
Effect of long-term feeding with a plant protein mixture based diet on growth and body/fillet quality traits of large rainbow trout (*Oncorhynchus mykiss*)

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Abstract:

Two diets based on fish meal (FM) or on a mixture of plant protein sources supplemented with free amino acids (PP) were fed to rainbow trout having a mean initial body weight of 162.5 g reared at 17±1 °C for 24 weeks to evaluate the effect of plant protein sources on fish growth, morphological and body quality traits. At the end of the growth study, samples were withdrawn for chemical analyses as well as measurements of different parameters of quality.

Compared to the FM group, the trout fed diet PP had lower growth rates (mean final body weight 663 vs. 800 g), feed efficiency (0.95 vs. 1.07) and protein efficiency ratio (1.96 vs. 2.13). Trout fed diet PP were also characterised by a lower dressed weight (89.45% vs. 91.07%), hepato-somatic index (0.92% vs. 1.01%) and fillet lipid content (5.50% vs. 6.98%), but had a higher mesenteric fat (5.65% vs. 4.08%), viscero-somatic index (10.55% vs. 8.93%) and fillet crude protein content (20.53% vs. 19.93%). The muscle fatty acid composition reflected that of the diet with a higher level of polyunsaturated (n-6) fatty acids in trout fed diet PP and a higher content in polyunsaturated n-3 fatty acids and n-3/n-6 ratio in trout fed diet FM. Sensory analysis was carried out at 5 days *post-mortem* on microwave-cooked dorsal portions of fillets stored at 1 °C with ice covering. The results of the triangular test showed differences between groups ($P<0.01$) in organoleptic characteristics as well as in visual colour. Descriptive tests pointed out that fillets of trout fed diet PP exhibited higher hardness, less sweetness and less odour intensity than trout fed diet FM.

In summary, data show that long-term feeding a diet in which fish meal was totally replaced by a mixture of plant protein sources significantly affects growth and quality criteria such as morphometric traits, fat deposits, fillet chemical composition and organoleptic characteristics of large commercial size rainbow trout.

1 Effect of long-term feeding with a plant protein mixture based diet on growth and body/fillet quality
2 traits of large rainbow trout (*Oncorhynchus mykiss*)

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10

11 **Abstract**

12 Two diets, either based on fish meal (FM) or on a mixture of plant protein sources supplemented
13 with free amino acids (PP) were fed to rainbow trout having a mean initial body weight of 162.5 g
14 reared at 17±1 °C for over 24 wks, in order to evaluate the effect of plant protein sources on fish
15 growth, morphological and body quality traits. At the end of the growth study, samples were
16 withdrawn for chemical analyses as well as measurements of different parameters of quality.

17 Compared to the FM group, the trout fed diet PP had lower growth rates (mean final body weight
18 663 vs 800 g), feed efficiency (0.95 vs 1.07) and protein efficiency ratio (1.96 vs 2.13). Trout fed
19 diet PP were also characterised by a lower dressed weight (89.45 vs 91.07 %), hepato-somatic index
20 (0.92 vs 1.01 %) and fillet lipid content (5.50 vs 6.98 %), but had a higher mesenteric fat (5.65 vs
21 4.08 %), viscero-somatic index (10.55 vs 8.93 %) and fillet crude protein content (20.53 vs 19.93
22 %). The muscle fatty acid composition reflected that of the diet with a higher level of
23 polyunsaturated (n-6) fatty acids in trout fed diet PP and a higher content in polyunsaturated n-3
24 fatty acids and n-3/n-6 ratio in trout fed diet FM. Sensory analysis was carried out at 5 days post-
25 mortem on microwave-cooked dorsal portions of fillets stored at 1°C with ice covering. The results
26 of the triangular test showed differences between groups (P<0.01) in organoleptic characteristics as

1 well as in visual colour. Descriptive tests pointed out that fillets of trout fed diet PP exhibited higher
2 hardness, less sweetness and less odour intensity than trout fed diet FM.

3 In summary, data show that long-term feeding with a diet in which fish meal was totally replaced by
4 a mixture of plant protein sources significantly affects growth and quality criteria such as
5 morphometric traits, fat deposits, fillet chemical composition and organoleptic characteristics of
6 large commercial size rainbow trout.

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8 Keywords: Oncorhynchus mykiss; Plant protein; Body traits; Flesh; Chemical composition;
9 Quality; Sensory evaluation

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2 **1. Introduction**

3 It is generally well recognised that over reliance on marine fishery resources is not a sustainable
4 practice for aquaculture development (Hardy, 1999; Naylor et al., 2000; New and Wijkstroem,
5 2002). Substitution of fish meal by alternate protein sources has been the object of study since more
6 than three decades (Fowler, 1980; Dorsa et al., 1982; Tacon et al., 1985; Smith et al., 1988),
7 possibly reducing production costs but definitely having a beneficial impact on environmental
8 loads, locally with regard to phosphorus discharge (Watanabe and Pongmaneerat, 1993; Ballestrazzi
9 et al., 1994; Hardy, 1995; Burel et al., 1998; Riche and Brown, 1999; Barrias and Oliva-Teles,
10 2000) and globally over the preservation of wild fishery resources. In spite of the lower protein
11 content, the deficiency in some indispensable amino acids (IAA) and the presence of anti-nutritional
12 factors (ANFs) frequently found in most plant protein sources (Kaushik, 1990; Francis et al., 2001),
13 many studies have demonstrated the possibility to incorporate such ingredients in feeds for
14 salmonids (Gomes et al., 1993, 1995; Oliva-Teles et al., 1994; Hardy, 1996; Forster et al., 1999;
15 Refstie et al., 2000; Burel et al., 2000; Vielma et al., 2000). Generally it has been observed that a
16 partial substitution of fish meal by plant protein sources is well supported by fish, so currently more
17 than 30% is already replaced in most commercial fish feeds (Teskeredzic at al., 1995; Forster et al.,
18 1999; Burel et al., 2000; Vielma et al., 2000). Under conditions of partial replacement of fish meal,
19 reasonably good growth performance has been observed with minor effects on metabolism (Gomes
20 and Kaushik, 1990; Kaushik and Mambrini, 1994; Sanz et al., 1994; Carter and Hauler, 1995;
21 Gomes et al., 1995; Robaina et al., 1995; Teskeredzic at al., 1995; Kissil et al., 2000; Refstie et al.,
22 1997; Médale et al., 1998; Refstie et al., 2000). Fish meal free diets have also been used with
23 success in some studies with rainbow trout (Kaushik et al., 1995; Watanabe et al., 1998).

24 The consequences of fish meal substitution on flesh quality traits have been investigated to some
25 extent. Smith et al. (1988) working with different strains of rainbow trout fed either fish meal-based
26 or plant protein-based diets did not find any differences in organoleptic quality or flesh acceptability

1 associated with either strain or diet. Kaushik et al. (1995) did not find any major effects of total
2 substitution of fish meal by a soy protein concentrate. Even in marine fish, Aoki et al. (1996) did
3 not find any difference in flesh quality between adult red sea bream fed with or without fish meal as
4 the dietary protein source. Other studies (Lopez-Bote et al., 2001) suggest that peroxidative
5 characteristics of fish muscle are susceptible to be affected by dietary protein sources. In most of
6 the above-mentioned studies, the duration of growth trials were relatively short. The aim of this
7 work was to study the long term effect of the total replacement of fish meal by a mixture of plant
8 protein sources supplemented with free amino acids to suit the IAA composition of trout muscle on
9 growth of rainbow trout and on several quality attributes.

10

11 **2. Materials and methods**

12 2.1. Diets

13 Two isoproteic (50% crude protein, CP) and isoenergetic (23 kJ/g dry matter, DM) diets,
14 containing either fish meal as the major protein source (diet FM), or a mixture of plant protein
15 sources (corn gluten meal, wheat gluten, extruded peas, rapeseed meal) adequately supplemented
16 with free amino acids (diet PP) were used (Table 1). Both diets were formulated to meet the
17 nutritional requirements of rainbow trout (NRC, 1993) and also contained synthetic astaxanthin as a
18 pigment source. All dry ingredients were mixed and pelleted dry (4 mm diameter) using a Simon-
19 Heese (Holland) pelleting machine. Dietary amino acid and fatty acid profiles are provided in tables
20 2 and 3.

21

22

23 2.2. Growth trials

24 Triploid rainbow trout (*Oncorhynchus mykiss*) of the same lot (initial average body weight
25 162.5 g) were reared in the INRA experimental fish farm (Donzacq, Landes, France) in four 1000 l
26 fiber glass tanks (2x2 m; water temperature 17±1 °C; oxygen level 8.5 ppm). Duplicate groups were

1 hand-fed to visual satiety twice daily the respective diets over 157 days. Fish were group weighed
2 every three weeks to follow growth and feed intake. At the end of the growth trial, different growth
3 and feed utilisation parameters were calculated.

4 2.3. Body and fillet quality determinations

5 After the final weighing, fish were kept unfed for two days, were cold stunned, slaughtered
6 by a blow on the head and stored on ice at 1 °C. All fish were weighed and the following
7 measurements made using an orthometric meter: total and standard length (cm), head length (cm),
8 and maximum height (cm). From linear and weight measures, morphometric indexes, as condition
9 factor = $(100 \times \text{body weight} / \text{total length}^3)$, agility index (distance between caudal plane and
10 maximum height plane / maximum height), cranial index (head length / total length), relative profile
11 (maximum height / total length), were computed. On the whole, 80 fish (40 fish per diets) were
12 completely dissected to weigh the main body components and to calculate the relative weights (%
13 of body weight) of fins, gills, head, fillets, viscera, digestive tract, mesenteric fat, liver, and dressed
14 weight. The fillet yield (%) was computed as twice the right fillet weight. The hepato-somatic index
15 (HSI) = $[100 \times (\text{liver weight} / \text{body weight})]$ and the viscero-somatic index (VSI) $[100 \times (\text{total}$
16 $\text{viscera weight} / \text{body weight})]$ were also computed.

17 During dissection, we observed the presence of 21 diploid trout (12 % of fish, n = 12 FM and n = 9
18 PP) in an advanced state of sexual maturity, therefore the corresponding data were removed and not
19 considered in the statistical analysis.

20 39 fillets and 11 livers were vacuum packed and stored on dry ice prior to chemical composition
21 analysis: moisture, crude protein, ash and phosphorus according to AOAC (1995) and total lipids
22 according to Folch et al. (1957). Fatty acid composition of fillets (n= 18 FM and n= 21 PP) and
23 livers (n=5 FM and n=6 PP) was analysed by quantitative gas chromatography (utilising C23:0 as
24 internal standard) and total cholesterol (Boach et al., 1988) and malonaldehyde-TBA complex
25 (Raharjo et al. 1993), were determined in the fillets.

1

2 2.4. Instrumental colour measurement of fillets

3 On 75 intact right fillets (FM n=36 and PP n= 39), withdrawn at the moment of the analysis from
4 whole fish stored at 1 °C, colorimetric measurements were made using a CR-200 Chroma Meter
5 (Minolta, U. K.) at three sites (dorsal, ventral and caudal) and each measure was replicated twice.
6 Data were expressed using the L* a* b* system, representing lightness, redness and yellowness as
7 indicated by the CIE (1976); in addition, the values of chroma [$\sqrt{(a^{*2} + b^{*2})}$], which defines the
8 saturation of colour and the angle of hue [$\tan^{-1}(b^* / a^*)$] were calculated.

9

10 2.5. Sensory evaluation

11 Sensory evaluation on cooked fillet was made after 5 days post mortem on fillets withdrawn
12 from whole fish stored with ice at 1 °C. The epaxial portion of each fillet with skin was divided into
13 5 portions, wrapped in a special microwave oven paper and cooked in a microwave oven (Moulinex
14 Optiquick Compact), at 500 watt for 70 seconds. A trained panel consisting of 10 judges evaluated
15 the fillets in air-conditioned individual boxes designed for sensory analysis (ISO, 1988) by
16 triangular and descriptive tests. The triangular test (ISO, 1983) and the descriptive tests were
17 replicated twice. As the triangular test was able to discriminate between diets, it was followed by
18 the descriptive test to evaluate the following sensory attributes: odour (intensity, rancid), flavour
19 (intensity, fresh, sweet, fresh oil, metallic, fat, slight herring like, rancid), hardness, compactness,
20 juiciness and roughness (mouth feeling of fibres). All attributes were evaluated on a continuous
21 intensity scale, going from 1 (no intensity) to 9 (high intensity), using a computerised system for
22 direct recording (PSA System III Oliemans Punter & Partners®).

23

24 2.6. Statistic analysis

25 Data were analysed by a one way ANOVA (diet). The fatty acid profile was analysed also
26 including the total fillet lipid content in the model. As the malonaldehyde content was determined at

1 different time after the death, the statistical analysis of this parameter was made including in the
2 model the number of days post-mortem. As colour measurements were made at different time
3 intervals after death and in different sites of the fillet, the statistical analysis of this parameter was
4 made including in the model the diet as a fixed factor, with the days, the site and the interaction site
5 x diet as variable. The sensory evaluation was computed by Generalised Procrustes Analysis (GPA)
6 (Gower, 1975) carried out with Senstool (Oliemans Punter & Partners®), to take into account the
7 variation among judges, then the data were analysed by a one way ANOVA to evaluate the effect of
8 diet on all the descriptors.

9

10 **3. Results**

11

12 3.1 Growth performance and body traits

13 Changes in mean body weights of both groups over the entire period is presented in figure 1.
14 Numerical data on growth, feed intake and utilisation are reported in table 4. While feed intake did
15 not differ between the two fish groups, significant differences were observed for all other
16 parameters, all being higher in trout fed diet FM than in those fed diet PP.
17 As reported in table 5 trout fed diet FM reached a significantly higher final body weight (800 vs 664
18 g) and total length compared to those fed diet PP. Trout fed diet PP had consequently a higher
19 condition factor (1.27 vs 1.22). We did not observe any differences in agility index, cranial index
20 and relative profile. Trout fed diet FM had significantly lower relative weight of gills, while fins,
21 head, filleting remnant and digestive tract incidences did not differ. The hepato-somatic index was
22 higher in trout fed diet FM than in those fed diet PP. No differences were observed in fillets
23 percentage between the fish groups, while the significantly higher ($P < 0.01$) viscero-somatic index
24 observed in trout fed diet PP, was principally due to higher mesenteric fat percentage leading to
25 lower dressed weight yield.

26

1 3.2. Chemical composition

2 Concerning fillet composition (table 6), moisture and phosphorous contents were similar in
3 both groups, while trout fed diet PP had slightly higher protein and ash and a lower lipid percentage
4 and cholesterol content than trout fed diet FM. No difference in malonaldehyde content was
5 observed between groups. As regards liver, only the moisture and crude protein percentages were
6 significantly different, with trout fed diet PP showing higher values in comparison to trout fed diet
7 FM.

8 The fatty acid composition of fillets is reported in table 7, expressed as percentages of total fatty
9 acids and as g / 100 g fillet. Saturated (SFA), monounsaturated (MUFA) and polyunsaturated n-3
10 (PUFA n-3) fatty acids and the n-3 / n-6 ratio were significantly higher in trout fed diet FM, while
11 polyunsaturated n-6 (PUFA n-6) fatty acids were significantly higher in trout fed diet PP. As
12 regards individual fatty acids, the main difference observed was the higher incidence and content of
13 linoleic acid in PP fillet in comparison to FM one, while there was no difference for the
14 eicosapentaenoic and the docosahexaenoic acid levels. The liver fatty acid profile (table 8) was also
15 characterised by the higher incidences and quantities of linoleic acid and n-6 PUFA in trout fed diet
16 PP in comparison to that in the liver of trout fed diet FM. However, there was no difference
17 between groups as regards total n-3 PUFA levels.

18 19 3.3 Fillet colour

20 As regards fillet colour, some differences were observed at each of the three points of
21 measure. In dorsal and caudal sites, the values of redness, of yellowness and of hue were different
22 between the diets (Table 9). At all the considered sites (dorsal, ventral and caudal) the dietary
23 treatments did not result in any significant difference for chroma or lightness except for the ventral
24 site. Statistical analysis of differences between sites indicated a significant site effect for all the
25 colour parameters.

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3.4 Sensory analysis

The triangular test made at 5 days after death indicated differences in organoleptic characteristics between groups, as 7 out of 10 judges identified the different samples, in particular for the clear difference in colour between the two groups. Consequently a descriptive test was carried out the same day, the taste panel working in individual boxes with red colour lamp to overcome any colour effect. The descriptive test revealed some significant differences between groups: Flesh from trout fed diet PP had higher hardness, less sweetness, less odour intensity and a trend ($P < 0.06$) to lower juiciness than flesh from trout fed diet FM (Figure 2).

4. Discussion

Growth rates of both groups were high, although in spite of the supplementation with synthetic amino acids to improve amino acid balance, the total substitution of the fish meal with a plant protein mixture did not lead to equivalent final weights at the end of the nearly 6 months growth trial. It is worth noting that the growth rates became significantly different only after 12 weeks (Figure 1), confirming the need for long term studies. Since feed intakes did not differ between groups, feed and protein efficiencies were also reduced. Whether this is due to inadequate amino acid supply to meet the high growth rates over the long run or due to accumulative effects of unidentified anti-nutritional factors remains to be investigated. It has been suggested that the length of a feeding trial can bring out growth differences in a significant manner (Weatherup and McCracken, 1999). Given the long term study as undertaken here, it is also difficult to compare data from literature, where the studies have been of shorter duration. However, the main objective of this work was to obtain trout with a final body weight of commercial size, and to compare the effects of dietary protein sources on flesh quality parameters.

1 One of the problems related to plant protein ingredients is the relatively high carbohydrate
2 contents in these ingredients which are generally not well digested by salmonids (Singh and Nose,
3 1967) but this can be improved by technological treatments (Luquet and Bergot, 1976; Bergot,
4 1993). In fact, the starch content as measured in diet PP was lower than in diet FM, mainly due to
5 the inclusion of extruded whole wheat which is generally known to have high starch digestibility.
6 Plant ingredients contain varying levels of non-starch polysaccharides (Malathi and Devegowda,
7 2000) whose possible adverse effects are little known in fish (Francis et al. 2001). This needs
8 further exploration.

9 It is quite possible that other ANFs could be responsible for the poor growth rate observed in
10 the trout fed the plant protein mixture diet. Preliminary control has shown that the anti-trypsinic
11 factors were low. We also found the phytoestrogen (genistein and daidzein) contents to be
12 extremely small (Bennetau – Pelissero et al., 2003).

13
14 As regards body morphological traits, mean condition factor value observed in both groups
15 of trout (1.2) was slightly lower than the value of 1.4 reported by Bonnet et al. (1999) in triploid
16 trout with a mean body weight of 837 g. Trout fed diet PP, even with 20% lower body mass, were
17 characterised by a higher condition factor, possibly mainly due to the high mesenteric fat content,
18 suggesting higher fat synthesis and deposition. But, trout fed diet based on fish meal had a higher
19 fillet fat content, possibly related to the higher body weight. The possible metabolic or endocrine
20 mechanisms involved in such differences in tissue lipid deposition are under scrutiny. One possible
21 reason is the higher supply of some of the dispensable amino acids (DAA) such as glutamic acid in
22 excess in the diet PP.

23
24 As is well known (Watanabe, 1982; Sargent, 2002), dietary fatty acid composition strongly
25 influenced flesh fatty acid composition. While it is common to see such changes when dietary fatty
26 acid profiles are modified through changes in dietary lipid sources, there is little information as

1 regards the effects of changes in fatty acid profiles as affected by dietary protein sources. Indeed,
2 total replacement of fish meal by plant protein ingredients modifies the fatty acid profiles to a
3 certain extent (table 3) with the consequent changes seen in liver and muscle fatty acid profiles.
4 Fillet of trout fed diet FM was richer in saturated fatty acids, monoenes, and n-3 PUFAs and those
5 of fish fed diet PP markedly richer in n-6 PUFAs, above all in C18:2 n-6. The differences in fillet
6 fatty acid profiles of the two groups of trout were confirmed even when corrected for differences in
7 total lipid content of fillets found between the two groups. Only the difference in C22:5 n-3, higher
8 in fillet of fish fed fish meal diet disappeared, indicating that it was strictly related to the different
9 total lipid content. The quantitative differences in C14:0, C16:0, C18:0, C20:5 n-3, C22:1 n-11,
10 C22:5 n-3, C22:6 n-3, SFA, MUFA, and PUFA n-3 were due to the higher lipid content in FM,
11 while the higher content of PUFA n-6 in PP fillet was confirmed also when evaluated at the same
12 lipid content. This result was consistent with data obtained by Gomes et al. (1993), they observed
13 an increased level of n-6 series fatty acids in muscle of rainbow trout fed diets with an increasing
14 level (5, 10, 15 and 45%) of colzapro. It is reassuring to note that, at the same lipid level, the
15 content in eicosapentaenoic (EPA) and in docosahexaenoic (DHA) acids did not show differences
16 in the two groups of trout. Pereira et al. (1998) reported that when broodstock rainbow trout were
17 fed diets in which fish meal was totally replaced by plant protein sources, egg fatty acid profiles
18 were modified reflecting dietary changes, still maintaining high levels of long chain n-3 PUFAs.
19 We see here that despite increases in n-6 fatty acids, the total replacement of fish meal with plant
20 protein sources did not influence the quantity of n-3 PUFA considered as the most interesting in
21 terms of human health. In an earlier study, Lopez- Bote et al. (2001) found that under conditions of
22 forced peroxidation in vitro, muscle of fish fed diets containing fish meal as the main source of
23 protein showed higher susceptibility to peroxidation than muscle from fish fed plant protein based
24 diets. This is in contrast to observations made here, where we did not see any differences in the
25 levels of malonaldehyde. Whether this is due to methodological differences remains to be verified.

26

1 As regards pigmentation, which remains a major flesh quality attribute for salmonids, the
2 differences in data obtained through instrumental measurements were consistent with the
3 observation of the panellists during the panel test, when they recognised as different the two PP and
4 FM fillet first of all by the colour. In the study by Kaushik et al. (1995), there was also a distinction
5 based on this colour attributes between trout fed fish meal or soy protein concentrate; but this was
6 largely due to the fact that the diets did not contain any added astaxanthin, except that which was
7 supplied by the fish meal. Dietary soybean meal does not seem to interfere with astaxanthin
8 absorption or deposition (Bjerkeng et al. 1997). Our results show in all the three sites a higher value
9 of yellowness in trout fed diet PP and a higher redness in trout fed diet FM. The differences
10 observed here could partially be attributed to the presence of corn gluten meal in diet PP as
11 suggested by Hardy (1996). Indeed, in an earlier work, we have found that even in the presence of
12 adequate amount of astaxanthin in the diet, muscle colour attributes are modified and muscle
13 retention of astaxanthin is lowered in the presence of very high levels of corn gluten meal (Kaushik
14 et al., 2002). Another interesting point is the consistency in the variability of the intra-fillet colour.
15 There was no interaction between the site and the diet, indicating that all the fillets, presented the
16 same colour difference in the different sites analysed. The highly ($P < 0.01$) significant effect of the
17 measurement location is in agreement with literature data (Choubert, 1997). Nickell and Bromage
18 (1998) proposed that this was due to the difference in fillet thickness between the examined regions,
19 and that a relation between muscular fibre size and astaxanthin retention would be implied.

20 In agreement with Kaushik et al. (1995), who reported that the panellists in the sensory analysis
21 were able to distinguish two groups of trout fed two different diets, fed either 100% fish meal diet
22 or a 100% of soy protein concentrate based diet, the sensory analysis made here indicated
23 organoleptic differences between the trout fed the two diets.

24 Juiciness is an important contributor to eating quality although the relationship between this food
25 characteristic and the objective measurement is not completely clear (Ruiz-Carrascal et al. 2000).
26 The higher juiciness and odour intensity of trout fed diet FM perceived at 5 days after death, can be

1 related to the different fatty acid profile and consequent minor changes in lipid oxidative status, as
2 reported by Johansson et al. (2000). The presence of a higher quantity of intramuscular fat in FM
3 fillet could have also stimulated the production of saliva resulting in a higher sensation of juiciness
4 (Johansson et al. 1991).

5

6 Summing up, the total replacement of fish meal by a mixture of plant protein sources and
7 adequately balanced with essential amino acids, affects growth of rainbow trout in the long term,
8 inducing changes in body traits, chemical composition and fatty acid profile of fillets and led to
9 changes in some organoleptic properties of flesh.

10

11 **Acknowledgements**

12

13 The authors thank Denise Blanc, Christiane Vachot, Frédéric Vallée and Antonio Bonelli for their
14 technical support, and the staff of the INRA Experimental Fish Farm at Donzacq (Yves Hontang,
15 Frédéric Terrier and Franck Sandres) for the care and feeding of the fish. This research was
16 financed by the European project QLRT-1999-30068 "Perspectives of plant protein use in
17 aquaculture".

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Table 1. Ingredient and chemical composition of the experimental diets.

	FM	PP
Ingredients (g/kg)		
Fish meal (Herring, Norway)	638.0	0.0
Corn gluten meal	0.0	232.4
Wheat gluten	0.0	200.0
Extruded peas (Aquatex, France)	0.0	163.3
Rapeseed meal (Primor 00, France)	0.0	100.0
Extruded whole wheat	203.4	0.0
Fish oil	128.6	158.7
Binder (Na Alginate)	10.0	10.0
Mineral premix ¹	10.0	10.0
Vitamin premix ¹	10.0	10.0
CaHPO ₄ ·2H ₂ O	0.0	40.0
Amino acid mix ²	0.0	75.7
Astaxanthin ³	0.01	0.01
Analytical composition		
Dry matter (DM, g/kg)	944	916
Protein (g /kgDM)	515	486
Lipid (g/kg DM)	197	192
Starch (g/kg DM)	138	105.
Ash (g/kg DM)	76	59
Phosphorus (g/kg DM)	19	13
Energy (kJ/g DM)	22.7	23.6

¹According to NRC (1993)

² Arg: 12.5; His: 4.0; Lys: 27.6; DL-Mét: 4.1; Trp: 2.7; Thr: 8.3; Ile: 7.1; Val:9.4

³ Carophyll Pink®, Hoffman LaRoche, Basel, Switzerland

1 Table 2. Amino acid composition (g / 100 g DM) of the experimental diets fed to rainbow trout
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Amino acid	Diet FM	Diet PP
ARG	3.6	3.3
LYS	4.0	3.5
HIS	0.9	1.3
ILE	1.8	2.2
LEU	3.4	4.2
VAL	2.2	2.8
MET	1.2	1.0
PHE	1.7	2.0
THR	2.4	2.3
TYR	1.4	1.6
Asp	4.3	2.1
Glu	7.5	12.9
Ser	2.1	2.1
Pro	2.1	3.3
Gly	3.1	1.4
Ala	3.0	2.0
Sum AA	44.7	46.7
Sum IAA	22.6	23.2
Sum DAA	22.1	23.5

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2 Table 3. Total lipids (% on wet weight) and fatty acid¹ (% of total fatty acids) composition of FM
3 and PP diets.
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	FM	PP
Lipids %	18.12	18.19
C14:0	6.3	6.0
C16:0	14.3	14.1
C16:1 n-7	5.4	4.7
C18:0	1.8	1.7
C18:1 n-9	13.3	14.8
C18:2 n-6	2.2	10.6
C18:3 n-3	1.1	1.6
C18:4 n-3	3.4	3.2
C20:1 n-9	9.1	7.3
C20:5 n-3	11.0	9.3
C22:1 n-11	12.5	11.1
C22:6 n-3	12.7	10.0
Σ SFA	23.3	22.6
Σ MUFA	41.9	38.8
Σ PUFA n-6	3.3	11.5
Σ PUFA n-3	30.3	25.8
n3 / n6 ratio	9.2	2.3

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6 ¹The fatty acids C12:0, C15:0, C16:2 n-4, C16:3 n-3, C16:4 n-1, C17:0, C18:2 n-4, C18:3 n-4, C18:3 n-6, C20:0, C20:1
7 n-7, C20:2 n-6, 20:3 n-3, C20:3 n-6, C 20:4 n-3, C20:4 n-6, C22:1 n-9, C21:5 n-3, C22:4 n-6, C22:5 n-6, in percentage
8 less than 1%, were considered in the composite fractions but not reported in the table for brevity.
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1 Table 4. Growth performance, feed intake and feed efficiency of rainbow trout fed the FM and PP
2 diets ¹.

	FM	PP
Initial body weight (IBW) (g)	163.0 ± 4.2	162.0 ± 2.8
Final body weight (FBW) (g)	844.9 ± 29.8 ^a	666.2 ± 14.1 ^b
Specific growth rate (SGR) (%)	1.05 ± 0.01 ^a	0.90 ± 0.03 ^b
Daily growth index (DGI) (°%)	2.54 ± 0.04 ^a	2.09 ± 0.06 ^b
Feed intake (FI) (DM / kg ABW* / d ⁻¹)	8.20 ± 0.28	8.19 ± 0.06
Feed efficiency (FE)	1.07 ± 0.01 ^a	0.95 ± 0.02 ^b
Protein efficiency ratio (PER)	2.13 ± 0.03 ^a	1.96 ± 0.03 ^b

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5 ¹ Means are indicated with standard deviation. Different superscript letters in the same row indicate significant
6 statistical differences (a, b: P<0.05).

7 * ABW = average body weight.

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9 $SGR = 100 \times [(\ln FBW - \ln IBW) / \text{duration}]$

10 $DGI = 100 \times [(FBW)^{1/3} - (IBW)^{1/3}] / \text{duration};$

11 ABW = average body weight

12 FE = wet weight gain / dry feed intake

13 PER = wet weight gain / crude protein intake.

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Table 5. Body morphological traits in trout fed diets based on fish meal (FM) or on a mixture of plant protein sources (PP) ¹.

	N°	FM	N°	PP	rsd
Body morphological traits					
Body weight (g)	73	800.27 ^A	77	663.50 ^B	148.72
Total length (cm)	73	40.16 ^A	77	37.21 ^B	2.48
Condition factor	73	1.22 ^B	77	1.27 ^A	0.10
Agility index	31	2.85	32	2.77	0.24
Cranial index	31	0.18	32	0.18	0.01
Relative profile	31	0.23	32	0.24	0.02
Body components					
Fins (%)	31	1.40	32	1.41	0.21
Gills (%)	31	2.11 ^a	32	2.24 ^b	0.20
Head (%)	31	12.58	32	12.02	1.20
Filleting remnant (%)	31	9.84	32	9.80	1.42
Hepato-somatic index (HSI, %)	45	1.01 ^a	47	0.92 ^b	0.18
Digestive tract (%)	45	4.02	47	3.99	0.92
Mesenteric fat (%)	43	4.08 ^B	47	5.65 ^A	1.36
Viscero-somatic index (VSI, %)	45	8.93 ^B	47	10.55 ^A	1.32
Dressed weight (%)	45	91.07 ^A	47	89.45 ^B	1.32
Fillets (%)	45	56.78	47	55.55	4.12

¹Means are indicated with rsd. Different superscript letters in the same row indicate significant statistical differences (a, b: P<0.05; A, B: P<0.01).

Table 6. Fillet and liver chemical composition (on wet weight basis) in rainbow trout fed diets based on fish meal (FM) or on a mixture of plant protein sources (PP) ¹.

	N°	FM	N°	PP	rsd
<i>Whole fillet composition</i>					
Moisture (%)	18	71.96	21	72.87	1.61
Crude protein (%)	5	19.93 ^b	6	20.53 ^a	0.40
Lipids (%)	18	6.98 ^A	21	5.50 ^B	1.54
Ash (%)	5	1.24 ^b	6	1.29 ^a	0.03
Phosphorus (%)	5	0.14	6	0.16	0.04
Cholesterol (mg / 100g)	5	51.04 ^a	6	47.74 ^b	2.11
Malonaldehyde (mg / kg)	13	0.058	15	0.073	0.026
<i>Liver composition</i>					
Moisture (%)	4	71.31 ^b	6	74.40 ^a	2.13
Crude protein (%)	4	15.30 ^B	6	16.50 ^A	0.54
Lipids (%) ²	5	5.15	6	4.23	1.14
Ash (%)	4	1.15	6	1.23	0.10

¹ Means are indicated with rsd. Different superscript letters in the same row indicate significant statistical differences (a, b: P<0.05; A, B: P<0.01).

² For the analysis of liver lipid, samples different from those utilised for the analysis of the other chemical components were utilised.

Table 7. Fatty acid composition of fillets from rainbow trout fed diets based on fish meal (FM) or on a mixture of plant protein sources (PP) ¹.

	FM (n=18)	PP (n=21)	Rsd	FM (n=18)	PP (n=21)	rsd
	% of total fatty acids			(g / 100 g fresh tissue)		
<i>Fatty acids</i> ²						
C14:0	4.75 ^A	4.45 ^B	0.13	0.182 ^a	0.146 ^b	0.042
C16:0	17.85 ^A	17.29 ^B	0.45	0.687 ^a	0.567 ^b	1.666
C16:1	6.25	6.35	0.55	0.242	0.208	0.063
C18:0	3.30 ^A	2.86 ^B	0.21	0.128 ^A	0.095 ^B	0.033
C18:1	20.12	21.07	1.56	0.780	0.699	0.228
C18:2 n-6	2.46 ^B	8.59 ^A	0.28	0.094 ^B	0.282 ^A	0.059
C18:3 n-3	0.87 ^B	1.03 ^A	0.05	0.033	0.034	0.009
C18:4 n-3	1.64	1.65	0.10	0.063	0.054	0.016
C20:1 n-9	7.63 ^A	5.79 ^B	0.21	0.294 ^A	0.191 ^B	0.065
C20:4 n-3	1.44 ^A	0.86 ^B	0.09	0.055 ^A	0.028 ^B	0.011
C20:5 n-3	6.05	5.59	1.06	0.235 ^a	0.182 ^b	0.065
C22:1 n-11	5.69	4.94	1.86	0.236 ^a	0.165 ^b	0.082
C22:5 n-3	1.64 ^a	1.46 ^b	0.27	0.064 ^A	0.048 ^B	0.018
C22:6 n-3	14.93	14.86	2.63	0.576 ^a	0.479 ^b	0.145
Σ SFA	26.58 ^A	25.25 ^B	0.60	1.023 ^a	0.829 ^b	0.246
Σ MUFA	41.46 ^A	38.55 ^B	1.40	1.601 ^a	1.269 ^b	0.400
Σ PUFA n-6	3.46 ^B	9.92 ^A	0.30	0.132 ^B	0.325 ^A	0.069
Σ PUFA n-3	27.87 ^A	25.43 ^B	1.99	1.065 ^A	0.828 ^B	0.232
n3 / n6 ratio				15.739 ^A	4.052 ^B	4.369

¹ Means are indicated with rsd. Different superscript letters in the same row indicate significant statistical differences (a, b: P<0.05; A, B: P<0.01).

² The fatty acids C12:0, C15:0, C16:2 n-4, C16:3 n-3, C16:4 n-1, C17:0, C18:2 n-4, C18:3 n-4, C18:3 n-6, C20:0, C20:1 n-7, C20:2 n-6, C20:3 n-3, C20:3 n-6, C20:4 n-6, C22:1 n-9, C21:5 n-3, C22:4 n-6, C22:5 n-6, in percentage less than 1%, were considered in the composite fractions but not reported in the table for brevity.

Table 8. Fatty acid composition of livers from trout fed diets based on fish meal (FM) or on a mixture of plant protein sources (PP)¹.

<i>Fatty acids</i> ²	FM (n=5)	PP (n=6)	rsd	FM (n=5)	PP (n=6)	rsd
	% of total fatty acids			(g / 100 g fresh tissue)		
C14:0	2.01 ^a	1.62 ^b	0.25	0.049	0.030	0.017
C16:0	15.05	15.99	1.46	0.347	0.294	0.073
C16:1	4.62	3.19	1.46	0.120	0.061	0.058
C18:0	4.98 ^B	5.90 ^A	0.42	0.114	0.110	0.022
C18:1	23.21	16.56	6.26	0.591	0.316	0.265
C18:2 n-6	1.35 ^B	3.88 ^A	0.22	0.032 ^B	0.072 ^A	0.011
C20:1 n-9	5.84 ^a	3.12 ^b	1.46	0.147 ^a	0.059 ^b	0.058
C20:4 n-3	1.20 ^A	0.70 ^B	0.22	0.029 ^a	0.013 ^b	0.009
C20:5 n-3	6.03	6.67	0.96	0.133	0.123	0.015
C22:1 n-11	1.97 ^a	0.75 ^b	0.64	0.050 ^a	0.014 ^b	0.022
C22:5 n-3	2.18	1.98	0.37	0.050	0.037	0.011
C22:6 n-3	26.86	33.46	7.00	0.578	0.612	0.042
Σ SFA	22.42	23.94	1.54	0.519	0.442	0.111
Σ MUFA	36.14	23.85	9.81	0.921	0.455	0.406
Σ PUFA n-6	4.43 ^B	8.76 ^A	0.81	0.097 ^B	0.162 ^A	0.015
Σ PUFA n-3	36.80	43.25	7.87	0.802	0.793	0.061

¹ Means are indicated with rsd. Different superscript letters in the same row indicate significant statistical differences (a, b: P<0.05; A, B: P<0.01).

² The fatty acids C12:0, C15:0, C16:2 n-4, C16:3 n-3, C16:4 n-1, C17:0, C18:2 n-4, C18:3 n-3, C18:4 n-4, C18:3 n-4, C18:3 n-6, C20:0, C20:1 n-7, C20:2 n-6, C20:3 n-3, C20:3 n-6, C20:4 n-6, C22:1 n-9, C21:5 n-3, C22:4 n-6, C22:5 n-6, in percentage less than 1%, were considered in the composite fractions but not reported in the table for brevity.

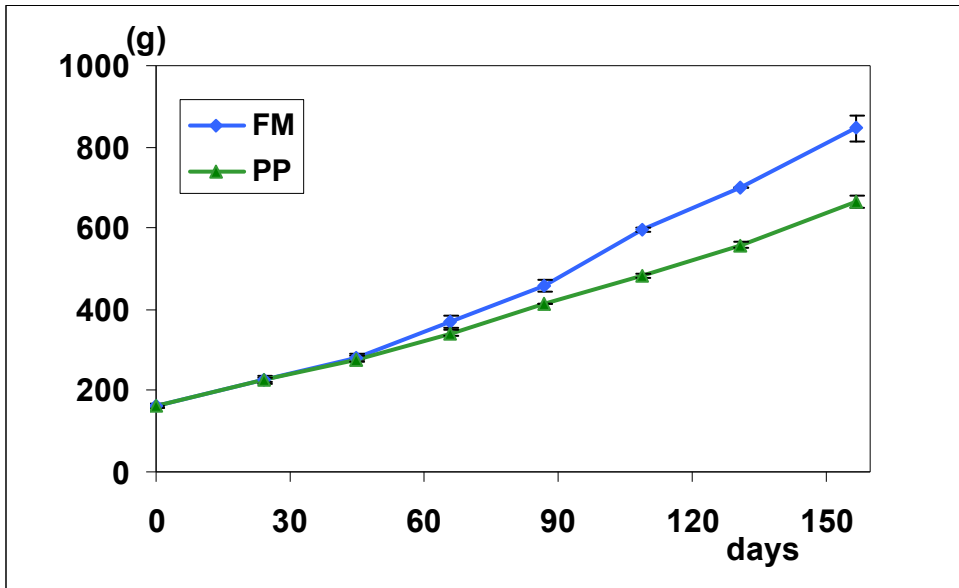
Table 9. Parameters of fillet colour in rainbow trout fed diets based on fish meal (FM) or on a mixture of plant protein sources (PP).

	FM (n= 36)	PP (n= 39)	rsd
<i>Parameters</i>			
Dorsal site			
Lightness	36.82	36.50	2.16
a	11.29 ^a	10.45 ^b	1.69
b	12.91 ^B	15.02 ^A	3.05
Chroma	17.18	18.35	3.23
Hue°	48.59 ^B	54.89 ^A	4.34
Ventral site			
Lightness	35.62 ^a	34.54 ^b	1.95
a	9.25 ^A	8.37 ^B	1.34
b	10.53 ^B	12.34 ^A	2.32
Chroma	14.09	14.95	2.37
Hue°	48.20 ^B	55.75 ^A	4.78
Caudal site			
Lightness	34.86	34.76	2.03
a	11.67 ^A	10.42 ^B	1.55
b	12.35 ^b	13.59 ^a	2.25
Chroma	17.07	17.20	2.44
Hue°	46.19 ^B	52.25 ^A	4.20

¹ Means are indicated with rsd. Different superscript letters in the same row indicate significant statistical differences (a, b: P<0.05; A, B: P<0.01).

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Fig. 1. Body weight changes in rainbow trout fed diet FM or PP



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Fig. 2. Sensory evaluation of cooked fillets at 5 days after death, results of the descriptive test (2= 2streplication, 2nd= 2 replication).

