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Simultaneous effects of nutritional and environmental factors on growth and flesh quality of *Perca fluviatilis* using a fractional factorial design study.

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Abstract

Fractional factorial design is a practical approach for studying multiple factors, with a minimum of experimental units. The objective of this work was to study the simultaneous effects of nutritional and environmental factors on both growth and flesh quality of common perch (*Perca fluviatilis*), a new inland aquaculture species. This study sought answering the two following questions: (i) which combinations of factors allow improving growth, food efficiency, and technological, sensorial and nutritional qualities? (ii) is it possible to simultaneously improve growth performances and flesh quality? In a first experiment, twelve factors (7 nutritional and 5 environmental factors) were each tested at 2 levels in a fractional factorial design in 24 independent recirculating 500 L tanks. The best 4 combinations identified in this first experiment were then validated in a second experiment. The first phase of the multifactorial approach used here allowed revealing emergent information: i) there is a combination of factors that allows reducing both the heterogeneity of the production and the losses of nitrates and phosphates, while preserving good characteristics of growth and quality of fillets; ii) it is possible to improve the quality of the aquatic production system, without decreasing significantly growth efficiency; iii) the effect of a given factor, even such an important one like diet, temperature or target biomass, depends on the levels of the other rearing factor levels, thus the usual reference optimum used for a given factor has no meaning theoretically and can be questioned according to the levels of the other factors which act altogether on the functioning of the rearing system.

The input factor combinations resulting in a significant enhancement of single output variables or several output variables were identified (e.g. improvement of feed efficiency, and/or fillet docosahexanoic acid content). Our results clearly demonstrate a strong interdependence of input factors into the animal rearing system, particularly between nutritional and environmental ones.

Keywords: Aquaculture; Nutrition; Growth; Quality; Fractional factorial design; System

1. Introduction

Numerous factors are known to influence both fish production and nutritional qualities of the finished product (e.g. fat level, levels of eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA)). Among these factors, several studies have focused on the effects of nutritional factors on growth and flesh quality of fish (e.g. Torstensen et al., 2001). However, the trophic environment constitutes only one of the elements of the fish rearing environment. Indeed, biological individuals and environmental factors define a system, which operates as an input and output transformation. Systems with biological components are complex because they are composed of numerous and various elements with high degrees of interactions (Weisbuch, 2000). Variations of input factors are responsible for modifications of both the system state and the value of the output variables. As in any biological system, these elements are interrelated, so that experimental approaches which take into account only one or a few factors do not fully allow (i) classifying the relative importance of these factors, (ii) evaluating their possible interactions and (iii) determining the combinations of factors that would be required to improve the features of either the production system or the final product or both.

A fractional factorial experimental design allows such a systemic approach, which includes two main phases: the first step corresponds to a screening of numerous and different factors and the second one aims at optimising the process using the highlighted factors of the first step. If the independent effect of each factor on output variables is usually relatively well known (e.g. the influence of temperature on growth), the influence of the combination between factors and their interactions are still largely unknown. It thus seems relevant to focus on these topics (1st phase of the method) and then search for optima.

In this study, the biological model used is perch, *Perca fluviatilis*. This species has been proposed for diversifying inland aquaculture production for human consumption. The rearing system is composed of recirculated water tanks to control the greatest number of environmental factors. The effects of 12 main factors (biotic and abiotic) were studied on 12 output variables during a first experiment. Then, the best 4 combinations identified in the first experiment were validated during a second experiment. This study sought answering the two following questions: (i) Are there combinations of factors (and their levels) which allow improving growth, food efficiency, technological, or sensorial and nutritional qualities? and (ii) Is it possible to improve simultaneously growth performances and flesh quality?

2. Materials and Methods

2.1. Experimental design

Thirty-six factors (likely to influence the aquatic system) have been studied by a meta analysis of bibliographical data (principal component analysis, 15 experiments, 33 variables and 172 experimental units) to select the main factors which should be studied in priority (unpublished data). The parameters of water quality have an effect on growth only when they reach extreme values. We measure them during the experimentation to prevent that they reach these extreme values. Thus they were not tested in this experiment. According to the results of this meta-analysis and the literature, twelve influencing factors, i.e., four nutritional factors at two levels defining 16 diets, three feeding factors and five environmental factors (Table 1) were tested in an initial experiment with a fractional factorial experimental design (Babiak et al., 2000; Ruohonen et al., 2001). The four nutritional factors are lipid content, dietary lipid source, protein source and astaxanthine enrichment. The two tested levels of each factor (Table 1) were defined from data available in the literature (Melard et al., 1996; Fontaine et al., 1997; Kestemont and Baras, 2001; Kestemont et al., 2001; Xu et al., 2001; Mathis et al., 2003). A full factorial study of 12 factors at 2 levels, entails 2^{12} (4,096) possible combinations. Fractional factorial studies have varying degrees of resolution, defining different aliasing structure (confounding between main effects and interactions effects; Butler, 2005). In the present study, a fractional factorial design of Resolution IV with 24 experimental units was selected. This experimental design enables an independent estimation of the constant terms and the main effects of factors as well as a group estimation of each of two-factor interactions (Chen and Cheng, 2000). Of the 4096 possible combinations, 24 were tested and thus the 4,072 remaining combinations were estimated *in silico* from the measured effects. The resolution IV used here was obtained by doubling its opposite, i.e. the resolution III of Plackett & Burman's design with 12 units at 2 levels (Kobilinsky and Monod, 1995). In practice, we detected discrepancies between the requested food and the actual food provided by the industrial (data not shown), thus we had to modify our experimental design, which resulted in the loss of both orthogonality and IV resolution. Consequently, factor effect coefficients were dependent, their estimation was less precise and there were some confusion between main effects and interactions. Consequently, the interpretation of the main factors effects will be realized in the form of hypotheses according to the probability that they correspond to main effects, alone, or to main effects confused with groups of interactions (thus they will not be presented here). Nevertheless, the first step of the study indicated above remained valid for both studying the global effect of the 12 combined factors on the 12 output variables and answering the two main questions above.

The first experiment was built without replication. In a second step, the reproducibility of the main results obtained in the first experiment was tested. Four factor combinations coming from the experiment 1 were chosen and tested again, with 4 replications.

2.2. Animals

All studies were conducted according to the French national legislation on animal care under a personal authorization to J. Brun-Bellut, delivered by the French Agricultural Ministry for conducting animal experimentation (Authorization 03890).

The perch used in the experiment 1, belonging to the same spawning, came from outdoor tanks (i.e. produced in natural conditions of temperature and photoperiod) located at the research station (CEFRA) at Tihange (Belgium). A single batch of 804 fish weighing between 30 g and 85 g (average 57.6 ± 0.5 g) was graded into 11 weight classes (5 g range). From these classes, 536 fish were distributed into the 24 experimental units (tanks), such that each unit contained fish of similar initial average weight, low or high initial weight heterogeneity and low (25 fish per tank) or high (42 fish per tank) total biomass (Target final biomass of 6 and 10 $kg.m^{-3}$). The target of maximum final biomass of 10 $kg.m^{-3}$ corresponds to the potential of these experimental units which are small and without contribution of oxygen. Each of the 24 experimental units was composed of a 500 L tank made of light blue PVC, operating independently in a recirculated circuit (Fontaine et al., 1996). These tanks were placed into four experimental rooms. Water temperatures were maintained either at 16 °C (air-conditioned rooms) or at 23 °C (heating resistors). Tanks were covered with opaque cages

80 cm high (isolating them from light and outside disturbances) and fitted with a 15 W neon light and a band automatic feeder.

At the end of the first experiment (116 days) fish were sacrificed by thermal shock combined with an overdose of anaesthetic (i.e. phenoxy-ethanol (3 mL L^{-1}) added to water at 0°C.). Measurements were carried out on 15 individuals randomly chosen in each tank in the population with a final weight ranging between the average ± 2 SD.

In the second experiment (129 days), fish at the larval stage were obtained from a fish farm in Lorraine (Pisciculture l'Huillier, Gellucourt, Moselle, France). They were then reared in our laboratory facilities until their mean weight was 38.3 ± 0.5 g. All other conditions are as the same to those presented above.

2.3. Diet

The 16 experimental diets corresponded to four crossed nutritional factors (lipid content, lipid source, protein source, astaxanthine enrichment (Table 2) with two levels for each factor. Two levels of feeding were established, 22.45% (limiting feeding) and 30.67% of body weight$^{-0.68}$ (ad lib feeding, Melard et al., 1996; Mathis et al., 2003). During the first experiment, growth was not very important (50 to 100g), so feeding rate remained unchanged. A regular adjustment of feeding quantities with time was made (three adjustments).

2.4. Measurements, calculations and analyses

Among the 47 outputs, only 12 which were the most explanatory in the principal component analysis are presented in the table 3. During rearing, water temperature and oxygen content were monitored daily after the first feeding period. Ammonium ion and nitrites ($N\text{-}NH^{4+}$ and $N\text{-}NO^{2-}$) were measured twice a week (Eaton et al., 1995) and nitrate and phosphate contents weekly. The contents ammonia, nitrites, nitrates, dissolved oxygen of each tank never exceeded the values threshold likely to disturb the growth of fish.

As both whole fish and flesh colour are important quality criteria for consumers, and Mathis et al. (2000) showed that reared and wild fish fillets are easily discriminated by colour differences, different measurements of colour were made on fresh fish using a chromameter (Minolta CR 300). The data obtained are expressed in Cartesian coordinates in the system L, a*, b* according to the method suggested by the International Lighting Committee (Kuehni 1976). Measurements were conducted on the inside surface, in the thickest antero-dorsal region of the fillet. Colour measurements were realized on two superimposed filets of the same fish (Mathis et al., 2003). Measures were taken twice in two parts separated by about 2 cm. For skin and fin colours, three series of duplicate measurements were realized: the first measure was done on the lower part of the caudal fin, the second on the 3rd stripe in the dorsal section, and the third between the 2nd and 3rd stripe, in the upper third of the fish.

Fillet samples were stored frozen under vacuum at –80 °C until analyses. Then, the fillets were ground and homogenized. Total lipids from diet and from tissues (muscle, liver, adipose tissue) were extracted in duplicate according to Folch et al. (1957) modified by Chen et al. (1981) using dichloromethane instead of chloroform as solvent. Fatty acid methyl esters from total lipids were prepared by acid-catalyzed transmethylation according to Santha and Ackman (1990) and analyzed using a gas chromatograph equipped with a DB Wax. Helium was used as carrier gas (0.9 mL min^{-1}). Fatty acid methyl esters were identified by comparison with known standard mixtures (Sigma, France) and quantified using a computer.

2.5. Statistical analyses

To analyze the global effect of combinations on output variables, we performed a first principal component analysis (PCA) using Spad v.6.5 software on the data comprising the system inputs (line = 24 combinations of rearing conditions; column = 47 outputs). Twelve outputs were selected and used in a final PCA (line = 24 combinations of rearing conditions; column = 12 outputs variables). This 12 outputs were (1) the final fish weight (Wf), (2) the produced biomass (Bio) and (3) the deltaCV, which reflect the volume and heterogeneity (recurring problems in aquaculture) of the production respectively (4) the feed efficiency (FE); (5) the gonadosomatic index (GSI) which reflects the gonadal development; (6) the fillet yield (Yff); (7) the losses of both nitrogen and (8) phosphorous (LN and LP, environmental variables); (9) the brightness of the fillet (Bf), (10) the caudal fin red-green component (a*c), (11) the lipid content in the fillet and (12) the DHA contents (%Lf , DHA; nutritional variables).

Each experimental combination of input factors was also assigned a global score of interest on the output variables. This global score was calculated from the results obtained on each of the 12 output variables of the system. The calculation was based on a transformation of the uncorrected result of each output, in centred reduced output.

The 4,072 non-tested combinations were estimated using aliases of significant estimations effects of factors and their interactions (Planor software, INRA).

Data of the second experiment were analysed by balanced one way ANOVA with 12 residual df (degrees of freedom) (GLM and Univariate Procedures, SAS® 9.1.3). Means were compared using the test of Newman-Keuls ($P < 0.05$) When non normality distribution was observed on the residual, Kruskal-wallis test was used.

3. Results

Fish survival between the 24 tanks was 93 ± 6%. It corresponds to usual results in our experimental conditions. Results of the experiment 1 are presented in the Table 4. The lowest variability is for the output DHA, Fillet final yield and especially Brightness fillet (CV ≤ 12 %). On the other hand the dispersal is very high for the following variables: caudal fin red-green component end, GSI, deltaCV and Loss of nitrogen (CV ≥ 78 %).

3.1. Results by combination

Results vary greatly according to the output considered. For example, combination C21 yielded the highest fillet level of DHA (51.1%), a desirable nutritional feature for human consumption, yet the growth performance of this group was very low (Table 4). The highest final fish weight was obtained in the combination C24, in which both the fillet lipid and DHA contents were also higher than the average of the 24 combinations. On the other hand, the nitrogen and phosphate losses were fairly high with an average fillet yield. The combination C1 had close characteristics and its 4th rank of produced biomass is obtained with a low level of initial biomass.

Combination C9 was among those that gave the highest growth (2^{d} highest fillet biomass) with the additional advantage of decreasing the variability of fish weight (-21%). On the opposite, combination C24 would be undesirable, because it increased the variability of fish weight by 42%. Furthermore, combination C9 had high fillet yields, and low nitrogenous water pollution. However, DHA fillet content of fish raised in the combination C9 was lower compared to the other combinations which had a high final weight.

3.2. Evaluation of the combinations, based on the global score of interest

The combination C9 is ranked 1st, particularly due to the large and homogeneous produced biomass, the high fillet yields and the low nitrogenous water pollution (Table 4). The combinations in 2d rank (Combination C8) had fairly similar results but with a high DHA content and a low produced biomass, due to the low level of initial biomass.

Combination C24, in 4th rank despite its 1st rank for growth, high feed efficiency and high fillet lipid content was penalized, like the combination C1, by both its high growth heterogeneity and its high nitrogenous water pollution. The combination C1 and C14 had the 5th and 7th produced biomass, respectively, despite a low level of initial biomass.

3.3. Evaluation of the global effect of combination by PCA

The plan 1-2 of the PCA explains 55% of the inertia (total variance). On the axis 1 (Fig. 1), the combinations C1, C7, C14, C16 and C24 were characterised by the vectors Bf, FE, Yff, LN, Wf, Bio, deltaCV and the modality 23°C and 16L/8D: they had high growth (Table 5) and high food efficiency, a brightness of the fillet higher than the mean, high final fillet yield except for C24, but the heterogeneity of growth increased during the experiment, and the nitrogenous losses were very high. On the opposite on this axis, the combinations C2, C4, C12, C13 and C19 were characterised by the vectors GSI, the modalities 16°C and 8L/16D: they had the best gonadic development and the opposite characters to the previous combinations.

On the axis 2 (Fig. 1), the combinations C3, C5, C9 and C11 were characterised by the vector Bf and the modality rapeseed oil lipid source (R): they had a brightness of the fillet higher than the mean (Table 6). On the opposite on this axis, the combinations C23 and C24 were characterised by the vectors LP, %Lf, DHA, deltaCV, Bio and the modality menhaden oil lipid source (M): they had high phosphorus losses, high lipid and DHA fillet contents with high increase of growth heterogeneity and high produced biomass, especially for the combination C24.

The plan 3-4 of the PCA explains 22% of the inertia. On the axis 3 (Fig. 2), the combinations C8, C10, C12, C16 were characterised by the vectors a*c, %Lf, the modality menhaden oil lipid source (M) and the modality 6 kg.m^{-3} target biomass (B4): they had a high red-green component caudal fin and a high fillet lipid content except for C16 (Table 7). In contrast on this axis, the combinations C17 and C22 were characterised by the vectors LP, the modalities rapeseed oil lipid source (R) and 10 kg.m^{-3} target biomass (B4): they displayed phosphorus losses higher than the mean.

On the axis 4 (Fig. 2), the combinations C9 and C8 were characterised by the vector Yff, DHA and a*c: they had a high fillet final Yield with **a** high red-green component of caudal fin and a high content of DHA except for C9 (Table 8). In contrast on this axis, the combinations C7 was characterised by the vectors deltaCV and LN even though C9 was characterised by GSI: C7 had higher increase of the weight heterogeneity and higher losses nitrogen even though C9 had very high gonadal development.

3.4. Temperature and diet effects

The simple fact of maintaining a high temperature (23°C) resulted in a blocking of sexual development (tank average GSI at 23°C = 1.2, n = 12 vs. 7.9 at 16°C, n= 12, Kruskal-Wallis test, P<0.05). When high rearing temperature was combined with a photoperiod of 16 hours of light, blocking was even more complete compared to the high temperature combined with a photoperiod of 8 hours of light (GSI = 0.5, n = 6 vs. 1.8, n=6, Kruskal-Wallis test, P<0.05). The only case where sexual development began was when a temperature of 16°C was combined with 8 hours of light (GSI = 11.4, n = 6 vs. 4.4 at 16°C and 8 hours of light, Kruskal-Wallis test, $P<0.05$). As a consequence fillet yields strongly decreased (23.1 vs. 33.3, Kruskal-Wallis test, $P<0.05$). A temperature of 23°C was found in the combinations that gave the 9 best results in terms of final fish weight (Table 4). On the other hand, three other combinations at 23°C (i.e. combinations C17, C3 and C22) resulted in lower weights than average. Finally, higher rearing temperature was associated with lower flesh lipid content (r = -0.6, n = 12).

Because of the use of 16 different diets among the 24 combinations tested, eight diets were used in duplicate (Fig. 3). For example, diet 1 was distributed in the combinations C7 and C15. Fish raised in these combinations were widely divergent with respect to final weight, feed conversion efficiency, and fillet lipid content. Others also differed on DHA content (e.g. diet 3, 6, 9, 15). For these variables, an ANOVA on the diet factor (ddf factor=7 vs ddf residual=8) showed no significant difference while the distances between averages were raised (31 in 72 % of the average). The within variability was very important (30 in 83 % of the total variability) which demonstrates the dominating effects of the rearing factors on the diet effects.

3.5. Reproducibility of the results

The second experiment was initiated to confirm the reproducibility of the initial results and thus four combinations were tested (Table 9, Fig. 4). These combinations were C9 and C24 from the first experiment, plus two "calculated" combinations, resulting from the extrapolation of the tested combinations of the first study. Combination C24 was selected because it had given both the highest individual live weights and produced biomass, and both high fillet lipid and DHA contents. Combinations C9 was interesting because it had strongly limited the heterogeneity of production (compared to the beginning of the experiment), and also displayed low nitrogen loss, despite a high rearing density. The third combination (Cs/n) optimized the ratio average on variance (signal noise ratio, Box et al., 1988) for final live weight, GSI, fillet yield, brightness, and caudal fin red green component. The fourth combination (Cest) was selected as it was the best among the 4072 calculated (untested) combinations. These calculations for an output variable used the average of this variable, the effect of the significant factors and their interactions calculated with the experimental matrix. This approach was carried out for the main variables which characterised growth (weight, heterogeneity of the weight and biomass), technological (fillet yield) and nutritional quality (% Lipids, n-3/n-6 ratio). The selected combination was calculated to approach target values fixed on each selected output variable.

Concerning the physiological state of fish, a temperature at 23°C coupled to a photoperiod of 16L:8D blocked sexual maturity as in the experiment 1, whatever the combination of other tested input factors. C24, which maximized growth performance during the first multifactorial experiment, was confirmed in this second experiment (best results for the final weight, feed efficiency, produced biomass, fillet lipid and DHA contents, Fig. 4). Combination C9, selected for its low final weight coefficient of variation in experiment 1, also showed this particular characteristic in the second experiment, while resulting in a high fillet biomass production (Fig. 4). Comparing to the two other combinations, Cs/n and Cest have not particular characteristics.

4. Discussion

The average specific growth rate in our two experiments (i.e. 0.4 to 0.6% d^{-1}) was lower than most of the values already described for perch (Baras et al., 2000; Mandiki et al., 2004), albeit these authors worked with lower average weight fish. Other factors such as the choice of individuals in the population at the beginning of the experiment, and fairly low rearing densities could also explained these lower performances. The growth rates were lower than those initially expected, thus the proposed food was not always entirely consumed, even with the lowest rate. On the other hand inter combination growth rates variability remained high (CV=31%) and a technology transfer of these main results in industrial rearing conditions with a high SGR (SGR>3%) and high biomass confirmed them (AQS F7 2001 report, Ministère de la Recherche, France). Thus we can conclude that the main results of this study are not affected by the experimental low growths.

4.1. Aims to improve fish process

The improvement of fish production system could concern either total production, productivity, environmental impacts, technological or nutritional qualities of final products. For species which, like perch, stored all their energy in the viscera, estimation of growth performances based on the produced fillet is therefore more relevant at the production level than performances based on total weight. Limiting the heterogeneity of the final product would also be an asset. The current environment of animal production is influenced by both concerns about the environmental impact of agricultural systems, the animal welfare and the requirements of the society. For these reasons, decrease of N and P discards in effluvia from production systems, or restricted density of animals in rearing systems, may be added to the list of aims to be taken into account. The product must be attractive, and in this case the colour features of the whole fish and fillets are influent factors for consumers. Finally, fatty acid composition of food has recently become a very high issue for consumers, since some fatty acids cause potential health problems (e.g. trans fatty acids) while others display possible health benefits (e.g. long-chain-polyunsaturated fatty acids). All these features could be taken into account with such a multifactorial approach.

The data produced here enables the selection of pertinent production input variables according to a given set of specific aims. For example, combinations C24 and C9 provided the best growth performance and low growth heterogeneity, respectively. If the main objective is to have a high weight and nutritional value, combination C24 should be chosen. If the aim is to obtain a high produced biomass with more homogeneous products and better fillet yield while limiting the nitrogenous releases, the combination C9 should be used. For high fillet DHA content, it would be necessary to choose the combination C21. In terms of efficiency of the rearing system, the combination C9 had very competitive growth performances, with decreased weight heterogeneity, high fillet yield and low nitrogenous losses. Its fillet lipid and DHA levels were near the average of the experiment. The combination C21 had the highest DHA content, but this was gained at the expense of poorer growth than the average. It thus seems possible to have excellent growth and production characteristics without too much sacrifice of DHA levels, yet not vice versa.

Solutions like the combination C24 or C9 were not unique, the combinations C1, C7, C16 and C14 appeared near to C24 on the axis 1 (Fig. 1) and had the same major characteristics. For example, in a context of preserving fish welfare or extensive livestock picture, the combinations C14 or C16 could be an alternative since the fish biomass per unit of volume is lower than results obtained with the combinations C7 and C24. Likewise the combination C8 represents an alternative with low rearing density as compared to the combination C9 (Fig. 2).

4.2. Interactions among production inputs

Each tested combination in the current study represents a complex set of linked inputs. To our knowledge, this is the first multifactorial study ever done on fish rearing to test simultaneously so many factors. Thus, it is not easy to compare our results to other more traditional results obtained in fish or other animal species.

Torstensen et al. (2001) realized the same approach but with only nutritional factors on Atlantic salmon (*Salmo salar*) for the investigation of effects of dietary lipid content and pro- and antioxidants on lipid composition: the FA composition did not differ significantly between the 16 diets and none of the measured responses were affected significantly by the two-factor interaction effects. Our results showed that nutritional effects are strongly dependent to other environmental factors. They revealed potential interactions between nutritional and non-nutritional factors. Different rearing conditions may alter chiefly the outcomes in groups of fish fed identical diets. One of the most striking results obtained in the present study is the degree to which performance of any diet was conditioned by other features of the production system. It was indeed possible to observe either excellent or bad growth for a given diet, or to obtain fish raised on a given diet displaying widely divergent product quality or nutritional features. Interactions between nutritional and environmental factors as highlighted in the present study may explain some contradictory results in the literature where only one or a few factors are tested (e.g. López-Bote et al., 2001, Kaushik et al., 2004).

The interdependence of the many input factors was also demonstrated concerning the relation usually proposed between temperature and growth. The temperature accepted for optimal growth of perch is 23°C (Mélard et al., 1996), but our results stemming from a multifactorial approach allowed demonstrating that this is conditional and dependent on other factors. Indeed, some combinations at 16°C (combinations C6 and C23) yielded very high produced biomass (>1070 g). By contrast, combinations C3, C17, and C22, with the same objective of target biomass, at 23°C, showed inferior growth performance (<820g, ANOVA1 P<0.05). Thus the best combinations at 16°C were better than many combinations at 23°C, and thus clearly demonstrated that the effect of any given factor, such as temperature, is dependent on the levels of other factors. Nevertheless, low temperature did not block sexual development and this effect was more marked with a limited photoperiod, in accordance with the available literature (Migaud et al., 2002). There is, in this case a risk of gonadal development that could compete with growth.

In the future, other factors may be tested. For example, light intensity has been considered to play a little role in the performance of growing perch (Jourdan et al., 2003), however this does not guarantee that this factor cannot interact with others in the same manner as observed here for temperature. More than two levels for each factor could be tested, taking into account that the effect of each factor is not always linear. Our fractional factorial approach with multiple factors at two levels represents the first step of screening, in a series of more advanced and detailed experiments to find the optimal operational conditions. This could require either testing the most relevant factors but with a number of levels higher than two (second phase by response surface design). Another way would be by modelling the results of these effects to simulate the behaviour of this system of rearing and carry out in this way a virtual experiment to facilitate targeting specific methods to be checked *in vivo*. The analysis of significant effects and interactions would thus allow taking into account the relevance of tested levels in this initial approach.

5. Conclusion

This study showed that it is possible to improve the quality of the aquatic production system, without too much decreasing growth efficiency. The multifactorial approach used here allowed revealing emergent information: i) there is a combination of the factors particularly interesting which enable reducing the heterogeneity of the production and the losses of N et P, while preserving good characteristics of growth and quality of fillets; ii) the effect of a given factor, even such an important one like diet, temperature or target biomass, depends on the levels of the other rearing factor levels, thus the usual optima for a given factor have no meaning theoretically and can be questioned according to the levels of the other factors which act on the functioning of the rearing system.

The generic multifactorial approach applied here to an aquaculture system could be used to other reared animal species.

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Fig. 1. Projection of outputs and combinations (C1 to C24) on the plan 1-2 of the principal components analysis of the table of the 12 output variables and 24 combinations. Axis 1 (a1) inertia 42%, represents brightness of fillet (Bf), fillet final Yield (Yff), feed efficiency (FE), final body weight (Wf), loss nitrogen (LN), producted biomass (Bio=Biomass final –Biomass initial), differences of coefficient of variation of body weight (deltaCV=CVfinal-CVinitial), Temperature 23°C, Photoperiod 16L/8D and in contrast gonado somatic index (GSI), loss phosphorus (LP), Temperature 16°C and Photoperiod 8L/16D. Axis 2 (a2) inertia 14%, represents brightness of fillet (Bf), rapeseed oil food Lipid source (R) and in contrast fillet lipid content (%Lf), DHA and menhaden oil food Lipid source (M).The characters in bold are those that are carried by the axes 1 or 2. ■ combination contributing to the axis 1, □ ouput modality contributing to the axis 1, ▲ combination contributing to the axis 2, △ ouput modality contributing to the axis 2, ◆ combination contributing to the axis 1 and 2. The size of the symbols is proportional to the contribution of the variables or modality in the construction of the axis.

Fig. 2. Projection of outputs and combinations (C1 to C24) on the plan 3-4 of the principal components analysis of the table of the 12 output variables and 24 combinations. Axis 3 (a3) inertia 11%, represents red-green component caudal fin (ac), fillet lipid content (%Lf), menhaden oil food Lipid source (M) and objective of final biomass 4kgm^{-3} (B4) and in contrast loss phosphorus (LP), rapeseed oil food Lipid source (R) and objective of final biomass 12kgm^{-3} (B12). Axis 4 (a4) inertia 11%, represents fillet final Yield (Yff) and DHA and in contrast gonado somatic index (GSI), loss nitrogen (LN) and differences of coefficient of variation of body weight (deltaCV=CVfinal-CVinitial). The characters in bold are those that are carried by the axes 1 or 2. ■ combination contributing to the axis 1, □ ouput modality contributing to the axis 1, ▲ combination contributing to the axis 2, △ ouput modality contributing to the axis 2, ◆ combination contributing to the axis 1 and 2. The size of the symbols is proportional to the contribution of the variables or modality in the construction of the axis.

Fig. 3. Evolution of the output according to the replicat for 8 feed used in duplicat. (A), final weight; (B) feed efficiency; (C) fillet lipid content; (D) fillet DHA content.

Fig. 4. Results of the experiment 2. C24 = factor combinations which bester body weight; C9 = factor combinations which lower body weight heterogeneity; Cs/n = signal noise ratio; Cest = estimated combination from 4072 combinations; Wf = final weight; Biof = fillet biomass; CVwf = coefficient of variation of final weight; Yff = fillet final yield; FE = food efficiency; %Lf = fillet lipid content (%); DHA = DHA lipid content (%); CV RMSE = Coefficient of variation of root mean square error (%). Means without a common superscript differ ($P<0.05$).

Table 1. The twelve influencing factors evaluated in the present rearing.

Factor	Level +1	Level -1
Temperature (°C)	23	16
Ration level (%biomass)	Low: 22.45.Weight$^{-0.68}$	High: 30.67.Weight$^{-0.68}$
Lipid content of diet (%)	21	17
Protein source of diet	Fish meal + Soybean meal + Wheat	Fish meal + Wheat
Lipid source of diet	Rapeseed oil	Menhaden oil
Astaxanthine enrichment (%)	0.4	0
Target final biomass (kg.m^{-3})	10	6
Feeding mode	2 meals	continuous
Initial weight heterogeneity (CV initial weight %)	30	15
Photoperiod (Light:Darkness)	16L:8D	8L:16D
Light spectra	Industrial white	Pinkish
Feeding day.week^{-1}	7	6

Table 2. Diet composition (%)

N° Diet	Fish meal	Soybean meal	Wheat	Menhaden oil	Rapeseed oil	Astaxan-thine	Wheat meal	Vitaminized premix*	Lecithin
1	43	30	14.9	.	10.5	.	0.78	0.42	0.4
2	43	30	14.9	10.5	.	.	0.78	0.42	0.4
3	43	30	14.5	.	10.5	0.4	0.78	0.42	0.4
4	43	30	14.5	10.5	.	0.4	0.78	0.42	0.4
5	43.5	30	8.4	.	16.5	.	0.78	0.42	0.4
6	43.5	30	8.4	16.5	.	0.4	0.78	0.42	0.4
7	43.5	30	8	.	16.5	0.4	0.78	0.42	0.4
8	43.5	30	8	16.5	.	.	0.78	0.42	0.4
9	60	.	28.4	.	10	0.4	0.78	0.42	0.4
10	60	.	28.4	10	.	0.4	0.78	0.42	0.4
11	60	.	28	.	10	.	0.78	0.42	0.4
12	60	.	28	10	.	0.4	0.78	0.42	0.4
13	61	.	21.9	.	15.5	0.4	0.78	0.42	0.4
14	61	.	21.9	15.5	.	.	0.78	0.42	0.4
15	61	.	21.5	.	15.5	0.4	0.78	0.42	0.4
16	61	.	21.5	15.5	.	0.4	0.78	0.42	0.4

* Vitamin = 0.3%; Minerals = 0.12%. Detailed composition (identical between all feed tested) not available due to industrial property.

Table 3. Measured output variables

Growth variables (mean by tank)
Wf = final body weight (g)
Bio = produced biomass = (Wf - Wi)number of fish
deltaCV = Coefficient of variation of final body weight (%) - Coefficient of variation of initial body weight (%)
Physiological variables (mean by tank)
GSI = Gonado somatic index = 100gonad weight . We^{-1} (%)
Feeding variables (mean by tank)
FE = Food efficiency = g biomass gain . g $food^{-1}$
Technological variables (mean by tank)
Yff = fillet final Yield = 100fillet Weight final . Wf^{-1}
Environmental variables (mean by tank)
LN = Loss nitrogen = g distributed nitrogen – (g N biomass + g N in water)
LP = Loss phosphorus = g phosphorus distribute – (g biomass . %P + g P in water)
Colour variables (mean by tank)
Bf = Brightness fillet
a*c = caudal fin red-green component
Nutritional variables (mean by tank)
%Lf = fillet lipid content (%)
DHA = docosahexanoic acid = C22:6(n-3)ΣFatty $Acid^{-1}$ (%)

Table 4. Results for each of the 24 combinations (C1-C24)

Combination of the factors	Diet	Temperature (°C)	Ration level	Lipid content of diet (%)	Protein Source	Lipid source	Astaxanthine enrichment (%)	Target final biomass (kg/m3)	Feeding mode	Initial weight heterogeneity (CV%)	Photoperiod (Light H)	Light spectra	Feeding day (day/week)	Final body weight (Wf, g)	Coefficient of Variation final - Coefficient variation initial (deltaCV)	Producted biomass (Bio, g)	Feed efficiency (FE, g gain/g food)	Gonado somatic index (GSI)	Fillet final Yield (Yff, %)	Loss phosphorus (LP, g)	Loss nitrogen (LN, g)	Brightness fillet (Bf)	Red-green component caudal fin (a*c)	Fillet lipid content (%Lf)	DHA (%)	Global Score	Rank of the global note
c24	14	23	L	21	F	M	0	10	2m	30	16	W	7	134.1	13	2360	0.62	0.5	43.9	38	558	43.4	4.4	1.62	41.0	6.2	4
c1	6	23	L	21	FS	M	0	6	C	15	16	P	6	123.8	14	1082	0.62	0.6	45.5	15	555	43.0	2.2	1.49	38.9	3.6	5
c14	3	23	H	17	FS	R	0.4	6	2m	30	16	P	7	116.9	9	1057	0.61	0.6	45.5	17	695	43.9	17.2	1.06	33.9	2.9	6
c16	16	23	H	21	F	M	0.4	6	C	15	16	W	7	116.7	5	893	0.74	0.5	44.8	16	567	43.1	25.8	1.37	43.7	7.6	3
c9	11	23	L	17	F	R	0.4	10	C	30	16	P	6	112.8	-7	1482	0.53	0.4	46.4	31	5	43.1	26.1	1.11	38.0	8.2	1
c7	1	23	H	17	FS	R	0	10	2m	15	16	W	6	106.5	20	1448	0.55	0.6	44.3	22	1035	43.5	9.1	1.16	36.7	-1.6	16
c18	10	23	L	17	F	M	0	6	2m	15	8	P	7	103.2	8	750	0.40	1.5	47.3	37	200	42.8	2.3	1.47	42.8	1.5	9
c8	8	23	L	21	FS	M	0.4	6	2m	30	8	W	6	102.2	0	577	0.42	1.8	46.9	20	78	43.1	23.3	1.49	45.9	7.6	2
c11	9	23	H	17	F	R	0	6	C	30	8	W	6	99.3	5	575	0.57	2.0	45.8	15	335	43.5	1.9	1.21	36.1	1.5	10
c6	7	16	H	21	FS	R	0.4	10	C	30	16	W	6	94.5	2	1074	0.44	3.2	43.7	26	37	42.0	21.5	1.53	38.3	2.6	7
c5	13	16	H	21	F	R	0	6	2m	30	16	P	6	92.2	8	248	0.48	3.7	43.7	19	62	43.7	4.0	1.41	33.1	-0.9	13
c23	8	16	L	21	FS	M	0.4	10	2m	15	16	P	7	92.0	14	1115	0.24	5.3	43.4	47	63	41.0	14.6	1.66	43.8	-3.4	20
c10	12	16	L	17	F	M	0.4	6	2m	15	16	W	6	90.6	3	150	0.34	2.9	44.8	34	-146	41.7	23.2	1.90	40.5	2.0	8
c17	3	23	L	17	FS	R	0.4	10	C	15	8	W	7	88.7	8	804	0.29	2.1	45.9	64	-34	43.2	12.4	1.00	41.2	-2.9	19
c4	2	16	L	17	FS	M	0	10	2m	30	8	P	6	88.4	-1	773	0.24	11.1	42.0	49	17	41.5	3.7	1.40	39.5	-6.3	22
c15	1	16	L	17	FS	R	0	6	C	30	16	W	7	87.5	2	464	0.25	5.9	43.7	32	30	42.8	5.2	1.56	37.7	-1.9	18
c3	15	23	H	21	F	R	0.4	10	2m	15	8	P	6	87.1	7	787	0.38	1.9	44.9	27	153	43.3	22.3	1.12	37.7	0.4	11
c20	9	16	H	17	F	R	0	10	C	15	16	P	7	86.9	9	768	0.34	5.1	44.1	26	8	42.4	0.2	1.36	45.4	-1.4	14
c12	4	16	H	17	FS	M	0.4	6	C	15	8	P	6	86.8	4	422	0.37	13.8	40.6	20	34	42.2	26.6	1.65	44.6	-0.7	12
c22	5	23	H	21	FS	R	0	10	C	30	8	P	7	86.4	5	813	0.32	1.6	46.5	37	73	42.5	2.5	1.09	41.1	-1.7	17
c19	15	16	L	21	F	R	0.4	6	C	30	8	P	7	84.2	-3	286	0.21	12.4	39.9	34	26	42.2	14.7	1.36	28.6	-8.1	24
c21	6	16	H	21	FS	M	0	6	2m	15	8	W	7	83.5	2	408	0.30	11.0	41.2	25	8	42.8	5.9	1.43	51.1	-1.4	15
c2	13	16	L	21	F	R	0	10	C	15	8	W	6	78.1	0	347	0.18	9.5	40.3	45	44	41.9	4.0	1.56	37.9	-7.8	23
c13	11	16	H	17	F	R	0.4	10	2m	30	8	W	7	77.9	9	538	0.21	10.8	40.7	40	5	42.4	26.5	1.37	38.6	-5.9	21
							Mean							96.7	5.6	801	0.4	4.5	44.0	30.6	184	42.7	12.5	1.4	39.8		
							SD							14.9	5.9	486	0.2	4.4	2.2	12.5	288	0.7	9.7	0.2	4.7		
							CV%							15	105	61	39	97	5	41	157	2	78	16	12		

Ration level: L= low, H=height; Protein source: F=fish+wheat, FS=fish+wheat+soybean meal; Lipid source: M=menhaden oil, R= rapeseed oil; Feeding mode: 2m=2 meals, C=continuous; Light spectra: W= Industrial white, P=Pinkish.

Global Score: note of interest for each combination from the results obtained on each of 12 output variables of the system. Rank: rank of combinations on the global score.

The grey lines correspond to a study temperature of 23°C.

Table 5. Characteristics of the combinations by the axis 1

Combination of the factors	Temperature (°C)	Photoperiod (Light H)	**Final body weight (Wf, g)**	**Coefficient of Variation final - Coefficient variation initial (deltaCV)**	**Producted biomass (Bio, g)**	**Feed efficiency (FE, g gain/g food)**	**Gonado somatic index (GSI)**	**Fillet final Yield (Yff, %)**	Loss phosphorus (LP, g)	**Loss nitrogen (LN, g)**	**Brightness fillet (Bf)**	Red-green component caudal fin (a*c)	Fillet lipid content (%Lf)	DHA (%)
c1	23	16	**123.8**	**14**	**1082**	**0.62**	0.6	**45.5**	15	**555**	**43.0**	2.2	1.49	38.9
c7	23	16	**106.5**	**20**	**1448**	**0.55**	0.6	**44.3**	22	**1035**	**43.5**	9.1	1.16	36.7
c14	23	16	**116.9**	**9**	**1057**	**0.61**	0.6	**45.5**	17	**695**	**43.9**	17.2	1.06	33.9
c16	23	16	**116.7**	**5**	**893**	**0.74**	0.5	**44.8**	16	**567**	**43.1**	25.8	1.37	43.7
c24	23	16	**134.1**	**13**	**2360**	**0.62**	0.5	**43.9**	38	**558**	**43.4**	4.4	1.62	41.0
c2	16	8	78.1	0	347	0.18	**9.5**	40.3	45	44	41.9	4.0	1.56	37.9
c4	16	8	88.4	-1	773	0.24	**11.1**	42.0	49	17	41.5	3.7	1.40	39.5
c12	16	8	86.8	4	422	0.37	**13.8**	40.6	20	34	42.2	26.6	1.65	44.6
c13	16	8	77.9	9	538	0.21	**10.8**	40.7	40	5	42.4	26.5	1.37	38.6
c19	16	8	84.2	-3	286	0.21	**12.4**	39.9	34	26	42.2	14.7	1.36	28.6
Mean			96.7	6	801	0.40	4.5	44.0	31	184	42.7	12.5	1.39	39.8
SD			14.9	6	486	0.16	4.4	2.2	13	288	0.7	9.7	0.22	4.7

Bold characters correspond to the essential characteristics of combinations.

Table 6. Characteristics of the combinations by the axis 2

Combination of the factors	Lipid source	Final body weight (Wf, g)	**Coefficient of Variation final - Coefficient variation initial (deltaCV)**	**Producted biomass (Bio, g)**	Feed efficiency (FE, g gain/g food)	Gonado somatic index (GSI)	Fillet final Yield (Yff, %)	**Loss phosphorus (LP, g)**	Loss nitrogen (LN, g)	**Brightness fillet (Bf)**	Red-green component caudal fin (a*c)	**Fillet lipid content (%Lf)**	**DHA (%)**
c3	R	87	7	787	0.38	1.9	44.9	27	153	**43.3**	22.3	1.12	37.7
c5	R	92	8	248	0.48	3.7	43.7	19	62	**43.7**	4.0	1.41	33.1
c9	R	113	-7	1482	0.53	0.4	46.4	31	5	**43.1**	26.1	1.11	38.0
c11	R	99	5	575	0.57	2.0	45.8	15	335	**43.5**	1.9	1.21	36.1
c23	M	92	**14**	**1115**	0.24	5.3	43.4	**47**	63	41.0	14.6	**1.66**	**43.8**
c24	M	134	**13**	**2360**	0.62	0.5	43.9	**38**	558	43.4	4.4	**1.62**	**41.0**
Mean		96.7	6	801	0.40	4.5	44.0	31	184	42.7	12.5	1.39	39.8
SD		14.9	6	486	0.16	4.4	2.2	13	288	0.7	9.7	0.22	4.7

Bold characters correspond to the essential characteristics of combinations.

Table 7. Characteristics of the combinations by the axis 3

Combination of the factors	Lipid source	Target final biomass (kg/m3)	Final body weight (Wf, g)	Coefficient of Variation final - Coefficient variation initial (deltaCV)	Producted biomass (Bio, g)	Feed efficiency (FE, g gain/g food)	Gonado somatic index (GSI)	Fillet final Yield (Yff, %)	**Loss phosphorus (LP, g)**	Loss nitrogen (LN, g)	**Brightness fillet (Bf)**	Red-green component caudal fin (a*c)	**Fillet lipid content (%Lf)**	DHA (%)
c8	M	6	102.2	0	577	0.42	1.8	46.9	20	78	**43.1**	23.3	**1.49**	45.9
c10	M	6	90.6	3	150	0.34	2.9	44.8	34	-146	**41.7**	23.2	**1.90**	40.5
c12	M	6	86.8	4	422	0.37	13.8	40.6	20	34	**42.2**	26.6	**1.65**	44.6
c16	M	6	116.7	5	893	0.74	0.5	44.8	16	567	**43.1**	25.8	**1.37**	43.7
c17	R	10	88.7	8	804	0.29	2.1	45.9	**64**	-34	43.2	12.4	1.00	41.2
c22	R	10	86.4	5	813	0.32	1.6	46.5	**37**	73	42.5	2.5	1.09	41.1
Mean			96.7	6	801	0.40	4.5	44.0	31	184	42.7	12.5	1.39	39.8
SD			14.9	6	486	0.16	4.4	2.2	13	288	0.7	9.7	0.22	4.7

Bold characters correspond to the essential characteristics of combinations.

591 **Table 8.** Characteristics of the combinations by the axis 4

Combination of the factors	Final body weight (Wf, g)	**Coefficient of Variation final - Coefficient variation initial (deltaCV)**	Producted biomass (Bio, g)	Feed efficiency (FE, g gain/g food)	**Gonado somatic index (GSI)**	**Fillet final Yield (Yff, %)**	Loss phosphorus (LP, g)	**Loss nitrogen (LN, g)**	Brightness fillet (Bf)	**Red-green component caudal fin (a*c)**	Fillet lipid content (%Lf)	**DHA (%)**
c8	102.2	0	577	0.42	1.8	**46.9**	20	78	43.1	**23.3**	1.49	**45.9**
c9	112.8	-7	1482	0.53	0.4	**46.4**	31	5	43.1	**26.1**	1.11	38.0
c7	106.5	**20**	1448	0.55	0.6	44.3	22	**1035**	43.5	9.1	1.16	36.7
c19	84.2	-3	286	0.21	**12.4**	39.9	34	26	42.2	14.7	1.36	28.6
Mean	96.7	6	801	0.40	4.5	44.0	31	184	42.7	12.5	1.39	39.8
SD	14.9	6	486	0.16	4.4	2.2	13	288	0.7	9.7	0.22	4.7

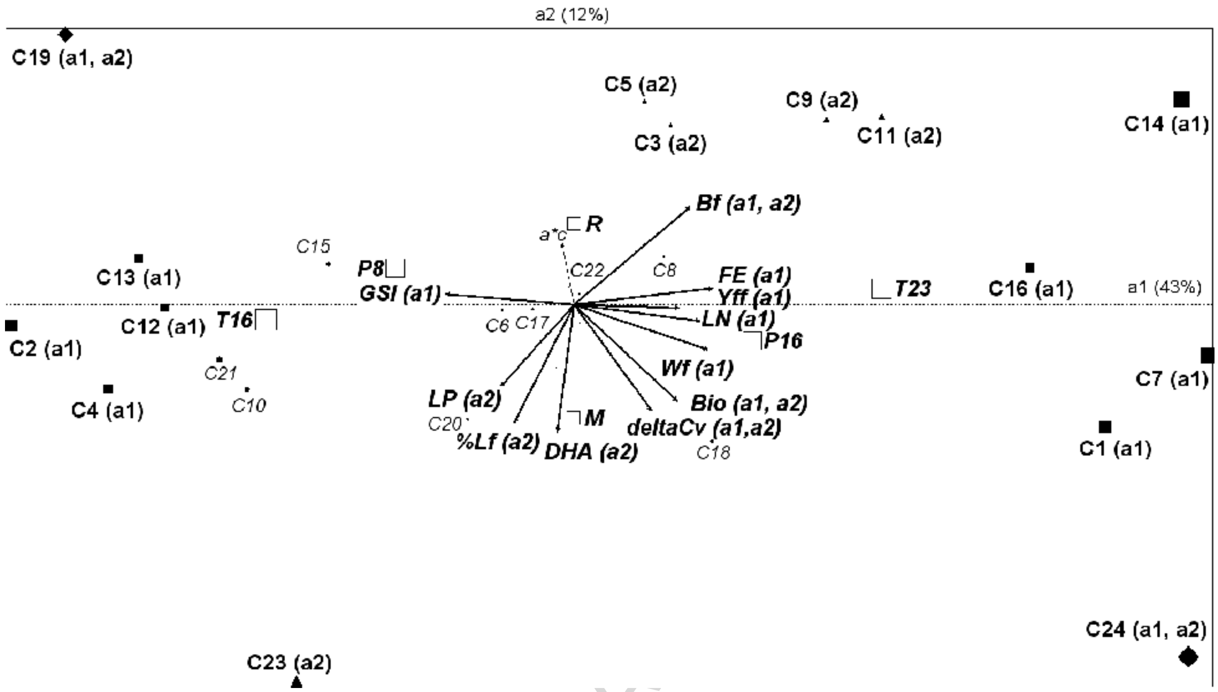
592

593 Bold characters correspond to the essential characteristics of combinations.

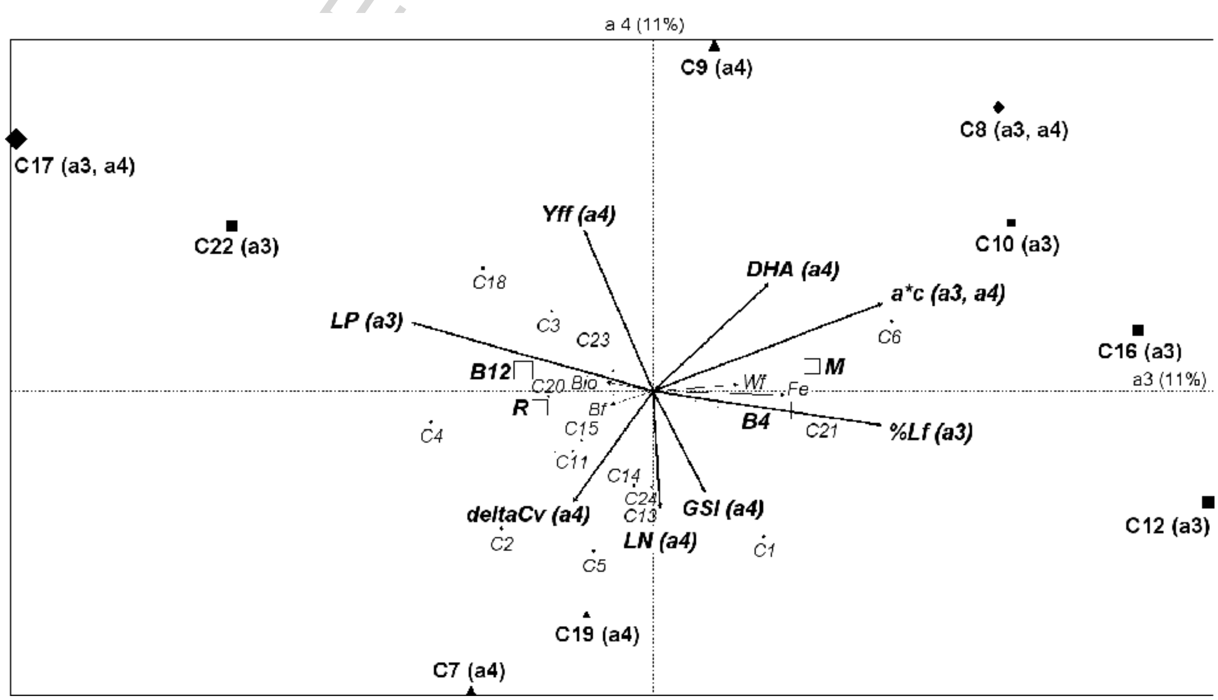
Table 9. Level of every factor tested in experimentation 2

Combination of the factors	Diet	Temperature (°C)	Ration level	Lipid content of diet (%)	Protein Source	Lipid source	Astaxanthine enrichment (%)	Target final biomass (kg/m3)	Feeding mode	Initial weight heterogeneity (CV%)	Photoperiod (Light H)	Light spectra	Feeding day (day/week)
c24	14	23	L	21	F	M	0	10	2m	30	16	W	7
c9	11	23	L	17	F	R	0.4	10	C	30	16	P	6
Cs/n	13	23	L	21	F	R	0	10	C	30	16	P	7
Cest	10	23	L	17	F	M	0	10	C	30	16	W	6

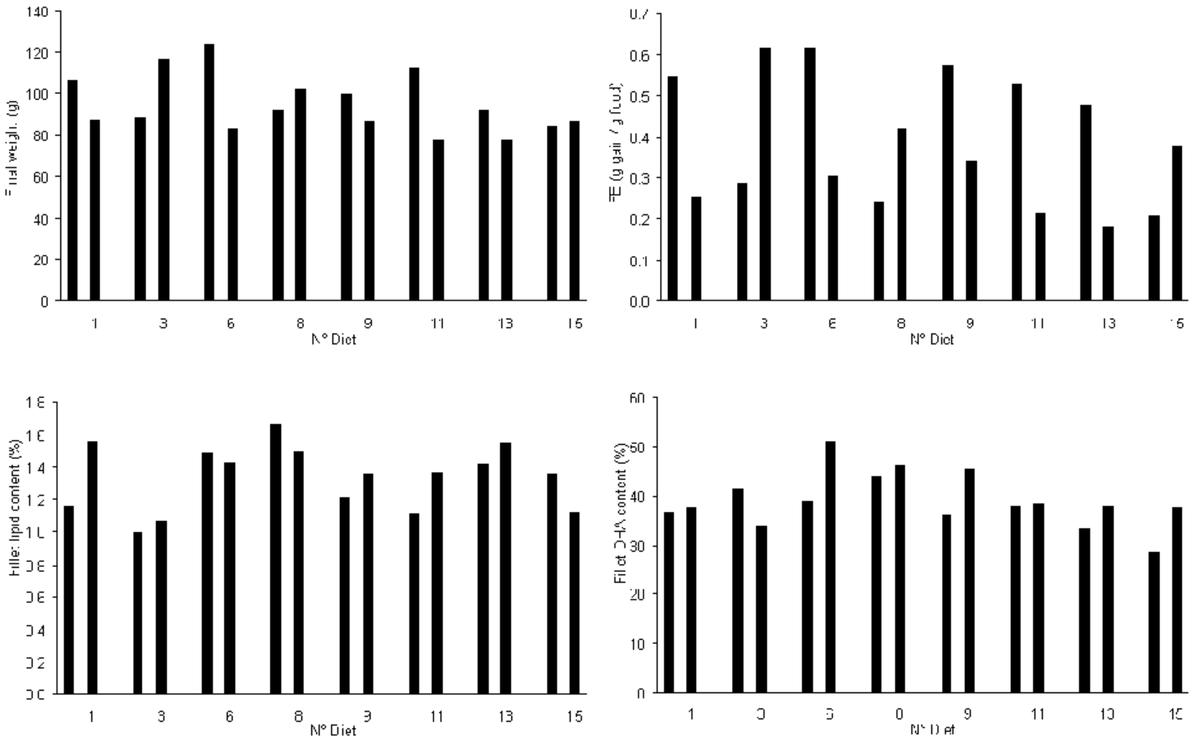
Ration level: L= low; Protein source: F=fish+wheat; Lipid source: M=menhaden oil, R= rapeseed oil; Feeding mode: 2m=2 meals, C=continuous; Light spectra: W= Industrial white, P=Pinkish.

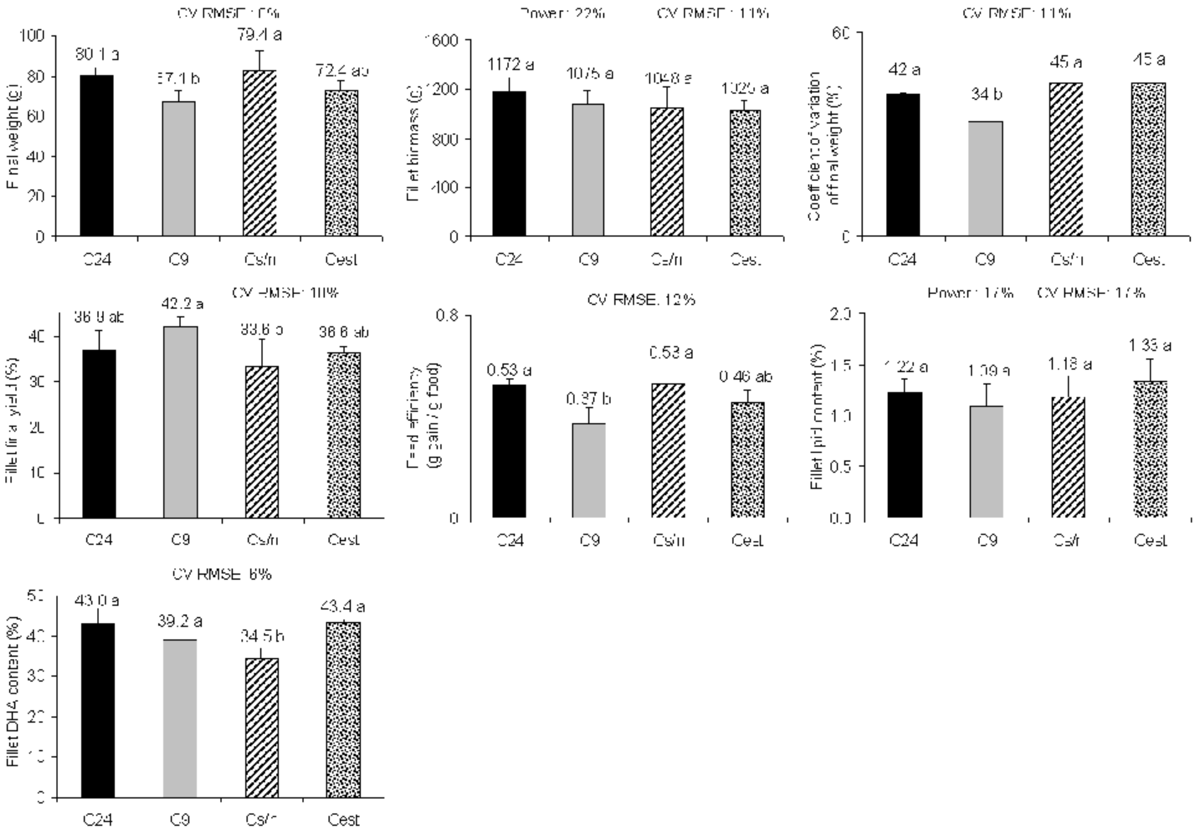


a2 (12%)
a1 (43%)
C19 (a1, a2)
C5 (a2)
C9 (a2)
C14 (a1)
C3 (a2)
C11 (a2)
Bf (a1, a2)
R
a*c
C15
C22
C8
P8
FE (a1)
C13 (a1)
GSI (a1)
Yff (a1)
T23
C16 (a1)
C12 (a1)
T16
C6
C17
LN (a1)
P16
C2 (a1)
C21
Wf (a1)
C7 (a1)
C10
LP (a2)
Bio (a1, a2)
C4 (a1)
M
deltaCv (a1,a2)
C20
%Lf (a2)
DHA (a2)
C18
C1 (a1)
C24 (a1, a2)
C23 (a2)



a 4 (11%)
a3 (11%)
C9 (a4)
C8 (a3, a4)
C17 (a3, a4)
Yff (a4)
C22 (a3)
C10 (a3)
DHA (a4)
C18
a*c (a3, a4)
LP (a3)
C3
C6
C23
C16 (a3)
M
B12
C20
Bio
Wf
Fe
R
Bf
B4
C15
C21
%Lf (a3)
C4
C11
C14
C24
GSI (a4)
deltaCv (a4)
C13
C12 (a3)
C2
LN (a4)
C1
C5
C19 (a4)
C7 (a4)


N° Diet
N° Diet
N° Diet


CV RMSE: 15%
Final weight (g)
80.1 a
67.1 b
79.4 a
72.4 ab
Power: 22%
CV RMSE: 11%
Fillet biomass (g)
1172 a
1075 a
1048 a
1025 a
CV RMSE: 11%
Coefficient of variation of final weight (%)
42 a
34 b
45 a
45 a
CV RMSE: 10%
Fillet final yield (%)
36.9 ab
42.2 a
33.6 b
36.6 ab
CV RMSE: 12%
Feed efficiency (g gain / g food)
0.53 a
0.37 b
0.53 a
0.46 ab
Power: 17%
CV RMSE: 17%
Fillet lipid content (%)
1.22 a
1.09 a
1.18 a
1.33 a
CV RMSE: 6%
Fillet DHA content (%)
43.0 a
39.2 a
34.5 b
43.4 a
C24
C9
Cs/n
Cest

617