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Complete List of Authors:	Gai, Francesco; CNR ISPA, Peiretti, Piergiorgio; CNR ISPA Brugiapaglia, Alberto; University of Torino, Department of Agriculture, Forestry, and Food Sciences Gasco, Laura; University of Turin,

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Effects of Dietary Protein Source and Feeding Regime on Growth Performance, Nutrient Digestibility, Fatty Acids and Fillet Quality Traits in Rainbow Trout (*Oncorhynchus mykiss*)

FRANCESCO GAI,[1] PIER GIORGIO PEIRETTI

Institute of Science of Food Production, National Research Council, Grugliasco, Italy

ALBERTO BRUGIAPAGLIA, LAURA GASCO

Department of Agriculture, Forestry, and Food Sciences, University of Torino, Grugliasco, Italy

[1]**Corresponding Author**: Francesco Gai; Institute of Science of Food Production, National Research Council, Largo Braccini 2, Grugliasco (TO), Italy, telephone: +39 0116709232; fax: +39 0116709297; e-mail address: francesco.gai@ispa.cnr.it

Abstract

The aim of this study was to investigate the effects of feeding regime and of bacterial protein meal (BPM), pea protein concentrate (PPC) and a mixture thereof (MIX) compared to a control fish meal-based diet on growth performance, nutrient digestibility, fatty acid (FA) profile and fillet quality traits in rainbow trout. A stock of 1200 juvenile rainbow trout were individually weighed (mean weight 114.6±0.2 g) and randomly distributed into 24 fibre-glass tanks (4 diets x 3 replications x 2 feeding regimes). Statistical differences appeared among the diets in terms of crude protein digestibility, while no differences appeared for dry matter, ether extract and gross energy digestibility. Growth performance and somatic indexes were significantly affected by the diet effect, while only the condition factor was influenced by the feeding rate effect. None of the parameters appeared to be affected by the interaction effects. Differences appeared between the FA profiles of the dorsal muscle. Oleic, linoleic, α-linolenic, and docosahexaenoic acid contents were influenced by diet, while only minor FAs were influenced by feeding regime. Consumer tests showed that fillets of trout fed the MIX diet <u>ad libitum</u> were the most preferred. A similar ranking was obtained with the trout fed rationed diets.

The use of alternative protein sources in aquaculture as fishmeal substitutes is an extensively studied subject (Bakke-McKellep and Refstie 2008; Médale and Kaushik 2008), since fishmeal will be a limited resource for fish feedstuff production in the future. In the past decade, in fact, a great deal of research into aquaculture nutrition has dealt with fishmeal and fish oil substitution with alternative sources. Various microbes (algae, fungi and bacteria) have been used to produce a wide range of single cell protein varieties (Anupama and Ravindra 2000). They can be used for fish or shellfish as a substitute for fish meal (4-5% substitution) and have been investigated as a feed ingredient in diets for rainbow trout (Øverland et al. 2006; Aas et al. 2006), Atlantic halibut (Aas et al. 2007), and Atlantic salmon (Storebakken et al. 2004).

Among the protein concentrates derived from fermentation bacteria, noted for its use as an attractant in fish food and shellfish, Protorsan is a bacterial protein meal (BPM) which is a by-product of fermentation conducted by Corynebacterium melassecola that led to the production of L-glutamic acid fermentation carried out using plant substrates, usually from beet molasses and/or starch hydrolysates. Fermentation takes place anaerobically, under optimal pH and temperature conditions for the growth of Corynebacterium melassecola, for about 36 hours, followed by heat treatment of fermentation broth at 75 C for 30 minutes to deactivate the bacteria. The bacterial mass is then separated from the liquid phase by centrifugation and subjected to washing and drying. The resulting product is used for animal feed. Protorsan contains 12% L-glutamic acid, around 7-7.5% of total nucleotides and 4.5% betaine, a methyl donor with high palatability. It also contains high levels of peptidoglycan as components of the bacterial cell wall. Protorsan has been tested as a feed stimulant in diets for sea bream (Chatzifotis et al. 2009), while no studies have been performed in rainbow trout (Oncorhynchus mykiss).

As far as alternative protein sources are concerned, the best growth performances have been achieved using plant protein concentrates and plant protein mixture (De Francesco et al. 2004, 2007). Among the plant protein sources, field peas (Pisum sativum) have reported some success for different fish species such as Atlantic salmon (Øverland et al. 2009), rainbow trout (Thiessen et al. 2003), hybrid sturgeon (Sicuro et al. 2012), common carp (Davies and Gouveia 2010), gilthead seabream (Sánchez-Lozano et al. 2009, 2011), sea bass (Tibaldi et al. 2005, Tulli et al. 2007), African catfish (Davies and Gouveia 2008), and Nile tilapia (Schulz et al. 2007).

Øverland et al. (2009) showed that 20% air-classified pea protein concentrate (PPC) could replace 20% of high-quality fish meal protein in feed without any adverse effect on growth performance, carcass composition or distal intestine histology in Atlantic salmon. By contrast, in another study, as PPC at high inclusion levels was shown to induce enteropathy in the distal intestine of Atlantic salmon, the authors concluded that caution should be used when including PPC in formulated feeds for Atlantic salmon (Penn et al. 2011).

Feeding PPC has been reported to support acceptable weight gain, feed intake, and feed conversion in both Atlantic salmon (Carter and Hauler 2000) and rainbow trout (Thiessen et al. 2003).

The aim of this study was to investigate the effects both of BPM, PPC and a mixture thereof compared to a control fish meal-based diet and of feeding regime on growth performance, nutrient digestibility, fatty acid (FA) profile and fillet quality traits in rainbow trout (Oncorhynchus mykiss).

Materials and Methods

Experimental plan

Three experimental diets were obtained by including BPM, PPC or a mixture of both protein concentrates (MIX), respectively, replacing fish meal. These experimental diets were tested against a control fish meal (FM)-based diet; all the diets were isonitrogenous (CP 45 %) and isoenergetic (22 MJ/kg DM).

The feeds were manufactured in the laboratory at the Experimental Station of the Department of Agriculture, Forestry, and Food Sciences of the University of Torino by means of a pelleting process using a 3.5 mm diameter. Pellets were dried in a stove overnight at 50 C and then refrigerated at 6 C until utilization.

Digestibility trial

A stock of juvenile rainbow trout was obtained from a private hatchery (Bassignana, Cuneo, Italy) and transferred to the facility at the Department of Agriculture, Forestry, and Food Sciences at the University of Torino. An in vivo digestibility experiment was performed in order to determine the apparent digestibility coefficient (ADC) of the diets following the experimental design adopted in a previous study reported by Palmegiano et al. (2006). The ADCs were measured using the indirect acid-insoluble ash (AIA) method; 1% celite® (Fluka, Switzerland) was added to the diets as an inert marker. The faeces were collected from each tank using a continuous automatic device, as reported by Palmegiano et al. (2006), six days per week. The faeces were collected daily and frozen (-20 C) for three consecutive weeks. The faeces were then dried in a stove in order to determine the dry matter (DM) content.

The ADC of the DM was calculated as follows:

$$ADC_{DM}\ (\%) = (1\text{-}A/B) \times 100$$

in which A and B represent the AIA concentrations in the feed and faeces, respectively.

The ADCs of the crude protein (CP), ether extract (EE) and gross energy (GE) were calculated as follows:

$$ADCs (\%) = [1-(A/B) x (SB/SA)] x 100$$

in which SA and SB represent the CP, EE or GE concentrations in the feed and faeces, respectively.

Growth trial

A selection of 1200 juvenile rainbow trout (initial mean body weight 114.6±0.2 g) were individually weighed to obtain a homogeneous stock of fish and randomly distributed into 24 fibre-glass tanks (0.5 m^3) supplied by an open-water circuit with a water flow rate of 25 l/min and a temperature of 13 ± 1 C while dissolved oxygen was 7.0 ± 0.5mg/l.

The adopted experimental design was balanced, bi-factorial with four diets x three replicates x two feeding regimes (4x3x2). The feeding trial lasted 77 days, after a 2-week period of acclimatisation to the tanks and diets. The feedstuff was distributed by hand, 6 days per week, twice a day with a daily feeding rate of 1.4% of the wet biomass or ad libitum, respectively. Feed intake was checked each time and no feed reject events were recorded during the trial. The biomass tanks were weighed in bulk every 15 days, in order to update the daily feeding rate.

Sampling and chemical analysis

At the end of the feeding trial, the fish were starved for one day, then the fish tanks were weighed for final mean body weight. In order to determine the somatic indexes, five trout per tank, with a body weight close to the mean body weight, were sampled and killed. The gut and liver were separated from the rest of the body and weighed. The dorsal muscle tissues from the same fish body were sampled and frozen until the subsequent chemical determinations. The diet and fish muscle samples were freeze-dried before analysis. All the diets were analyzed to determine proximate composition and AIA concentration according to standard methods (AOAC 1990). GE content was determined using an adiabatic calorimetric bomb (IKA C7000, Staufen, Germany).

Gas-chromatographic analysis of the fatty acids

FA composition was determined on the diets and fish flesh samples. The lipid extraction of the samples was performed according to Peiretti and Meineri (2008); the extract was expressed as crude fat and used for the trans-methylation of the FAs. The FA methyl esters in hexane were then injected into a gas chromatograph (Dani Instruments S.P.A. GC1000 DPC; Cologno Monzese, Italy) equipped with a flame ionization detector (FID). The separation of the FA methyl esters was performed using a Famewax™ fused silica capillary column (30m×0.25mm [i.d.], 0.25 µm) (Restek Corporation, Bellefonte, PA, USA). The peak area was measured using a Dani Data Station DDS 1000. Each peak was identified and quantified on the basis of pure methyl ester standards (Restek Corporation, Bellefonte, PA, USA).

pH and Color flesh measurements

pH (pH_{24}) was measured on muscles by means of a Crison MicropH 2001 (Crison Instruments, Barcelona, Spain) equipped with a combined electrode and an automatic temperature compensator.

The flesh colour measurements were taken on the inside fillet portion using a bench colorimeter Chroma Meter CR-400 Konica Minolta Sensing (Minolta Sensing Inc, Osaka, Japan) in the CIELAB colour space (CIE 1976). The lightness (L*), redness (a*) and yellowness (b*) were recorded. Three readings were taken on each portion of the fillet and averaged.

Consumer tests

A sensory panel of 36 untrained PhD students and staff members from the campus of the University of Torino and of the Italian National Research Council of Torino, 21 males and 15 females, ranging in age from 25 to 60 years, participated in this study. Panelists were regular consumers of fish flesh and were already involved in surveys on fish flesh preference/acceptability tests. Consumer tests were carried out in 6 distinct evaluation sessions over three days in the Sensory Evaluation Facility of the Department of Agriculture, Forestry and Food Science of Torino. In each session, a preference ranking test was performed to evaluate the preference of cooked fillets from trout fed with the four experimental diets offered ad libitum or rationed. Between sessions, panelists took a 15 min break. Sixteen trout (two fish from each diet), homogeneous for size and weight, were filleted. The fillets were wrapped in aluminum foil and cooked without additives in an air convection oven at 200 C until the core temperature reached 70 C (about 15 min). After cooking, the fillets were cut into equal portions.

Each panelist received four warm samples corresponding to the 4 diets. Samples were labelled with three-digit numbers, and were offered using a Williams design to balance the order of presentation (MacFie et al. 1989).

Panelists were asked to rank the samples from trout fed with the four diets in order of preference (most preferred =1; least preferred =4). Tap water was offered to the panelists to rinse their mouths between samples.

Statistical analysis

Statistical analyses were performed using the SPSS software package (version 11.5.1 for Windows, SPSS Inc., USA). Growth performance, FA profile and fillet quality traits were analysed by two-way ANOVA by considering dietary protein source, feeding regime and their interaction as the main effects. The data were presented as the means for each group, together with the significance levels of the main effects and interactions.

Analysis of variance was used to evaluate the diet effect on ADC. These data were presented as the means for each group and the standard deviation (SD). Significance was established at $P < 0.05$ for all data.

The results of the sensory analysis were analysed by Friedman's test. The Friedman rank sum was performed to determine whether the panellists were able to discriminate between samples. Then, the least significant ranked difference values were calculated to ascertain which samples were significantly preferred to the others (Meilgaard et al. 1991). The ranking data were analysed by box-plots and correspondence analysis.

Results and Discussion

Composition and fatty acid profile of the diets

The ingredients and chemical composition of the four diets are shown in Table 1, while the FA patterns for the four experimental diets are reported in Table 2.

The experimental diets were similar as concerned CP, crude fibre and GE, while the dietary concentration of EE was lower in the BPM diet than in the other diets. Ash and nitrogen-free extracts were higher and lower, respectively, in the FM diet.

The concentration of crude fat in the bacterial protein resembles that of fish meal, while the composition of the lipid is different (Storebakken et al. 2004). Phospholipids are the main lipid components of bacterial protein, consisting mainly of phosphatidylethanolamine and phosphatidylglycerol (Müller and Skrede, 2003) with predominantly SFA and MUFA and no PUFA.

The experimental diets showed a similar FA profile with slightly high values of C20:5n-3 (EPA) and C22:6n-3 (DHA) in the FM diet. This diet also showed a slightly higher saturated FA (SFA) and PUFA content than the other diets, while the lowest content of MUFA was found in the MIX diet.

Digestibility of the experimental diets

As far as digestibility is concerned (Table 3), statistical differences appeared among the diets for CP, while no differences appeared for DM, EE and GE. The lowest CP digestibility coefficients were recorded in the BPM and MIX groups, both fed diets containing the bacterial protein meal. Similar ADC of nitrogen was found in studies carried out by Storebakken et al. (2004) and Øverland et al. (2006) in Atlantic salmon and rainbow trout, respectively. These authors recorded lower nitrogen digestibility with increasing BPM inclusion compared to a fish meal-based diet, with values of 87% and 83% in fish fed diets containing 193 and 147 g of bacterial protein meal per kg of diet, respectively. In contrast, even though this was lower than fish meal-based diets, higher values (91 and 88%) of ADC of nitrogen were found in trials with rainbow trout and Atlantic halibut fed diets containing 270 and 180 g of bacterial protein meal per kg of diet, respectively (Aas et al. 2006, 2007). The lower nitrogen digestibility values recorded in the BPM and MIX groups of this study and in the other trials utilising bacterial protein meal diets, could be due to a negative effect of bacterial membrane and cell wall components on protein digestibility, as observed in previous studies in rainbow trout fed single-cell proteins from brewer’s yeast (Rumsey et al. 1991; Kiessling and Askbrandt 1993). Burel et al. (2000) found that extruded peas showed lower protein digestibility in trout (88%) than in turbot (92%).

Growth performance and somatic indexes

As far as the growth performance traits and the somatic indexes reported in Table 4 are concerned, all the parameters investigated were significantly affected by the diet effect while only the condition factor (CF) was influenced by the feeding rate effect. None of the parameters showed to be affected by the interaction between diet and feeding rate effect.

Compared to the FM group, the trout fed alternative protein source diets had lower weight gain and specific growth rates (SGR). Similar results were found by de Francesco et al. (2004) in a long-term feeding study where large rainbow trout were fed with a plant protein mixture-based diet. In contrast, de Francesco et al. (2007) found similar weight gain and SGR in gilthead sea bream fed with a plant protein high-level fish meal replacement diet. In our trial, trout fed alternative protein source diets were characterised by a higher viscero- and hepato-somatic index but a lower carcass yield. A decrease in dressed carcass and fillet yield was also observed in a study carried out in trout fed plant proteins and guar gum as fish meal replacements (Brinker and Reiter, 2011).

A similar effect on CF was observed in a study carried out in Atlantic salmon fed to satiation or moderately reduced rations of high or low energetic feeds, in which the rationed fish showed the lowest CF values (Johnsen et al. 2011).

<u>Fatty acid profile of the fillet</u>

While it is common to see changes in the FA profile when dietary fat is modified through changes in dietary lipid sources, there is little information as regards the effects of changes in FA content as affected by dietary protein sources. Indeed, total replacement of fish meal by plant protein ingredients modifies FA profiles to a certain extent with the consequent changes seen in muscle FA profiles (Tables 5 and 6). As far as FA composition of different fillets is concerned, C18:1n-9, C18:2n-6, C18:3n-3, and DHA contents and some minor FAs (such as C15:0, C16:2n-4, C17:0, C16:3n-4, C18:3n-6, C20:1n9, C20:4n-6, C21:0 and C22:1n-9) were influenced by diet, while only minor FAs (such as C16:1n-9, C16:2n-4, C16:3n-4, C18:3n-4, C20:1n9 and C22:1n-9) were influenced by feeding regime (Table 5). An interaction was found only for two minor FAs (C16:2n-4 and C22:1n-9).

In common with other studies on rainbow trout (De Francesco et al. 2004; Morris et al. 2005) the FA content of trout muscle in the present study was significantly influenced by that of the feed. In fact, it is well known that dietary FA composition strongly influences flesh FA composition in fish (Sargent et al. 2002). As shown by Palmegiano et al. (2006), who evaluated the use of rice protein concentrate as a potential substitute of fish meal in rainbow trout, fillet FA profile reflects diet composition, but some FAs are not present in the same proportion and this induces one to suppose that an elongation and desaturation process has occurred. Numerous FAs were present at higher proportions in the flesh lipids than in the feeds, including C18:1n-9 and DHA. However, C18:2n-6, C18:3n-3 and EPA were all present at lower relative percentages in the flesh than in the feeds. Preferential accumulation and/or retention of selected FAs, including C18:2n-6, C20:4n-6 and DHA, has previously been recorded in rainbow trout (Greene and Selivonchick 1990).

In the present study, MUFA and PUFA content (total PUFA, PUFA n-3, PUFA n-6 and their ratio) were influenced by diet treatment, while only MUFA content was influenced by feeding regime without interaction between factors (Table 6), as previously demonstrated in rainbow trout fed with a plant protein mixture-based diet (De Francesco et al. 2004). They reported that SFA, MUFA and PUFA n-3 and the n-3/n-6 ratio were significantly higher in trout fed diet based on fish meal, while PUFA n-6 (above all in C18:2n-6) were significantly higher in trout fed diet based on mixture of plant protein sources (corn gluten meal, wheat gluten, extruded peas, and rapeseed meal), while the main difference observed in single FA was the higher incidence and content of C18:3n-3 in fillet of trot fed diet based on mixture of plant protein sources in comparison to those fed the fish meal, no differences were found for eicosapentaenoic (EPA) and the docosahexaenoic (DHA) acid levels. This result was consistent with the data obtained by Gomes et al. (1993), who observed an increased level of PUFA n-6, in particular of C18:2n-6, in muscle of rainbow trout fed diets with increasing levels (5%, 10%, 15% and 45%) of a co-extruded plant protein (rapeseed and peas). It is reassuring to note that at the same lipid level, EPA and DHA were no different between the two groups of trout.

Morris et al. (2005) reported that, with the exception of five individual FAs (C16:2n-6; C20:4n-6; C20:4n-3; DHA and C24:1n-9), the FA profile of the rainbow trout flesh responded linearly to changing proportions of individual FAs in the feed formulated with extracted soya (7.5%) and full-fat soya (0–25%). Although the percentage of DHA and PUFA n-3 in the fillet was not significantly influenced by the level of soya in the feeds, the relative proportions of the fish and soybean derived PUFA n-3 shifted towards the latter, i.e. higher relative percentages of C18:3n-3, in response to a higher proportion of soya-derived fat in the feeds.

The challenges of creating new plant-based feed ingredients for salmonid diets are providing high-quality protein and providing a source of PUFA n-3 (Drew et al. 2005). Fish oil is the most widely-used source of PUFA n-3, required by salmonids to maximize growth potential and maintain the PUFA n-3 content of the fish carcass desired by consumers. Rainbow trout can elongate and desaturate C18:3n-3 into EPA and DHA (Owen et al. 1975), but most plant oils are poor sources of C18:3n-3.

Fillet quality traits

Parameters of fillet pH and colour are reported in Table 7; all the parameters investigated were significantly affected by the treatments except redness for the diet effect and yellowness for the feeding rate effect, respectively. None of the parameters appeared to be affected by the interaction between diet and feeding rate effect.

The fillet pH level after 24h was slightly higher for fish fed BPM and MIX diets while similar values were found for the FM and PPC groups. Brinker and Reiter (2011) observed a reduction in pH 24h post mortem in fillets of trout fed a mixture of fish meal and plant protein-based feeds and they attribute the observed reduction in pH to the differences in fillet energy stores. The same authors found that the pure plant diet appears to increase undesirable yellowness in the trout fillets, in agreement with the results of the present trial where we found a similar trend in fish fed the PPC and MIX diets.

In our trial, the feeding rate modified the redness values of the trout fillets with the higher values recorded in rationed fish while satiation feeding induced higher red colour intensity (a*-value) compared to restricted feeding in a study carried out in Atlantic salmon (Johnsen et al. 2011). The same authors did not find any differences in the lightness values, in contrast to the findings from the present trial where lightness values decreased in fillets from the rationed groups.

Fillet composition in terms of DM, CP, EE, and ash content is reported in Table 8. The results for fillet composition showed that only CP content was affected by the diet effect with an increased content in fish fed fish meal-alternative protein sources. A similar increase was also observed by De Francesco et al. (2004) in large rainbow trout fed with plant protein mixtures in replacement of fish meal. The same effect was also reported in rainbow trout fed diets where 25, 50, 75, or 100% of the fish meal protein was replaced with a mixture of rendered animal protein ingredients (Lesiow et al. 2009). DM and ash content were affected by feeding rate effect, fillets from rationed fish groups showed an increased content of DM and a decreased ash content compared to ad libitum groups.

Consumer tests

The results of the preference ranking test concerning the trout fillets fed ad libitum with the four diets are reported in Fig. 1. Fillets of trout fed the MIX diet ad libitum obtained the highest number of most preferred votes as well as the best median value (Fig. 2). Fillets of trout fed the FM diet ad libitum obtained the largest number of least preferred and the smallest number of most preferred votes. Fillets of trout fed the BPM and PPC diets ad libitum had the majority of votes in 2^{nd} and 3^{th} preference votes, 75% and 53% respectively.

The Friedman's test showed that there was a significant difference ($P \leq 0.05$) in preference between the fillets (Table 9). Fillets of trout fed the MIX diet ad libitum were the most preferred (rank sum = 72), followed by the BPM diet ad libitum (rank sum = 89), PPC diet ad libitum (rank sum = 90) and FM diet ad libitum (rank sum = 109).

A similar ranking was obtained with the rationed trout, although no significant difference in preference between the four fillets was observed (Table 9).

Fillets of trout fed the rationed MIX diet obtained the highest number of most preferred votes (Fig.3). Fillets of trout fed the rationed PPC diet obtained the same number of most and least preferred votes. The rationed BPM diet had the majority of 2^{nd} preference votes while the rationed FM diet received the largest number of least preferred votes. The rationed FM and PPC diets showed the worst median values (Fig. 2).

The overall results can be represented in the correspondence analysis plot (Fig. 4). Dimensions 1 and 2 explain 58% and 39% of the inertia, respectively.

Fillets of trout fed the MIX diet ad libitum and 1^{st} preference votes, and, fillet of trout fed the rationed FM diet and 4^{th} preference votes, showing the highest deviation from the origin, gave the main contribution to the inertia of dimension 1 and dimension 2, respectively. According to the distribution of samples in the plane, it can be seen that the samples preferred by the consumers are found to the right in the plot and near the 1^{st} preference votes. The next two groups, rationed BPM diet and BPM diet ad libitum, are found on the left near the 2^{nd}

preference votes. Less- preferred fillets were from FM diet ad libitum and rationed FM diet near the 4th preference votes, while rationed PPC diet and PPC diet ad libitum, near the 3th preference votes, are found between the 1st and 4th preference votes.

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TABLE 1. Ingredients and proximate composition of experimental diets.

Diets	FM	BPM	PPC	MIX
Ingredients (%)				
Herring fish meal[a]	50.00	25.00	25.00	25.00
Protorsan[b]	0.00	25.00	0.00	12.50
Pea protein concentrate[c]	0.00	0.00	30.00	15.00
Corn meal	23.00	24.00	12.00	17.50
Fish oil	10.00	11.00	12.50	12.00
Corn gluten	8.00	6.00	12.00	9.00
Lignum sulphate	6.00	6.00	6.00	6.00
Mineral mixture[d]	1.50	1.50	1.25	1.50
Vitamin mixture[e]	1.50	1.50	1.25	1.50
Proximate composition (%DM)				
Dry matter (% fresh matter)	96.6	92.5	95.9	96.1
Crude protein	45.4	45.9	45.2	45.2
Ether extract	17.3	14.6	16.5	17.0
Ash	12.0	8.9	9.2	9.2
Crude fiber	1.9	2.0	1.9	1.9
Nitrogen free extracts[f]	23.4	28.6	27.2	26.7
Gross energy (MJ/kg DM)[g]	21.5	21.8	22.2	22.1

[a] Mangimi Monge, Torre San Giorgio, Italy: DM 91.2%, CP 69%, EE 8.2%, ash 9.6%, CF 0.5%.

[b] Mazzoleni Prodotti Zootecnici, Cologno al Serio, Italy: DM 92%, CP 67%, EE 6%, ash 3.8%, CF 1%.

[c] AgriMarin Nutrition, Stavanger, Norway: DM 90%, CP 55%, starch 9%, EE 2%, ash 6%.

[d] Mineral mixture (g or mg/kg diet): bicalcium phosphate 500 g, calcium carbonate 215 g, sodium salt 40 g, potassium chloride 90 g, magnesium chloride 124 g, magnesium carbonate 124 g, iron sulphate 20 g, zinc

sulphate 4 g, copper sulphate 3 g, potassium iodide 4 mg, cobalt sulphate 20 mg, manganese sulphate 3 g, sodium fluoride 1g, (Granda Zootecnica, Cuneo, Italy).

[e] Vitamin mixture (IU or mg/kg diet): DL-a tocopherol acetate, 60 IU; sodium menadione bisulphate, 5 mg; retinyl acetate, 15000 IU; DL-cholecalciferol, 3000 IU; thiamin, 15 mg; riboflavin, 30 mg; pyridoxine, 15 mg; B12, 0.05 mg; nicotinic acid, 175 mg; folic acid, 500 mg; inositol, 1000 mg; biotin, 2.5 mg; calcium panthotenate, 50 mg; choline chloride, 2000 mg (Granda Zootecnica, Cuneo, Italy).

[f] Calculated as 100-(%Crude protein +%Ether extract +%Ash +%Crude fiber).

[g] Determined by calorimetric bomb.

521 TABLE 2. Dietary main fatty acid (% of total fatty acid) composition.

	FM	BPM	PPC	MIX
C14:0	4.70	4.66	4.19	4.56
C15:0	0.34	0.32	0.30	0.32
C16:0	13.39	13.15	12.08	13.00
C16:1n-9	0.20	0.21	0.18	0.21
C16:1n-7	5.13	5.23	4.79	5.17
C16:2n-4	0.62	0.23	0.15	0.15
C17:0	0.27	0.24	0.24	0.24
C16:3n-4	0.69	0.69	0.71	0.61
C17:1n-7	0.18	0.17	0.19	0.22
C18:0	3.57	3.27	3.14	3.15
C18:1n-9	19.53	21.90	20.79	22.09
C18:1n-7	2.86	2.81	2.70	2.81
C18:2n-6	9.56	10.85	11.03	11.20
C18:3n-6	0.21	0.23	0.34	0.19
C18:3n-4	0.16	0.18	0.52	0.18
C18:3n-3	2.36	2.62	2.99	2.83
C18:4n-3	1.66	1.69	1.70	1.71
C20:0	0.26	0.26	0.25	0.30
C20:1n-9	2.24	2.49	2.55	2.66
C20:2n-6	0.44	0.48	0.49	0.47
C20:3n-3	0.14	0.15	0.41	0.11
C20:4n-6	0.67	0.59	0.62	0.62

C21:0	0.22	0.23	0.24	0.11
C20:4n-3	0.81	0.85	0.81	0.82
C20:5n-3	9.55	9.00	8.51	8.73
C22:1n-9	1.68	1.90	1.87	1.90
C22:2n-6	0.45	0.43	1.69	0.70
C22:5n-3	1.91	1.88	1.79	1.84
C22:6n-3	11.01	8.80	9.02	8.80
SFA[a]	22.74	22.14	20.44	21.69
MUFA[b]	31.83	34.72	33.07	35.05
PUFA[c]	39.81	38.24	39.11	38.27
PUFA n-3[d]	27.44	24.99	25.23	24.85
PUFA n-6[e]	11.34	12.58	14.19	13.17
n-3/n-6	2.42	1.99	1.78	1.89

[a] Saturated fatty acids.

[b] Monounsaturated fatty acids.

[c] Polyunsaturated fatty acids.

[d] Polyunsaturated fatty acids serie n-3.

[e] Polyunsaturated fatty acids serie n-6.

528 TABLE 3. Apparent digestibility coefficients (%) of nutrients and gross energy of the
529 experimental diets (means ± SD; n=9).

Diets	FM	BPM	PPC	MIX
Dry matter	69.97±0.67	69.23±0.25	70.33±1.29	67.33±1.92
Crude protein	90.30±0.28[a]	84.20±0.28[b]	88.90±0.28[a]	85.40±1.13[b]
Ether extract	97.35±0.78	96.90±0.28	95.70±0.28	96.35±0.21
Gross energy	79.93±1.62	77.00±2.26	78.50±3.04	74.87±2.97

530 In the row, different letters mean statistical difference at P≤0.05.

TABLE 4. Growth performance, feed utilization and whole body composition (n=5) in rainbow trout fed experimental diets.

	ad libitum				Rationed				Significance		
	FM	BPM	PPC	MIX	FM	BPM	PPC	MIX	Diet effect	F.R. effect	Interaction
WG[a]	113.5	39.5	99.4	102.5	100.4	40.6	99.8	91.8	0.000	0.082	0.250
SGR[b]	0.90	0.40	0.80	0.80	0.85	0.40	0.80	0.77	0.000	0.360	0.815
PER[c]	1.63	0.67	1.75	1.63	1.60	0.80	1.77	1.57	0.000	0.790	0.459
FCR[d]	1.40	3.53	1.25	1.33	1.40	2.70	1.23	1.40	0.000	0.246	0.213
VSI[e]	10.8	12.4	11.7	13.0	9.8	10.8	11.5	12.3	0.025	0.095	0.790
HSI[f]	1.02	1.17	1.08	1.40	1.04	1.13	1.20	1.25	0.007	0.803	0.418
CF[g]	1.20	1.15	1.26	1.26	1.09	0.99	1.18	1.22	0.002	0.005	0.577
CY[h]	88.2	86.4	87.2	85.6	89.1	88.1	87.3	86.5	0.015	0.107	0.774

[a] Weight gain, (g) =[FBW (final body weight, g) −IBW (initial body weight, g)].

[b] Specific growth rate (%) =[(lnFBW−lnIBW)/number of feeding days]*100.

[c] Protein efficiency ratio =[WG (weight gain,g)/total protein fed (g DM)].

[d] Feed conversion ratio=[total feed supplied (g DM)/WG (weight gain, g)].

[e] Viscerosomatic index, (%) =[gut weight (g)/ fish weight (g)]*100.

[f] Hepatosomatic index, (%) =[liver weight (g)/ fish weight (g)]*100.

[g] Condition factor, (%) =[fish weight (g)/ fish length $(cm)^3$]*100.

[h] Carcass yield, (%) =[carcass weight (g)/ fish weight (g)]*100.

TABLE 5. Fatty acids in fillets (n=5): composition (% of total fatty acid).

	ad libitum				Rationed				Significance		
	FM	BPM	PPC	MIX	FM	BPM	PPC	MIX	Diet effect	F.R. effect	Interaction
C14:0	3.35	3.33	2.94	3.47	3.17	3.11	3.15	3.23	0.375	0.374	0.523
C15:0	0.28	0.29	0.24	0.28	0.27	0.27	0.25	0.27	0.026	0.410	0.482
C16:0	14.60	14.70	14.06	15.06	14.56	14.34	14.82	14.50	0.618	0.783	0.085
C16:1n-9	0.25	0.24	0.24	0.26	0.14	0.25	0.23	0.19	0.332	0.032	0.136
C16:1n-7	4.28	4.20	3.83	4.39	4.01	4.06	4.01	3.94	0.590	0.231	0.484
C16:2n-4	0.27	0.21	0.39	0.41	0.21	0.15	0.14	0.17	0.000	0.000	0.000
C17:0	0.28	0.30	0.21	0.28	0.28	0.27	0.26	0.27	0.005	0.663	0.053
C16:3n-4	0.25	0.24	0.27	0.29	0.18	0.14	0.12	0.21	0.010	0.000	0.142
C18:0	3.29	3.26	3.14	3.37	3.20	3.40	3.21	3.33	0.279	0.805	0.611
C18:1n-9	19.23	20.06	18.96	20.30	18.19	19.57	17.59	19.95	0.022	0.082	0.849
C18:1n-7	2.80	2.68	2.54	2.05	2.68	2.64	2.53	2.63	0.300	0.514	0.387
C18:2n-6	12.01	13.79	16.13	12.88	12.41	14.71	15.82	13.11	0.001	0.603	0.904
C18:3n-6	0.27	0.34	0.35	0.29	0.30	0.29	0.34	0.31	0.005	0.714	0.117

C18:3n-4	0.34	0.38	0.40	0.37	0.29	0.28	0.29	0.31	0.731	0.000	0.576
C18:3n-3	1.69	2.11	1.93	2.00	1.70	2.05	1.71	1.91	0.000	0.068	0.400
C18:4n-3	0.99	1.14	0.95	1.05	0.93	1.04	1.00	1.04	0.101	0.441	0.586
C20:0	0.08	0.19	0.14	0.19	0.18	0.19	0.16	0.12	0.371	0.640	0.158
C20:1n-9	2.11	2.02	1.64	2.19	1.69	2.02	1.34	1.94	0.001	0.019	0.482
C20:2n-6	0.69	0.67	0.76	0.71	0.65	0.74	0.64	0.71	0.688	0.454	0.082
C20:3n-3	0.38	0.37	0.50	0.39	0.56	0.47	0.48	0.38	0.554	0.259	0.503
C20:4n-6	0.70	0.63	0.62	0.65	0.70	0.63	0.64	0.64	0.001	0.796	0.909
C21:0	0.21	0.21	0.18	0.22	0.15	0.17	0.04	0.21	0.006	0.003	0.162
C20:4n-3	0.95	0.93	0.91	1.01	0.96	0.92	0.84	0.85	0.299	0.049	0.188
C20:5n-3	5.59	5.77	5.17	5.59	5.48	5.39	5.56	5.44	0.749	0.727	0.479
C22:1n-9	1.04	1.13	0.76	1.10	0.82	0.29	0.21	0.28	0.000	0.000	0.001
C22:2n-6	0.46	0.48	0.34	0.40	0.91	0.38	0.37	0.38	0.210	0.466	0.366
C22:5n-3	2.15	2.11	2.07	2.11	2.15	2.03	2.24	1.98	0.293	0.810	0.139
C22:6n-3	18.60	15.44	17.38	16.31	19.77	16.08	18.32	17.46	0.013	0.189	0.993

TABLE 6. Relationship of fatty acids in fillets (n=5): composition (% of total fatty acid) to nutritional quality.

	ad libitum				Rationed				Significance		
	FM	BPM	PPC	MIX	FM	BPM	PPC	MIX	Diet effect	F.R. effect	Interaction
SFA[a]	22.09	22.28	20.90	22.87	21.82	21.73	21.90	21.91	0.147	0.504	0.126
MUFA[d]	29.71	30.34	27.97	30.30	27.52	28.82	25.91	28.94	0.049	0.019	0.971
PUFA[c]	44.87	44.12	47.84	44.05	46.30	44.92	48.16	44.51	0.004	0.299	0.945
PUFA n-3[d]	30.34	27.87	28.91	28.46	31.56	27.97	30.15	29.05	0.037	0.294	0.934
PUFA n-6[e]	14.13	15.91	18.21	14.93	14.96	16.76	17.82	15.14	0.002	0.536	0.868
n-3/n-6	2.18	1.76	1.64	1.91	2.15	1.69	1.70	1.93	0.003	0.952	0.962

[a] Saturated fatty acids.

[b] Monounsaturated fatty acids.

[c] Polyunsaturated fatty acids.

[d] Polyunsaturated fatty acids series n-3.

[e] Polyunsaturated fatty acids series n-6.

TABLE 7. Parameters of fillet pH (n=12) and colour (n=24) in rainbow trout fed experimental diets.

	ad libitum				Rationed				Significance		
	FM	BPM	PPC	MIX	FM	BPM	PPC	MIX	Diet effect	F.R. effect	Interaction
pH_{24}	6.56	6.76	6.58	6.63	6.65	6.74	6.67	6.75	0.000	0.000	0.063
L [a]	44.9	46.4	47.8	48.9	44.2	45.5	45.6	44.8	0.004	0.000	0.051
a [b]	1.14	1.84	1.00	0.84	1.84	1.83	1.33	1.84	0.066	0.007	0.234
b [c]	5.08	3.12	6.71	6.33	4.33	3.14	7.19	5.53	0.000	0.435	0.460

[a] Lightness.

[b] Redness.

[c] Yellowness.

555 TABLE 8. Composition (% dry matter) of fillets (n=5).

	ad libitum				Rationed				Significance		
	FM	BPM	PPC	MIX	FM	BPM	PPC	MIX	Diet effect	F.R. effect	Interaction
Dry matter[a]	24.1	23.0	23.4	24.5	25.9	24.3	26.9	24.8	0.480	0.017	0.451
Crude protein	89.7	92.3	90.3	90.2	88.5	92.0	88.1	88.9	0.021	0.085	0.836
Ether extract	8.0	14.5	10.8	8.6	9.8	7.7	10.1	10.6	0.440	0.357	0.018
Ash	6.7	7.6	7.5	7.3	6.3	6.5	6.1	6.8	0.056	0.000	0.076

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TABLE 9. Preferences of flesh expressed as Rank sums.

	ad libitum				Rationed			
	MIX	BPM	PPC	FM	MIX	BPM	PPC	FM
Rank	1	2	3	4	1	2	3	4
Rank sum	72a	89ab	90ab	109b	79	90	91	100

Rank sum of the preference ranking test for each trout flesh.

Rank sums with different superscripts indicate significant differences among treatments (P≤0.05).

FIGURE 1. Results from ranking test of fillets from trout fed <u>ad libitum</u> (where 1 = most preferred and 4 = least preferred). <u>Legend:</u> FM = Fish meal diet; BPM = Bacterial protein diet; PPC = Pea protein diet; MIX = Bacterial protein diet+Pea protein diet.

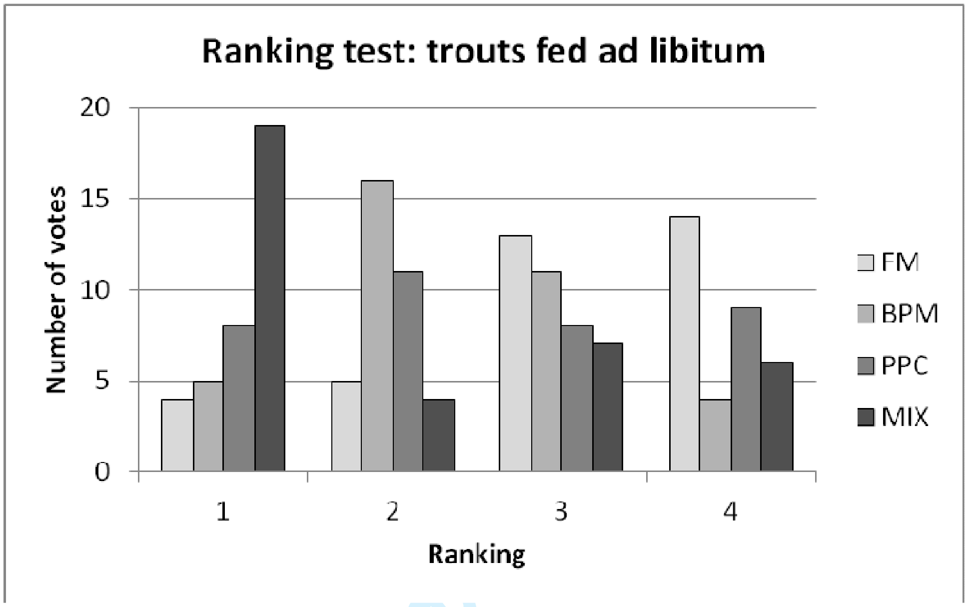
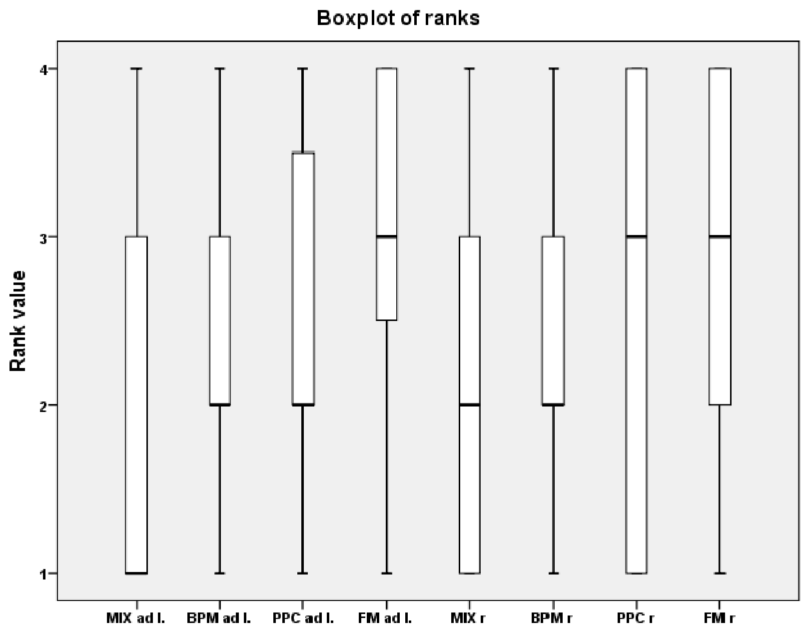


FIGURE 2. Box-plot of ranks. Legend: Feeding regime ad libitum: FM ad l. = Fish meal diet; BPM ad l. = Bacterial protein diet; PPC ad l. = Pea protein diet; MIX ad l. = Bacterial protein diet+Pea protein diet. Rationed feeding regime: FM r =Fish meal diet; BPM r =Bacterial protein diet; PPC r = Pea protein diet; MIX r = Bacterial protein diet+Pea protein diet.



573 FIGURE 3. Results from ranking test of fillets from trout fed rationed diets (where 1 = most preferred and 4 = least preferred). Legend: FM = Fish meal diet; BPM = Bacterial protein diet; PPC = Pea protein diet; MIX = Bacterial protein diet+Pea protein diet.

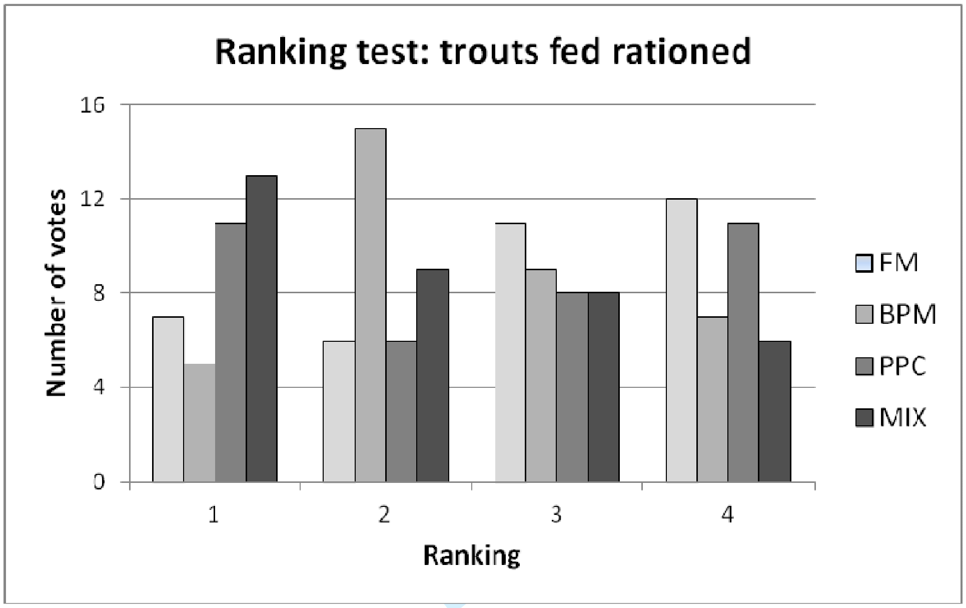


FIGURE 4. Correspondence analysis of sensory data. Position of the fillets of trout and ranks in the plane formed by the first two dimensions. . Legend: Feeding regime ad libitum: FM ad l. = Fish meal diet; BPM ad l. = Bacterial protein diet; PPC ad l. = Pea protein diet; MIX ad l. = Bacterial protein diet+Pea protein diet. Rationed feeding regime: FM r =Fish meal diet; BPM r =Bacterial protein diet; PPC r = Pea protein diet; MIX r = Bacterial protein diet+Pea protein diet.