AN ABSTRACT OF THE THESIS OF

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Title: SHRIMP PROCESSING WASTE AS A PIGMENT SOURCE FOR RAINBOW TROUT (SALMO GAIRDNERI)

Abstract approved: Dr. David L. Crawford

A suitable method for the utilization of shrimp processing waste was investigated. The shrimp waste, which consisted of shell, viscera, and residual meat particles, was dried into a pink meal. This meal contained 5.4 percent moisture, 23.5 percent ash, 4.1 percent crude lipid, 36.3 percent actual protein, and 9.5 percent chitin nitrogen. Its mineral analysis revealed 1.37 percent phosphorus, 11.50 percent calcium, 5.30 percent potassium, 1.28 percent sulphur, 3.0 ppm cobalt, 11.9 ppm copper, 412 ppm iron, 9.5 ppm manganese, and 75.0 ppm zinc.

The shrimp waste meal was used as a dietary supplement for rainbow trout (Salmo gairdneri). It was incorporated into a purified diet at a level of 15 percent of the dry mix. After a feeding period of 24 weeks, the shrimp waste meal increased the pigmentation in the skin and muscular tissue of trout nearly 13 fold when comparisons were made to control fish. Sensory evaluations indicated that fish fed the

diet containing shrimp waste meal were rated significantly ($P > .01$) higher in flavor and desirability than trout fed the control diet.

An effective method to achieve pigmentation in the skin and muscular tissue of trout was investigated. Five pigment-rich diets were fed to separate groups of trout for 34 weeks. The pigmentation produced by each diet was compared to a sixth group of trout that received a control diet.

The diet which contained 15 percent shrimp waste meal or a pigment-lipid extract of the meal proved the most effective in achieving muscular pigmentation. External pigmentation in the fins, skin, and operculum was produced more rapidly by the diet which contained a pigment-lipid extract of the shrimp waste meal. The carctenoid canthaxanthin did not produce pigmentation when fed in a crystalline form. Pigmentation was produced in the muscular tissue when canthaxanthin was fed in a water-dispersible form.

Sensory evaluations showed that fish which received the shrimp waste meal or the pigment-lipid extract of the meal were rated significantly ($P > .05$) higher in firmness, color, and overall desirability than control trout. Trout that received the water-dispersible form of canthaxanthin were rated significantly ($P > .05$) higher than control trout only with regard to color.

Shrimp Processing Waste as a Pigment Source for Rainbow Trout (Salmo gairdneri)

by

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SHRIMP PROCESSING WASTE AS A PIGMENT SOURCE FOR RAINBOW TROUT (SALMO GAIRDNERI)

INTRODUCTION

The pink Pacific shrimp is a crustacean scientifically classified as Pandalus jordani (29). In Oregon and Washington the pink shrimp fishery dates back to 1956 (34). The shrimp fishery in Oregon alone is a million dollar-plus industry. Up to the month of August, 1970, shrimp boats had landed 7.9 million pounds in Oregon's coastal ports and estimations set landings at 10 million pounds at the completion of the 1970 season (26).

New innovations are springing up in the shrimp industry with one of the most unique seen in the processing phase. The mechanized peeling of the shrimp is an intriguing operation and, as in many examples in the food industry, a versatile application of engineering skills.

To use the seemingly cumbersome movements of a machine to remove the hard carapace which surrounds the delicate shrimp meat is not an easy task. It is understandable why processing plants have employed the adept hands of individuals to perform this task.

During the mechanized peeling operation, the shrimp are fed into an apparatus consisting of rows of rubber rollers located side by side with a pair of frames containing many metal fingers which completely cover these rollers. These intermittently press the shrimp gently

against the rollers to break the adhesion between the shell and the body. The rubber rollers then grip the shell particles and pull them off the meat, much like an old clothes wringer might snatch the sleeve from your arm.

It is at this point in the shrimp processing operation where the interest for this thesis originated. With mechanization, product output has increased and as a consequence so has the waste material. Since the waste material accounts for approximately 70 percent of the whole shrimp, there arises a timely problem in our age of environmental pollution and ecological awareness.

In essence, the investigation of a suitable method for the utilization of shrimp processing waste was the underlying purpose of this research.

LITERATURE REVIEW

Shrimp Waste Utilization

Scientific research and technological skills have made significant advances possible in the area of the food industry which deals with the utilization of waste material from processing operations. It has even been said that in pork processing everything is used except the "squeal".

The fishing industry might be slightly antiquated in this respect, yet trends are starting where the waste material is being processed into utilizable by-products. The shellfish industry could also utilize its waste material if it was proven economically feasible. It is apparent that the waste material from the shrimp industry would contain everything except the edible shrimp meat and consist primarily of shell and visceral material.

Chemical Composition of Crustacean Waste

Previous research dealing with crustacean waste has been directed primarily toward its nutritional value and chemical composition. When compared to most fish meals, crustacean wastes generally contain larger amounts of chitin and minerals.

Kirk et al. (22) showed that air-dried shrimp meal contained 27.2% chitin, 34.4% chitin-free protein, 1.0% ether extractable lipid,

3.3% nitrogen-free extract, and 25.3% ash. The ash fraction was shown to contain 42.3% calcium and 10.7% phosphorus. As reported by Lovell et al. (23), crayfish meal contained 14.1% chitin, 32.2% chitin-free protein, 4.9% ether extract, 14.2% crude fiber, 18.1% calcium and 29.0% total ash. Kifer and Bauersfeld (21) determined the chemical composition of king crab meal and blue crab meal. King crab meal was shown to contain 39% chitin-free protein, 8% ether extractable lipid, 7% calcium, 1% phosphorus, and 30% total ash. Analysis showed blue crab meal to be composed of 29% chitin-free protein, 2% ether extractable lipid, 18% calcium, 1% phosphorus and 48% total ash.

The amino acid profile of vacuum-dried shrimp meal was compared to the shrimp meat by Burkholder et al. (4). They found that the shells contained more glycine and valine while the meat was higher in cystine, histidine, methionine, and tryptophan.

Animal Diet Supplementation with Shrimp Waste Meal

Research of an applied nature began as early as 1934 when Francisco et al. (10) used shrimp meal as a supplement in poultry diets. This was a comparative study and indicated that shrimp meal was superior to meat scraps, tankage, or fish meal in securing high yields of eggs. Fronda and Kabigting (13) showed that 25% was an optimum level for the supplementation of diets for growing chicks.

Fronda and Campos (11) later implicated it as a factor for improving poultry production. Improvements in duck egg production has been shown with diets that contained shrimp meal (12). Shrimp meal has also been used as a supplement in rations for growing pigs (1) and steers (22).

Trout Diet Supplementation with Shrimp Waste Meal

Although present feeds for hatchery or farm-raised trout are reported to produce top quality fish (6), the trout's natural coloration deserves further attention. Trout that spend their lifetime in a natural environment often are more colorful and asthetically appealing than hatchery-raised trout. Assuming that one of the important variables between the natural trout and the hatchery-raised trout is their diet, it would seem likely to consider that the natural trout derives its colorful pigmentation from its food source.

In 1948 Niort (25) noted that trout feeding largely on crustacea acquired a pinkish tint which had implications of increasing the trout's economic value. Several other workers have reiterated the belief that crustacean pigments are responsible for the natural coloration of trout (37, 9, 15, 3).

In 1960, Rousseau (30) investigated the pigment content of shrimp meal and implied that it showed promise as an effective pigment source for trout. In 1968, a Danish report (41) showed that feeding a

commercial shrimp meal (high-temperature processed) did not improve flesh color. The report also stated that high quality vacuum-dried shrimp meal improved trout taste and color.

In 1968, Evans (7) reported on the establishment of a shrimp-waste industry in Maine directed by Robert Curren. Curren conducted work which consisted of feeding trout shrimp-waste in the form of a frozen paste. The trout which received the shrimp waste were reported to be brilliant in color. The results also suggested that the shrimp waste could possibly be an important nutritional ingredient for trout. Curren presumed that the drying and subsequent storage of shrimp waste would cause chemical changes which could make it ineffective as a coloring agent for fish.

In 1970, Saito and Regier (31) explained the desirability of having moderately pink tissue in salmonoids and described a method for determining the amount of pigmentation in their flesh. This report briefly mentioned that a diet containing 20% dried shrimp waste would impart a desirable color to the fish if fed for a 12 week period. These investigations were still incomplete.

Pigmentation Inducement in Fish

Several other pigment-rich sources of food have proven successful in achieving coloration in certain species of fish. Sumner and Fox (38) studied the pigmentation of Fundulus parvipinnis and

reported increased tissue coloration after the ingestion of carotenoid-rich diets. Tunison et al. (40) described the pigments responsible for coloration in trout and fed carotenoid-rich diets, including shrimp, paprika, and herring gull egg extracts, to successfully increase the coloration in the trout's flesh. Grangaud et al. (16) fed the pigmented tissue of Aristeus antennatus to rainbow trout and produced coloration within four months. Hirao et al. (19) showed that various carotenoid-rich diets would turn goldfish and fancy colored carp red. In 1966, Peterson et al. (28) described feeding trials with brook, brown, and rainbow trout. Their results showed that extracts of crayfish as well as other pigment sources could successfully achieve a desirable pigmentation in the tissue of these fish. An undesirable yellow pigmentation was produced in the skin of the trout which were fed paprika pepper extracts.

Stevens (36) reported no effect upon trout coloration after feeding the carotenoids lutein and astacin. Hirao et al. (18) fed beta-apo-2-carotenal to trout and reported a brownish-orange tint acquired in the flesh. In 1969, Schmidt and Baker (33) fed salmon, cutthroat trout, and rainbow trout a commercial preparation of the carotenoid canthaxanthin. The pigmentation which was achieved was indicated as being more stable to heat processing than the natural pigmentation of these fish. In 1970, Savolainen and Gyllenberg (32) fed yeast preparations which were rich in non-specific carotenoids, such as torularhodin and torulene, to produce tissue coloration in rainbow trout.

EXPERIMENTAL PROCEDURE

Shrimp Waste Processing

The waste material from a Pacific shrimp (Pandalus jordani) mechanical peeling operation was processed into a dry pink meal. The shrimp waste consisted of shell, residual meat, and visceral material. The waste material was obtained from Pacific Shrimp, Inc. of Warrenton, Oregon. The shrimp waste was collected in porous plastic baskets that were placed under a disposal outlet located at the water separation point in the operation. At this point, jets of water sprayed the meat to remove adhering shell and visceral material. A generalized schematic diagram, which illustrates the shrimp processing operation and the point where the waste material was collected is shown in Figure 1.

The shrimp waste was then dewatered by hand pressing over porous screens. It was then ground into a thick slurry using an Urschel Mill (Model MG) which had a rotating head with 0.012 inch openings. An antioxidant (Tenox IV) was thoroughly mixed into the slurry at a level of 0.05 percent (wet weight). The slurry was poured on the steam jacketed drums of a Stokes atmospheric double drum dryer (Model 214-1) for initial drying. The steam pressure was maintained at approximately 80 p.s.i.g. which produced a product temperature of 190° F. on the surface of the drums. The drums were spaced 0.004 inch

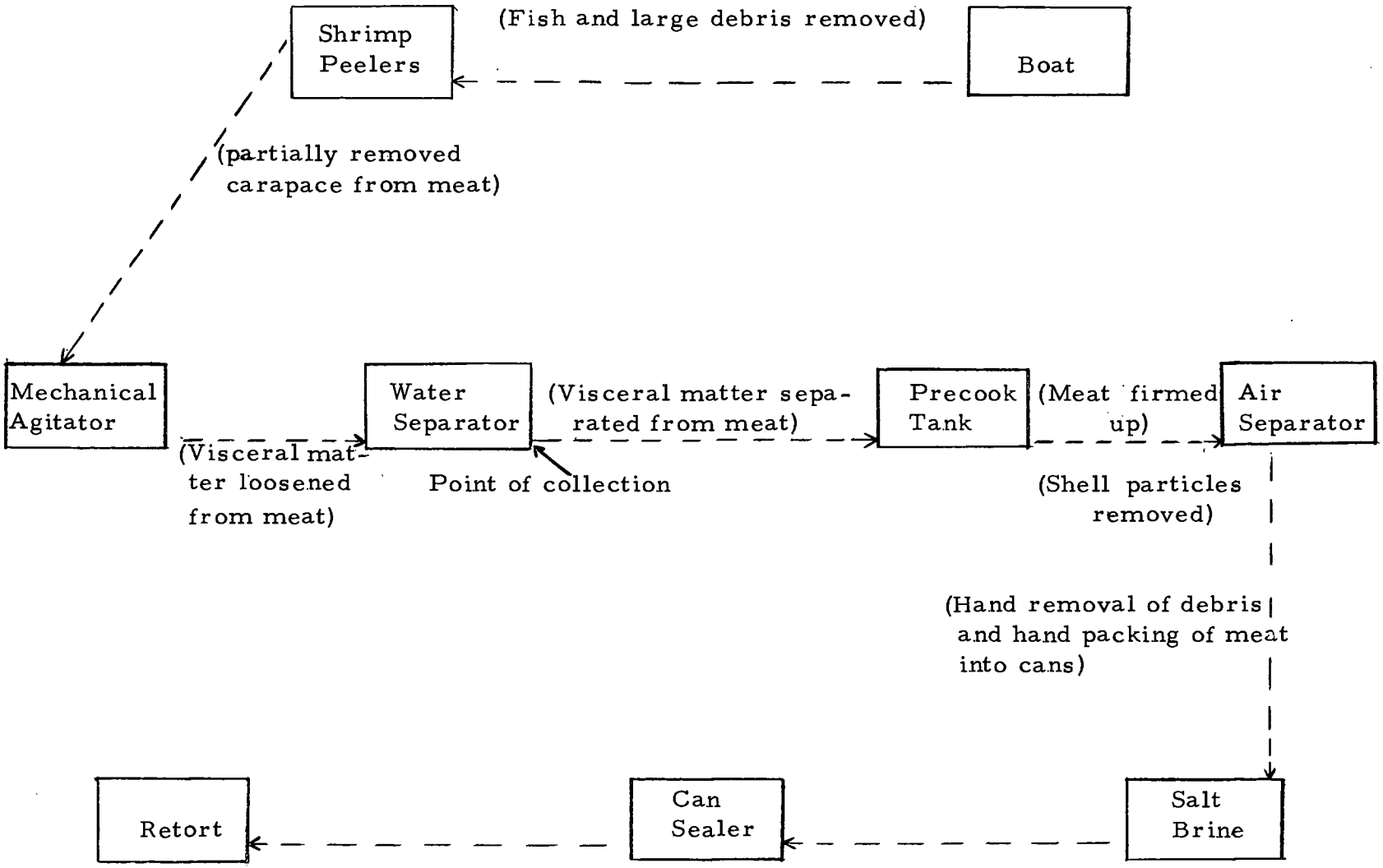


Figure 1. Schematic diagram of shrimp processing operation.

apart and were set at a speed of 5 r.p.m. A ventilation system over the drum drying unit aided in the removal of water vapor.

The drum drying operation was carried out immediately after the shrimp waste was ground into a slurry. If the ground waste was held in a cold room (approximately 40°F) for processing at a later time (approximately 24 hours), deteriorative changes occurred which resulted in its putrification. This was most likely caused by the rise in temperature during the grinding and the consequent increase in enzymatic activity associated with the enzyme systems present in the shrimp waste.

After drum drying, a secondary drying operation was used to remove additional moisture. This involved drying the drum-pressed flakes on flat porous screens located in a chamber which was equipped to circulate warm air. These flakes were dried at 130°F. for approximately six hours.

The flakes were then milled into a fine meal using a Mikro-Pulverizer (Type SH) hammer mill that was fitted with a screen possessing 0.020 inch openings. The shrimp waste meal was vacuum-sealed in cans and stored at 0°F.

An average yield from the shrimp waste processing operations was determined. Gravimetric methods were employed to determine the percentage yield of shrimp waste meal from the drum drying phase

through the final milling phase. This was determined on four separate batches of shrimp waste.

Characterization of the Shrimp Waste Meal

To fully evaluate the shrimp waste meal as a dietary supplement, it was necessary to determine the content of some of its nutritional components. These components were determined according to the methods of analysis of the A.O.A.C. (2).

The percentage of moisture in the shrimp waste meal was determined gravimetrically using a drying-oven method [23.003]. Crude lipid in the shrimp waste meal was determined using an acid hydrolysis-solvent extraction method [18.013]. The percentage of crude protein was determined on the basis of total Kjeldahl nitrogen [2.044]. The amount of chitin nitrogen in the shrimp waste meal was estimated using a method which involved a stepwise technique of lipid extraction, decalcification, and nitrogen assay with a modified Kjeldahl procedure. A detailed outline of this procedure is shown in Appendix II. The actual protein was calculated on the basis of total nitrogen less chitin nitrogen times 6.25. The meal was assayed for its total ash content using a procedure which involved ignition of the sample in a muffle furnace [18.008]. The amount of carbohydrate (which included glucosamine from chitin) was calculated as the difference between 100 and the percent of other components.

The shrimp waste meal was also assayed for several minerals including phosphorus [22.073], calcium [41.001], potassium [41.001], sulfur [2.123], cobalt [2.086], copper [2.086], iron [2.086], manganese [2.086], and zinc [2.086].

The pigment content of the shrimp-waste meal was quantitatively extracted with chloroform. The absorbance of the resulting solution was measured at 487 nm using a Beckman DB spectrophotometer and the pigment content was reported on an $E^{1\%}_{1cm}$ basis. Details of this particular analysis are shown in the Appendix II.

Trout Diets Supplemented with Shrimp Waste Meal

Feeding Trial Design

The fish used in this research were rainbow trout (Salmo gairdneri) of the Mount Shasta strain. They were spawned from brood fish in January of 1968. The brood fish and the resulting progeny were reared at the Food Toxicology and Nutrition Laboratory of the Department of Food Science and Technology in Corvallis, Oregon. Each fish weighed approximately 37 grams and had been reared to that size on the Oregon Test Diet. This diet was fed in the form of a soft cube containing 35 percent dry mix and 65 percent water. The dry mix formulation for this diet is shown in Table 1.

The fish were placed on experimental diets at the beginning of

August, 1968. The fish were maintained in 200 gallon fiberglass tanks supplied by well water at a constant 54°F. The water was jetted in each tank to maintain an oxygen level of approximately 10.5 ppm. . . and a flow rate of four gallons per minute.

Table 1. Dry Mix Formulation for Oregon Test Diet.

Ingredient	Percentage
Casein (NBC vitamin-free)	49.5
Gelatin	8.7
Dextrin	15.6
Mineral mix[1]	4.0
Carboxy methyl cellulose	1.3
Alpha-cellulose (Alphacel - NBC)	7.7
Vitamin E (supplies 660 IU/kg)	0.2
Choline chloride (70%)	1.0
Vitamin mix[2]	2.0
Salmon oil	10.0
Total	100.0

[1] Bernhart-Tomerelli salt mix formulation is shown in Appendix I.

[2] Vitamin mix formulation is shown in Appendix I.

Four tanks were used for this particular investigation with 50 fish placed in each tank. Two tanks were designated as controls and the fish in these tanks received a control diet while the fish in the two remaining tanks received a diet supplemented with shrimp-waste meal.

The shrimp-waste meal was incorporated into the dry mix at a level of 15%. Initially both diets were fed as a firm pellet containing 64 percent dry mix to 36 percent water. The pelletizing of these diets

was conducted at the Seafoods Laboratory using apparatus designed by Charles Jow and Professor Duncan Law.

After two weeks of feeding, it was noticed that feeding enthusiasm for both diets was poor and attributable to the firmness of the pellets. The formulation of these diets was altered at this time in a manner which would allow them to be fed as a soft cube containing 35 percent dry mix and 65 percent water. Their dry mix formulations are shown in Table 2. These diets were fed ad libitum for the duration of the six month feeding period.

Fish were analyzed periodically for the amount of extractable pigment which was present in the skin, fins, and muscular tissue. The procedure involved the masceration of the fish tissue in a chloroform-methanol solvent system followed by suitable filtration and clarification procedures. The absorbance of the resulting solution was then determined at 487nm using a Beckman DB spectrophotometer. The extractable pigmentation was expressed on an $E^{1\%}_{1cm}$ basis. A more detailed description of this analysis is presented in the Appendix II.

Sensory Evaluation of Experimental Trout

At the termination of the feeding period, the fish were subjected to sensory evaluations. A flavor panel comprised of 23 staff members of the Department of Food Science and Technology evaluated the cooked fish samples in the Department's flavorium. These samples were

Table 2. Dry Mix Formulations for Experimental Diets.

Ingredient	Control Diet	Shrimp Meal Diet
Casein (NBC vitamin-free)	49.4%	45.8%
Gelatin	8.7%	8.7%
Dextrin	15.6%	15.6%
Menhaden oil	10.0%	10.0%
Mineral mix[1]	4.0%	
$CaCO_3$	0.9%	
Carboxy methyl cellulose	1.3%	1.3%
Alpha-cellulose (Alphacel - NBC)	6.5%	
Vitamin mixture[2]	2.0%	2.0%
Choline chloride	1.0%	1.0%
Vitamin E (supplies 660 IU/kg)	0.6%	0.6%
Shrimp meal		15.0%
Total	100.0%	100.0%
Caloric value[3]	4.0 $\frac{cal}{gm}$	4.1 $\frac{cal}{gm}$

[1] Bernhart-Tomerelli salt mix formulation is shown in Appendix I.

[2] Vitamin mix formulation is shown in Appendix I.

[3] Caloric values based upon calculations from amount of casein (protein=4.27 cal/gm), gelatin (protein=4.27 cal/gm), dextrin (carbohydrate=4.0 cal/gm), menhaden oil (lipid=9.0 cal/gm) and proximate analysis of shrimp meal (protein=35% and lipid=4.0%). (39)

rated on a hedonic scale ranging from one to nine according to their texture, juiciness, off-flavor, and desirability. The fish samples were randomly selected from the two experimental treatments consisting of the control diet and the shrimp waste meal diet. The samples were prepared using two cooking methods. One preparation involved frying the fish in a shallow pan with a thin layer of oil until the meat was tender and separated easily from the bone. The other method involved baking the fish in an oven at 400°F for approximately 55 minutes. The samples were served warm to the panel members who rated them under the illumination of red and orange lights.

The ballot which was used by the panel members is shown in Figure 2. The individual scores from panel members were tabulated in a frequency table and analyzed statistically utilizing a three-factor analysis of variance (17). This analysis was programmed for use on Oregon State University's CDC Model 3300 computer (27). Further statistical analysis of the computer results was accomplished using an F-test (17). This indicated whether the treatment means, the preparation means, and the treatment-preparation interaction were different at specific significance levels.

Name _____ Fish _____

Point Scale	Texture	Juiciness	Off-Flavor	Desirability
9.0	Tender	Juicy	None	Extremely Desirable
8.0	Normal			Very Desirable
7.0	Slightly Tough	Slightly Dry	Slight	Moderately Desirable
6.0				Slightly Desirable
5.0	Moderately Tough	Moderately Dry	Moderate	Neutral
4.0				Slightly Undesirable
3.0	Very Tough	Very Dry	Large	Moderately Undesirable
2.0				Very Undesirable
1.0	Extremely Tough	Extremely Dry	Extreme	Extremely Undesirable

Figure 2. Flavor Panel Ballot.

An Effective Method of Achieving Trout Pigmentation

Diet Preparation

Five pigment-rich diets were formulated to evaluate an effective mode of dietary pigment transfer in trout. The pigmentation produced by these diets was compared to a sixth diet which was designated as a control.

Diet 1 was the control diet and contained no pigment-rich materials.

Diet 2 contained shrimp-waste meal at a level of 15 percent in the dry mix. The shrimp-waste meal was prepared in the same manner as described in the previous investigations. Sodium phosphate was added to this diet to maintain a calcium to phosphorus ratio of three to one which was equivalent to diet 1.

Diet 3 contained ground whole shrimp (Pandalus jordani) in order to evaluate natural crustacean pigments similar to what a trout might consume in the wild. Approximately 100 pounds of fresh shrimp were obtained from Pacific Shrimp, Inc. and ground through an Urschel mill. Tenox IV was added to the ground shrimp at a level of 0.05 percent (wet weight). The ground shrimp was vacuum-sealed in 1 lb. flat cans in portions of approximately 250 grams per can and stored at 0^{o}F. In order to incorporate this material into diets on an isocaloric basis, it was analyzed for moisture, crude lipid, crude protein, and

chitin nitrogen. The amount of actual protein and carbohydrate (including glucosamine from chitin) were calculated by difference. Brief descriptions of these procedures were covered in the section dealing with the shrimp-waste meal characterization. The ground shrimp was incorporated into a diet according to its extractable pigment levels in amounts calculated to achieve a pigment content which was equal to diet 2. This amounted to a level of 14.2 percent (dry weight) in the dry mix. Due to the enzyme systems present in the ground shrimp, the gelatin was hydrolyzed and rendered ineffective for setting-up the diet. Kraystay T.M., a carrageen extract, was partially effective at producing a firm diet if added to the dry mix at a level of five percent. The firmness of diet 3 was still less than the other diets and for feeding purposes this diet was frozen and broken up into small chunks. Sodium phosphate was also added to diet 3 in order to maintain a calcium to phosphorus ratio of three to one.

Diet 4 contained a pigment-lipid extract of the shrimp waste meal. The extract was obtained by batch (50 lbs.) extraction of the shrimp waste meal with chloroform. This was followed by concentration on a 10 liter concentrator, evaporation of the solvent with a rotary evaporator, and elimination of residual solvent by nitrogen stripping on a steam bath. The extract was analyzed for its pigment content spectrophotometrically by measuring the absorbance of the pigment in a chloroform solution at 487 nm. The pigment value was expressed on

an $E^{1\%}_{1cm}$ basis. The extract was then diluted with menhaden oil. The menhaden oil-extract mixture was added at a level of 7.0 percent of the dry mix in diet 4. This produced a diet with a pigment content equal to diet 2.

Diet 5 also contained a pigment-lipid extract of the shrimp waste meal. This extract was added at a level of 7.0 percent of the dry mix. Menhaden oil-extract dilutions were used which produced a diet that contained approximately twice the pigment as diet 2.

Diet 6 contained a commercially produced carotenoid known as canthaxanthin. It was added to the diet as a crystalline pigment dispersed in menhaden oil at a level of 190 mg/kg dry mix. This, as assayed, produced a pigment content that was approximately 4.5 times that of diet 2 or diet 4, and 2.5 times that of diet 5. At the 23rd week in the feeding period it was decided to alter this diet and add canthaxanthin as a 10% pigment in a dry water-soluble form dispersed in a carrier of gelatin, sugar, and starch (Roxanthin T. M. Red 10) instead of the crystalline form. The water-dispersible form was dissolved in water and added to the diet as a portion of the water phase. When this form of canthaxanthin was added at a level of 1680 mg/kg diet (dry wt.), it was equivalent in pigment content to the original diet 6 formulation. All the canthaxanthin derivatives were obtained from Hoffmann - La Roche Inc., Nutley, New Jersey.

All six diets were formulated on an isocaloric basis and contained

35 percent dry mix to 65 percent water. The dry mix formulations are shown in Table 3.

The diets were stored in plastic bags at freezer temperatures (approximately 0°F.). New batches were made on the average of every four to six weeks. The pigment content of each new batch of diet was determined.

Trout Feeding

The fish used in this phase of the research were rainbow trout (Salmo gairdneri) of the Mount Shasta strain. These fish were the progeny of brood fish which were spawned in February of 1969 at the Food Toxicology and Nutrition Laboratory.

Each fish weighed close to 25 grams and had been reared to that size on the Oregon Test Diet.

The trout were placed on the six experimental diets in the middle of August, 1969. They were maintained in 100 gallon fiberglass tanks which were supplied by well water at a constant 54°F. The water was jetted in at a flow rate of four gallons per minute which maintained the oxygen level close to 10.5 ppm.

Six tanks were used for these studies with 100 fish placed in each tank. Each group of fish was fed one of the six designated diets on an ad libitum basis for 34 weeks.

Table 3. Dry Mix Formulations for Experimental Diets.

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	(Altered) Diet 6
Casein (NBC vitamin-free)	49.4%	43.7%	41.4%	49.4%	49.4%	49.4%	49.4%
Gelatin	8.7%	8.7%	8.7%	8.7%	8.7%	8.7%	8.7%
Dextrin	15.6%	15.4%	14.5%	15.6%	15.6%	15.6%	15.6%
Menhaden oil	10.0%	10.0%	9.0%	3.0%	3.0%	10.0%	10.0%
Mineral mix[1]	4.0%			4.0%	4.0%	4.0%	4.0%
$CaCO_3$	0.9%			0.9%	0.9%	0.9%	0.9%
Carboxy methyl cellulose	1.3%	1.3%	1.3%	1.3%	1.3%	1.3%	1.3%
Alpha-cellulose (Alphacel - NBC)	6.5%			6.5%	6.5%	6.5%	6.5%
Vitamin mix[2]	2.0%	2.0%	2.0%	2.0%	2.0%	2.0%	2.0%
Vitamin E (supplies 660 IU/kg)	0.6%	0.6%	0.6%	0.6%	0.6%	0.6%	0.6%
Choline chloride	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%	1.0%
$NaH_2PO_4 \cdot 2H_2O$		2.3%	2.3%				
Kraystay			5.0%				
Ground whole shrimp			14.2%				
Shrimp meal		15.0%					
Pigmented lipid-extract				7.0%	7.0%		
Canthaxanthin (crystals)						19mg%	
Canthaxanthin (water-dispersible beadlets)							168mg%
Total	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Caloric value[3]/100gm	400.4cal	403.3cal	410.2cal	400.4cal	400.4cal	400.4cal	400.4cal

[1] Bernhart-Tomerelli salt mix formulation is shown in Appendix I.

[2] Vitamin mix formulation is shown in Appendix I.

[3] Caloric values based upon calculations from amount of casein (protein=4.27 cal/gm), dextrin (carbohydrate=4.0 cal/gm), menhaden oil (lipid=9.0 cal/gm), and proximate analysis of shrimp meal (protein=35% and lipid=4.0%). (39)

Pigment Analysis of Trout

The trout were periodically analyzed for the extractable pigmentation present in their skin, fins, and muscular tissue. These tissues were analyzed together using the method described in the Appendix II.

Up to the 20th week of the feeding period, the pigment analyses were performed on composite samples of fish. For the remaining 14 weeks, fish were analyzed individually to determine more accurately the range of tissue pigmentation produced by each diet. Individual sample weight and fish sex were recorded.

Occasionally the anterior section and posterior section of the trout were analyzed separately to determine variation within an individual fish. If the fish was lying flat, the anterior section was distinguished as the portion cut in a vertical direction from the anterior point of the dorsal fin. The posterior section was the portion cut in a vertical direction from the posterior point of the dorsal fin.

For comparative purposes various species of trout and salmon were also analyzed for their tissue pigmentation. These species included: searun cutthroat trout (Salmo clarki clarki) from the Necanicum River near Seaside, Oregon; brown trout (Salmo trutta) and rainbow trout (Salmo gairdneri) from the Deschutes River near Bend, Oregon; and coho salmon (Oncorhynchus kisutch) and chinook salmon

(Oncorhynchus tshawytscha) from the Columbia River near Astoria, Oregon.

Photography

At approximately the 30th week of the feeding period, photographs were taken of the observable pigmentation which was produced in each group of fish. These photographs were taken to illustrate the pigmentation in the skin, fins, operculum, and muscular tissue. The photographs of the muscular tissue showed the pigmentation produced in a portion of the anterior and posterior sections.

All the photographs were taken during days which seemed of equal brightness and the lighting conditions were monitored with a light meter. A Mimya-Flex C camera loaded with Kodachrome II ASA 40 or Kodacolor-X-ASA 80 film was used for all the photographic work.

A control photograph was taken under the same lighting conditions. This photograph included two Kodak charts (Kodak color control patches and Kodak gray scale) which were placed beside a trout. This photograph was taken for the purpose of matching the designated colors and shades of gray during the development process.

All the development was done by Color West Inc., of Portland, Oregon. Two consultations were held with their technical staff before the final development stage to insure that the photographs represented the natural coloration of the trout.

After each group of trout were photographed they were analyzed for the amount of extractable pigmentation to put the pigment values on a more meaningful basis.

Sensory Evaluation of Experimental Trout

At the end of the feeding period specific groups of fish were selected for flavor panel evaluations. Approximately 20 fish from each group were randomly selected for these studies.

A student panel consisting of 126 members evaluated the cooked fish samples in the Food Science and Technology Flavorium. They rated the desirability of each sample on a hedonic scale which ranged from one to nine. The fish samples that the panel members evaluated were from diet 1, diet 2, diet 5, and diet 6.

All the samples were wrapped in aluminum foil and baked at 400°F. for approximately 55 minutes. The samples were served warm to the judges. The judges who tasted in the morning rated the samples under the illumination of white lights while those who tasted in the afternoon rated the samples under red and orange lights. An illustration of the ballot used by the panel is shown in Figure 3.

Twenty-seven staff members from the Food Science and Technology Department participated in a more in-depth flavor panel evaluation. This panel was designed to evaluate the same groups of fish according to their texture, juiciness, natural trout flavor, color, and

Test No. _____ Judge No. _____

Product Fish Date _____

Name _____

Please write the sample number in the space following the statement which best describes your opinion of the sample.

9. Like extremely	_____	_____	_____	_____
8. Like very much	_____	_____	_____	_____
7. Like moderately	_____	_____	_____	_____
6. Like slightly	_____	_____	_____	_____
5. Neither like nor dislike	_____	_____	_____	_____
4. Dislike slightly	_____	_____	_____	_____
3. Dislike moderately	_____	_____	_____	_____
2. Dislike very much	_____	_____	_____	_____
1. Dislike extremely	_____	_____	_____	_____

Do you normally like and eat fish?

Comments:

Figure 3. Student Flavor Panel Ballot.

overall desirability. Samples were cooked in an identical manner to those prepared for the student panel. The samples were served warm to the panelists and the scoring was done under the illumination of white light. An illustration of the staff scoring ballot is shown in Figure 4.

The weight and sex of each fish submitted to sensory evaluation was recorded. Pigment analyses were performed on sections from each group of fish both before and after the cooking process. The tissue from each group of fish was also analyzed for its level of crude lipid and moisture. The Folch extraction method (8) was used for the determination of crude lipid. The solvent extract was dried over sodium sulfate and acetone was added during the final evaporation to azeotropically distill most of the water. The residue was placed in a drying oven (approximately 40°C.) for 15 minutes before the final weight was determined. The moisture of the tissue was determined on mascerated samples by an A.O.A.C. (2) drying oven procedure [23.003].

Statistical Analysis

The pigmentation and flavor panel results were statistically analyzed with the use of Oregon State University's CDC 3300 computer.

A linear regression analysis was performed on each set of pigmentation results (35). The significance of the results was established with an analysis of variance (17), an F-test (17), and a Wilcoxon test

Name _____

Scale	Texture	Juiciness	Natural Trout Flavor	Desirability	Color
9.0	_____Tender	_____Juicy	_____None	_____Extremely	_____Extremely
8.5	___ Normal	___	___	___ Desirable	___ Desirable
8.0	_____	_____	_____	_____Very	_____Very
7.5	___	___	___	___ Desirable	___ Desirable
7.0	_____Slightly	_____Slightly	_____Slight	_____Moderately	_____Moderately
6.5	___ Soft	___ Dry	___	___ Desirable	___ Desirable
6.0	_____	_____	_____	_____Slightly	_____Slightly
5.5	___	___	___	___ Desirable	___ Desirable
5.0	_____Moderately	_____Moderately	_____Moderate	_____Neutral	_____Neutral
4.5	___ Soft	___ Dry	___	___	___
4.0	_____	_____	_____	_____Slightly	_____Slightly
3.5	___	___	___	___ Undesirable	___ Undesirable
3.0	_____Very	_____Very	_____Large	_____Moderately	_____Moderately
2.5	___ Soft	___ Dry	___	___ Undesirable	___ Undesirable
2.0	_____	_____	_____	_____Very	_____Very
1.5	___	___	___	___ Undesirable	___ Undesirable
1.0	_____Extremely Soft	_____Extremely Dry	_____Extreme	_____Extremely Undesirable	_____Extremely Undesirable

Do you normally like and eat fish?

Comments:

Figure 4. Staff Flavor Panel Ballot.

(35). The analysis of variance and F-test determined the difference between the rates of pigment deposition in each group of trout. The Wilcoxon test showed the superiority between two sets of pigmentation results by making paired comparisons of values at each sampling point. These statistical analyses indicated both the levels and rates of pigment deposition achieved in each group of trout.

The student flavor panel scores were evaluated using an N-factor analysis of variance (17). F-values were calculated to establish the existence of any differences between the treatment (diet 1, diet 2, diet 5, or diet 6) mean scores and at what level of significance these differences could be stated. Fisher's LSD procedure (14) was then used to determine which treatment caused the significant difference.

The staff panel scores were evaluated using a two factor analysis of variance (17). The F-test and Fisher's LSD procedure were again used to determine which treatment mean scores were significantly different.

RESULTS AND DISCUSSION

Shrimp Waste Processing

After the final drying and pulverizing steps, a fine-grained (approximately 75 mesh) meal was produced which was pink in color. This meal appeared superior in color to the brownish-colored meals available from commercial drying operations. As shown in Table 4, the average yield of meal which resulted after the drum-drying and secondary drying steps was 12.1 percent.

Table 4. Yield From Shrimp Waste Processing.

Batch #	Starting Wt.	Ending Wt.	Percentage Yield
1	88.0 lbs.	11.6 lbs.	13.2
2	103.0 lbs.	14.8 lbs.	14.4
3	138.0 lbs.	15.4 lbs.	11.2
4	205.0 lbs.	22.4 lbs.	11.0
Average	133.5 lbs.	16.1 lbs.	12.1

Characterization of Shrimp Waste Meal

Commercial shrimp meals and fish meals are generally sold on a protein basis. It is interesting to note that the crude protein content of the experimental shrimp waste meal was shown to be 45.0 percent. The results of the various analyses are shown in Table 5. The actual protein content was 36.3 percent since chitin (a polymer of glucosamine)

Table 5. Analysis of Shrimp Waste Meal.[1, 2]

Component	Percentage (Wet Wt. Basis)
Moisture	5.4
Ash	23.5
Crude lipid	4.1
Crude protein[3]	45.8
Actual protein[4]	36.3
Chitin nitrogen	9.5
Carbohydrate[5]	21.2

[1] Mineral analysis: Phosphorus 1.37%, calcium 11.50%, potassium 5.30%, sulfur 1.28%, cobalt 3.0 ppm, copper 11.9 ppm, iron 413.0 ppm, manganese 9.5 ppm, zinc 75.0 ppm.

[2] Pigment analysis: $E^{1\%}_{1cm}$ @ 487nm=0.30.

[3] Total Kjeldahl N x 6.25.

[4] (Total Kjeldahl N-chitin N) x 6.25.

[5] Calculated by difference (100 percent - total percent of other components).

contributed almost 10 percent nitrogen to the meal. The actual protein level might have been higher than some commercial shrimp meals. The experimental shrimp waste meal was prepared from material collected at the water separation phase of the processing operation (Figure 1). At this collection point, the waste material was composed of small meat, viscera, and shell particles. If the waste had been collected directly under the peeling machine, it would have been composed of a larger proportion of shell particles. This would have consequently increased the amount of chitin present and lowered the actual protein content.

The carbohydrate content of the meal amounted to 21 percent. This figure was calculated by the subtraction of the total percentage of the other components from 100 and included the amount of glucosamine contributed by the chitin.

The calcium content of the shrimp meal was shown to be 11.5%. This amounted to nearly 49 percent of the total ash content. The high calcium content of shrimp waste meal may explain why supplementation of poultry rations with this material has been shown to increase yields of eggs from poultry (10, 11, 12). Possibly shrimp waste meal could be used to increase egg production in other avian species as well.

The phosphorus content of the shrimp meal was shown to be 1.87 percent. This relatively high calcium content resulted in a calcium to phosphorus ratio of 8.394. The addition of phosphate to diets

that contained shrimp meal or whole shrimp (Table 3) was necessary to maintain a calcium to phosphorus ratio equivalent to the 3:1 ratio in the control diet.

Values of 34.4 percent actual protein, 27.2 percent chitin, and 25.3 percent ash which were reported by Kirk et al. (22) for air-dried shrimp meal were in close agreement with the values shown in Table 5. Their value of 42.3 percent calcium was also close to the 49 percent level which was calculated for the experimental shrimp waste meal on the basis of total ash. Burkholder et al. (4) reported higher values for ash and fat when only the shrimp shells were analyzed.

Trout Fed Shrimp Waste Meal

Pigment Analysis of Trout

A comparison between the extractable pigmentation of the fish that received the control diet and the diet which contained 15 percent shrimp waste meal is shown in Figure 5. At the end of the 24 week feeding period, the fish on the meal diet contained approximately 12.5 times more extractable pigmentation in their tissue than the fish receiving the control diet.

To make the pigmentation values more meaningful a photograph was taken at approximately the 16th week of the feeding period. The observable pigmentation in the muscular tissue of the fish which received the shrimp waste meal (A) was compared to the pigmentation

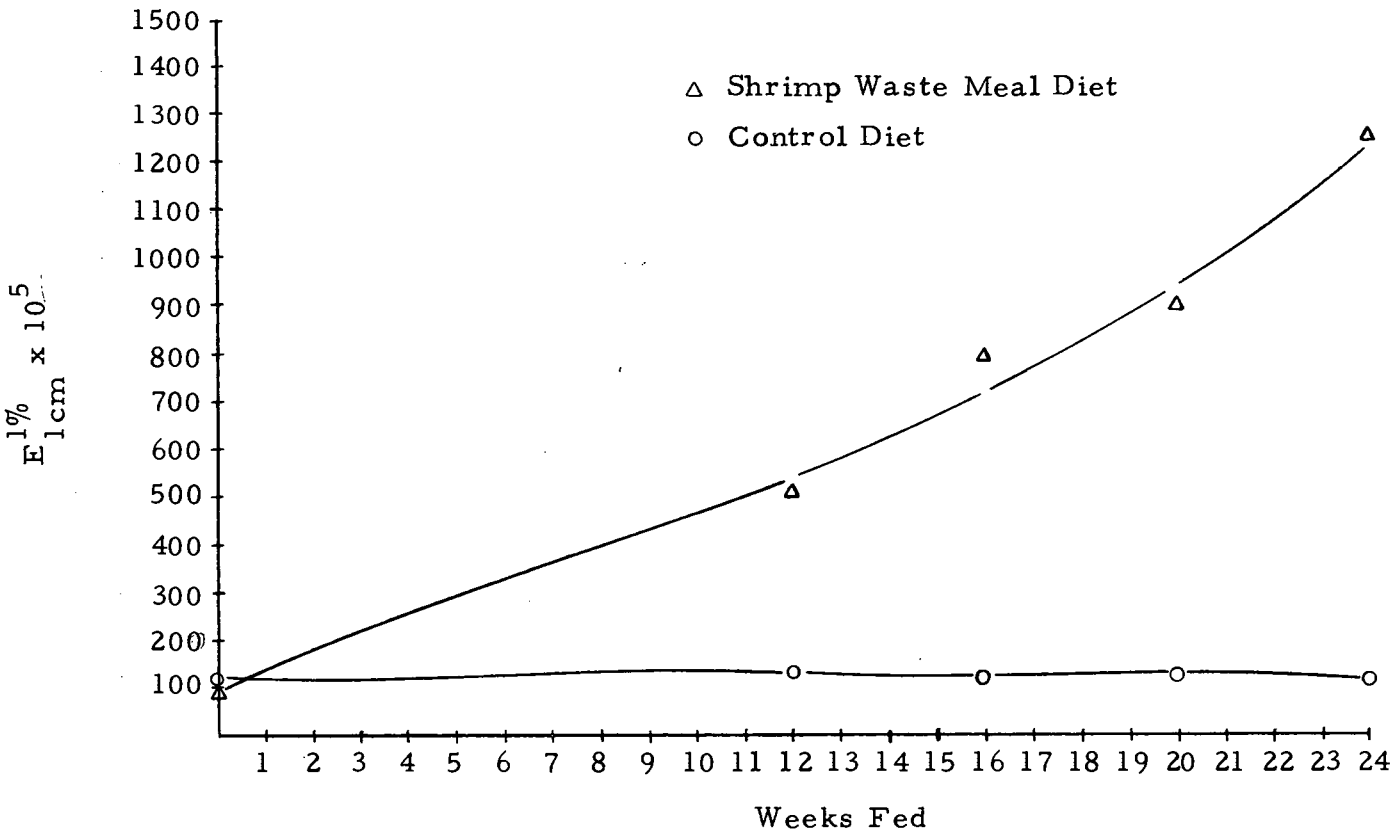


Figure 5. Comparison of Pigment Deposition Between Trout Fed Control Diet and Trout Fed Shrimp Meal Diet.

in the control fish (B). These results are illustrated in photograph 1.

Sensory Evaluation of Trout

The results of sensory evaluations by judges composed of Department staff members are summarized in Tables 6 and 7. Scoring frequency, mean scores, and the positive or negative deviations from control mean scores are listed.

When only the mean scores that showed a 0.50 difference from the scores for the control fish were considered, it appeared that the baked samples of meal fed fish showed trends toward a higher rating with regard to flavor and desirability. The fried samples of fish fed the shrimp waste meal showed a trend toward a better flavor.

To place these results on a firm statistical basis, the scores were analyzed using a three-factor analysis of variance. The computer output for this analysis and the calculations from the F-test are shown in Table 8.

The table of F-values (24) at the .05 and .01 levels of significance, with one and 22 degrees of freedom, were used for comparisons to the calculated F-values. Values of 4.30 and 7.95 (tabular F-values at .05 and .01 respectively) had to be exceeded in order to confirm the existence of a significant difference. These comparisons were made between the two treatment mean scores for each characteristic evaluated.

Photograph 1: Comparison of Pigmentation Between the Muscular Tissue of Control Trout and Trout Fed Shrimp Waste Meal.

Sample A = Fish fed diet supplemented with 15% shrimp waste meal.
Sample B = Fish fed control diet.
Feeding Time: Four months.

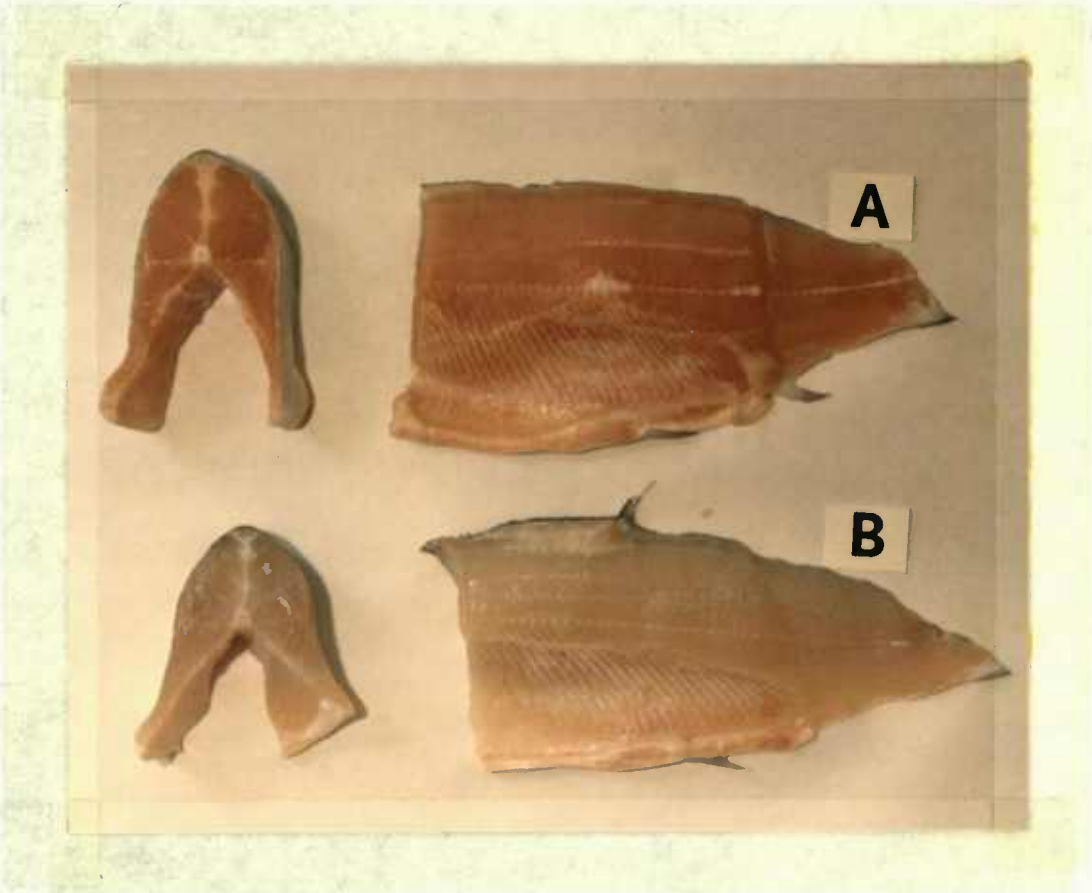

A
B

Table 6. Frequency Table of Flavor Panel Scores for Baked Samples.

	Texture		Juiciness		Off-Flavor		Desirability	
Scale	Control Diet	Shrimp Meal Diet	Control Diet	Shrimp Meal Diet	Control Diet	Shrimp Meal Diet	Control Diet	Shrimp Meal Diet
9.0	3	2	2	4	2	8	1	1
8.5	3	3	1	1	5	2		1
8.0	2	2	2	3	1	3	3	3
7.5	3	5	5	5	1	1	3	3
7.0	7	3	8	3	6	3	4	8
6.5				2	1	1	1	1
6.0		2		2			2	2
5.5	1	1	1		1	2		1
5.0	2	2			1	2	2	
4.5	1		1		2		1	
4.0			1				2	2
3.5							1	
3.0		1		1	1		1	
2.5			1					
2.0							1	
1.5								
1.0		1		1	1			
Sum[1]	160.0	149.5	152.5	156.0	148.0	169.0	133.0	152.0
Mean	7.22	6.80	6.93	7.09	6.73	7.68	6.05	6.91
Difference[2]		-0.47		+0.16		+0.95		+0.86

[1] n = 22

[2] From control diet

Table 7. Frequency Table of Flavor Panel Scores for Fried Samples.

	Texture		Juiciness		Off-Flavor		Desirability	
Scale	Control Diet	Shrimp Meal Diet	Control Diet	Shrimp Meal Diet	Control Diet	Shrimp Meal Diet	Control Diet	Shrimp Meal Diet
9.0	6		4		5	7		1
8.5	2	4	2	1	1	4		1
8.0	2	2	1	6	3	4	4	5
7.5	3	2	3	4	4	1	5	
7.0	5	6	5	6	5	7	2	5
6.5	1	3	1		1			
6.0	1	1	2	1			5	6
5.5	1		3				1	
5.0	1	1	1	1	3		2	2
4.5		1	1					
4.0				2			1	2
3.5								
3.0	1	1		1	1		2	
2.5								
2.0								1
1.5		1						
1.0		1		1			1	
Sum[1]	170.5	159.5	163.0	151.5	167.0	185.5	140.0	148.5
Mean	7.41	6.93	6.59	6.59	7.26	8.07	6.09	6.46
Difference[2]		-0.48		-0.50		+0.81		+0.37

[1] n = 23

[2] From control diet

Table 8. Statistical Analysis of Sensory Evaluations.

		Analysis of Variance							
		Texture		Juiciness		Flavor		Desirability	
Source of Variation	Degrees of Freedom	Mean Square	F-Value	Mean Square	F-Value	Mean Square	F-Value	Mean Square	F-Value
Treatment	1	0.978	2.900	0.033	0.962	2.057	9.946*	1.079	9.387*
Preparation	1	0.004	0.025	0.013	0.851	0.457	1.358	0.046	0.139
Judge	22	0.558	0.505	0.545	2.063	0.309	0.107	0.586	1.480

	Mean Scores			
Diet				
Control	7.39	7.05	6.99	6.12
Shrimp Meal	6.74	6.94	7.94	6.80
Preparation				
Fried	7.09	6.96	7.69	6.39
Baked	7.04	7.03	7.24	6.53

* Significant at P > .01

Flavor panel scores for off-flavor and desirability were shown to vary significantly at the .01 level. The preparations (baked or fried) and the treatment-preparation interaction were not different at the .05 level.

These statistical analyses confirm that samples of fish from the group fed the diet supplemented with shrimp waste meal were rated significantly higher in flavor and desirability than control fish.

If the treatment mean scores for both cooking methods were averaged and then compared, the samples of fish fed shrimp waste meal scored 0.33 higher in juiciness, 0.83 higher in flavor, 0.63 higher in desirability, and 0.47 firmer in texture than control fish. If the scores which exhibited over 0.50 difference from the control scores were the only ones considered significant, then these results were in close agreement with the statistical analyses. Therefore, the frequency tables provided a valid criteria for evaluating the flavor scores even though these tables were not based on more sophisticated statistical methods.

An Effective Method to Achieve Pigmentation in Trout

An effective mode of dietary pigment transfer in trout was investigated during this phase of research. Five pigment-rich diets were evaluated on the basis of the pigmentation produced in separate groups of trout. The extractable tissue pigmentation in each group

was quantified during a 34 week feeding period. Comparisons were made to a sixth group of trout which received a control diet.

Analysis of Diets and Pigment Ingredients

A description of these diets and the pigment value for each pigment rich ingredient is shown in Table 9.

Table 9. Summary of Diets and Pigment Rich Ingredients.

Diet	Pigment Ingredient	Pigment Value of Ingredients (Dry Wt.) ($E^{1\%}_{1cm} \times 10^3$)
1	None-control	
2	Shrimp meal	300
3	Ground whole shrimp	320
4	Extract of shrimp meal	640
5	Extract of shrimp meal	1280
6	Canthaxanthin	4560

The results of the analyses performed on the ground whole shrimp are shown in Table 10. These analyses were performed in order to formulate a diet with the whole shrimp which would be isocaloric to the other diets. If dry weight comparisons were made to the shrimp waste meal, the whole shrimp contained higher percentages of lipid and protein and lower percentages of chitin nitrogen, ash, and carbohydrate. The values of 12.0 percent fat, 23.4 percent ash, and 65.6 percent total protein were reported for dried whole shrimp by Burkholder et al. (4). These values are in close agreement with the

results shown in Table 10.

Table 10. Proximate Analysis of Ground Whole Shrimp.

Component	Percentage (Wet Wt.)	Percentage (Dry Wt.)
Moisture	78.1	
Crude lipid	3.5	16.0
Ash	3.3	15.0
Crude protein[1]	13.4	61.1
Actual protein[2]	12.8	58.4
Chitin nitrogen	0.6	2.7
Carbohydrate[3]	1.7	7.9

[1] Total Kjeldahl N x 6.25.

[2] (Total Kjeldahl N-chitin N) x 6.25.

[3] By difference (100 percent - total percent of other components).

Pigment analyses were performed on each batch of diets that was made during the 36 week feeding period. The average pigment value for each diet is listed in Table 11. There was a certain amount of interference from dry mix components. This was indicated by the pigment value of the control diet which should theoretically have been zero. The theoretical pigment value of each diet on a dry weight basis is also shown. These theoretical pigment values corresponded to extrapolations made on the basis of the pigment content (Table 9) of each diet's pigment-rich ingredient.

Considering these values it would seem that diet 6 would have produced the highest pigmentation in the fish followed by diets 5, 3, 2, 4, and 1.

Table 11. Pigment Analysis of Diets.[1]

Diet	Pigment Value ($E^{1\%}_{1cm} \times 10^3$)		
	Wet Wt.	Dry Wt.	Theoretical (Minus Value of Control)
1	5.5	16.0	0.0
2	21.3	61.8	45.8
3	23.3	67.6	51.6
4	20.1	58.3	42.3
5	36.3	105.3	89.3
6	90.6	262.7	246.7

[1]The figures reported for each diet are the average value for seven separate batches made during the feeding period.

Pigment Analysis of Trout

The deposition of pigment in the tissue of trout from each experimental group during the feeding period is shown in Figure 6. Pigment values reported for the period after the 23rd week of feeding show the range of pigmentation observed for each group of fish.

Fish fed the diet containing the highest level of pigment, which was derived from a meal-extract, produced fish with the highest tissue pigment levels. This diet also yielded the greatest range in pigment levels among individual fish. The average pigment values, however, were close to those produced by diet 2 which contained 15% shrimp waste meal. It was noted that after two to three months, the observable pigmentation in the skin, fins, and operculum of the group of fish fed diet 5 was more pronounced than any other group. Diet 4, which

was supplemented with a meal-extract to yield a dietary pigment level approximately one-half that of diet 5, produced a pigmentation which was lower than that of diet 5 or of diet 2, which contained 15 percent shrimp waste meal. The rate of pigment deposition seemed to follow a similar pattern for diets 2, 4, and 5. The pigmentation produced by diet 3 was slightly lower than diet 4. However, it should be recalled that diet 3 was supplemented with whole shrimp and fed as frozen chunks which had a tendency to disperse in water. Dispersion of this diet in the water may have prevented fish from receiving comparable quantity of pigment-rich material. This physical difference should be considered.

The diet containing canthaxanthin (diet 6) produced the lowest level of pigmentation. The pigment content of fish receiving this diet was roughly equivalent to that of fish receiving the control diet (diet 1) up to the 23rd week. At this point, diet 6 was altered by replacing the crystalline pigment dispersed in oil with a water-dispersible form of canthaxanthin. Pigment uptake by trout upon addition of this water-dispersible form was quite rapid. Evidently the pure crystalline form of canthaxanthin dispersed in the oil fraction of the diet was not biologically available to the fish.

The significance of the differences in the rates of pigment deposition in fish receiving the various diets was partially established using linear regression analysis. This expressed the pigmentation

results for each group of fish as a linear function and straight lines were derived from this function. The slopes of the straight lines were compared using an F-test in order to demonstrate the existence of any differences between the slopes at specific significance levels. The results of these analyses and F-tests are shown in Figure 7.

A Wilcoxon test was used to further investigate the significance of each set of pigmentation results. This test evaluated two sets of pigmentation results and made paired comparisons of mean pigmentation values at specific sampling points to determine if a superiority existed. The pigmentation values for each group of fish were expressed as smooth curves that corresponded closely to the linear regression functions. The results of the Wilcoxon tests are shown in Figure 8.

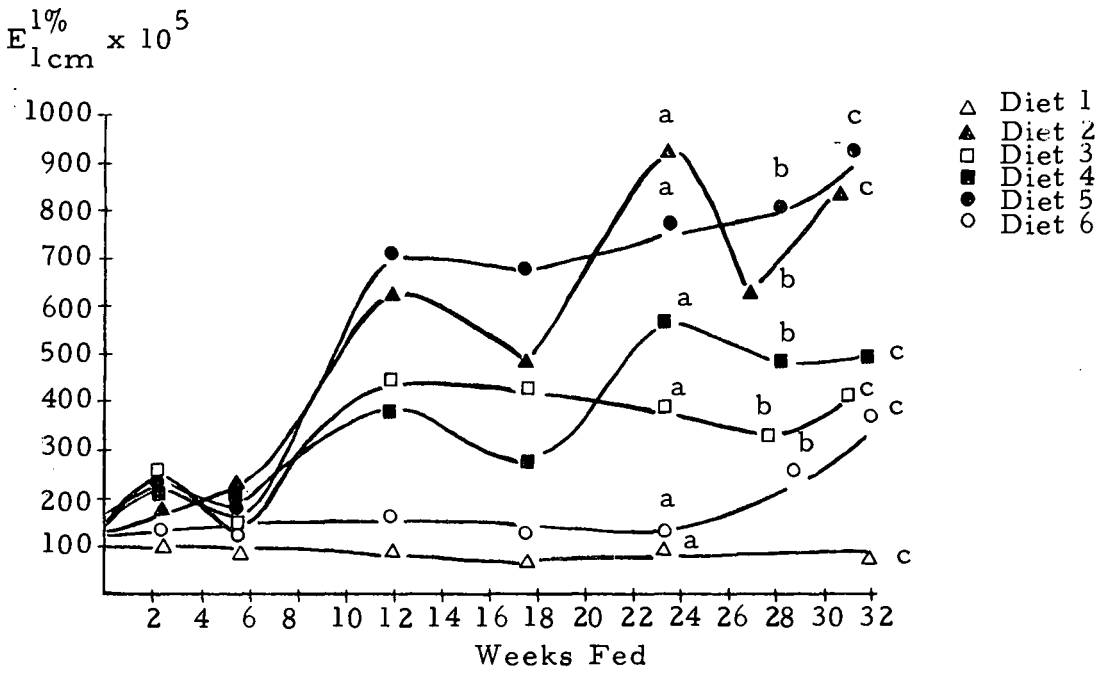
Linear regression analyses and the analyses of variance showed that the rates of pigment deposition in the groups of fish fed the shrimp meal (diet 2), and the two levels of pigment added as an extract (diets 4 and 5) were the same. This result is better shown in Figure 6 where the pigment deposition pattern for these three diets appear to be in close agreement. The Wilcoxon test, however, showed that the level of pigmentation produced by diet 2 and diet 5 was the same, but significantly superior to diet 4. This result may indicate that pigment derived from dietary shrimp waste meal was more biologically available than from the extract. Diet 4 contained a pigment level equal to diet

Figure 6. Pigment Deposition Produced by the Experimental Diets.

	Range of Pigment Values from 23rd Week on:		
Diet 1	[a] 80-125	[b] ---	[c] 0-129
Diet 2	[a] 625-1130	[b] 350-1060	[c] 370-1415
Diet 3	[a] 340-425	[b] 140-660	[c] 189-678
Diet 4	[a] 430-690	[b] 190-690	[c] 235-839
Diet 5	[a] 590-930	[b] 310-1290	[c] 286-2100
Diet 6	[a] 60-110	[b] 20-550	[c] 187-526

Figure 7. Linear Regression Analysis of Pigment Deposition Curves.

The diet which was underlined once exhibited no significant difference when compared to the diet listed below it according to the F-Test. The diet which was underlined twice showed no difference from the 2nd diet listed below it. The diet which was not underlined exhibited a significant difference from all the diets listed below it.


$E^{1\%}_{1cm} \times 10^5$
1000
900
800
700
600
500
400
300
200
100
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
Weeks Fed
Diet 1
Diet 2
Diet 3
Diet 4
Diet 5
Diet 6

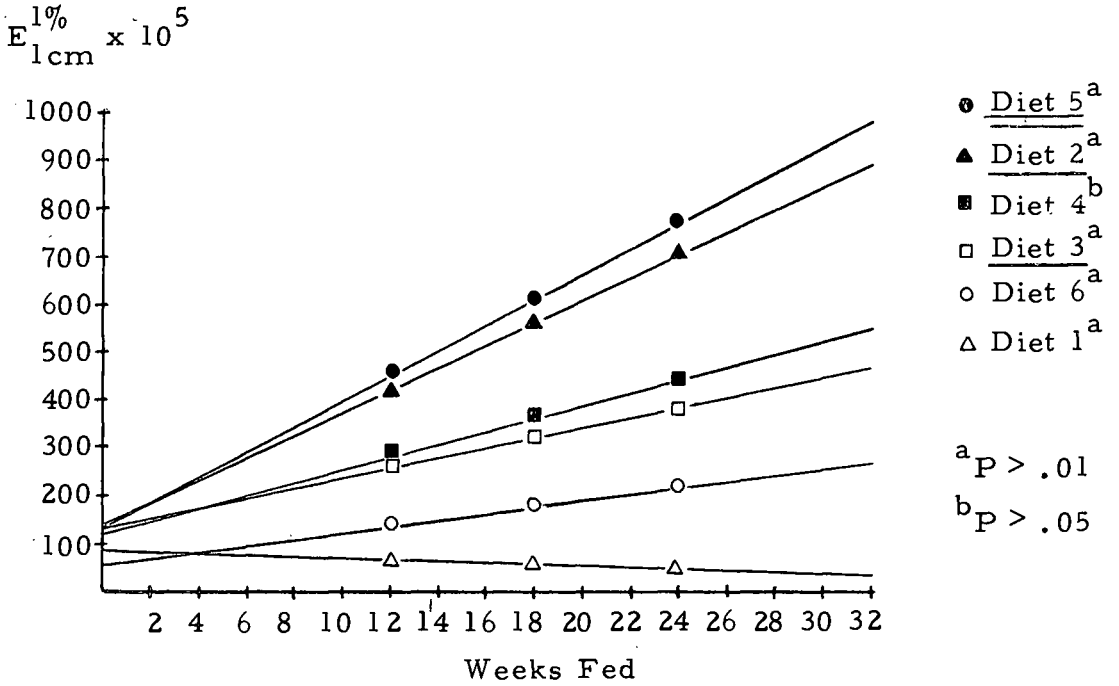
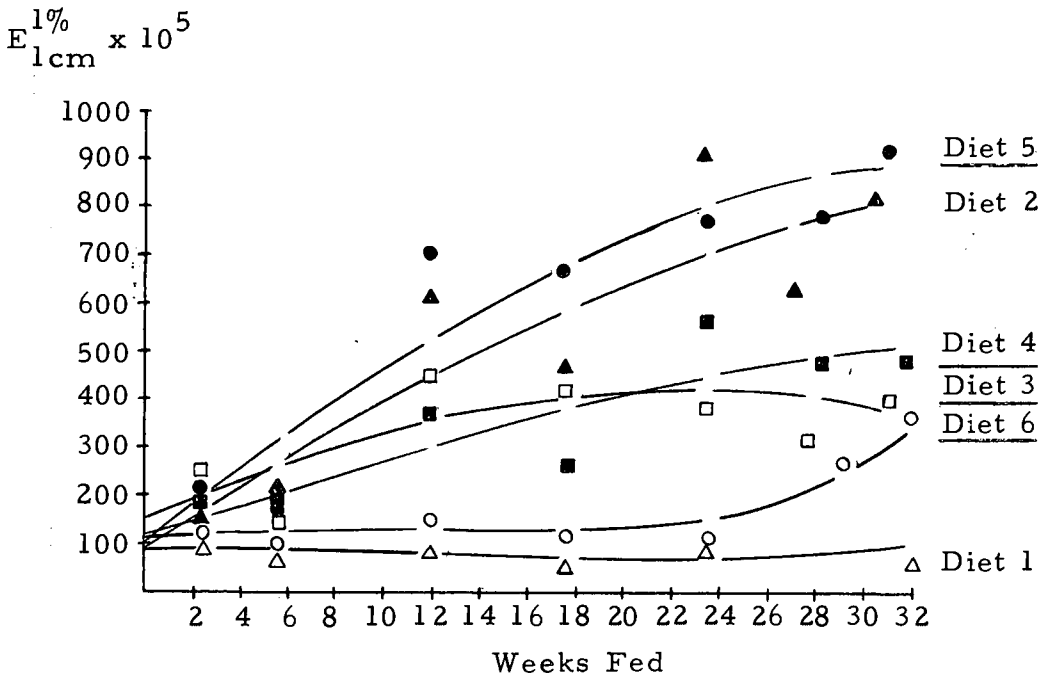

$E^{1\%}_{1cm} \times 10^5$
1000
900
800
700
600
500
400
300
200
100
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
Weeks Fed
Diet 5[a]
Diet 2[a]
Diet 4[b]
Diet 3[a]
Diet 6[a]
Diet 1[a]
[a]P > .01
[b]P > .05

Figure 8. Pigment Deposition Curves for Each Group of Experimental Trout.

The diet which was underlined once exhibited no superiority in the pigmentation it produced when compared to the diet listed below it according to the Wilcoxon test. The diet which was not underlined showed a superiority in pigmentation when compared to all the diets listed below it. These comparisons were made at the .025 significance level.


$E^{1\%}_{1cm} \times 10^5$
1000
900
800
700
600
500
400
300
200
100
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
Weeks Fed
Diet 5
Diet 2
Diet 4
Diet 3
Diet 6
Diet 1

2, while diet 5 contained nearly twice the pigment level. Both of these diets were supplemented with the meal extract.

Diet 4 produced a faster rate of pigment deposition than the diet containing whole shrimp (diet 3), but the levels of tissue pigmentation achieved by fish receiving these diets were shown to be the same. The fish which were fed diet 3 and the diet containing canthaxanthin (diet 6) exhibited the same rate and level of pigment deposition. The fish which received diet 6 were superior in their rate of pigment uptake when compared to the fish fed the control diet (diet 1), however, both of these diets produced tissue pigment levels which did not vary significantly. The results obtained for diet 6 may not be completely valid, since the portion of the results obtained after the 23rd week of feeding represent a different set of dietary parameters. If the water-dispersible canthaxanthin had been fed from the beginning of the feeding period, it might have produced a faster rate of pigment deposition and achieved a higher level of pigmentation.

The curves shown in Figure 8 corresponded closely with the linear regression lines and possibly were more representative of the actual pigment deposition in each group of fish.

Pigmentation in Experimental Trout and Other Fish

For comparative purposes certain species of trout and salmon were analyzed to determine their level of tissue pigmentation. These

values are summarized in Table 12 and comparisons were made to the pigmentation level achieved in each group of experimental trout at the termination of the feeding period.

Table 12. Comparison of Pigmentation Between Experimental Trout and Other Fish.

Fish	Pigmentation ($E^{1\%}_{1\,cm} \times 10^5$)	
	Mean	Range
Brown trout	332	326-337
Brown trout	615	585-644
Rainbow trout	256	221-291
Rainbow trout	274	272-276
Searun cutthroat trout	2260	2140-2370
Searun cutthroat trout	2410	2060-2850
Chinook salmon	2203	2024-2382
Coho salmon	2355	2346-2364
Rainbow trout from Diet 1	31	0-129
Rainbow trout from Diet 2	821	370-1415
Rainbow trout from Diet 3	401	189-678
Rainbow trout from Diet 4	497	235-839
Rainbow trout from Diet 5	932	286-2100
Rainbow trout from Diet 6	369	187-526

The searun cutthroat trout and the two species of salmon showed a higher tissue pigmentation than the other samples of fish. These were anadromous fish which were able to derive their pigmentation from the abundant source of crustaceans available in the ocean. The samples of experimental trout fed diet 5 showed the highest pigmentation levels. The range of pigmentation in this group of fish reached a level which was comparable to the searun cutthroat trout and the two species of salmon. The other groups of experimental trout which

were fed pigment-rich diets achieved pigmentation levels which were higher than the samples of wild rainbow trout and comparable in average pigmentation levels to the samples of brown trout.

The experimental trout which were fed the control diet (diet 1) showed the lowest pigmentation level. The pigmentation level in the wild rainbow trout was even higher than the control fish. This would suggest that the wild trout ingested a certain amount of carotenoid-rich food.

In feeding experiments conducted by Peterson et al. (28), rainbow trout that were fed crayfish extracts achieved a pigmentation in the muscle tissue of 1.36 μg/gm tissue expressed as β-carotene ($E^{1\%}_{1cm}$ = 2500). This resulted after feeding the crayfish extracts for a period of seven weeks. Similarly, the group of fish which received diet 5 supplemented with the extract from shrimp meal showed a pigmentation level of 1.30 μg/gm tissue (fins, skin, and muscle) after seven weeks of feeding.

Savolainen and Gyllenberg (32) reported a level of 177 μg canthaxanthin/5 gm tissue in rainbow trout maintained on a diet rich in canthaxanthin. The canthaxanthin was added as carophyll red at a level of 4000 μg/5 gm food. The values reported by Savolainen and Gyllenberg seem unusually high since a level of 8.5 μg/5 gm tissue was calculated for the fish fed the water-dispersible canthaxanthin ($E^{1\%}_{1cm}$ = 2200) for nine weeks. The highest pigmentation level in the

fish from diet 5 was 45.5 μg/5 gm tissue expressed as canthaxanthin.

After the analyses of several species of Pacific salmon, Kanemitsu and Aoe (20) reported values 0.47 mg% for chinook salmon, 1.35 mg% for coho salmon, and 3.86 mg% for blueback salmon expressed as astaxanthin ($E^{1\%}_{1cm}$ = 2200). The values for salmon samples shown in Table 12 contained 1.00 mg% and 1.07 mg% for the chinook and coho, respectively. These values are in agreement with the levels reported by Kanemitsu and Aoe.

Photography

Photographs were taken of each group of fish at approximately the 30th week of the feeding period. The observable pigmentation in the skin, fins, and muscular tissue of the trout is shown in photographs 2 through 13. A portion of the anterior and posterior sections of the fish is also shown in these photographs. These portions were assayed for their pigmentation level and these values are listed on the page which preceeds each photograph.

To fully evaluate the observable pigmentation level achieved by each group of trout, photograph 14 was included. The gradation of muscular tissue pigmentation which was produced by the various diets is clearly shown by this photograph.

It should be noted that in several cases the pigmentation in the anterior sections was lower than the posterior sections. The

photographs and measured pigment values show this observation. In several instances during the sampling, anterior sections were analyzed separately from posterior sections. Some of these samplings are shown in Table 13. Frequently the pigmentation level of the anterior section was one-half the level of the posterior section.

In photographs 5 and 7 precocious males were included in order to show the external pigmentation in the skin, especially the lateral line, belly, operculum, and fins. These fish had reached sexual maturity and illustrated two interesting observations. First, the muscular pigmentation level was lower than most of the other fish, while their external pigmentation was more pronounced. Second, the anterior sections contained a higher pigmentation level than the posterior sections which is contrary to what was normally found.

Crozier (5) reported that male sockeye salmon increase their external pigmentation as they approach spawning age. Crozier also showed that this pigmentation probably resulted from the transfer of color from the muscular tissue.

Photographs of trout from diet 2 are more sharply highlighted than the other photographs. This is explained by the fact that the prints of the diet 2 fish were made from slides while the prints of the other fish were made directly from negatives.

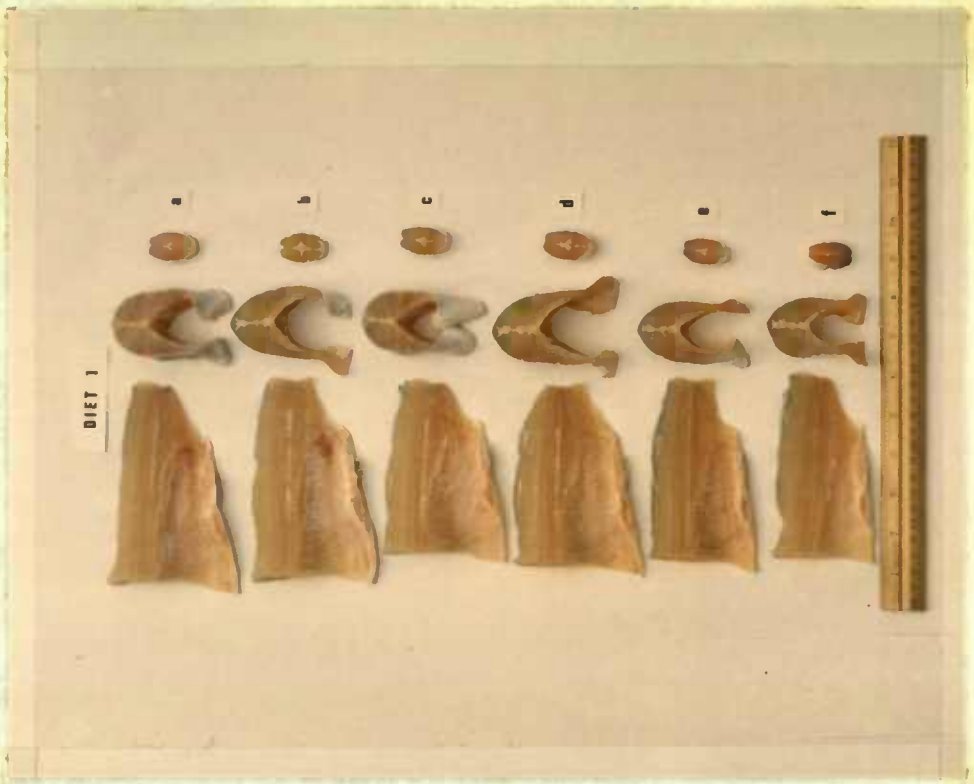
Fish Samples from Diet 1

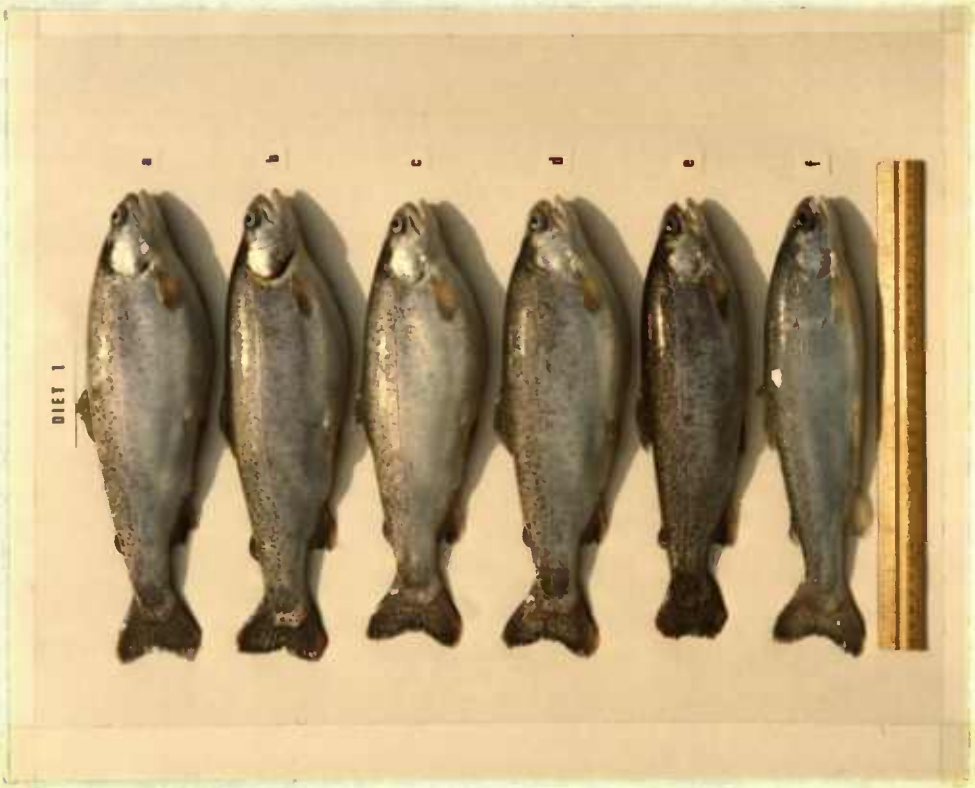
Photograph 2: Pigmentation in muscular tissue.

Fish #	Pigment Value ($E^{1\%}_{1\ cm} \times 10^5$)	
	Anterior Section	Posterior Section
a	69	58
b	27	32
c	35	54
d	41	17
e	8	16
f	5	10
Ave	31	

Photograph 3: Fish size and pigmentation in skin tissue, lateral line, operculum, and fins.

Fish #	Sex	Weight
a	Male	467 gms
b	Female	448 gms
c	Female	357 gms
d	Female	371 gms
e	Male	269 gms
f	Female	247 gms


DIET 1
a
b
c
d
e
f


DIET 1
a
b
c
d
e
f

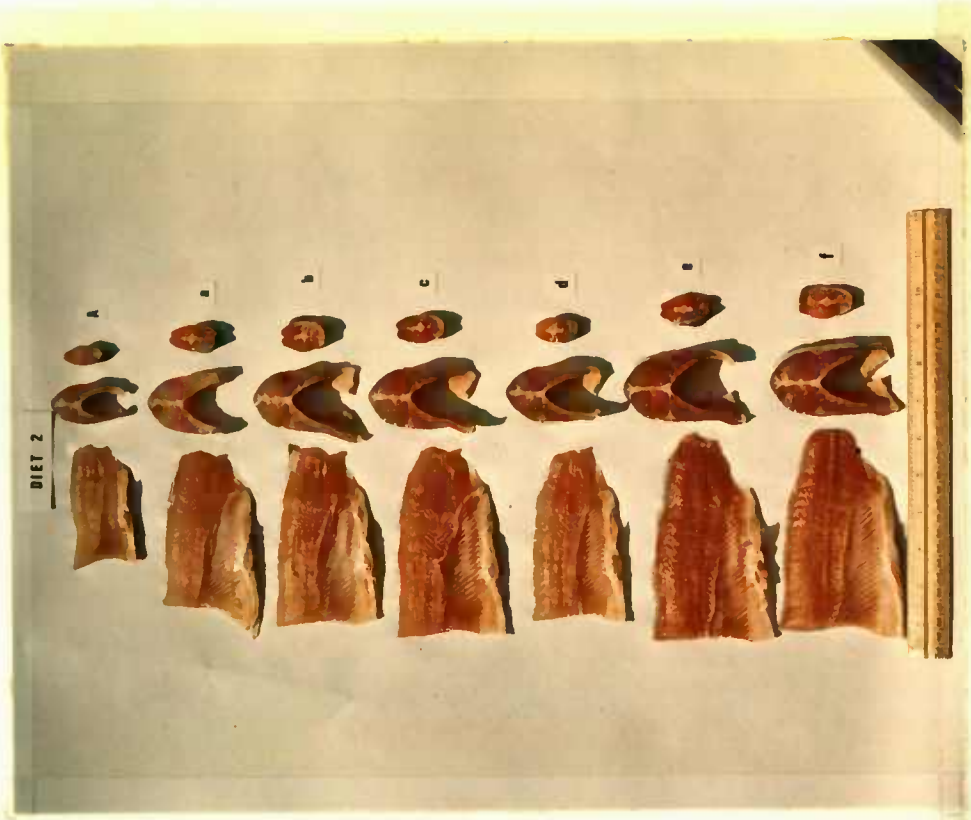
Fish Samples from Diet 2

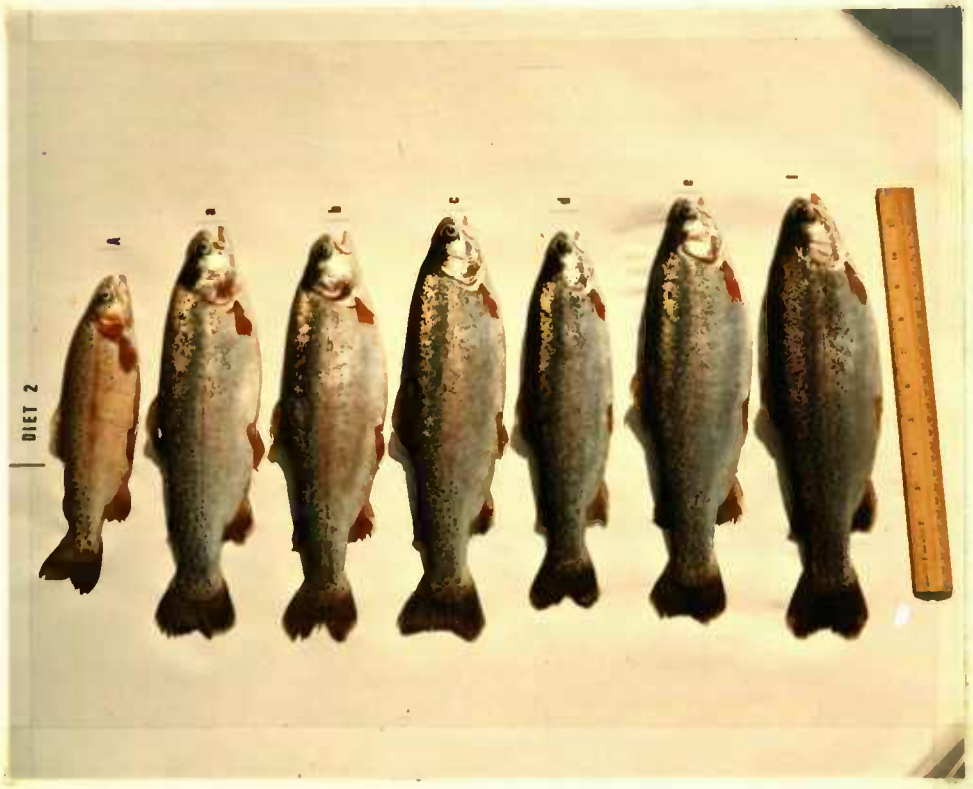
Photograph 4: Pigmentation in muscular tissue.

	Pigment Value ($E^{1\%}_{1\ cm} \times 10^5$)	
Fish #	Anterior Section	Posterior Section
A	391	761
a	405	1035
b	464	1014
c	825	1110
d	370	625
e	919	1415
f	645	1021
Ave	787	

Photograph 5: Fish size and pigmentation in skin tissue, lateral line, operculum, and fins.

Fish #	Sex	Weight
A	Precocious male	135 gms
a	Male	316 gms
b	Female	353 gms
c	Female	373 gms
d	Male	267 gms
e	Female	412 gms
f	Male	460 gms
Ave		331 gms


DIET 2


DIET 2

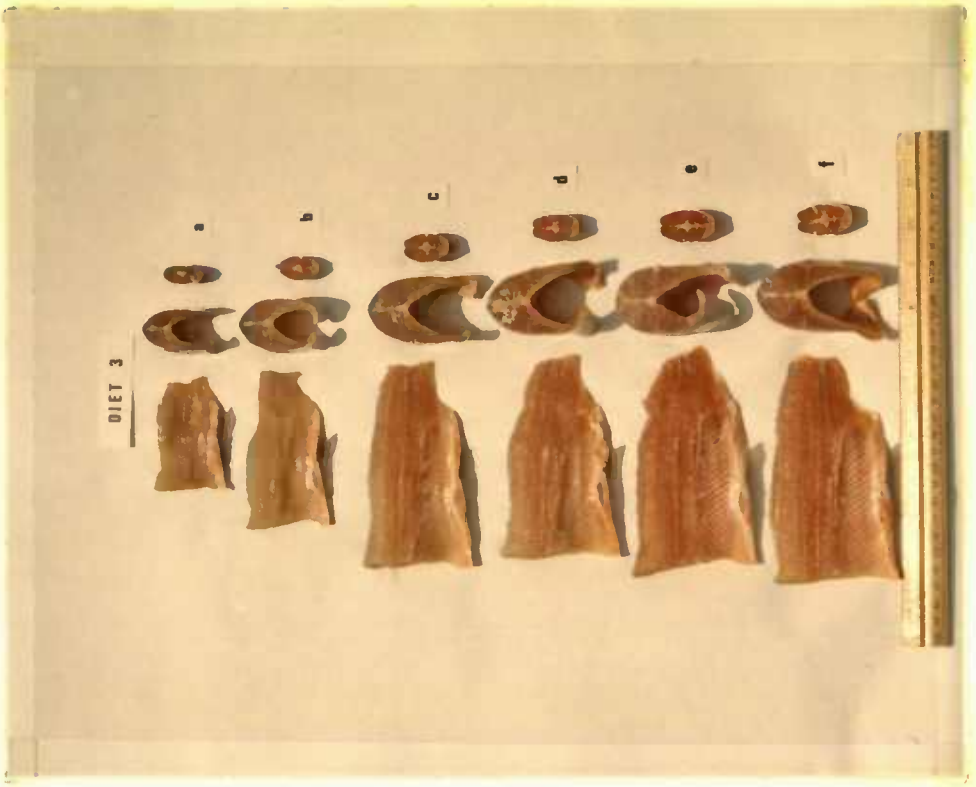
Fish Samples from Diet 3

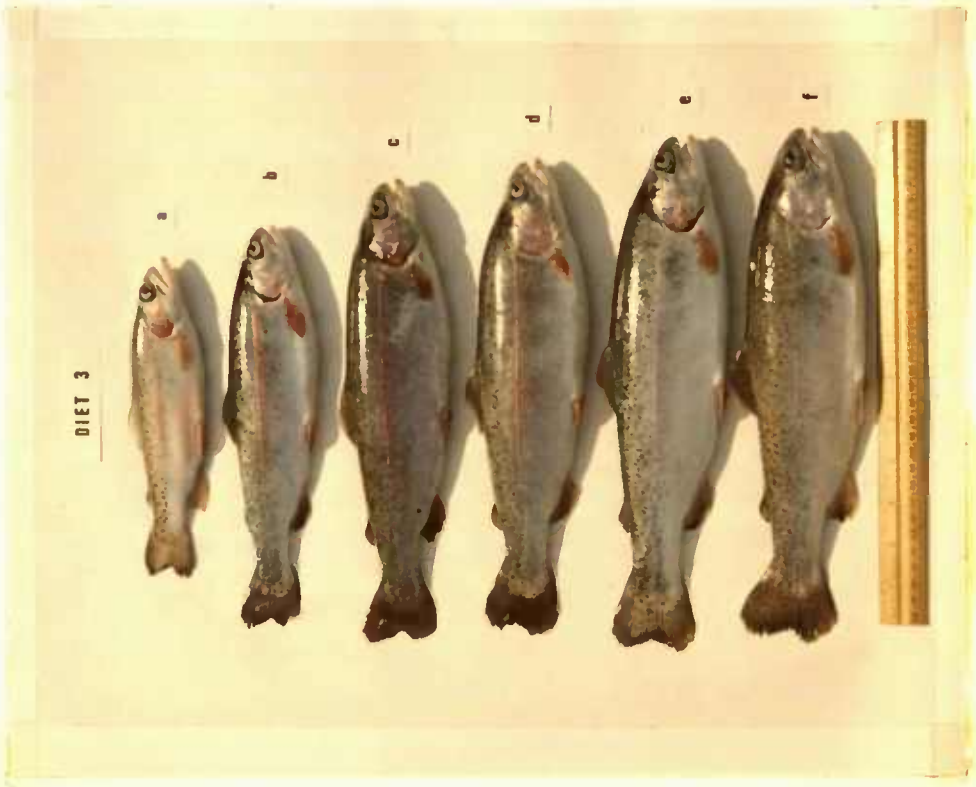
Photograph 6: Pigmentation in muscular tissue.

	Pigment Value ($E^{1\%}_{1cm} \times 10^5$)	
Fish #	Anterior Section	Posterior Section
a	421	180
b	195	189
c	322	623
d	346	678
e	498	508
f	265	579
Ave	401	

Photograph 7: Fish size and pigmentation in skin tissue, lateral line, operculum, and fins.

Fish #	Sex	Weight
a	Precocious male	100 gms
b	Male	180 gms
c	Male	284 gms
d	Male	363 gms
e	Female	353 gms
f	Male	342 gms
Ave		270 gms


DIET 3
a
b
c
d
e
f


DIET 3
a
b
c
d
e
f

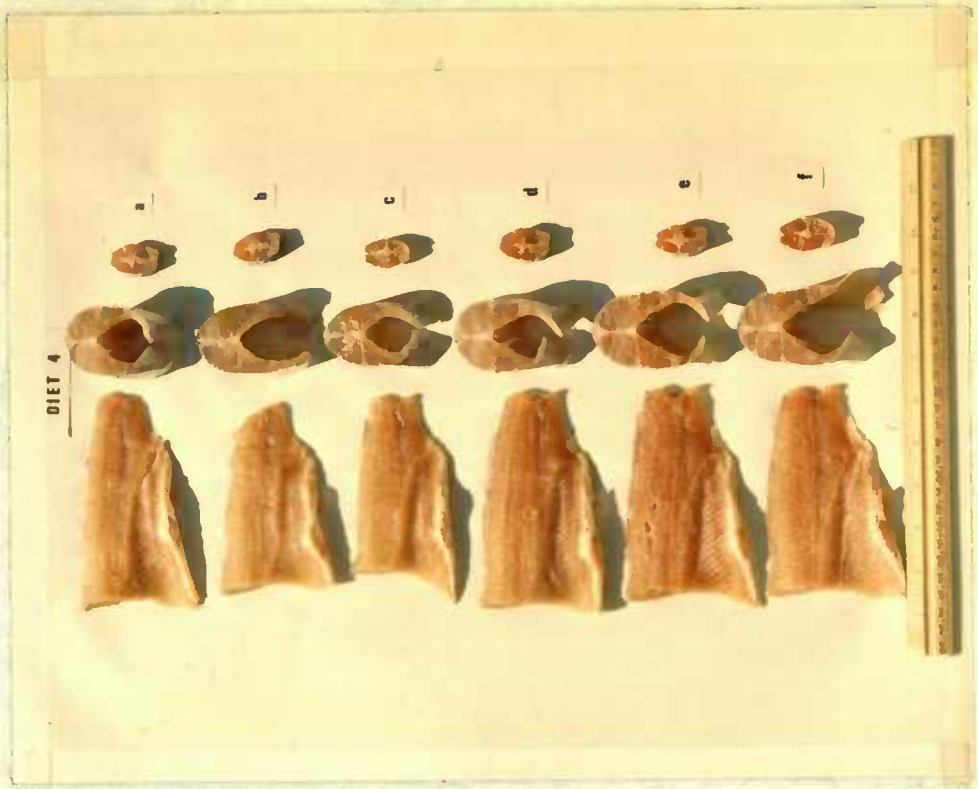
Fish Samples from Diet 4

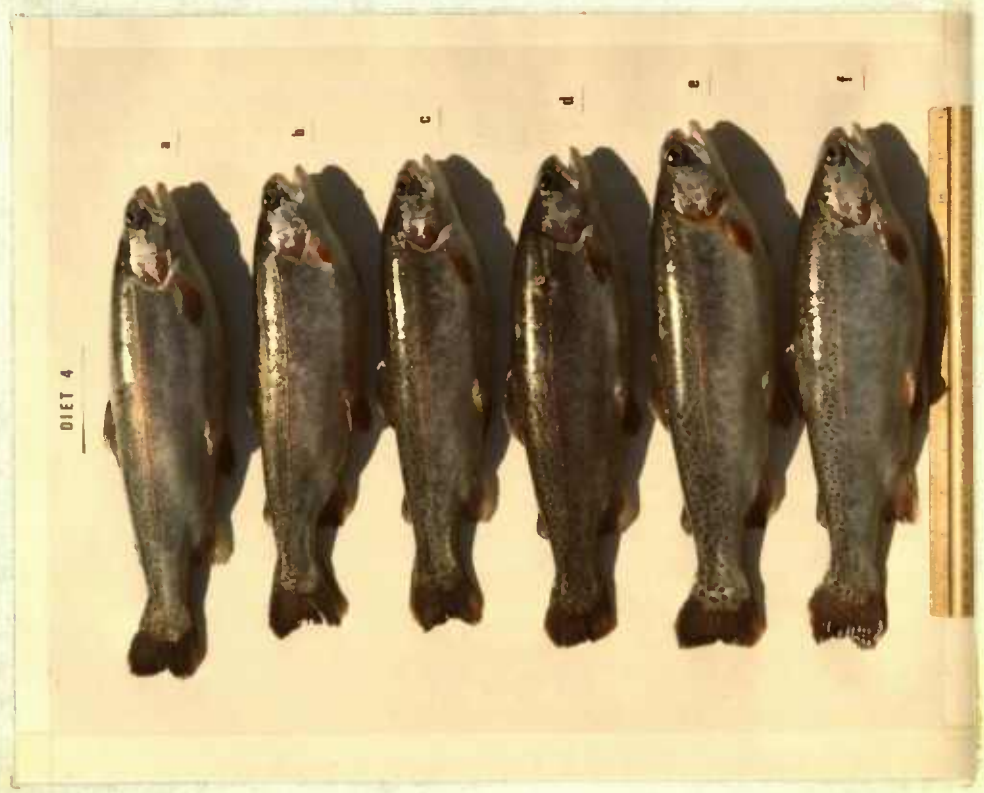
Photograph 8: Pigmentation in muscular tissue.

	Pigment Value ($E^{1\%}_{1cm} \times 10^5$)	
Fish #	Anterior Section	Posterior Section
a	377	560
b	315	395
c	235	493
d	344	683
e	643	839
f	334	688
Ave	497	

Photograph 9: Fish size and pigmentation in skin tissue, lateral line, operculum and fins.

Fish #	Sex	Weight
a	Male	441 gms
b	Male	353 gms
c	Male	320 gms
d	Female	398 gms
e	Male	400 gms
f	Female	382 gms
Ave		382 gms


a
b
c
d
e
f
DIET 4


a
b
c
d
e
f
DIET 4

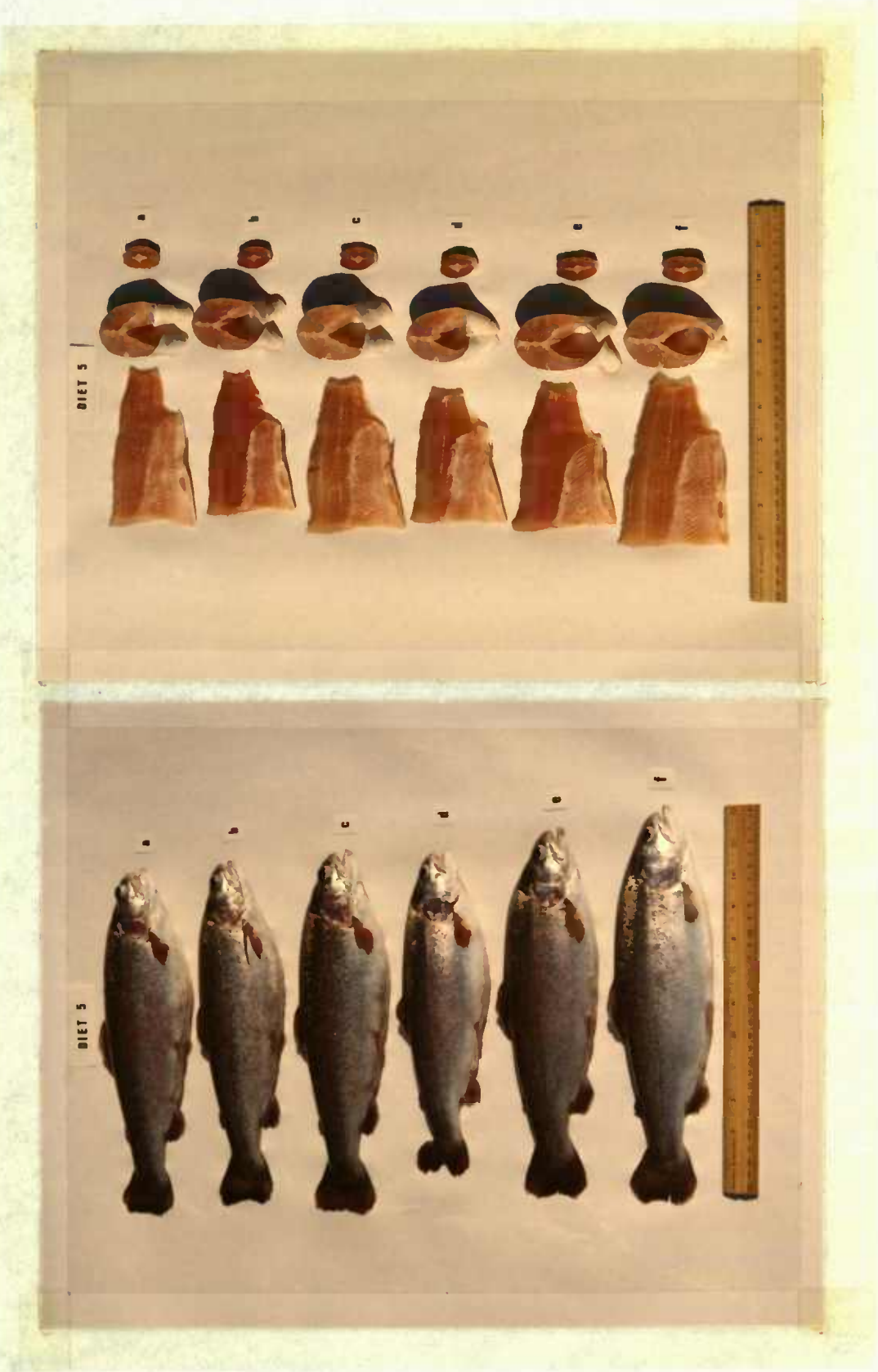
Fish Samples from Diet 5

Photograph 10: Pigmentation in muscular tissue.

	Pigment Value ($E^{1\%}_{1cm} \times 10^5$)	
Fish #	Anterior Section	Posterior Section
a	720	1357
b	1193	2100
c	376	781
d	752	1140
e	726	1214
f	286	535
Ave	932	

Photograph 11: Fish size and pigmentation in skin tissue, lateral line, operculum, and fins.

Fish #	Sex	Weight
a	Male	433 gms
b	Female	368 gms
c	Male	408 gms
d	Female	308 gms
e	Male	371 gms
f	Male	400 gms
Ave		381 gms


DIET 5
DIET 5

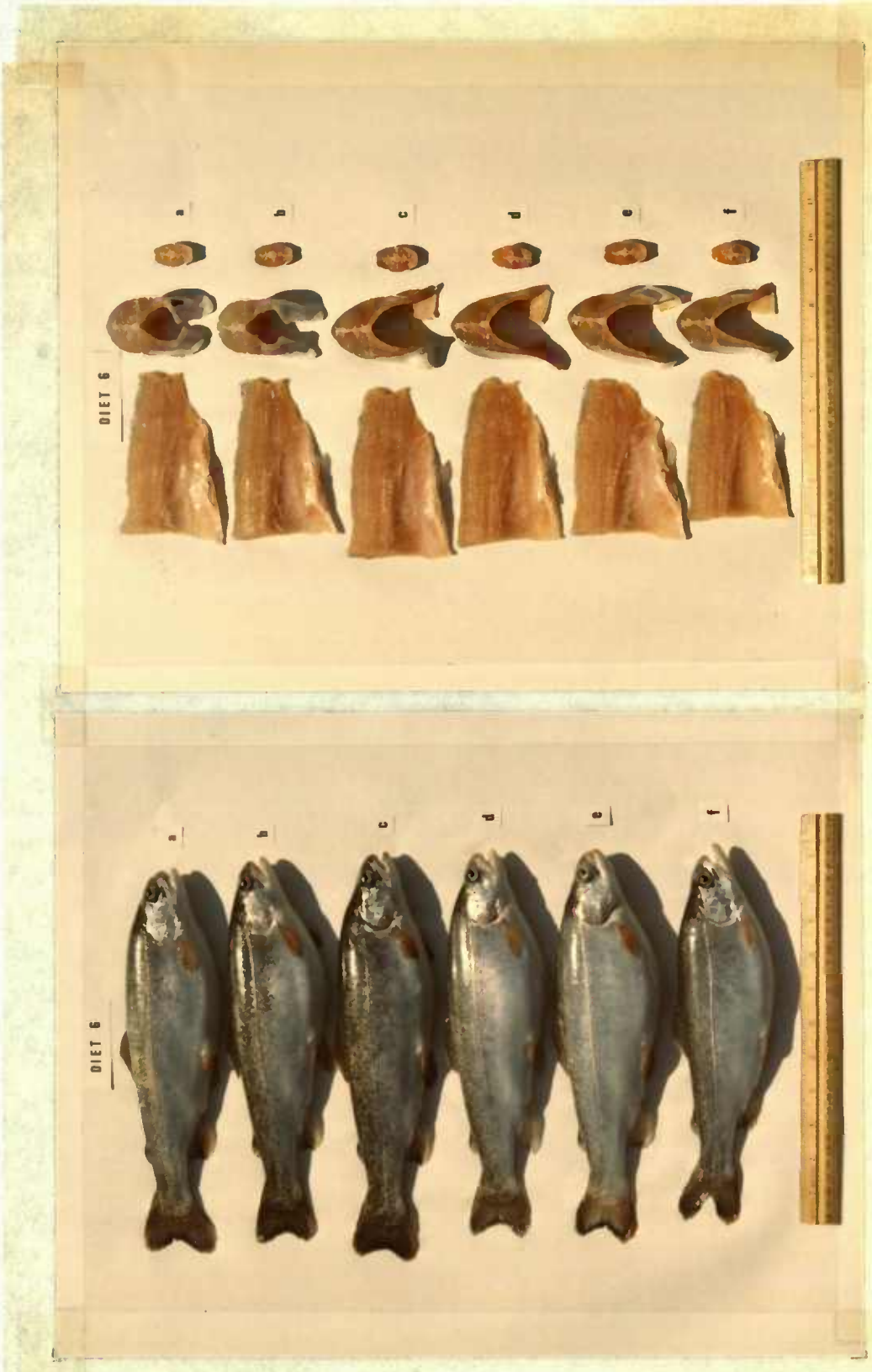
Fish Samples from Diet 6

Photograph 12: Pigmentation in muscular tissue.

	Pigment Value ($E^{1\%}_{1cm} \times 10^5$)	
Fish #	Anterior Section	Posterior Section
a	263	358
b	187	338
c	378	425
d	485	526
e	361	433
f	263	410
Ave	369	

Photograph 13: Fish size and pigmentation in skin tissue, lateral line, operculum, and fins.

Fish #	Sex	Weight
a	Male	382 gms
b	Male	362 gms
c	Female	376 gms
d	Female	327 gms
e	Female	346 gms
f	Male	263 gms
Ave		343 gms


a
b
c
d
e
f
DIET 6
a
b
c
d
e
f
DIET 6

Fish Samples from all Six Diets

Photograph 14: Pigmentation in muscular tissue.

Fish Sample	Weight	Sex	Pigment Value ($E^{1\%}_{1\,cm} \times 10^5$)
Diet 1	285 gms	Female	29
Diet 4	411 gms	Male	241
Diet 3	262 gms	Male	345
Diet 6	357 gms	Male	350
Diet 2	414 gms	Male	997
Diet 5	444 gms	Male	923

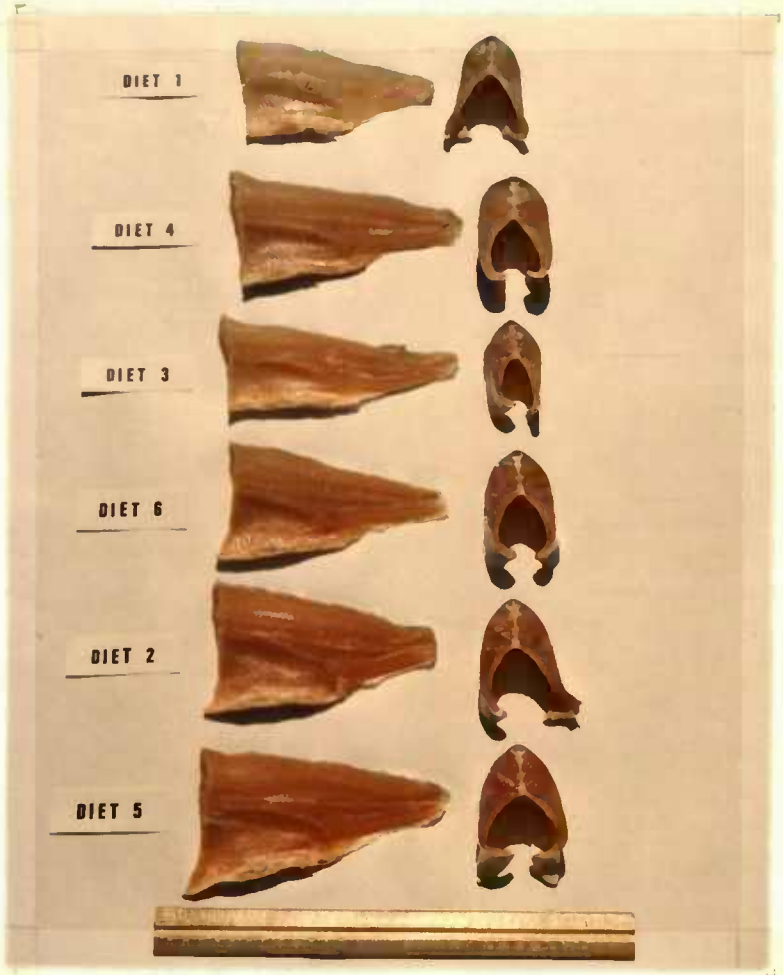

DIET 1
DIET 4
DIET 3
DIET 6
DIET 2
DIET 5

Table 13. Comparison of Pigmentation in Anterior and Posterior Sections of Trout.

Fish Sample	Sex	Weight (gms)	Pigment Value ($E^{1\%}_{1cm} \times 10^5$)	
			Anterior Section	Posterior Section
Diet 2	Male	265	341	642
Diet 2	Male	240	688	911
Diet 2	Male	301	380	714
Diet 2	Male	412	482	1064
Diet 3	Female	142	297	396
Diet 3	Female	137	315	671
Diet 3	Male	240	305	605
Diet 4	Female	295	269	581
Diet 4	Male	430	404	688
Diet 5	Male	199	755	1219
Diet 5	Male	260	316	592
Diet 5	Male	411	505	772

Sensory Evaluations

Student Panel

The frequency of scores from the student panel who rated the desirability of trout fed diets 1, 2, 5, and 6 are shown in Table 14. The difference from the mean score for control fish (diet 1) is shown at the bottom of the table. If the means which differed by an amount greater than 0.50 are considered, the trout receiving diet 5 supplemented with the highest level of meal extract showed trends toward a higher desirability score than control trout when rated under white lights. When the students rated the samples under colored lights, the fish from diet 2 (which contained 15% shrimp waste meal) and diet 5 showed higher scores for desirability than control trout.

These scores were analyzed statistically and the results of this analysis are shown in Table 15. Taste panel scores for fish fed the various diets evaluated under the illumination of white light were shown not to vary significantly from each other at the .05 level.

Conversely, the same fish evaluated during the afternoon of the same day under the illumination of red and orange lights were shown to vary significantly at the .01 level. Scores for fish fed diets containing 15% shrimp waste meal (diet 2) and the highest level of added meal extract (diet 5) were shown to be significantly higher at the .01 level than those for control fish (diet 1). Fish fed the diet containing

Table 14. Frequency Table of Sensory Evaluations by the Student Panel.

Score	Judgements Under White Lights[1]				Judgements Under Colored Lights[2]			
	Diet 1	Diet 2	Diet 5	Diet 6	Diet 1	Diet 2	Diet 5	Diet 6
9	2	7	4	1		6	8	1
8	11	11	12	12	6	17	15	10
7	12	10	17	22	18	16	15	14
6	10	14	14	9	17	7	12	15
5	10	3	6	8	10	4	9	13
4	8	6	3	1	9	11	5	10
3	3	4	2	4	6	5	2	2
2	1	2	1			1		2
1		1		1	1		1	
Sum	345	361	385	370	381	425	441	397
Mean	5.94	6.22	6.64	6.38	5.69	6.34	6.58	5.93
Difference[3]		+0.28	0.70	+0.44		+0.65	+0.89	+0.24

[1] n = 58

[2] n = 67

[3] From diet 1

Table 15. Statistical Analysis of Student Panel Evaluations.

Source of Variation	Analysis of Variance: Judgements Under White Lights — Degrees of Freedom	Mean Square	F-Value	Judgements Under Colored Lights — Degrees of Freedom	Mean Square	F-Value
Treatment	3	2.900	1.422	3	12.200	6.054*
Judge	60	5.524	---	64	5.485	---
Error	180	2.039	---	192	2.015	---
Total	243			259		
		LSD				
P=0.05		0.508			0.489	
P=0.01		0.658			0.642	
		Mean Scores[1]				
Diet						
1		6.05[a]			5.66[a]	
2		6.21[a]			6.37[b]	
5		6.56[a]			6.59[b]	
6		6.38[a]			5.85[a]	

*Significant at P > .01

[1] Mean scores in a column with same exponent letter did not vary significantly (P > .05) from each other.

canthaxanthin (diet 6) yielded scores that were significantly lower than those for diets 2 and 5 at .05 and .01 levels, respectively. Comparison of scores between fish fed diets 1 and 6 or diets 2 and 5 showed that neither of these two groups of fish varied significantly at the .05 level.

These results indicate that judges who could not distinguish a color difference between any of the fish samples (due to masking by colored illumination) rated trout fed diets 2 and 5 significantly higher in desirability than those fed diets 1 and 6. This suggests there was a true taste difference when the highly pigmented trout, which were fed diets 2 and 5, were compared to the control trout.

Peterson et al. (28) evaluated the taste difference between trout fed diets supplemented with crayfish extracts and those fed a control diet. Taste tests performed by seven blindfolded panel members yielded scores which were not statistically different. Their fish, however, were fed for a shorter period of time and consequently were not as highly pigmented as the fish fed diets 2 and 5 in this experiment.

The difference between the desirability scores for fish under white lights and those under colored lights is not readily explainable. Such factors as the variation among the student judgements, the difference in the tasting times, or the lighting conditions were variables which could be considered.

If the lighting condition was the sole factor responsible for the scoring difference, then the results suggest that students had a tendency to rate all of the samples statistically the same when the pigmentation was distinguishable. The subject of whether or not highly colored trout produce a psychological taste difference has not been clearly established. In these flavor panel results a tendency was shown toward lowering the scores of the observably colored trout to the same level as the control trout. This decrease in the desirability of colored fish was contrary to what would normally be expected.

In an attempt to provide an explanation for the scoring difference, 15 staff judges participated in a panel designed to rate the samples under the influence of both illumination sources.

The frequency of scoring by the judges is shown in Table 16. The differences in the mean scores from the control fish indicate that the scoring trends were nearly the same under both sources of illumination. The statistical analyses of these scores are shown in Table 17. The interaction of lighting on flavor panel scores was shown not to be significant at the .05 level. These judges were staff members who are experienced in flavor panel work. This points to the possibility that the differences in student scoring under the two lighting conditions were due to a variation among judgements since the students were untrained in sensory evaluations.

Table 16. Frequency Table of Sensory Evaluations by a Staff Panel Under Two Lighting Conditions.

Score	Judgements Under White Lights[1]				Judgements Under Colored Lights[1]			
	Diet 1	Diet 2	Diet 5	Diet 6	Diet 1	Diet 2	Diet 5	Diet 6
9.0	1	1	1			3	3	
8.5		1	2	1				
8.0		6	2	2	2	3	6	2
7.5	1	2	3	1				
7.0	6	1	4	1	4	3	2	3
6.5	1		2	2				
6.0	1			2	5	2	3	8
5.5	2			2				
5.0	1			3	2		1	1
4.5		1						
4.0		1			2	3		1
3.5				1				
3.0	2	1				1		
2.5								
2.0		1						
1.5			1					
1.0								
Sum	92.0	101.0	107.0	93.5	92.0	99.0	112.0	94.0
Mean	6.13	6.73	7.13	6.13	6.13	6.60	7.47	6.23
Difference[2]		+0.60	+1.00	0.00		+0.47	+1.34	+0.10

[1] n = 15

[2] From diet 1

Table 17. Statistical Analysis of Staff Panel Evaluations Under Two Lighting Conditions.

Analysis of Variance			
Source of Variation	Degrees of Freedom	Mean Square	F-Value
Treatment	3	10.024	---
Judge	14	4.470	---
Trmt x Judge	42	2.535	---
Lights	1	1.752	1.681
Trmt x Lights	3	0.308	---
Judge x Lights	14	1.042	---
Trmt x Lights x Judge	42	0.598	---
Total	119		
LSD			
p=0.05		0.529	
Mean Scores[1]			
Lighting			
White	6.56[a]		
Colored	6.62[a]		

[1]Mean scores in a column with same exponent letter did not vary significantly ($P > .05$) from each other.

When all of the 126 student scores were combined and analyzed (Table 18), a comparison of mean scores indicated that trout fed diets 2 and 5 were rated significantly higher in desirability than the control trout at the .05 and .01 levels, respectively. The trout fed diet 5 yielded a mean score which was significantly higher at the .01 level than the trout fed diet 6. Trout from diets 2 and 6 were rated the same at the .05 level of significance. No significant difference could be shown when the mean scores for trout fed diets 1 and 6 or diets 2 and 5 were compared.

Staff Panel

Fish fed the experimental diets were subjected to a more detailed sensory evaluation by 27 trained judges composed of Department staff members. The frequency of scoring is listed in Tables 19 and 20. It would appear that trout fed diets 2 and 5 were rated higher in firmness, color, and overall desirability. These trout also showed trends toward a more natural trout flavor than the control trout. The results of statistical analyses of these flavor panel evaluations are shown in Table 21.

When the mean scores for texture were compared, the LSD procedure indicated that fish fed formulated diets containing shrimp meal (diet 2) and the highest level of meal extract (diet 5) were significantly higher than control samples at the .01 level. Fish fed canthaxanthin

Table 18. Statistical Analysis of Total Evaluations by the Student Panel.

Analysis of Variance			
Source of Variation	Degrees of Freedom	Mean Square	F-Value
Treatment	3	11.722	5.752*
Judge	125	5.494	
Error	375	2.038	
Total	503		

LSD	
P=0.05	0.349
P=0.01	0.459

Mean Scores[1]	
Diet	
1	5.85[a]
2	6.29[bc]
5	6.57[b]
6	6.10[ac]

*Significant at $P > .01$

[1]Mean scores with same exponent letter did not vary significantly ($P > .05$) from each other.

Table 19. Frequency Table of Sensory Evaluations by the Staff Panel. Texture and Juiciness.

	Texture				Juiciness			
Scale	Diet 1	Diet 2	Diet 5	Diet 6	Diet 1	Diet 2	Diet 5	Diet 6
9.0	3	6	4	2	1	3	1	
8.5	3	5	5	2	5	3	3	4
8.0	4	4	6	5	3	11	4	6
7.5	1	6	6	6	7	2	7	5
7.0	6	3	3	6	3	3	5	2
6.5	1	2	2	1	2	3	1	2
6.0	2			2	2		2	1
5.5	2	1		2	1	1		2
5.0	5		1		2	1	3	2
4.5								
4.0							1	
3.5								2
3.0					1			1
2.5								
2.0				1				
1.5								
1.0								
Sum[1]	188.5	213.0	210.5	181.5	192.5	206.5	191.5	183.5
Mean	6.98	7.89	7.80	6.72	7.13	7.65	7.09	6.80
Difference[2]		+0.91	+0.82	-0.26		+0.52	-0.04	-0.33

[1] n = 27

[2] From diet 1

Table 20. Frequency Table of Sensory Evaluations by the Staff Panel. Natural Trout Flavor, Color and Desirability.

	Natural Trout Flavor				Color				Desirability			
Scale	Diet 1	Diet 2	Diet 5	Diet 6	Diet 1	Diet 2	Diet 5	Diet 6	Diet 1	Diet 2	Diet 5	Diet 6
9.0							4	1		5	1	1
8.5			1	1	1	1	2			2	4	2
8.0	1			2	1	16	8	3	5	7	5	4
7.5					2	3	1	1	1	6	5	3
7.0	4		1	2	2	3	8	5	8	1	7	1
6.5						1	2	5	1	1	5	5
6.0	1	4	1	1	2	2	1	8	4	1		4
5.5					1				3			2
5.0	1	1	4		6				2			4
4.5	2	1	1	3	1			2		1		
4.0	3	2	2	2	5				1	2		
3.5	1	1	1	4	1			2				
3.0	5	3	4	3	3				1	1		
2.5	2	2	3	2								
2.0	3	7	4	2	1				1			
1.5		1	5	4								1
1.0	4	4	1	1	1							
Sum[1]	101.5	85.5	86.5	105.0	133.0	202.5	203.5	174.5	169.5	198.0	202.0	176.5
Mean	3.76	3.17	3.20	3.89	4.93	7.50	7.54	6.46	6.28	7.33	7.48	6.54
Difference[2]		-0.59	-0.56	+0.13		+2.57	+2.61	+1.53		+1.05	+1.20	+0.26

[1] n = 27

[2] From diet 1

Table 21. Statistical Analysis of Staff Panel Evaluations.

Analysis of Variance						
		Texture	Juiciness	Color	Flavor	Desirability
Source of Variation	Degrees of Freedom	Mean Square	Mean Square	Mean Square	Mean Square	Mean Square
Treatment	3	5.237**	3.216*	40.509**	3.928	9.401**
Judge	26	2.381	3.627	1.847	10.589**	2.791
Error	78	1.001	1.131	1.900	1.731	1.754
Total	107					
			LSD			
P=0.05		0.534	0.567	0.735	0.702	0.707
P=0.01		0.702	0.746	0.966	0.922	0.929
			Mean Score[1]			
Diet						
1		7.04[a]	7.11[ab]	4.89[a]	3.83[a]	6.28[a]
2		7.93[b]	7.59[a]	7.50[b]	3.17[a]	7.33[b]
5		7.80[b]	7.06[ab]	7.44[b]	3.24[a]	7.48[b]
6		7.19[a]	6.76[b]	6.52[c]	3.89[a]	6.54[a]

* Significant at P > .05 ** Significant at P > .01

[1]Mean scores with same exponent letter in a column did not vary significantly (P > .05) from each other.

(diet 6) were shown to be significantly lower than those fed diets 2 and 5 at the .05 and .01 levels, respectively. Comparisons between fish fed diets 1 and 6 or diets 2 and 5 were shown not to vary at the .05 significance level.

Scores for juiciness of the fish fed diet 2 were shown to be significantly higher at the .01 level than the trout fed diet 6. Scores for fish fed the other diets did not vary significantly. Scores for flavor did not vary significantly (.05 level). Flavor scores were shown to range from 3.17 to 3.89. It should be noted these were acceptable scores, since the ballot ranged from 9.0, "no natural trout flavor", to, 1.0, "extreme natural trout flavor" (Figure 4).

Trout fed diets 2 and 5 yielded significantly higher scores for color than fish fed diet 1. Scores for trout fed diet 6 were shown to be higher than those for control trout at the .01 significance level. Scores for the color of trout fed diets 2 and 5 were higher than those for fish fed diet 6 at the .05 and .01 levels, respectively. These subjective findings are varified by the average pigment values for the raw and cooked flesh of samples submitted to taste panel evaluations listed in Table 22.

Trout fed diets 2 and 5 received the highest scores for desirability. Scores were shown to be significantly (.01 level) higher than those for control fish (diet 1) and higher (.05 and .01 levels, respectively) than those for trout fed diet 6. Scores for fish fed diets 1 and 6 were not significantly (.05 level) different.

Table 22. Pigment Analysis of Fish Used in Sensory Evaluations.

Fish Sample	Average Weight (gms)	Pigment Value ($E^{1\%}_{1cm} \times 10^5$) Raw Tissue[1]	Raw Tissue[2]	Cooked Tissue[3]
Diet 1	282	63	44	61
Diet 2	444	934	951	1029
Diet 5	389	1074	788	998
Diet 6	327	385	443	582

[1]Ave. value for all 20 fish used for flavor study.

[2]Ave. value for samples analyzed before cooking.

[3]Ave. value for same samples analyzed after cooking.

Since fishermen generally claim that brightly colored wild trout taste better and are firmer than paler hatchery-released trout, these flavor panel results would favor the belief that these claims are not just psychological. There have been indications that shrimp meal (41) or other crustacean derivatives (28) may improve the color, flavor, or texture of trout. If all the flavor panel results are considered, then this observation has been shown to be statistically valid.

Trout fed formulated diets containing shrimp meal and meal extracts possessed muscle tissue that was firmer in texture than fish fed the control diet. This improved textural quality must have been associated with a difference in the structural or chemical characteristics of the fish muscle.

In an attempt to provide an explanation for this particular

difference, the lipid and moisture content were determined from sections taken from the fish which were used for the flavor panel evaluations. The results of these analyses are shown in Table 23.

Table 23. Lipid and Moisture Analysis of Fish Used in Sensory Evaluations.

Fish Sample	Percent Moisture	Range	Percent Lipid	Range
Diet 1	72.6	±0.3	6.1	±0.2
Diet 2	72.4	±0.1	5.2	±0.4
Diet 5	73.1	±0.1	5.1	±0.2
Diet 6	73.2	±0.1	4.9	±0.1

There was less than a 1.0 percent difference among the moisture content of any of the samples and less than a 1.5 percent difference among the lipid content. This may not prove significant, since the deviation between replicate samples reached nearly 0.5 percent. Possibly a correlation between the flavor panel scores and these results was shown for the trout fed diet 6. These fish had a lower lipid content and were shown to yield significantly lower scores for juiciness than trout fed diet 2. Fish fed diets 2 and 5 showed a slightly lower lipid value than the control samples. Trout fed diets 2 and 5 were also judged significantly firmer in texture than the control trout.

Diet 2 contained 15% shrimp meal, while diet 5 was supplemented with a pigment-rich meal extract. Both of these formulations yielded trout with significantly better texture. Although both contained the

pigment-lipid components of shrimp meal, diet 5 lacked the minerals, chitin, etc. associated with the meal. The more favorable textural characteristics of trout appear to be associated with the pigment-lipid fraction of shrimp waste meal.

Only slight chemical differences, which would normally be associated with texture, were detected in the tissue. The reason for the texture differences might lie in a structural difference of the tissue or a difference which was intrinsically associated with the pigmentation in the muscular tissue. It would seem unlikely that the gross tissue structure was different among any of the fish samples, although this is worthy of consideration. The color of the muscular tissue was due to the deposition of a lipid soluble pigment. The texture differences might be associated with the trout's ingestion of this pigment and its consequent deposition. The physiological aspects of the pigment metabolism and transport across tissue membranes may have affected the texture of the flesh. These results merit further study.

The color improvement and increase in the desirability of the trout would also be of interest to the trout producer and others involved in aquaculture.

SUMMARY AND CONCLUSIONS

Research was directed primarily toward the investigation of a suitable method to utilize shrimp processing waste. It was found through suitable processing methods, that the waste material could be dried into a meal without destruction of the carotenoid pigments.

Investigations were carried out to determine if the shrimp waste meal could be utilized by rainbow trout (Salmo gairdneri) as a source of pigmentation. When added to the dry mix of a diet at a level of 15 percent, the shrimp waste meal produced nearly a 13-fold increase in the tissue pigmentation of trout when compared to a control group after a 24 week feeding period. Sensory evaluations were performed on the trout at the termination of the feeding trials. Fish which were fed the shrimp waste meal received scores significantly higher in flavor and desirability than control fish.

An effective method for achieving pigmentation in trout was then investigated by feeding five pigment-rich diets for 34 weeks. These results indicated that shrimp waste meal or a pigment-lipid extract of the meal were the most effective in achieving muscular pigmentation. External coloration was produced more rapidly by a diet which included a pigmented-lipid extract of the shrimp waste meal. Canthaxanthin supplemented diets did not produce pigmentation when fed in a crystalline form. It did produce muscular pigmentation when fed in a water-dispersible form, but little external coloration.

Sensory evaluations were also conducted on these fish at the termination of the feeding trials. Trout which received shrimp waste meal or its pigment-lipid extract were rated significantly higher in firmness, color, and overall desirability than control fish. Dietary factors responsible for improving organoleptic characteristics appear to be related to the pigment-lipid fraction of the shrimp waste meal. The pigmented trout from the diet with water-dispersible canthaxanthin were rated significantly higher in color than control fish.

These research results have pointed to a suitable and potentially economical means for utilizing shrimp processing waste. The implications dealing with the improvement in the marketability of trout could be of significant value to the aquaculture industry.

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APPENDICES

APPENDIX I

Dietary Components

Table 1. Vitamin Mix

Vitamin	% in Mix
Thiamin (HCl)	0.3200
Riboflavin	0.7200
Niacinamide	2.5600
Biotin	0.0080
Ca-pantothenate $(D)_4$	1.4400
Pyridoxine (HCl)	0.2400
Folic acid	0.0960
Menadione	0.0800
B_{12} (Cobalamine 3,000 μgm/gm)	0.2667
i-inositol (meso)	12.5000
Ascorbic acid	6.0000
Para-amino-benzoic acid	2.0000
Vitamin D_2 (500,000 USP/gm)	0.0400
Butylated hydroxy anisole	0.0750
Butylated hydroxy toluene	0.0750
Vitamin A (250,000 IU/gm)	1.0000
Celite	67.4052

Table 2. Modified Bernhart-Tomarelli Salt Mix (NaF and $CoCl_2$ added).

Ingredient	% in Mix
CaCO3	2.100
$Ca(PO_4)_2$	73.500
Citric acid	0.205
Cupric citrate ($2Cu_2C_6H_5O_7 \cdot 5H_2O$)	0.046
Ferric citrate ($FeC_6H_5O_7 \cdot 5H_2O$)	0.558
MgO	2.500
$Mn_3(C_6H_5O_7)_2$	0.835
KI	0.001
K_2HPO_4	8.100
K_2SO_4	6.800
NaCl	3.060
$Na_2HPO_4 \cdot 2H_2O$	2.140
$Zn_3(C_6H_5O_7)_2 \cdot 2H_2O$	0.133
NaF	0.002
$CoCl_2$	0.020

APPENDIX II

Pigment Analysis

Extraction of Tissue Carotenoids

A method similar to the Folch lipid extraction (8) was used for the pigment extraction from all the tissue samples.

1. Mascerated samples of tissue (including muscle, skin, and fins) were placed in a Virtis blender with a solvent system of chloroform:methanol (2:1) and mixed for two minutes.

2. The mixture was filtered into a graduate cylinder and the volume noted.

3. Water was added to the filtered solution at a level of 20 percent of the total volume and the two solutions were mixed.

4. The mixture was centrifuged at 1800 rpm for five minutes and the water-methanol was removed.

5. The chloroform layer was clarified by adding 10-20 ml of the chloroform:methanol (2:1) solution and the final volume was recorded.

Spectrophotometric Examination

1. The clarified solution was placed in a cuvette.

2. The absorbance at 487 nm was determined against a chloroform blank using a Beckman DB recording spectrophotometer.

Calculations for Tissue Pigmentation Level

$$\frac{0.01 \times \text{Final Volume} \times \text{Absorbance}}{\text{Sample Weight}} = E^{1\%}_{1\text{cm}}$$

Chitin Nitrogen Determination

1. A 5-gm sample of shrimp meal was placed in a 250 ml centrifuge bottle fitted with a reflux condenser.

2. 100 ml of acetone was added. After refluxing for 45 minutes, the mixture was centrifuged and the supernatant liquid discarded.

3. The material was washed with 70% acetone, centrifuged, and the supernatant discarded.

4. 100 ml of 90 percent formic acid was added and the stoppered bottle was shaken for 18 hours. The mixture was centrifuged and the supernatant discarded.

5. The material was washed with acetone, centrifuged, and the supernatant discarded.

6. Step 5 was repeated using 70% acetone.

7. 100 ml of 1% (w/v) NaOH was added and the mixture was refluxed for 90 minutes. The material was filtered through a sintered glass funnel and the filtrate discarded.

8. The residue was washed with boiling water and transferred quantitatively to a 250 ml Kjeldahl flask using a small amount of water. The volume of water was evaporated to 5 ml and a Kjeldahl determination was conducted using a six-hour digestion.

9. The percent apparent protein contributed by the chitin was calculated by multiplying the amount of nitrogen determined by the factor 6.25.