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Evaluation of the Crawfish Chitin-Carotenoid Complex and Its Nutritional/Pigmentation Effect in Poultry Diets.

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Lee, Keun Soo, Ph.D.

The Louisiana State University and Agricultural and Mechanical Col., 1990

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EVALUATION OF THE CRAWFISH CHITIN-CAROTENOID COMPLEX AND ITS NUTRITIONAL/PIGMENTATION EFFECT IN POULTRY DIETS

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Food Science

by

Keun Soo Lee
B.S., Kyung Hee University, 1980
M.S., Louisiana State University, 1985
December, 1990

DEDICATION

This dissertation is dedicated in loving memory to my late grandmother Kyu Ho Cho, to my father Seok Heon Lee, and my brother Sung Lak Lee.

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ABSTRACT

The present investigation involved utilization of the chitin-carotenoid complex and the whole meal derived from crayfish (Procambarus clarkii) composite byproduct, in controlled broiler diets to determine the biological value of these substrates as potential sources of growth promoters, red intensifying agents, and protein supplements. Included are basic studies of the unique characteristics of the chitin-protein and chitin-carotenoid complexes.

The crawfish chitin-carotenoid substrate did not exhibit any deleterious effects on broiler growth performance in terms of animal weight gain and feed efficiency at levels up to 5% of the total diet. Controlled chitin addition to the diet indicated no apparent relationship with the degree of chitinoclastic activity in the gizzard and intestine of broilers. Similar results were observed between specific chitinase activity and overall broiler growth rates. Astaxanthin pigment in the chitin-carotenoid substrate was demonstrated to impart significant red intensifying effects to broiler skin and shank pigmentation, even at levels as low as 0.52 mg/kg feed.

Compared with whole crawfish meal, sieved meal gave a 170% increase in corrected protein, and decreases of 23% in

calcium, 24% in ash, and 27% in chitin. The protein quality of whole crawfish meal was comparable to that of soybean meal based on broiler weight gain, feed conversion, and protein efficiency ratio, using a non-protein nitrogen corrected value. The biological availability of the crawfish astaxanthin pigment in the meal was confirmed with practical broiler skin and shank coloration analysis.

The protein in the crawfish chitin-protein complex was demonstrated to be associated with chitin by covalent bonding. The ratio of chitin to covalently bound protein was 33.6 to 1, contrasting significantly with data from other crustacean meal studies. Higher astaxanthin stability in the chitin-carotenoid complex was observed compared with earlier studies on pigment stability in crawfish meal, with and without antioxidant treatment. Data suggest this may be attributed to the firmly bound carotenoid/chitin compared with the bonding strength of pigment to calcium and protein in the composite complex. The research confirms the significant physiological availability of astaxanthin from the crawfish chitin-carotenoid complex.

CHAPTER I

INTRODUCTION

Over one hundred million pounds of crawfish are harvested from pond-farming and wild-catch in Louisiana, the world's largest crustacean farming industry. With expansion of this industry, estimated at an annual growth rate of 10%, the increasing volume of composite byproduct ("waste"), comprising 85% of the whole animal, has traditionally created serious disposal problems. As of January, 1985 disposal via landfill dumps was prohibited. A long series of investigations conducted in the LSU Food Science Department have provided a new outlook and direction to the waste disposal problem (Meyers, et al., 1990).

Earlier studies (Chen, 1981; Chen and Meyers, 1982, 1983; Meyers and Bligh, 1981), which focused on characterization of the carotenoid pigment and its subsequent recovery have demonstrated the value of this renewable resource and have facilitated the establishment of a commercial pigment recovery plant. Utilization of crawfish pigment in its various forms in laying hen diets (Lee, 1985) and the oil extract of carotenoid in aquatic diets (Meyers and Thibodeaux, 1984) have been evaluated for their intensifying effect on egg yolk pigmentation and

enhancement of external fish coloration. Crawfish astaxanthin pigment showed a noteworthy efficiency in its deposition in both egg yolk and fish flesh. When supplied to laying hen diets, concentrations of astaxanthin as low as 1 mg/kg imparted consumer acceptable coloration to egg yolk within 9 to 10 days, and allowed replacement of 30% yellow corn, the major traditional pigment source in such a ration. In addition to its value as a natural pigment, recent research (No et al, 1989; No and Meyers, 1989a, 1989b) has explored the isolation of crawfish chitin, the unique functional aspects of this biopolymer, and the potential feasibility of use of derived crawfish chitosan as a coagulant for seafood processing waste water treatment and for organic byproduct recovery.

Since crawfish processing byproducts are rich in pigment, 153 mg/kg, as well as in chitin-free protein, 32.2%, and in chitin, 14.1%, early interest was shown in their potential as a source of protein supplements and pigment in animal feed (Lovell et al, 1968; Meyers and Rutledge, 1971). The nutritional value of other crustacean byproducts in a prepared meal form already has been demonstrated in poultry feeds (Jarquin et al., 1972; Kifer and Bauersfeld, 1969; Mangold and Damkohler, 1938; Parkhurst et al., 1944). Compared with fish meal or a commercial corn-soybean diet, at equal levels of supplementation after calcium and phosphate levels were

adjusted, performance of chickens fed crab meal showed no significant differences in growth rate, feed efficiency, mortality, and feathering. Crustacean meal also has received attention for its natural red pigment value in broiler pigmentation (Chawan and Gerry, 1974) as well as for enhancement of fish integument and flesh color (Saito and Reiger, 1971; Simpson, 1978). Due to the intensifying effect of red pigment upon yellow pigments (De Groote, 1964), a small amount of red astaxanthin can replace the relatively high amount of yellow pigment in broiler diets needed to produce desirable skin and shank color (Nelson and Baptist, 1968; Braeunlich, 1974). Differences in biological availability of astaxanthin depends on its existing form for pigmentation of egg yolk and rainbow trout (Lee, 1985; Waddy, 1990). In egg yolk pigmentation, astaxanthin in crawfish shell showed greater efficacy than did the whole meal or pigment-enriched oil at equivalent levels of pigment (Lee, 1985). Elsewhere, it has been observed that astaxanthin associated with protein imparted superior coloration to rainbow trout than did the free form (Waddy, 1990). This is probably due to the efficient physiological transfer of pigment protected by the associated protein from digestive enzyme processes.

In addition to the pigment and protein value of crustacean byproduct, application of a small amount of chitin from crustacean exoskeleton added to poultry diets

promoted the digestion of lactose-rich cheese whey in chickens lacking lactase activity (Austin et el., 1981; Zikakis et al., 1982). The chitin monomer, N-acetyl glucosamine, stimulated growth of the lactase-producing bacterium, Bifidobacterium bifidus var. pennsylvanicus present in the gut of the chickens (Kahagias et al., 1977). However, evidence for existence of Bifidobacteria in the microflora of normal chickens is not evident (Spreen et al., 1984). Another growth promoting effect, i.e., an increase in feed efficiency by 7% and carcass weight by 12%, and a decrease in feed consumption by 2%, also was reported when 0.5% chitin was added to commercial chicken diets (Ramachandran Nair et al., 1986). Chitin also has been shown to have considerable potential as a dietary dye carrier in feeding trials with gerbil (Watkins and Knorr, 1983). When 0.1% dye was added to the diet, with and without chitin, the presence of chitin resulted in significantly lower concentrations of dye extracted indicating the protective effect of chitin in the digestive system. This may be attributed to either the binding properties of chitin and/or the effect of chitin on reductive cleavage of the azo bond of the dye.

Based on the aforementioned, the major objectives of the present investigation were as follows:

1. Evaluation of an improved (Ca-reduced) crawfish meal as a nutritional substrate specifically for

its protein source and astaxanthin pigment value in broiler feeding trials.

2. Examination of crawfish-derived chitin/carotenoid as a potential growth promotant and as an effective pigment source for use in broiler diets.
3. Examination of the covalent bonding between chitin and protein in the crawfish matrix and the stability of astaxanthin pigment in the crawfish chitin-carotenoid complex during its drying and storage.

Results are presented in the format of three separate, but related, chapters to facilitate subsequent publication of each of the experiments. For purposes of taxonomical correctness, the term "crayfish" specifically is used with the taxon Procambarus clarkii, while the commonly used lay term "crawfish" refer to both the composite byproduct recovered as well as commercial food-grade products from this industry. In view of current developments, the term "composite byproduct" is used in place of "waste", these reflecting its present and future economic importance. Individual byproducts recovered are referred to as "separated byproducts". Reports of studies prior to 1950 with shrimp and crab meal used in poultry diets are cited. Unfortunately, there is almost a complete lack of information on uses of crustacean meals in poultry diets over the past four decades, giving further justification to

the present study. A complete literature cited section is given at the end of the dissertation along with an appendix detailing the statistical analyses.

CHAPTER II

Evaluation of the crawfish chitin-carotenoid complex and its nutritional/pigmentation effect in poultry diets

ABSTRACT

A chitin-carotenoid complex, prepared from crawfish shell, was studied to ascertain its value both as a potential growth promoter and as a pigmenter in broiler feeding trials. The chitin-carotenoid substrate was fed at levels up to 5% of the total ration with graded levels of crawfish-derived astaxanthin, ranging from 0 to 5.2 mg/kg feed, over a seven week period. No significant differences in terms of body weight and feed efficiency were found between the broiler groups on the chitin-free control diet and the two experimental diets supplemented with 1% and 2.5% chitin-carotenoid. Poorest body weight and feed efficiency were with broilers fed a 5% cellulose diet, followed by those supplied with 0.5% and 5% chitin, respectively. No apparent relationship was observed between specific chitinase activity in the gizzard and gut and in overall broiler growth rates. Astaxanthin in the crawfish chitin-carotenoid complex was demonstrated to be an effective red intensifier in eliciting a significant increase in broiler skin and shank pigmentation. These results confirm earlier studies with egg yolk pigmentation.

INTRODUCTION

In view of the increasing utilization of crustacean composite byproduct, astaxanthin-rich proteinaceous crawfish processing byproduct is rapidly being recognized as a valuable source of natural astaxanthin pigment and chitin (Meyers et al., 1981; Meyers and Thibodeaux, 1984; No, 1987). Recent research (No et al., 1989; No and Meyers, 1989a, 1989b) has demonstrated the unique functional aspects of crawfish chitin and chitosan, and their potential industrial value as coagulants in organic compound recovery from seafood processing discharge streams.

There has been increasing interest in the application of chitin in poultry feed (Austin et al., 1981, 1982; Ramachandran Nair et al., 1986; Zikakis et al., 1982). Austin et al. (1981) and Zikakis et al. (1982) found that the addition of a small amount of chitin was effective in promoting the digestion of high lactose cheese whey in chickens that lack lactase activity. This confirms results reported by Kahagias et al. (1977) that growth of the lactase producing bacterium, Bifidobacterium bifidus var. pennsylvanicus, can be stimulated by the N-acetyl glucosamine, a chitin monomer. However, there is no conclusive evidence that Bifidobacteria exist in the usual microflora of chickens; thus, diet manipulation is

necessary to increase the bacterial population (Spreen et al., 1984). This was supported by poultry studies that failed to show biochemical evidence of B. bifidus var. penn. activity in chicken fed diets with 15% whey and 2% chitin without bacterium inoculum. One interesting observation from these studies was two contrasting results in growth performance of chicken fed the diet supplemented with 2% chitin. Compared with birds on the control diet, the work of Austin et al. (1981) showed significant differences in weight gain, whereas that of Zikakis et al. (1982) failed to demonstrate differences in weight gain or increased feed efficiency. Another growth-promoting effect of chitin also was reported in that addition of 0.5% chitin increased feed conversion rate by 7% and carcass weight by 12%, but produced a decrease in feed consumption of 2% compared with chickens on a chitin-free diet. However, no explanation was given for the growth-stimulating effect of chitin. Ramachandran Nair et al. (1986) reported that use of chitinous substrates resulted in an average 70% profit increase to poultry farmers in India.

Due to the fact that chitin is a polymer of N-acetylglucosamine (NAG) linked by β-1,4 glucoside bonds comparable to cellulose, it has been long considered indigestible in chickens and other nonruminants (Scott et al., 1982). Chitin was postulated to lower the nutritive value of the composite shrimp and crawfish meal (Lovell et

al., 1968; Oke et al., 1978). Therefore, to evaluate true protein digestibility of crustacean meal, use of corrected protein value was recommended by subtracting chitin nitrogen from crude nitrogen value (Watkins, 1976).

High chitinolytic activity in gizzard and guts of chicken (Gallus gallus L.) was reported by Smirnoff (1978). In chickens, the glandular stomach seems to be the only site of chitinase secretion. Jeuniaux and Cornelius (1978) suggested that chitin digestion in vivo depends mainly on the following: chitinase concentration in the gut; the optimum pH of this enzyme complex; the physical and chemical state of the chitin provided with the diet; intestinal transit; and perhaps the presence or absence of the enzyme chitobiase. Actual chitin digestion in young and old chickens (Gallus gallus L.) has been reported (Jeuniaux and Cornelius, 1978), with a digestibility coefficient of 23.5-31%, using chitin obtained from shrimp meal. However, chitinase activity of bacterial origin, involved with digestion in chickens, has not been indicated.

Chitin from crawfish shell contains significant amounts of astaxanthin pigment and must be bleached with various reagents to obtain the final white product (No et al., 1989). The bioavailability of the valuable pigment has been already demonstrated in fish and poultry (Lee, 1985; Meyers and Thibodeauex, 1984; Simpson, 1978).

Previous research (Lee, 1985) has shown the superior pigmentation value of crawfish shell in coloration of egg yolks compared with pigment rich soy oil and whole meal at an equivalent level of astaxanthin. This may be explained in part by the physicochemical nature of the basic structure of the crustacean shell associated with pigment.

In crustacean shell, there are two distinct structural units (Lee, 1988): an acidic polypeptide fraction with a strong affinity for calcium ions called a mineralization matrix associated with pigment, so called calcareous carotenoid (Fox et al., 1969); a high molecular weight chitinoprotein complex associated with carotenoid, so called chitinocarotenoid (Fox, 1973) without affinity for calcium. It can be postulated that the latter can be utilized only via possible chitinolytic enzyme action resulting in pigment release from the chitin structure. Simpson (1978) noted the difficulty of pigment extraction from crustacean meal, thus, making accurate analysis of pigment levels difficult.

Based on the aforementioned, the composite crawfish chitin-carotenoid complex was examined as a growth promoter and an effective pigment source for possible use in broiler diets.

MATERIALS AND METHODS

Chitin-Carotenoid Complex Preparation

The chitin isolation process for crayfish (Procambarus clarkii) developed by No (1989) was adapted for the large scale production of chitin-protein complex needed for the present study. The ground crawfish exoskeleton, obtained from Acadiana Processors, Inc. (Henderson, LA), was washed with cold running water for 3 hrs. to remove soluble organics and adherent protein. Excessive moisture was removed by centrifugation (Centrifugals 40-950, Louis Allis Co.). To eliminate the possible deleterious effect of high temperature on astaxanthin during the drying period, wet shell was used throughout the entire process following determination of its moisture content.

The shell was deproteinized with 3.5% NaOH with a solid to solvent ratio of 1:10 (w/v) at 65°C for 2 hrs. with continuous stirring using a commercial blender. The deproteinized shell was washed to neutrality in running tap water and centrifuged as above.

Deproteinized shell was demineralized with 1 N HCl using a solid to solvent ratio of 1:15 at ambient temperature for 30 min. under conditions described previously. After washing and removing water, the complex of chitin-carotenoid was dried at 20°C in an air-forced dryer until the moisture content reached less than 2 %.

To obtain homogeneous uniform sized product, dried chitin-carotenoid complex was ground through a Wiley mill (Standard Model No. 3) with 2 mm mesh screen and mixed. The chemical properties of the chitin-astaxanthin complex were determined to evaluate its quality. The dried product was placed into doubled black polyethylene bags and stored at ambient temperature.

Broiler Feeding Regimen

Three hundred and sixty, one day-old Arbor Acres broilers were obtained from the Louisiana State University Poultry Research Farm, Baton Rouge, LA. and floor-reared to 7 weeks of age at the same farm. Thirty birds per pen (2.26 X 1.50 m) were randomly placed in an open-sided broiler house with 12 pens, 6 pens on each side and temperature controlled ventilation fans. Each pen was equipped with automatic waterer, tubular feeder, and heating lamp. During the first 10 days of brooding, a feeder tray was used, subsequently replaced with tubular feeder. Feed and water were supplied ad *libitum*. Body weight and feed consumption data were recorded weekly.

A total of 5 experimental diets and one control diet, based on different ratios of chitin-astaxanthin complex, were prepared. The six different diets were randomly assigned within 6 pens at each side of the broiler house. The ration formula of starter for the first 4 weeks, and

finisher for the last 3 weeks of control and experiment diets are given in Table 1. These were based on the nutrient requirements of broilers (NRC, 1984). The experimental diets were obtained by adding the chitin-astaxanthin complex at levels of 0, 0.5, 1.0, 2.5, and 5.0 % with cellulose supplementation. The calculated amount of astaxanthin in the chitin-astaxanthin complex was 0.051 mg/g sample.

Feeding experiments were conducted using a randomized complete block design (RCBD) with split-plot arrangement. Each six pens comprised a block. The six diet treatments were randomly assigned within the block. At seven weeks of age, six birds (3 males and 3 females) from each pen were randomly selected and properly identified by means of wing bands. Birds were withdrawn from water and diets 8 hours before slaughtering. A total of seventy-two birds from 12 pens were slaughtered and dressed according to standard commercial practices. The carcasses were kept in ice water for 30 min. and drained of excess water. Carcass, intestines, and legs, below the middle of the hock joint, from each bird were placed separately into polyethylene bag with identification card and stored at -20^{0}C until used.

Chemical Analysis

Official methods of analysis (AOAC, 1980) were used

for chemical analysis. Nitrogen was determined by a semiautomated method; ash, fat, and phosphorus by standard methods. Calcium was analyzed with a Perkin-Elmer atomic absorption spectrometer. All analytical values reported are the average of five replicates. Data are expressed on a dry weight basis.

Acetyl Value

To determine the acetyl value, the distillation method of Lemieux and Purves (1947) was used. The degree of deacetylation was estimated using the difference between the theoretical chitin acetyl value of 21.2 % and the experimental value obtained.

Amino Acid Analysis

Twenty mg of sample were hydrolyzed with 1 ml 6 N HCl at 110^{0}C for 24 hrs under nitrogen in a sealed vial. Following this, the hydrolysate was cooled and 9 ml H_2O (HPLC grade) was added. A total volume of 10 ml diluted hydrolysate was centrifuged at 3000 rpm for 10 min. and 50 µl of supernatant was taken for derivatization. Through the three basic steps of derivatization, i.e., drying, redrying, and coupling, amino acid samples were derivatized with phenylisothiocyanate (PITC). The phenylthiocarbamyl amino acid (PTC-AA) mixture 25 µl was used for analysis on a Pico-Tag Amino Acid Analyzer (Waters, Milford, MA) under

the following conditions of 1 ml/min flow rate: Pico-Tag reverse phase column, temperature control module set at 40°C, and UV-detector at 254 nm.

Astaxanthin Determination

The solvent extraction procedure of Lee (1985), modified from that of Chen (1981) was used for extraction of astaxanthin from the chitin-astaxanthin pigment complexes. Total astaxanthin was calculated by the formula of Kelly and Harmon (1972).

Formula 1.

$$\text{gm astaxanthin/gm crawfish meal} = AD/100\ G\ d\ E^{1\%}_{1cm}$$

where A = absorbance at maximum wavelength (467 nm in petroleum ether), D = dilution volume (ml), G = crawfish meal (gm), d = cell width (1 cm), and $E^{1\%}_{1cm}$ = 2400 in petroleum ether.

Chitinase Extraction

The frozen chicken intestines, including small intestine, ceca, large intestine, and cloaca, were thawed at 4°C prior to use, and separated from adhered fat. After removal of the intestinal contents, intestines were chopped and mixed. Five grams of sample were homogenized in 25 ml of 1% NaCl containing 1 mM of disodium ethylenediamine tetraacetate (2Na-EDTA) solution using a tissue grinder

(Tekmar Tissumizer, Cincinnati, OH). The homogenate was centrifuged at 12,000xg (Sorvall RC-5C automatic superspeed refrigerated centrifuge) for 20 min. The supernatant was filtrated through Whatman No.1 filter paper to remove residual lipid. The filtrate was dialyzed against distilled water for 12 hrs, then the dialyzate was centrifuged at 12,000xg for 20 min. The supernatant was used as a test solution for the crude enzyme assay.

Determination of Chitinase Activity

The method of Lindsay (1984) was used to determine chitinase activity in chicken intestines. A 0.5 ml test solution, prepared as above, was mixed with 0.5 ml of citrate-HCl buffer, pH 4.5, and 0.5 ml of chitin azure (6 mg/ml), a Remazol Brilliant Violet 5R dyed chitin (Sigma Chemical Co.). The mixture in a 5 ml centrifuge reaction tube was incubated in a shaking water bath (120 strokes/min., Blue M, Model No. B2729Q) at 37°C for 6 hrs and then centrifuged at 3000xg for 30 min. The supernatant, free of unsolubilized chitin, was measured at 510 nm and compared with a standard curve (Fig. 1) to determine the amount of chitin solubilized and to calculate chitinolytic activity. The standard curve shown in Fig. 1 was prepared by dissolving chitin azure in 21 N sulfuric acid and immediately determining the optical density at 510 nm in the range of 0-0.95 mg of chitin azure per ml.

Pigmentation Analysis

Prior to sample preparation, carcass and shank were thawed at 4^0C. After cutting one side of the shank, the epithelial and cutaneous layers were separated from the bone. The skin of four different parts, i.e., breast, wings, back, and thigh, was removed and the excess subcutaneous skin fat was eliminated. Each skin from the different parts was placed on a white 8 x 8 cm poster board to facilitate pigmentation analysis. The Roche Yolk Color Fan, with 15 color grades (Vuilleumier, 1969), and the Hunterlab Tristimulus Colorimeter (Model D 25 M-9) were used for visual comparison of pigmentation differences.

Statistical Analysis

The data were analyzed using analysis of variance in the General Linear Model (GLM) procedure of SAS (1982). Least Squares (LS) means were used to compare effects of treatments using an option of PDIFF of SAS.

Table 1. Composition of control and experimental broiler diets

	Starter		
Ingredients (%)	1	2	3
Ground yellow corn	57.50	44.37	44.37
Soybean meal-44[1]	26.44	28.98	28.98
Menhaden fish meal-61[1]	10.00	10.00	10.00
Crawfish chitin	0.00	0.00	0.50
Cellulose[2]	0.00	5.00	4.50
Tallow	4.53	10.12	10.12
DL methionine	0.10	0.12	0.12
Oyster shell flour	0.93	0.91	0.91
Mineral mix[3]	0.25	0.25	0.25
Vitamin mix[4]	0.25	0.25	0.25
Total	100.00	100.00	100.00
Calculated analysis			
Crude protein (%)	23.00	23.00	23.00
Fat (%)	7.91	13.01	13.01
Fiber (%)	2.84	7.73	7.73
Calcium (%)	1.00	1.00	1.00
Astaxanthin (mg/Kg)	0.00	0.00	0.52
Metabolic energy (Kcal/lb)	1451.52	1451.52	1451.52

[1] Protein content of ingredient (%)

[2] United States Biochemical Co.

[3] Amount per kg of diet: sodium, 0.13 mg; zinc, 90.8 mg; copper, 9.08 mg; manganese,108.96 mg; iron, 45.4 mg; cobalt, 0.18 mg; selenium, 0.18 mg.

[4] Amount per kg of diet: vitamin A, 7264 IU; vitamin D_3; 1816 IU; vitamin E, 9 IU; vitamin B_{12}, 0.0091 mg; riboflavin, 4.54 mg; vitamin K, 1.82 mg; niacin, 18.16 mg; pantothenic acid, 8.17 mg; choline, 317 mg; folacin, 0.45 mg; biotin, 0.091 mg; pyridoxine, 1.82 mg.

Table 1. (Continued) Composition of control and experimental broiler diets

	Starter		
Ingredients (%)	4	5	6
Ground yellow corn	44.37	44.37	44.37
Soybean meal-44[1]	28.98	28.98	28.98
Menhaden fish meal-61[1]	10.00	10.00	10.00
Crawfish chitin	1.00	2.50	5.00
Cellulose[2]	4.00	2.50	0.00
Tallow	10.12	10.12	10.12
DL methionine	0.12	0.12	0.12
Oyster shell flour	0.91	0.91	0.91
Mineral mix[3]	0.25	0.25	0.25
Vitamin mix[4]	0.25	0.25	0.25
Total	100.00	100.00	100.00
Calculated analysis			
Crude protein (%)	23.00	23.00	23.00
Fat (%)	13.01	13.01	13.01
Fiber (%)	7.73	7.73	7.73
Calcium (%)	1.00	1.00	1.00
Astaxanthin (mg/Kg)	1.04	2.60	5.20
Metabolic energy (Kcal/lb)	1451.52	1451.52	1451.52

[1] Protein content of ingredient (%)

[2] United States Biochemical Co.

[3] Amount per kg of diet: sodium, 0.13 mg; zinc, 90.8 mg; copper, 9.08 mg; manganese,108.96 mg; iron, 45.4 mg; cobalt, 0.18 mg; selenium, 0.18 mg.

[4] Amount per kg of diet: vitamin A, 7264 IU; vitamin D_3; 1816 IU; vitamin E, 9 IU; vitamin B_{12}, 0.0091 mg; riboflavin, 4.54 mg; vitamin K, 1.82 mg; niacin, 18.16 mg; pantothenic acid, 8.17 mg; choline, 317 mg; folacin, 0.45 mg; biotin, 0.091 mg; pyridoxine, 1.82 mg.

Table 1. (Continued) Composition of control and experimental broiler diets

	Finisher		
Ingredients (%)	1	2	3
Ground yellow corn	57.06	43.94	43.94
Soybean meal-44[1]	33.41	35.95	35.95
Menhaden fish meal-61[1]	-	-	-
Crawfish chitin	-	0.00	0.50
Cellulose[2]	-	5.00	4.50
Tallow	6.89	12.48	12.48
DL methionine	0.08	0.09	0.09
Oyster shell flour	2.06	2.04	2.04
Mineral mix[3]	0.25	0.25	0.25
Vitamin mix[4]	0.25	0.25	0.25
Total	100.00	100.00	100.00
Calculated analysis			
Crude protein (%)	20.00	20.00	20.00
Fat (%)	9.42	14.52	14.52
Fiber (%)	3.15	8.04	8.04
Calcium (%)	0.90	0.90	0.90
Astaxanthin (mg/Kg)	0.00	0.00	0.52
Metabolic energy (Kcal/lb)	1451.52	1451.52	1451.52

[1] Protein content of ingredient (%)

[2] United States Biochemical Co.

[3] Amount per kg of diet: sodium, 0.13 mg; zinc, 90.8 mg; copper, 9.08 mg; manganese,108.96 mg; iron, 45.4 mg; cobalt, 0.18 mg; selenium, 0.18 mg.

[4] Amount per kg of diet: vitamin A, 7264 IU; vitamin D_3; 1816 IU; vitamin E, 9 IU; vitamin B_{12}, 0.0091 mg; riboflavin, 4.54 mg; vitamin K, 1.82 mg; niacin, 18.16 mg; pantothenic acid, 8.17 mg; choline, 317 mg; folacin, 0.45 mg; biotin, 0.091 mg; pyridoxine, 1.82 mg.

Table 1. (Continued) Composition of control and experimental broiler diets

	Finisher		
Ingredients (%)	4	5	6
Ground yellow corn	43.94	43.94	43.94
Soybean meal-44[1]	35.95	35.95	35.95
Menhaden fish meal-61[1]	-	-	-
Crawfish chitin	1.00	2.50	5.00
Cellulose[2]	4.00	2.50	0.00
Tallow	12.48	12.48	12.48
DL methionine	0.09	0.09	0.09
Oyster shell flour	2.04	2.04	2.04
Mineral mix[3]	0.25	0.25	0.25
Vitamin mix[4]	0.25	0.25	0.25
Total	100.00	100.00	100.00
Calculated analysis			
Crude protein (%)	20.00	20.00	20.00
Fat (%)	14.52	14.52	14.52
Fiber (%)	8.04	8.04	8.04
Calcium (%)	0.90	0.90	0.90
Astaxanthin (mg/Kg)	1.04	2.60	5.20
Metabolic energy (Kcal/lb)	1451.52	1451.52	1451.52

[1] Protein content of ingredient (%)

[2] United States Biochemical Co.

[3] Amount per kg of diet: sodium, 0.13 mg; zinc, 90.8 mg; copper, 9.08 mg; manganese,108.96 mg; iron, 45.4 mg; cobalt, 0.18 mg; selenium, 0.18 mg.

[4] Amount per kg of diet: vitamin A, 7264 IU; vitamin D_3; 1816 IU; vitamin E, 9 IU; vitamin B_{12}, 0.0091 mg; riboflavin, 4.54 mg; vitamin K, 1.82 mg; niacin, 18.16 mg; pantothenic acid, 8.17 mg; choline, 317 mg; folacin, 0.45 mg; biotin, 0.091 mg; pyridoxine, 1.82 mg.

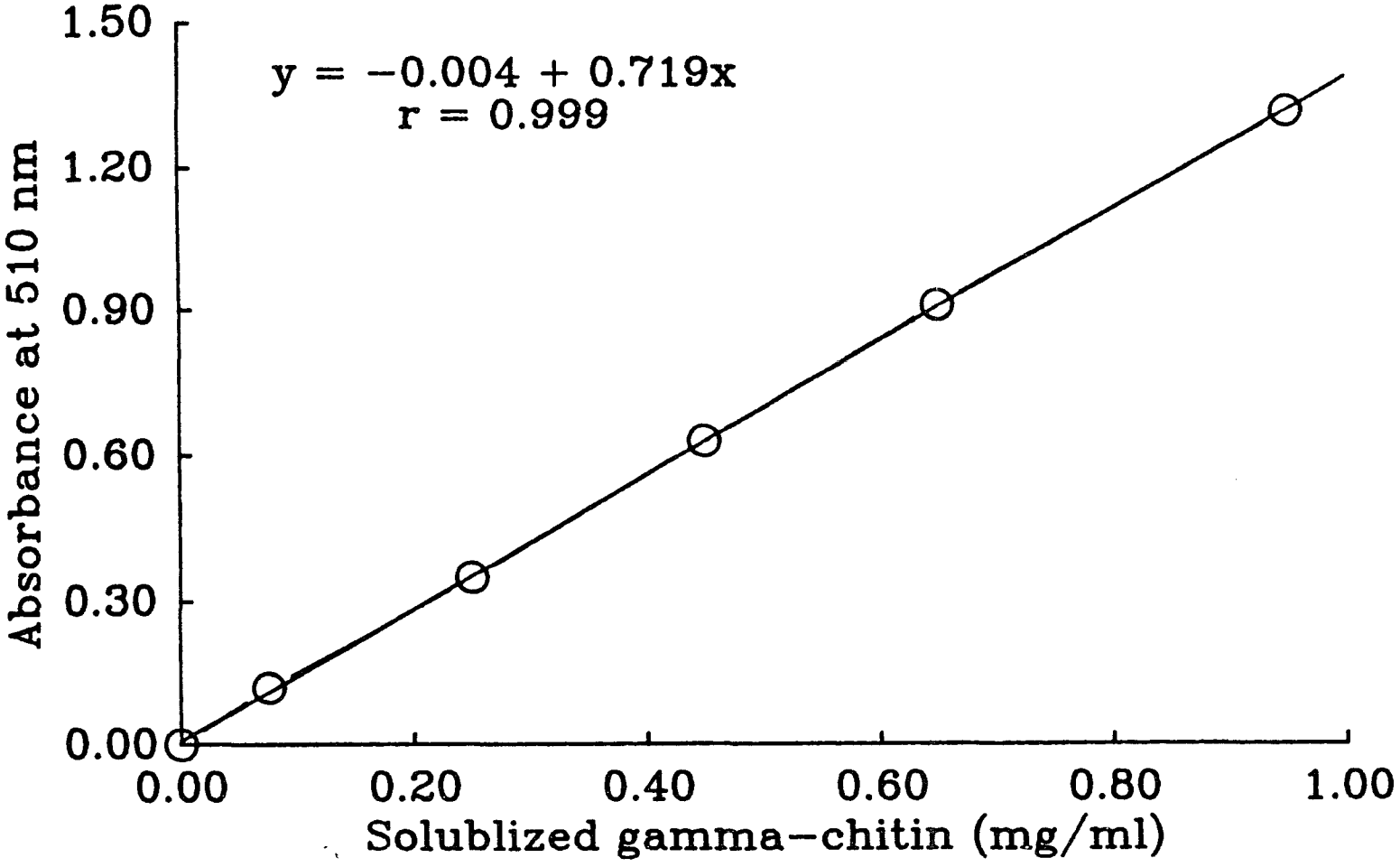

Figure 1. Standard curve of chitinase activity

Chitin Characterization

The results of crawfish chitin characterization are shown in Table 2. The nitrogen value of crawfish chitin was 7.04 (S.E. of 0.04) in the present investigation. No (1987) reported a similar nitrogen value (7.01). A theoretical nitrogen value of 6.9 has been reported for pure chitin. A nitrogen value higher than the theoretical value of 6.9 can be attributed to either deacetylation or the presence of protein not removed during isolation of the chitin (Rutherford and Austin, 1978). This is presumably due to the presence of protein residues in crawfish chitin associated with covalent bonds (Attwood and Zola, 1967; Brine and Austin, 1981; Hackman, 1972; Herzog et al., 1975; Karlson et al., 1969; No, 1987). Therefore, preparation of pure chitin without some residual protein present is almost impossible. Furthermore, exhaustive chemical treatment of the complex to remove trace amounts of protein will have a deleterious effect, allowing breakage of the chitin polymeric linkage.

The value of deacetylation indicated 9.2% of chitin, based on the theoretical acetyl value for chitin of 21.19%, exists in the form of chitosan, i.e., deacetylated chitin. Muzzarelli (1973) reported that the ratio of one deacetylated monomer for every five or six acetylated

monomer is common with chitin. Hackman and Goldber (1965) postulated that this is a natural occurrence and is correlated with the site of protein bonding. Einbrodt and Stober (1960) reported an acetyl value of 21.9% of chitin isolated from crayfish without acids or alkali treatment.

Based on the initial dried crawfish shell, No (1987) reported a value of 23.48% chitin for the chemical composition of shell. The value of 25.8% obtained in the present investigation reflects the presence of protein residues. This is slightly higher than the 25.35% obtained by No (1987), and is probably due to the large volume of chitin produced and variations between different batches of shell used for chitin extraction.

Crawfish chitin contains a relatively high concentration, 104 mg/kg, of astaxanthin compared with the amount of pigment presented in crustacean meals of shrimp origin (Bligh, 1978; Meyers and Bligh, 1981). No (1987) reported astaxanthin concentration of 78 and 108 mg/kg for whole crawfish meal and shell, respectively. Table 2 demonstrates crawfish chitin is a excellent source of astaxanthin. Chan and Meyers (1982), and Lee (1985) have all shown that crawfish waste is a noteworthy source of naturally-occurring astaxanthin, with realistic commercial possibilities for its incorporation into aquatic and poultry dietary formulations.

Table 3 presents the residual amino acid composition

of crawfish chitin compared with that of shell reported by No (1987). The value of 26.02 mg/g shows that significant amounts of amino acids are covalently bound to chitin. Serine was the predominant amino acid in chitin, whereas No (1987) reported that tyrosine was the major amino acid present. Reasons for these differences are not evident, but possibly may be attributed to the dissimilar methods of analysis, i.e., amino acid analyzer vs HPLC method. Significant species-related variations in residual amino acids in chitin have been reported earlier (Hackman, 1960; Karlson et al., 1969).

Effect of crawfish chitin in broiler performance

The effect of the various levels of crawfish chitin in the diet on weekly growth rate is shown in Table 4. The growth trends of all groups of chickens fed various diets were comparable. There were significant differences in body weight at 4, 6, and 7 weeks of age (Appendix Table 1). Block effects also were found at 4 and 6 weeks of age. However, based on overall performance of broiler chickens at 7 weeks of age, a highly significant effect ($p<0.01$) was shown only for the diet treatments. Maximal final body weight was obtained in the group of chickens fed the control diet and 2.5% and 1% chitin diet. Minimal growth was in the group on 5% cellulose-fortified feed, probably attributed to the non-nitrogenous value of cellulose.

However, Insko and Culton (1949) reported that broiler diets containing 5% fiber from either oats, alfalfa meal, or wheat bran gave maximum broiler growth. A diet with 6 to 7% fiber from oat hulls increased growth rate compared to that containing 2 to 3% fiber (Wilcke and Hammomnd, 1940). LS mean comparison (Table 5) indicated no significant difference ($p>0.05$) in final body weight among the groups on control, 1% chitin and 2.5% chitin diet. Comparable results with double-sheared chitin and ground chitin were reported by Zikakis et al. (1982). However, there was no evidence that single addition of chitin depresses weight gain (Austin et al., 1981; Lee and Mo, 1985).

One broiler in the group fed 5% chitin, one fed 5% cellulose, and two in the group on a 0.5% chitin diet developed crooked toes outward which can be caused by riboflavin deficiency. This was also found in the chickens fed 20% whey in diet (Austin et al., 1981; Lee and Mo, 1985). These workers did not observed any apparent riboflavin deficiency in the diets since all experimental diets met the nutritional requirements of poultry (NRC, 1984). Austin et al. (1981) also mentioned that curved toe condition is common in commercially raised broilers. During seven weeks of experiments, the control group (treatment 1) had two mortalities and the groups fed 2.5% chitin diet and 5% cellulose diet had one mortality each.

No differences were observed in feathering compared with the control group.

Values for body weight were reflected in the feed efficiency ratio (Table 5). No significant effect ($p>0.05$) of chitin was seen on feed efficiency at 4 weeks of age, whereas, a significant effect ($p<0.05$) was found at 7 weeks of age. The trend of descending order in feed efficiency for each group is correlated with the descending order in body weight gain. The lowest ratio was obtained with the 5% cellulose diet. The chickens supplied 0.5% chitin gave the second lowest results in both body weight and feed efficiency. The 7% percent increase in feed conversion and 5% increase in weight gain by 0.5% chitin addition found by Ramachandran Nair et al. (1986) was not evident in the present experiment.

Effect of chitinase on broiler performance

Chitinase activities in the chicken gizzard and gut at the conclusion of the experiment are shown in Figure 2. Data do not show a distinctive pattern expected in an animal that might digest chitin, namely, high chitinase activities in the stomach and low activities in the intestine (Lindsay et al., 1984). So far, chickens have been known to secrete chitinase only by the gastric mucosa (Jeuniaux and Cornelius, 1978). However, as shown in Figure 2, comparable activities also were found in the gut.

There are several reports showing chitinase activity of bacterial origin involved in chitin digestion in fish intestine (Kono et al., 1987a, 1987b). Similar to that reported by Jeuniaux and Cornelius (1978) for mouse, rat, hamster, and guinea pig, broilers do not appear to modify their secretion of chitinase when the diet is changed for a few weeks. Chitinase activity was not apparently enhanced in either the gizzard or gut. However, enzyme activity in broilers fed 2.5% chitin diet, which gave the best results in feed efficiency and weight gain compared with those on other experimental diets, was higher than average in the gizzard and gut in both male and female birds. Five percent cellulose diet, which gave the poorest results in feed efficiency and weight gain, also showed the lowest average chitinase activity, even less than that of the control diet.

However, since chitinase activity was measured from the whole gizzard and gut, instead of from the mucosa of the glandular stomach, activity was significantly lower, less than 130 µg chitin/g hr, compared to 1350-1780 µg chitin/g hr in mucosa of chicken (Gallus gallus) (Jeuniaux and Cornelius, 1978). However, since chitinase production is a reflection of the particular chitin source, i.e., native vs acid-washed, it is entirely possible that increased chitinolytic activity could be obtained if a different substrate type had been used. Comparable

observation have been noted by Hood and Meyers (1977) in degradation of chitin in aquatic system.

Based on the aforementioned, it is difficult to make definitive conclusions whether chitinase in broilers is involved in digestion of chitin although Jeuniaux and Cornelius (1978) reported 23.5-31.7% digestibility of chitin in chicken. However, it should be noted that chitin in the diet did not cause a negative effect on feed efficiency and weight gain, whereas, 5% cellulose alone in the diet depressed broiler growth.

Effect of crawfish chitin on broiler skin and shank pigmentation

The results of broiler pigmentation by the chitin/carotenoid crawfish complex are presented in Tables 7 and 8. A total of 40% of astaxanthin pigment in crawfish shell was lost during the isolation process used to obtain the chitin/carotenoid complex. The total amount of pigment in the complex was 104 mg/kg. This is relatively higher than the value reported by No (1987) for whole crawfish meal, 78 mg/kg, and comparable to shell, 108 mg/kg. All data used in statistical analyses, particularly for skin pigmentation, are based on the average of four different parts, i.e., breast, wings, back, and thigh. This is due to the uniformity of broiler skin color and other visual components such as gloss and translucency (Fletcher, 1981).

In view of the nature of the experiment, there was no variation in the level of yellow corn used which is the only source of yellow carotenoid in all of the diets. Lutein content in yellow corn was 11.5 mg/kg. Control and experimental diets for finisher had 57.06 and 43.94% of yellow corn, providing a total of 6.56 and 5.05 mg lutein per kg of feed, respectively. The level of crawfish astaxanthin in each diet is listed in Table 6.

As shown in Tables 7 and 8, redness (a) value increased as the amount of chitin/carotenoid complex increased in diets 3-6, while lightness (L) in both skin and shank, are decreased and yellowness (b) in skin is steady. Unusual remark was the increase of yellowness in shank as redness increased due to the increased concentration of pigment complex. The pattern of yellowness change in skin agrees with previous reports of Fletcher and Halloran (1981) and Lee (1985) but the reason for the patter of yellowness change in shank is not clear. However, there were highly significant differences ($p<0.01$) in L, a, and b values except for yellowness in skin between diets, with and without chitin/carotenoid complex (Appendix Table 2). This is probably due to the astaxanthin pigment, the primary influencing factor, since the level of lutein is constant throughout the experimental diets. No significant differences ($p>0.05$) were observed in L, a, b values and visual scores between male and female birds,

except for lightness in the shank.

As seen in Table 8, a shank visual score of 1 occurred in both control diet with 57.06% yellow corn and 5% cellulose diet with 43.94% yellow corn, providing only 6.56 and 5.05 mg lutein per kg of feed, respectively. This value was doubled by the addition of astaxanthin at levels as low as 0.52 mg/kg feed. The five percent chitin diet containing 5.2 mg astaxanthin and 5.05 mg lutein/kg feed gave 4.3 in shank score, obtained by 60% yellow corn ration with 3.5% alfalfa meal, providing a total of 26.0 mg yellow oxycarotenoid/kg feed.

The earlier report of De Groote (1964) showed that supplemental amounts of red pigment had an intensifying effect on yellow pigments present in the feed ingredient. Braeunlich (1974) and Chawan and Gerry (1974) confirmed the intensifying effect of red oxycarotenoid on yellow pigment on broiler pigmentation. The visual values of astaxanthin in this study were much higher than those obtained by colorimetry. To explain high utilization of red pigment, Hinton et al. (1973) suggested that the addition of relatively small amounts of red pigment produces apparent utilization when evaluated visually, due to the difference in light absorption of yellow and red pigment. There were significant differences ($p<0.05$) in shank visual score among astaxanthin levels of 0.52, 1.04, 2.60, and 5.2 mg/kg. This did not agree with results from the previous

crawfish meal research on broiler pigmentation. These differences probably can be attributed to the visual response affected by the combination of yellow and red pigment rather than to a single red pigment.

In comparison with the earlier crawfish meal study on broiler pigmentation, less astaxanthin and lutein in the chitin/carotenoid diet gave an almost similar level of pigmentation in both skin and shank. This was reported previously by Lee (1985), i.e., astaxanthin in crawfish shell showed higher pigment utilization in egg yolk than similar levels of pigment from other crawfish sources, i.e., pigmented oil and whole meal. This is probably due to the chitinase activity in gizzard and gut in broilers which may effectively release carotenoid from the chitin complex. Since it has been known that carotenoids are firmly bonded to the chitin matrix (Fox, 1973), active chitinolytic enzymes are needed to break the bond between chitin and carotenoid.

Table 2. Characterization of crawfish chitin

Specification	Percentage (%)
Nitrogen[a]	7.04 %
Fat	0.04 %
Ash	0.16 %
Acetyl	19.24[b] %
Deacetylation	9.20 %
Yield	25.80 %
Residual amino acid	26.02 mg/g
Astaxanthin	104 mg/kg

[a] Calculated on a moisture-free and ash-free basis

[b] Theoretical value = 21.19%

Table 3. Total amino acid composition of crawfish shell and chitin

Composition	Content (mg/g)	
	Shell*	Chitin
Arginine	2.63	0.36
Histidine	0.67	3.62
Isoleucine	1.40	0.35
Leucine	2.50	0.35
Lysine	2.19	0.31
Methionine	0.28	0.09
Cystine	0.18	0.01
Phenylalanine	2.35	0.04
Tyrosine	28.43	4.05
Threonine	2.07	0.72
Valine	2.07	0.43
Aspartic acid	4.28	0.72
Serine	2.40	13.14
Proline	3.76	0.45
Glutamic acid	5.06	0.52
Alanine	2.62	0.37
Glycine	4.06	0.49
Total	67.05	26.02

* No, 1987

Table 4. Growth rates of broilers fed various levels of crawfish chitin in experimental diet

	Weight of broiler*(g)					
Time	Treatment					
(wk)	1	2	3	4	5	6
0	40.5	40.5	40.0	40.0	40.5	40.0
1	122.0	125.5	125.5	126.0	122.5	120.5
2	269.0	268.0	272.0	283.5	267.5	253.5
3	542.0	522.0	530.0	550.0	546.0	490.5
4	921.5	815.0	879.0	921.0	913.0	831.5
5	1165.0	1001.0	1151.0	1124.0	1131.0	1041.0
6	1429.0	1250.5	1291.0	1403.0	1401.5	1308.0
7	1668.0	1482.0	1550.5	1627.0	1643.5	1584.5

* LS mean weight

Table 5. The effect of crawfish chitin on performance of broilers at 4 and 7 weeks of age

	4 wk		7 wk	
Treatment	Body Weight (g)	Feed Efficiency (feed/gain)	Body Weight (g)	Feed Efficiency (feed/gain)
1	921.5[a]	2.16[a]	1668.0[a]	2.21[a]
2	815.0[b]	2.34[a]	1482.0[b]	3.01[b]
3	879.0[ab]	2.05[a]	1550.5[c]	2.63[c]
4	921.0[a]	1.92[a]	1627.0[ae]	2.30[a]
5	913.0[a]	2.04[a]	1643.5[a]	2.26[a]
6	831.0[b]	2.14[a]	1584.5[ce]	2.50[c]

[a,b,c,d,e] LS means with different superscripts in the same column are significantly different ($p<0.05$)

Table 6. The level of astaxanthin in experimental diet

Diet	Astaxanthin (mg/kg)
1. Control	0.00
2. 95% basal + 5.0% CL*	0.00
3. 95% basal + 4.5% CL + 0.5% CH**	0.52
4. 95% basal + 4.0% CL + 1.0% CH	1.04
5. 95% basal + 2.5% CL + 2.5% CH	2.60
6. 95% basal + 5.0% CH	5.20

Control and basal contains 57.06% and 43.94% yellow corn, respectively, for finisher rations.
*Cellulose (USB Co.), **Chitin

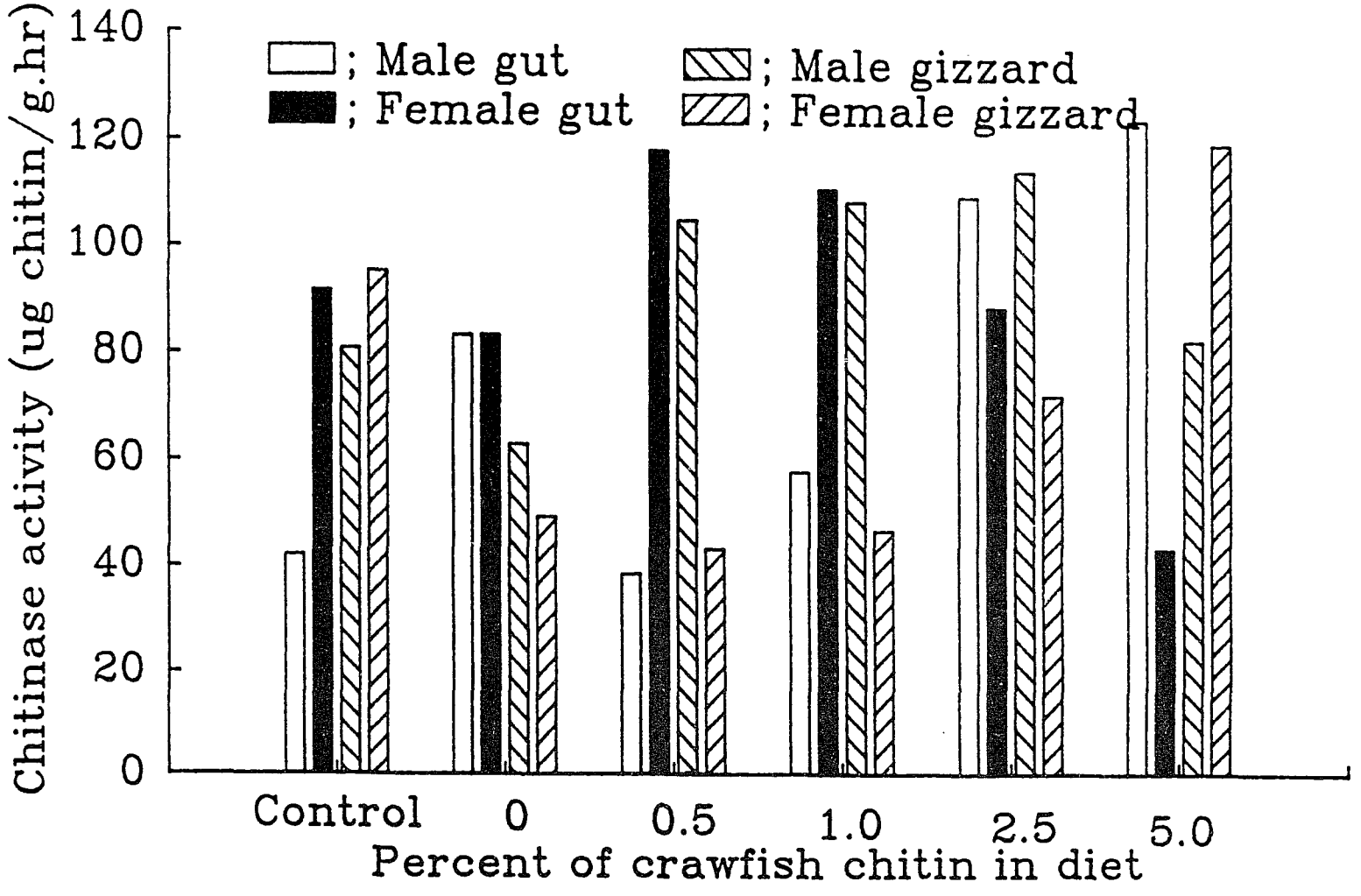

Figure 2. Chitinase activities in the gizzard and gut of broilers at 7 weeks of age

Table 7. Lightness (L), redness (a), and yellowness (b) value of skin of male (M) and female (F) broilers fed chitin diet at 7 weeks of age

Treat.	Sex	L	a	b
1	M	81.10[a]	1.78[a]	17.44[a]
2	M	79.98[a]	1.60[a]	17.06[a]
3	M	77.44[b]	3.80[b]	16.61[a]
4	M	74.09[c]	5.40[c]	16.81[a]
5	M	73.39[c]	6.03[c]	16.84[a]
6	M	73.26[c]	7.00[d]	16.90[a]
1	F	81.50[a]	1.44[a]	16.63[a]
2	F	78.92[a]	1.40[a]	16.43[a]
3	F	74.82[b]	4.69[b]	17.21[a]
4	F	74.10[b]	5.45[bc]	18.21[a]
5	F	73.26[b]	6.27[cd]	17.07[a]
6	F	72.93[b]	6.50[d]	17.50[a]

[a,b,c,d] LS means with different superscripts in the same column are significantly different ($p<0.05$)

Table 8. Lightness (L), redness (a), and yellowness (b) value and visual score (V) of shank of male (M) and female (F) broilers fed chitin diet at 7 weeks of age

Treat.	Sex	L	a	b	V*
1	M	80.40[a]	2.29[a]	21.42[a]	1.0[a]
2	M	79.24[a]	2.04[a]	20.58[a]	1.0[a]
3	M	76.35[b]	4.15[b]	26.22[b]	2.0[b]
4	M	75.52[b]	5.53[c]	26.13[b]	2.7[c]
5	M	75.09[b]	6.11[d]	27.21[b]	3.3[d]
6	M	74.11[b]	6.83[e]	27.46[b]	4.0[d]
1	F	81.32[a]	2.22[a]	20.82[a]	1.0[a]
2	F	80.08[a]	2.13[a]	20.17[a]	1.0[b]
3	F	75.81[bc]	4.32[b]	25.18[b]	2.3[c]
4	F	75.02[bc]	5.25[c]	25.95[b]	2.7[d]
5	F	73.41[c]	6.29[d]	26.59[b]	3.7[e]
6	F	73.13[c]	6.43[d]	26.91[b]	4.3[f]

* Roche Color Fan with 1-15 grade was used

[a,b,c,d,e,f] LS means with different superscripts in the same column are significantly different ($p<0.05$)

CHAPTER III

Evaluation of crawfish meal as a dietary ingredient for broilers and its nutritive properties

ABSTRACT

Composite crawfish meal was evaluated as a poultry feedstuff, serving as a partial protein substitute and a skin and shank pigmenter in broiler rations. For effective utilization, a sieving method, employing various mesh screens was utilized to improve the quality of crawfish meal, especially in reduction of ash and calcium. Compared with the whole meal, separation with a 35 mesh screen resulted in a 170 % increase in corrected protein, and decreases of 23% in calcium, 24% in ash, and 27% in chitin. In feeding trials, broilers were supplied isonitrogenous and isocaloric diets over 7 weeks, supplemented with a 35 mesh-sieved meal at levels up to 10%. This contained concentrations of astaxanthin of 0 to 8.3 mg/kg feed. No significant differences ($p>0.05$) in body weight, feed efficiency, and PER were found between broiler groups on the control corn-soybean meal diet compared with the five experimental diets. The quality of modified crawfish meal was comparable to that of soybean meal using a non-protein nitrogen corrected value. The astaxanthin present in crawfish meal was demonstrated to be an effective red intensifier, producing a significant increase in broiler skin and shank pigmentation.

INTRODUCTION

Tonnages in excess of 100 million pounds of proteinaceous recoverable composite byproduct are produced annually by the rapidly expanding Louisiana crawfish industry (Meyers, 1987). This now forms the basis of a recently established byproduct recovery industry. Earlier studies from our laboratory have focused on basic quality of carotenoid pigment, astaxanthin, 3,3' dihydroxy-4,4'-diketo-β-carotene, in crawfish processing composite byproduct (Meyers and Bligh, 1981; Chen and Meyers, 1982, 1983) and its application in aquatic diets for enhancement of external fish pigmentation (Chen and Meyers, 1983; Meyers and Thibodeaux, 1984) and in intensification of egg yolk color in laying hen diets (Lee, 1985). In addition to its use as a source of natural red pigment, crawfish processing composite byproduct has been evaluated for its chemical composition and nutritional value for possible use in animal diets (Lovell et al., 1968).

Various researchers (Jarquin et al., 1972; Kifer and Bauersfeld, 1969; Mangold and Damkohler, 1938; Parkhurst et al., 1944; Titus et al., 1930; Watkins, 1976) have demonstrated the nutritional value of crustacean meals, such as shrimp and crab, as protein supplement in poultry rations. Compared with menhaden meal with equal amounts of supplementation at any level tested, chicken fed blue crab

meal showed no significant differences in growth rate and exhibited rates of weight gain comparable to those receiving a commercial corn-soybean diet (Kifer and Bauersfeld, 1969). Parkhurst et al.(1944) also reported comparable results of higher biological value of crab meal than fish meal in performance of chicks as the ratio of calcium and phosphorus in the ration was adjusted. No significant differences in mortality, feed efficiency, or feathering were observed. The unidentified growth factor reported in fish meal has also been postulated for crustacean meal (Combs et al., 1954). Normal storage condition and drying temperature (Sullivan et al., 1960) did not have a deleterious effect on the meal.

Crustacean meal also has received attention especially for its red pigment value in broiler pigmentation comparable to that of salmon and trout pigmentation (Chawan and Gerry, 1974; Parkhurst et al., 1944; Saito and Reiger, 1971; Simpson, 1978). De Groote (1964) reported that additional amounts of red pigment imparted an intensifying effect on yellow pigments provided by the feed ingredient. The use of specific amounts of red and yellow carotenoid for proper broiler pigmentation has been discussed (Braeunlich, 1974; Wagstaff, 1984). By adjusting the level of plant xanthophylls and shrimp meal in the ration, different intensities of skin pigmentation were obtained. Due to its higher biological activity, a small amount of

astaxanthin can replace the large amount of yellow pigment used in poultry diets, when used in combination with lutein, to produce a desirable deep yellow color for egg yolk and broiler skin (Nelson and Baptist, 1968; Braeunlich, 1974). Furthermore, it has been proposed that astaxanthin of crustacean origin may be more readily absorbed into fish tissues than that from synthetic sources (Meyers, 1981).

A problem in effective utilization of crawfish and other crustacean waste as a dried whole meal has been the large amount of exoskeleton present, the latter consisting of chitin, protein-associated chitin, and calcium carbonate. According to studies of over 50 years ago, the digestibility of crustacean meal by poultry was lower than that of fish meal in spite of its protein quality (Manning, 1939; Mangold and Hock, 1938; Mangold and Damkohler, 1938). This lower efficiency is probably due in part to indigestible N-containing chitin incorrectly interpreted as protein nitrogen by many early poultry researchers. For accurate evaluation of crustacean meal, corrected protein values must be used to compensate for the non-protein nitrogen. High amount of calcium may adversely affect the nutritional value of crustacean meal (Meyers and Rutledge, 1971). Depressed weight gain and feed efficiency, and increased mortality were reported when feeding high calcium levels in poultry rations (Fangauf et al., 1961; Smith and

Taylor, 1961; Urbanyi, 1959). Levels of calcium above 2.5%, fed between 8 and 20 weeks of age, caused nephrosis, visceral gout, and calcium urate deposits (Scott et al., 1982).

However, a simplified method of physical separation by grinding the meal through a Wiley mill and subsequent sieving with No. 12 U.S.standard mesh screen, reduced calcium as much as 68% in crawfish meal, and 25% in crab meal (Muralidhara, 1985; Rutledge, 1971). The impact generated in the mill was sufficient to reduce the protein constituents to a finer mesh than the shell portion. Crustacean meal processed by physical means, compared with chemical processing, was superior in terms of protein quality and pigment value. A sieved shrimp meal, containing less calcium, gave superior mean weight gain in broilers of 25 days of age compared with control soybean meal and untreated whole meal (Watkins, 1976).

Among the several procedures to utilize whole recovered crawfish composite byproduct, its use in a dried meal form in poultry feed is highly recommended as a nutritionally acceptable, low-cost substitute, allowing increased flexibility and economics of feed formulation. Furthermore, the short growth span of the broiler produces an ideal test animal for examining the effect of diet modifications with new supplemental ingredients. In the present investigation, possible use of dried crawfish meal

has been evaluated as a nutritional substrate for its protein and astaxanthin pigment content in broiler diets.

Materials and Methods

Sample Collection and Preparation

Composite byproduct from the crayfish (Procambarus clarkii) dried at between 107 and 121°C, and passed through a commercial hammermill, was obtained from Acadiana Processors Inc. (Henderson, LA) and designated as whole meal. The meal included the intact cephalothorax, abdominal exoskeleton, and viscera. Material was placed into doubled black polyethylene bags and stored at -20°C. For the analysis of crawfish meal fractions, the meal was sifted with sieves (US Standard ASTM E 11-61) with various mesh sizes, i.e., 10, 20, 25, 35, 45, and 60, for 20 min. on a portable Tyler sieve shaker (Model RX-8). After an evaluation of crawfish meal with different particle sizes, based on chemical composition and total yield after sieving, a 35-mesh sieve was selected as most suitable for the large volume of crawfish meal needed for this study.

Birds and Diets

Three hundred and sixty, day-old Arbor Acres broilers were obtained from the Louisiana State University Poultry Research Farm, Baton Rouge, LA. and floor-reared to 7 weeks of age at the same farm. Thirty birds per pen (2.26 X 1.50 m) were randomly placed in an open-sided broiler house with 12 pens, 6 on each side and temperature controlled

ventilation fans. Each pen was equipped with automatic waterer, tubular feeder, and heating lamp. For the first 10 days of brooding, a feeder tray was used and subsequently replaced with a tubular feeder. Feed and water were supplied ad *libitum*. Body weight and feed consumption data were recorded weekly.

A total of 6 experimental diets, based on different ratios of crawfish meal, were prepared. The processed crawfish meal was incorporated into formulated feeds at various levels for its evaluation as a specific ingredient for pigmentation properties and as a protein supplement. The formula of experimental rations are listed in Table 1. These are based on the nutrient requirements of broilers (NRC, 1984). The ratio of crawfish meal was varied at levels of 0, 1.0, 2.5, 5.0, 7.5, and 10.0 %, with substituted diet supplementation. The formulation of the substituted diet is listed in Table 2. This is based on the composition of crawfish meal.

Feeding experiments were conducted using a randomized complete block design (RCBD) with split-plot arrangement. Each six pens was a block. The six diet treatments were randomly assigned within the block. At seven weeks of age, six birds (3 males and 3 females) from each pen were randomly selected and then properly identified by means of wing bands. Birds were withdrawn from water and diets eight hours before slaughtering. A total of 72 birds from

12 pens were slaughtered and dressed according to standard commercial practices. The carcasses were kept in ice water for 30 min. and drained of excess water. Carcass, intestines, and shanks, of each bird were separately placed into polyethylene bags with identification cards and stored at -20^{0}C until use.

Chemical Analysis

Official methods of analysis (AOAC, 1980) were used for chemical analysis. Nitrogen was determined by a semiautomated method; ash, fat, and phosphorus by standard methods. Calcium was analyzed with a Perkin-Elmer atomic absorption spectrometer. All analysis values reported are the average of five replicates on a dry basis. All data are expressed on a dry weight basis.

Amino Acid Analysis

The Pico-Tag method (Bidlingmeyer et al., 1984) was used for amino acid analysis. Twenty mg of sample were hydrolyzed with 1 ml 6 N HCl at 110^{0}C for 24 hrs under nitrogen in sealed vial. The hydrolysate was cooled and 9 ml H_2O (HPLC grade) was added. A total volume of 10 ml diluted hydrolysate was centrifuged at 3000 rpm for 10 min. and 50 µl of supernatant taken for derivatization. Through the three basic steps of derivatization, i.e., drying, redrying, and coupling, amino acids sample was derivatized

with phenylisothiocyanate (PITC). A 25 µl aliquot of the phenylthiocarbamyl amino acid (PTC-AA) mixture was used for analysis on a Pico-Tag Amino Acid Analyzer (Waters, Milford, MA) under the following conditions of 1 ml/min flow rate, Pico-Tag reverse phase column, temperature control module set at 40°C, and UV-detector at 254 nm.

Astaxanthin Determination

The solvent extraction procedure of Lee (1985), modified from the method of Chen (1981), was used for determination of astaxanthin in the crawfish meal. The total amount of astaxanthin was calculated by the formula of Kelly and Harmon (1972).

Formula 1.

$$\text{gm astaxanthin/gm crawfish meal} = AD/100\ G\ d\ E^{1\%}_{1cm}$$

where A = absorbance at maximum wavelength (467 nm in petroleum ether), D = dilution volume (ml), G = crawfish meal (gm), d = cell width (1 cm), and $E^{1\%}_{1cm}$ = 2400 in petroleum ether.

Xanthophyll Determination

The official method of analysis of the Association of Official Analytical Chemists (AOAC, 1980) was used for extraction and determination of total xanthophyll from yellow corn. The xanthophyll content of ground yellow corn

was analyzed by the Feed and Fertilizer Laboratory at Louisiana State University.

Pigmentation Analysis

Prior to sample preparation, carcasses and shanks were thawed at 4^{0}C. After cutting a side of shank, the epithelial and cutaneous layers were separated from the bone. The skin from four different parts, i.e., breast, wings, back, and thigh, was removed and the excess subcutaneous fat of skin was excised. Each skin was placed on a white 8 x 8 cm poster board to facilitate pigmentation analysis. The Roche Yolk Color Fan with 15 color grades (Vuilleumier, 1969), and the Hunterlab Tristimulus Colorimeter (Model D 25 M-9) were used for visual comparison of pigmentation differences.

Statistical Analysis

The data were analyzed using analysis of variance in the General Linear Model (GLM) procedure of SAS (1982). Least Squares (LS) means were used to compare effects of treatments using an option of PDIFF of SAS.

Table 1. Composition of experimental broiler diets

Ingredients (%)	Starter 1	Starter 2	Starter 3
Ground yellow corn	46.39	46.39	46.39
Soybean meal-44[1]	24.75	24.75	24.75
Menhaden fish meal-61[1]	7.79	7.79	7.79
Crawfish meal	0.00	1.00	2.50
Substituted meal[2]	10.00	9.00	7.50
Tallow	8.97	8.97	8.97
Defluorinated phosphate[3]	0.67	0.67	0.67
DL methionine	0.23	0.23	0.23
Cellulose	-	-	-
Oyster shell flour	0.69	0.69	0.69
Salt	0.01	0.01	0.01
Mineral mix[4]	0.25	0.25	0.25
Vitamin mix[5]	0.25	0.25	0.25
Total	100.00	100.00	100.00
Calculated analysis			
Crude protein (%)	24.43	24.43	24.43
Fat (%)	11.77	11.80	11.84
Fiber (%)	2.96	3.00	3.08
Calcium (%)	1.28	1.36	1.45
Astaxanthin (mg/Kg feed)	0.00	0.83	2.08
Metabolic energy (Kcal/lb)	1451.54	1451.54	1451.54

[1] Protein content of ingredient (%)
[2] See Table 2.
[3] Contains 17% P and 33% Ca
[4] Per kg of diet: sodium, 0.13 mg; zinc, 90.8 mg; copper, 9.08 mg; manganese,108.96 mg; iron, 45.4 mg; cobalt, 0.18 mg; selenium, 0.18 mg.
[5] Per kg of diet: vitamin A, 7264 IU; vitamin D_3; 1816 IU; vitamin E, 9 IU; vitamin B_{12}, 0.0091 mg; riboflavin, 4.54 mg; vitamin K, 1.82 mg; niacin, 18.16 mg; pantothenic acid, 8.17 mg; choline, 317 mg; folacin, 0.45 mg; biotin, 0.091 mg; pyridoxine, 1.82 mg.

Table 1. (Continued) Composition of experimental broiler diets

	Starter		
Ingredients (%)	4	5	6
Ground yellow corn	46.39	46.39	46.39
Soybean meal-44[1]	24.75	24.75	24.75
Menhaden fish meal-61[1]	7.79	7.79	7.79
Crawfish meal	5.00	7.50	10.00
Substituted meal[2]	5.00	2.50	0.00
Tallow	8.97	8.97	8.97
Defluorinated phosphate[3]	0.67	0.67	0.67
DL methionine	0.23	0.23	0.23
Cellulose	-	-	-
Oyster shell flour	0.69	0.69	0.69
Salt	0.01	0.01	0.01
Mineral mix[4]	0.25	0.25	0.25
Vitamin mix[5]	0.25	0.25	0.25
Total	100.00	100.00	100.00
Calculated analysis			
Crude protein (%)	23.43	23.43	23.43
Fat (%)	11.91	11.98	12.05
Fiber (%)	3.08	3.22	3.41
Calcium (%)	1.63	1.80	1.98
Astaxanthin (mg/Kg feed)	4.15	6.23	8.30
Metabolic energy (Kcal/lb)	1451.54	1451.54	1451.54

[1] Protein content of ingredient (%)
[2] See Table 2.
[3] Contains 17% P and 33% Ca
[4] Per kg of diet: sodium, 0.13 mg; zinc, 90.8 mg; copper, 9.08 mg; manganese,108.96 mg; iron, 45.4 mg; cobalt, 0.18 mg; selenium, 0.18 mg.
[5] Per kg of diet: vitamin A, 7264 IU; vitamin D_3; 1816 IU; vitamin E, 9 IU; vitamin B_{12}, 0.0091 mg; riboflavin, 4.54 mg; vitamin K, 1.82 mg; niacin, 18.16 mg; pantothenic acid, 8.17 mg; choline, 317 mg; folacin, 0.45 mg; biotin, 0.091 mg; pyridoxine, 1.82 mg.

Table 1. (Continued) Composition of experimental broiler diets

	Finisher		
Ingredients (%)	1	2	3
Ground yellow corn	62.82	62.82	62.82
Soybean meal-44[1]	8.24	8.24	8.24
Menhaden fish meal-61[1]	12.71	12.71	12.71
Crawfish meal	0.00	1.00	2.50
Substituted meal[2]	10.00	9.00	7.50
Tallow	4.15	4.15	4.15
Defluorinated phosphate[3]	0.47	0.47	0.47
DL methionine	0.04	0.04	0.04
Cellulose	0.67	0.67	0.67
Oyster shell flour	0.20	0.20	0.20
Salt	0.20	0.20	0.20
Mineral mix[4]	0.25	0.25	0.25
Vitamin mix[5]	0.25	0.25	0.25
Total	100.00	100.00	100.00
Calculated analysis			
Crude protein (%)	20.06	20.06	20.06
Fat (%)	7.89	7.92	7.96
Fiber (%)	2.34	2.38	2.46
Calcium (%)	1.58	1.66	1.75
Astaxanthin (mg/Kg feed)	0.00	0.83	2.08
Metabolic energy (Kcal/lb)	1451.54	1451.54	1451.54

[1] Protein content of ingredient (%)
[2] See Table 2.
[3] Contains 17% P and 33% Ca
[4] Per kg of diet: sodium, 0.13 mg; zinc, 90.8 mg; copper, 9.08 mg; manganese,108.96 mg; iron, 45.4 mg; cobalt, 0.18 mg; selenium, 0.18 mg.
[5] Per kg of diet: vitamin A, 7264 IU; vitamin D_3; 1816 IU; vitamin E, 9 IU; vitamin B_{12}, 0.0091 mg; riboflavin, 4.54 mg; vitamin K, 1.82 mg; niacin, 18.16 mg; pantothenic acid, 8.17 mg; choline, 317 mg; folacin, 0.45 mg; biotin, 0.091 mg; pyridoxine, 1.82 mg.

Table 1. (Continued) Composition of experimental broiler diets

Ingredients (%)	Finisher 4	5	6
Ground yellow corn	62.82	62.82	62.82
Soybean meal-44[1]	8.24	8.24	8.24
Menhaden fish meal-61[1]	12.71	12.71	12.71
Crawfish meal	5.00	7.50	10.00
Substituted meal[2]	5.00	2.50	0.00
Tallow	4.15	4.15	4.15
Defluorinated phosphate[3]	0.47	0.47	0.47
DL methionine	0.04	0.04	0.04
Cellulose	0.67	0.67	0.67
Oyster shell flour	0.20	0.20	0.20
Salt	0.20	0.20	0.20
Mineral mix[4]	0.25	0.25	0.25
Vitamin mix[5]	0.25	0.25	0.25
Total	100.00	100.00	100.00
Calculated analysis			
Crude protein (%)	20.06	20.06	20.06
Fat (%)	8.04	8.10	8.18
Fiber (%)	2.46	2.60	2.79
Calcium (%)	1.95	2.10	2.28
Astaxanthin (mg/Kg feed)	4.15	6.23	8.30
Metabolic energy (Kcal/lb)	1451.54	1451.54	1451.54

[1] Protein content of ingredient (%)
[2] See Table 2.
[3] Contains 17% P and 33% Ca
[4] Per kg of diet: sodium, 0.13 mg; zinc, 90.8 mg; copper, 9.08 mg; manganese,108.96 mg; iron, 45.4 mg; cobalt, 0.18 mg; selenium, 0.18 mg.
[5] Per kg of diet: vitamin A, 7264 IU; vitamin D_3; 1816 IU; vitamin E, 9 IU; vitamin B_{12}, 0.0091 mg; riboflavin, 4.54 mg; vitamin K, 1.82 mg; niacin, 18.16 mg; pantothenic acid, 8.17 mg; choline, 317 mg; folacin, 0.45 mg; biotin, 0.091 mg; pyridoxine, 1.82 mg.

Table 2. Composition of substituted meal (SM) and crawfish meal (CM)

Ingredients	SM (%)	CM (%)
Soybean meal (44% protein)	77.88	-
Chitin[1]	8.50	-
Cellulose[2]	6.81	-
Oyster shell flour	6.81	-
Total	100.00	
Calculated analysis		
Crude Protein (%)	42.75	42.75
Corrected protein (%)	35.05	35.05[3]
Fat (%)	0.70	3.90
Calcium (%)	2.83	9.77
Chitin (%)	8.50	8.50
NaCl (%)	0.00	0.00
Astaxanthin (mg/kg)	-	83.00
Metabolic energy (Kcal/lb)	794.47	363.00

[1] From crab shells (practical grade), Sigma Chemical Co.

[2] United States Biochemical Co.

[3] Corrected protein = (total nitrogen - chitin N) x 6.25
chitin nitrogen based on 6.9% N present in chitin

Chemical composition of whole crawfish meal

Chemical analysis, indicative of the potential nutritional quality of whole crawfish meal, is shown in Table 3. Throughout the decade of research on crawfish processing composite byproduct from the LSU Food Science Department, the range of crude protein content of whole crawfish meal has been reported by between 26.5% and 40% on a dry weight basis. This variability may possibly be attributed to the crawfish growth stages, i.e., degree of calcification, and to the nutritioned status of the animal, or more likely to the magnitude of the actual crawfish processing operation. Astaxanthin concentration, 89 ppm, found in the meal was lower than with the 153 ppm level reported by Meyers and Bligh (1981). The difference may be due to the variability of astaxanthin levels in crawfish waste affected by the processing method and the seasonality of resource. The effect of drying on carotenoid levels of crustacean meal has been studied by Kamata et al. (1976). Carotenoids also are susceptible to oxidation by light, heat, and atmospheric oxygen. Regardless of the lower concentration, Lee (1985) demonstrated effectiveness of crawfish astaxanthin in egg yolk pigmentation.

Sodium chloride content was almost undetectable in crawfish meal. This contrasts with data from crustacean

meals of marine origin. The practical sodium requirement is 0.15% for starting chicks and 0.1% for growing chicks (Scott et al., 1982). Increasing sodium chloride in feed from 0.4% to 1% showed no adverse effect on bird weight gain and feed efficiency (Proudfoot et al., 1985). Nevertheless, sudden death syndrome among female chickens was reported (Proudfoot et al., 1985) to be related to increased dietary sodium probably causing hypertension. Since sun-dried shrimp meal was used in poultry diet initially in 1934 in Louisiana, maximum salt content was regulated to 7% by state regulation (Upp, 1935). The Association of American Feed Control Officials (1944) noted that if crab meal as well as shrimp meal contains more than 3% salt, the amount of salt must constitute a part of the brand name. In any event, salt content should not exceed 7%.

Effect of physical separation on chemical composition of crawfish meal

The effect of physical separation on chemical composition of crawfish meal is summarized and compared with whole crawfish meal and 1 N HCl-treated decalcified meal (Table 4). Considerable variability in chemical composition was observed with differential sieving. With decreased particle size, percentages of protein and fat increased, while fiber and ash decreased. In addition,

calcium level decreased along with a slight increase in potassium. No differences were found in phosphorus levels. Astaxanthin content decreased with reduced particle size. However, compared with shrimp meals, i.e., vacuum dried meal, 76.2 ppm, (Simpson, 1978) and shrimp head meal, 31 ppm, (Meyers, 1980) the lowest pigment content in crawfish meal sieved with 60 mesh screen is comparable to that present in shrimp whole meal. Compositional variability by various particle size separation probably was due to the separation of the calcium portion from the meal with decreased particle size (Rutledge, 1971). Table 4 also shows percentage sieving yield of each particle size. Total yield was significantly decreased with increased mesh size.

Based on these results, sieved crawfish meal with 35 mesh screen was selected to improve the quality of meal for its evaluation in poultry diets. The main consideration was the level of protein, more than 40%, calcium, less than 10%, and astaxanthin content, more than 80 ppm, for comparison with shrimp meal. After processing with a 35 mesh screen, the percentage improvement of meal quality compared with whole meal was as follows; 170% increase in corrected protein; 135% increase in crude protein; 23% reduction in calcium; 24% in ash; and 27% in chitin.

Amino acid composition of crawfish meal

The amino acid profiles of whole and sieved crawfish meal were compared with published data (Crawford, 1975; Kifer and Bauersfeld, 1969) of blue crab and shrimp meal (Table 5). By physical separation, the total amount of amino acid increased 17% in sieved crawfish meal. Glutamic acid and serine account for 22% of the total amino acids present in crawfish meal, with all essential amino acids present. The level of serine in crawfish meal was particularly noteworthy, 20.4 mg/g, compared with other crustacean meals. Scott et al. (1982) reported that most rapid growing chicks under some conditions may not synthesize sufficient serine. Tryptophan was not determined due to the destruction in the acid hydrolysis. However, unpublished investigation, (Meyers and Rutledge, 1977) revealed high levels of tryptophan in crawfish meal. Levels of all amino acids in sieved crawfish meal are higher than shrimp meal, except for cystine. Compared with shrimp and crab meal, sieved crawfish meal shows a good essential amino acid balance, ultimately resulting in a superior protein quality. This is particularly important for a protein with high biological value. However, amino acids should be available and in sufficient amounts required by poultry (Patrick and Schaible, 1980). Level of valine present is almost seven times higher than in blue crab meal, but the level of cystine is 50% of that in the

latter.

The limiting amino acids in crawfish meal were calculated based on recommended practical levels of amino acid in feeds for chickens (Table 6). Cystine is the primary limiting amino acid, followed by methionine plus cystine, and lysine.

Effect of crawfish meal on broiler performance

The effect of the various levels of crawfish meal on weekly body weight expressed in LS mean weight for 7 weeks are presented in Table 7. No significant weight differences among treatments in each week was found due to the different addition rates of crawfish meal (Appendix Table 1). The summarized information of the experimental diets are listed in Table 8. Compared with control corn-soybean meal diet, the growth rates of broiler fed five experimental diets, with 1, 2.5, 5, 7.5, and 10% crawfish meal, respectively, showed comparable patterns of growth curves. This does not agree with a much earlier study (Titus et al., 1930) in which it was reported that during the first 10 weeks, chickens fed 10% shrimp meal gave the poorest growth rates although during the last 6 weeks, weight gain exceeded that in other groups supplied white fish meal, meat meal, and menhaden meal. Such studies did not differentiate true protein from nonprotein chitin nitrogen, thus negating much of the early observations.

The results of feed efficiency between 4 and 7 weeks of age are given in Table 10. There was no significant difference ($p>0.05$) in feed efficiency of the six diets in starter and finisher rations, even between male and female birds (Appendix Table 2). In the comparison of body weight, statistical interaction between block and treatment was observed. Since there was no significant difference in feed efficiency among treatments, the possible explanation for the difference in weight gain was related to the amount of feed consumed, and the palatability of a particular diet (Fronda et al., 1933). Other explanations may be unidentified growth factors, or a favorable ratio of Ca and P in the diet (Simco et al, 1961; Waldroup et al., 1962).

The final weights of male and female birds tended to be higher and lower, respectively, in both control and experimental groups. Differential growth responses between sexes have been reported previously, although a valid reason has not been given.

In the present study, nitrogen was used to evaluate the practical quality of crawfish meal. Therefore, the corrected crawfish protein content, 35% in sieved meal with 35 mesh screen, instead of the uncorrected protein value contributed by chitin nitrogen, was used. To eliminate the possible growth stimulating effect of chitin reported by Ramachandran et al. (1986), an equivalent level of chitin in crawfish meal was added to the substitute meal. Protein

efficiency ratio (PER) of each diet is almost identical. This is expected since there was no significant difference in feed conversion ratio, and all diets have equivalent corrected protein and are isonitrogenous and isocaloric (Table 11). The maximum contribution of protein from crawfish meal (treatment 6) is 15.0% and 17.5% in starter and finisher diet, respectively. Compared with soy protein, it is notable than crawfish meal did not show apparent differences in protein quality, and even gave higher body weight at 4 weeks of growth.

In protein digestibility, the uncorrected crawfish protein was approximately 82.5% compared with soy protein in rats (Lovell, 1968). However, as protein value was corrected for chitin nitrogen, the biological availability of crawfish protein was comparable to that of soy protein. Other unpublished digestibility studies for catfish rations conducted at LSU indicated that crawfish meal could readily substitute for fish and meat meal in formulated rations for catfish (Personal Communication). The aforementioned significant observation on the comparative nutritional value of crawfish meal may be due in part, or entirely, to its use in a decalcified ($CaCO_3$ removed) state.

The ratio of calcium and phosphorous in experimental diets increased gradually as crawfish meal increased (Table 9). No significant differences ($p>0.05$) were found in growth performance attributed to the various ratios of Ca

and P. This may be due to the fact that when an adequate level of Ca and P is present in the diet, the effect of changing the Ca and P ratio is decreased (Damron, 1976). However, Parkhurst et al. (1943) reported that the performance of crab meal was less satisfactory when the mineral balance was adjusted in the diet. No apparent differences in feathering were observed in the experimental lot compared with the broilers fed the control diet throughout the test period.

The performance of broilers in body weight, feed efficiency, and PER on crawfish meal was comparable to those on the control diet with soybean meal supplementation. However, due to the lower metabolic energy, 363 Kcal/lb, the maximum level of crawfish meal should be less than 20% of the ration. Higher levels of meal requires more tallow in the diet, which is not practical because this makes it difficult for broilers to pick at the feed.

Effect of crawfish meal on broiler skin and shank pigmentation

The pigmentation results of broilers subjected to colorimetric evaluation are presented in Tables 12 and 13 for skin and shank, respectively. All data used in statistical analysis, particularly for skin pigmentation, are based on the average of 4 different parts, i.e.,

breast, wings, back, and thigh, due to the uniformity of broiler skin color and other visual components such as gloss and translucency (Fletcher, 1981). Due to the nature of the experiment, no variation was observed in the level of yellow corn, which is the only source of yellow carotenoid in all diets. Lutein content in yellow corn was 11.5 mg/kg. All experimental finishing diets including control, had 62.82% of yellow corn, providing a total of 7.2 mg lutein per kg of feed. The level of crawfish astaxanthin in each diet is listed in Table 8.

As shown in Tables 12 and 13, redness (a) and yellowness (b) values increased as the amount of crawfish meal was increased, while lightness (L) decreased in both skin and shank. Similar results of the effect of red pigment on the lightness has been reported (Fletcher and Halloran, 1981; Lee, 1985). However, contrasting data also are reported by Lee (1985), in which it was shown in egg yolk that yellowness decreased sharply with increased concentration of astaxanthin in the range above 5 mg/kg with 10 to 40% yellow corn in the diet. This trend of yellowness increase with increased concentration of red pigment was shown only in the range of less than 5 mg/kg astaxanthin with higher levels of yellow corn. In the current experiments, yellowness was steady rather than decreasing in the range above 4.15 mg/kg of astaxanthin.

Highly significant differences ($p<0.01$) were seen in

all three values of L, a, b, and visual score (Appendix Table 4 and 5). However, the only difference between male and female birds was in redness in both the skin and shank. This may be due to the females having an additional body organ, the ovaries to deposit the pigment compared with males since the deposition of red oxycarotenoid is not affected by even high levels of xanthophyll in feed (Braeunlich, 1974). In redness, skin was more sensitive than the shank in the range above 4.15 mg/kg astaxanthin. However, no significant difference was seen below the latter carotenoid level and in the yellowness response.

Visual evaluation alone is not sufficient to evaluate utilization of red pigment effectiveness, although visual scoring is adequate for commercial purposes (Hinton et al., 1973). Thus, the objective reflectance colorimetric method was used for skin analysis due to the lack of uniformity of broiler skin color. Therefore, direct comparison with other published data was possible only by visual score of the shank with Roche Color Fan with 1-15 grade. As noted in Table 13, control diet with 62.82% yellow corn, providing only 7.2 mg/kg lutein per kg of feed, showed a score of 1 in shank visual perception. This was accomplished with only 25% yellow corn ration providing 3.0 mg lutein/kg (Marusich and Bauernfeind, 1970). However, these workers also noted highly significant variations in shank pigmentation. This showed that a

visual shank score of 2.2 was accomplished by feeding 6 mg lutein/kg in 50% yellow corn ration for 4 weeks. In contrast, a visual shank score of 2.4 was obtained with 11 mg lutein/kg in 60% yellow corn ration for 6 weeks. The addition of astaxanthin in concentrations as low as 0.83 mg/kg in 1% crawfish meal, increased the shank score from 1 to 2.2 in males and 1.8 in females without xanthophyll addition.

Significant enhancement of visual score by addition of small amounts of red pigment in broiler rations also was obtained by Chawan and Gerry (1974) and Marusich and Bauernfeind (1970). A shank visual score of 4.3 was achieved by the addition of 15 mg/kg yellow oxycarotenoid in 3.5% alfalfa meal to 60% yellow corn ration, providing 11 mg/Kg lutein. A visual score of 4.8 was obtained by the addition of 8.3 mg/kg astaxanthin in 10% shrimp meal to 62.8% yellow corn ration, providing only 7.2 mg/kg lutein. No significant difference ($p>0.05$) occurred in shank visual score among astaxanthin levels of 0.83, 2.08, and 4.15 mg/kg in males and between 2.08 and 4.15 mg/kg in females. As reported earlier (Herrick et al., 1970), the relationship between shank and skin pigmentation was not very clear, but both showed the same general trend in affecting color change.

Table 3. Chemical composition of whole crawfish meal

Composition	Percentage (%)
Crude protein	31.5 %
Corrected protein	20.4 %
Fat	4.0 %
Fiber	12.6 %
Chitin	12.3 %
Ash	39.9 %
Minerals	
Ca	12.8 %
P	0.9 %
K	0.5 %
Mg	0.6 %
Fe	1576 ppm
Mn	490 ppm
Astaxanthin	83 ppm
NaCl	0 %

Table 4. Effect of physical separation with various size of siever[1] on the chemical composition of crawfish meal[2]

Composition			Screen size (mesh/inch)					
(%)	WM[3]	Decal[4]	10	20	25	35	45	60
Protein	31.5	63.7	30.8	37.2	40.6	42.8	43.8	45.0
Fat	4.0	7.9	2.3	3.2	3.6	3.9	4.3	4.2
Fiber	12.6	25.0	13.5	11.7	10.1	9.2	8.3	7.5
Ash	39.3	-	40.4	36.7	33.2	30.2	29.5	28.5
Ca	12.8	0.1	14.4	12.3	10.4	9.8	9.6	9.1
P	0.9	0.5	1.0	1.0	1.0	1.0	1.0	1.0
K	0.5	0.0	0.4	0.5	0.6	0.6	0.6	0.6
Asta-xanthin (ppm)	89	-	135	124	95	83	72	54
Yield[5]	100.0	60.0	84.7	46.0	32.0	23.3	22.2	14.7
Internal mesh width in mm			2.00	0.841	0.707	0.500	0.354	0.250

[1] US Standard ASTM E 11-61

[2] Average of 5 determinations

[3] Crawfish whole meal

[4] Decalcification with 1 N HCl for 30 min. at room temp. solid : solvent = 1 : 15

[5] Total % amount of meal passed through screen

Table 5. Total amino acid composition of whole crawfish meal (WCM), sieved meal with 35 mesh screen[1](SCM), shrimp meal[2](SM), and crab meal[3](CM)

	Content (mg/g)			
Composition	WCM	SCM	SM	CM
Arginine	12.8	16.6	13.8	18.8
Histidine	5.1	5.7	5.7	7.3
Isoleucine	7.4	8.2	9.0	10.5
Leucine	14.0	15.3	12.8	14.6
Lysine	8.4	10.1	12.7	14.6
Methionine	3.7	4.1	4.4	5.2
Cystine	0.5	1.0	3.5	2.1
Phenylalanine	8.2	9.8	-	11.5
Tyrosine	7.0	9.0	8.6	10.5
Threonine	8.7	11.3	8.5	10.5
Valine	8.2	9.6	11.5	1.4
Aspartic acid	10.0	12.6	20.1	23.0
Serine	23.6	20.4	9.7	9.4
Proline	8.9	11.3	10.3	13.6
Glutamic acid	18.8	23.6	27.8	31.4
Alanine	10.9	12.9	11.9	14.6
Glycine	11.0	13.7	11.9	18.8
Total	167.2	195.2	182.2	217.8

[1] Average of 5 determinations

[2] From pacific shrimp waste meal (Crawford, 1975)

[3] From blue crab (Kifer and Bauersfeld, 1969)

Table 6. Limiting amino acids in crawfish meal[a] for broilers

Amino acid	Requirement[b] %/protein	Crawfish meal % of AA	Crawfish meal %/protein	Ratio***
Arginine	5.0	1.66	4.7	0.95[10]
Histidine	2.0	0.57	1.6	0.82[8]
Isoleucine	4.0	0.82	2.3	0.59[4]
Leucine	6.0	1.53	4.4	0.73[5]
Lysine	5.0	1.01	2.9	0.58[3]
Methionine	2.0	0.41	1.2	0.59[4]
Cystine	1.6	0.10	0.3	0.19[1]
Met + Cys	3.6	0.51	1.5	0.41[2]
Phenylalanine	3.2	0.98	2.8	0.88[9]
Tyrosine	3.2	0.90	2.6	0.80[6]
Threonine	3.2	1.13	3.2	1.01[11]
Valine	3.2	0.82	2.3	0.81[7]
Tryptophan	0.9	-	-	-

[a] Crawfish meal with 35 mesh size: Corrected protein 35.05%

[b] From Nutrition of The Chicken (Table 9.1b) (Scott et al., 1982)

[c] Tryptophan not determined in Pico-Tag system due to destruction.

*** Number: Sequence of limiting amino acids

Table 7. Growth rates of broilers fed various levels of crawfish meal in experimental diets

Time (wk)	Weight of broiler*(g) Treatment 1	2	3	4	5	6
0	40.9	40.1	40.5	41.0	40.4	40.3
1	142.6	139.9	144.6	146.7	148.5	130.9
2	352.1	346.7	353.4	366.8	373.6	332.9
3	702.1	646.3	661.7	700.4	711.7	669.7
4	977.5	997.9	992.1	1040.0	1022.2	1005.7
5	1365.1	1300.5	1424.7	1371.2	1301.0	1357.0
6	1758.3	1625.7	1737.0	1683.7	1720.9	1761.5
7	2103.9	2074.9	2067.6	2106.0	2077.1	2141.5

* LS mean weight

Table 8. Summarized experimental design for crawfish meal study

Treatment	Crawfish Astaxanthin (mg/kg)
1. Control, 90% basal* + 10% SM**	0
2. 90% basal + 9.0% SM + 1.0% CFM	0.83
3. 90% basal + 7.5% SM + 2.5% CFM	2.08
4. 90% basal + 5.0% SM + 5.0% CFM	4.15
5. 90% basal + 7.5% SM + 2.5% CFM	6.23
6. 90% basal + 10.% SM + 0% CFM	8.30

* Basal contains 46.39% and 62.82% yellow corn for starter and finisher rations, respectively.

** Substitute meal listed in Table 2.

Table 9. The ratio of calcium and phosphorus in starter and grower diets.

		Treatment					
Diet	Ca/P	1	2	3	4	5	6
Starter		2.72	2.83	2.96	3.20	3.40	3.60
Finisher		2.93	3.02	3.13	3.36	3.50	3.68

Table 10. The effect of crawfish meal on performance of broilers at 4 and 7 weeks of age

	Body weight* (g)		Feed efficiency* (feed/wt. gain)	
Treatment	M	F	M	F
4 WEEKS				
1	1077.7[a]	877.2[b]	1.66[c]	1.79[c]
2	1086.3[a]	909.5[b]	1.64[c]	1.79[c]
3	1095.9[a]	888.3[b]	1.61[c]	1.78[c]
4	1128.4[a]	951.5[b]	1.59[c]	2.16[c]
5	1109.4[a]	935.3[b]	1.64[c]	1.71[c]
6	1093.3[a]	918.1[b]	1.65[c]	1.70[c]
7 WEEKS				
1	2289.9[a]	1866.1[b]	2.00[c]	2.07[c]
2	2257.2[a]	1888.7[b]	2.03[c]	2.04[c]
3	2276.7[a]	1845.0[b]	2.00[c]	2.08[c]
4	2298.9[a]	1937.1[b]	2.02[c]	1.98[c]
5	2238.1[a]	1887.8[b]	2.04[c]	2.04[c]
6	2300.0[a]	1931.4[b]	2.03[c]	2.03[c]

[a,b,c] LS means with different superscripts with the same response parameter are significantly different ($p<0.05$)

Table 11. Protein efficiency ratio (PER) assay of crawfish meal in 4 weeks-old male (M) and female (F) broilers

	Weight gain (g)		PER	
Treatment	M	F	M	F
1	1036.8[a]	836.3[b]	2.59[c]	2.40[c]
2	1046.2[a]	869.4[b]	2.61[c]	2.49[c]
3	1055.4[a]	847.8[b]	2.66[c]	2.45[c]
4	1087.4[a]	910.5[b]	2.69[c]	2.58[c]
5	1069.0[a]	894.9[b]	2.61[c]	2.50[c]
6	1053.0[a]	877.8[b]	2.64[c]	2.52[c]

[a,b,c] LS means with different superscripts with the same response parameter are significantly different ($p<0.05$)

Table 12. Lightness (L), redness (a), and yellowness (b) value of skin of male (M) and female (F) broilers at 7 weeks of age

Treat.	Sex	L	a	b
1	M	80.69[a]	2.36[a]	15.92[a]
2	M	79.61[ab]	3.14[b]	16.45[a]
3	M	78.26[b]	4.37[c]	17.80[ab]
4	M	75.11[c]	5.81[d]	19.28[b]
5	M	73.10[d]	7.21[e]	19.73[b]
6	M	71.04[e]	8.23[f]	19.30[b]
1	F	81.82[a]	1.40[a]	16.40[a]
2	F	80.55[a]	2.57[b]	16.60[a]
3	F	77.38[b]	4.08[c]	19.05[b]
4	F	76.25[b]	4.89[d]	19.11[b]
5	F	72.17[c]	7.17[e]	19.55[bc]
6	F	72.80[c]	7.08[e]	20.86[c]

[a,b,c,d,e,f] LS means with different superscripts in the same column are significantly different ($p<0.05$)

Table 13. Lightness (L), redness (a), and yellowness (b) value and visual score (V) of shank of male (M) and female (F) broilers at 7 weeks of age

Treat.	Sex	L	a	b	V*
1	M	80.27^{a}	2.26^{a}	23.65^{a}	1.00^{a}
2	M	76.55^{b}	3.46^{b}	24.12^{ac}	2.17^{b}
3	M	76.79^{b}	4.26^{c}	27.54^{bcd}	2.50^{b}
4	M	76.71^{b}	5.58^{d}	26.91^{ad}	2.50^{b}
5	M	74.33^{b}	6.09^{e}	29.10^{bd}	3.67^{c}
6	M	73.90^{b}	6.73^{f}	28.75^{bd}	4.83^{d}
1	F	80.66^{a}	2.31^{a}	22.59^{a}	1.00^{a}
2	F	76.79^{b}	3.98^{b}	23.04^{a}	1.83^{b}
3	F	75.84^{b}	4.48^{bc}	29.40^{b}	2.50^{c}
4	F	74.28^{bc}	4.79^{c}	28.14^{b}	2.67^{c}
5	F	72.74^{c}	6.20^{d}	28.15^{b}	3.83^{d}
6	F	73.57^{c}	6.90^{d}	28.65^{b}	4.67^{e}

* Roche Color Fan with 1-15 grade was used

^{a,b,c,d,e,f} LS means with different superscripts in the same column are significantly different ($p<0.05$)

CHAPTER IV

Studies on various complexes of crawfish chitin

ABSTRACT

Separated dried crawfish shell was subjected to analysis of chitin-protein and its chitin-carotenoid complex. Protein fractions were obtained from the crawfish chitin-protein complex under varying conditions of extractions, i.e., disodium ethylenediaminetetraacetic acid (EDTA) and urea for determination of physical association of protein to chitin, and various concentrations of NaOH for analysis of covalently bound protein. Total levels of protein, chitin, and covalently bound chitin/protein in the shell were 6.5%, 23.5%, and 0.7%, respectively. Serine was determined to be the major amino acid in the crawfish chitin. This specific amino acid bonding contrasts notably with comparable analyses of other crustacean chitin/protein complexes. The effect of drying temperature and storage on astaxanthin stability in the chitin-carotenoid complex was investigated. Factors of both drying temperature and time affected pigment retention. Increased oven drying to 24 hr at 60°C and 100°C, and sun-drying resulted in losses of 39.5%, 67.6%, and 43.9% astaxanthin, respectively.

Introduction

Chitin, a long straight chain polymer of N-acetylglucosamine linked by β 1, 4 bonds, is one of the most abundant natural polysaccharides, especially in the exoskeleton of crustacea. Crustacean shell comprises composites of chitin, polypeptides or proteins, and calcium carbonate. Chitin in the shell has been reported to be in association with protein and carotenoid (Attwood and Zola, 1967; Austin et al., 1981; Fox, 1973). The degree of interaction between chitin/protein and chitin/carotenoid has been classified on the basis of the solubilization of proteins and the recovery of carotenoid from such complexes as: not bound; loosely bound by Van der Waal's forces; hydrogen bonded; and firmly (covalently) bonded (Hackman, 1972; Fox, 1973).

In the chitin-protein association, there are several potential sites on the polysaccharide polymer where covalent binding can occur. However, binding to these sites has not been confirmed (Lee, 1988). Nevertheless, the form of linkage between chitin and protein has not been characterized completely. Herzog et al. (1975) reported that proteins are covalently bound to chitin according to the categorization as aforementioned by Hackman (1972) in chitin-protein complex obtained from crayfish (Astacus fluviatilis) after a series of treatments with lithium rhodanide solution, urea solution, formic acid, pronase,

papain, and soda lye. The glycine content is especially notable in the chitin-protein complex treated with pronase and papain. However, Herzog et al. (1975) could not determine whether glycine contributes to the chitin-protein binding. Variations in percentage, i.e., 0.7 to 34.8 mol%, of the covalently bound amino acids of chitin isolates of various crustacea have been reported (Austin et al., 1981). The most prevalent amino acids found in residual chitin were aspartic acid, serine, and glycine, which could be involved in the covalent linkage between chitin and protein.

Austin et al. (1981) reported that the ratios of chitin to covalently bound protein, remaining after a series of acid and alkali treatments, varied greatly with particular species, i.e., from 3.1% of the dry shell in the red crab (Geryon quirauedons) to 27.9% in horseshoe crab (Limulus polyphemus). Comparable species-related variation also was obtained by Lee et al. (1984). The ratio of chitin to covalently bound protein varied from 2.1% in the crab (Charybdis bimaculata) to 9.9% in shrimp (Metapenaeus intermedius). Both groups of investigators concluded that each species has its own characteristic protein binding matrix and there was no simple relationship between chitin content and the amount of covalently bound protein in the chitin-protein complex.

Carotenoids in crustacea are associated with protein, calcium, and chitin by forming different chemical bonds

(Cheesman et al., 1967; Fox, 1972, 1973; Fox and Wilkie, 1970). In comparison with calcareous carotenoids and carotenoprotein, only limited studies on identification of the bonding form of chitinocarotenoids have been reported. Cheeseman et al. (1967) noted that all of the pigments are not recoverable from some crustacean chitinous material by usual simple solvent extraction, with and without protein denaturants, unless the shell has first been decalcified. After decalcification with aqueous solutions of citric acid or Na_2EDTA, astaxanthin pigment in red kelp crab (Taliepus nuttallii) was observed to be firmly associated with chitin itself (Fox, 1973).

Carotenoid stability depends on the processing method and storage conditions involved, i.e., excessive heat, exposure to light, and atmospheric oxygen (Meyers and Perkins, 1977). A series of investigations in the LSU Food Science Department has documented biochemical degradation of astaxanthin in dried crawfish meal and pigmented oil extract (Bligh, 1978; Chen and Meyers, 1982; Meyers and Bligh, 1981).

The objective of the study reported here was to examine covalent bonding between crawfish chitin and protein and to determine the retention of the astaxanthin pigment in crawfish chitin-carotenoid complex during drying and storage of the derived chitin.

MATERIALS AND METHODS

For convenience in presentation and description of the two different studies conducted, the following is arranged into two sections.

A. Isolation of a Chitin-Protein Complex

Sample Collection and Preparation

Live crayfish (Procambarus clarkii) were obtained from local seafood market. Whole animals were placed into heavy duty freezer bags, frozen, and stored at -20°C until used.

Prior to use, samples were thawed at room temperature. From the whole crawfish, the tail meat, intact cephalothorax, viscera and claw were removed using only the shell portion. This was washed with deionized water and then air-dried. The dried shell was ground through a Wiley Mill (Standard Model No. 3, Arthur H. Thomas Co., PA) with 2 mm mesh screen and sieved with 0.84 mm mesh screen using a portable sieve shaker (Model RX-8, the W.S. Tyler Co., Mentor. OH). This uniform sized shell was stored in opaque plastic bottles at room temperature.

Isolation of Protein Fractions in Chitin-Protein Complex

The procedure of Austin et al. (1981) was used to determine the protein binding type fractions present in the

chitin-protein complexes of crawfish shell. The dried sample was treated with a neutral, phosphate-buffered formalin solution saturated with disodium ethylenediaminetetraacetic acid (EDTA) at ambient temperature for 12 hrs. This treatment is very effective for decalcification of the crawfish shell and elimination of physically admixed and salt-bound protein. To remove the hydrogen bonded protein, the shell was extracted with 7 M urea. The series of extractions with NaOH were accomplished in different concentration of 0.01 N solution at ambient temperature for 5 hrs., 1.0 N at 50^{0}C for 6 hrs. and 1.0 N at 100^{0}C for 48 hrs. sequentially to remove the covalently bound protein fraction .

Protein Determination

To determine the protein content of the various binding type fractions in the chitin-protein complexes, Bio-Rad assay (Bradford, 1976) was used. Total protein content of crawfish shell was determined separately by extraction with 1 N NaOH for 48 hrs. The residual protein in chitin was calculated by subtracting the total weight losses in each extraction step as described above from the total protein content of the chitin-protein complex.

Amino Acid Analysis

The amount of residual protein remaining in the chitin

after the final alkali treatment was determined by amino acid analysis using the Pico-Tag method (Bidlingmeyer et al., 1984). The phenylthiocarbamyl amino acid (PTC-AA) mixture 25 µl was used for analysis on a Pico-Tag Amino Acid Analyzer (Waters, Milford, MA).

Chitin Determination

The process for isolation of crawfish chitin described by No (1987) was used for chitin determination.

B. Carotenoid Stability of a Chitin-Carotenoid Complex

Sample Collection and Preparation

Crawfish exoskeleton separated from heat-processed crawfish composite byproduct through a commercial vertical hammermill, was collected from Acadiana Processors, Inc. (Henderson, Louisiana). Material, placed into double black polyethylene bags to exclude light, was transported to the LSU Food Science Dept. and stored at -20^{0}C until used.

The frozen shell was thawed at room temperature, and washed with cold water to remove soluble organics and adherent protein. The excessive moisture was removed by filtration with vacuum. The shell was dried at ambient temperature in a commercial forced-air dryer to minimize the decomposition of carotenoids. The dried shell was

ground through a Wiley mill with 2 mm mesh screen, and sifted with a 20 mesh screen on a portable Tyler sieve shaker (Model RX-8) to obtain the homogeneous sample.

Isolation of Chitin-Carotenoid Complex

The chitin isolation procedure with three basic steps of demineralization, deproteinization, and decoloration (No, 1987) was slightly modified to isolate chitin-carotenoid complexes. This included elimination of the decoloration step with acetone and sodium hypochlorite solution. The schematic of the overall process is given in Figure 1.

Biochemical Degradation of Carotenoid in Chitin-Carotenoid Complex

Effect of Drying Condition: After removal of excessive moisture with vacuum filtration, the chitin-carotenoid complex was dried for periods up to 24 hrs. by oven-drying at 60 and 100^{0}C in a forced-air oven, and via sun-drying. Triplicated samples of each treatment were assayed for carotenoid content with time intervals of 0, 2, 4, 6, 8, 12, and 24 hours, respectively.

Effect of Temperature of Storage: Prior to storage, the sample was dried at ambient temperature in a commercial

forced-air dryer until the moisture content was less than 5%. The sample was divided into four parts, and each one stored at temperatures of -20, 4, 20, and 40°C. After 1, 2, 3, and 4 weeks, samples were analyzed to determine the carotenoid retention based on its initial concentration.

Carotenoid Determination

To determine the extractable astaxanthin content of the chitin-carotenoid complexes, the mixed solvent procedure of Lee (1985) was adapted using the formula of Kelly and Harmon (1972).

Formula

$$\text{gm astaxanthin/gm crawfish meal} = AD/100\ G\ d\ E^{1\%}_{1cm}$$

where A = absorbance at maximum wavelength (467 nm in petroleum ether), D = dilution volume (ml), G = crawfish meal (gm), d = cell width (1 cm), and $E^{1\%}_{1cm}$ = 2400 in petroleum ether.

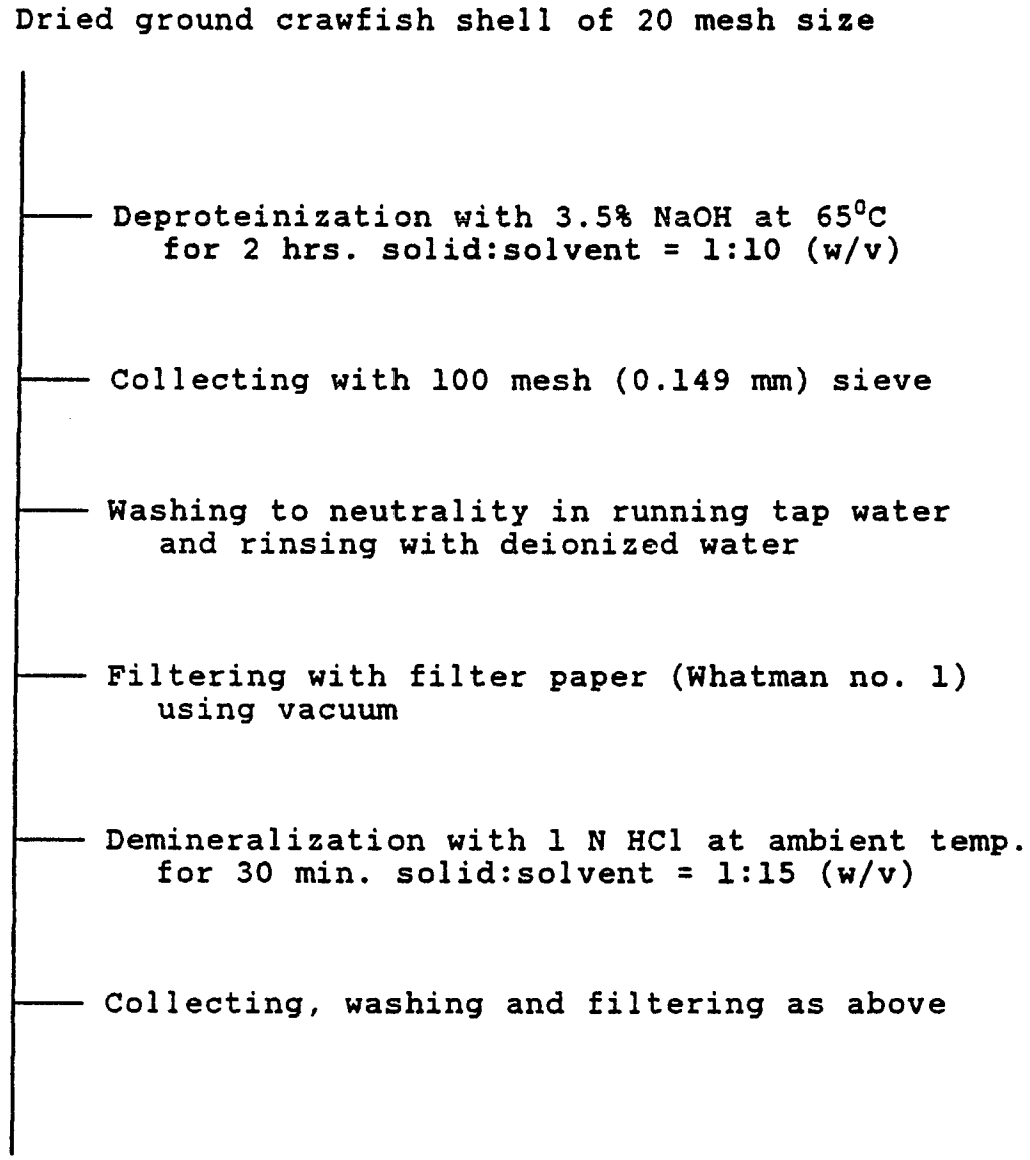

Figure 1. Flow chart of modified procedure of chitin isolation of crawfish shell (No, 1987)

Results and Discussion

Isolation of chitin-protein complex

The results of the protein fractionation are shown in Table 1. A series of successive hydrolytic treatments was followed to identify chemical bonds present in the chitin-protein complex. In crawfish, 10.3% of the protein is covalently bound to chitin and the remaining 89.7% is in the form of ionically and hydrogen-bonded protein. Compared with data reported by Austin et al. (1981) and Lee et al. (1984), crayfish (Procambarus clarkii) showed the lowest value of 0.7% in covalently bound protein, but exhibited the highest value of 2.5% in residual chitin protein. The highest value of 27.9% for covalently bound protein was in horseshoe crab. Data on total content of chitin in crawfish shell, the total protein, and the ratio of chitin to bound protein are presented in Table 2. Compared with other crustaceans, significant differences exist in the ratio of chitin to bound protein, demonstrating the characteristic protein binding matrix of different species.

The quantitative analysis of covalently bound amino acids of the chitin-protein complex is given in Table 3. Herzog et al. (1975) reported that hydrolysis with 6N HCl at 110^{0}C for 24 hr. is sufficient to release all amino acids from chitin-protein complex. In crawfish, serine was the

major amino acid, followed by histidine. In contrast, glutamic acid, aspartic acid, and glycine were the major amino acids in Astacus fluviatilis. Significantly higher percentages of serine, i.e., 76.6% of the total amino acids, are noticeable. Comparable results in the level of serine found were obtained by Lee (1990). However, No (1987) reported that tyrosine was the prevalent amino acid in crawfish chitin. No clear explanation of these differences are evident except possibly for the differences in the analytical instruments used, i.e., HPLC and amino acid analyzer. Even though serine is one of amino acids acting as a bridge between polysaccharide and protein chains (Herzog et al., 1975), it cannot be determined whether serine contributes to a chitin-protein binding. There is a strong possibility that protein is bound by covalent bonds to the crawfish chitin through the serine residue. The total amount of amino acids in crawfish chitin-protein complex is relatively lower than that reported for stone crab, red crab, and brine shrimp (Austin et al., 1981).

Effect of drying temperature on carotenoid retention in crawfish chitin-carotenoid complex

Drying temperature had a considerable effect on the astaxanthin stability (Table 4). In oven-dried samples, increasing drying time to 24 hr at 60°C and 100°C resulted

in notable losses in astaxanthin, 39% and 68%, respectively. Results with sun-drying, 44%, were comparable to those obtained with oven-drying at 60°C. The highest retention of pigment in chitin-carotenoid complex was with oven-drying at 60°C, followed by sun-drying and oven-drying at 100°C. The complex was dried to less than 5% moisture with oven-drying at 60°C and sun-drying for 6 hr, and oven-drying at 100°C for 4 hr, representing 74%, 65%, and 68% astaxanthin retention, respectively. Although sun-drying gave comparable results in pigment retention, oven-drying is recommended to minimize the deleterious effect of exposure to light as well as heat.

Effect of storage time at various temperatures

Results of pigment stability of the chitin-carotenoid complex are given in Figure 2. Apparent differences were observed in storage time and at various temperatures. As storage temperature increased, the rate of astaxanthin pigment retention decreased. At high storage temperatures, pigment decomposition rate is considerably higher. As much as 38% astaxanthin in the complex was decomposed within 4 weeks storage at 40°C. The highest retention of astaxanthin, 94%, based on initial concentration of pigment, was obtained at -20°C, whereas the lowest was 62% retention at 40°C. Comparable results in storage of crawfish meal, with and without antioxidant treatments,

were reported by Meyers and Bligh (1981). The addition of 1% butylated hydroxyanisole (BHA) and Santoquin in crawfish meal at 50°C showed 44% and 53% retention of astaxanthin, respectively, after 3 weeks of storage. A lower temperature of storage appears to be more effective in pigment retention than antioxidant treatments at a high temperature of 50°C. No apparent differences was seen between storages at 40° and 50°C without antioxidant treatment.

Table 1. Fraction of chitin-protein complexes of crayfish (Procambarus clarkii)

Total* protein (%)	Physical association (%)**		Covalently bound protein (%)**			
	EDTA 20°C 12 hr	7M Urea 20°C 48 hr	0.01N NaOH 20°C 5 hr	1N NaOH 50°C 6 hr	1N NaOH 100°C 48 hr	Residual in chitin
6.5	88.9	0.8	0.1	4.8	2.9	2.5

* Total protein in dry shell was determined independently by extraction with 1N NaOH for 48 hr.

** Percent of weight loss after each extraction/total weight loss

Table 2. Percentages of chitin and protein content of dry crawfish shell

Chitin	Total protein	Covalently bound protein*	Ratio of chitin to bound protein
23.5	6.5	0.7	33.6 to 1

* Total covalently bound protein (Table 1) as percent of total dry shell

Table 3. Total amino acid composition of chitin-protein complexes of crayfish (*Procambarus clarkii* and *Astacus fluviatilis*[*])

Composition	Content (mg/g)	
	Procambarus	Astacus
Arginine	0.6	5.4
Histidine	10.5	3.7
Isoleucine	0.2	8.7
Leucine	-	15.2
Lysine	0.4	4.9
Methionine	0.2	17.9
Cystine	0.2	-
Phenylalanine	-	14.5
Tyrosine	2.5	8.8
Threonine	2.4	10.3
Valine	0.9	11.9
Aspartic acid	0.2	23.8
Serine	67.1	10.8
Proline	1.9	12.5
Glutamic acid	0.2	26.7
Alanine	0.3	12.7
Glycine	-	19.8
Total	87.6	207.6

[*] Herzog et al. (1975)

Table 4. Effect of drying temperature on astaxanthin level in crawfish chitin-carotenoid complex

Drying time (hr)	Drying temperature		
	60°C	100°C	Sun-dried
	Astaxanthin conc. (mg/Kg)*		
0	114	114	114
2	96	78	87
4	89	64	78
6	84	54	74
8	76	48	74
12	72	44	71
24	69	37	64

* On a dry weight basis

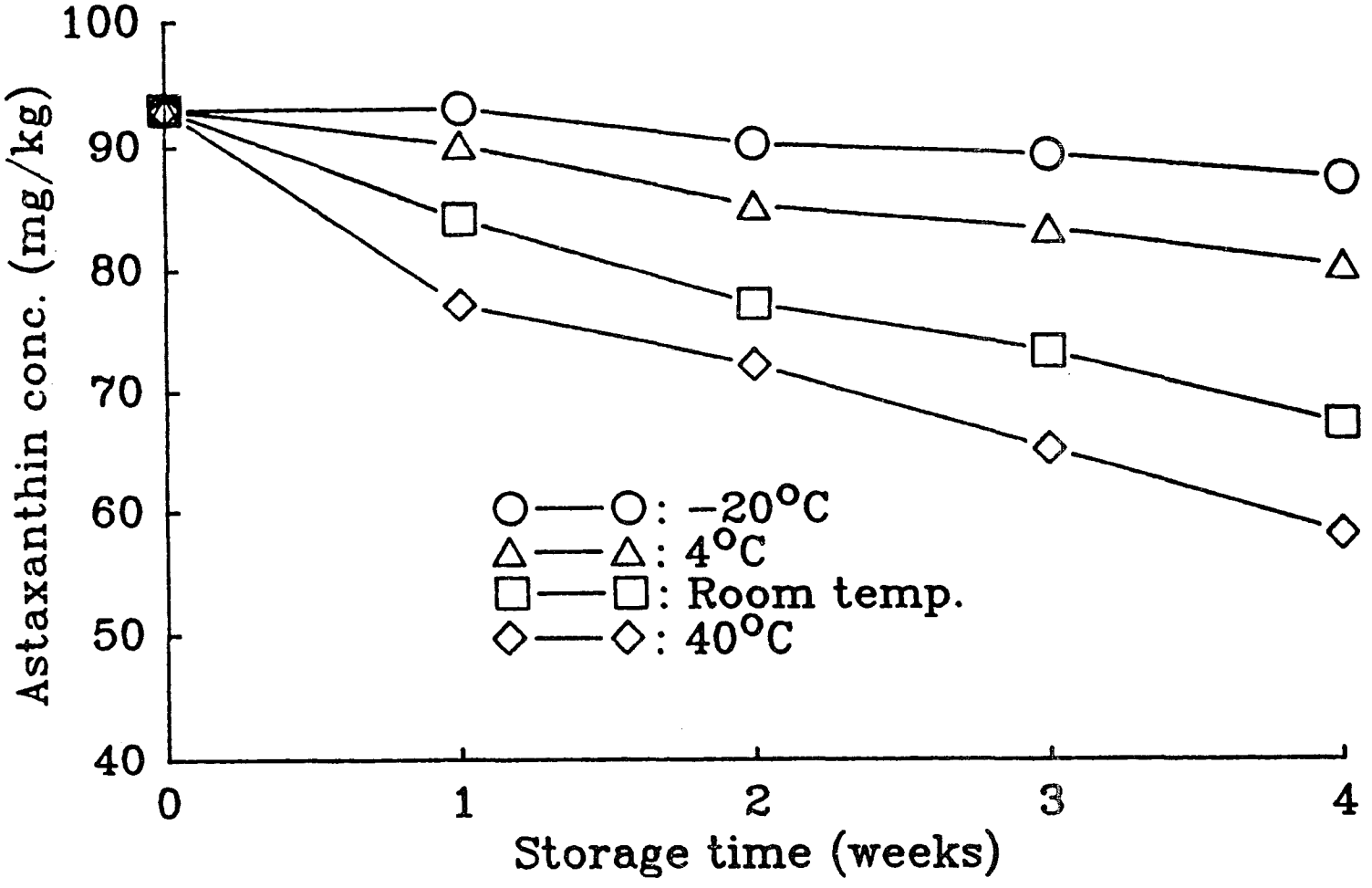

Figure 2. Effect of storage time at various temperatures on stability of the chitin-carotenoid complex

CHAPTER V

Summary and Conclusions

This investigation, as well as previous studies by the LSU Food Science Department, illustrates the magnitude and potential of utilization of crawfish processing composite byproduct, rich in astaxanthin pigment, protein, and chitin. As emphasized at a recent international conference (Meyer et al.,1990), fishery processing byproducts should not be considered as discardable waste but rather as sources of potentially valuable items of commerce. A total composite byproduct recovery and utilization schematic has been proposed (Figure 1), facets of which already are part of a commercial industry in Louisiana. The pigment recovery aspect has been documented by Meyers and Chen (1985) and in the other publication cited throughout this dissertation.

Meyers et al. (1990) have summarized these studies and have emphasized the importance of basic research dealing with composition of crawfish shell and meal, preservation of composite byproduct using acid ensilage techniques, recovery of natural pigment, utilization of crawfish astaxanthin in animal feed, isolation and application of crawfish chitin/chitosan, and identification of volatile flavor compounds in the separated byproduct. This latter

aspect is well documented in two flavor-related publications from the LSU Food Science Department (Tanchotikul and Hsieh, 1989; Vejakhan et al., 1988). A total of 70 volatile compounds have been characterized in the tailmeat, and over 117 identified in the processing composite byproduct. Some of the more desirable aromas may be contributed by the claw meat component of the separated byproduct. In any event, careful selection and recovery of important authentic crawfish flavor components from the abundant byproduct resource may further enhance its composite economic value.

The present investigation has focused on the biological evaluation of both the crawfish chitin/carotenoid complex and the whole meal in broiler diets. These compounds could serve as potential sources of growth promoters, a red pigmenter to intensify broiler skin and shank color and as a protein supplement. Further work also has involved basic studies of the covalent bonding between chitin and protein and the stability of astaxanthin in the chitin/carotenoid complex. The following summary statements covering results from the broad scope of this dissertation can be listed as following:

Chapter II

1. Crawfish shell is a excellent source of chitin, representing 25.80% of the dry weight.

2. Crawfish chitin, isolated from shell without decoloration, contains relatively high concentrations, 104 mg/kg, of astaxanthin compared with 84 mg/kg presented in the whole meal.
3. Physicochemical characteristics of crawfish chitin are as follows: nitrogen (7.04%), fat (0.04%), ash (0.16%), acetyl value (19.24%), deacetylation (9.20%), residual amino acids (26.02 mg/g), color (red-pink), and yield (25.80%).
4. The predominant amino acid in chitin is serine, whereas tyrosine is the major amino acid in the composite crawfish shell.
5. Based on overall performance of broilers at 7 weeks of age, the diet treatments had a highly significant effect ($p<0.01$) on overall composite body weight. The group on the corn-soy control diet and the 2.5% and 1% chitin diet gave comparable results. The lowest body weight was obtained with the 5% cellulose diet, followed by the 0.5% and 5% chitin diet, respectively.
6. No significant effect ($p>0.05$) of chitin on feed conversion rate was noted at 4 weeks of age, but was seen after 7 weeks growth of the broilers.
7. No differences in feathering were observed between the test and control groups.
8. Chitinase activity itself was not notably enhanced in either the gizzard or gut by the addition of increasing

amounts of chitin in the diet. However, the group of broilers fed 2.5% chitin, showed the best feed conversion rate and body weight among groups fed experimental diets, and gave the highest enzymatic activity in the gizzard and gut in both male and female birds. Birds fed 5% cellulose diet, which gave the poorest results in terms of overall performance, also showed the lowest average chitinase activity, even less than that of the control diet without cellulose and chitin.

9. Significantly, no deleterious or adverse effect was found by addition of any level of chitin to the diet, whereas, 5% cellulose alone in the diet depressed growth of birds.
10. In broiler pigmentation, "a" value increased as the amount of astaxanthin increased in experimental diets No. 3-6, while "L" and "b" were steady in both skin and shank. No significant differences ($p>0.05$) were observed between male and female birds in all three values of L, a, b, and visual score, except for L in the shank.
11. The addition of astaxanthin at concentrations as low as 0.54 mg/kg of feed increased two-fold the shank visual score of the control and 5% cellulose diets, containing 57.06% and 43.94%, respectively.

Chapter III

12. The quality of crawfish whole meal was improved by physical separation. With decreased particle size, percentage of protein and fat increased, while fiber and ash decreased. Sieved crawfish meal (35 mesh screen), selected for processing, showed a significant percentage improvement in meal quality compared with whole course meal, i.e., 170% increase in corrected protein,, 135% increase in crude protein, 17% increase in total amino acids, 23% decrease in calcium, 24% in ash, and 27% in chitin. The concentration of astaxanthin was 83 mg/kg.
13. Major amino acids in sieved meal were glutamic acid (23.6 mg/g) and serine (20.4%). Compared with other crustacean meals, this level of serine was particularly noteworthy.
14. Sieved crawfish meal shows a essential amino acid pattern compared with that of shrimp and crab meals. The limiting amino acids in order are cystine, methionine plus cystine, and lysine.
15. No significant differences ($p>0.05$) in the performance of broiler chicken body weight, feed efficiency, and PER were found based on the different addition rates of crawfish meal. The performance of broilers on crawfish meal was comparable to those on the control diet with soybean meal supplementation.

16. Results indicated that the maximal level of crawfish meal in broiler diets should be less than 20%, due to the lower metabolic energy present, i.e., 363 Kcal/Ib.
17. When the amount of crawfish meal in the diet was increased, the value of "a" and "b" increased, while "L" decreased in both skin and shank. Highly significant differences ($p<0.01$) were seen in all three values of L, a, b, and visual score. No significant difference ($p>0.05$) was seen between sexes, except in "a" value.
18. The addition of astaxanthin in concentrations as low as 0.83 mg/kg in 1% crawfish meal, increased the shank score from 1.0 to 2.2 in males and to 1.8 in females, without supplemental xanthophyll addition.
19. No apparent relation between shank and skin pigmentation was found, but the overall response by the crawfish-supplemental diets in affecting color change was comparable.

Chapter IV

20. The total levels of protein, chitin, and covalently bound chitin/protein in crawfish shell were 6.5%, 23.5%, and 0.7%, respectively.
21. In the chitin/protein complex, the major amino acid was serine, followed by histidine. A notably higher percentage of serine, 76.6% of total amino acids, was

present.

22. Increasing drying time of the chitin/carotenoid complex to 24 hr at 60°C and 100°C, and sun-drying resulted in losses of astaxanthin of 39.9% and 67.6%, and 43.9%, respectively.
23. At different storage temperatures of -20°C, 4°C, room temperature, and 40° for 4 weeks, the percentage retention of astaxanthin in the chitin/carotenoid complex was 94%, 86%, 72%, and 62%, respectively, compared with those with an initial pigment concentration of 93 mg/kg.

In summary, the research has used broilers as a target test animal to analyze biochemical features of selected crawfish-derived products and their overall physiological effect on animal growth and pigmentation. However, the implications involved are considerably greater, as demonstrated by the current utilization of crawfish astaxanthin in specific commercial finfish aquaculture. Further interest certainly will be shown in the chitin-related aspects of this research as worldwide attention to industrial application of the biopolymers chitin and chitosan increases. Additional work is needed on the physicochemical characteristics of both the complexes of chitin/carotenoid and chitin/protein. This has been demonstrated in the present study, as well as in the

previous research of the investigator (Lee, 1985).

Clearly, the term "waste" as applied to seafood processing byproducts in general is no longer applicable. Similar observations, recently noted by Jaswal (1990) and Shiau and Chai (1990), are being made throughout the seafood processing industry.

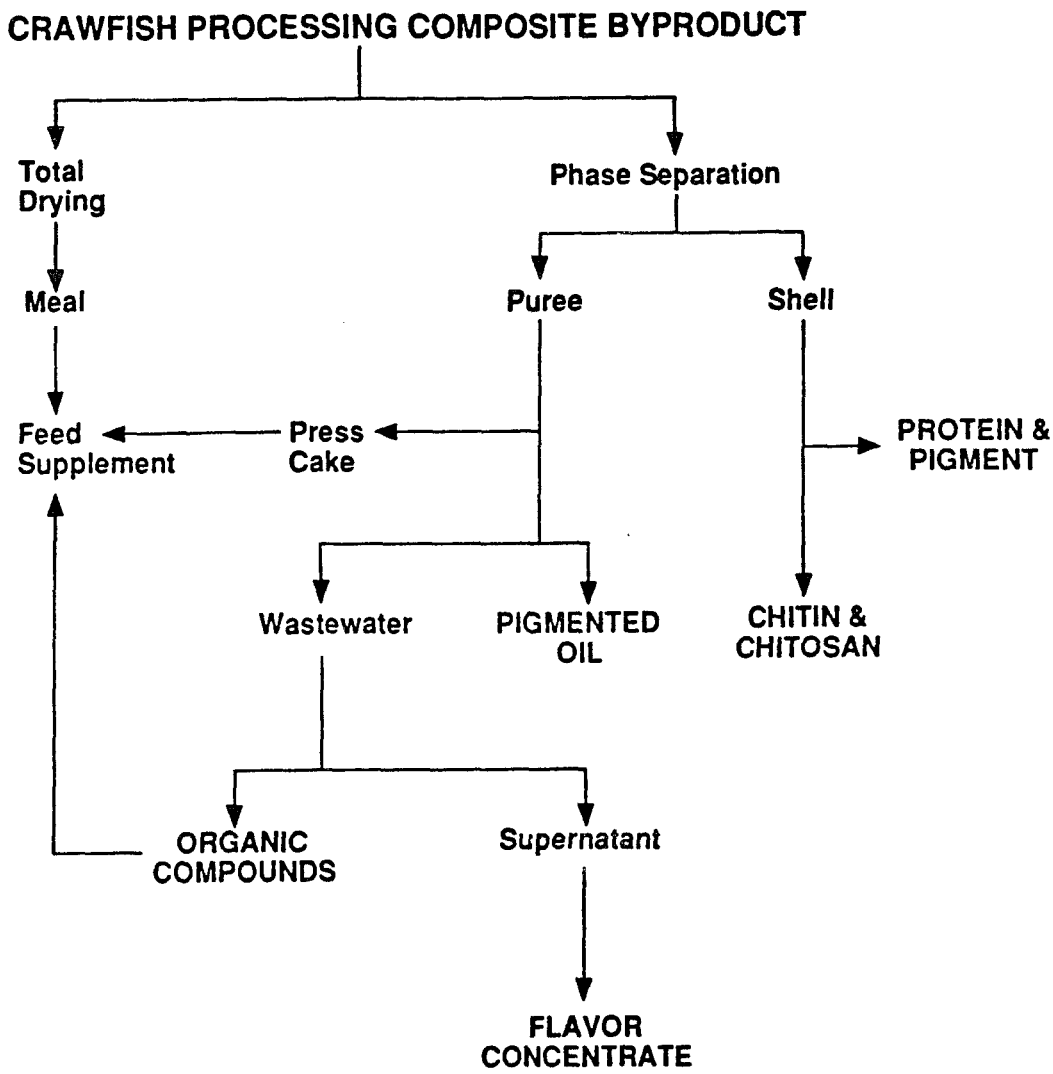

Figure 1. Total utilization of crawfish composite byproduct.

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APPENDIX

Chapter II

Appendix Table 1. ANOVA of growth rates of broilers

Time: 0 WK

Source	DF	SS	PR > F
Block	1	0.0833	0.6109
Treatment	5	0.7500	0.7490
Error	5	1.4167	
Corrected Total	11	2.2500	

Time: 1 WK

Source	DF	SS	PR > F
Block	1	0.0000	1.0000
Treatment	5	52.6667	0.7753
Error	5	108.0000	
Corrected Total	11	160.6667	

Time: 2 WK

Source	DF	SS	PR > F
Block	1	52.0833	0.3502
Treatment	5	925.4167	0.0858
Error	5	245.4167	
Corrected Total	11	1222.9167	

Time: 3 WK

Source	DF	SS	PR > F
Block	1	768.0000	0.1366
Treatment	5	4832.6667	0.0789
Error	5	1223.0000	
Corrected Total	11	6823.6667	

Appendix Table 1. (Continued) ANOVA of growth rates of broiers

Time: 4 WK

Source	DF	SS	PR > F
Block	1	11408.3333	0.0102
Treatment	5	22140.6667	0.0330
Error	5	3550.6667	
Corrected Total	11	37099.6667	

Time: 5 WK

Source	DF	SS	PR > F
Block	1	13668.7500	0.1056
Treatment	5	43291.7500	0.1723
Error	5	17561.7500	
Corrected Total	11	74522.2500	

Time: 6 WK

Source	DF	SS	PR > F
Block	1	11285.3333	0.0235
Treatment	5	53598.6667	0.0127
Error	5	5445.6667	
Corrected Total	11	70329.6667	

Time: 7 WK

Source	DF	SS	PR > F
Block	1	2002.0833	0.0616
Treatment	5	47059.4167	0.0013
Error	5	1737.4167	
Corrected Total	11	50798.9167	

Appendix Table 2. ANOVA of the effect of crawfish chitin on performance of broilers

Time: 4 WK Weight

Source	DF	SS	PR > F
Block	1	11408.3333	0.0102
Treatment	5	22140.6667	0.0330
Error	5	3550.6667	
Corrected Total	11	37099.6667	

Time: 7 WK Weight

Source	DF	SS	PR > F
Block	1	2002.0833	0.0616
Treatment	5	47059.4167	0.0013
Error	5	1737.4167	
Corrected Total	11	50798.9167	

Time: 4 WK Feed Conversion

Source	DF	SS	PR > F
Block	1	0.2187	0.0509
Treatment	5	0.2053	0.4139
Error	5	0.1673	
Corrected Total	11	0.5913	

Time: 7 WK Feed Conversion

Source	DF	SS	PR > F
Block	1	0.7400	0.0001
Treatment	5	0.9216	0.0004
Error	5	0.0208	
Corrected Total	11	1.6824	

Appendix Table 3. ANOVA of Lightness (L), redness (a), and yellowness (b) values and visual scores of skin[1] and shank[2] of male (M) and female (F) broilers fed chitin diet at 7 weeks of age

SKIN Lightness (L)

Source	DF	SS	PR > F
Block	1	0.0578	0.8701
Treatment	5	589.4598	0.0001
Block X Trt	5	8.2378	0.5760
Sex	1	14.4364	0.0124
Sex X Trt	5	29.5471	0.0285
Sex X Trt(Block)	6	23.1741	0.1180
Error	48	102.6920	
Corrected Total	71	767.6050	

Tests of hypotheses using the type III MS for Block X Trt as an error term

Block	1	0.0578	0.8588
Treatment	5	589.4598	0.0001

Tests of hypotheses using the type III MS for Sex X Trt(Block) as an error term

Sex	1	14.4364	0.1014
Sex X Trt	5	29.5471	0.3077

Appendix Table 3. (Continued) ANOVA of Lightness (L), redness (a), and yellowness (b) values and visual scores of skin[1] and shank[2] of male (M) and female (F) broilers at 7 weeks of age

SKIN Redness (a)

Source	DF	SS	PR > F
Block	1	0.4513	0.3226
Treatment	5	229.1097	0.0001
Block X Trt	5	3.0359	0.2622
Sex	1	0.1233	0.6037
Sex X Trt	5	3.6104	0.1787
Sex X Trt(Block)	6	0.7686	0.9420
Error	48	21.6870	
Corrected Total	71	258.7862	
Tests of hypotheses using the type III MS for Block X Trt as an error term			
Block	1	0.4513	0.4280
Treatment	5	229.1097	0.0001
Tests of hypotheses using the type III MS for Sex X Trt(Block) as an error term			
Sex	1	0.1233	0.3644
Sex X Trt	5	3.6104	0.0287

Appendix Table 3. (Continued) ANOVA of Lightness (L), redness (a), and yellowness (b) values and visual scores of skin[1] and shank[2] of male (M) and female (F) broilers at 7 weeks of age

SKIN Yellowness (b)

Source	DF	SS	PR > F
Block	1	0.5134	0.6585
Treatment	5	8.0907	0.6825
Block X Trt	5	16.9007	0.2787
Sex	1	3.5734	0.2464
Sex X Trt	5	7.9518	0.6905
Sex X Trt(Block)	6	6.8069	0.8503
Error	48	124.5638	
Corrected Total	71	168.4007	
Tests of hypotheses using the type III MS for Block X Trt as an error term			
Block	1	0.5134	0.7128
Treatment	5	8.0907	0.7810
Tests of hypotheses using the type III MS for Sex X Trt(Block) as an error term			
Sex	1	3.5734	0.1263
Sex X Trt	5	7.9518	0.3429

Appendix Table 3. (Continued) ANOVA of Lightness (L), redness (a), and yellowness (b) values and visual scores of skin[1] and shank[2] of broilers at 7 weeks of age

SHANK Lightness (L)

Source	DF	SS	PR > F
Block	1	2.2720	0.2885
Treatment	5	401.1331	0.0001
Block X Trt	5	35.2503	0.0079
Sex	1	5.5057	0.1018
Sex X Trt	5	10.8830	0.3709
Sex X Trt(Block)	6	3.2042	0.9479
Error	48	94.6615	
Corrected Total	71	552.9098	
Tests of hypotheses using the type III MS for Block X Trt as an error term			
Block	1	2.2720	0.5948
Treatment	5	421.1331	0.0092
Tests of hypotheses using the type III MS for Sex X Trt(Block) as an error term			
Sex	1	0.5057	0.0183
Sex X Trt	5	10.8830	0.0584

Appendix Table 3. (Continued) ANOVA of Lightness (L), redness (a), and yellowness (b) values and visual scores of skin[1] and shank[2] of broilers at 7 weeks of age

SHANK Redness (a)

Source	DF	SS	PR > F
Block	1	0.1043	0.5984
Treatment	5	169.6388	0.0001
Block X Trt	5	1.4626	0.3631
Sex	1	0.0027	0.9325
Sex X Trt	5	1.2357	0.6508
Sex X Trt(Block)	6	0.9571	0.8553
Error	48	17.8057	
Corrected Total	71	191.2069	
Tests of hypotheses using the type III MS for Block X Trt as an error term			
Block	1	0.1043	0.5765
Treatment	5	169.6388	0.0001
Tests of hypotheses using the type III MS for Sex X Trt(Block) as an error term			
Sex	1	0.0027	0.9009
Sex X Trt	5	1.2357	0.3028

Appendix Table 3. (Continued) ANOVA of Lightness (L), redness (a), and yellowness (b) values and visual scores of skin[1] and shank[2] of broilers at 7 weeks of age

SHANK Yellowness (b)

Source	DF	SS	PR > F
Block	1	14.9605	0.1005
Treatment	5	307.8054	0.0001
Block X Trt	5	17.4566	0.8248
Sex	1	1.9536	0.6256
Sex X Trt	5	7.6213	0.9657
Sex X Trt(Block)	6	35.2578	0.5691
Error	48	388.8677	
Corrected Total	71	777.9230	

Tests of hypotheses using the type III MS for Block X Trt as an error term

Block	1	14.9605	0.0932
Treatment	5	307.8054	0.0034

Tests of hypotheses using the type III MS for Sex X Trt(Block) as an error term

Sex	1	1.9536	0.6045
Sex X Trt	5	7.6213	0.9343

Appendix Table 3. (Continued) ANOVA of Lightness (L), redness (a), and yellowness (b) values and visual scores of skin[1] and shank[2] of broilers at 7 weeks of age

SHANK Visual score

Source	DF	SS	PR > F
Block	1	0.0000	1.0000
Treatment	5	82.2778	0.0001
Block X Trt	5	0.0000	1.0000
Sex	1	0.5000	0.1402
Sex X Trt	5	0.5000	0.8112
Sex X Trt(Block)	6	0.0000	1.0000
Error	48	10.6667	
Corrected Total	71	93.9444	

Tests of hypotheses using the type III MS for Block X Trt as an error term

Block	1	0.0000
Treatment	5	82.2778

Tests of hypotheses using the type III MS for Sex X Trt(Block) as an error term

Sex	1	0.5000
Sex X Trt	5	0.5000

Chapter III

Appendix Table 1. ANOVA of growth rates of broilers

Time: 0 WK

Source	DF	SS	PR > F
Block	1	0.0008	0.9518
Treatment	5	1.3742	0.3814
Error	5	1.0342	
Corrected Total	11	2.4092	

Time: 1 WK

Source	DF	SS	PR > F
Block	1	31.0408	0.5535
Treatment	5	395.4242	0.4890
Error	5	385.3042	
Corrected Total	11	811.7692	

Time: 2 WK

Source	DF	SS	PR > F
Block	1	669.0133	0.1553
Treatment	5	2101.8767	0.2755
Error	5	1195.9367	
Corrected Total	11	3966.8267	

Time: 3 WK

Source	DF	SS	PR > F
Block	1	533.3333	0.5283
Treatment	5	6921.6100	0.4264
Error	5	5813.4467	
Corrected Total	11	13268.3900	

Appendix Table 1. (Continued) ANOVA of growth rates of broilers

Time: 4 WK

Source	DF	SS	PR > F
Block	1	1469.6533	0.2536
Treatment	5	4977.9467	0.4494
Error	5	4417.2367	
Corrected Total	11	10864.8367	

Time: 5 WK

Source	DF	SS	PR > F
Block	1	11982.7200	0.0724
Treatment	5	22180.7567	0.2477
Error	5	11625.5600	
Corrected Total	11	45789.0367	

Time: 6 WK

Source	DF	SS	PR > F
Block	1	16747.7408	0.1924
Treatment	5	27008.7042	0.6301
Error	5	36927.8842	
Corrected Total	11	80684.3292	

Time: 7 WK

Source	DF	SS	PR > F
Block	1	16702.9408	0.1587
Treatment	5	7689.3642	0.9215
Error	5	30465.2242	
Corrected Total	11	54857.5292	

Appendix Table 2. ANOVA of the effect of crawfish meal on performance of broilers

Time: 4 WK Weight

Source	DF	SS	PR > F
Block	1	2926.0417	0.0328
Treatment	5	9975.5483	0.0345
Block X Trt	5	8844.1083	0.0450
Sex	1	205757.2017	0.0001
Sex X Trt	5	1098.5083	0.7217
Error	6	2303.8300	
Corrected Total	23	230905.2383	

Tests of hypotheses using the type III MS for Block X Trt as an error term

Block	1	2926.0417	0.0328
Treatment	5	9975.5483	0.0345

Appendix Table 2. (Continued) ANOVA of the effect of crawfish meal on performance of broilers

Time: 7 WK Weight

Source	DF	SS	PR > F
Block	1	44229.9204	0.0020
Treatment	5	13173.5670	0.2900
Block X Trt	5	50418.9771	0.0237
Sex	1	885235.2704	0.0001
Sex X Trt	5	5961.4870	0.6294
Error	6	9870.2275	
Corrected Total	23	1008889.4496	

Tests of hypotheses using the type III MS for Block X Trt as an error term

Block	1	44229.9204	0.0904
Treatment	5	13173.5670	0.9165

Appendix Table 2. (Continued) ANOVA of the effect of crawfish meal on performance of broilers

Time: 4 WK Feed Conversion

Source	DF	SS	PR > F
Block	1	0.1148	0.1737
Treatment	5	0.1235	0.7601
Block X Trt	5	0.2263	0.5176
Sex	1	0.1908	0.0938
Sex X Trt	5	0.1945	0.5840
Error	6	0.2893	
Corrected Total	23	1.1392	

Tests of hypotheses using the type III MS for Block X Trt as an error term

Block	1	0.1148	0.1721
Treatment	5	0.1235	0.7388

Appendix Table 2. (Continued) ANOVA of the effect of crawfish meal on performance of broilers

Time: 7 WK Feed Conversion

Source	DF	SS	PR > F
Block	1	0.0081	0.0770
Treatment	5	0.0052	0.7142
Block X Trt	5	0.0217	0.1536
Sex	1	0.0024	0.2891
Sex X Trt	5	0.0100	0.4387
Error	6	0.0107	
Corrected Total	23	0.0579	

Tests of hypotheses using the type III MS for Block X Trt as an error term

Block	1	0.0081	0.2308
Treatment	5	0.0052	0.9288

Appendix Table 3. ANOVA of protein efficiency ratio (PER) assay of crawfish meal at 4 weeks old broilers

Source	DF	SS	PR > F
Block	1	0.0260	0.0204
Treatment	5	0.0422	0.0961
Block X Trt	5	0.0924	0.0177
Sex	1	0.1247	0.0005
Sex X Trt	5	0.0106	0.5908
Error	6	0.0160	
Corrected Total	23	0.3119	
Tests of hypotheses using the type III MS for Block X Trt as an error term			
Block	1	0.0260	0.2888
Treatment	5	0.0422	0.7947

Appendix Table 4. ANOVA of Lightness (L), redness (a), and yellowness (b) values of skin of broilers at 7 weeks of age

SKIN Lightness (L)

Source	DF	SS	PR > F
Block	1	0.3003	0.7369
Treatment	5	884.2342	0.0001
Block X Trt	5	7.1328	0.7433
Sex	1	5.0297	0.1732
Sex X Trt	5	19.6251	0.2101
Sex X Trt(Block)	6	12.9229	0.5609
Error	48	123.2821	
Corrected Total	71	1055.5272	

Tests of hypotheses using the type III MS for Block X Trt as an error term

Block	1	0.3003	0.1773
Treatment	5	884.2342	0.2428

Tests of hypotheses using the type III MS for Sex X Trt(Block) as an error term

Sex	1	5.0297	0.1773
Sex X Trt	5	19.6251	0.2428

Appendix Table 4. (Continued) ANOVA of Lightness (L), redness (a), and yellowness (b) values of skin of broilers at 7 weeks of age

SKIN Redness (a)

Source	DF	SS	PR > F
Block	1	0.1284	0.6415
Treatment	5	321.3038	0.0001
Block X Trt	5	1.3160	0.8110
Sex	1	7.6441	0.0007
Sex X Trt	5	2.8131	0.4501
Sex X Trt(Block)	6	0.6046	0.9830
Error	48	28.0575	
Corrected Total	71	361.8675	

Tests of hypotheses using the type III MS for Block X Trt as an error term

Block	1	0.1284	0.5161
Treatment	5	321.3038	0.0001

Tests of hypotheses using the type III MS for Sex X Trt(Block) as an error term

Sex	1	7.6441	0.0001
Sex X Trt	5	2.8131	0.0294

Appendix Table 4. (Continued) ANOVA of Lightness (L), redness (a), and yellowness (b) values of skin of broilers at 7 weeks of age

SKIN Yellowness (b)

Source	DF	SS	PR > F
Block	1	3.5200	0.2173
Treatment	5	160.6408	0.0001
Block X Trt	5	4.7300	0.8325
Sex	1	4.9718	0.1439
Sex X Trt	5	8.0144	0.6177
Sex X Trt(Block)	6	21.4895	0.1706
Error	48	108.1198	
Corrected Total	71	311.4864	

Tests of hypotheses using the type III MS for Block X Trt as an error term

Block	1	3.5201	0.1116
Treatment	5	160.6408	0.0007

Tests of hypotheses using the type III MS for Sex X Trt(Block) as an error term

Sex	1	4.9718	0.2833
Sex X Trt	5	8.0144	0.8023

Appendix Table 4. (Continued) ANOVA of Lightness (L), redness (a), yellowness (b) values and visual scores of shank of broilers at 7 weeks of age

SHANK Lightness (L)

Source	DF	SS	PR > F
Block	1	11.5200	0.0960
Treatment	5	392.7712	0.0001
Block X Trt	5	43.8726	0.0702
Sex	1	6.7100	0.2013
Sex X Trt	5	10.6612	0.7498
Sex X Trt(Block)	6	9.7417	0.8713
Error	48	191.8548	
Corrected Total	71	667.1315	

Tests of hypotheses using the type III MS for Block X Trt as an error term

Block	1	11.5200	0.3037
Treatment	5	392.7712	0.0156

Tests of hypotheses using the type III MS for Sex X Trt(Block) as an error term

Sex	1	6.7100	0.0883
Sex X Trt	5	10.6612	0.3702

Appendix Table 5. ANOVA of Lightness (L), redness (a), yellowness (b) values and visual scores of shank of broilers at 7 weeks of age

SHANK Redness (a)

Source	DF	SS	PR > F
Block	1	0.0378	0.7846
Treatment	5	165.2808	0.0001
Block X Trt	5	1.1060	0.8170
Sex	1	0.1050	0.6490
Sex X Trt	5	2.0378	0.5455
Sex X Trt(Block)	6	1.6971	0.7560
Error	48	24.0298	
Corrected Total	71	194.2944	

Tests of hypotheses using the type III MS for Block X Trt as an error term

Block	1	0.0378	0.6964
Treatment	5	165.2808	0.0001

Tests of hypotheses using the type III MS for Sex X Trt(Block) as an error term

Sex	1	0.1050	0.5646
Sex X Trt	5	2.0378	0.3317

Appendix Table 5. (Continued) ANOVA of Lightness (L), redness (a), yellowness (b) values and visual scores of shank of broilers at 7 weeks of age

SHANK Yellowness (b)

Source	DF	SS	PR > F
Block	1	2.6523	0.6429
Treatment	5	408.5851	0.0001
Block X Trt	5	28.6613	0.7964
Sex	1	0.0003	0.9866
Sex X Trt	5	24.5822	0.8440
Sex X Trt(Block)	6	33.1695	0.8388
Error	48	584.9695	
Corrected Total	71	1082.6238	

Tests of hypotheses using the type III MS for Block X Trt as an error term

Block	1	2.6528	0.5266
Treatment	5	408.5851	0.0056

Tests of hypotheses using the type III MS for Sex X Trt(Block) as an error term

Sex	1	0.0035	0.9808
Sex X Trt	5	24.5822	0.5415

Appendix Table 5. (Continued) ANOVA of Lightness (L), redness (a), yellowness (b) values, and visual scores of shank of broilers at 7 weeks of age

SHANK Visual score

Source	DF	SS	PR > F
Block	1	0.1250	0.5255
Treatment	5	104.5694	0.0001
Block X Trt	5	0.7917	0.7612
Sex	1	0.0139	0.8321
Sex X Trt	5	0.5694	0.8649
Sex X Trt(Block)	6	0.2500	0.9908
Error	48	14.6667	
Corrected Total	71	120.9861	

Tests of hypotheses using the type III MS for Block X Trt as an error term

Block	1	0.1250	0.4150
Treatment	5	104.5694	0.0001

Tests of hypotheses using the type III MS for Sex X Trt(Block) as an error term

Sex	1	0.0139	0.5847
Sex X Trt	5	0.5694	0.1265

VITA

The author was born in Daegu, Korea, on November 11, 1957. He entered Posung High School in Seoul and was graduated in January, 1976. In January, 1980, he graduated from Kyung Hee University with a Bachelor of Science degree in Food Science. After graduation, he taught mathematics and physics in Unbong Industrial High School, Incheon, Korea, for one and half years. He entered Graduate School in Food and Agriculture, Korea University in March, 1980 and had studied for the major of Fermentation Chemistry until December, 1981.

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DOCTORAL EXAMINATION AND DISSERTATION REPORT

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Major Field: Food Science

Title of Dissertation: Evaluation of the Crawfish Chitin-Carotenoid Complex and Its Nutritional/Pigmentation Effect in Poultry Diets

Approved:

Major Professor and Chairman

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October 5, 1990