AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523

BIBLIOGRAPHIC INPUT SHEET

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1. SUBJECT CLASSIFICATION

A. PRIMARY
Agriculture

B. SECONDARY
Fisheries

2. TITLE AND SUBTITLE
Red crab processing-waste as a carotenoid source for rainbow trout

3. AUTHOR(S)
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4. DOCUMENT DATE	5. NUMBER OF PAGES	6. ARC NUMBER
1976	5 p.	ARC

7. REFERENCE ORGANIZATION NAME AND ADDRESS
International Center for Marine Resource Development.
University of Rhode Island, Kingston, Rhode Island 02881

8. SUPPLEMENTARY NOTES *(Sponsoring Organization, Publishers, Availability)*
(In Alimenta, v.15, p.47-51)
(In Proceedings, 4th Internat'l Symposium on Carotenoids, Berne, 1975)

9. ABSTRACT

The dried red crab waste contained 27.5% protein, 13.8% crude lipid, and 0.008% carotenoids of which 95% was in some form of astaxanthin. Rainbow trout were fed diets consisting of 20% whole crab waste or a pigment extract from crab waste adsorbed on a commercial trout ration. The fish fed the pigment extract containing 0.2 mg of carotenoids/g diet were highly pigmented after seven weeks. The fish fed the 20% crab meal contained a much lower level of astaxanthin in the skin and flesh than would have been expected on the basis of the level of dietary carotenoids. It was concluded that red crab waste could be used for the pigmentation of trout.

10. CONTROL NUMBER	11. PRICE OF DOCUMENT
PN-AAC-152	

12. DESCRIPTORS

Carotenoids
Crabs
Food processing
Organic wastes
Trout
Waste disposal

13. PROJECT NUMBER

14. CONTRACT NUMBER
CSD-2455 211(d)

15. TYPE OF DOCUMENT

AID 590-1 (4-74)

Red Crab Processing Waste as a Carotenoid Source for Rainbow Trout

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Introduction

The agricultural and food processing industries are faced with the problems of disposing of waste products or converting this waste material into economic by-products. Disposal operations will have to meet increasingly more stringent ecological standards as well as attempt to recover valuable protein and other products. These waste products must compete economically with products that they replace or create their own markets. In some food industries the very existence of the plant may be dependent on the economic and ecological disposal of waste material.

It is not uncommon in the shellfish industries to have waste represent greater than 80 % of the landings. Thus it has been estimated that solid waste from the crab industries is now in excess of a million kg/year in the USA (Meyers and Rutledge [10]).

With the establishment of a crab processing facility at Galilee, Rhode Island and more recently a larger and more modern plant at New Bedford, Ma., we attempted to find new food uses for the waste. The crab, *Geryon quinquedens,* is found on the Continental Shelf from Nova Scotia to the Gulf of Mexico as well as in the Eastern Atlantic off the coast of New England. It is captured with pots in 300 to 800 meter depths where the water temperature is 4–5 °C. About 80 % of the weight of crab is lost as waste material which consists of raw carapace, shells of cooked appendages, viscera and meat residue, etc. Only live crabs are used and thus the dead crabs also contribute to the waste problems. One possible use of this waste is as a source of carotenoids, protein and lipid for the diet of salmonids raised in aquaculture. While the protein and lipids can be supplied from other sources, there is no source of the carotenoids astaxanthin other than from dietary crustacea.

In this study, different diets containing 20 % crab waste and extracted pigments were fed to rainbow trout *(Salmo gairdneri)* to evaluate the effect of the crab waste on pigmentation and growth.

Materials and methods

Crab meal:

The waste material of red crab *(Geryon quinquedens)* was obtained from the Galilee Offshore Marine Inc., Rhode Island, USA. The crab waste material was dipped for 10 seconds in a solution containing 10 % citric acid and 0.5 % sodium bisulfite to prevent discoloration due to enzymatic oxidation [2, 9]. The wet crab waste was processed into a dry, pink meal every month through grinding, freeze-drying and pulverization. An antioxidant (BHA) was added to the wet slurry prior to freeze-drying at a level of 0.05 % w/w.

The crab meal was found to contain 27.5 % protein, 13.8 % crude lipid, 36 % ash, 21 % chitin and 1.7 % nitrogen free extract on a dry weight basis. The ash fraction was shown to contain 30.5 % calcium and 4.3 % phosphorus. These components were determined according to the methods of analysis of the A.O.A.C. [1] and Steel [16].

Carotenoids in crab meal:

The carotenoids of crab meal were extracted with acetone then transferred to light petroleum by the addition of water. The absorption spectrum showed a single peak at 468 nm in light petroleum and the amount of total carotenoids was calculated as astaxanthin ($E^{1\%}_{1\,cm}$ = 2000). Total carotenoid was about 8 mg per 100 g crab meal. Over 95 % of the total carotenoids was in some form of astaxanthin [7]).

Fish diets:

1. Control diet

Commercial trout feed manufactured by Zeigler Bros. Feed Mills, Inc., Pennsylvania, USA, was used for feeding the control fish. This diet was analyzed and found to contain 35.3 % crude protein, 6.3 % crude lipid, 4.9 % fiber, 10.4 % ash, 10.0 % moisture and 33.1 % nitrogen free extract. Small amounts of carotenoids were found in this diet – 1 μg β-carotene, 0.4 μg α-cryptoxanthin, 0.1 μg zeaxanthin and 0.8 μg lutein per gram diet. Astaxanthin was not isolated from this feed.

2. 20 % crab meal diet

Crab meal was mixed with other nutrient components to constitute 20 % of the mixture by weight. This dry mix contained the same amount of protein, lipid, etc. as the Oregon test diet for rainbow trout [8]. The protein, lipid and minerals of the crab waste were used to replace part of the casein, oil and minerals used in the Oregon test diet. The formula of the 20 % crab meal diet dry mix is shown in Table 1. For each 60 g of dry mix, 40 ml warm tap water was added. The diet was mixed thoroughly and made into 'spaghetti' by forcing it through a grinder that was fitted with a plate possessing many small openings. The 'spaghetti' shaped diet was air dried then broken into small pellets manually. The diet was stored in the refrigerator until use. New batches were made on the average of every two weeks.

Table 1
Dry mix formula of 20 % crab meal diet

Ingredient	Percentage
Casein (vitamin free)	42.66
Gelatin	8.70
Dextrin	15.60
Corn oil and linolenic acid*	7.24
Carboxymethyl cellulose	1.30
Vitamin mix**	2.00
Vitamin E (250 IU/g)	0.26
Choline chloride	0.70
KH_2PO_4	1.54
Crab meal	20.00

* Linolenic acid (100 %) was 1 % of the diet.
** Vitamin mix was prepared according to Steel [16].
All the chemicals were obtained from Nutritional Biochemicals Corporation.

3. Pigmented diet

The desired amount of carotenoid extracted from crab meal was dissolved in a small amount of light petroleum and a weighed amount of control diet was added to reach the level of 0.2 mg and 0.1 mg carotenoid per gram of control diet. The mixture was shaken and dried in a vacuum rotary evaporator (40 °C) for even adsorption of the pigment.

Fish culture:

Two sets of different size rainbow trout *(Salmo gairdneri)* were supplied by local hatcheries. The small fish had a mean weight of 25 g per fish obtained from Perryville Trout Hatchery, R. I. Department of Natural Resources. The large fish had a mean weight of 85 or 132 g obtained from American Fish Culture Company, Carolina, Rhode Island. The fish were held in 125-gallon (475 liter) fiberglass tanks (Sims Fiberglass, Corvallis, Oregon) with aerated, flowing water (1–1/2 gallons (2–4 liter) per minute). The water temperature was 11 °C. The oxygen level was 8 ppm. The laboratory aquaria were similar to those used by Lee et al. [8]. The small fish were distributed evenly in two tanks and were fed on control and 20 % crab meal diets respectively. The larger fish were distributed evenly in tanks and were fed on control and pigmented diets respectively. The fish were fed at the rate of approximately 3 % of their body weight per day.

Analysis of carotenoids of fish:

The fish were sacrificed after the feeding period. The fish were cut into pieces and ground in a Waring blender with glass beads, Hyflo Supercel and acetone. The acetone extract was transferred to light petroleum by adding water, then dried over anhydrous sodium sulfate. Carotenoid pigments were separated on Microcel C columns with light petroleum and 1–4 % acetone in light petroleum as developing solvents. Each pigment fraction was saponified by adding an equal volume of 20 % potassium hydroxide in methanol to the light petroleum pigment solution. The mixture was left overnight under nitrogen at room temperature in the dark. After saponification was completed, the pigment was transferred to diethyl ether by the addition of water. Acetic acid was added to bring astacene to the epiphase. The pigment solution was dried over sodium sulfate. Silica gel G Sheets (Eastman Chromagram Sheet 13179) were used for further purification with 3 % methanol in benzene as the developing solvent. A Cary 15 recording spectrophotometer was used to measure the light absorption spectrum of the pigments. The $E^{1\%}_{1\,cm}$ values for lutein, canthaxanthin and astacene were 2500, 2200 and 2000 respectively in diethyl ether. The pigments were identified in the same manner as in the crab pigments (Kuo [7]).

Results

Rainbow trout fed on the 20 % crab meal diet:

Fish were sacrificed after 15 and 23 week feeding periods (small fish) and 4 week feeding periods (large fish). The fish fed on the 20 % crab meal diet had a red rainbow streak and faintly pink colored flesh whereas the control fish had a grayish white flesh and no clear rainbow streak. The nutrient contents of the commercial feed were not the same as those of the 20 % crab meal diet. These diets could only be compared on the basis of color as initial efforts to take the casein control diet failed. The control fish were found to be slightly bigger than the crab meal fed fish. Crab meal fed fish as well as the controls exhibited similar mortality rates over the feeding period.

In the control fish, small amounts of lutein ester, trace amounts of α-cryptoxanthin and a few unidentified carotenoids were found. The experimental fish had a mean weight of 34 g per fish after 15 weeks (2.21 μg total carotenoids per fish) and 51 g per fish after 23 weeks (3.162 μg total carotenoids/per fish) of feeding on the 20 % crab meal diet (15.4 μg crude pigment/g diet). Astaxanthin ester, free astaxanthin and astacene were isolated from these fish. In addition, the plant pigment lutein ester was also isolated (Table 2). The

Table 2 Carotenoid content of fish fed 20 % crab waste diet

Pigments	Concentration of carotenoids (μg/g)				
	control* diet 15 weeks	crabmeal diet 15 weeks	crabmeal diet 23 weeks	control** diet 4 weeks	crabmeal diet 4 weeks
Total astaxanthin	–	0.051	0.052	–	0.056
Zeaxanthin	–	–	–	0.26	0.23
Lutein	0.052	0.014	0.01	0.33	0.26
Canthaxanthin	–	–	–	0.01	trace

* 25 g fish at the beginning of the experiment
** 132 g fish at the beginning of the experiment

amount of total carotenoids per gram fish was almost the same in the fish after 15 and 23 week feeding periods – 0.065 and 0.062 μg total carotenoids per gram respectively. Larger fish (132 g) were fed the 20 % crab meal diet to assess the effect of size on the uptake of carotenoids from the crab meal. After 4 weeks the control (167 g) and the crab fed fish (165 g) had increased in size but the level of astaxanthin was the same as in the smaller fish (Table 2).

A strict comparison between the two lots of fish is difficult because of the residual levels of plant carotenoids contained in the fish.

Rainbow trout fed on control and pigmented diet:

Large fish (85 g and 132 g) were fed commercial trout pellets on which pigment extracts (0.1 and 0.2 mg/g diet) were adsorbed. After 4 or 7 weeks on this experimental diet, the trout had a strong orange-pink flesh and fins and vivid red streak on the skin, whereas the control [illegible] flesh remained grayish white in color and only a faint yellow-orange streak was present on the skin. The pigmented diet fed fish as well as the controls exhibited a similar mortality over the feeding periods.

A second experiment was run with larger fish (132 g with 0.1 mg/g diet) over a 4 week period (Table 4). The final average weight of the 5 fish sets were 167 g for the control and 169 g for the extract fed fish. The larger fish were found to be rapidly pigmented over the 4 week period with 0.1 μg/g carotenoid content added to the feed.

The fish in one study were of a nearly uniform size at the start of the feeding experiments. After a 4 week adaption period on a control diet and a 7 week period on the pigmented diet, the fish went from an average weight of 85 g to a range of 90 g to 172 g. The color of the flesh ranged from a faint pink to a strong orange-pink. The large fish contained the greatest concentration of pigment. The fish clearly obtained astaxanthin from the diet as had been suggested by Steven [17, 18].

Goodwin [6] and Fox [4] reviewed the subject of fish carotenoids. They reported that integumentary xanthophylls were esterified in most of the fish and the xanthophylls of flesh and ovaries were unesterified. The largest and most pigmented fish (172 g) in this experiment also showed the highest concentration of free astaxanthin (Table 3).

The ratios of astaxanthin and astaxanthin ester were different in three sets of pigmented trout analyzed (Table 3). This suggested that rainbow trout *(Salmo gairdneri)* had the ability not only to absorb the crab pigment but also to make some changes in the pigment in regard to esterification, and then deposit it depending on their own genetic characteristics.

Table 4

Carotenoids in the rainbow trout fed on 0.1 mg/g diet for 4 weeks

	Concentration (μg carotenoid/g fish)	
	Control	0.1 mg/g
Total astaxanthin	–	0.88
Zeaxanthin	0.26	0.21
Lutein	0.33	0.27
Canthaxanthin	0.013	0.008
α-Cryptoxanthin	trace	trace
Initial weight: 132 g	Final weight: 167 g	169 g

The total astaxanthin present in red crab waste (7.6 mg/100 g dried crab waste) is higher than that present in the snow crab waste (0.64 mg/100 g dried crab waste) which was obtained by Saito and Regier [13]. In their experiment, the snow crabs were cooked twice before the carapace and leg shells were removed and dried in a vacuum oven. The combination of heat treatment and low levels of carotenoids in the snow crab probably resulted in low concentration of astaxanthin in the test diets, therefore Saito and Regier failed to pigment the trout by feeding 20 % snow crab waste meal. A combination of minimal heat treatments and higher levels of astaxanthin in the red crab resulted in some deposition of astaxanthin in the skin and flesh of rainbow trout. The greatest incorporation of pigment was obtained from pigment extracts in shorter feeding periods. A 20 % crab meal was found

Table 3

Carotenoids of rainbow trout which were fed on pigmented diet (0.2 mg carotenoids/g diet) for seven weeks

experiment	no. of fish	wt. per fish (g)	color of flesh	main carotenoids (μg/g fish)				
				astaxanthin ester	astaxanthin	astacene	lutein ester	lutein
1	2	91.5	faint pink	0.5	0.3	0.18	1	trace
2	2	115	pink	1.1	0.39	0.20	0.1	0.08
3	1	172	strongly orange-pink	0.61	1.57	0.09	0.56	0.02

The conc. of carotenoids was calculated in saponified forms.

not to contribute significant amounts of pigments to fish of the 50 g size.

Discussion

The red or yellow color of fins, skin and flesh in wild rainbow trout as well as in other kinds of salmonid fish is due to the carotenoids. The carotenoids which occur most commonly are astaxanthin, canthaxanthin, lutein and to a lesser extent, β-carotene [11, 12, 15, 18]. It has been established that carotenoids from ingested crustaceans and insects are responsible for the pink coloration of trout in nature [4, 6, 18, 12].

Trout and salmon reared on most commercial feeds lack the natural red color because these red pigments are not contained in the feeds.

Peterson et al. [11] reported that natural coloration of brook, brown and rainbow trout (12 to 15 months of age) was obtained after feeding extracts of fresh, raw crayfish (0.048 μg/g feed) for one to two months to low pigmented fish. The same pattern of xanthophylls existed in the original extract. Lutein, when fed to trout, was deposited in the fish without modification. Trout fed β-carotene at a level of 0.44 mg of pigment per gram feed resulted in no deposition of pigments after four weeks.

Deufel [3], Schmidt and Baker [15], and Saito and Regier [12] had shown that canthaxanthin could be used to color the skin, flesh and eggs of salmon and trout. No evidence was presented to show that canthaxanthin was converted to astaxanthin in trout.

Savolainen and Gyllenberg [14] fed rainbow trout dried yeast cells *(Rhodotorula sanneii)*. The major pigments of this yeast are torularhodin and torulene with small amounts of γ-carotene and β-carotene. After feeding the yeast to the trout, lutein and a small amount of canthaxanthin were isolated from the fish. Saito and Regier [13] fed year old brook trout for 12 weeks on diets containing 20 and 30 % shrimp waste and 20 % snow crab waste. The 20 % shrimp waste fed fish were ranked first in skin appearance, flesh appearance, flesh odor and taste. The crab waste was found to have only a slight effect on the pigmentation of the trout. The pigments found in the shrimp extracts were mainly astaxanthin (10 mg/100 g), astacene (0.91 mg/100 g) and an unidentified keto carotenoid (0.24 mg/100 g). In dried crab waste, the pigments found were a lutein-like carotenoid (0.01 mg/ 100 g), astaxanthin (0.47 mg/100 g), astacene (0.17 mg/100 g) and a trace amount of an unidentified keto carotenoid.

Steel [16] fed rainbow trout a diet containing 15 % shrimp waste meal over a 24 week period. The shrimp waste meal increased the pigmentation in the skin and muscular tissue of trout nearly 13 fold over the control fish. The shrimp waste fed trout had a higher rating with regard to flavor and desirability. Sensory evaluation of trout studied by Steel [16] showed that fish which received the shrimp processing waste meal or the shrimp pigment extract meal were rated significantly higher in firmness, color and overall desirability than control fish.

In the present study it was shown that the pigments extracted from red crab waste were readily incorporated into the flesh and skin of trout. Thus, efforts are continuing to develop economic methods for the extraction of this valuable pigment for fish raised in aquaculture.

Summary

The dried red crab waste contained 27.5 % protein, 13.8 % crude lipid and 0.008 % carotenoids of which 95 % was in some form of astaxanthin.

Rainbow trout were fed diets consisting of 20 % whole crab waste or a pigment extract from crab waste adsorbed on a commercial trout ration. The fish fed the pigment extract containing 0.2 mg of carotenoids/g diet were highly pigmented after 7 weeks. The fish fed the 20 % crab meal contained a much lower level of astaxanthin in the skin and flesh than would have been expected on the basis of the level of dietary carotenoids.

It was concluded that red crab waste could be used for the pigmentation of trout.

Acknowledgement

This work was supported by grant no. 04-5-158-6 of the NOAA office of Sea Grant, U.S. Department of Commerce, and National Science Foundation grant no. GF-42495. Rhode Island Agricultural Experiment Station Contribution no. 1662.

The authors wish to extend their sincere gratitude to the Galilee Offshore Marine Inc., Rhode Island for their cooperation and for their supply of crab waste.

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