Shrimp pigmentation with natural carotenoids

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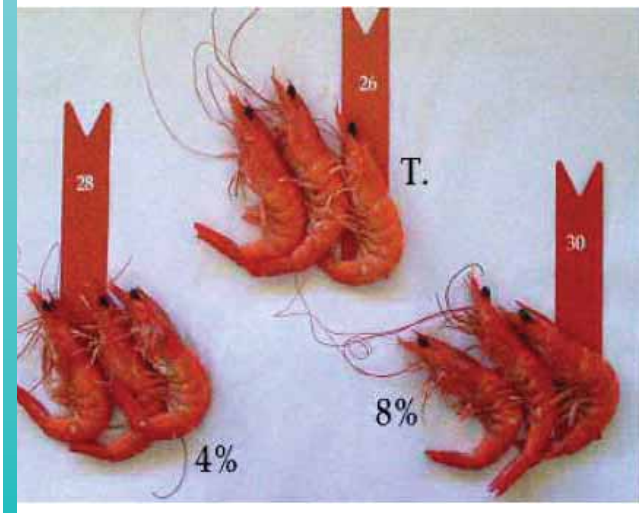
Natural alfalfa concentrate in finisher feed fed during the last month of the grow-out cycle significantly increases the reddish colouration of shrimp after cooking.

Dr Gerard Cuzon and the IFREMER team in Tahiti, French Polynesia studied the effect of adding a natural source of carotenoids from alfafa concentrate on shrimp pigmentation. The product is obtained by the dehydration of this widely used forage. The extraction process uses water and heat application.

In a series of trials, conducted in clear water tanks with controlled parameters (36ppt and 26°C), the effect of the alfalfa concentrate Pigmentech (Aqua Techna, France) was tested on marine shrimp *Penaeus stylirostris*, at three inclusion rates T (0% Pigmentech), A (4% Pigmentech) and B (8% Pigmentech). There were 6 replicates.



Variation of the colouration between the three batches after 21 days of experimentation

Shrimp were individually weighed at the start of the experiment and fed 3-4 times/day at 5% body weight per day. Shrimp were sampled on day 7 and day 21 and two shrimp were sampled from each tank per time (6 replicates and thus 12 shrimp per treatment). The analysed parameters were:

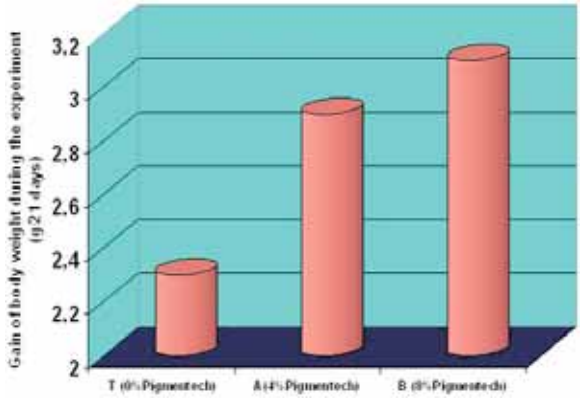
Pigmentation: For this purpose, 6 shrimp from each treatment were immersed in boiling water (5g/l of salt) for 3 minutes. Subsequently, the shrimp were placed on a black area and/or a white zone in order to evaluate colouration using the Roche index: SalmoFan™ (a graduated pink color chart from 20 to 34, see photo). Colour was evaluated by a team of 10 people.

Table 1. Changes in colouration after 21 days of experiment

T (0% inclusion)*	A (4% inclusion)*	B (8% inclusion)*
26.4 ± 1.3	28.3 ± 1.1	30.2 ± 0.9

**Percent inclusion of the alfafa concentrate Pigmentech in diet of shrimp*

Figure 1. Effect on the shrimp colouration



Zootechnical: The growth rate, survival and number of molts were analysed. After 7 days of experiment, there was no statistical difference between treatments. However, at day 21, the results were as shown in Table 1 and after 21 days of experiment, colour was significantly enhanced as seen in cooked shrimp.

The addition of the alfafa concentrate also showed a very positive effect on animal responses, especially in terms of growth as shown in Figure 2. This improvement in the growth rate may be explained by the digestibility of the proteins contained in the alfalfa. However, it could also be attributed to the high content of natural antioxidants and

Figure 2. Effects on the growth

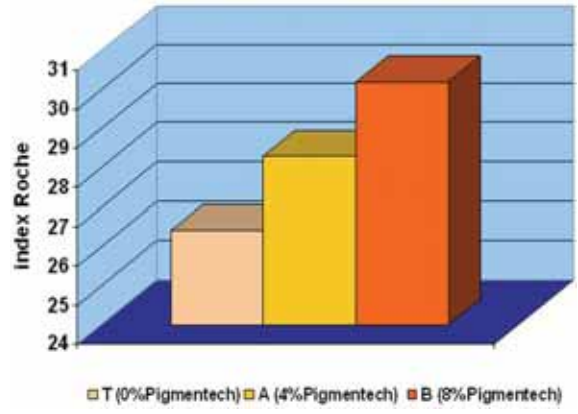


Table 2. Concentration in total carotenoids ((μg) in the tissues of the shrimp sample

Shell	Number of shrimp	Q (μg)	C (μg/g)
T (0%)	5	0.6 ± 0.1	18.7 ± 10.8
A (4%)	6	0.7 ± 0.2	12.2 ± 2.3
B (8%)	6	0.8 ± 0.3	20.8 ± 10.7
P (from pond culture)	6	0.8 ± 0.4	13.9 ±6.3
Epidermis	**Number of shrimp**	**Q (μg)**	**C (μg/g)**
T (0%)	5	24.8 ± 2.6	510 ± 141
A (4%)	6	32.8 ± 12.1	890 ± 221
B (8%)	6	42.2 ± 4.8	1,383 ± 318
P (from pond culture)	6	27.8 ± 9.9	781 ± 235
Hepatopancreas	**Number of shrimp**	**Q (μg)**	**C (μg/g)**
T (0%)	6	18 ± 10	28 ± 15
A (4%)	6	81 ± 66	119 ± 95
B (8%)	6	104 ± 78	156 ± 115
P (from pond culture)	6	340 ± 90	1,144 ± 520

Q is the quatity of carotenoids as μg/g in the sample (shell and epidermis, 3.6cm² per sample and the total hepatopancreas). C is the concentration of carotenoids as μg/g of dry weight of tissue after extraction.

Table 3. Distribution of the various forms of astaxanthin in the hepatopancreas and in the epidermis of the shrimp sample

Percent of total carotenoids in the hepatopancreas			
	Treatments		
	T (0%)	A (4%)	B (8%)
Free astaxanthin	43.9	31.9	29.4
Mono-ester astaxanthin	8.7	24.8	24.0
Di-ester astaxanthin	12.0	15.0	18.0
Total astaxanthin	64.6	71.7	71.4
Percent of total carotenoids in the epidermis			
	Treatments		
	T (0%)	A (4%)	B (8%)
Free astaxanthin	48.4	55.3	48.5
Mono-ester astaxanthin	17.1	18.6	26.4
Di-ester astaxanthin	18.4	12.3	10.9
Total astaxanthin	83.9	86.2	85.8

essential fatty acids. Nevertheless, no effect was observed in terms of molting rate or survival, during this period.

This first study showed that shrimp colouration was enhanced by the use of the concentrate in the feed, even at low rates of inclusion (4 and 8%). This showed that shrimp fed with these diets could accumulate the carotenoid pigments and demonstrate similar beneficial effects. Similar results were obtained with *Penaeus vannamei* by separate teams in Thailand and China.

Analysis of carotenoid contents

The purpose of the second work carried out by O. Berticat, R. Castillo and G. Nègre-Sadargues from the Laboratory of Ecophysiology of the Invertebrate, University of Montpellier, France was to investigate factors controlling the nature of the pigments involved in pigmentation of shrimp. The pigments present in the concentrate are mainly yellow (lutein) and orange (carotene). Thus, the objective was to determine the nature of the pigments obtained after bioconversion by the shrimp.

During the experiment carried out in Tahiti, six shrimp in each treatment T, A and B, were sampled and deep frozen at -80°C. At the same time, shrimp (group P) were sampled from semi-intensive earthen ponds in a farm in Tahiti.

For each individual animal, the total carotenoid inclusion level and the qualitative analysis of these pigments were carried out by chromatography on tissues. These results in Table 3 showed an increase in the carotenoid concentration in the epidermis from group T to B. The same tendency could be noted in the hepatopancreas.

In each group, the exoskeleton was near transparent with a very low concentration of carotenoids. The epidermis is the richest tissue in pigments. This concentration was directly related to the level of pigments present in feed. The pigment content in the hepatopancreas showed significant individual variations. It also showed increasing concentrations with the increases in the level of pigment in food.

In spite of the small number of analyses carried out, the results indicated that *P. stylirostris* fed pellets supplemented with carotenoids absorbed the pigment in the feed, which after bioconversion, accumulated in the hepatopancreas before being transferred to the epidermis during the formation from the new integuments during the premolt stages.

The concentration in the carapace of shrimp from the earth pond (batch P) showed contents of pigments comparable with those of the three other treatment groups. The hepatopancreas was pigmented much more than the epidermis and is similar to that of group A. Compared to this "wild" batch, the control animals (0% carotenoid) were de-pigmented and those fed with pellet enriched with 4% of Pigmentech preserved the initial content of pigment, while those where the feed enriched with 8% have the highest carotenoid level. In treatment groups, the pigments from the alfafa concentrate appear to be better absorbed than the natural pigments available in the earthen pond resulting in a better colouration of the tissues.

The various forms of astaxanthin of which the quantity increases in the epidermis and the hepatopancreas of supplemented shrimp came from yellow carotenes and xanthophylls provided by the supplementation in the feed.

Economical aspects

The alfalfa concentrate is also one of the least expensive sources of carotenoids available on the market. If we compare the cost/g of active ingredient, Pigmentech can be the most price competitive product compared to most common products used as colour enhancers, such as synthetic astaxanthin, paprika or the *Spirulina* based pigments.

In the above trials, we were only considering the effects on pigmentation through the inclusion of carotenoids in the pelleted feeds. There are, however, other additional benefits of the inclusion of the alfalfa-based pigment as a source of limiting amino acids, essential fatty acids and antioxidants, all of which have effects on economically important variables such as growth and survival. Moreover the high level of protein in the concentrate allow its use as an ingredient to substitute for other, more expensive dietary components such as fish meal.

Conclusion

These studies showed that the incorporation of the natural alfalfa concentrate at between 4 to 8% and fed during the last month of the grow-out significantly increases the reddish colouration of shrimp after cooking. Subsequent analysis showed a significant increase in carotenoids, particularly in astaxanthin, in the shrimp tissue.