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Evaluation of Shell Fish Waste As A Feed For Fish

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It is becoming increasingly important that waste products from the agricultural and food processing industries be used in an ecomomic and ecologically acceptable manner. In some food industries the very existence of the plant may be dependent on the disposal of waste material. The disposal may be dictated by economic or legal constraints. Any waste product must compete economically with products that they replace or create their own markets. The introduction of a product derived from waste materials would be greatly facilitated if the new product could be shown to be better and safer than existing products.

Ocean quahogs have long been an underutilized clam species on the east coase of the USA. A market has not existed in the past becaous of the abundance and ease of harvest of surf clams. Since 1976 the surf clam resource has marketly declined and a shift to the use of ocean quahogs as a substitute has resulted. The ocean quahog is a different type of animal and requires different processing conditions. As a result more visceral waste is generated. Although unusable for clam meat applications, preliminary studies indicated that the waste material could be used as a base for fish feed. This utilization would be preferable to its disposal in a sanitary land fill.

It has been estimated that as many as 90 tons of ocean quahogs may be landed per year. Of this amount, 50% may be eviscerated for use in a milk-based clam chowder.

Key wards : Shellfish, Waste products, Fish Feed, Salmonid, Evaluation.

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If 5% of the total solids were recovered this would amount to 5000 kilograms of recoverable waste.

One potential use of the clam waste solids would be as a protein supplement of a trout ration. The solid clam waste could be dried into a fish meal or utilized in a moist pellet. Commercially, both dry and moist feeds are available. Generally speaking, the dry feeds are more readily available due to their lower spoilage potential. The Oregon Moist Pellet and, later, the Abernathy Moist Pellet were developed in the Pacific Northwest of the USA for salmonids which better accept the softer and more palatable moist diet. Ghittino[1)] suggested that there would be a shift to the moist diets. Ghttino further states that the food convertion of wet diets average 5:1 whereas the dry pellet is 1.5-2:1. However, he states that the production costs to rear one kilogram of trout on a moist diet is about half that of rearing trout on a dry diet. This suggests that the economics of feeding either diet are similar.

Other advantages of the moist diet could include better acceptability, superior growth and better nutrient retention. Obvious disadvantages would include perishability in shipment and storage, induction of diseases such as kidney diseases, nocardiosis and ichthyophoniasis. Often a moist diet dose not bind well, such that more food is released on impact with water. The dry feeds are also not free of problems and have caused hepatoma from aflatoxins as well as nutritional disease from nutrient deficiency due to formulation or loss during storage.

The goal in fish farming is neither to produce or use the most ideal food but to attain the best growth, survival and quality at the lowest possible costs. More often than not, the primary concern has been to obtain good growth and survival without regard for quality.

The pigment of fish is a critical area since this is a primary visual means by which the consumer judges quality.

The red crab, *Geryon quinquidens*, has been found to be a potential source of protein and the pigment astaxanthin. The red crab is found along the edges of the continental shelf from Nova Scotia to Cuba and has also been reported on the eastern side of the Atlantic. They live at depths ranging from 350 to 600 meters at water temperatures of 3-10℃. The size of the resource is not known with accuracy in terms of sustainable yield. Much of the technology for the processing of red crab has been adapted from other products, such as Snow or King crab. About 80% of the red crab is discarded although it has potential as a protein and pigment source. The pigment astaxanthin is necessary for the proper pigmentation in Salmon.

The objectives of this research was to further develop the clam waste moist pellet with a goal to improving the fatty acid and amino acid profiles and to lower the advantitious contamination (PCB's, etc). Then to compare this diet with commercial wet and dry diets under laboratory conditions for growth and survival of salmonids. Feeding trials were conducted in commercial and state hatcheries to compare this moist diet with presently used dry diet . In addition, some work is reported on the extraction of red crab waste pigments and their feeding to trout.

MATERIALS AND METHODS

1. Formulation of waste diets

The clam process waste was removed from the discharge of the slicing and grinding operation at Blount Seafood Corporation, Warren, RI. USA. Removal is by a hydrocyclonics roto-strainer-essentially a 1.5m screen with a 0.5 mm open mesh, rotating in a horizontal plane. The solids are further de-watered with a reciprocating screen and collected in a hopper.

The clam diet was mixed and pelletized with a Hobart commercial meat grinder using a 3/32" dye. The feed was stored in the frozen state due to the high moisture content. The size and shape of the frozen pellet was comparable to a commercial pellet of 3/32" size.

The waste material of red crab was obtained from the Galilee Offshore Marine Inc.. 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. 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).

Table-1 Proximate Analysis of Fish Diets

	Clam meal	Crab meal	Clam Diet	OMP	Silver Cup
Crude Protein	21.0	27.5	43.9	37.0	38.0
Crude Lipid	13.8	13.8	15.1	12.7	6.5
Ash	6.0			8.0	15.0
Fiber	67.0		15.3	29.1	7.0
Chitin		21.0	1.7		
N-Free Extract		1.7		13.2	

An anlysis of the meals and the clam waste diet is given as Table 1. 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[2]. 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-2. For each 60g of dry mix, 40ml 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-2 Composition of test diet

Composition	OMP	Clam Blount	Crab
Fish meal	28.00	47.50	20.00
Crab meal	30.00		42.66
Clam waste		19.80	
Casein			
Shirimp meal	4.00		15.60
Dextrin			
Wheat meal		9.90	
Cottonseed meal	17.00		
CMC			1.30
Wheat germ meal	4.00		
Herring meal			
Corn distillers' dried solibles	5.00		
Fish oil	4.00	9.90	
Corn oil	6.00		7.24
Vitamin pre-mix	1.50	1.00	2.00
Vitamin E			0.26
Cholime chloride	0.50		0.70
KH_2PO_4			1.54
Water		9.90	
Gelatin		2.00	8.70
	100.00	100.00	100.00

2. Fish culture systems

The diet testing was conducted at the URI facility which consisted of 12 500liters fiberglass tanks (Sims Fiberglass). Each tank had a center stand pipe and as outer pipe with holes at the bottom. This allowed uneaten food if any to be flushed from the tank. Flow rate was maintained at 8 liters ／min.

Clam waste Experiment

Rainbow trout, *Salmo gairdneri*, (average weight 50.0g) were purchased from American Fish Culture, Carolina, RI. After the one month acclimation period, 140 fish (average weight 71.0g) were divided into the ten fish tanks. The culture system was operated according to Kuo *et al.* [3] Water temperature was kept at 12.0-12.5℃, oxygen level was 8.0±0.3 ppm and pH was at 6.58±0.15 during the three-month feeding period.

Tank #2, 8 and 10 were fed the Blount B diet, Tnak #3, 7 and 9 were fed Blount E diet, tank #1 and 5 were fed Silver Cup and tank #4 and 6 were fed Oregon Moist Pellet (OMP). The fish were fed twice a day, in the morning and in the evening, at a rate of 3% body weight (dry weight basisi) per day. The amount of diet to be fed was re-calculated every two weeks. At the end of the trials, each treatment was analyzed for its amino acid, fatty acid profiles and level of pesticides and PCB's.

Since raceways were not available at the RI State Hatchery, Perryville, a wooden cage was constructed. The divided cage was placed within the main rearing pond of the hatchery. The cage was constructed so as to stand within the pond system. One hundred fifty rainbow trout were placed in each cage. The fish in one side were fed the Blount diet and the other, Rangen's Trout Feed. Feeding was at the rate of 3% (dry weight) of the fish body weight.

Fish culture: Red Crab

Two sets of different size rainbow trout were supplied by local hatcheries. The small fish had a mean weight of 25g per fish obtained from Perrybille Trout Hatchery, RI Department of Natural Resources. The large fish had a mean weight of 85g or 132g obtained from American Fish Culture Company. The fish were held in fiberglass tanks with aerated, flowing water (2-4 liter per minute). The water temperature was 11℃. The oxygen level was 8ppm. The small 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.

3. Amino acid analysis

The protein hydrolysis and amino acid analyses were performed in the same manner as described by Seidel *et al.*[4].

The samples from diets and fish were hydrolyzed with 6.0N HCl at 110℃ for 22 hours. The hydrolyte was filtered and rotary-evaporated to remove any HCl from the residue. The free amino acid residue was then dissolved in pH 2.2 sodium citrate buffer.

Amino acid analysis were performed on a NC-2P Technicon Auto Analyzer equipped with a 25 x 0.5 cm cation exchange column packed with Chromobead-Resn, type C-3 (ion exchange capacity of 5 meq／mg : 8% cross linked). The column was operated at 55℃ with flow rate of 0.5 ml／min. The absolute amount of each amino acid was determined by the method used by Seidel *et al.*[4].

4. Lipid extraction and analysis

Total lipid extractions and fatty acid analyses were performed in the same manner as described by Schauer and Simpson[5]. Briefly, this involved lipid extractions by the Bligh and Dyer technique as modified by Kates[6] with subsequent methylation with 14% boron trifluoride methanol (w／v). Fatty acid esters (FAME) were identified with a flame ionization detector in a single column, Varion Aerograph 1200 gas-liquid chromatography unit, isothermally operated at 180℃. FAME were separated on a 3% ethylene glycol succinate polyester-Z column and identified and quantified with an electronic integrator (Hewlett Packard 3380 A).

5. Chlorinated hydrocarbon analysis

The analysis of chlorinated hydrocarbon in the diet and fish was performed as described by Olney *et al.*[7]. The weighed sample was blended with anhydrous sodium salfate and then extracted with petroleum ether in a Soxhlet apparatus. The extracts were chromatographed on Woelm alumina, activity grade III, to remove lipids, pigments and other co-extractives. The eluents were then fractionated into four fractions (HCB, PCB's, pesticides and toxaphens) on silicic acid[8]. Individual column fractions were analyzed by dual column electron capture gas chromatography (Tracor MT-220 gas chromatograph, Ni-63 detectors, 180 x 0.4cm glass columns packed with 1.5% OV-17／1.95% QF-1 or 4% SE-30／6% Qf-1 on 100／120 mesh Supelcon AW-DCMS). Peaks were identified by retention time and quantitated using peak height as compared with standards.

6. Carotenoid pigment analysis

The carotenoid pigments were extracted with acetone in a Waring blender, then transferred to petroleum ether. Carotenoid pigments were separated on Microcel-C columns with petroleum ether and 1-4% acetone in petroleum ether as developing solvents. Each pigment fraction was saponified. Silica gel-G sheets (Eastman Chromatogram 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%／1cm values for lutein, canthaxanthin and astaxanthin were 2500, 2200 and 2000 respectively in petroleum ether. The pigments were identified in the same manner as in the crab pigments[8].

RESULTS AND DISCUSSION

Table-1 gives the proximate composition of some of the diets tested; the Blount diet was higher in crude protein and crude fat than Oregon Moist Pellet (OMP). The diets were fed on a dry content basis. Table-2 shows the composition of the Blount diet and the OMP, the crab waste and Silver Cup.

Table-3 shows the amino acid composition of the diets and the fish fed the diets (URI study). All of the diets showed a remarkable similarity in their amino acid composition. As expected, the variations were greater in the diets than in the fish. For example, methionine appeared to be low in the Silver Cup diet yet the fish level of this amino acid was high. The two Blount diets (B and E), which varied only in the source of the wheat midlings, showed only minor differences and were very similar to the OMP.

Table-4 shows the fatty acid profiles of the various diets and the fish fed the diets. Large differences were noted between the fatty acid profiles of the various diets and the fish. The two Blount diets and Blount fish showed lower levels of 18:2ω6 than Silver Cup and the OMP. The level of 20:5ω3 was higher in the Blount diet than the two commercial diets. The OMP showed a slightly lower 22:6ω3 than the other diets. The ratio of ω3／ω6 was thus higher in the Blount dist than the other diets.

Lasker and Theilacker[9] showed that the marine Pacific sardine generally deposited and utilized natural foods unchanged if the dietary fatty acids were present. The rainbow trout diets accentuated nutritional disease such as heart myopathy, pale and fatty livers, shock syndrome and fin erosion. Maximum fish growth was exhibited when the 18:3 fatty acid was the sole source of fatty acid. However, the feed efficiency was better

Table-3 Amino acid profiles of the Diets, Blount B, Blount E, Silver Cup and OMP, and the Fish cultured with Each Diet (Expressed as gram amino acid per 100 gram protein)

Amino	Blount B +		Blount E +		OMP		Silver Cup		Chinook
Acid	Diet	Fish	Diet	Fish	Diet	Fish	Diet	Fish	Salmon Req.
Asp.	10.2	13.0	11.2	11.4	10.9	11.3	9.7	11.8	
Thr.	4.5	5.7	5.1	5.4	5.0	5.3	4.2	5.3	
Ser.	5.0	4.9	5.3	4.7	5.3	4.3	5.1	4.4	
Glu.	16.8	17.1	17.8	18.0	18.2	16.8	17.7	17.7	
Pro.	6.3	4.1	6.1	4.3	4.4	4.3	4.8	4.1	
Gly.	10.0	6.0	9.7	5.7	7.3	5.4	5.8	6.0	
Ala.	8.6	6.9	9.5	7.4	7.7	7.2	7.4	7.4	
1/2Cys	Trace	–	–	–	0.8	–	0.4	–	
Val.	5.3	6.0	6.4	6.2	5.5	6.1	6.8	6.1	3.3
Met.	2.7	3.4	2.9	3.4	2.6	4.0	1.8	3.7	1.5**
Ile.	3.1	3.4	3.8	3.5	3.7	4.4	3.2	4.1	2.5
Leu.	7.1	8.2	7.4	8.0	7.1	8.6	8.4	8.5	4.0
Tyr.	2.3	3.3	2.5	3.3	2.8	3.6	3.1	3.4	
Phe.	3.9	4.0	3.5	4.2	4.0	5.0	5.6	4.3	5.3***
His.	2.6	3.7	2.1	3.9	2.4	4.1	3.4	3.9	1.8
Lys.	9.0	10.8	7.4	11.4	7.1	11.0	6.2	10.5	5.0
Arg.	7.6	7.1	7.4	6.6	7.3	6.2	7.0	7.1	6.3
NH_3	11.5	8.8	9.3	8.8	14.3	8.7	15.7	7.7	

* Minimum whole body amino acid requiement levels of Chinnok salmon
** Without Cys.
*** Without Tyr.
\+ B and E represent 2 samples of wheat middlings.

when $\omega 3/\omega 6$ fatty acid were present in the proper ratio. Neverthless, dry commercial diets cotinue to utilize 18:2-rich vegetable oils as their primary energy source because of their purity, availability and low cost. The stability of a polyunsaturated oil on a dry pellet would, of course, present a rancidity problem.

Yone[10], in culturing red sea bream, found that a vegetable oil, when supplemented with a pollock oil in a diet gave a higher feed efficiency and resulted in substantially greater growth rates. The pollock oil supplied long chain fatty acids. There is also some question that the pollock oil may have provided a vitamin A supplement.

Our work with the fish *Menidia* tends to bear out the need for a balanced fatty acid content. A recognized freshwater salmon diet was found to give a fatty acid ratio $\omega 3/\omega 6$ of 0.12. Artemia gave a ratio of 2.19, whereas marine oils gave ratios of 6-8.

Table-4 Percent Composition of the Fatty Acids of the Test Diets, Bolunt B, Blount E, Silver Cup and OMP, and the Fish Cultured with Each Diet for 12 weeks

	Blount B		Blount E		Silver Cup		OMP	
FAME	Diet	Fish	Diet	Fish	Diet	Fish	Diet	Fish
12:00	0.06	0.04	0.05	0.04	0.09	0.04	0.05	0.03
13:0	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01
14:0	5.73	4.07	5.55	3.97	2.78	2.25	2.50	1.98
15:0	0.50	0.32	0.36	0.34	0.32	0.33	0.28	0.29
15:1	0.06	0.04	0.05	0.02	0.03		0.03	
16:0	19.05	17.01	19.55	17.81	20.26	20.41	17.35	15.84
16:1	9.05	7.38	8.10	6.60	4.67	4.40	4.65	3.71
16:2ω7	1.08	0.81	1.02	0.81	0.15	0.17		0.07
16:2ω4	1.34	0.85	1.39	0.76	1.52	0.85	1.30	0.30
16:4ω1	1.13	0.51	1.12	0.34	0.49	0.23	0.25	0.51
18:0	4.22	4.22	4.41	4.44	4.59	6.74	3.49	5.72
18:1ω9	21.58	24.02	21.81	24.90	17.12	25.29	23.24	25.33
18:2ω6	5.94	7.06	5.99	6.98	28.56	17.22	25.70	23.71
18:3ω6						0.53		0.18
18:3ω3	0.74	1.33	1.13	1.30	3.36	1.75	2.87	2.38
18:4ω3	2.25	1.71	2.18	1.68	0.75	0.64	0.71	0.68
20:1ω9	3.54	3.28	3.63	3.56	0.92	1.94	3.33	3.16
20:2ω6		0.40		0.23		1.03		1.50
20:3ω6	0.50		0.48		0.51	1.08	0.63	0.93
20:5ω3	11.05	6.79	10.76	7.09	4.77	2.01	4.42	1.63
21:0		0.90		0.97		0.68		0.77
22:1ω11	2.58	1.69	2.64	2.21	0.57	0.48	2.97	1.83
22:4ω6		0.13		1.01				0.36
22:5ω6	0.05		0.04		0.21	0.24	0.42	0.06
22:5ω3	1.20	1.52	1.32	1.34	0.53	0.39	0.25	0.27
22:6ω3	7.73	15.44	7.74	14.21	7.37	11.46	5.72	9.44
24:1ω9		0.49		0.24		0.18		0.01
ω3／ω6 ratio	3.54	3.54	3.22	3.55	0.57	0.54	0.52	0.53

In these same studies, the amino acid composition of the diet was less critical as long as the level of protein was high.

Table-5 shows an analysis of the diets and fish for chlorinated hydrocarbons. It can be seen that the Blount diets using eastern fish oil (herring oil) showed much higher contamination level than the other two commercial diets. A similar result was observed in the fish fed the test and the control diets. The fish fed the Blount diets showed much higher levels of PCB's than the fish fed Silver Cup and OMP.

The level of chlorinated hydrocarbon in the herring oil and the clam waste was analyzed. It was found that the herring oil contained a high level of PCB's and pesticides (9.2 ppm, 7.4 ppm for PCB's) and some PCB's and pesticides (0.25 ppm) were detected from the clam waste. Thus the higher level of contamination in the Blount diets was mainly caused by the herring oil although the clam waste contained some pollutants.

Table-5 Pesticide Residues and PCB's in the Blount Diet B and E, Silver Cup and OMP Diets and the Fishsh fed Each Diet for 12 Weeks.

(μg/g dry weight)

	Blount B		Blount E		Silver Cup		OMP	
Pesticides	Diet	Fish	Diet	Fish	Diet	Fish	Diet	Fish
HCB	0.009	0.004	0.01	0.004	0.001	0.001	0.001	0.001
α−BHC	ND	0.002	ND	0.001	ND	ND	ND	ND
t−Chlodane	0.037	0.095	0.048	0.111	0.003	0.006	0.007	0.005
c−Chlordane	0.08	0.1	0.129	0.115	0.003	0.005	0.009	0.008
ppDDE	0.242	0.175	0.283	0.225	0.06	0.1	0.112	0.1
ppDDD	0.082	0.026	0.081	0.025	0.04	0.05	0.06	0.05
ppDDT	.0.007	0.005	0.02	0.009	0.007	0.07	0.005	0.007
PCBs*	3.49	1.6	3.84	1.85	0.126	0.09	0.17	0.101

* Sum of Aroclor 1242, 1248, and 1254

The fish were fed on the basis of 3% body weight/dry (dry weight basis). Table-6 shows that the four diets were essentially equal in providing growth for the fish over the 12-week period. The survival on Silver Cup, OMP and Blount E was greater than 92%. The lower level of survival in Blount B (76%) was due to a loss of 6 fish in tank #8 that was not experienced in the other two tanks.

Table-6 Growth Levels of the Fish fed the Blount Diets, Silver Cup and OMP for 12 Weeks (grams)

Weeks	Blount B	Blount E	Silver Cup	OMP
0	70.9	71.0	70.4	72.0
2	93.9	92.8	91.0	94.4
4	111.8	114.5	110.6	111.1
6	135.5	137.4	132.8	130.5
8	161.1	156.5	159.3	154.5
10	187.0	189.3	174.9	185.8
12	203.1	198.9	204.9	199.3

One specific ingredient in the two Blount diets was checked for contamination. Two samples of wheat midlings were used which were designed B and E. Neither were found to containe aflatoxins and were low in chlorinated hydrocarbons. Therefor, the slightly higher mortalities with the Blount B diet are neither explained by the chlorinated hydrocarbon levels nor the presence of aflatoxins.

The feed conversion levels for the four diets are shown in Table-7. Again, the values of the various diets are very similar on the average. There are variations between tanks receiving the same diet.

Table-7 Feed Conversion of Test Diets

Tank #	10 weeks	12 weeks
1 (S. Sup)	2.04 (±0.45)	2.05 (±0.45)
2 (Blount B)	1.84 (±0.42)	2.64 (±1.77)
3 (Blount E)	2.25 (±1.38)	3.29 (±2.64)
4 (OMP)	2.07 (±0.47)	2.51 (±1.07)
5 (S. Cup)	1.95 (±0.49)	2.88 (±2.12)
6 (OMP)	1.76 (±0.23)	2.50 (±1.67)
7 (Blount E)	1.77 (±0.33)	2.70 (±2.11)
8 (Blount B)	2.03 (±0.53)	2.43 (±1.04)
9 (Blount E)	1.94 (±0.52)	2.89 (±2.19)
10 (Blount E)	1.84 (±0.52)	2.20 (±0.94)
Average		
Silver Cup	1.995 : 1	2.465 : 1
OMP	1.915 : 1	2.505 : 1
Blount B	1.903 : 1	2.423 : 1
Blount E	1.987 : 1	2.960 : 1

It was shown in Table-5 that the Blount diets were somewhat contaminated with PCB's. The values detected in the Blount diets (3.5 ppm for Blount B and 3.8 ppm for Blount E) were more than 25 times higher than that of Silver Cup (0.13 ppm) and 20 times greater than that of OMP (0.17 ppm).

A similar result was observed in the fish fed the test and the control diets. The fish fed the Blount diets showed much higher levels of PCB's than the fish fed Silver Cup and OMP.

The effects of pesticides (especially DDT and PCB's) on fish have been studied. It has been shown that PCB's and DDT inhibit K^+, Na^+, Mg^{++} -ATPase[11]. Hansen *et al* [12]

studied the effects of dietary Aroclor 1242 on channel catfish and reported that channel catfish fingerlings fed a diet containing 20 ppm Aroclor 1242 for 20 weeks responded with a reduced weight gain and liver hypertrophy when compared to controls. They found no histopathological lesions in fish fed PCB's. Both Blount diets contained much higher PCB's levels (3.5 and 3.8 ppm) than the two commercial diets. However, these PCB's and pesticides levels did not appear to affect the growth rates of the rest of the test fish over the 12-week feeding period.

Mortalities consisted of 8 fish on the Blount side of 5.3%, while no mortalities were experienced for the Range's diet. The dead fish were examined by the Department of Animal Pathology, URI. The posted fish showed no gross abnormalities. At this time, we are unable to determin a cause for the mortalities.

Two series of experiments were conducted to determine the effect of crab waste as a pigment source for fish. In the first experiments fish were fed 20% crab waste in their diet. The growth of the fish was comparable. The carotenoid content of the fish is given in Table-8.

Table-8 Crotenoid Content of Fish fed 20% Crab waste Diet

Pigments	Control Diet* 15 Weeks	Crabmeal Diet* 15 Weeks	Crabmeal Diet* 23 Weeks	Control Diet** 4 Weeks	Crabmeal 4 Weeks
Total Astaxanthin	——	0.051	0.052	——	0.056
Zeaxanthin	——	——	——	0.26	0.23
Lutein	0.052	0.014	0.014	0.33	0.26
Canthaxanthin	——	——	——	0.01	Trace
Total Pigment	0.052	0.064	0.066	0.6	0.446

* 25 g fish at the beginnig of the experiment

** 132 g fish at the beginning of the experiment

The pigment was extracted from the crab waste and the extract was added to the feed. Table-9 shows that the fish took up a large amount of pigment and that this was related to size.

The red or tellow color of fins, skin and flesh in wild rainbow trout as well as in other kinds of slmonid fish due to the carotenoids. The carotenoids which occur most commonly are astaxanthin, canthaxanthin, lutein and to a lesser extent, β-carotene. It has been established that carotenoids from ingested crustaceas and insects are

responsiblefor the pink coloration of trout in nature. Trout and salmon reared on most commercial feeds lack the natural red color because these red pigments are not contained in the feeds.

Table-9 Crotenoid Content of Fish fed on Pigmented diet (0.2 mg Carotenoid/g Diet) for seven Weeks

		weight per		Main	Carotenoid (μg/g fish)	
Experiment	No. of Fish	Fish (g)	Color of Fish	Astaxanthin	Astacene	Lutien
1	3	91.5	Faint Pink	0.8	0.18	1.01
2	3	115	Pink	1.49	0.2	0.15
3	3	172	Storongly Pink	2.18	0.1	0.62

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.

This study also showed that the trout could not extract much pigment from the meal but could readily deposit the extracted pigment in the flesh and skin.

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