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Enhancement of quality of rainbow trout (*Oncorhynchus mykiss*) flesh incorporating barley on diet without negative effect on rearing parameters

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Abstract Barley concentrations ranging from 0 to 32% (0B, 40B, 80B, 160B, and 319B) were incorporated into rainbow trout, *Oncorhynchus mykiss* (Walbaum) diets. The experiment started with an initial average fish weight of 127.72 ± 5.65 g and finished when they reached commercial weight (final weight between 312 and 330 g) after 84 days. The inclusion of barley in the diets did not show a significant effect on growth and biometric parameters, fat and carbohydrate digestibilities; however, protein digestibility decreased significantly with the incorporation of barley on diets. Glucose levels increased significantly with barley concentration in the diet, and lactate and cortisol levels were also significantly affected after a stress period regardless of the diet. Meat quality was influenced as well by barley concentration. Lower water activity values and an enhancement in textural and color properties were observed in fish fed with the diet containing the highest barley concentration. Trout fed feed with higher concentrations of barley (160B) showed lower lipid oxidation levels than those fed with lower concentrations (control and 40B). The sensory panel found that fish fed with diets higher than 8% in barley content (80B) exhibited a brighter red color in the gills and a better texture; also, meat color became redder with a higher barley inclusion (160B and 319B), being all these sensory parameters correlated with fish freshness. Thus, results indicate that barley can be substituted for wheat fraction without any detrimental effect on production efficiency and enhancing fish quality.

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Introduction

In the course of just a few decades, fish farming has evolved into a highly productive and efficient industry in animal protein production for human consumption (Caballero et al. 2002). Rainbow trout, *Oncorhynchus mykiss* (Walbaum) is one of the most important freshwater cultured fish worldwide. European rainbow trout production represents 21% (176.983 metric tons in 2012; APROMAR 2014) of the world production and Spain holds 10% of this production (14.009 metric tons in 2015, MAGRAMA). Aquaculture requires nutrition optimization in order to raise fish with food production purposes efficiently (Hixson 2014).

Incorporation of novel ingredients needs to balance economic and product quality aspects (Pratoomyot et al. 2010; Valente et al. 2015) without compromising sensory attributes and consumer acceptance. Cereals are usually incorporated in extruded diets of rainbow trout as a carbohydrate and starch source. Wheat is the cereal traditionally used as a carbohydrate source in commercial trout diet (Sealey et al. 2008, Gaylord et al. 2009); however, barley has not been used widely as an ingredient in aquaculture feed, although a few studies showed that its incorporation into fish feed did not have any detrimental effect on growth parameters (Sealey et al. 2008). Probably one of the reasons of the scarce use of barley is due to the presence of anti-nutritive components in its composition, such as phytic acid (Cheng and Hardy 2003). The presence of phytic acid limits the absorption of some minerals in diets such as phosphorus, zinc, and calcium caused by the formation of insoluble salts (Cheng and Hardy 2003; Overturf et al. 2003; Gaylord et al. 2009; Kumar et al. 2012). However, in order to decrease the presence of phytates, new varieties low in phytic acid levels have been developed (Overturf et al. 2003; Gaylord et al. 2009). Another limiting factor for the use of barley is the low protein content compared to that found in other different sources (wheat, soy, corn, etc).

However, barley presents many advantages due to its β-glucan content (Sealey et al. 2008; Meena et al. 2013). β-Glucans in nature are in the cell walls of several plants such as barley, oats, rye, and wheat at concentrations ranging from 2 to 7 and <1% respectively. However depending the variety of barley, β-glucan content can range from 4 to 11% (Gatlin et al. 2007). The acceptance of β-glucans as a functional, bioactive ingredient has increased their popularity (Lazaridou and Biliaderis 2007) and potential due to their immunostimulant effect. Different studies have been carried out to evaluate the beneficial effects of β-glucans on the growth and survival rates (Hai and Fotedar 2009; Lin et al. 2011), disease resistance and protection against pathogens (Dalmo and Bøgwald 2008; Lokesh et al. 2012), and immune system enhancement (Gu et al. 2011) in a wide range of aquaculture species (Sealey et al. 2008; Meena et al. 2013). In particular, several studies on trout have reported the growth enhancement when β-glucans were added on fish feed (Heidarieh et al. 2012; Ghaedi et al. 2015). Jeney et al. (1997) observed that low doses of β-glucans (0.1%) in the feed may prevent stress caused by transport.

The objective of the present work was to study the effect of the inclusion of barley, as an alternative ingredient in rainbow trout, *O. mykiss* (Walbaum) diets, and evaluate the impact on growth performance, apparent digestibility, response to stress, and final fish meat quality parameters.

Material and methods

Production system

The trial was conducted in 20 cylindrical fiberglass tanks (500 L) within a freshwater recirculation system (RAS). Throughout the experiment temperature remained constant at 13.58 ± 1.06 °C and so were dissolved oxygen levels, kept between values of 9.18 ± 1.35 mg L^{-1}. All tanks were equipped with aeration and an oxygen probe. Water pH was 8.03 ± 0.07 and ammonia and nitrites concentration in water were 0.16 ± 0.23 and 0.15 ± 0.11 mg L^{-1} respectively. Water flow was 12.2 ± 0.5 L h^{-1}. The photoperiod consisted on 12-h light and 12-h dark intervals and all tanks had identical lightning conditions.

Fish and experimental design

A total of 500 rainbow trout from a commercial trout farm (IPEASA, Fuentidueña, Segovia, Spain) were used. Fish were randomly allocated in 20 tanks, 25 fish in each tank (initial stocking density 6.7 ± 0.4 kg m^{-3}). Prior to the feeding trial, all fish were acclimated to the indoor rearing conditions for 2 weeks and fish were fed once a day (8:00) to apparent satiation using exclusively a control diet. The study lasted 84 days.

Rearing parameters (growth (final weight, biomass increment, survival, and SGR), nutritional parameters (FI and FCR) and biometric indexes (CF, VSI and HSI), and meat quality (proximate composition, water activity, color, texture, and sensory analysis)) were evaluated approximately every 28 days. All fish were starved for 24 h and anesthetized with (MS222®; 200 mg L^{-1}) prior to taking weight and length measurements. Fish were randomly sampled from each tank to determine rearing and meat quality parameters during the growth period (0, 28, 56, and 84 days). At day 44, fish were controlled stressed by decreasing the concentration of oxygen from 8 to 4 mg L^{-1}. The concentration of oxygen was decreased by lowering water level to a volume of 50 L and removing the aeration. When the levels of dissolved oxygen in water reached 4 mg L^{-1}, it is started to count 10 min in these conditions, reaching levels of <2 mg L^{-1}. Biochemical parameters in blood plasma (glucose, lactate, and cortisol levels) were determined.

Diets and feeding

Five isoproteic (40% crude protein) and isolipidic diets (18% crude lipid) were developed containing different barley levels (0B (0% barley, 0% β-glucans); 40B (4% barley, 0.14% β-glucans); 80B (8% barley, 0.22% β-glucans); 160B (16% barley, 0.53% β-glucans); 319B (31.92% barley, 1.5% β-glucans)). Control diet (0B) has been prepared with the same ingredients as experimental diets but without barley on the formulation. This diet was not a commercial diet. There were five feeding treatment groups each in four replicates (n = 4).

The barley which was used corresponded to an H13 genetically modified and bred variety of Merlin and VOLGA varieties, harvested in the 2012/2013 period and commercially known as GALIS. This barley is bare with a β-glucan content of 5.2%.

The formulation and composition of the diets are shown in Table 1. Diets were prepared by an extrusion process using a semi-industrial twin-screw extruder (CLEXTRAL BC-45. St. Etienne, France). Raw material was processed at a speed of 100 rpm, at 110 °C and a pressure of 40–50 atm.

Table 1. Formulation and proximate composition of the experimental diets

	Diets[a]				
	0B	40B	80B	160B	319B
Ingredients (g kg^{-1})—international feed number in parentheses					
Fish meal (5-02-000)	220	220	220	220	220
Wheat (4-05-268)	318	278	238	159	0
Barley	0	40	80	160	319
Wheat gluten	192	186	182	177	160
Meat meal	103	111	116	120	140
Soybean oil (4-07-983)	91	89	88	88	85
Fish oil (4-08-048)	45	45	45	45	45
Maltodextrin (4-08-023)	11	11	11	11	11
Multivitamin and minerals mix[b]	20	20	20	20	20
Analyzed composition (% dry matter)					
Dry matter	90.30	90.90	90.40	90.90	90.40
Crude protein (% CP)	38.70	39.30	39.80	40.20	39.40
Crude fat (% CF)	17.60	17.10	17.10	16.90	15.90
Ash (%)	7.50	7.70	7.90	8.00	8.40
Carbohydrate (% CHO)[c]	36.20	35.90	35.20	34.90	36.30
β-Glucans (%)	0.00	0.14	0.22	0.53	1.50

[a] Different experimental diets: 0B (0% barley, 0% β-glucans), 40B (4% barley, 0.14% β-glucans), 80B (8% barley, 0.22% β-glucans), 160B (16% barley, 0.53% β-glucans), and 319B (31.92% barley, 1.5% β-glucans)

[b] Vitamin and mineral mix (values are g kg^{-1} except those in parenthesis): Premix: 25; Choline, 10; DL-a-tocopherol, 5; ascorbic acid, 5; $(PO_4)_2Ca_3$, 5. Premix composition: retinol acetate, 1,000,000 IU kg^{-1}; calciferol, 500 IU kg^{-1}; DL-a-tocopherol, 10; menadione sodium bisulfite, 0.8; thiamine hydrochloride, 2.3; riboflavin, 2.3; pyridoxine hydrochloride, 15; cyanocobalamin, 25; nicotinamide, 15; pantothenic acid, 6; folic acid, 0.65; biotin, 0.07;ascorbic acid, 75; inositol, 15; betaine, 100; and polypeptides 12

[c] Carbohydrate (% CHO) were calculated by difference for all the nutrients: %CHO = 100 − (% CP + % CF + % Ash)

Fish were fed twice a day (8:00 am and 15:00), 6 days per week to apparent satiation level during the whole experimental period. Pellets were distributed manually to allow all fish to eat. The amount of pellets not consumed for fish were collected to determine feed intake (FI).

Apparent digestibility coefficients

Simultaneously to the feeding trial, digestibility studies were conducted. After fish were fed for a second time, tanks were completely cleaned and feces were collected in a settling column (Cho et al. 1982), which was emptied in the following morning at 8:00 hours. Wet fecal content was then collected and dried at 60 °C for 48 h prior to analysis (crude protein (CP), crude fat (CF), carbohydrates (CHO), and acid-insoluble ashes (AIA)). Over the whole experimental period, samples of feces were collected from each tank (n = 4).

The *apparent digestibility coefficients (ADCs)* of protein, fat, and carbohydrates in the diets tested were calculated according to the following formula:

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

where PN is the percentage of nutrient.

Biochemical parameters in blood plasma

To determine the stress response (hypoxia conditions, <4 mg L^{-1} per 10 min), three fish per tank ($n = 3$) were alternatively captured before stress conditions (basal levels), during stress condition, and after 1 and 2 weeks, to measure their ability to recover basal levels.

Blood samples were withdrawn from the caudal vein using 1-mL syringes (BD Plastipak) with ethylenediaminetetracetic acid (EDTA) as anticoagulant, 0.5 mL were centrifuged (Hettich Zentrifugen, Universal 320 R, Germany) at 5000 rpm for 20 min at 4 °C, and the plasma was extracted to measure cortisol, glucose, and lactate levels. Samples were stored at −80 °C till analysis.

Concentration of plasma cortisol was determined using the method described by Thomas (1992), using an enzyme-linked immunosorbent assay (DEMEDITEC CORTISOL ELISA® Ref. DE1887). Briefly, aliquots (20 μL) from plasma were dispensed into appropriate wells and incubated with 200 μL of enzyme conjugate solution for 60 min at room temperature. After incubation, the wells were rinsed three times with wash solution (400 μL per well) and incubated with 100 μL substrate solution for 15 min at room temperature. The enzymatic reaction was stopped by adding 100 μL of stop solution, and the absorbance was measured at 450 nm with an absorbance microplate reader (Bibby Scientific Limited, Jenway 7315, UK).

Concentration of glucose and lactate were measured by an enzymatic colorimetric assay, in particular by GOD-POD (SPINREACT® Ref. 1001191) and LOD-POD (SPINREACT® Ref. 1001330) method respectively (Kaplan and Pesce 1984). Briefly, aliquots (5 μL) from plasma samples were mixed with 500 μL of reactive and incubated for 10 min for glucose determination and 5 min for lactate determination at 37 °C in the dark. The absorbance was determined at 490 nm in a 96-well microplate reader (Bibby Scientific Limited, Jenway 7315, UK).

Quality markers of fish meat

Proximate composition analysis

Proximate analyses (moisture, crude protein, crude fat and ash, % of dry weight) were evaluated from ingredients, diets, and feces obtained from the digestibility trial and from fish flesh ($n = 4$ for flesh, one fish per tank). Analyses were determined according to AOAC (1990) procedures: dry matter (60 °C to constant weight), ash (incinerated at 550 °C to constant weight), crude protein (N × 6.25 and nitrogen was analyzed by Dumas principle, TruSpec CN; Leco Corporation, St. Joseph, MI, USA), and crude lipid content using the Soxhlet extraction method. AIA was used as an indicator for the ADC and was analyzed according to the method described by Atkinson et al. (1984) with some modifications. Briefly, 5 g of sample was ashed for 5 h at 550 °C to ensure complete combustion of the organic material in the sample. The resulting ash was boiled till dryness in 75 mL of HCl (2 N) and boiled in other 75 mL HCl during 15 min. Samples were filtered hot through ashless filter paper and washed in boiling destilled water till neutralized the samples. Finally as Atkinson et al. (1984) method, samples were ashed for 5 h at 550 °C.

β-Glucan content was measured in barley, control, and all experimental diets. β-Glucan content on barley and different diets were evaluated using McCleary method (Megazyme mixed-linkage beta-glucan assay procedure K.BGLU04/06). Briefly, 0.5 g of sample was mixed with 1 mL ethanol (50% *v/v*) and 5 mL of sodium phosphate buffer (20 mM, pH 6.5). It was incubated in a water bath during 5 min. It was cooled at 40 °C and mixed with 0.2 mL of liquenase (10 U) during 1 h at 40 °C. After this time, the mixture was centrifuged at 100×*g* during 10 min. 0.1 mL of supernatant is transferred and mixed with 0.1 mL of sodium acetate buffer (50 mM pH 4) and 0.1 mL of β-glucosidase (0.2 U). The mixture was incubated during 15 min at 40 °C for the determination of β-glucan. The absorbance was determined at 510 nm in a 96-well microplate reader (Bibby Scientific Limited, Jenway 7315, UK).

Water activity (a_w)

Water activity (a_w) was instrumentally measured using an Aqualab 4TE (Decagon Devices inc., Pullman, WA, USA). Measurements were taken directly from the muscle. Six measurements were made in each flesh at three different locations (front, central, and tail). The study was evaluated in four independent fish flesh ($n = 4$).

Color

CIELAB parameters (lightness (L^*), redness (A^*), yellowness (B^*)) were evaluated using a portable colorimeter (Minolta CM-2002, Osaka, Japan). Hue and Chroma were calculated using the formulas.

$$\text{Chroma} = \left(a^{*2} + b^{*2}\right)^{1/2}$$

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

Measurements were taken directly over the muscle. Six measurements were evaluated randomly over skinless fish meat. The study was evaluated in four independent fish flesh ($n = 4$).

Texture analysis

Texture was determined using a texture analyzer TA-XT2i (ANAME, Stable Micro System, Vienna Court, Lammas Road, Godalming, Surrey, UK). A texture profile analysis (TPA) was carried out using a penetration probe of 4-mm diameter at speed of 1 mm s^{-1} with a 5-mm distance, and the instrument was equipped with a 25-kg-load cell. The time delay between cycles was 5 s. Previous to analysis, samples were peeled manually and texture was analyzed in the front, middle, and tail parts. Fish flesh were evaluated in the same position (with the muscle fibers perpendicular) to the test probe. The study was evaluated in four independent fish flesh per treatment ($n = 4$).

Curves were evaluated and the following parameters were determined: *hardness* (g) (maximum force required to compress the sample), *cohesiveness* (capacity of the sample to deform before rupture (A_2/A_1, where A_1 is the total energy required for the first compression and A_2 is the total energy required for the second compression)), *elasticity* (mm) (capacity of the sample to recover its original shape after the deformation force ends), and *gumminess* (g) (strength to disintegrate a sample to a constant state of swallowing (hardness × cohesiveness)).

Thiobarbituric acid reactive substances

TBA as an indicator of lipid oxidation was evaluated using the methodology described by Vyncke (1975). Briefly, 10 g of samples was mixed with 30 mL of 7.5% TCA. The mix was homogenized and centrifuged for 5 min at 4 °C and 4000 rpm, and then filtered with Whatman no. 1 filters (Prat Dumas, France). Five milliliters of the filtrate was mixed with 5 mL 0.02 M TBA, incubated at 90 °C in a water bath during 40 min and then read in spectrophotometer (Fluostar® Omega, BMG labtech, The microplate reader company, Germany) at 530 nm. One fish per tank was analyzed during the entire experiment ($n = 4$), and results were expressed as micromoles malondialdehyde (MDA) per kilogram of fresh muscle.

Sensory analysis

All sensory analysis were performed according to ISO standards (ISO 2001, 2008) in a sensory room compliant with (ISO 2007) by a panel of 8 people (4 male and 4 female aged between 25 and 50) with previous experience in sensory analysis of food products. Nonetheless, in order to familiarize the panel with the sensory assessment of fish products and optimize the tables used for sensory evaluation, the panel were trained in the main characteristics we wished to study.

Sensory analysis comprised fresh whole fish and fish meat samples ($n = 4$). Whole fish were evaluated using the quality index method (QIM), and fish flesh were analyzed using a quality descriptive method (QDM). Panelists were trained to perform both analyses. QIM was assessed following the guideline of QIM Eurofish (Martinsdóttir et al. 2001). Freshness was evaluated by giving demerit points according to certain aspects associated with general appearance such as skin, stiffness, odor, gills pots color and odor, belly, and eye brightness and shape. The trained judges scored ranked from 0 to 3 for each attribute. The maximum score of 3 corresponded to the fish with the worst quality parameters.

For the QDM, panelists were trained to discriminate color, texture, odor, and acceptability of fish meat. A continuous non-structured scale (1–10) was used for evaluation. The left side of the scale corresponded to the lowest intensity (value 1: white, soft, fresh odor, and acceptable sample) whereas the right side corresponded to the highest intensity (value 10: dark, hard, rancid odor, and non-acceptable sample).

Panelists evaluated one fish per treatment every 28 days during the whole experiment. Five samples, in pairs of whole fish and flesh of each treatment, were individually presented in porcelain dishes to each panelist. Samples were coded with random numbers and maintained at room temperature during evaluation.

Statistical analyses

Statistical analysis was performed using SAS version 9 (SAS Institute Inc., Cary, North Carolina, USA) by a GLM procedure for the variance analysis (ANOVA). In the rearing parameters, the initial weight (Wi) was included as covariable.

For the rearing parameters and digestibility, diet was included as the only fixed factor. For biochemical parameters, in order to evaluate the differences between diets at a certain moment and at different moments within a same diet, the fixed factors of diet and time and their interaction were included in the model. For proximal composition, color, sensorial analysis, thiobarbituric acid reactive substances (TBARS), water activity (a_w), and texture in the GLM model, only the diet was included as fixed effect. In addition, for a_w and texture, the section

where these parameters were measured (front, middle or tail of the fish flesh) was also included as a fixed effect.

When the ANOVA revealed a significant effect, values were compared using the Student *t* test and were considered to be significant at $p < 0.05$. When the interaction was proven to be significant the data correspond to the double interaction and are presented as least-squares means (LSM) ± the standard error of the mean (SEM).

Ethical statement

The rainbow trout study complied with the European Union Council Directive 2010/63/UE, which provides the minimum standards for animal protection, and was also in accordance with the Spanish national legislation (Spanish Royal Decree 53/2013) based on animal protection in experimentation and other scientific practices and approved by the Animal Ethics Committee of Agro-Technological Institute of Castilla y León (Spain).

Fish in tanks were checked on a daily basis. Every 4 weeks, fish were weighed individually and their health status was assessed by observation, after sedation with MS222 dissolved in water (MS222®; 200 mg L^{-1}) to minimize animal suffering.

Animals were euthanized by excess of MS222 (300 mg L^{-1}) or with ice (when quality samples were taken), and then fish were dissected.

Results

Rearing parameters: growth and biometric analysis

The experiment started with an initial average fish weight of 127.72 ± 5.65 g and finished when fish reached commercial weight (range 312–330 g). Every 28 days, fish were weighed and length measured to determine the growth and biometric indexes (Table 2). The study did not show significant differences in any of the parameters studied.

Apparent digestibility coefficients

The results showed that *protein digestibility* of fish fed with the control and 40B diets was significantly higher (98.28%) ($p < 0.05$) than that of fish fed with higher barley concentrations. The *fat* and *carbohydrate ADC* on experimental diets was not significantly affected by diet (Table 3).

Biochemical parameters

Higher concentrations of barley on the diet showed higher concentration of glucose in blood plasma in the moment of stress. But no significant effects were observed in lactate and cortisol levels (Fig. 1). When the stress response results were analyzed, a significant increase ($p < 0.05$) of glucose, lactate, and cortisol was observed under stress, recovering basal levels of cortisol and lactate after 7 days, while hyperglycemia persisted 7 days more (Fig. 1). An interactive effect was only observed in glucose levels on the different experimental diets. Changes in glucose levels have been significantly ($p < 0.05$) affected by the inclusion of barley and the effect of stress.

Table 2. Growth and biometric indexes of rainbow trout fed with different experimental diet for 84 days (values are least-squares means ± SEM, $n = 4$)

	Diets[a]					
	0B	40B	80B	160B	319B	SEM
Initial weight (g)	125	125	127	131	131	2.62
Final weight (g)	328	312	330	328	330	11.64
Biomass increment (g)	4354	4215	4444	4415	4387	233.85
Survival (%)	91	99	96	97	95	2.16
SGR (% day^{-1})[b]	1.26	1.19	1.24	1.19	1.20	0.04
FI (g 100 g $fish^{-1}$ day^{-1})[c]	1.23	1.15	1.16	1.14	1.16	0.03
FCR[d]	1.17	1.13	1.11	1.11	1.14	0.02
CF (g cm^{-3})[e]	0.93	0.93	0.93	0.96	0.94	0.01
VSI (%)[f]	10.75	9.94	10.36	10.71	10.80	0.35
HSI (%)[g]	1.63	1.60	1.68	1.72	1.58	0.11

Absence of letters indicate no significant differences between treatments ($p > 0.05$)

[a] Diets' explanation as in Table 1

[b] Specific growth rate (% day^{-1}). SGR = 100 × ln(final weight/initial weight)/days

[c] Feed intake ratio (g 100 g $fish^{-1}$ day^{-1}). FI = 100 × feed consumption (g)/average biomass (g) × days

[d] Feed conversion ratio. FCR = feed intake (g)/weight gain (g)

[e] Condition factor (g cm^{-3}). CF = 100 × final weight/$length^3$

[f] Viscerosomatic index (%). VSI = 100 × visceral weight/final weight

[g] Hepatosomatic index (%). HSI = 100 × liver weight/final weight

Quality markers of fish meat

Proximate composition

Results showed that barley increased significantly ($p < 0.05$) crude fat and ash content on meat proximate composition (Table 4) while moisture and crude protein were not affected. At the

Table 3. Apparent digestibility coefficients (ADC) of protein, fat, and carbohydrates in rainbow trout fed with five experimental diets differing on the source of carbohydrate (wheat and barley) (values are least-squares means ± SEM, $n = 3$)

	Diets[a]					
	0B	40B	80B	160B	319B	SEM
Apparent digestibility coefficient (ADC)						
Protein ADC	98.28b	98.28b	96.61a	96.41a	96.49a	0.45
Fat ADC	96.88	97.93	96.17	95.90	97.20	1.20
Carbohydrate ADC	84.76	88.89	79.23	80.67	83.48	3.04

Data in the same row with different letters indicate significant differences between treatments ($p < 0.05$). Absence of letters indicate no significant differences between treatments ($p > 0.05$)

[a] Diets' explanation as in Table 1

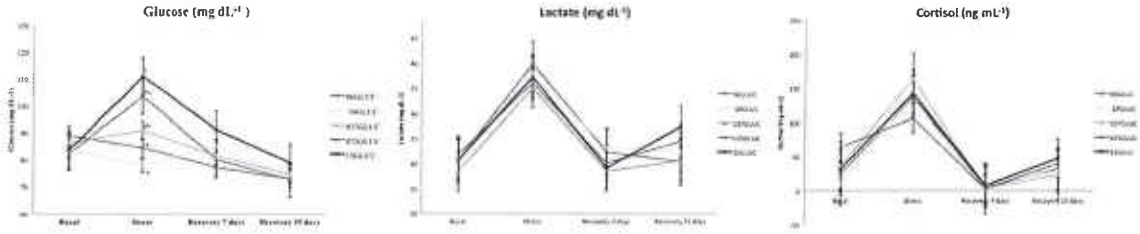

Fig. 1. Effect of hypoxia challenge on the biochemical parameters (glucose, lactate, and cortisol) of trout fed different experimental diets measured along the time (basal levels, just after the stress, 1 week of recovery, and 2 weeks of recovery) (values are least-squares means ± SEM, $n = 3$). *Small letters* indicate significant differences between diets ($p < 0.05$). *0B* (0% barley, 0% β-glucans), *40B* (4% barley, 0.14% β-glucans), *80B* (8% barley, 0.22% β-glucans), *160B* (16% barley, 0.53% β-glucans), and *319B* (31.92% barley, 1.5% β-glucans) are the different experimental diets

end of the experimental growth, crude fat content of fish fed 160B diets increased significantly ($p < 0.05$), while ash content decreased significantly with the concentration of barley.

Water activity (a_w)

a_w was significantly ($p < 0.05$) affected by the different diets (Fig. 2). Lower a_w values were observed in fish fed with diets high in barley (319B) at the end of the experimental growth period and in the front and middle parts of the filet (results not shown).

Color

No significant differences were observed despite barley concentration in meat of fish fed the different diets (Table 5).

Texture

The results obtained in the present study showed that barley concentration on the diet had a significant effect on meat gumminess (Table 6 and Fig. 3). Compared to control

Table 4. Proximate composition of rainbow trout meat fed with increasing levels of barley at the end of the experimental period (data are expressed as % of dry matter) (values are least-squares means ± SEM, $n = 4$)

	Diets[a]						
	Initial	0B	40B	80B	160B	319B	SEM
Analyzed composition (% dry matter)							
Moisture	75.80	73.60	75.50	75.50	74.30	76.00	1.30
Crude protein (CP)	17.22	17.30	16.30	16.60	17.00	17.00	0.68
Crude fat (CF)	5.74	7.84c	6.81b	6.39ab	7.78c	5.71a	0.28
Ash	1.08	2.85c	2.57b	2.57b	2.69b	2.30a	0.05

Data in the same row with different small letters indicate significant differences between treatments ($p < 0.05$). Absence of letters indicate no significant differences between treatments ($p > 0.05$)

[a] Diets explanation as in Table 1

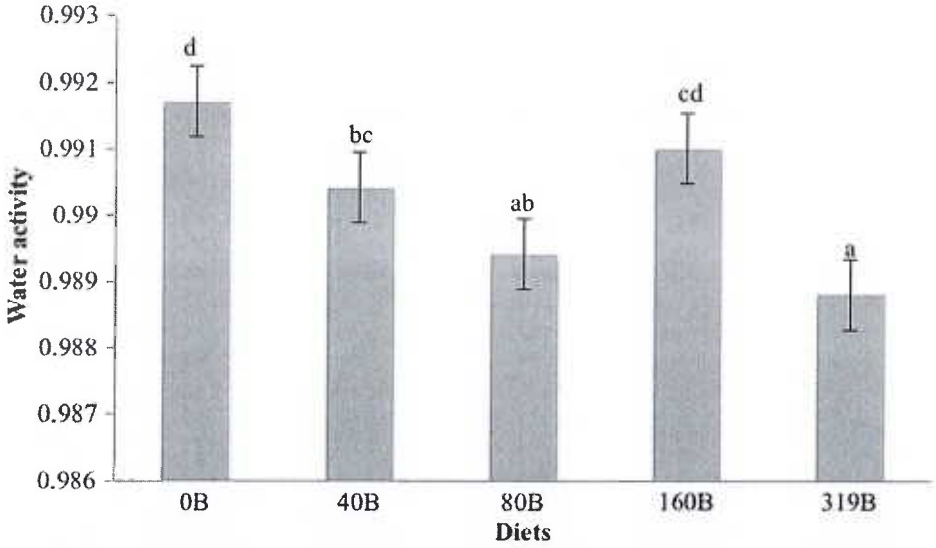

Fig. 2. Effect of barley concentration on water activity (a_w) of fish meat at the end of the experimental growth period. Data are presented as least-squares means ± standard error of the mean (n = 4); significant differences ($p < 0.05$) are indicated with *different letters* above the column. *0B* (0% barley, 0% β-glucans), *40B* (4% barley, 0.14% β-glucans), *80B* (8% barley, 0.22% β-glucans), *160B* (16% barley, 0.53% β-glucans), and *319B* (31.92% barley, 1.5% β-glucans) are the different experimental diets

diet (0B) the increase of barley on diet produced a significant decrease of meat gumminess. Hardness, cohesiveness, and elasticity were not significantly affected by the diet. Different sections of the fish flesh were also studied (front, middle, and tail). Results showed the tail region was the stiffest part of the flesh (results not shown).

Thiobarbituric acid reactive substances

The inclusion of barley in the diet had an inhibitory effect. Trout fed with diets higher in barley concentration had a lower level of TBARS in meat than those obtained from trouts fed with lower barley concentrations diets (Fig. 4).

Table 5. Effect of barley concentration on the CIELAB parameters of fish meat at the end of the experimental growth period (values are least-squares means ± SEM, n = 4)

	Diets[a]						
	Initial	0B	40B	80B	160B	319B	SEM
L^*	53.86	57.83	57.54	57.16	57.32	55.13	0.76
A^*	3.01	−0.84	−0.24	−0.52	−0.75	−0.46	0.30
B^*	12.50	7.82	7.46	7.57	7.02	6.41	0.52
Hue	0.96	−0.65	−0.16	−0.33	−0.48	−0.28	0.27
Chroma	13.05	7.98	7.66	7.77	7.34	6.55	0.49

[a] Diets' explanation as in Table 1

Absence of letters indicate no significant differences between treatments ($p > 0.05$)

Table 6. Effect of barley concentration on hardness, cohesiveness, elasticity, and gumminess of fish meat fed with diets with increasing concentrations of barley at the end of the experimental growth period (values are least-squares means ± SEM, $n = 4$)

	Diets[a]						
	Initial	0B	40B	80B	160B	319B	SEM
Hardness	198.18	36.78	55.16	42.53	44.81	45.53	7.80
Cohesiveness	0.12	0.20	0.21	0.21	0.22	0.21	0.01
Elasticity	4.63	4.82	4.83	4.71	4.78	4.68	0.09
Gumminess	20.35	7.46a	12.01b	8.74ab	9.75ab	6.26a	1.27

Data in the same row with different small letters indicate significant differences between treatments ($p < 0.05$). Absence of letters indicate no significant differences between treatments ($p > 0.05$)

[a] Diets' explanation as in Table 1

Sensory analysis

Results from QIM showed that barley concentration significantly affected ($p < 0.05$) gill color (Fig. 5). Gills became pale on fish fed with 40B diets but diets with higher barley concentrations enhanced the redness, so barley with a β-glucan content of 0.22% (80B) or higher enhanced fish freshness by making gills appear redder.

On the other hand, QDM was evaluated on fish flesh. Experimental diets showed a significant ($p < 0.05$) effect on meat color (Fig. 6). Fish color was redder in those fish fed with diets at higher barley concentrations (0.53%, 160B). Texture was also affected by diets; fish fed with 80B showed a higher hardness than those fish fed with diets higher in barley concentrations (Fig. 7). When acceptability was analyzed, no significant differences were observed regardless of barley concentration, so fish samples were considered to be acceptable compare to control.

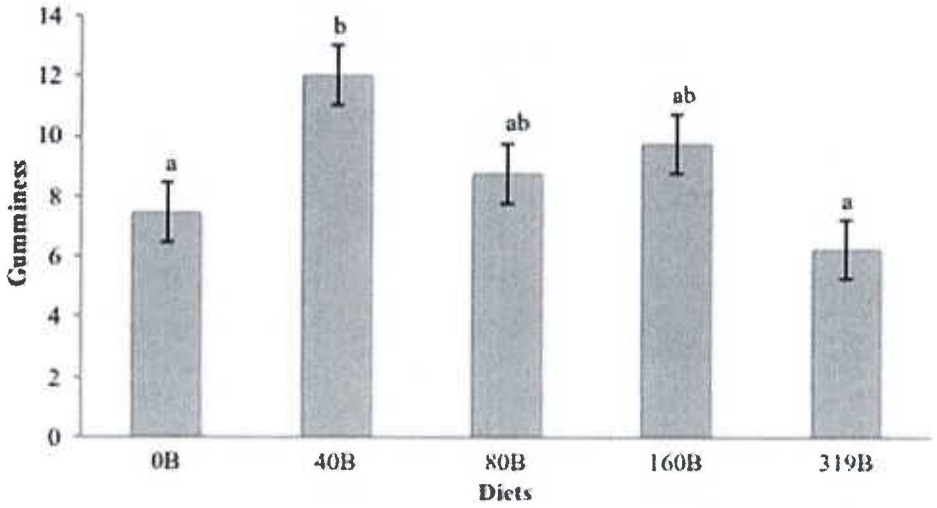

Fig. 3. Effect of barley on meat gumminess of fish fed different experimental diets. Data are presented as least-squares means ± standard error of the mean ($n = 4$); significant differences ($p < 0.05$) are indicated with *different letters* above the column. *0B* (0% barley, 0% β-glucans), 40B (4% barley, 0.14% β-glucans), *80B* (8% barley, 0.22% β-glucans), *160B* (16% barley, 0.53% β-glucans), and *319B* (31.92% barley, 1.5% β-glucans) are the different experimental diets

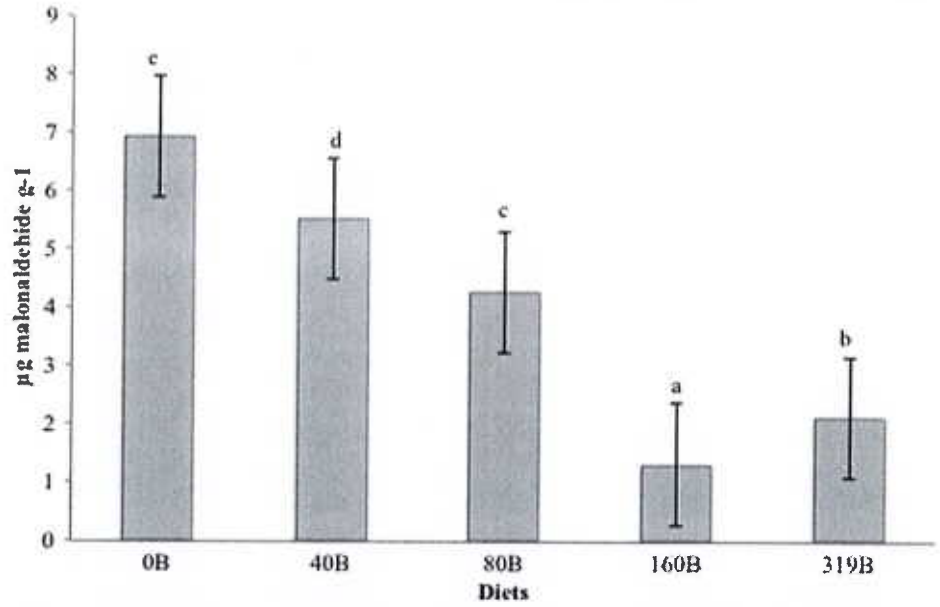

Fig. 4. Effect of barley concentration on lipid oxidation (TBARS) measured as micrograms malonaldehide per gram of fish meat at the end of the experimental growth period. Data are presented as least-squares means ± standard error of the mean ($n = 12$); significant differences ($p < 0.05$) are indicated with *different letters* above the column. *0B* (0% barley, 0% β-glucans), *40B* (4% barley, 0.14% β-glucans), *80B* (8% barley, 0.22% β-glucans), *160B* (16% barley, 0.53% β-glucans), and *319B* (31.92% barley, 1.5% β-glucans) are the different experimental diets

Discussion

The present study was mainly focused on evaluating the effect of increasing levels of barley, as an ingredient rich in β-glucans, on rainbow trout diets. The findings concerning growth performance and digestibility obtained on the present study have showed the potential use of barley on commercial diets for rainbow trout. In the actual study, substituting wheat for

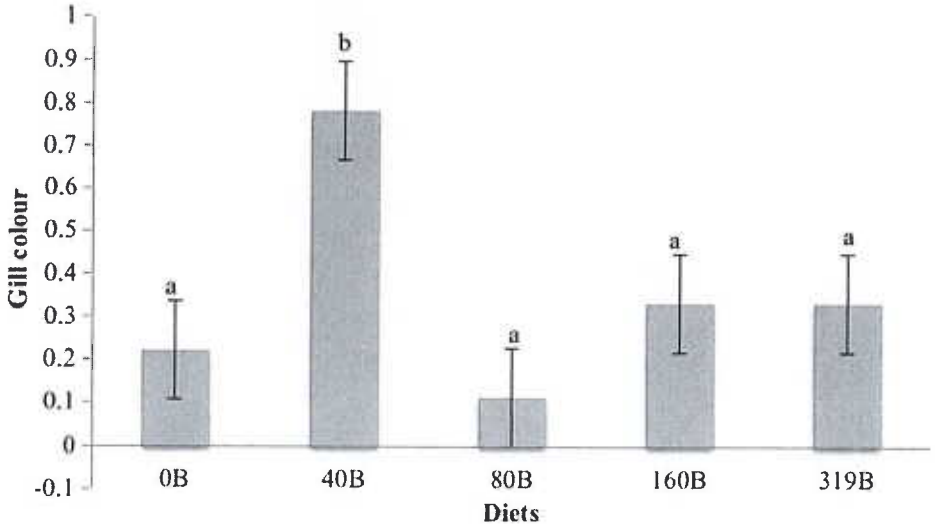

Fig. 5. Effect of barley concentration on the gill color of fish from sensory analysis (QIM) at the end of the experimental growth period. Data are presented as least-squares means ± standard error of the mean ($n = 4$); significant differences ($p < 0.05$) are indicated with *different letters* above the column. *0B* (0% barley, 0% β-glucans), *40B* (4% barley, 0.14% β-glucans), *80B* (8% barley, 0.22% β-glucans), *160B* (16% barley, 0.53% β-glucans), and *319B* (31.92% barley, 1.5% β-glucans) are the different experimental diets

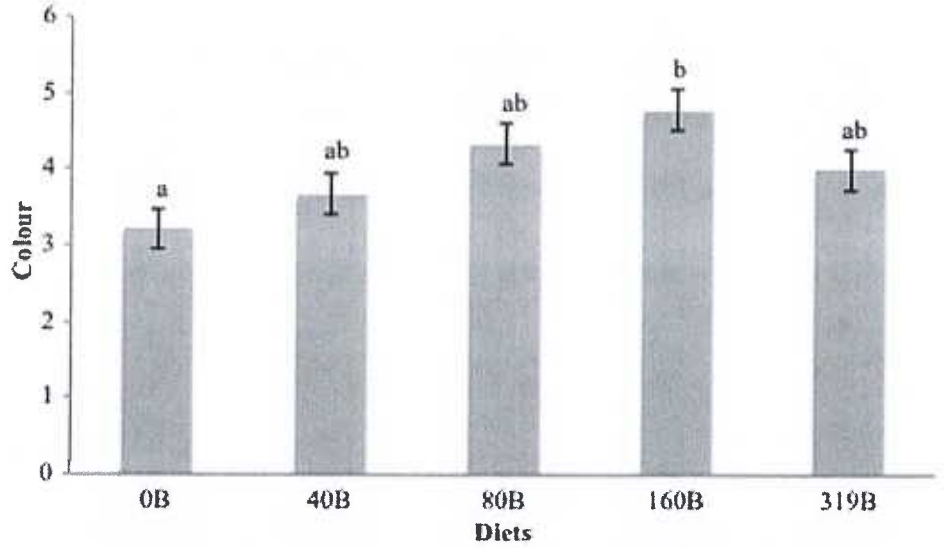

Fig. 6. Effect of barley concentration on the color of fish meat from sensory analysis (QDM) at the end of the experimental growth period. Data are presented as least-squares means ± standard error of the mean (n = 4); significant differences ($p < 0.05$) are indicated with *different letters* above the column. *0B* (0% barley, 0% β-glucans), *40B* (4% barley, 0.14% β-glucans), *80B* (8% barley, 0.22% β-glucans), *160B* (16% barley, 0.53% β-glucans), and *319B* (31.92% barley, 1.5% β-glucans) are the different experimental diets

barley did not substantially altered growth performance of rainbow trout suggesting that both cereals could be equally used even though barley contains more dietary fiber than wheat. However, barley, in contrast to wheat, enhanced fish meat quality. Similar results were obtained by Sealey et al. (2008), who studied the effect of three barley genotypes on growth performance of rainbow trout and did not observed significant differences on final weight regardless barley concentration. The fact that growth has not been disadvantaged could also

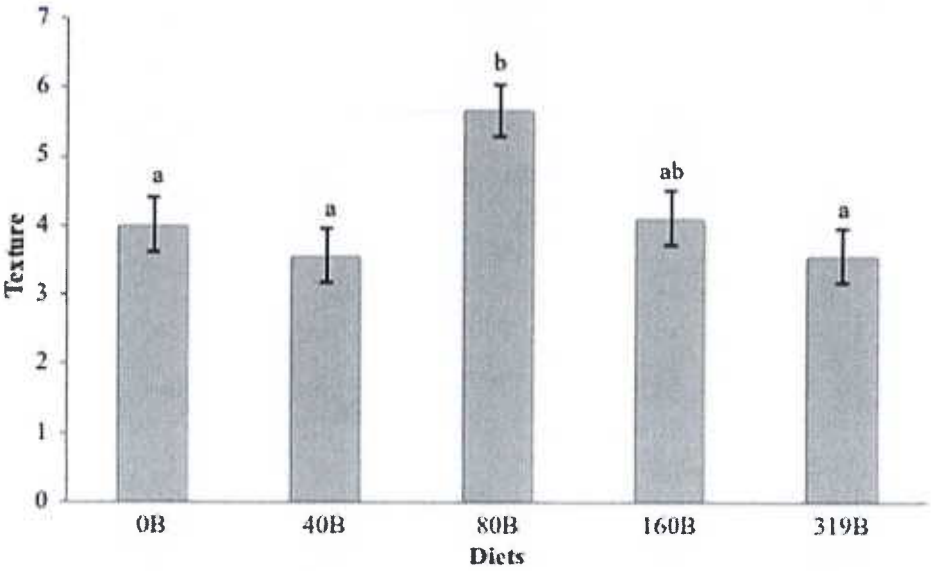

Fig. 7. Effect of barley concentration on the texture of fish meat from sensory analysis (QDM) at the end of the experimental growth period. Data are presented as least-squares means ± standard error of the mean (n = 4); significant differences ($p < 0.05$) are indicated with *different letters* above the column. *0B* (0% barley, 0% β-glucans), *40B* (4% barley, 0.14% β-glucans), *80B* (8% barley, 0.22% β-glucans), *160B* (16% barley, 0.53% β-glucans), and *319B* (31.92% barley, 1.5% β-glucans) are the different experimental diets

been explained that the phytic acid content of this barley variety had not a negative effect on the growth of rainbow trout, as it has also been reported by other authors (Overturf et al. 2003; Gaylord et al. 2009). Despite barley has more dietary fiber than wheat it was not observed a greater feed intake (FI). It is common that when fiber levels are very high, digestive transit is faster and FI increase, possibly as a result that this higher fiber level is not harmful for rainbow trout. Results did not show significant differences on survival rate. Probably one of the reasons of this could be that experimental diets studied in the present study were very well balanced nutritionally. However, it has been reported a significant increase on survival rates with the incorporation of β-glucan in other fish species: croaker (*Pseodosciena crocea*) (Ai et al. 2007), Pacific white prawns (*Penaeus monodon*) (Chang et al. 2003), and juvenile western king prawns (*Penaeus latisulcatus kishinouye*) (Hai and Fotedar 2009). In the case of Chang et al. and Ai et al., they tested immunity, so the survival rate is related to resistance of fish to a disease factor.

In the present work, it has been observed that diets with barley and wheat were properly digested by rainbow trout, since all apparent digestibility coefficients were high. It is true that protein digestibility coefficient was slightly lower in trouts fed with diets containing higher barley levels, but considering the high percentage of this coefficient, it is not possible to conclude a negative effect of this ingredient in the overall digestibility of diets. The ability of salmonids to digest fiber is rather limited due to the low α-amylase activity and the large amounts of undigested starch in the intestinal content which it would reduce digestibility of other macronutrients (Skrede et al. 2002; Stone 2003; Krogdahl et al. 2005; Couto et al. 2016). The concentration of undigested carbohydrate in the gut has been related to reduction in fat digestibility in rainbow trout (Storebakken et al. 1998; Morken et al. 2011). In the present study, the ADC of fat on experimental diets was not significantly affected by diet; fat digestibility was higher than values reported in other carnivorous fish species studies: rainbow trout (*O. mykiss*) (Storebakken et al. 1998), atlantic salmon (*Salmo salar*) (Skrede et al. 2002), and gilthead seabream (*Sparus aurata*) (Couto et al. 2016). The ADC for protein and fat were higher than 80%; values in agreement with the results reported by Cheng and Hardy (2002, 2003) who reported ADC for protein and fat in barley were also higher than 80% for rainbow trout. Starch digestibility decreased with the increase of wheat and barley levels in the diets, in accordance with previously reported data (Grisdale-Helland and Helland 1997; Skrede et al. 2002). Skrede et al. (2002) performed a study with lactic acid fermentation of both barley and wheat, reporting a higher starch digestibility in the case of barley. Results which were similar to those obtained in the present study, indicating that barley would be an interesting ingredient in extruded diets for rainbow trout.

For rainbow trout, high glucose values following feeding with high levels of available carbohydrates were observed (Walton 1986; Krogdahl et al. 2004). When the stress response results were analyzed, a significant increase of all parameters (glucose, lactate, and cortisol) were observed under stress, recovering basal levels of cortisol and lactate in 7 days, while hyperglycemia persisted 7 days more. Rainbow trout, as a carnivorous fish, has limited capability to digest fiber (Skrede et al. 2002; Stone 2003, Krogdahl et al. 2005; Couto et al. 2016), which will explain why plasma glucose levels increased significantly with the inclusion of barley in the diet. During any type of stress, cortisol levels can reach up to more than 100 ng mL^{-1} and later drop to 10–20 ng mL^{-1}, their basal level (Flores-Quintana 2002). Changes in cortisol levels during hypoxia produced a hyperglycemia due to glucogenolysis and gluconeogenesis pathways (Hemre et al. 2002). Changes in cortisol and glucose plasma levels occurred at different kinetics (Mommsen et al. 1999); that is why the hyperglycemia

persisted for 14 days while basal cortisol levels were reached in 7 days. Lactate is produced by glucose from anaerobic glycolysis, and as glucose, it incremented significantly at the time stress occurred, but recovered basal levels in 7 days. Hemre (1992) reported in the case of Atlantic cod, that even 96 h after transport stress, sustained hyperglycemia was detected only in fish adapted to high dietary starch levels, while adaptation to a low starch diet resulted in a lower glucose peak coupled with a shorter recovery period to establish basal levels. This adaptation also influenced muscle and liver ability to regulate plasma glucose levels after peaking, assuming that the space for glycogen storage can be modified by an adaptation diet, in agreement with studies on glucose space in halibut (*Hippoglosus hippoglossus*) (García-Riera and Hemre 1996) and Atlantic salmon (*S. salar*) (Hemre and Krogdahl 1996).

Proximate composition values were similar to those reported by other authors (Yildiz 2004; Popelka et al. 2014). Substituting wheat for barley did not significantly affect proximate composition of rainbow trout flesh, results that were in accordance with those reported by Sealey et al. (2008). Lower a_w values were observed on fish fed with diets at high barley concentration (319B) at the end of the experimental growth period. The reduction of a_w would help to reduce lipid oxidation process and microbial growth. For this reason, the incorporation of barley seems to have a positive effect on shelflife.

The appearance of food products is of major importance to consumers, both from the acceptability and preference point of view. The color of rainbow trout is generally considered as one of the most relevant quality parameters. Therefore, color plays a decisive characteristic during quality evaluation of the product at the point of sale (Ortiz et al. 2013). No significant differences were observed despite barley concentration at different diets. These results differed from the studies obtained from sensory analysis where in the QDM analysis it was observed that when fish reached commercial weight, fish fed with 319B diets were significantly redder than fish fed with control diets.

Fish muscle texture is based on many intrinsic biological factors such as collagen or fat content. Some autolytic enzymes and microbiological effects could be induced to degradation, which made muscles less elastic and softer (Asghari et al. 2014; Xu et al. 2015). Casas et al. (2006) reported cohesiveness as a parameter to measure muscle elasticity since it describes the ability of the muscle to recover from deformation and its resistance to subsequent deformation. If cohesiveness is <1, the deformation suffered by the first compression is partly irrecoverable. In the present samples, the deformation along the experimental growth period was <1 for every experimental diet. Different sections of the fish flesh were also studied (front, middle, and tail). Results showed the tail region to be the stiffest part of the flesh also in accordance with the results obtained by Casas et al. (2006).

Lipid oxidation of fish meat was measured through TBARS indicators. Lakshmanan (2000) proposed a range of 1–2 mg malonealdehyde per kilogram of sample as the limit of acceptability, when TBARS index is above this value it affects to the fish. At the end of the experimental period, TBARS index was between the range proposed by Lakshmanan (2000) and fish fed with 80B and diets higher in barley concentration reached those TBARS index levels. Trout fed with diets higher in barley concentration had a lower level of TBARS in meat than those obtained from trouts fed with lower barley concentrations diets. This decrease on the TBARS index was correlated with the lower water activity of fish fed with diets higher in barley concentrations at the end of the experimental growth period, which probably reduced microbial and enzymatic activity and probably with a positive effect of different compounds of barley which act as endogenous antioxidants.

Barley is a cereal with bioactive components, not only β-glucans but also phenolic acids, polyphenols, and non polar compounds such as tocols that can enhance growth and quality parameters; however, with the obtained data, we cannot claim those improvements to be associated to the combined effect of all of these components or just to one of them, and so further studies should be done to evaluate the cause of those beneficial effects on rainbow trout. β-Glucans are potential immunostimulant components; thus, some immunological studies should be carried out to explain their efficiency in growth and quality parameters.

Conclusions

Results indicated that wheat can be substituted by barley without any significant detrimental effect on rearing parameters and with a positive enhancing effect on fish quality, lower water activity values, as well as an enhancement in textural and color properties, were observed in fish fed with the diet containing the highest barley concentration. Trout fed feed with higher concentrations of barley showed lower lipid oxidation levels than those fed with lower concentrations. The sensory panel found that fish fed with diets higher than 8% in barley content exhibited a brighter red color in gills and a better texture; also, filet color became redder with a higher barley inclusion, being all these sensory parameters correlated with fish freshness. Considering the total of the results obtained and taking into account that the product quality (fish flesh) is a balance between rearing parameters (fish health) and quality of fish (fish flesh) is considered that barley concentrations of 31.9 g kg^{-1} is a suitable concentration to achieve this balance.

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