Director's Digest
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#310

RENDERED NON-MARINE ANIMAL BY-PRODUCTS AS SHRIMP FEED COMPONENTS

Project 99 B-2

FINAL REPORT

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MATERIALS AND METHODS

Ingredient characterization

Three samples of meat and bone meal (MBM) were obtained from different sources and were subjected to analysis for proximate composition (AOAC, 1990), particle size distribution, energy content, amino acid and elemental analysis (Tables 1, 2, 3; Appendix I). The three MBMs, designated as A, B, C, had the following composition: MBM-A 35% beef, 35% pork, 30% poultry; MBM-B 90% beef, 5% pork, 5% poultry; and, MBM-C 50% beef, 50% pork).

Experiment #1: Nutrient Digestibility of Meat and Bone Meal for Shrimp

A trial was conducted to ascertain the relative apparent digestibility coefficients of three sources of MBM. This trial was conducted in the digestibility laboratory of the Oceanic Institute consisting of twelve polyethylene tanks (600 l volume) designed for automated collection of fecal matter through a conical tank bottom and a PVC settling tube. Each tank was supplied with a continuous supply of flow-through seawater.

Four dry diets were manufactured (Table 4): a fish meal control diet, and three diets in which 30% of the fishmeal protein was replaced by one of three meat and bone meals (10% of the control diet). All diets contained 0.5% chromic oxide as an indigestible marker. The test diets were analyzed for proximate composition (see Table 4), chromic oxide content, pellet stability and pellet durability.

Each tank was stocked with 96 juvenile shrimp (initial weight±standard deviation 5.09±0.05 g/shrimp), a stocking density equivalent to 100 shrimp/m^2. A 72-h stress replacement period after stocking was observed to avoid bias in the treatments.

The shrimp were acclimated to the experimental conditions and diets for seven days before fecal collections began. Shrimp were fed a fixed ration continuously throughout the day with an automatic feed dispenser. The daily feed amount was calculated according to shrimp size and temperature-dependent feeding rates (Appendix II).

After the acclimation period, the feeding regimen was modified slightly and the shrimp were fed continuously throughout the night and into the morning (1700 to 1030 H daily). One hour after the final feeding, the collection tubes were scrubbed and flushed to remove feces and uneaten diet. The cleaning process was then repeated to ensure complete removal of fecal matter and diet residues.

At 1200 H, sampling vials were attached to the collection tubes on each tank for a period of 60 min. Fecal matter was collected continuously during this period, after which the sampling vials were removed and replaced with clean vials. The collected fecal matter was poured into an aspirated filtering funnel lined with hardened, ashless filter paper (Whatman #541, Whatman International, Ltd., Maidstone, UK) and rinsed with distilled water. The rinsed feces were placed into a labeled petri dish and stored at –20°C. This collection process was repeated four times/day at 1300, 1400, 1500, and 1600 H. After a sufficient quantity of fecal matter was collected from all tanks, the feces were lyophilized and submitted for analysis. The weight of the animals was measured at the end of the collection period (total of four weeks).

Experiment #2: Shrimp Grow-out with Graded Inclusion Levels of Three Meat and Bone Meals

A growth trial was conducted to ascertain the relative ability of three sources of MBM to replace fish meal in diets for shrimp. This trial was conducted in an indoor clearwater laboratory (ICL) at the Oceanic Institute. Ten dry diets were manufactured (Table 5): one fish meal control diet and nine experimental diets formulated to contain one of three sources of MBM at 25, 50, and 75% replacement of fish meal. The diets were formulated to contain 35.3% crude protein and 9.3% crude lipid on an as fed basis. Fish oil was adjusted to maintain equal lipid levels among the diets. The test diets were analyzed for proximate composition (Table 5), pellet stability and pellet durability.

Each aquarium was stocked with 14 juvenile shrimp (initial weight±standard deviation of 0.80±0.01 g/shrimp), a stocking density equivalent to 50 shrimp/m^2. Analysis of variance procedures revealed no significant variation in stocking weight among dietary treatments. A 72-h stress replacement period after stocking was observed to avoid bias in the treatments.

Each diet was fed to three randomly selected aquaria for 8 wk. Shrimp were fed daily a fixed ration 8 times/day (0800, 1100, 1400, 1700, 2000, 2300, 0200, and 0500 H), by an automatic feed dispenser. The daily ration was calculated according to shrimp size and temperature-dependent feeding rates (Appendix II) and used as an estimate of the feeding requirement. Feeding rates were then adjusted daily following observations of the quantity of feed residue present in each tank to determine whether rations were excessive, sufficient, or insufficient. Each tank was siphoned before the first feeding of the day to remove diet residues and fecal matter.

Water temperature was recorded daily, while salinity, dissolved oxygen, and pH were measured weekly with laboratory-calibrated handheld instruments in one randomly selected aquarium per dietary treatment.

The shrimp in each group were bulk weighed initially and every two weeks using an electronic top-loading balance accurate to 0.01g. Weight and feed intake data were used to calculate feed conversion ratios (dry diet fed/live shrimp weight gain (g/g)).

Chemical and Physical Methods

All proximate analyses (moisture, crude protein, crude lipid, ash) were conducted in duplicate (Divakaran, 1999). The gross energy contents of the MBMs were determined using a Parr 1261 Isoperibol Bomb Calorimeter (Parr Instrument Co, St. Moline, IL 61265, USA) using benzoic acid as the standard. Mineral analysis of MBMs and diets were undertaken by Inductively Coupled Plasma Atomic Emission Spectroscopy using a Model Atomscan 16 radial configuration instrument (Thermo Jarrel Ash, TJA Solutions, Franklin, MA, USA), after first ashing the samples at 600 °C for 6 h and then dissolving the ash in 3N HCl prior to analysis (AOAC, 1990).

Pellet stability was determined by leaching 2 g of pellets for 2 h in salt water (25 °C temperature and 3.4 g/l salinity) using OI's horizontal shaking method. The leached and original feed samples were dried at 105 °C for 24 h and cooled in a desiccator before analysis. Pellet stability was calculated as the ratio of leached feed dry matter to original feed dry matter, multiplied by 100% (Obaldo and Tacon, 2000).

The pellet durability index is a measure of the relative ability of the feed pellet to resist breaking up in a bulk handling system (Obaldo et al., 1998). A 500 g sample of pellets was placed into the Durability

Pellet Tester (Continental-Agra Grain Equipment, Newton, KS) and spun for 10 min. The sample was then passed over a screen (ASTM #10-mesh) and retained pellets were weighed. Pellet durability index was calculated as the ratio of retained pellets to original pellets, multiplied by 100% (ASAE, 1997).

Statistics

The data from the digestibility and growth trials were submitted to one-way analysis of variance procedures. The significance of the differences between results obtained for the fish meal control and experimental diets were ascertained by Tukey's test (Zar, 1999). All statistical procedures were conducted using a computer software package (SigmaStat v. 2.03, SPSS Inc., Chicago, IL). Differences were considered significant at the 5% level of probability.

RESULTS

Analysis of Meat and Bone Meal samples

The three samples of meat and bone meal were similar in crude protein content (4.3% range), but varied in crude lipid (61.4% range), ash (26.3% range) and gross energy (9.6% range) (Table 1). There was little variability among the MBM sources for each amino acid (Table 2). With the exception of iron, the mineral composition of these sources was similar (Table 3). The iron content of MBM-C was found to be more than twice as high as that of MBM-A. The particle size distribution and density of these products were similar (Table 1).

Experiment #1: *Diet Characteristics and Nutrient Digestibility of Meat and Bone Meal for Shrimp*

The pellet stability of the diets containing the MBM was slightly lower than the fish meal control diet (Table 4), with MBM-A being the lowest. The pellet durability of the diets containing MBM, however, was higher than for the control diet (Table 4).

The growth and survival of the shrimp over the four weeks of the digestibility trials was within the range of expectation for these animals reared under these conditions (Table 6), and there were no significant differences among the treatments in these parameters. This indicates the suitability of the culture environment and feeding methodology employed in this trial.

The apparent digestibility of dry matter was somewhat lower for the diets containing MBM, but the difference was only statistically significant for the MBM-C (Table 7a). The apparent digestibility coefficients for protein were also marginally lower for the diets containing MBM, relative to the fish meal control, but the differences were only statistically significant for MBM-B and MBM-C. The apparent digestibility of the amino acids of the diets was consistent with the crude protein digestibility. The digestibility coefficients for phosphorus and calcium were negative for all diets (data not shown), indicating the unsuitability of this method for measuring mineral availability (shrimp reared in salt water drink copious amounts and this interferes with the accurate assessment of the fecal mineral content resulting from dietary intake).

Experiment #2: Shrimp Grow-out with Graded Inclusion Levels of Three Meat and Bone Meals

There was a positive correlation between MBM inclusion level and pellet stability (Table 5), indicating the positive binding characteristics of these ingredients.

The values for growth survival and feed conversion ratio of the best performing groups in the 8 wk feeding trial (Table 8) were consistent with what we have observed within the culture conditions of this type of trial. Growth, as measured by final weight and weight gain, of the shrimp was affected by dietary treatment (Table 8, Figure 1). For all MBM sources, there was a general trend toward lower growth among the shrimp fed the diets containing levels of MBM above 25% replacement of fish meal. The final weight of the shrimp fed MBM-A was significantly lower than those fed the control diet at levels of 50% fish meal replacement and higher, whereas there were no significant differences in the growth of the shrimp fed the diets containing MBM-C at the 50% replacement level or less. The differences in growth of the shrimp fed the diets containing MBM-B were not significantly different than those fed the control diet at all levels of fish meal replacement.

There were no significant differences among the treatments for survival and feed conversion efficiency (Table 8).

DISCUSSION

The results of this study indicate that there is variability in the ability of MBM to replace fish meal in diets for Pacific white shrimp. In all sources examined in this study, at least 25 % of the fish meal and as much as 75% could be replaced without a statistically meaningful reduction in growth. MBM-B, which contained 90% beef, exhibited the best nutritional quality for shrimp, in terms of its ability to replace fish meal. MBM-C, which contained 50% beef, was able to replace higher percentage of fish meal than was MBM-A, which contained only 35% beef. These findings suggest that beef products are more effective in meeting the nutritional needs of shrimp than are either pork or poultry products. More study is needed to verify this, and to establish what remediation is possible to improve the utilization of other sources of MBM.

It is interesting to note that the ranking of the MBMs on the basis of digestibility was quite different (A>B>C), indicating that the differences found between the MBMs in the growth trial were not due to protein digestibility. The consistency of the amino acid apparent digestibility with the crude protein apparent digestibility is consistent with this (i.e., an inconsistent variability in the digestibility of any of indispensable could control protein quality independently of the overall protein digestibility). Instead, other factors (e.g., amino acid balance and palatability) were more important in affecting the overall results.

Rendered products have been used as animal feeds since the middle of last century. Research has shown that poultry by-product meal, feather meal, blood meal, meat and bone meal, meat meal, and fats could be successfully used for growing animals as protein and energy sources.

Tacon et al. (1983) found that hexane-extracted meat and bone meal or a meat and bone meal:bone meal (4:1 mixture) supplemented with methionine successfully replaced up to 50% fish meal protein within diets containing 45% crude protein fed to Nile tilapia *Oreochromis niloticus* fry over a six-week period. By contrast, in the same study, feather meal supplemented with methionine, histidine and lysine could replace only 30% of the fish meal. When ratios that are more appropriate are maintained between the dietary components used, however, even better results can be achieved, as reported by Davies et al. (1989). These authors found that optimum meat and bone meal:bone meal ratios could effectively replace up to 75% of the fish meal in diets fed to Mozambique tilapia *O. mossambicus* fry over a seven-week period. In fact, diets containing meat and bone meal or high meat and bone meal:bone meal ratios (3:1 and 2:3) were found to be superior to fish meal, even at a 100% substitution level; when bone meal was used as a total replacement for fish meal, fish growth was still comparable to the control diet (Davies et al., 1989).

Rodriguez-Serna et al. (1996) studied the use of commercially defatted animal by-product meal, a combination of bone meal, meat and bone meal, hydrolyzed feather meal and fish meal, with soybean oil or a soybean oil:fish oil mixture (1:1), as a replacement for fish meal within feeds for *O. niloticus* fry over a 7-week period. These authors reported that animal by-product meal could replace up to 75% of the fish meal within the control diet tested, and that animal by-product meal supplemented with soybean oil could totally replace fish meal within the diets with no loss in fish performance.

Long-term feeding trials (120-days) of Otubusin (1987) with cage-reared *O. niloticus* fingerlings (3 g initial body weight) concerning the use of bone meal as a fish meal replacement found that dietary bone meal inclusion levels above 50% of the fish meal protein significantly reduced fish performance (10% replacement level being the most efficient).

Lawrence and Castille (1991) reported that up to 5% and possibly 10% feather meal, poultry by-product meal, or meat and bone meal could be used in commercial feeds for *L. vannamei* (30–70 mg initial weight) without significantly decreasing animal growth performance, provided that the diet contained 35% crude protein, and also that 3% fish oil is adequate for supplying the C20 and C22 unsaturated fatty acids. In their experiment, three levels of fish, meat and bone, feather, and poultry by-product meals replaced 2.5, 5.0, and 10% of the protein of the purified diets containing 35% protein. The authors concluded that protein quality in terms of essential amino acids for meat and bone, feather, and poultry by-product meals representing up to 10% protein in shrimp feeds is not limiting for growth of juvenile *L. vannamei*.

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Table 1. Proximate composition, energy content and particle size distribution of three sources of meat and bone meal (MBM-A,B,C) (values are expressed on an as is basis).

	MBM-A	MBM-B	MBM-C
Moisture (%)	6.79	3.92	3.29
Crude Protein (%)	53.30	55.61	53.51
Crude Fat (%)	17.12	10.61	15.06
Ash (%)	19.00	24.09	22.28
Energy (cal/g)	4668	4259	4546
Particle size range[1]	488 (256-932)	355 (167-757)	500 (288-869)

1. Average size of particles in ingredients in microns. The numbers within brackets () indicate the size range of particles containing 68% of the sample. See Appendix I for more details of this analysis.

Table 2. Amino acid composition of rendered by-product meals (values expressed on an as fed basis). The amino acids in bold type are considered indespensible (essential) for shrimp.

	MBM-A (%)	MBM-B (%)	MBM-C (%)
Alanine	3.867	4.095	4.240
Arginine	**3.453**	**3.766**	**3.455**
Aspartic acid	4.188	4.657	4.510
Cystine	0.694	0.445	0.419
Glutamic acid	6.257	7.060	6.696
Glycine	6.177	6.430	7.011
Histidine	**1.179**	**1.335**	**1.287**
Isoleucine	**1.696**	**1.977**	**1.617**
Leucine	**3.407**	**3.946**	**3.340**
Lysine	**2.667**	**3.498**	**2.768**
Methionine	**0.835**	**1.108**	**0.862**
Phenylalanine	**1.874**	**2.120**	**1.783**
Serine	2.434	2.177	1.815
Taurine	0.128	0.076	0.068
Threonine	**1.844**	**2.104**	**1.723**
Tryptophan	**0.334**	**0.413**	**0.323**
Tyrosine	1.554	1.907	1.487
Valine	**2.555**	**2.691**	**2.419**

Table 3. Mineral composition of rendered by-product meals (values expressed on an as fed basis).

	MBM-A	MBM-B	MBM-C
P (%)	3.14	3.66	4.11
K (%)	0.42	0.26	0.50
Ca (%)	5.98	8.15	7.84
Mg (%)	0.14	0.21	0.17
Na (%)	0.68	0.44	1.65
Mn (ppm)	11.92	10.21	14.98
Fe (ppm)	470.33	872.60	998.78
Cu (ppm)	9.34	9.74	9.71
Zn (ppm)	56.43	119.91	81.29
B (ppm)	4.57	4.51	6.66

Table 4. Diet formulation and chemical characteristics for digestibility evaluation of three rendered by-product meals (values expressed on an as fed basis).

	% inclusion			
Ingredient	**Control**	**MBM-A**	**MBM-B**	**MBM-C**
Fishmeal – LT 94	24.50	17.15	17.15	17.15
Wheat, whole	46.50	46.50	46.50	46.50
Squid meal	2.50	2.50	2.50	2.50
Vital wheat gluten	4.00	4.00	4.00	4.00
Brewers yeast	5.00	5.00	5.00	5.00
Wheat starch	2.94	1.19	0.49	0.99
Soybean meal	6.50	6.50	6.50	6.50
MBM-A	0.00	10.00	0.00	0.00
MBM-B	0.00	0.00	10.00	0.00
MBM-C	0.00	0.00	0.00	10.00
Soy lecithin	2.00	2.00	2.00	2.00
Fish oil	3.00	2.10	2.80	2.30
Cholesterol	0.234	0.234	0.234	0.234
Mineral Premix	0.060	0.060	0.060	0.060
Vitamin Premix	0.400	0.400	0.400	0.400
Choline chloride	0.115	0.115	0.115	0.115
Stay C-35 (35% AA potency)	0.071	0.071	0.071	0.071
Potassium phosphate, dibasic	0.560	0.560	0.560	0.560
Sodium phosphate, dibasic	0.560	0.560	0.560	0.560
Calcium phosphate, monobasic	0.560	0.560	0.560	0.560
Chromic oxide	0.500	0.500	0.500	0.500
Total	100.00	100.00	100.00	100.00
Moisture (%as fed)	6.09	6.30	6.64	6.19
Crude Protein (% as fed)	36.42	35.14	35.08	34.99
Crude Lipid (% as fed)	8.45	8.07	8.22	8.14
Ash (% as fed)	6.01	7.10	7.92	5.42
Chromic Oxide (% as fed)	0.49	0.41	0.40	0.42
Pellet Stability	73.9	61.9	71.6	64.9
Pellet Durability	84.4	92.0	97.0	96.7

Table 5. Formulations and chemical characteristics of diets for growth trial conducted to evaluate three meat and bone meals (MBM-A,B,C) at inclusion levels of 25%, 50%, and 75% of fish meal replacement (values expressed on an as fed basis).

		Inclusion (%)								
Ingredient	Control	MBM-A 25%	MBM-A 50%	MBM-A 75%	MBM-B 25%	MBM-B 50%	MBM-B 75%	MBM-C 25%	MBM-C 50%	MBM-C 75%
Fishmeal - LT 94	24.500	18.375	12.250	6.125	18.375	12.250	6.125	18.375	12.250	6.125
Wheat, whole	43.500	43.500	43.500	43.500	43.500	43.500	43.500	43.500	43.500	43.500
Vital wheat gluten	4.000	4.000	4.000	4.000	4.000	4.000	4.000	4.000	4.000	4.000
Brewers yeast	5.000	5.000	5.000	5.000	5.000	5.000	5.000	5.000	5.000	5.000
Squid liver powder	2.500	2.500	2.500	2.500	2.500	2.500	2.500	2.500	2.500	2.500
Wheat starch	6.339	4.518	2.636	0.834	4.254	2.139	0.004	4.362	2.355	0.368
Soybean meal	6.500	6.500	6.500	6.500	6.500	6.500	6.500	6.500	6.500	6.500
MBM-A	--	8.827	17.653	26.480	--	--	--	--	--	--
MBM-B	--	--	--	--	8.460	16.920	25.380	--	--	--
MBM-C	--		--	--	--	--	--	8.792	17.584	26.376
Soy lecithin	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000	2.000
Fish oil	3.100	2.220	1.400	0.500	2.850	2.630	2.430	2.410	1.750	1.070
Cholesterol	0.234	0.234	0.234	0.234	0.234	0.234	0.234	0.234	0.234	0.234
Mineral Premix	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060	0.060
Vitamin Premix	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400	0.400
Choline chloride	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.115	0.115
Stay C-35 (35% AA potency)	0.071	0.071	0.071	0.071	0.071	0.071	0.071	0.071	0.071	0.071
Potassium phosphate, dibasic	0.560	0.560	0.560	0.560	0.560	0.560	0.560	0.560	0.560	0.560
Sodium phosphate, dibasic	0.560	0.560	0.560	0.560	0.560	0.560	0.560	0.560	0.560	0.560
Magnesium phosphate	0.560	0.560	0.560	0.560	0.560	0.560	0.560	0.560	0.560	0.560
Total	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000
Moisture (% as fed)	8.87	7.80	8.33	7.93	2.84	6.98	7.04	7.21	7.92	7.82
Crude Protein (as fed)	35.10	38.11	35.82	36.04	35.91	36.40	36.32	36.36	35.91	35.93
Crude Lipid (as fed)	8.19	8.94	8.44	8.37	9.17	8.68	8.51	8.25	7.62	8.87
Ash (as fed)	5.34	6.10	7.37	8.12	7.56	8.57	10.46	7.12	8.08	9.97
Pellet Stability	65.9	70.5	75.8	84.3	65.1	66.0	76.8	46.4	73.2	76.0
Pellet Durability	95.1	92.4	92.3	97.9	92.9	94.6	94.4	93.5	96.5	96.0

Table 6. Growth and survival of *L. vannamei* fed a control fish meal based diet and three diets in which 30% of fish meal was replaced by one of three meat and bone meals (MBM-A,B,C) during a 28-day indoor digestibility trial. Values are means of three observations. There were no significant differences among the treatments for each of the parameters (P>0.05).

Dietary Treatment	Initial Weight (g)	Final Weight (g)	Weekly Growth (g/wk)	Weight Gain (%)	Survival (%)
Control	5.14	9.17	1.01	78.5	93.1
MBM-A	5.09	8.96	0.97	75.9	87.9
MBM-B	5.04	8.84	0.95	75.3	89.9
MBM-C	5.10	8.95	0.96	75.4	94.4
SEM[1]	0.02	0.17	0.04	3.4	1.8

1. SEM = pooled standard error of treatment means.

Table 7a. Apparent digestibility coefficients of dry matter, and crude protein of a fish meal control diet and diets containing three sources of meat and bone meal (MBM-A,B,C) in replacement of 30 % of fish meal in the control diet fed to *L. vannamei*. Values are means of three observations. Values within a column with different superscripts are significantly different (P<0.05).

	Dry Matter (%)	Crude Protein (%)
Control	78.46a	92.63a
MBM-A	74.25ab	87.84ab
MBM-B	68.08ab	85.08bc
MBM-C	63.60b	81.04c
SEM[1]	3.10	1.47

1. SEM = pooled standard error of treatment means.

Table 7b. Apparent digestibility coefficients of amino acids of a fish meal control diet and diets containing three sources of meat and bone meal (MBM-A,B,C) in replacement of 30 % of fish meal in the control diet fed to *L. vannamei*. Values are means of three observations. The amino acids in **bold** type are considered indispensable (essential) for shrimp.

	Control	MBM-A	MBM-B	MBM-C
Alanine	92.3	85.9	82.2	77.3
Arginine	**94.4**	**88.2**	**86.3**	**81.5**
Aspartic acid	91.6	87.4	83.6	76.9
Cystine	84.7	74.6	78.1	76.3
Glutamic acid	95.7	92.9	91.2	87.6
Glycine	89.1	77.4	73.2	69.8
Histidine	**91.3**	**89.9**	**85.9**	**83.2**
Isoleucine	**93.4**	**89.6**	**87.7**	**81.8**
Leucine	**93.4**	**89.0**	**87.0**	**81.0**
Lysine	**95.4**	**92.7**	**88.6**	**84.5**
Methionine	**92.4**	**91.0**	**86.7**	**81.5**
Phenylalanine	**92.5**	**88.3**	**86.7**	**81.7**
Serine	90.7	82.4	83.6	78.9
Taurine	94.3	93.3	92.3	88.6
Threonine	**90.3**	**85.7**	**82.8**	**76.9**
Tryptophan	**91.2**	**91.0**	**87.2**	**81.5**
Tyrosine	90.9	87.8	85.6	80.3
Valine	**92.5**	**86.8**	**85.4**	**79.6**
Average	92.0	87.4	85.2	80.5

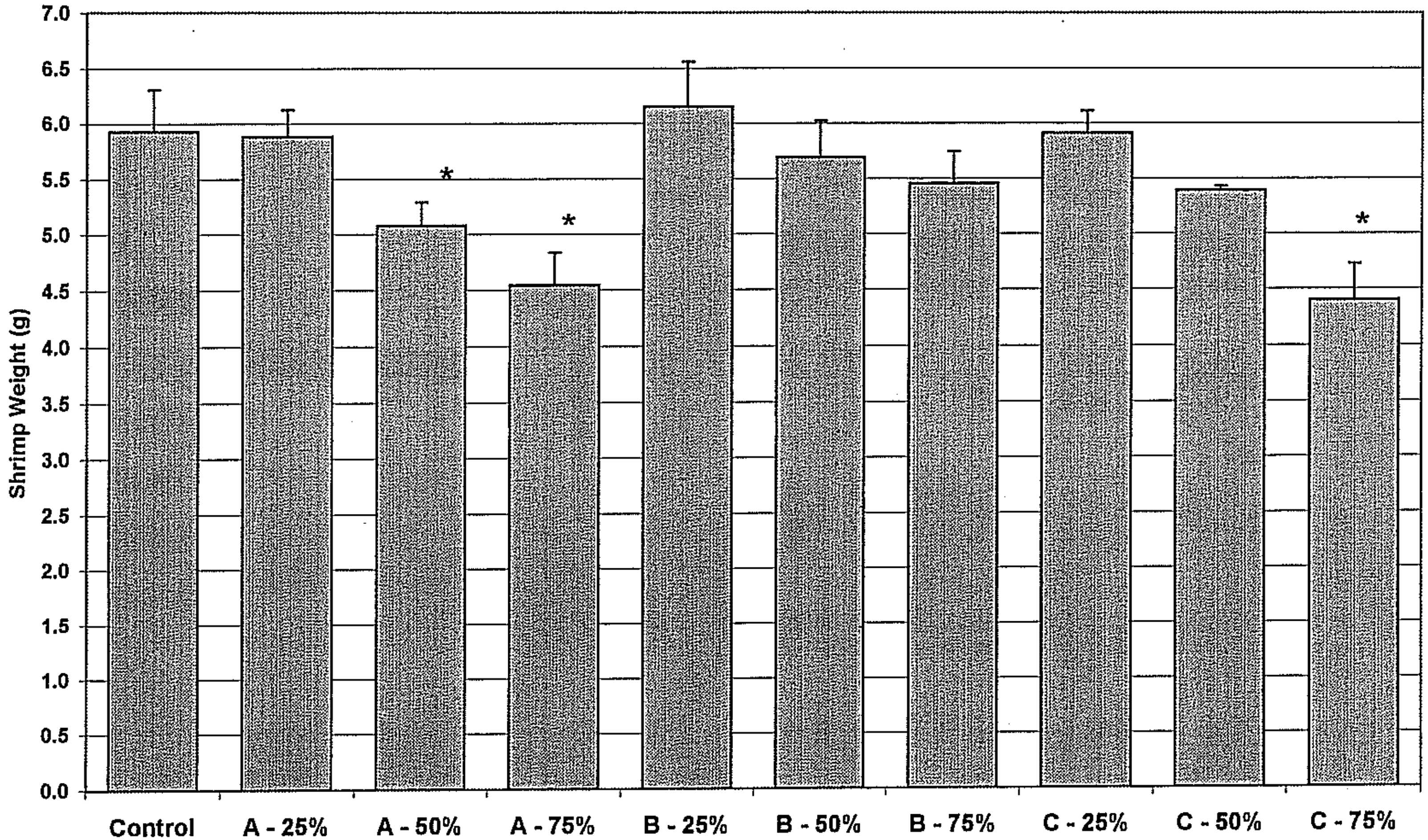
Table 8. Summary of shrimp performance of 8-week indoor growth trial evaluating three meat and bone meals (MBM) at inclusion levels of 25%, 50%, and 75% of fish meal replacement.

Dietary Treatment	Diet 1 Control	Diet 2 MBM-A 25%	Diet 3 MBM-A 50%	Diet 4 MBM-A 75%	Diet 5 MBM-B 25%	Diet 6 MBM-B 50%	Diet 7 MBM-B 75%	Diet 8 MBM-C 25%	Diet 9 MBM-C 50%	Diet 10 MBM-C 75%	SEM
Shrimp Weight											
Initial body weight (g)	0.80 [a]	0.81 [a]	0.81 [a]	0.80 [a]	0.79 [a]	0.80 [a]	0.79 [a]	0.80 [a]	0.80 [a]	0.79 [a]	0.01
Final body weight (g)	5.93 [a]	5.88 [ab]	5.08 [bc]	4.55 [c]	6.15 [a]	5.70 [ab]	5.45 [ab]	5.91 [ab]	5.39 [ab]	4.40 [c]	0.17
Shrimp Feed Intake[2]											
Mean daily feed intake (g/shrimp/day)	0.14	0.14	0.13	0.12	0.15	0.14	0.14	0.14	0.14	0.12	
Shrimp Growth Response											
Total weight gain (%)	644.3 [a]	629.7 [ab]	530.3 [bcd]	470.1 [cd]	677.1 [a]	611.1 [ab]	586.6 [ab]	638.7 [a]	571.3 [abc]	453.6 [d]	21.21
Mean Weekly weight gain (g/shrimp/week)	0.64 [a]	0.63 [ab]	0.53 [bcd]	0.47 [cd]	0.67 [a]	0.61 [ab]	0.58 [ab]	0.64 [a]	0.57 [abc]	0.45 [d]	0.02
Specific growth rate (%/day)	3.58 [a]	3.55 [ab]	3.29 [bc]	3.11 [c]	3.66 [a]	3.50 [ab]	3.44 [ab]	3.57 [a]	3.40 [ab]	3.05 [c]	0.06
Shrimp Feed Utilization											
Food conversion ratio (as fed)	1.57 [a]	1.59 [a]	1.77 [a]	1.79 [a]	1.56 [a]	1.61 [a]	1.69 [a]	1.54 [a]	1.69 [a]	1.81 [a]	0.07
Tank Production											
Total food fed (g) (as fed)[2]	192.5	185.1	180.4	167.0	193.3	198.6	186.8	182.9	183.4	160.8	
Tank food conversion ratio	1.67 [a]	1.73 [a]	1.93 [a]	1.96 [a]	1.70 [a]	1.71 [a]	1.85 [a]	1.67 [a]	1.85 [a]	2.00 [a]	0.09
Survival (%)	85.7 [a]	82.1 [a]	85.7 [a]	89.3 [a]	83.3 [a]	90.5 [a]	85.7 [a]	83.3 [a]	84.5 [a]	88.1 [a]	3.85

[1] Fixed feeding rates dependent on shrimp weight and water temperature were used with all tanks. Feeding rates ranged from 6% to 4.5% of estimated tank biomass.

[a, b] mean values for components with the same superscripts are not significantly different ($P<0.05$).

Figure 1. Final weight of shrimp during an 8-week indoor growth trial evaluating three meat and bone meals (MBM-A,B,C) at three replacement levels of fish meal (25,50, and 75%). Values represent means (+1 standard deviation) of three replicate observations. The columns with an asterisk (*) are significantly lower than the control (P<0.05).



Appendix I

Table and Figure 1. Particle size distribution of rendered by-product meal "A".

AQUAFAN INGREDIENT PARTICLE SIZE ANALYSIS

TEST N0. 2000-2 **DATE** 3/13/00 **MATERIAL** Feed Ingredient - MBM A

TYLER SCREEN NO.	d Microns	Wi Grams	Pi (%)	Sum Pi (% less than)	log di	Wi log di	(log di - Log dgw)	Wi(log di-log dgw)^2
4	4760	0.00	0.00	100.00	3.753	0.000	1.064	0.000
6		0.00	0.00	100.00	3.602	0.000	0.913	0.000
8	2380	0.75	0.75	99.25	3.452	2.589	0.763	0.437
10	1680	0.51	0.51	98.73	3.301	1.684	0.612	0.191
14	1190	10.46	10.51	88.22	3.149	32.939	0.460	2.216
20	841	12.71	12.77	75.45	3.000	38.130	0.311	1.232
28	595	10.46	10.51	64.94	2.849	29.801	0.160	0.269
35	420	14.23	14.30	50.64	2.699	38.407	0.010	0.002
48	297	30.92	31.07	19.57	2.549	78.815	-0.140	0.603
65	210	12.05	12.11	7.47	2.398	28.896	-0.291	1.018
100	149	5.49	5.52	1.95	2.248	12.342	-0.441	1.066
150	105	1.57	1.58	0.37	2.097	3.292	-0.592	0.550
200	74	0.19	0.19	0.18	1.944	0.369	-0.745	0.105
270	53	0.12	0.12	0.06	1.799	0.216	-0.890	0.095
400	37	0.06	0.06	0.00	1.646	0.099	-1.043	0.065
pan	0	0.00	0.00	0.00	1.435	0.000	-1.254	0.000
SUMMATION		99.52	100.00			267.58		7.85

DGW MICRONS	488	SGW	1.91
g/cc	1.26		
SURFACE AREA(CM^2)/GRAM	120.2	PARTICLES / GRAM	44,758
PARTICLE SIZE RANGE (68%)	256	TO	932

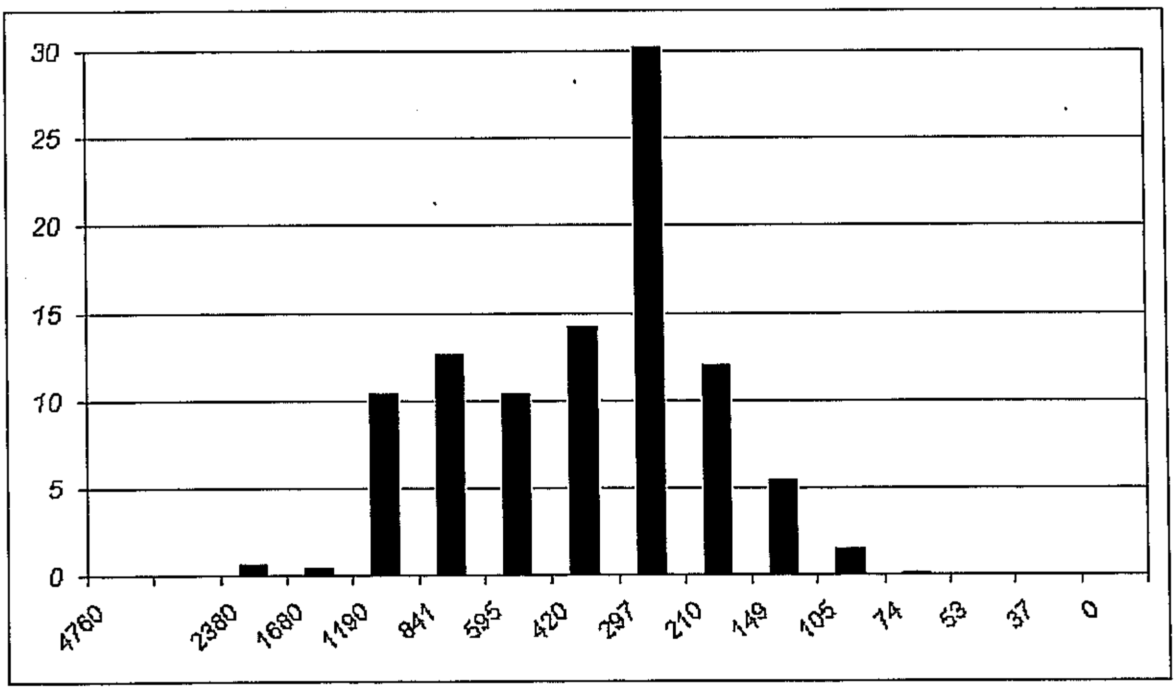


Table and Figure 2. Particle size distribution of rendered by-product meal "B".

AQUAFAN INGREDIENT PARTICLE SIZE ANALYSIS

TEST N0. 2000-1 **DATE** 3/13/00 **MATERIAL** Feed Ingredient - MBM B

TYLER SCREEN NO.	d (µm)	Wi (g)s	Pi (%)	Sum Pi (% less than)	log di	Wi log di	(log di - Log dgw)	Wi(log di- log dgw)^2
4	4760	0.00	0.00	100.00	3.753	0.000	1.202	0.000
6	3360	0.00	0.00	100.00	3.602	0.000	1.051	0.000
8	2380	0.02	0.02	99.98	3.452	0.069	0.901	0.016
10	1680	0.86	0.86	99.12	3.301	2.839	0.750	0.484
14	1190	6.90	6.90	92.22	3.149	21.728	0.598	2.471
20	841	8.29	8.29	83.92	3.000	24.870	0.449	1.675
28	595	10.71	10.72	73.20	2.849	30.513	0.298	0.954
35	420	13.36	13.37	59.84	2.699	36.059	0.148	0.295
48	297	14.34	14.35	45.49	2.549	36.553	-0.002	0.000
65	210	14.11	14.12	31.37	2.398	33.836	-0.153	0.328
100	149	20.55	20.56	10.81	2.248	46.196	-0.303	1.881
150	105	8.47	8.48	2.33	2.097	17.762	-0.454	1.742
200	74	1.97	1.97	0.36	1.944	3.830	-0.607	0.725
270	53	0.35	0.35	0.01	1.799	0.630	-0.752	0.198
400	37	0.01	0.01	0.00	1.646	0.016	-0.905	0.008
pan	0	0.00	0.00	0.00	1.435	0.000	-1.116	0.000
SUMMATION		99.94	100.00			254.90		10.78

DGW MICRONS	355		SGW	2.13
g/cc	1.26			
SURFACE AREA(CM^2)/GRAM	178.4	PARTICLES / GRAM		231,962
PARTICLE SIZE RANGE (68%)	167	TO	757	

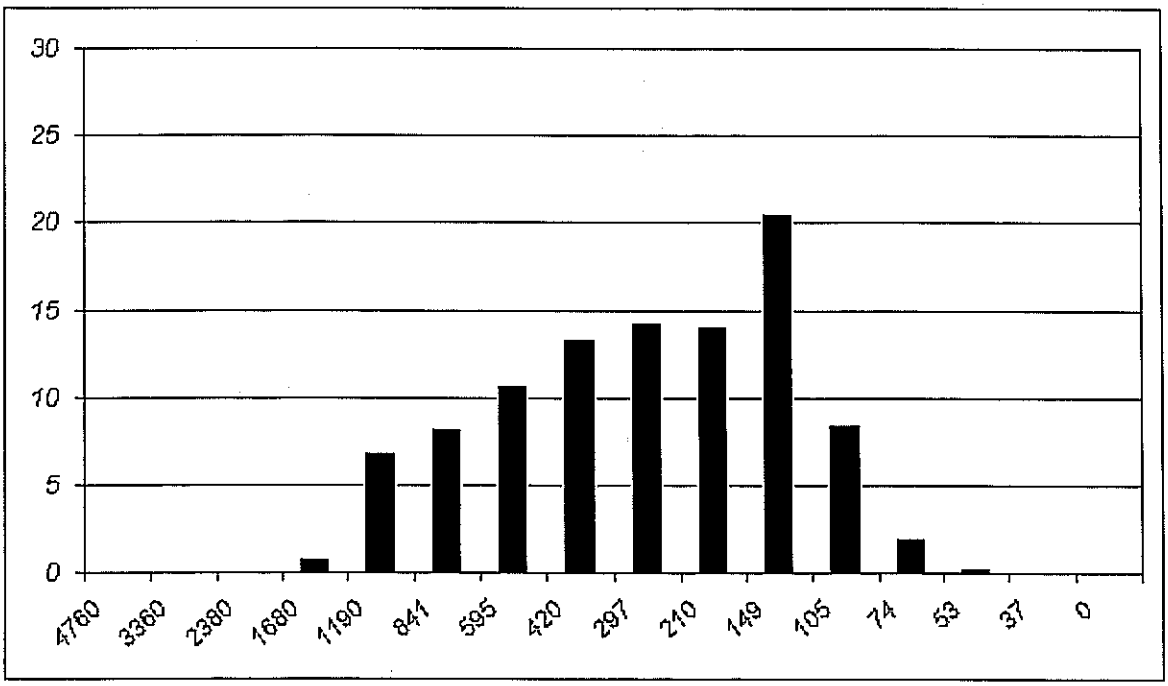


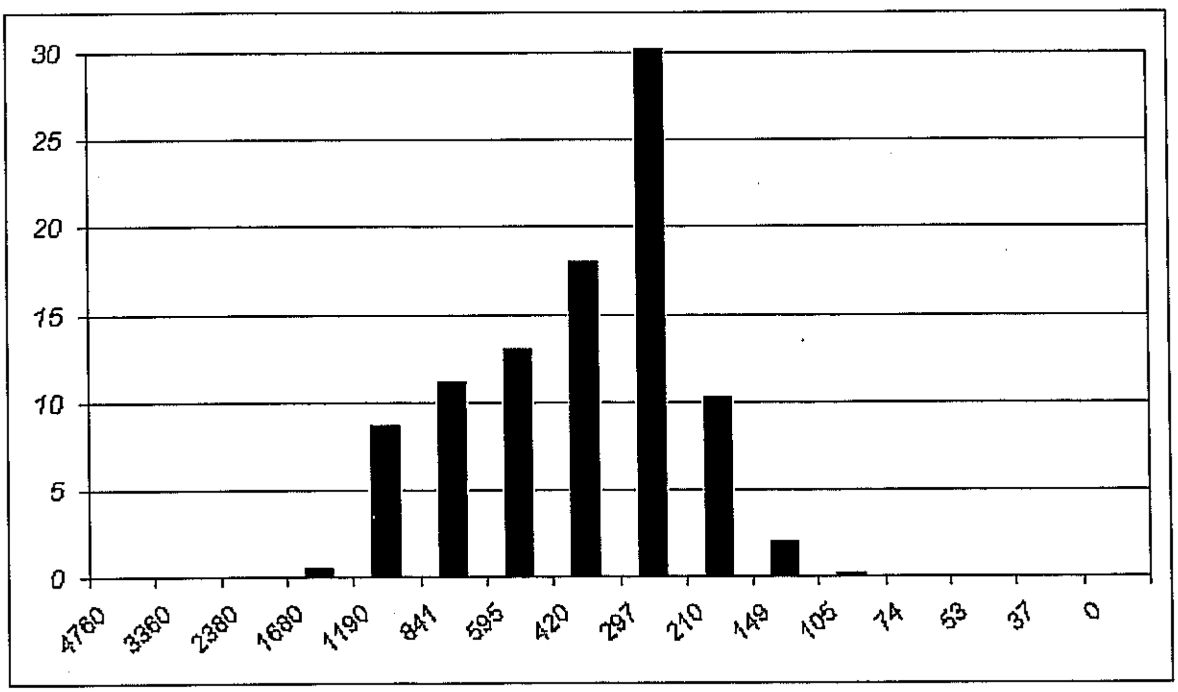
Table and Figure 3. Particle size distribution of rendered by-product meal "C".

AQUAFAN INGREDIENT PARTICLE SIZE ANALYSIS

TEST NO. 2000-3 **DATE** 3/13/00 **MATERIAL** Feed Ingredient - MBM C

TYLER SCREEN NO.	d Microns	Wi Grams	Pi (%)	Sum Pi (% less than)	log di	Wi log di	(log di - Log dgw)	Wi(log di-log dgw)^2
4	4760	0.00	0.00	100.00	3.753	0.000	1.054	0.000
6	3360	0.00	0.00	100.00	3.602	0.000	0.903	0.000
8	2380	0.00	0.00	100.00	3.452	0.000	0.753	0.000
10	1680	0.64	0.64	99.36	3.301	2.113	0.602	0.232
14	1190	8.84	8.84	90.52	3.149	27.837	0.450	1.790
20	841	11.22	11.22	79.30	3.000	33.660	0.301	1.017
28	595	13.15	13.15	66.14	2.849	37.464	0.150	0.296
35	420	18.13	18.13	48.01	2.699	48.933	0.000	0.000
48	297	34.92	34.93	13.08	2.549	89.011	-0.150	0.786
65	210	10.36	10.36	2.72	2.398	24.843	-0.301	0.939
100	149	2.16	2.16	0.56	2.248	4.856	-0.451	0.439
150	105	0.35	0.35	0.21	2.097	0.734	-0.602	0.127
200	74	0.12	0.12	0.09	1.944	0.233	-0.755	0.068
270	53	0.09	0.09	0.00	1.799	0.162	-0.900	0.073
400	37	0.00	0.00	0.00	1.646	0.000	-1.053	0.000
pan	0	0.00	0.00	0.00	1.435	0.000	-1.264	0.000
SUMMATION		99.98	100.00			269.85		5.77

DGW MICRONS	500	**SGW**	**1.74**
g/cc	1.26		
SURFACE AREA(CM^2)/GRAM	111.0	**PARTICLES / GRAM**	**25,132**
PARTICLE SIZE RANGE (68%)	288	**TO**	**869**



Appendix II

Table 1. Shrimp size and temperature-dependent feeding rates used in growth trial.

Mean Body Weight (g)	% of Estimated Tank Biomass		
	21 – 24°C	24 – 28°C	28 – 32°C
1 - 3	6.0	7.0	8.0
3 - 5	5.0	6.0	7.0
5 - 7	4.5	5.5	6.5
7 - 9	4.0	5.0	6.0
9 – 11	3.5	4.5	5.5
11 - 13	3.0	4.0	5.0
13 - 15	2.5	3.5	4.5
15 - 17	2.5	3.0	4.0
17 – 30	2.0	2.5	3.0