerevisiae*, on rainbow trout (*Oncorhynchus mykiss*) growth

613   performance, enzymatic activities and gastrointestinal structure. Aquac Nutr 1-6.

614

615   Hemre G-I. (1992). Studies on carbohydrate nutrition in Cod (*Gadus morhua*). Dr.

616   scientiarum Thesis. Institute of Nutrition, University of Bergen, Norway.

617

618   Hemre G-I. & Krogdahl Å. (1996). The effect of handling and fish size on the

619   secondary changes in carbohydrate metabolism in Atlantic salmon (*Salmo salar*). Aquac

620   Nutr 2, 249-252.

621

622   Hemre G-I., Mommsen T.P. & Krogdahl Å. (2002). Carbohydrates in fish nutrition:

623   effects on growth, glucose metabolism and hepatic enzymes. Aquac Nutr 8, 175-194.

624

625   Hixson S. M. (2014). Fish nutrition and current issues in aquaculture: the balance in

626   providing safe and nutritious seafood, in an environmentally sustainable manner. J

627   Aquac Res Dev 5: 234 doi: 10.4172/2155-9546.1000234

628

629   ISO 8586-1:2001 (2001). Sensory analysis – General guidance for the selection, training

630   and monitoring of assessors – Part 1: Selected assessors (International Organization for

631   Standardization).

632

633   ISO 8586-2: 2008 (2008). Sensory analysis – General guidance for the selection,

634   training and monitoring of assessors – Part 2: Expert sensory assessors (International

635   Organization for Standardization).

636

637   ISO 8589: 2007 (2007). Sensory analysis – General guidance for the design of test

638   rooms (International Organization for Standardization).

639

640   Jeney G., Galeotti M., Volpatti D. & Anderson D. P. (1997). Prevention of stress in

641   rainbow trout (*Oncorhynchus mykiss*) fed diets containing different doses of glucan.

642   Aquaculture 154, 1-15.

643

644   Kaplan L. A. & Pesce (1984). Clin Chem The CV Mosby Co. St Louis. Toronto.

645   Princeton; 1032-1036.

646

647   Krogdahl Å., Sundby A. & Olli J.J. (2004). Atlantic salmon (*Salmo salar*) and rainbow

648   trout (*Oncorhynchus mykiss*) digesti and metabolize nutrients differently. Effects of

649   water salinity and dietary starch level. Aquaculture 229, 335-360.

650

651   Krogdahl Å., Hemre G.I. & Mommsen T.P. (2005). Carbohydrates in fish nutrition:

652   digestion and absorption in postlarval stages. Aquac Nutr 11, 103-122.

653

654   Kumar V., Sinha A. K., Makkar H. P. S., De Boeck G. & Becker K. (2012). Phytate and

655   phytase in fish nutrition. Review Article. J Anim Physiol An N 96, 335-364.

656

657   Lakshmanan P. T. (2000). Fish spoilage and quality assessment. In: Lyre TSG,

658   Kandoran MK, Thomas M, Mathew PT (eds) quality assurance in seafood processing,

659   Cochin: society fisher techno (India), 26-40.

660

661   Lazaridou A. & Biliaderis C. G. (2007). Molecular aspects of cereal β-glucan

662   functionality: Physical properties, technological applications and physiological effects. J

663   Cereal Sci 46, 101-118.

664

665   Lin S., Pan Y., Luo L. & Luo L. (2011). Effects of dietary β-1,3-glucan, chitosan or

666   raffinose on the growth, innate immunity and resistance of loi (*Cyprinus carpio koi*).

667   Fish Shellfish Immun 31, 788-794.

668

669   Lokesh J., Fernandes J. M. O., Korsnes K., Bergh Ø. & Brinchmann M. F. (2012).

670   Transcriptional regulation of cytokines in the intestine of Atlantic cod fed yeast derived

671   mannan oligosaccharide or β-Glucan and challenged with *Vibrio anguillarum*. Fish

672   Shellfish Immun 33, 626-631.

673

674   MAGRAMA. Ministerio de Agricultura, Alimentación y Medio Ambiente. Gobierno de

675   España.

676   Available at: http://www.magrama.gob.es/es/

677

678   Martinsdóttir E., Sveinsdóttir K., Luten J., Schelvis-Smit R. & Hyldig G. (2001). La

679   evaluación sensorial de la frescura del pescado. Manual de referencia para el sector

680   pesquero. Icelandic Fisheries Laboratories.

681   Avalilable at: QIM Eurofish. URL http:// qim-eurofish.com

682

683   Meena D. K., Pronob Das, Shailesh Kumar, Mandal S. C., Prusty A. K., Singh S. K.,

684   Akhtar M. S., Behera B. K., Kundan Kumar, Pal A. K. & Mukherjee S. C. (2013). Beta-

685   glucan: an ideal immunostimulant in aquaculture. Fish Physiol Biochem 39, 431-457.

686

687   Mommsen T.P., Vijayan M.M. & Moon T.W. (1999). Cortisol in teleosts: dynamics,

688   mechanisms of action and metabolic regulation. Rev Fish Biol Fisher 9, 211-268.

689

690   Morken T., Kraugerud O.F., Barrows F.T., Sørensen M. Storebakken T. & Øverland M.

691   (2011). Sodium diformate and extrusion temperature affect nutrient digestibility and

692   physical quality of diets with fish meal and barley protein concentrate for rainbow trout

693   (*Oncorhynchus mykiss*) Aquaculture 317, 138-145.

694

695   Ortiz J., Lemus-Mondaca R., Vega-Gálvez A., Ah-hen K., Puente-Díaz L., Zura-Bravo

696   L. & Aubourg S. (2013). Influence of air-drying temperatura on drying kinetics, colour,

697   firmness and biochemical characteristics of Atlantic salmon (*Salmo salar* L.) fillets.

698   Food Chem 139, 162-169.

699

700   Overturf K., Raboy V., Cheng Z. J. & Hardy R. W. (2003). Mineral availability from

701   barley low phytic acid grains in rainbow trout (*Oncorhynchus mykiss*) diets. Aquac Nutr

702   9, 239-246.

703

704   Popelka M., Marcinčák S., Maskaľová I., Guothová L. & Čertík M. (2014).

705   Comparison of the chemical composition and nutritional values of fresh and frozen

706   rainbow trout. Slov. Vet. Res. 51 (2) 73-80.

707

708   Pratoomyot J., Bendiksen E. Å., Bell J. G. & Tocher D.R. (2010). Effects of increasing

709   replacement of dietary fishmeal with plant protein sources on growth performance and

710   body lipid composition of Atlantic salmon (*Salmo salar* L.). Aquaculture 305, 124-132.

711

712   Sealey W. M., Barrows F. T., Hang A., Johansen K. A., Overturf K., LaPatra S. E. &

713   Hardy R. W. (2008). Evaluation of the ability of barley genotypes containing different

714   amounts of β-glucan to alter growth and disease resistance of rainbow trout

715   *Oncorhynchus mykiss*. Anim Feed Sci Tech 141, 115-128.

716

717   Skrede G., Storebakken T., Skrede A., Sahlstrøm S., Sørensen M., Shearer K.D. &

718   Slinde E. (2002). Lactic acid fermentation of wheat and barley whole meal flours

719   improves digestibility of nutrients and energy in Atlantic salmon (*Salmo salar* L.) diets.

720   Aquaculture 210, 305-321.

721

722   Storebakken T., Shearer K.D., Refstie S., Lagocki S. & McCool J. (1998). Interactions

723   between salinity, dietary carbohydrate source and carbohydrate concentration on the

724   digestibility of macronutrients and energy in rainbow trout (*Oncorhynchus mykiss*).

725   Aquaculture 163, 347-359.

726

727   Stone D. A. J. (2003). Dietary carbohydrate utilization by fish. Rev Fish Sci 11(4): 337-

728   369.

729

730   Thomas L. (1992). Labor und Diagnose, 4. Auflage.

731

732   UNE 87017-1992. Sensory analysis. Methodology. Flavor profile methods.

733

734   UNE-EN ISO 8586-2014. Sensory analysis. General guidelines for the selection ,

735   training and monitoring of selected assessors and expert sensory assessors (ISO

736   8586:2012).

737

738   USDA, 1987. Compositions of foods 15. Fish and shellfish. Agricultural Handbook

739   Number 8. US Government Printing Office, Washington D. C.

740

741   Valente L. M. P., Rema P., Ferraro V., Pintado M., Sousa-Pinto I., Cunha L. M.,

742   Oliveira M. B. & Araújo M. (2015). Iodine enrichment of rainbow trout flesh by dietary

743   supplementation with the red seaweed *Gracilaria vermiculophylla*. Aquaculture 446,

744   132-139.

745

746   Vyncke W. (1975). Evaluation of the direct thiobarbituric acid extraction method for

747   determining oxidative rancidity in mackerel (*Scomber scombrus* L.). Fette, Seifen,

748   Anstrichmittel, 77(6), 239-240.

749

750   Walton M.J. (1986). Metabolic effects of feeding a high protein/low carbohydrate diet

751   as compared to a low protein/high carbohydrate diet in rainbow trout (*Salmo gairdneri*).

752   Fish Physiol Biochem 1:1, 7-15.

753

754   Xu Y., Liu Y., Zhang C., Li X., Yi S. & Li J. (2015). Physicochemical responses and

755   quality changes of turbot (*Psetta maxima*) during refrigerated storage. Int J Food Prop

756   DOI: 10.1080/1094.2912.2015.1022260. In press.

757

758   Yildiz M. (2004). The study of fillet quality and the growth performance of rainbow

759   trout (*Oncorhynchus mykiss*) fed with diets containing different amounts of vitamin E.

760   Turk J Fish Aquat Sc 4: 81-86.