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Effects of extruded diets with different energy levels on fat Body composition and profile of fatty acids of Rainbow trout (*Oncorhynchus mykiss*) fed extruded diets with different energy levels

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ABSTRACT:

We have evaluated the influence of energy level on the ratio of digestible protein and digestible energy on the body composition and fatty acid profile composition of fillet in rainbow trout. Two extruted diets with different energy levels were used. The comparison of the two foods with different formulations is perfomed in isoenergetic conditions. Following this study, two diets were formulated: the extruded diet A with 41,4% crude protein, 27,4% lipids and 20,4% carbohydrate while the extruded food B with 39.7% CP, 24,4% fat and 15,7 carbohydrates with digestible energy of 21.32 Mj kg^{-1} and 19.32 Mj kg^{-1} respectively. The initial average weight of the trouts was 100 g from the same batch of eggs which were divided randomly into six fiberglass conical tanks at open circuit.

The diet was assigned to six tanks of 50 fish each with three replicates for each diet and the experiment was conducted for 127 days. The ratio DP/DE of body composition influenced by diet and profile of fatty acid and their distribution in the fillet of fish was calculated. The test focused on the ventral fillet of fish. Our results demonstrated that the total fatty acids expressed in g/100g wet matter (WM) reveal significant variations ($p<0.05$). The fatty acid composition of the fish muscle varied with the high level fat for the low ratio DP/DE, and the best ratio n3/n6 ($p<0.05$) is found out. The lipid content increased with increasing dietary lipid levels and depended on the protein content in feeds and the ratio DP / DE. Fatty acid (FA) composition of the muscle reflected the fat levels in the diet.

Keywords:
Fat, fatty acid, protein digestible, energy digestible, fillet, n-3/n-6 Ratio, rainbow trout.

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INTRODUCTION

The success of the Fish culture is based on the provision of feed supplied in rations containing optimal levels of energy and nutrients for growth and body composition (Hardy and Barrows, 2002). The optimization of the ratio protein / energy (P: E) has been therefore having an important role in protein and energy utilization (Kaushik, and Medale, 1994).

So, one of the factors affecting optimization of the efficiency of food is the balance between digestible protein (availability of amino acids) and digestible energy non-protein food. This balance is represented by the ratio of digestible protein (DP) and digestible energy (DE) of the food (DP / DE). To get a better optimization of the ratio DP / DE, the rate of this ratio can be reduced if an additional power source (fat or digestible carbohydrates) is provided to save the protein. (Aba *et al.*, 2012). Many studies have shown that increasing dietary DE by increased non-protein energy food resulted in better retention of protein and a decrease in the excretion of ammonia nitrogen, (Dias *et al*., 1999, Watanabe *et al.*, 2001; Bureau *et al*., 2002, Aba *et al*., 2011).

Fish and fish products are considered as valuable foodstuffs because of their nutritional properties that are beneficial for human health. The nutritional benefits of fish stems, from its exceptionally advantageous fatty acid profile. In recent years increasing attention has been focused on significance the of n-3 polyunsaturated fatty acids (PUFAs) in nutritional food consumed by humans (Hossain, 2011). As the world's fish stock is limited, farmed fish is being proposed as an alternative (Medale, 2010).

There are variations in lipid content with varying quatity of fat deposits in the muscle in diferent species. Thus, in salmonids, the ventral part of the net is usually fat than the dorsal, and the anterior oilier than the posterior (Katikou *et al*., 2001; Testi *et al*., 2006).

Fish is the main food source of omega 3 and omega 6PUFAs (n3, n6), in the human diet (Simopoulos, 2008). So, numerous studies have revealed that n-3 fatty acids are essential for the best development of the body and are beneficial for human health (Dewailly *et al*., 2001; Demers, 2001). Polyunsaturated fatty acids, omega 3 are effective against cardiovascular problems. They have been shown to have beneficial effect in a range of cardiovascular risk factors, which result in primary cardiovascular prevention, reduction in total and cardiovascular mortality (Calder and Yaqoob, 2010), and various inflammatory (Wall, 2010) conditions such as arthritis, asthma, psoriasis and bowel diseases (Dewailly *et al*., 2007; Gogus and Smith, 2010), and are essential for brain development (Blanchet *et al*., 2005). The n3 play a role in improving learning ability (Yonekubo, 1992) and visual function (Birch *et al*., 2000). Long chain n3 PUFAs cannot be synthesized by humans and must be obtained through diet (Alasalvar *et al*.,2002)

The n3/n6 ratio has been suggested to be a useful indicator for comparing relative nutritional values of fish oils (Piggot and Tucker,1990).Today's western diet is generally deficient in n-3 fatty acids and excessive in n-6 resulting in a low n-3/n-6 ratio. It was proposed in general that human diet should have the n-3/n-6 ratio close to 1 whereas in the western diet it exceeds 1:15 (Simopoulos, 2008).

Rainbow trouts are members of Salmonidae family. It is also widely used as farmed fish in many countries because of its rapid growth and value as a food. Freshwater fishes have a greater capacity to desaturate and elongate short chain fatty acids than marine fishes (Moreira *et al*., 2001).

Rainbow trout (*Oncorhynchus mykiss*) is one of the most popular fish species in nature, but in many countries it is also recognized and accepted as cultivated/ farmed fish species, due to its fast growth and excellent nutritional quality (Tikeogly, 2000).

The quality assessment of rainbow trout must be considered from two aspects. One important attribute is its nutritional value as a source of n-3 PUFA but sensory attributes like colour, need to be taken into account as well. (Aba *et al*., 2012)

There isn't much researches in Morocco that gives information on the whole nutritional value of rainbow trout. In view of these facts, the objective of the present study is to determine the, fat, fatty acid, compositions of the rainbow trout. And the aim of the present study is to investigate the impact of the chemical composition of feed (feeds with different levels of energy and lipid) on the fat content of body composition and dietary quality of fillets of rainbow trout.

MATERIALS AND METHODS

Experimental design

The experiment was conducted between June 1, 2010 and October 5, 2010 at the National Center of Hydrobiology and Fish Culture (NCHP) in Azrou (Morocco).

This test was conducted in fiberglass conical tanks of 0,8 m^3 volume at open circuit with an initial load of 5 kg fed with spring water at a constant temperature of around 14°C ± 0.2 and a flow rate of 1,6 m^3/h, with a time renewal of water two times per hour with oxygen levels above 80% saturation. The average content of dissolved oxygen in the outlet of the ponds was 7.1 ppm, and pH around 7.

Biological materials

300 juveniles' trout females triploid of average weight of 100 g ± 3g from the same batch of eggs were divided randomly into six fiberglass conical tanks.

The test was conducted in triplicate culture, the fish were fed manually and the daily ration was split into two meals distributed at 09 am and 03 pm, seven days a week for 127 days, according to the feeding table provided by the supplier of food (LeGouessant). Every two weeks eight fish of each batch have been anesthetized after 24 h of fasting in order to measure the size and the weight of each fish. The quantities of food distributed were weighed to estimate the consumption by fish between two weighings.

The rate of feeding

The experimental test was aimed at comparing the effect of two non-isoenergetic foods of different formulations on the growth performance of fish and their flesh quality in isoenergetic condition. The amount of food distributed is consistent with the feeding tables of the tow extruded foods (A,B) that have different digestible energies 21.32 Mj kg^{-1},19.32 Mj kg^{-1}, respectively. These rates of rationing depend on the temperature of the water close to the site. We have set the rates according to the temperature of the site which is about 14°C so that the quantitative ratio for the same food energy intake is: amount of food extruded (ExA) 1.10 = amount of extruded (ExB) food.

Gross energy was calculated using the following values: crude protein = 23.73 Mj Kg^{-1} crude lipids = 39.5 Mj kg^{-1} and carbohydrate = 17.2 Mj kg^{-1}proposed by Brett and Groves (1979). The calculation of digestible energy is obtained by the coefficient of digestibility of protein, fat and carbohydrates (gelatinized or raw) (Guillaume and Medale, 2001).

Proximate composition of experimentals diets are shown in Table 1.

Parameters	Extruded diet A	Extruded diet B
Dry matter	94.4 %	96.1 %
Protéins	41.1%	39.7 %
Lipids	27.4%	24.4 %
carbohydrates	20.4%	15.7 %
Moisture	5.6 %	3.9 %
Gross Energy (GE, Mj Kg^{-1})	23.73	21.70
Digestible energy (DE, MJ Kg^{-1})	21.32	19.32
DP / DE (g MJ^{-1}) (DP:Digestible Protein)	17.35	18.48
Ratio P /L	41/27	40/24

Chemical analysis of fat

Four fish in each group were randomly selected, filleted and each fillet was divided into four discrete sections; antero ventral part, antero dorsal part, postero dorsal part, postero ventral part.

These fillets were divided into four parts (Fig 1): Each part of fillets from all fish was pooled. The pooled fillet parts were ground and homogenized in a blender and then the proximate composition of the fillets was analyzed. Crude lipid analysis was conducted by ether extraction, and total lipid was extracted after homogenization in chloroform/methanol (2/1 v/v) containing 0.01% butilated hydroxytoluene as antioxidant basically according to Folch *et al*., (1957).

Chemical analysis of fatty acids

Total lipid was extracted from fish fillets and feed samples, by homogenization in chloroform/ methanol (2/1, v/v) containing 0.01% butylated hydroxytoluene (BHT) as antioxidant, according to the method of Folch *et al*., (1957).Fatty acid methyl esters were prepared from total lipid by acid-catalyzed transesterification using 2 mL of 1% H_2SO_4 in methanol, plus 1 mL of toluene, as described by Christie (24), and fatty acids were analyzed by gas liquid chromatography (Auto System XL Perkin Elmer) using a 30 × 0.25 mm capillary column (FID detector CP-2330 Supelco, Deutschland). The conditions of the method were: carrier gas, helium; flame ionization detection temperature, 220°C; split rate: 1/50, oven temperature programmed to rise from 120°C/2 min to 220°C/15 min at a rate of 5°C/1 min; injector temperature, 240°C. The identification of the individual methyl esters Were achieved by comparison of their retention times with commercial standards (Sigma-Aldrich Chemie GmbH, Deutschland).

Levels of polyunsaturated fatty acids (PUFAs), the total content and the ratio of n3 and n6 fatty acids were determined and expressed in grams per 100 g fillet of wet matter. All analytical determinations were done in triplicate.

Table 2 : content of fat and fatty acids n3 and n6 for two diets (g/ 100g of fillet WM)

Parameters	Extruded diet A	Extruded diet B
Fat	$8.50^b \pm 0.02$	$7.03^a \pm 0.03$
Omega 3	$2.8^b \pm 0.06$	$1.90^a \pm 0,05$
Omega 6	$2.6^b \pm 0.04$	$2.10^a \pm 0.02$
Ratio n 3 / n 6	$1.08^b \pm 0.03$	$0.90^a \pm 0.02$

Statistical studies

Results are expressed as mean (± SD). Our results are compared statistically (Development Core Team R, 2011). All parameters of growth and yield were subjected to Analysis of Variance test (ANOVA). Tukey's multiple procedure was used to compare the differences among mean values. Differences were regarded as significant, when P<0.05.

RESULTS

Values are means of four replications. Data are expressed as mean ± SD. Values in a row with different superscripts are significantly different from each other (P<0.05).

The present study shows, food with different fat and this energy concentrations, has a significant impact on body composition of rainbow trout which is expressed as the percentage of the total of 100g of fillet. The values of fat are significantly the highest in the group of fish receiving feed with the lowest DP/DE (P < 0.05).

The content of polyunsaturated fatty acids n3 and n6 are limited to the anterior ventral part of fillets of rainbow trout expressed in g/100 g fillet of wet matter. Fatty acid compositions of fish were well reflected by the content composition of dietary lipids. The content of the PUFAs from n-3 fatty acids in the fillets of fish from fed by the extruded diet A was higher than that fed with the diet B (P<0.05, Table 2),and the content of the n-6 fatty acids is lower for the diet B than the diet A (P<0.05). The ratio n-3 / n-6 is affected by the content of fat in this part (P<0.05). The values of the n3/n6 index were the most advantageous in the fillets of fish fed with the extruded diet A.

DISCUSSION

The content of fat in foods reflects significant the lipid content in the ventral part of the fillets of fish, particularly salmonids that are recognized by excessive deposition at this part (Corraze and Kaushik, 1999; Médale 2010)

Lipid content in fish fillets of rainbow trout increased along with increases in the lipid content and energy of feeds. The distribution of lipids in the fillet of fish is uneven and depends on the species, and location (Katikou *et al*., 2001) reported differences in the lipid content between dorsal and ventral as well as anterior and posterior sections of *Salmo salar* , the anterior parts contain more fat than the posterior parts and the same for the ventral and dorsal parts . Thakur *et al*., (2003) determined varying amounts of lipid in the anterior dorsal, dorsal, and tail sections of muscle in cultivated *Seriola quinqueradiata* .

The studies in Brown trout of Toussaint *et al*., (2005) have shown that differences in lipid contents exist in the fillet of this fish .Feeding a high-energy diet resulted in significantly higher lipid deposition, especially in the anterior ventral region of the muscle (Toussaint *et al*., 2005). It is evident from our results, that different levels of fat in parts of the fillet result of the increased ability of the fish to capture more fatty acids from the diet A, diet with high lipid content and low a ratio of DP/DE. The activity of the Lipoprotein lipase (LPL) at preferential sites of storage was higher when the dietary fat intake was high. (Arantzamendi, 2002; Kolditz *et al*., 2008) .

In recent years fish nutrition has foccused on one main objective, the content of polyunsaturated fatty acids n-3 and n-6 acid (Wall *et al*., 2010). In general, lipid content in the different fillet portions was correlated to PUFA content (Medale, 2010). The fatty acid composition of fillets is reported in table 2, as percentages of total fatty acids (g/100 g fillet). Polyunsaturated n-3 (PUFA n-3) fatty acids and the n-3 / n-6 ratio were significantly higher in the trout fed with diet A.

Freshwater fish normally contain more omega 6 PUFAs, whereas marine fish are rich in omega 3 fatty acids (Tocher,2003).

The lipids of the muscle from rainbow trout examined in the present study consisted mainly of polyunsaturated fatty acids (PUFA). The n-3 FAs and n-6 FAs are two bio-chemical families within the PUFAs, and they also have different biological effects (James and Cleland, 1996).

The omega 3/omega 6 ratio has been an indicator to be useful for comparing the relative nutritional value of different fish species. The n-3/n-6 ratio depends on the type of diet consumed (Steffens and Wirth 2005). The Omega-3/Omega-6 ratio has been suggested to be a useful indicator for comparing the relative nutritional value of fishes. (Sargent *et al*., 2002).

The ratio of n-3 ⁄ n-6 PUFAs is an important index that enables a comparison of the relative nutritive value of lipids. According to Valfre *et al*. (2003), in freshwater fish species, the ratio is varied from 1 to 4. In general n-3 ⁄ n-6 PUFAs ratios are higher in wild fish species than in the farmed ones (Grigorakis *et al*., 2002). The ability to elongate and desaturate fatty acids is not the same in all the fish species, and freshwater fish such as rainbow trout is capable of this bioconversion. (Tocher, 2003). The current recommendation regarding the consumption of PUFA in human nutrition is 1:4 (the ratio of n-3 to n-6 PUFA) (Ralph, 2000).

Flesh quality can be influenced by the biochemical composition of fish fillets (Hernández *et al*., 2002). A n 3 /n 6 ratio of 1:1 is considered to be optimal for nutritional purposes but ratio within 1:5-1:0.68 would constitute a healthy human diet (Simopoulos and Cleland, 2003). The ratio of n3 /n6 in total lipids of freshwater fishes changes mostly between 0.55 and 5.60, whereas for marine fishes is between 4.7-14.4 (Henderson and Tocher, 1987; Hearn *et al*., 1987). Hence, our findings are in accordance with those earlier studies. Our studies indicate that the values of the n3/n6 ratio were the most advantageous in the fillets of fish fed with the extruded diet A, therefore the ratio of the extruded diet A is favourable for health compared to the extruted diet B.

Our studies indicate that the values of ratio n3/n6 were more favourable in the fish fillets fed with the extruded diet A. Our results for the ratio n3 / n6 showed a rate of 1.08 and 0.90 respectively for the extruded foods A and B. The values are higher than the values found by Stancheva and Merdzhanova (2011) with a ratio of 0.84, despite the fact that the ratio of omega-3 / omega-6 is in freshwater fish ranging from 0.55 to 5.60. So, from this study it is evident that extruded diet A is rich in n 3 and n6 when compared to the extruded diet B. The diet A has a large deposit at the fish fillets, which generated high levels of omega 3 and a better ratio n3/n6 (Kolditz,2008; Medale, 2010).

CONCLUSION

Fish is a source of protein and fat and its consumption is recommended for its beneficial effects on health. In fact, fish is the main source of polyunsaturated fatty acids long chain (PUFA) n-3 (or omega 3) in human food, but its contents of these products depend mainly on the feed, the content of fat in food, the ratio of DP/DE , by its energy content and its composition will reflects the levels of lipids and fatty acids in the flesh of fish.

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