



## Efficacy of Dietary Inclusion Levels of Astaxanthin in the Feed on the Flesh of Rainbow Trout

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### Abstract

The main aim of this study is to understand the influence of pigmentation of three types of Compound feeds (A, B and C) physical and organoleptic quality of rainbow trout flesh by instrumental and sensory measurements. This test compares the effects of different pigmentation levels on rainbow trout fillets.

The three feeds have the following compositions;

- Feed A with 40 ppm of Astaxanthin,
- Feed B with 40 ppm of Astaxanthin,
- Feed C with 0 ppm of Astaxanthin

The results of this study showed that extruded feed B is characterized by appearance, physical color of the flesh which is a quality factor sought by fishermen, unlike feed A which has an orange color as a result of feed C which has no salmonization of the flesh.

### Keywords

Astaxanthin, Pigmentation, Salmonization, Fillet, Level, and Rainbow trout.

### Introduction

Carotenoids form the largest and most widely distributed group of natural pigments in living organisms. More than 650 carotenoids found in their natural form have been identified. While most of the natural synthesis of carotenoid pigments is actually carried out by plants, some microorganisms such as bacteria and yeasts also possess the ability to synthesize them. Aquaculture species, on the other hand, are unable to produce carotenoids *de novo*; however, most species can modify their molecular structure through certain metabolic reactions

[1]. The carotenoid pigments found in fish therefore come from their diet. They are fat-soluble polyene substances characterized by a large number of conjugated double bonds, giving them a coloration ranging from orange to red (Figure 1) [2]. This original chemical structure enables the existence of a large number of stereoisomers, giving each molecule unique chemical (oxidation) and physical (light absorption) properties. The presence of numerous double bonds is also the cause of the instability that characterizes these pigments. Carotenoids are easily destroyed and become colorless under the action of high temperatures, light, oxygen and acid [3]. In plants, they are accessory pigments for photosynthesis. They can act by transferring absorbed light energy in the violet-red range of the visible spectrum to chlorophyll, or by capturing chlorophyll energy, particularly in the event of excess light (photoprotective role). Carotenoids are divided into two main groups:

- 1) Carotenes, consisting solely of carbon and hydrogen, and
- 2) Xanthophylls, which also contain oxygen atoms [4].

Astaxanthin, lutein and zeaxanthin belong to the xanthophylls and are the molecules under study in this project. Each has 40 carbon atoms and two cyclic structures at the ends of the molecule. Astaxanthin is a pigment widely used in salmonid farming. Its main role is to give the flesh an orange-red color characteristic of certain salmonid species. Astaxanthin is the most widely used type of pigment in the aquaculture industry worldwide. In 1990, Goycoolea began large-scale production of synthetic astaxanthin, and since then has dominated the global pigment production market, now valued at \$175 million.

One of the particularities of aquaculture compared to fishing is that it is possible to obtain a certain amount of control over the flesh quality of fish intended for consumption. Although there is no simple definition of flesh quality factors, nutritional value, safety, flavor, color, preservation and type of processing are all paramount [5]. Salmonids are renowned for their characteristic orange-red color. Consumers use color as a sign of product quality (flavor and freshness) [6]. In the wild, salmonids obtain their color by ingesting crustaceans, while in farmed fish, synthetic pigments are added to the compound feed. This is where astaxanthin and canthaxanthin come into their own. The use of this type of red pigment has been highly documented for over 25 years [7-10]. There are two types of synthetic red pigment on the market. The first is astaxanthin, available under the brand name Carophyll Pink™. Canthaxanthin, meanwhile, is also used as a pigment under the name Carophyll Red™.

Astaxanthin is preferred to canthaxanthin, as it is more efficiently deposited in muscle, probably due to better intestinal absorption [11,12]. Among the many factors that can influence fish flesh pigmentation is feed composition. Fish pigmentation can be influenced very differently depending on the sources of carotenoid pigments added to the feed. Plants and yeast are highly concentrated in carotenoids, but they are not always usable by fish. Manufacturers of compound feeds for fish mainly use pure, stabilized carotenoids produced by chemical synthesis. Tissue pigmentation will be greatly influenced by the nature and molecular form of the carotenoids added to the feed, even if these are synthetic. A study by Torrissen

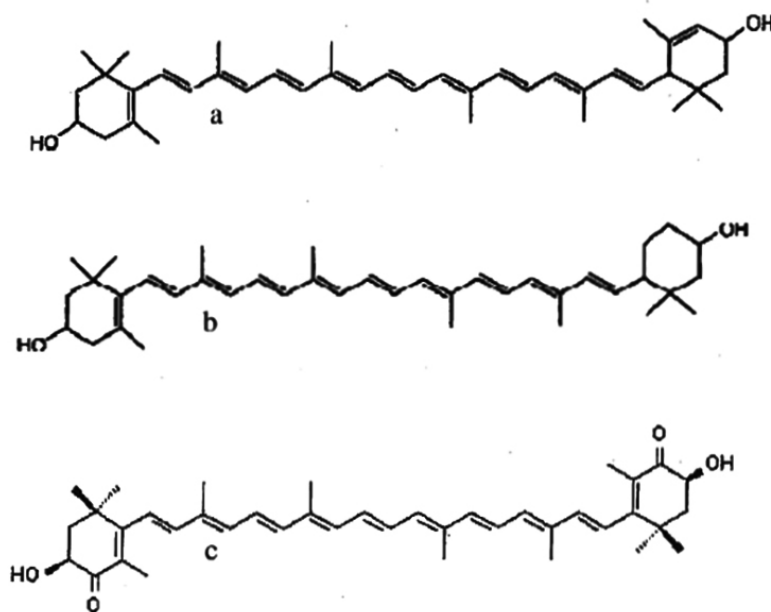


Figure 1: (a) Lutein-yellow molecule, (b) zeaxanthin-yellow molecule, (c) Astaxanthin-red molecule.

showed that astaxanthin is more efficiently deposited in flesh than canthaxanthin. Molecular conformational dissimilarities are responsible for the different levels of absorption and deposition of the two types of pigment [11,12].

## Materials and Methods

At the Azrou rainbow trout farm, we investigated the effect of three types of compound feed with different astaxanthin incorporation rates on the physical appearance of the flesh and salmonization of the finished product. 3 batches of rainbow trout (10 trout fed each type of feed) fed for 222 days on three compound feeds containing identical pigmentation levels for feeds A and B (40 mg/kg) and zero for feed C (0 mg/Kg). Feed A with 40ppm Astaxanthin, Feed B with 40 ppm Astaxanthin, Feed C with 0 ppm Astaxanthin. The fish were fasted for three days before being harvested to evacuate waste from the intestines and avoid feed wastage. The fish used were caught without anaesthetization, bled at the gill arch and placed in a tank containing water for 15 minutes to allow total elimination of blood from the body and washing of the fish (Figure 2). Trout are individually weighed before and after evisceration, with viscera and liver weighed to calculate morphometric parameters, and stored in cold storage at 0°C-4°C until processing.

Fish fed with the three feeds A, B and C undergo the final product processing stages, including drying, salting and smoking.

Using a salmo Fan Tm Lineal ruler, for salmonids pigmented with carophyll Pink (Astaxanthin), the degree of salmonization was measured on the finished product (Figure 2). Biochemical analysis of couponed feed tested according to suppliers' data sheets. Table 1 below shows the biochemical composition (proteins, lipids and fatty acids) of the test diets according to the three suppliers data sheets.

## Experimental regimes

The fish are put on a long diet for a period of three days. Trout

are usually put on a diet to allow the digestive tract to empty before slaughter, in order to increase the quality and freshness of the fish (3 day diet, no feed is found in the digestive tract).The fish is filleted into two pieces after removal of viscera, skin and head. Each person was asked to complete the questionnaire, indicating these parameters (visual appearance, odour, taste and texture) opposite the sample number, with a quality rating for each aspect ranging from 1 to 9 (Table 2).

The test focused on raising awareness among staff, especially marketing staff, of certain sensory analysis concepts, and in particular of the main organoleptic characteristics of products (visual appearance, smell, taste and texture). Each taster was given a questionnaire for recording tasting responses for each sample (PFA, PFB and PFC). Three samples are then presented. Each taster is asked to complete the questionnaire, indicating these parameters (visual appearance, smell, taste and texture) opposite the sample number, with a quality rating for each aspect ranging from 1 to 9.

## Results

According to Figure 3 we observe that the samples of three fish fillets fed with the three compound feeds are not pigmented except the fish fillets fed with feed B. These results show that the feed categories have an effect on the color index of the experimental fish. In fact, fish fed with feed A and B obtained higher degrees of coloration than those fed with feed C.

Feed B has an excellent aspect of coloring, the flesh is very salmon-colored compared to feed A which has an orange color contrary to feed C which shows no salmonization. For this experiment, a certain number of tasters evaluated the fish on the various criteria 171 listed according to an evaluation grid varying from 1 to 9. Table 3 presents the acceptability means for the criteria of colour, odour, texture and taste 186 for rainbow trout fed the three feeds tested. For this experiment, a certain number of tasters evaluated the fish on the various criteria 188 listed according to an evaluation grid varying

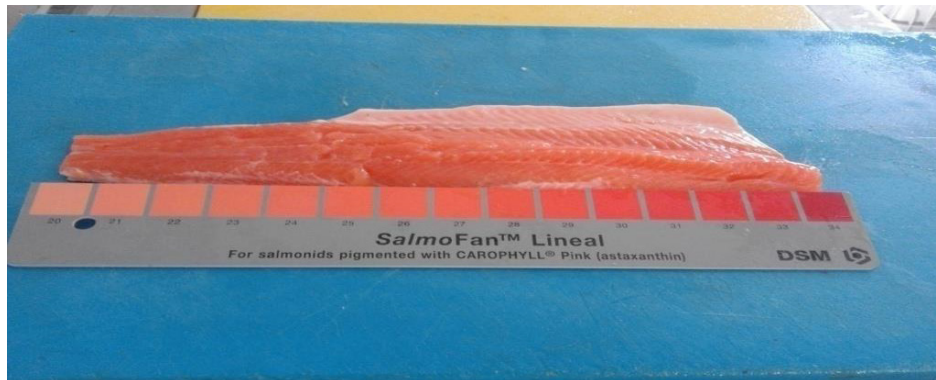


Figure 2: Measurement of salmon staining.

Composition	Experimented Feed compounded		
	Feed A	Feed B	Feed C
Protein	39	41	40
Fat	27	24	26
Fatty acid	16	-	-
Fatty acid W3	17	-	17,5
Fatty acid W6	14	-	15
EPA + DHA	1,2	-	8
Pigmentation (40 mg/kg Astaxanthin)	40	40	-

Table 1: Percentage of biochemical composition of experimental diets according to the three suppliers.

	Sensory characteristics	1	2	3	4	5	6	7	8	9	Average
		Feed B	Visual	7	3	-	-	-	-	-	
Odor	7		3	-	-	-	-	-	-	-	-
Taste	7		3	-	-	-	-	-	-	-	-
Texture	9		0	-	-	-	-	-	-	-	-
Feed A	Visual	9	1	-	-	-	-	-	-	-	-
	Odor	6		-	1	-	-	-	-	-	-
	Taste	7	3	-	-	-	-	-	-	-	-
	Texture	7	2	-	-	-	-	-	-	-	-
Feed C	Visual		2	-	-	3	-	2	-	-	5
	Odor	1	2	-	1	1	1	1	-	-	3
	Taste		2	1	4	-	-	-	-	-	3
	Texture	1	5	1	1	1	-	-	-	-	1

Table 2: Sensory parameters questionnaire.

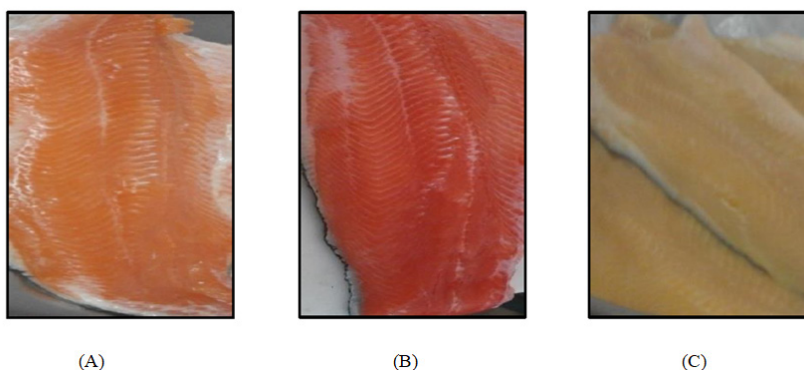


Figure 3: Degrees of salmonization of fish fillets fed with the three compounded feeds tested (A, B and C).

	Sensory characteristics	1	2	3	4	5	6	7	8	9	Average
Feed B	Visual	7	3	-	-	-	-	-	-	-	-
	Odor	7	3	-	-	-	-	-	-	-	-
	Taste	7	3	-	-	-	-	-	-	-	-
	Texture	9	0	-	-	-	-	-	-	-	-
Feed A	Visual	9	1	-	-	-	-	-	-	-	-
	Odor	6		-	1	-	-	-	-	-	-
	Taste	7	3	-	-	-	-	-	-	-	-
	Texture D	7	2	-	-	-	-	-	-	-	-
Feed C	Visual i	-	2	-	-	3	-	2	-	-	5
	Odor S	1	2	-	1	1	1	1	-	-	3
	Taste C	-	2	1	4	-	-	-	-	-	3
	Texture	1	5	1	1	1	-	-	-	-	1

**Table 3:** Presents the acceptability means for the criteria of colour, odour, texture and taste 186 for rainbow trout fed the three feeds tested.

from 1 to 9.

## Discussion

Color is one of the most important factors that motivate consumer purchase and 193 acceptability of a fish product [13-15]. The pigmentation of the skin and flesh of salmonids, a quality factor sought by 196 fishermen and gourmets as well as by fish farmers wishing to improve their products. The color of fish flesh can be assessed by sensory analysis using a jury trained in 198 descriptive tests, by analysis of the concentration of carotenoid pigments or by instrumental analysis such as a colorimeter.

According to Sheehan and O'connor the degree of pigmentation of the flesh of fish fed with Danish feed B is identical to the degree of salmonization of the flesh of fish fed with feed B that we used in our experiment. The color of fish flesh does not appear to be affected by the diets tested. However, differences are highlighted on the external color of the smoked fillet. The value (indication of redness) increases with the fat content of the diet [16]. These results are different from what we found in our experiment. Indeed, feed B, which has low lipid content, produces a very salmomy fillet with a degree of salmonization of  $29.75 \pm 0.63$ .

The lower salmon color of feed A can be explained by the phenomenon of peroxidation. Due to their Poly Unsaturated Fatty Acids (PUFAs) content, fish are very sensitive to oxidation and peroxidation phenomena [16]. PUFAs can be altered, like all polyunsaturated acids because the presence of double bonds makes them sensitive to oxidation, it is characterized by the production of a peroxide then an oleic acid, giving the harmful effects of lipid peroxidation, a destruction of vitamins which can lead to a vitamin E deficiency, a modification of the 216 texture due to dystrophy of the flesh, a reduction in the color of the flesh and especially an alteration of the flavor by rancidity [17]. This is marked in the fish fed with food A knowing that both feeds A and B have the same amount of Astaxanthin but the flesh of feed B is more salmon  $\{B=29.75 \pm 0.63\}$  than feed A  $\{A=26.25 \pm 0.26\}$ . Feed C shows no salmon color probably due to the lack of 221 pigment incorporation in the feed.

## Conclusions

The results of this study show that the extruded feed B characterized by its physical 226 aspect of the coloring of the flesh,

a quality factor sought by both fishermen and 227 gourmets. Unlike food A which has an orange colour. Conversely to food C which does 228 not present any salmonization but it is rich in fatty acids of the n-3 and n-6 series. On the other hand, feed A, also with 40 ppm of Astaxanthin, resulted in fillets with an orange color, indicating some degree of oxidation and peroxidation due to the high polyunsaturated fatty acids content.

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## Conflicts of Interest

The authors declare no conflict of interest.

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