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Processing of meat meal for utilisation in aquaculture diets M.744

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Processing of Meat Meal for Utilisation in Aquaculture Diets

Final Report to MRC

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1. Summary

A protein enriched, reduced ash meat meal was evaluated for potential application in aquaculture diets.

In the first phase of the study, inclusion at up to 50% within a diet of rainbow trout (Oncorhynchus mykiss), showed no significant growth penalty. To validate this, two 'commercial' growth trials were undertaken in freshwater and saltwater systems. Food conversion rates, growth factors and environmental parameters were not compromised with the meat meal substituting for 30% of the protein meal content of the diet. Sensory evaluation of the flesh of these fish failed to identify any negative taste, odour and other consumer factors. It was concluded that the modified meat meal was very suitable for application to trout diets from a nutritive and product quality perspective.

When the meat meal was incorporated into diets for black tiger prawns (Penaues monodon), growth similar to a standard fish meal based prawn food occurred, when an inclusion level of 30% was set. However, at a 50% level, performance penalties occurred. This is in keeping with previous studies by other researchers, both locally and overseas. A pond study in the current project further supported this substitution opportunity.

The study conclusively demonstrated that high quality low ash meat meals may adequately substitute, albeit partially, for fish meal in the diets of rainbow trout and tiger prawns. One could argue that opportunity also exists for such a substitution into the Australian Salmon industry. If competitively priced and of high ongoing quality as much as 2,500 tonnes might be consumed within current locally manufactured aquaculture feed products. However, this is unlikely to occur, if a retail price is set for this modified meat meal, akin to that of FAQ grade fish meal of around 68% fish proteins.

2. Background

The global annual growth rate of aquaculture systems is currently 9%, with value tripling over the last decade to US$36 billion. Commercial aquaculture feed production is expanding at 30% each year with global production around 4.2 million tonnes. While one third of the global fish/shellfish catch is processed as fish meal, production increased only 1.6% in the decade to 1993, while fish oil production decreased 24%. Anticipated demand for these products by the year 2000 is double current supply (Tacon A., FAO Rome - draft paper).

Clearly replacement of fish meal in aquacultural diets is a global challenge. Domestic production is variable and of recent only some 5000 tonnes of FAQ grade fish meal has been available annually. The majority of fish meal is imported from either South America or Scandinavian sources. Volatile prices over the past 2 years (up to AUS$1200/tonne for 68% protein product) has further enhanced the opportunity for meat meal products.

Meat meal and protein concentrate meals are abundant throughout Australia. Evaluation of a range of these has received significant attention in laboratory tank trials involving prawns (Penaeus monodon) (Smith 1995) and silver perch (Bidyarus bidyarus) (Allan 1994). Compositional analyses of some of these meals showed enormous variation around the typical specification for high quality Danish fish meal (Table 1).

Table 1

Comparison of proximal analyses for Tasmanian fish meal and Australian meat meals (Source: D. Smith 1995)

Component (%)	Tasmanian Fish meal	Australian Meat meals (Range)
Ash	14.3	9.4-38.6
Crude Protein	77.2	46.7-83.5
Total Lipid	9.2	6.3-17.1
Apparent DE (%)	88.5	55.2-63.9

*(Source: Fletchers, Dubbo; Aspen, Preston; Beef City, Toowoomba)

Noteworthy was the high ash content (except for the Aspen product), being a consequence of high bone content. This is directly correlated to the reduced apparent digestible energy (DE) values of the meat meals, when compared to fish meal.

High ash diets have proved to be detrimental to the growth of finfish. Consumption of diets containing high levels of ash derived from the ingredients has been shown to cause increased morality, reduced growth and identifiable pathological conditions including cataracts and skeletal abnormalities (Ketola, 1979; Satoh, Takeuchi and Watanabe, 1987 a,b). A deficiency in the availability of the element zinc has been proposed by these researchers as a major factor within the inter-relationship.

Richardson, Higgs, Beames and McBride (1985) and Gatlin & Phillips (1989) found that the binding of zinc and therefore its reduced availability was not a function of calcium and phosphorous in isolation, but a function of the interaction of phytate, a hexophospate of myoinositol, which is found in oilseed meals and cereals. Both of these class of ingredients find wide usage in the production of aquaculture feeds. It is for this reason that the use of modified animal by-product meals with low levels of ash show potential in the production of aquaculture species.

The present study was undertaken to evaluate the opportunity for a low ash (<10%), higher protein (>65%) meat meal in the diets of rainbow trout (Oncorhynchus mykiss) and the black tiger prawn (Penaeus monodon). In association with the aquaculture of Atlantic salmon, these 3 species represent the vast majority of demand for manufactured diets, for commercial species, in Australia.

3. Objectives

(i) Application to Rainbow Trout Diets

To determine the protein and lipid digestibility of a modified meat meal in a diet for rainbow trout.

To determine, by graded inclusion levels, applicable levels of a modified meat meal that can be included in practical diets for rainbow trout.

Evaluate under commercial conditions the effect of a modified meat meal on the growth and food conversion ration of rainbow trout in fresh and saltwater environments.

Evaluate impact of meat meal on flavour and textural characteristics of trout flesh.

(ii) Application of Giant Tiger Prawn Diets

Determine by graded inclusion, applicable levels of this meat meal for tiger prawns.

Substantiate the opportunity to include this modified meat meal in diets for prawns in commercial systems.

(iii) Recommend Appropriate Research Strategy and Product Development to:

Provide a cost effective substitute for fish meal at critical inclusion levels.

Investigate the impact of substitution on flesh quality (consumer preference) within prawn and trout flesh.

4. Methods

4.1 Trout Graded Inclusion Trial

4.1.1 Methods

Facilities

Growth and digestibility experiments were conducted simultaneously in twelve 380 litre circular polypropylene tanks over a period of 8 weeks at the Ridley AgriProducts Narangba (Queensland) facility. Each tank was supplied with recirculated water at a rate of approximately 7 litres per minute. Aeration and circulation was achieved by the use of tangential sprays of influent water into each tank. This created a circular water flow forcing particulate matter to the centre of each tank. Effluent was drained from the centre of each tank and passed through two settling areas. The first tank was a 750mm X 100mm PVC cylinder, that tapered to a 25mm ball valve, removing all of the faecal and uneaten food particles. From this faecal collection chamber, samples were taken for digestibility analyses. The second, a 380 litre circular tank, was designed to ensure particular material was removed before water entered the biological filter. The biological filter comprised a 380 Lt tank filled with plastic shavings as media. Twenty five percent of the total water from each tank was exchanged daily with dechlorinated town water. Tanks were cleaned daily. Settling containers were cleaned and cleared of uneaten food and faeces before and after feeding (ie four times daily). Temperature, pH and salinity were recorded daily.

Fish

Rainbow trout(O. mykiss) used in the experiment were purchased from the Ballarat Fish Acclimatisation Society, Ballarat, Victoria. Fish were air transported to the Narangba facility. Fish were maintained in holding tanks for two weeks to ensure all remaining fish had recovered from transportation stresses.

Fish were selected randomly and were placed in one of six tanks, and allowed to acclimatise for one week. The initial fork length and total weights were determined for each trout. At the end of the first week, tanks were randomly allocated either the reference diet or one of the two experimental diets. Every two weeks the entire tank population was reweighed and measured. Throughout the experiment mortalities were recorded daily.

Diet Formulation

Modified meat meal, prepared by MIDCOAST Protein Pty Ltd, Wirrimbi, Via MACKSVILLE NSW 2447 (PO Box 40), was included at two levels, 30% and 50%, replacing fish meal in the diet. The diets, reference and trial were based on the formulae proposed by Cho, Slinger and Bayley (1982). Chromic oxide was used as the inert digestibility marker at an initial calculated inclusion level of 1% of the total diet.

Diet Preparation

Trout feed ingredients were mixed and extruded using the commercial milling equipment at the Ridley AgriProducts, Narangba Feed Mill, Queensland.

Feeding

All fish were fed at rates (% body weight as dry feed/day) according to commercial recommendations. Fish were fed to satiation twice daily at 0830 and 1600. Feeding rations were adjusted weekly to allow for mortalities, growth and changes in water temperature. One hour after feeding had ceased all uneaten food was drained from settling chambers, dried for 6hrs at 105°C and weighed.

Faecal Collection and Digestibility

Trout faecal material was collected each morning at 08.00Hrs. Faecal samples were bottled, labelled and frozen, until required for analysis. Faeces collected was pooled. Faecal material was analysed for protein, fat, ash, phosphorous, calcium, fatty acid and chromic oxide content.

Fish Whole Body Composition

At the conclusion of the eight week trial, the entire fish populations were humanly euthanased using deep anaesthesia (Benzocaine @ 150 ppm). Fish were subsequently blended to a paste and analysed to determine water, protein, fat and ash content.

Biochemical analyses

Chromium analysis was performed as described by Cho et al, 1982. Nitrogen content was determined using the macro kjeldahl method. Fat was determined by standard Soxhlet extraction and Folch/gravimetric method. Fatty acid analysis was determined by gas chromatography. Calcium was determined by colourmetric methods and phosphorous levels by colourmetric methods. All of these methods were according to accepted laboratory practice.

Statistical Analysis

Fish growth and survival data were analysed using Analysis of Variance and Scheffe's Multiple Range Test.

4.2 Saltwater Trout Validation Trial

4.2.1 Methods

Facilities

The eight-week experiment was conducted in nine 1000 litre fibreglass raceways situated at Ridley's Cheetham Salt, Aquaculture Research Facility, Lara, Victoria. Each raceway was supplied with continuous flowthrough seawater at ambient temperature.

Seawater was diluted with freshwater where appropriate to maintain a salinity range of 28-32 parts per thousand (ppt).

Water flow rates were maintained at forty (40) litres per minute through each raceway. Supplementary aeration was provided in all tanks. Tanks were cleaned weekly.

Diets

Three diets were used in the experiment. Two of these were commercially available products; one product was a semi-floating extruded diet and the other a conventional steam pressed sinking diet. The third diet was the MRC trial diet, based on the trout reference diet proposed by Cho et. al. (1982), with the inclusion of 30% meat meal. The formula for this diet is presented in Table 2.

Table 2

Composition of MRC diet used in freshwater and saltwater growth trials (% diet DM).

Raw Material	Inclusion Level (%)
Fish meal	35.8
Wheat	20.0
Meat meal	30.0
Soya Bean Meal	7.0
Fish Oil	6.0
Chromic Oxide	1.0
Premix and Vitamins	0.2

For each diet three replications occurred, totalling nine tanks for the experiment. Allocation of diets to each raceway was randomised to avoid any inherent bias associated with position within the facility. All feeds were obtained immediately before the experiment commencement and were stored in sealed plastic bins at ambient temperature.

Fish

The rainbow trout used in the experiment were purchased from the Ballarat Fish Acclimatisation Society, Ballarat, Victoria. Fish were transported by road within insulated containers, Fish were placed into freshwater holding tanks and acclimatised to seawater over a 14 day period. Salinity was incremented by 2 ppt daily, until the experimental salinity level of 28 ppt was reached.

The day before the commencement of the experiment, fish were graded to obtain a uniform size and weight class for the trial. Small and large fish were excluded from the trial and returned to the separate holding tanks.

Two hundred and fifty randomly selected trout were added to each of the raceways, replicating stocking density used in commercial freshwater growout environments. This density coincided with the density used in the freshwater segment of the trial.
During the first day of the trial, 80 fish were randomly selected from the raceways and anaesthetised (MS222 @ 2.0g/2 litres). The fork length (+/- 5 mm) and liver weight (+/- 0.1g) for each fish was then recorded.

Every two weeks throughout the experiment a random sample of 2- fish from each raceway, were measured and weighed. Throughout the experiment any fish mortalities were recorded daily.

Feeding Rates

All fish were fed at rates according to their body weight, as a percentage dry feed/day basis. Fish were fed by hand to satiation twice daily at 0830 and 1600.

Feeding rates were adjusted weekly to allow for mortalities, growth and changes in water temperature.

Before feeding, all food was sieved to remove dust and small particles, according to commercial practice.

Water Quality

Air and water temperatures (degrees C), salinity (ppt) and dissolved oxygen levels (ppm) were measured daily. Meteorological values for rainfall (mm) cloud cover (10/10 scale) and wind strength (km/hr) were recorded at the same time (10:30Hrs) as water quality parameters.

Total nitrogen and phosphorus in influent and effluent waters were analysed on four occasions throughout the period of the experiment. Effluent water was taken prior to the later feeding event directly from the raceway. Influent water was taken from the supply pipe to the raceway systems. All samples were analysed by the Australian Government Analytical Laboratories, Melbourne, Victoria.

4.3 Freshwater Trout Validation Trial

4.3.1 Methods

An 8 week growout trial was contracted out of the Victorian Fisheries Research Institute, Snobs Creek, Victoria. A detailed description of the evaluation is appended as Attachment I.

Briefly, a 3 diet comparison occurred with the MRC experimental diet (Table 2) being compared to extruded and steam pressed commercial diets. Fish were stocked at 20kg/m^3, being a typical commercial density. Feeding was 6 times a day with adjustments according to growth and water temperature. Water quality parameters were logged during the trial, as were typical parameters of the fish viz: growth, fork length, food conversion efficiency, survival. These were recorded on a 2 week basis.

4.4 Graded Inclusion Trial for Black Tiger Prawns

4.4.1 Methods

Facilities

Growth and digestibility experiments were conducted simultaneously in nine 50 litre perspex tanks over a period of 5 weeks at the Ridley Narangba (Queensland) facility. Each tank was supplied with recirculated water at an exchange rate of approximately 1000% per day. Aeration and circulation was achieved by spraying influent water into each tank. This created a transit water flow forcing particulate matter to the bottom of each tank. Effluent was drained from the centre of each tank and passed into a settling cone packed in ice to minimise faecal degradation. From this faecal collection chamber, samples were drained off for digestibility analyses. Tanks were cleaned daily. Faecal collection containers were cleaned and cleared of uneaten food and faeces before and after feeding (ie two times daily). Temperature, pH and salinity were recorded daily.

Prawns

Tiger prawns (Penaeus monodon) used in the experiment were purchased from Gold Coast Marine Hatcheries, Woongoolba, Queensland. Animals were maintained in holding tanks for two weeks prior to the experiment, to ensure all had recovered from transportation stresses.

Prawns were then selected randomly and placed in one of 9 tanks and allowed to acclimatise for one week. The weight of each prawn was determined. At the end of this week, tanks were randomly allocated to either the reference diet or one of the two experimental diets. Every two weeks the entire tank population was reweighed and measured. Throughout the experiment mortalities were recorded daily.

Diet Formulation

Modified meat meal was included at two levels, 30% and 50%, replacing fish meal in the diet. The diets, reference and trial were based on commercial formulae used by Aquafeed Products Australia, Narangba.

Diet Preparation

Prawn feed ingredients were mixed and pressed using the commercial milling equipment at the Narangba site.

Feeding

All prawns were fed at rates (% body weight, as dry feed/day) according to commercial recommendations. Prawn were fed to satiation twice daily at 0830 and 1600. Feeding rations were adjusted weekly to allow for mortalities, growth and changes in water temperature. One hour after feeding had ceased all uneaten food was drained from settling chambers, dried for 6hrs at 105° C and weighed.

Faecal Collection and Digestibility

Faecal samples were bottled, labelled and frozen, until required for analysis. Faeces collected was pooled. Faecal material was analysed for protein, fat, ash, phosphorous, calcium and fatty acid content.

Prawn Whole Body Composition

At the conclusion of the eight week trial, the entire prawn population was euthanased using anaesthesia. Animals were subsequently blended to a paste and analysed to determine water, protein, fat and ash contents.

Biochemical Analyses

Nitrogen content was determined using the macro Kjeldahl method. Fat was determined by standard Soxhlet extraction and Folch/gravimetric method. Fatty acid composition was determined by gas chromatography. Calcium was determined by colourmetric methods and phosphorous levels by colourmetric methods. All of these methods were according to accepted laboratory practice.

Statistical Analysis

Prawn growth and survival data were analysed using Analysis of Variance and Scheffe's Multiple Range Test.

4.5 Prawn Growth Validation Trial

4.5.1 Methods

The "commercial" growth validation trial was conducted with a single crop of P. monodon prawns being finished within one pond at the Central Queensland University Applied Science Faculty, Rockhampton. Prawns were fed for a period of 5 weeks, with the MRC diet. Food was offered 5 times each day being: sunrise, 1000, 1400, Sunset and 2000. Two thirds of the daily amount of food on offer each day was fed at sunrise and sundown, with the remaining one third split between the remaining 3 feeding events. One other pond at the facility within the same production cycle, served as the control group.

Animals

Prawns used for the trial were 23 weeks of age at commencement, being in the ponds for the previous 21 weeks. Expected growth rate was 1 gram per week.

Measurements

The average body weight (ABW) was calculated weekly and at the start and conclusion of the trial, following the group weighing of 5 samples of 20 prawns from each pond. Daily measurements of environmental parameters including pH, conductivity, salinity, water temperature, turbidity and dissolved oxygen level occurred.

4.6 Sensory Evaluation

4.6.1 Methods

Sensory evaluation of the trout grown on the three different diets within the freshwater trial (4.3) was undertaken at the Queensland Department of Primary Industries Sensory Evaluation Unit, within the Centre for Food Technology (Brisbane).

The object of this study was to ensure no "off-flavour" characteristics had been imparted to the fish consuming a diet with moderate levels of meat meal.

Experimental Procedure

Fresh chilled "heads on gutted" trout (10) from each treatment group were air freighted, on ice, to Brisbane. A detailed description of the procedure is presented within Attachment II. Briefly, trained panellists evaluated cooked "head end" and "tail end" portions of trout flesh, from each diet group. Evaluators under "daylight equivalent" illumination systems ranked flesh according to colour, odour, flavour and texture.

5. Results

5.1 Trout Graded Inclusion Trial

Mean growth, food conversion efficiency (FCR) and survival rates for each of the three diets are identified in Table 3.

Table 3

Summary of growth, FCR and survival data for trout reared on diets containing 0%, 30% and 50% modified meat meal.

PARAMETER Mean (SE)	DIET Reference(0%)	 MRC (30%)	 MRC (50%)
Weight (g) Initial	15.8 (0.8)	14.3 (0.9)	14.6 (0.9)
Final	34.7 (2.6)	29.5 (2.1)	28.7 (2.5)
Increase	18.9	15.2	14.1
Gain (%)	120.8	107.0	97.0
Fork Length (mm)			
Initial	100.2 (2.3)	99.5 (1.9)	99 (2.4)
Final	129 (3.5)	122.4 (3.0)	123.8 (3.6)
Increase	28.8	22.5	24.8
Gain (%)	28.6	23.3	25.1
Food Conversion Ratio (FCR)	1.5: 1	1.8: 1	1.5: 1
Mean Survival (%)	86.6	96.6	86.6

Analysis of the three experimental diets according to gross nutritional parameters is presented in Table 4.

Table 4

Nutritional parameters of trout inclusion diets.

	Meat meal Inclusion Level		
Parameter Mean	0%	30%	50%
Protein (SE)	40.06 (0.06)	44.1 (0.12)	41.03 (0.08)
Fat (SE)	12.17 (0.05)	16.1 (0.01)	21.48 (0.04)
Ash (SE)	9.15 (0.01)	9.7 (0.01)	9.4 (0.01)
Calcium (SE)	1.4 (0.05)	1.7 (0.03)	2.06 (0.03)
Phosphorous	1.6	1.8	2.0
Chromic Oxide	0.79	0.86	0.56

The body composition values of trout feeding on the three diets are presented in table 5.

Table 5

Whole body composition of trout (dry matter basis) fed various levels of meat meal.

	Meat meal Inclusion Level		
Parameter Mean	0%	30%	50%
Protein (SE)	50.29 (0.05)	49.49 (0.06)	49.90 (0.03)
Fat (SE)	37.50 (0.21)	39.50 (0.25)	39.17 (0.30)
Ash (SE)	9.04 (0.12)	8.52 (0.04)	8.43 (0.09)
Moisture (SE)	75.73 (0.10)	75.83 (0.11)	74.04 (0.13)

Mean cumulative nett weight gains within the inclusion trial presented in Table 6 and Figure 1 respectively.

Table 6

Mean cumulative trout weight gain within the graded inclusion trial.

Mean Cumulative Weight Gain (grams)					
Inclusion Level	Week 0	Week 2	Week 4	Week 6	Week 8
0%	15.7	18.3	24.9	31.1	34.6
30%	14.3	17.3	22.8	26.7	29.5
50%	14.6	18.4	23.9	26.6	28.7

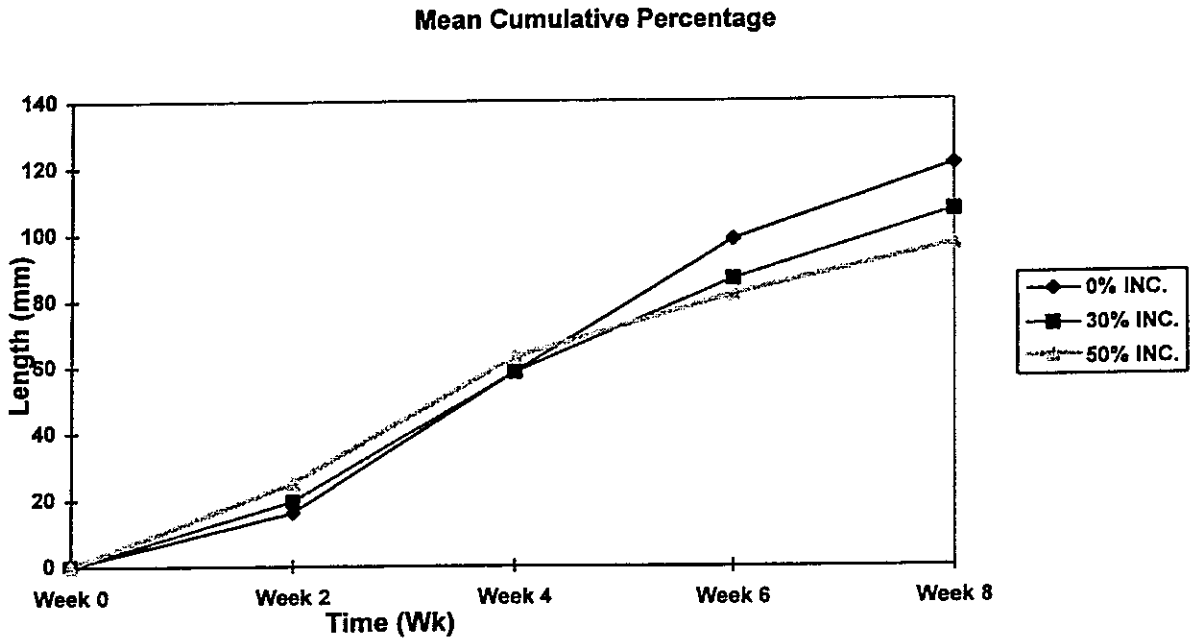

Figure 1

Mean cumulative weight gain (%) for each of the inclusion diets.

The mean cumulative length gain within the inclusion trial is presented on a nett and percentage basis in Table 7 and Figure 2 respectively.

Table 7

Mean cumulative length gain (gm) for trout in each of the inclusion diets.

Mean Cumulative Trout Length Gain (mm)					
Inclusion Level	Week 0	Week 2	Week 4	Week 6	Week 8
0%	100.2	110.4	117.6	126.2	128.8
30%	99.5	108.1	112.7	119.9	122.6
50%	99	109.5	115.3	121.3	123.7

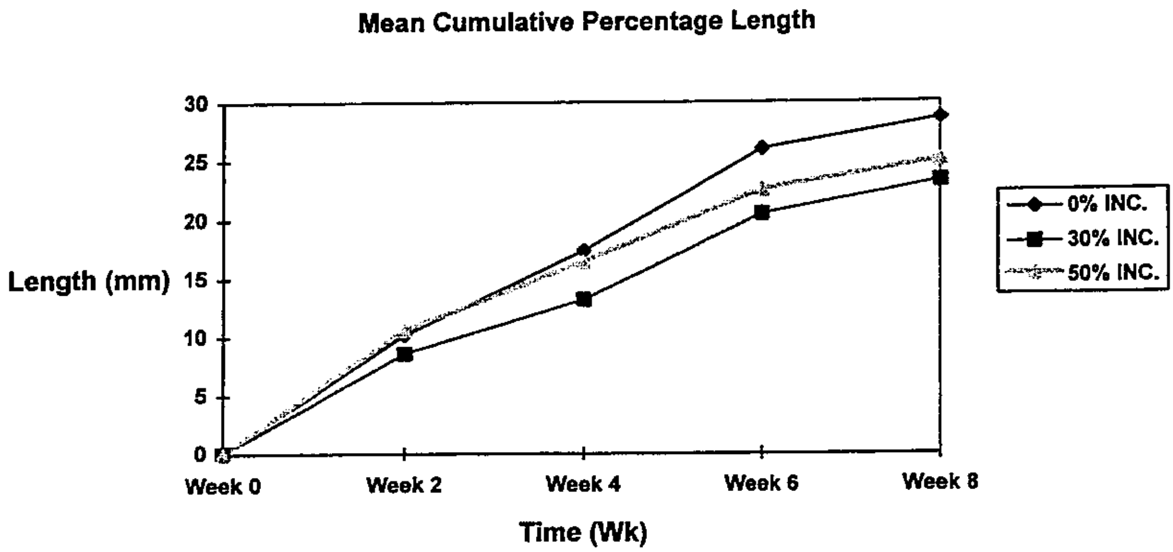

Figure 2

Mean cumulative length gain (%) for rainbow trout fed the 3 inclusion diets.

A summary of the statistical analyses is presented in Table 8.

Table 8

Summary of Statistical Analyses - Freshwater Inclusion Trial

Anova Trout Weight		
P=0.0742 F(2,3)=6.999		
Inclusion	Mean	Grouping
0%	34.7	A
30%	29.5	A
50%	28.7	A
Anova Trout Length		
P=0.2439 F(2,3)=2.342 Scheffe's 95% multiple range test		
Inclusion	Mean	Grouping
0%	128.8	A
30%	122.7	A
50%	123.8	A

Food Conversion Ratio	
Diet	Mean
0%	1.5:1
30%	1.8:1
50%	1.5:1

5.2 Saltwater Trout Validation Trial

The mean growth, feed conversion rates and survival of trout in saltwater environment are presented in Table 9.

Table 9

Summary of growth, FCR and survival data for trout reared in saltwater on commercial and experimental diets.

PARAMETER (MEAN)	DIET MRC	Extruded	Pressed
Wt(g): Initial	32.1	32.1	32.1
Final	137.1	91.9	104.9
Increase (g)	105	59.8	72.8
Gain (%)	427.1	286.2	326.7
Fork Length (mm)			
Initial	140	140	140
Final	214	194.2	200.2
Increase (mm)	74	54.2	60.2
Gain (%)	152.8	138.7	143.0
Food Conversion Ratio (FCR)	0.8:1	1.3: 1	1.1:1
Mean Survival (%)	96.0	95.6	94.0

The compositional values of overall parameters for the three diets used in the saltwater trial are presented in Table 10.

Table 10

Proximal analysis of trout diets used in the saltwater feeding trial.

Parameter (%)	MRC Diet	Extruded Diet	Pressed Diet
Protein	44	45	45
Fat	16	14	14
Ash	9.7	10	10

The mean live weight gains on a cumulative nett and percentage basis are presented in Table 11 and Figure 3, respectively.

Table 11

Cumulative weight gain for trout grown in saltwater on 3 different diets.

Mean Cumulative Trout Weight Gain (grams)					
Diet	Week 0	Week 2	Week 4	Week 6	Week 8
MRC	32.1	50.43	75.8	103.3	137.13
Extruded	32.1	48.23	66.93	82.8	91.9
Pressed	32.1	46.63	66.13	83.63	104.96

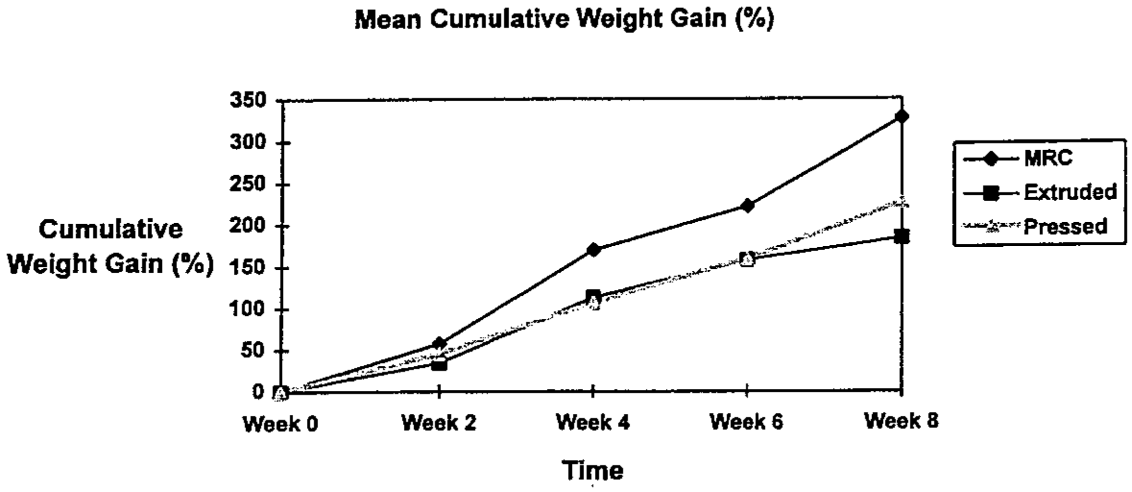

Figure 3

Mean cumulative weight gain for trout grown in saltwater on 3 different diets.

The mean length gains, expressed on a nett and cumulative basis for the trout in saltwater is presented in Table 12 and Figure 4, respectively.

Table 12

Cumulative gain in trout length grown in saltwater.

Mean Cumulative Trout Weight Gain (grams)					
Diet	Week 0	Week 2	Week 4	Week 6	Week 8
MRC	140.0	156.03	178.03	195.86	214.1
Extruded	140.0	156.26	174.16	189.1	194.1
Pressed	140.9	153.53	173.53	186.43	200.13

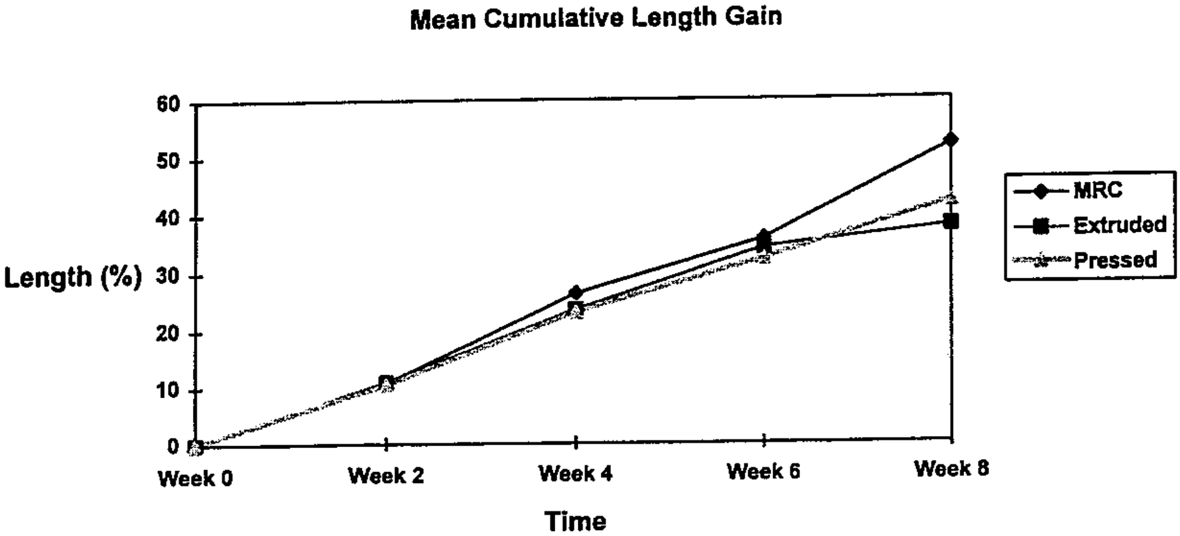

Figure 4

Mean cumulative percentage trout length gain - saltwater trial.

A summary of the statistical analyses for the saltwater trial is presented in Table 13.

Table 13

Summary of statistical analyses - saltwater trial.

Food Conversion Ratios					
Diet	Week 2	Week 4	Week 6	Week 8	Average
MRC	1.1:1	0.9:1	0.6:1	0.5:1	0.8:1
Extruded	1.1:1	1.3:1	1.1:1	1.9:1	1.3:1
Pressed	1.4:1	1.1:1	0.9:1	0.9:1	1.1:1

Anova Week 8 Weight - Saltwater Trial

P=0.0145
F(2,6)=9.316
Scheffe's Multiple Range Comparison 95% of mean weights for trout reared on three different diets

Grouping	Diet	Mean Weight
A	MRC	137.1
B	Pressed	105.0
C	Extruded	91.9

Anova Week 8 Length - Saltwater Trial P<0.0459 F(2,6)=5.381 Scheffe's Multiple Range Comparison 95% of mean lengths for trout reared on three different diets		
Grouping	**Diet**	**Mean Weight**
A	MRC	214.1
A	Pressed	200.1
A	Extruded	194.2

5.3 Freshwater Trout Validation Trial

A complete set of results is contained in the contract report in Appendix I.

The mean live weights of fish within the MRC diet treatment group and the extruded diet groups were not significantly different from each other, with mean incremental gains of 65 and 66 grams respectively. This represented a weight increase of 7.6 and 7.7% per week respectively. Food conversion efficiency ratios were 1.36 and 1.27 for MRC and extruded diets. Survival rates were very high for each treatment group and water quality parameters were generally within regulatory standards set by state authorities.

Fish growth for the MRC diet containing 30% modified meat meal appeared at least equal to industry standards.

<u>Table 14</u>

Summary of growth and FCR for trout on 3 different diets.

	Diet		
Parameter	**Extruded**	**Pressed**	**MRC (pressed)**
Weight(g) Initial	82	83	82
Final	148	129	147
Increase	66	46	65
% growth/wk	7.7	5.7	7.6
Length Initial	185	186	185
Final	218	216	219
Increase	30	30	34
FCR	1.27	1.41	1.36

5.4 Graded Inclusion Trial for Black Tiger Prawns

The gross nutritional parameters of the prawn diets is presented in Table 15. With higher inclusion levels the crude protein and fat levels both increased marginally while ash remained relatively constant.

Table 15

Nutritional parameters of prawn inclusion diets.

	Meat Meal Inclusion Level		
Parameter Mean	0%	30%	50%
Protein (SE)	47.6 (0.03)	50.1 (0.07)	53.3 (0.12)
Fat (SE)	9. 9 (0.15)	11.3 (0.20)	12.5 (0.30)
Ash (SE)	11.3 (0.04)	12.0 (0.02)	12.4 (0.01)
Calcium	1.9	2.0	2.3
Phosphorus	1.9	2.1	2.7

Whole body composition values for prawns fed either of the diets are presented in Table 16.

Table 16

Whole body composition of prawn (% DM basis) fed various levels of meat meal.

	Meat Meal Inclusion Level		
Parameter Mean	0%	30%	50%
Protein (SE)	73.6 (0.08)	73.5 (0.10)	73.1 (0.11)
Fat (SE)	3.91 (0.28)	4.26 (0.15)	4.15 (0.05)
Ash (SE)	9.6 (0.01)	9.88 (0.01)	12.4 (0.02)
Moisture (SE)	82.8 (0.11)	80.7 (0.07)	79.8 (0.10)

Prawn growth rate was comparable at the 30% inclusion level to that of a complete fish meal inclusion level, but deteriorated when present at the 50% level (Table 17).

Table 17

Mean whole body weights of prawns grown on diets containing 0, 30 and 50% modified meat meal.

	Meat Meal Inclusion Level		
Parameter Mean	0%	30%	50%
Weight (g) Initial (SE)	1.61 (0.11)	1.74 (0.14)	1.49 (0.13)
Final (SE)	2.66 (0.52)	2.78 (0.34)	2.43 (0.29)
Increase (SE)	1.05 (0.55)	1.04 (0.39)	0.94 (0.36)

5.5 Prawn Growth Validation Trial

Mean whole body weights for the prawns in each pond are presented in Table 18. No detrimental effects were recorded, with harvested prawns from both ponds, seen as dark grey-black in colour and a bright orange colour, cooked. High consumer appeal was deemed equal between the product from the 2 ponds.

Table 18

Mean weekly body weights for Panaeus monodon grown with the MRC and a commercial 'control' diet.

	Mean Body Weight (g)	
Time (Week #)	MRC Diet	Control Diet
21	25.5	13.0
22	27.5	14.9
23	29.4	15.9
24	31.9	17.5
25	34.9	19.0
26	37.2	21.0
Harvest Weight	38.7	22.8
Mean Growth /Week (g)	2.20 (a)	1.63 (b)

5.6 Sensory Evaluation

Trout fed the different diets were generally very similar on sensory evaluation. Colour differences were recorded but as various levels of artificial colouring had been added to each diet, according to commercial demand, this was of little consequence.

Odour profiles showed the MRC diet to produce a higher "fishy" smell but this was not statistically significant as was flavour and texture.

A detailed record of these findings is presented in the QDPI report in Appendix II.

6. Discussion

6.1 Applicability of Modified Meat Meal to Salmonoid diets

Food Conversion Ratio (FCR)

The food conversion ratios derived from this section of the evaluation have indicated that the use of modified meat meals in the feeding of rainbow trout does not have any detrimental effects on performance.

Results from the graded inclusion trials indicate that the incorporation of modified meat meal into trout diets can potentially be up to fifty percent. No significant differences were seen between the reference diet with zero inclusion of modified meat meal and either the thirty or fifty percent inclusion diets.

The trout reference diet returned an FCR of 1.5:1. In the experimental situation this is considered a very good rate and confirms the suitability of the reference diet. Of interest though is the FCR's for the diets with included modified meat meal of thirty and fifty percent. The rates were 1.8:1 and 1.5:1 respectively. The higher figure for the thirty percent included diet rather than the fifty percent diet may be explained by experimental variation.

Results from the saltwater validation trial produced an FCR for the thirty percent inclusion diet, of 0.8:1. Two commercially available diets were also trialed in this section of the project. A semi floating extruded diet and a steam pressed sinking diet produced FCR's of 1.3 and 1.1 respectively.

The freshwater commercial validation trial (appendix I) reported FCR's of 1.3:1 for the thirty percent inclusion diet, 1.2:1 for the extruded diet and 1.4:1 for the pressed diet.

Weight Gain

The freshwater validation trial (Appendix 1) determined that the weight gain of fish was within currently accepted limits for trout under commercial conditions. Therefore it can be concluded that the substitution of 30% modified meat meal does not adversely effect the weight gain of rainbow trout in fresh water conditions.

The salt water commercial validation trial determined a positive advantage through the addition of modified meat meals to trout diets under these conditions. Percentage total weight gain for extruded and pressed commercial diets was 286% and 326% respectively. However, the reference diet with 30% inclusion of modified meat meal returned a gain of 427%.

This data supports the findings of Allan (1994) who evaluated various meat products for use in silver perch (Bidyanus bidyanus) diets. With bone removed from the product, prior to rendering, low ash (3.0 to 12.1%) protein meals gave comparable food conversion efficiency to Peruvian fish meal. That study records a range of digestibility's for amino acids with vegetable and animal derived protein ingredients for aquaculture diets.

The value of digestible protein and lysine was also calculated by Allan (1994) for a high protein derived meat meal (PROVINE(R)). When priced at $775/tonne (1994 prices) this product was very competitive to Danish fish meal at $1200/tonne, for these two parameters. Within the current study the low ash modified meat meal cost $1785/tonne (1995 price) which prevented it's ability to substitute for even the highest grade of imported fish meal (e.g. Danish fish meal of 72% protein costing $1350/tonne, 1995 price).

6.2 Applicability of Modified Meat to Prawn Diets

Food Conversion Efficiency

When included to a level of 30% within the diet no apparent penalty in growth rate or food conversion efficiency occurred. However, when present at 50% of the diet a negative impact on these parameters was indicated. Further studies are warranted to substantiate this response.

Smith (1995) evaluated a number of meat meals which were put forward as potential substitutes for fish meal. When included at rates of up to 29% no significant reduction in growth performance was observed. At this level one half of the fish meal present in the juvenile P. monodon diets had been substituted. Lawrence and Castille (1988) had previously shown that P. vannamli juveniles on a 40% protein diet showed significant reductions in growth when >30% meat and bone meal was included. When included at 20% of the diet growth was reduced by only 6.4% which was not significant. The quality of the meat and bone meal was not discussed.

7. Recommendations

This study further substantiates previous local studies, that low ash, high protein animal sourced meat meals, are effective in sustaining the growth of rainbow trout and tiger prawns in commercial environments. Critical levels of substitution occur, but such meals at 30% inclusion appear to offer negligible physiological penalty.

However, cost of producing these meals is of paramount importance. Allan (1994) rated the PROVINE product highly @ $775/tonne (1994 price) for aquaculture diets while in this study the MIDCOAST product, of comparable proximal analysis, was more than twice of that material. The Australian Renders Association must have members introduce critical production systems and competitive pricing, if such meals are to be placed in the aquaculture industry.

8. References

Allan, G., 1994. Preliminary evaluation of meat meal in aquaculture diets for silver perch (Bidyanus bidyanus). Final Report to MRC.

MRC Final Report M.561

Cho,C.Y., Slinger,S.J. and Bayley,H.S., 1982. Bioenergetics of salmonid fishes: Energy intake, expenditure and productivity. *Comp. Biochem. Physiol.* 73B(1): 25-41.

Gatlin,D.M.III and Phillips,H.F., 1989. Dietary calcium, phytate and zinc interactions in channel catfish. *Aquaculture* 79: 259-266.

Ketola,G.H., 1979. Influence of dietary zinc on cataracts in rainbow trout (*Salmo gairdneri*) (= *Oncorhynchus mykiss*). *J. Nutr.* 109: 965-969.

Lawrence A.L. and Castille F.C., 1988 The Nutritional Response of Postlarval Penaeus vannamei to Meat and Bone Meal "Journal of the World Aquaculture Society, 19, 44A.

Richardson,N.L., Higgs,D.A., Beames,R.A. and McBride,J.R., 1985. Influence of dietary calcium, phosphorous, zinc and sodium phytate level on cataract incidence, growth and histopathology in juvenile chinook salmon (*Oncorhychus tshawytscha*). *J. Nutr.* 115: 553-567.

Satoh,S., Takeuchi,T. And Watanabe,T., 1987a. Availability to rainbow trout of zinc in white fish meals. *Nippon Suisan Gakkaish* 53:595-599.

Satoh,S., Takeuchi,T. And Watanabe,T., 1987b. Availability to rainbow trout of zinc contained in various types of fish meals. *Nippon Suisan Gakkaish* 53:1861-1866.

Smith D. 1995. Preliminary evaluation of meat meal in aquaculture diets for prawns (Penaues monodon) MRC Final Report, Part 2.

EVALUATION OF THE MEAT RESEARCH CORPORATION (MRC) TROUT DIET:

Comparison with Aquafeed and Barastoc diets, and quality of discharge water

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September, 1995

Prepared on behalf of: Ridley Agriproducts Pty Ltd and Cheetham Salts Ltd.

EVALUATION OF THE MEAT RESEARCH CORPORATION (MRC) TROUT DIET:

Comparison with Aquafeed and Barastoc diets, and quality of discharge water

SUMMARY

The following results were obtained from an eight week experiment to compare the growth and survival of rainbow trout reared on three different diets, and the quality of discharge waters in relation to inlet water quality.

1. There was a significant difference in the weight of fish. Overall trout reared on the MRC and Aquafeed diets were significantly heavier that trout reared on the Barastoc diet.
2. Growth (percent weight increase per week) of fish were 5.7% (Barastoc), 7.6% (MRC) and 7.7% (Aquafeed).
3. Food conversion ratios were 1.27 (Aquafeed), 1.36 (MRC) and 1.41 (Barastoc), but these figures should be treated with caution due to the relatively short period of time over which the experiment was run.
4. There were no significant differences in fork lengths of fish reared on the different diets.
5. Survival of fish was high (99.4-100%), and there were no significant differences.
6. Significant differences between the inlet water and discharge waters for the three diets were observed for pH, Total Ammonia Nitrogen (TAN) and Total Phosphorus (P). pH readings from discharge waters were within EPA requirements and net TAN concentrations rarely exceeded EPA median concentration requirements. However, net P concentrations in discharge waters often exceeded EPA median concentration requirements and occasionally exceeded EPA maximum concentration requirements.

INTRODUCTION

As part of the Meat Research Corporation (MRC) Project, "*Processing of Meatmeal for Utilisation in Aquaculture Diets*", the Victorian Fisheries Research Institute, Snobs Creek (VFRISC) was contracted to undertake an experiment to evaluate the MRC trout diet. Specifically, the growth and survival of rainbow trout (*Oncorhynchus mykiss*) reared on the MRC diet were compared against fish reared on either Barastoc or Aquafeed trout diets. In addition, the quality of the discharge water (pH, Total Ammonia (Nitrogen) and Total Phosphorus) was analysed for each of the three diets and compared against the inlet water.

This report outlines the results of this experiment and includes summation and analysis of fish growth (fork length and weight), survival and food conversion data, and water quality data from inlet and discharge waters.

MATERIALS AND METHODS

Facilities

The eight week experiment was conducted in nine 2,000 l fibreglass tanks situated at the VFRISC. Each tank was supplied with a continuous flow of water at ambient temperature without supplementary aeration, and at a rate of 50 l/minute. Tanks were cleaned once each day. Uneaten food was removed, the bottom and sides of the tanks were cleaned with a broom and the sumps were purged.

Diets

The three diets used for the experiment were:

1. Barastoc DF Special Grower No. 7
2. Aquafeed 4 mm Grower
3. Meat Research Corporation (MRC) test diet

For each diet, three tanks (replicates) were used, giving a total of nine tanks for the experiment. Allocation of a diet to each tank was randomised to avoid any inherent bias associated with tank position. All feeds were obtained immediately before the experiment commenced and were held in a coolroom at 4°C throughout the experiment.

Fish

Rainbow trout used in the experiment were on loan from the Narangi Trout Farm (based at the VFRISC). Prior to commencement of the experiment the trout were graded to remove smaller fish and were held in a concrete raceway. On the agreed start date (10 July 1995), 500 randomly selected rainbow trout were hand-counted into each of the nine tanks, which is a standard stocking density for a commercial operation (stocking density 20 kg/m^3). However, due to a delay in the arrival of one of the diets, the experiment did not commence until the 17th July 1995. On this day, random samples of 20 fish from each tank were anaesthetised and the fork length (FL) to the nearest mm and weight (Wt) to the nearest gram (g), were recorded for each fish. Every two weeks during the experiment a random sample 20 fish was measured from each tank, and at the end of the experiment a sample of 30 fish was measured from each tank. Throughout the experiment fish mortalities were recorded daily.

Feeding

All fish wear fed at rates (percent body weight as dry feed/day) provided by Ridley Agriproducts for each of the diets, which were based on digestible energy, water temperature and fish weight. Fish were fed by hand, 6 x daily. Feeding rations were adjusted weekly to allow for fish mortalities, fish growth (ie 5% increase per week) and changes in water temperature. Prior to feed weighing the daily rations, feeds were sieved to remove dust and small particles. Therefore, daily feed rates were based on sieved material for all diets.

Water quality

A TPS Data logger was used to record water temperature (°C), dissolved oxygen (D.O.) (mg/l), conductivity (µS/cm) and pH of the inlet water every two hours during the experiment. Water temperature and dissolved oxygen in each tank were also measured with a YSI meter daily. Total Ammonia Nitrogen (TAN) (Nessler Method), Total Phosphorus (P) (Acid Persulphate Digestion Method) and pH were measured 3 times each week. This was done by collecting a 200 ml sample of water from the discharge drain of each tank and then pooling the 3 samples (one from each replicate) for each diet. From the pooled samples triplicate readings of TAN and P were measured with a Hach DREL/2000 water analysis kit. In addition triplicate readings of these parameters were taken of inlet water.

Analysis

Fish growth data (FL and Wt) were analysed using the SAS Generalised Linear Models Procedure and Duncan's Multiple Range Test, fish survival data was analysed using Analysis of Variance, and water quality data was analysed using Analysis of Variance and Tukey's Multiple Range Test.

RESULTS AND DISCUSSION

Fish Growth and survival

A summary of the fish growth data, including initial and final FL and Wt, growth rates (percent increase in weight per week) and Food Conversion Ratios (FCR), for each diet is presented in Table 1.

There was a significant overall difference ($F_{2,6} = 6.85$; $P = 0.0282$) and a significant difference over time ($F_{2,24} = 3.04$; $P = 0.0164$) in weight of fish for each diet (Fig. 1). Duncan's Multiple Range Test showed that the mean weights of fish reared on Aquafeed and MRC diets were both significantly greater than the mean weight of fish reared on the Barastoc diet (Table 2), which is also apparent in Fig 1.

There were no significant differences in the fork lengths of fish for the three diets (Overall: $F_{2,6} = 1.10$; $P = 0.3909$. Interaction: $F_{2,24} = 1.10$; $P = 0.6398$) (Fig. 2).

Survival of fish in all replicates and treatment at the end of the experiment was high with 0-3 fish (99.4 - 100% survival) dying in any one tank over the 8 week period (Table 1). There was no significant difference in survival of fish for the three diets ($F_{2,6} = 0.36$; $P = 0.7095$).

FCR's for each diet are presented in Table 1. However, these data should be treated with caution due to the relatively short period of time over which the experiment was run.

At the completion of the experiment, 30 fish from each diet type (10 fish from each replicate) were held in tanks and fed on the respective diets for another week after which they were gilled and gutted, placed on ice and air-freighted to Brisbane for taste-testing analysis. All other fish used in the experiment were returned to Narangi Trout Farm.

Feeds

In general, the Barastoc and Aquafeed diets were readily taken by the fish. However, in tanks which were receiving the MRC diet there was often uneaten food left on the bottom.

Table 1. Summary of growth, FCR and survival data for trout reared on three different diets

Parameter		Diet	
	Aquafeed	Barastoc	MRC
Wt(g): Initial (mean ± s.e.*)	82 ± 1.7	83 ± 1.7	82 ± 1.4
Final (mean ± s.e.)	148 ± 2.8	129 ± 2.5	147 ± 1.9
Increase	66	46	65
Mean growth (% per week)	7.7	5.7	7.6
FL (mm): Initial (mean ± s.e.)	185 ± 1.4	186 ± 1.5	185 ± 1.1
Final (mean ± s.e.)	218 ± 1.4	216 ± 1.5	219 ± 1.0
Increase	30	30	34
Food Conversion Ratio (FCR)	1.27	1.41	1.36
Mean percent survival (%)	99.8	100	99.7

* s.e. = standard error

Table 2 Duncan's Multiple Range comparison of overall mean weights for trout reared on three different diets

Duncan's Groupings*	Diet	Mean weight (g)
A	MRC	114.5
A	Aquafeed	113.2
B	Barastoc	106.9

* Means with the same letter are not significantly different.

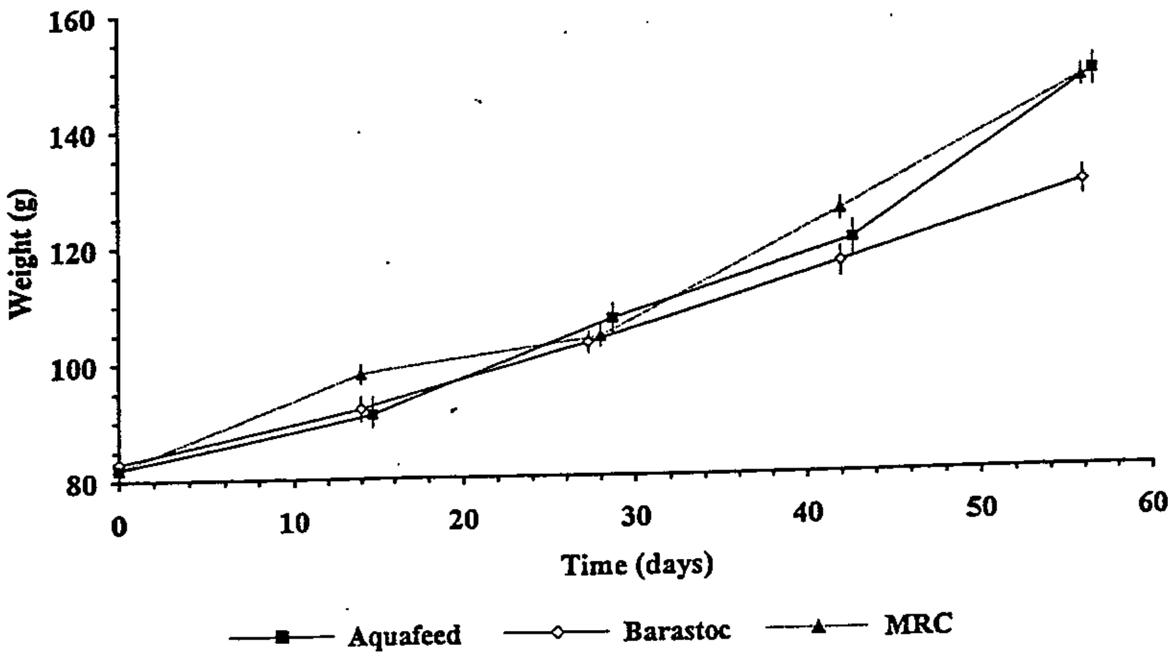

Fig 1. Change in weight of rainbow trout reared on three different diets (mean ± standard error)

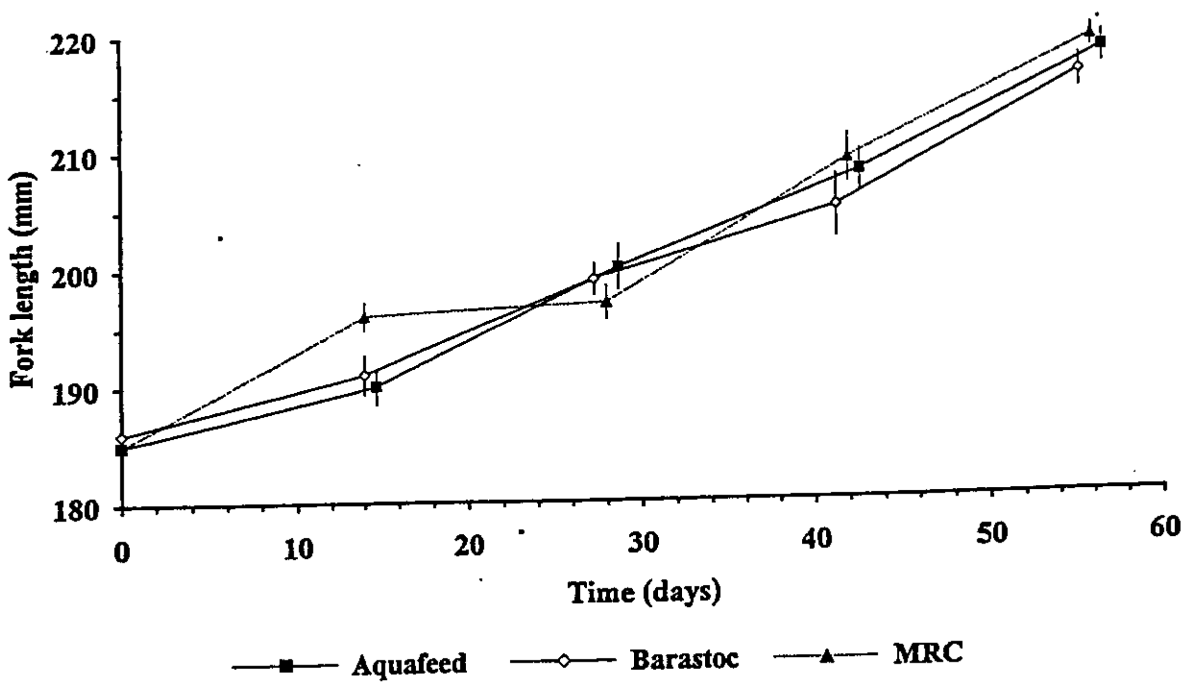

Fig 2. Change in fork length of rainbow trout reared on three different diets (mean ± standard error)

Quality of inlet water and discharge water

Logged readings of pH, water temperature, dissolved oxygen (D.O.) and conductivity for the inlet water are presented in Figs 3 and 4, and are summarised in Table 3. Missing data for pH and dissolved oxygen were due to probe failures.

A summary of the pH, TAN and P measurements recorded from the inlet water and discharge waters from each of the diets is presented in Table 4.

Table 3 Summary of logged water quality date for inlet water

Parameter	**Mean**	**Range** (minimum - maximum)
Temperature	7.96	5.8 - 11.2
pH	6.7	6.4 - 6.9
Dissolved Oxygen	10.9	9.7 - 12.4
Conductivity	18.6	14.5 - 23.0

Table 4. Mean and range of pH, TAN and P measurements recorded in the inlet water and discharge waters from each of the diets

Parameter		**Inlet**	**Aquafeed**	**Barastoc**	**MRC**
pH	Mean	6.8	6.5	6.5	6.4
	Range	6.5 - 7.0	6.3 - 6.7	6.3 - 6.8	6.3 - 6.6
Ammonia (TAN)	Mean	0.05	0.18	0.19	0.19
	Range	0.02 - 0.29	0.11 - 0.39	0.09 - 0.38	0.07 - 0.35
Net* Ammonia	Mean		0.12	0.13	0.14
	Range		0.06 - 0.18	0.03 - 0.2	0.01 - 0.24
Total Phosphorus (P)	Mean	0.03	0.18	0.16	0.16
	Range	0 - 0.07	0.05 - 0.27	0.08 - 0.28	0.04 - 0.26
Net Total Phosphorus	Mean		0.15	0.13	0.13
	Range		0.02 - 0.22	0.02 - 0.24	0.01 - 0.23

* Discharge concentration less inlet concentration

Dissolved oxygen in tanks

Dissolved oxygen concentrations for each tank, which was recorded daily during the experiment, ranged from 7.0 to 15.0 mg/l (mean: 10.8-11.5 mg/l).

pH of discharge water

The pH readings from the inlet water and discharge waters for the three diets were significantly different ($F_{3,192}$ = 1,379.77; P = 0.0001) (Fig. 5). Tukey's Multiple Range Test indicated that mean pH from the discharge waters for the three diets were significantly lower than the mean pH from the inlet water, and that the mean pH from the MRC discharge water was significantly less than mean values obtained for Aquafeed and Barastoc discharge waters (Table 5).

The EPA fish farm discharge licence requires that for pH there be no greater than one unit difference between inlet and discharge water. In this experiment, pH readings from the discharge water for all three diets were within EPA requirements.

Table 5 Tukey's Multiple Range comparison of mean pH readings

Tukey's Groupings*	Water source	Mean pH
A	Inlet	6.8
B	Aquafeed	6.5
B	Barastoc	6.5
C	MRC	6.4

* Means with the same letter are not significantly different.

Total Ammonia Nitrogen (TAN) of discharge water

TAN concentrations in the inlet water and discharge waters for the three diets were significantly different ($F_{3,190} = 798.26$; $P = 0.0001$) from each other (Fig. 6). Tukey's Multiple Range Test indicated that mean TAN concentrations in the discharge waters from each diet were significantly higher than the mean TAN concentration in the inlet water, and that the mean TAN from the Aquafeed discharge water was significantly lower than values for MRC and Barastoc discharge waters (Table 6).

The EPA fish farm discharge licence requires that net (discharge concentration less inlet concentration) concentrations of Ammonia (as Nitrogen) should not exceed a maximum of 0.4 mg/l and a median of 0.2 mg/l. In this experiment, net TAN concentration from the discharge water for all three diets rarely exceeded 0.2 mg/l (Fig. 7).

Table 6 Tukey's Multiple Range comparison of mean TAN concentrations

Tukey's Groupings*	Water source	Mean TAN
A	Barastoc	0.19
A	MRC	0.19
B	Aquafeed	0.18
C	Inlet	0.05

* Means with the same letter are not significantly different.

Total Phosphorus (P) of discharge water

Phosphorus concentrations in the inlet water and discharge waters for the three diets were significantly different ($F_{3,167} = 302.99$; $P = 0.0001$) from each other (Fig. 8). Tukey's Multiple Range Test indicated that mean Phosphorus concentrations in the discharge waters from the three diets were significantly higher than the mean concentration in the inlet water, and that mean Phosphorus concentration in the Aquafeed discharge water was significantly higher than mean concentrations in MRC and Barastoc discharge waters (Table 7).

The EPA fish farm discharge licence requires that net (discharge concentration less inlet concentration) concentrations of total Phosphorus should not exceed a maximum of 0.2 mg/l and a median of 0.1 mg/l. In this experiment, net total Phosphorus concentrations from the discharge water for all three diets often exceeded the median EPA value and occasionally exceeded the maximum EPA value (Fig. 9).

Table 7 Tukey's Multiple Range comparison of mean total Phosphorus concentrations

Tukey's Groupings*	Water source	Mean P
A	Aquafeed	0.18
B	Barastoc	0.16
B	MRC	0.16
C	Inlet	0.03

* Means with the same letter are not significantly different.

Inlet Water Quality

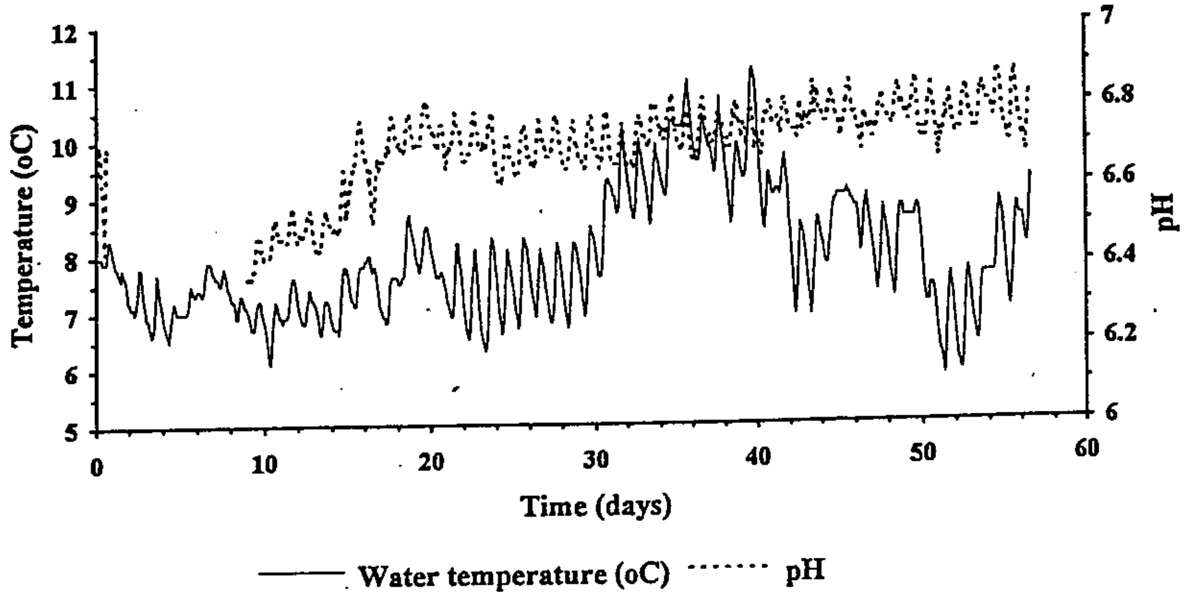

Fig 3. Water temperature and pH concentrations taken from the inlet water

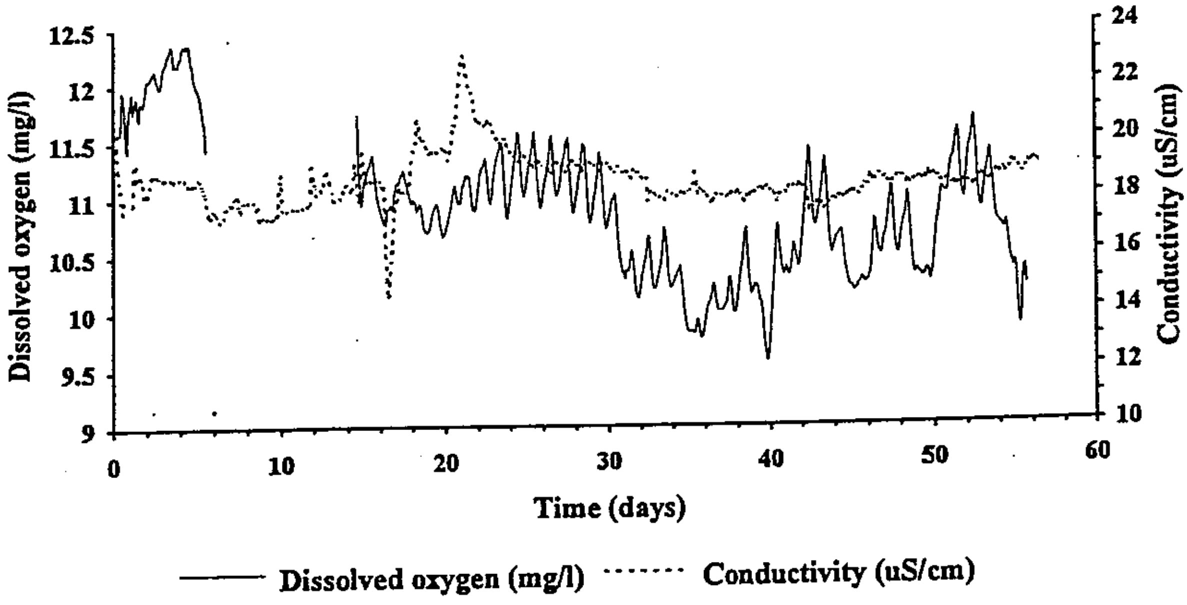

Fig 4. Dissolved oxygen and conductivity concentrations taken from the inlet water

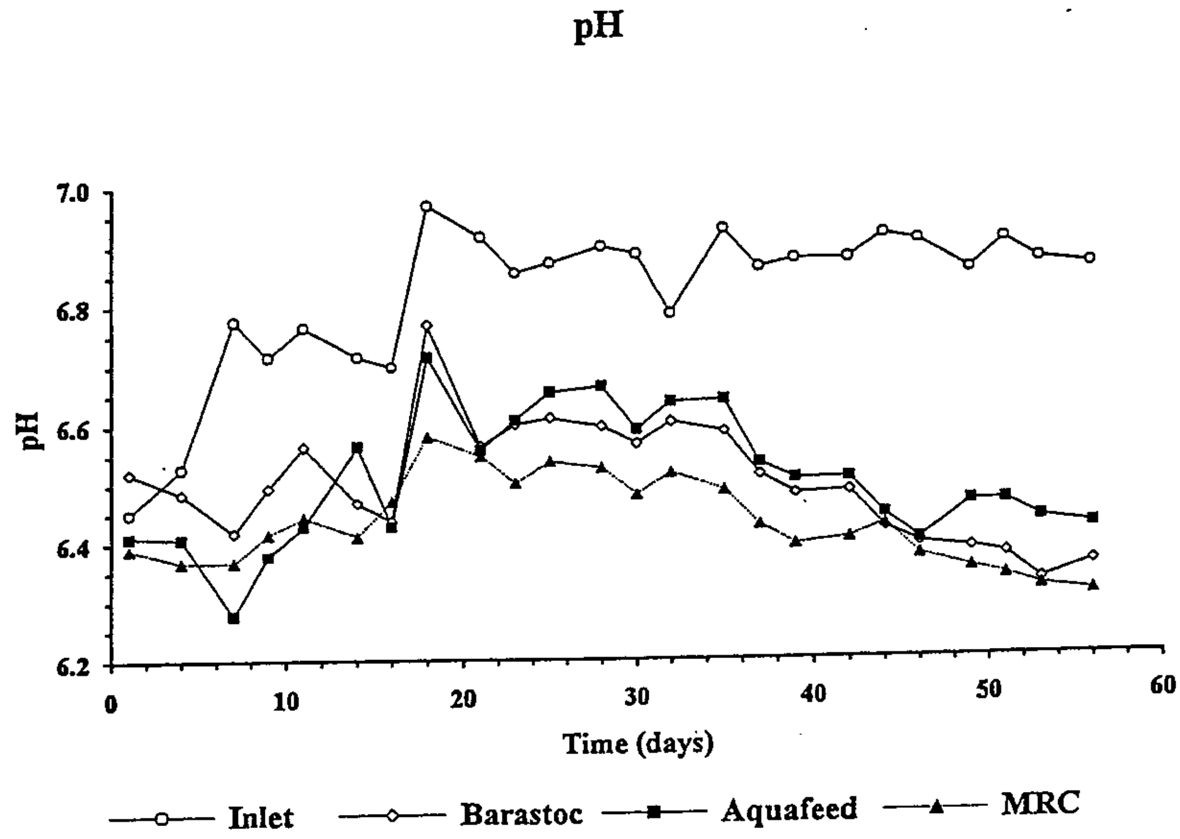

Fig 5. pH concentrations in the inlet water and discharge waters for each diet

Total Ammonia Nitrogen (TAN)

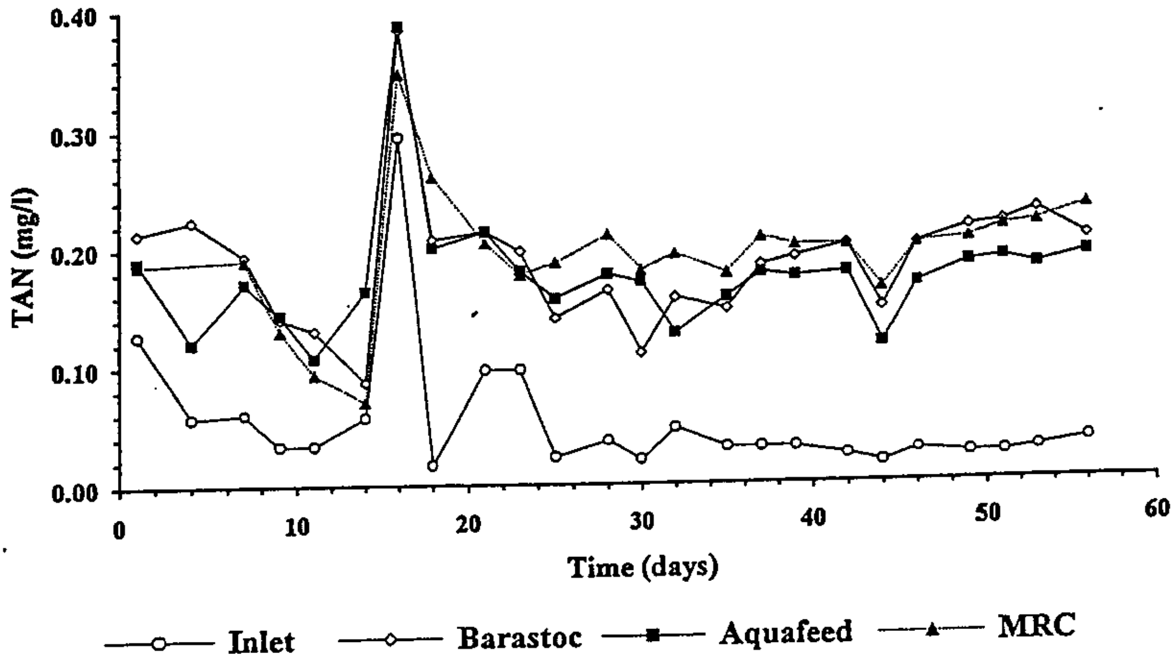

Fig 6. TAN concentrations in inlet water and discharge waters for each diet

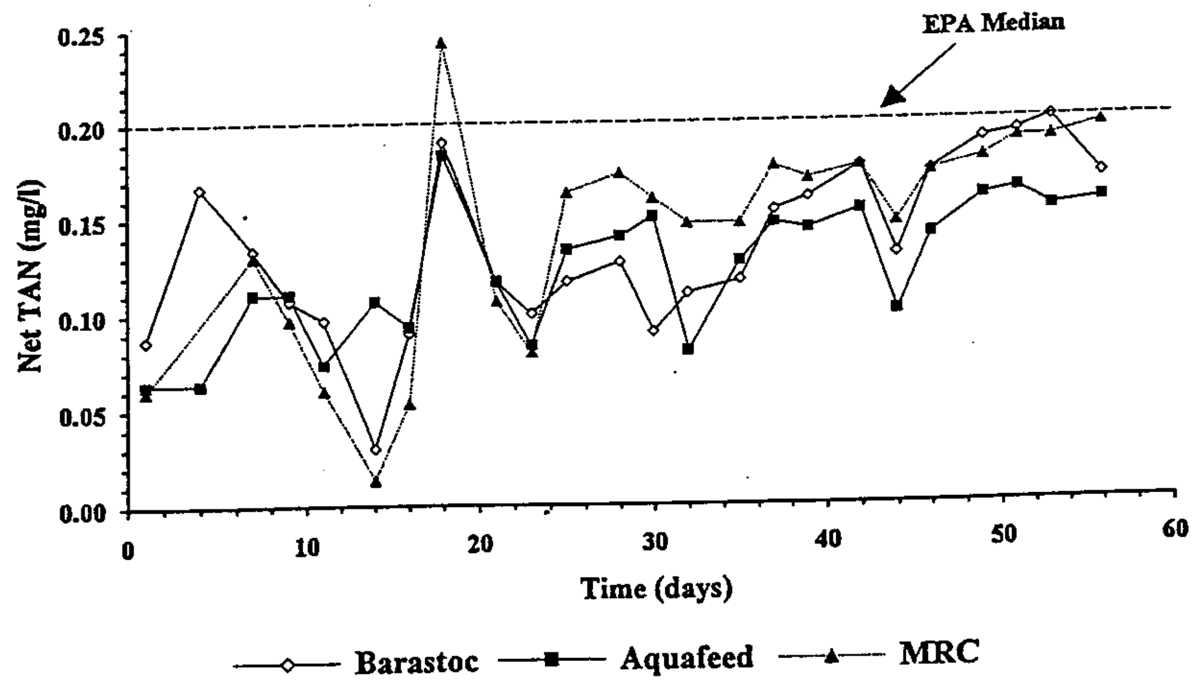

Fig 7. Net (discharge concentration less inlet concentration) TAN concentrations in inlet water and discharge waters for each diet

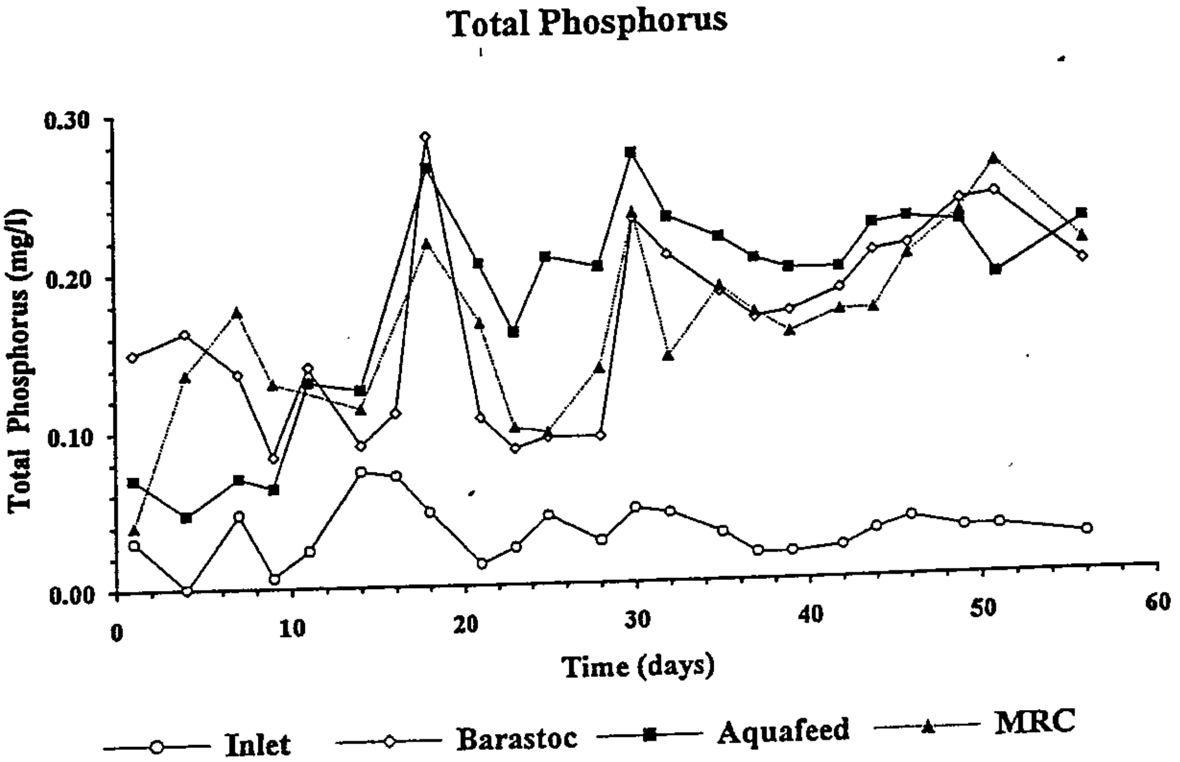

Fig 8. Total Phosphorus concentrations of the inlet water and discharge waters for each diet

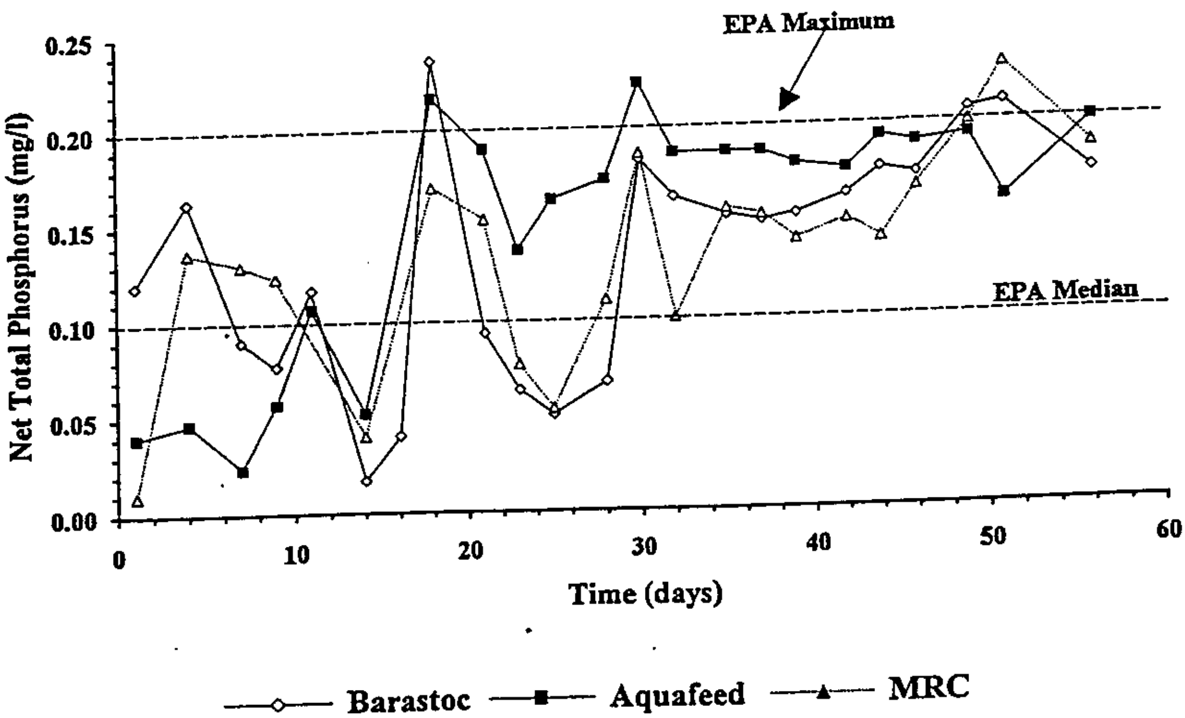

Fig 9. Net (discharge concentration less inlet concentration) total Phosphorus concentrations in inlet water and discharge waters for each diet

SENSORY PROFILING OF RAINBOW TROUT

for

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PO Box 329
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DPI QUEENSLAND DEPARTMENT OF PRIMARY INDUSTRIES AGRIBUSINESS

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IFIQ Sensory Evaluation

Brisbane 1995

ACKNOWLEDGMENTS

Thanks to Riantang Wansri for skilled technical assistance.

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EXECUTIVE SUMMARY

Whole, cleaned and gutted rainbow trout fed three different diets during a feeding trial in Victoria for Ridley Agriproducts, were airfreighted to IFIQ, Hamilton packed in plastic bags in ice. Sensory evaluation using a panel of thirteen experienced seafood tasters was conducted on the oven baked trout.

Odour, flavour and texture profiles were obtained for the fish to identify and quantify any differences between the three treatments. Colour of the flesh was also assessed.

In general the fish from the different diets were fairly similar. However, some small but significant differences in flavours were detected over all the diets. These occurred in "clean" and "weedy" characteristics at the 5% level of significance and in "sweet" and "mouldy/musty" characteristics (10% level). Trout from the Aquafeed diet had a noticeably pink flesh, whereas the other diets produced flesh described as white/grey/beige. No significant textural differences were noted.

OBJECTIVES

- To assess flavour and texture attributes of cooked rainbow trout in order to investigate any changes produced by the diets.
- To establish if colour differences attributable to diet exist in farmed rainbow trout.

METHODOLOGY

Products tested

Rainbow trout grown out under commercial conditions at Snob's Creek Freshwater Research Station, and fed one of three diets:

- Aquafeed
- Barastoc
- MRC

Tasters

Thirteen tasters were selected from the staff at IFIQ on the basis of their experience in seafood flavour profiling.

Experimental procedure

The trout had been cleaned and gutted prior to packing in plastic bags in ice and airfreighting to Brisbane. When received (20 September 1995) they were individually weighed and ranked within feed groups according to weight. They were rinsed in tap water, placed in rank order in clean plastic bags, immersed in ice, and stored in a refrigerated room at 1-2°C, until then were tested during the following two days, 21 and 22 September 1995.

Cooking

After removing the head fish were cut into two portions providing a "head end" and "tail end", individually wrapped in aluminium foil, and oven-baked at 200°C for eleven minutes. Samples were then held in a warming oven at 75°C. Throughout the cooking and testing procedure individual fish were identified by weight rank order so that fish of similar weights were compared from each diet.

Sensory Evaluation

Panellists tasted fish from all three diets at each session. Three replicate sessions were held based on fish size within dietary treatments. The panellists sat in individual booths which were illuminated with white light (daylight equivalent) and were asked to describe the flesh colour and to assess odour, flavour and textural characteristics of the trout using standard rating test procedures (AS2542.2.3). Sensory data was collected using Compusense 5 (Compusense Inc, Canada 1995). Panellists registered their opinions of the fish using a light pen to mark horizontal line scales on the computer screen, and the keyboard to add comments. Lists of descriptors used to profile the characteristics are in Appendix 1.

Fish were served on a white plastic tray with a knife and fork. They were served foil wrapped from the oven at 75°±5°C. Samples were identified with a random three digit code number and tasters received weight rank matched samples from the same end of each fish. Purified water at room temperature was freely available during tasting. The order of tasting of the dietary treatments was balanced across the individual tasters to counteract any bias due to sequential effects.

RESULTS AND DISCUSSION

The trout were received in excellent condition at 9.30 am on Wednesday 20 September.

Average weight of all fish received was 118.9g. Weights for specific diets are shown in Table 1.

Table 1

	Aquafeed	Barastoc	MRC
Mean	117.2	118.7	120.9
Minimum	74	74	87
Maximum	162	165	163

There was some difference in the external appearance of the fish. Those from the Aquafeed diet group appeared to have a darker pink stripe along their bodies, than those in the other groups.

Fish reached an internal temperature of approximately 80°C during cooking.

Sensory evaluation

Numerical scores of 0 and 100 were assigned to all scales on the questionnaire, with 0 representing the left hand end (= None) and 100 the right hand and (= Very much) of the attribute labelled on the profiling scales.

All the data was subjected to analysis of variance, and pairwise comparison of means for those attributes which showed a significant difference ($p<0.05$) between the diets. There were few significant differences at the 5% level, so probabilities quoted have been extended to 10% to include trends which may become significant if more extensive testing were undertaken.

Appearance

The flesh of the trout fed Aquafeed was noticeably pinker than that of the other fish. The flesh colour of 87% of samples tested was described as pink or orange, compared to 21% and 26% for Barastoc and MRC respectively. The flesh colour from these feeds was mainly described as white/grey (38% of each) or cream/beige (41% Barastoc, 36% MRC). (See Figure 1, Appendix 2.)

Odour

Scores for intensity of odour attributes are shown in Table 2 on the next page. The only *odour* attribute which showed any significant (p=0.09) difference was "fishy". The MRC diet produced slightly higher (but not significantly so) scores than either of the others as can be seen in the odour profiles in Figure 2, Appendix 2.

Flavour

In general the fish were considered to have a predominantly clean, sweet, chicken/meaty and fishy taste (see figure 3 Appendix 2). The mean taste panel scores are shown in Table 3. The only flavour characteristics showing a significant ($p<0.05$) overall difference between diets were "clean" and "weedy", but pairwise comparisons of treatment means using Tukey's LSD at the 5% level were all non significant. "Sweet" and "mouldy/musty" were significant at the 10% level, and all other flavour characteristics showed no significant differences. The greatest flavour differences, which are represented graphically in Figure 3 in Appendix 2, were between the Aquafeed and MRC diets, Flavour scores for "fishiness" showed the same rank order as those for fishy odour, but were not significantly different.

Texture

The flesh texture of all fish was rated soft, moist and slightly fibrous. No significant differences were detected between feed treatments. Mean scores are shown in Table 4 below and the profiles are depicted graphically in Figure 4, Appendix 2.

General

Tasters were asked to describe any "other" odours, flavours, or textures that they detected, and to make any general comments about the fish that they considered relevant.

These are listed in Appendix 2, Comments.

Table 2. Aquacultured rainbow trout mean taste panel scores (13 tasters) for odour

	Clean	Fishy	Chicken/meaty	Buttery	Mouldy/musty	Muddy/earthy	Weedy	Other
Aquafeed	23.3	30.1	33.0	8.0	7.1	3.7	9.4	4.7
Barastoc	20.6	27.2	30.4	8.8	7.9	4.7	8.6	4.7
MRC	22.9	32.6	31.2	9.1	6.4	3.4	8.6	1.9
Probability if ≤ 0.10	NSD	0.09	NSD	NSD	NSD	NSD	NSD	NSD

NSD = no significant difference   Scales: 0 = none 100 = very

Table 3 Aquacultured rainbow trout mean taste panel scores (13 tasters for flavour)

	Clean	Sweet	Fishy	Chicken /meaty	Oily	Buttery	Salty	Mouldy/ musty	Weedy	Bitter	Aftertaste	Other
Aquafeed	27.9	16.8	29.5	33.3	4.8	8.1	5.1	6.0	6.3	4.3	11.2	4.9
Barastoc	26.6	14.1	29.3	36.0	3.8	7.1	4.9	7.8	8.2	3.8	12.0	4.3
MRC	23.9	13.9	33.9	36.0	4.0	7.0	4.3	9.8	11.8	3.6	12.3	5.7
Probability if ≤ 0.10	0.04	0.08	NSD	NSD	NSD	NSD	NSD	0.08	0.02	NSD	NSD	NSD

NSD - no significant difference   Scales: 0 = none 100 = very

Table 4. Aquacultured rainbow trout mean taste panel scores (12 tasters) for texture

	Soft	Oily	Moist	Dry	Chewy	Fibrous	Chalky	Other
Aquafeed	48.7	4.2	41.9	13.7	9.1	11.4	4.1	3.5
Barastoc	49.2	4.1	40.4	16.7	11.1	11.0	4.7	4.8
MRC	49.2	4.7	43.4	13.8	9.6	12.9	5.4	3.6
Probability if ≤ 0.10	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD

NSD = no significant difference   Scales: 0 = none 100 = very

CONCLUSIONS

Fish from the three diets were quite similar in odour, flavour and texture when oven-baked whole. The most obvious difference was the pink colour of the flesh of the fish on the Aquafeed diet. The dietary treatments caused minor differences in the flavour profiles of the fish, and there were no significant differences in texture profiles.

Appendix 1
Methodology

Descriptors used for profiling odour, flavour and textural characteristics of rainbow trout

Odour

- Clean
- Fishy
- Chicken/meaty
- Buttery
- Mouldy/musty
- Muddy/earthy
- Weedy
- Other

Flavour

- Clean
- Sweet
- Fishy
- Chicken/meaty
- Oily
- Buttery
- Salty
- Mouldy/musty
- Weedy
- Bitter
- Aftertaste
- Other

Texture

- Soft
- Oily
- Moist
- Dry
- Chewy
- Fibrous
- Chalky
- Other

Appendix 2
Results

Frequency of Selection of Colour Descriptors of Rainbow Trout Flesh

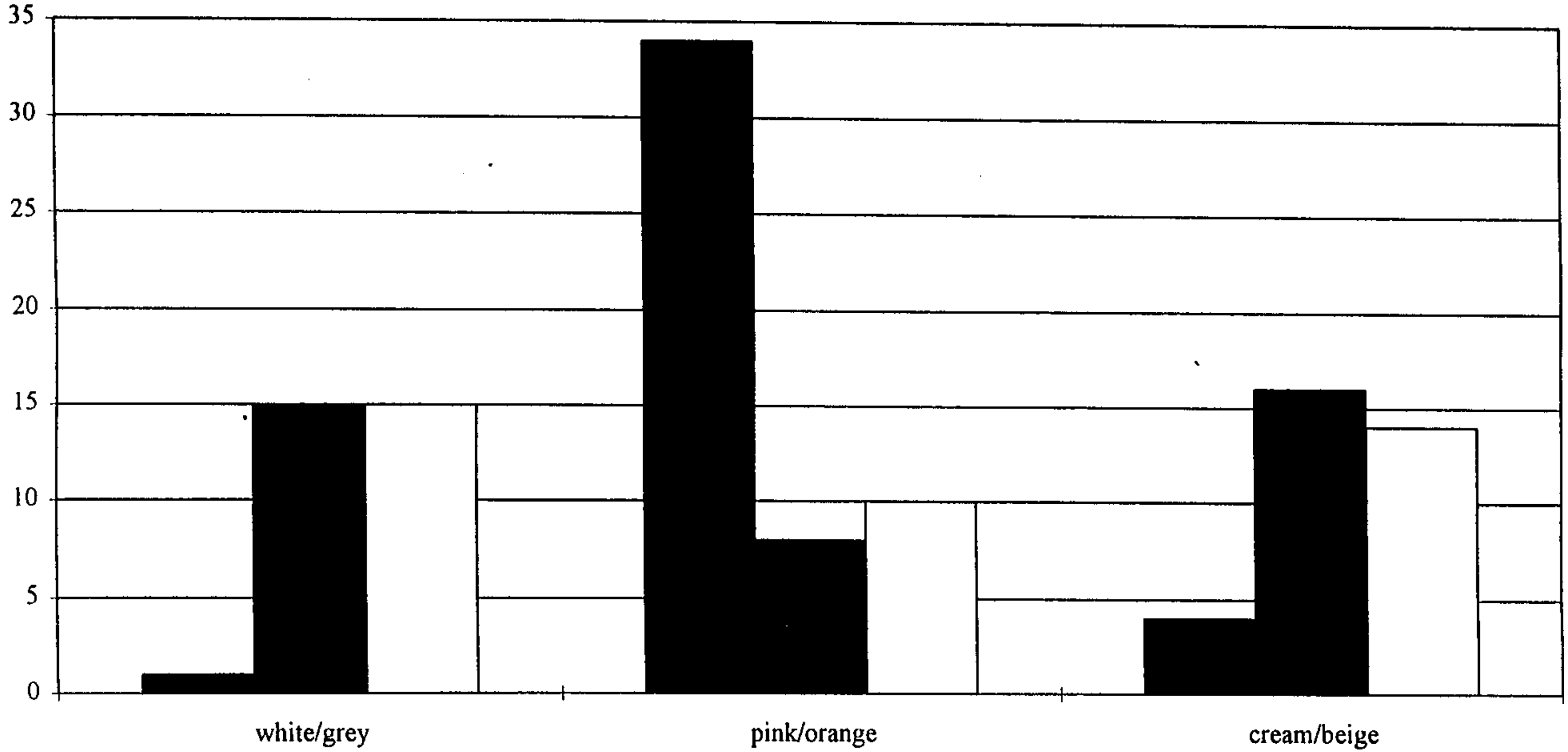

Figure 1 ■ Aquafeed ■ Barastoc □ MRC

Odour Profile of Rainbow Trout

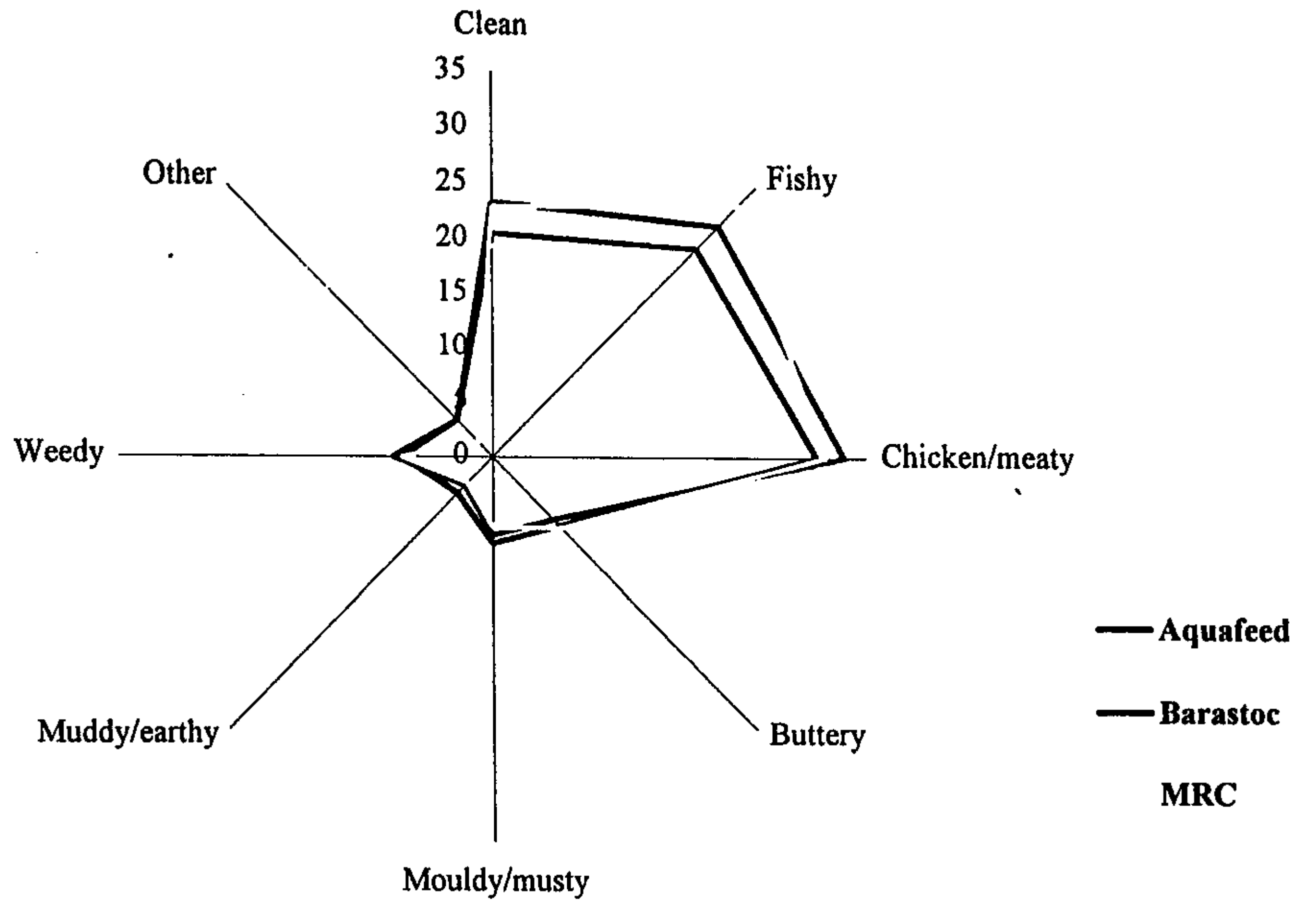

Figure 2

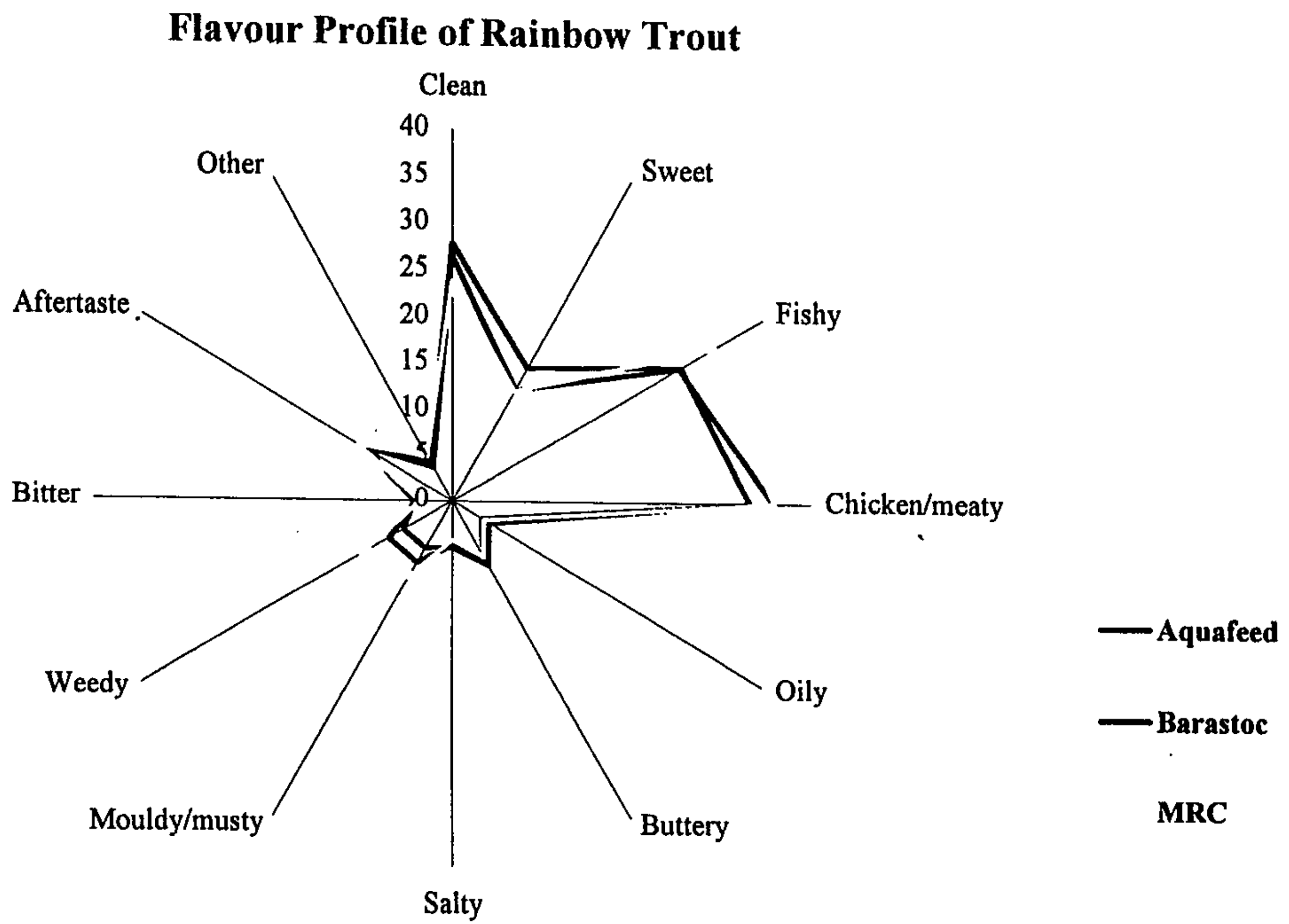
Flavour Profile of Rainbow Trout
Clean
40
35
30
25
20
15
10
5
0
Sweet
Fishy
Chicken/meaty
Oily
Buttery
Salty
Mouldy/musty
Weedy
Bitter
Aftertaste
Other
Aquafeed
Barastoc
MRC

Figure 3

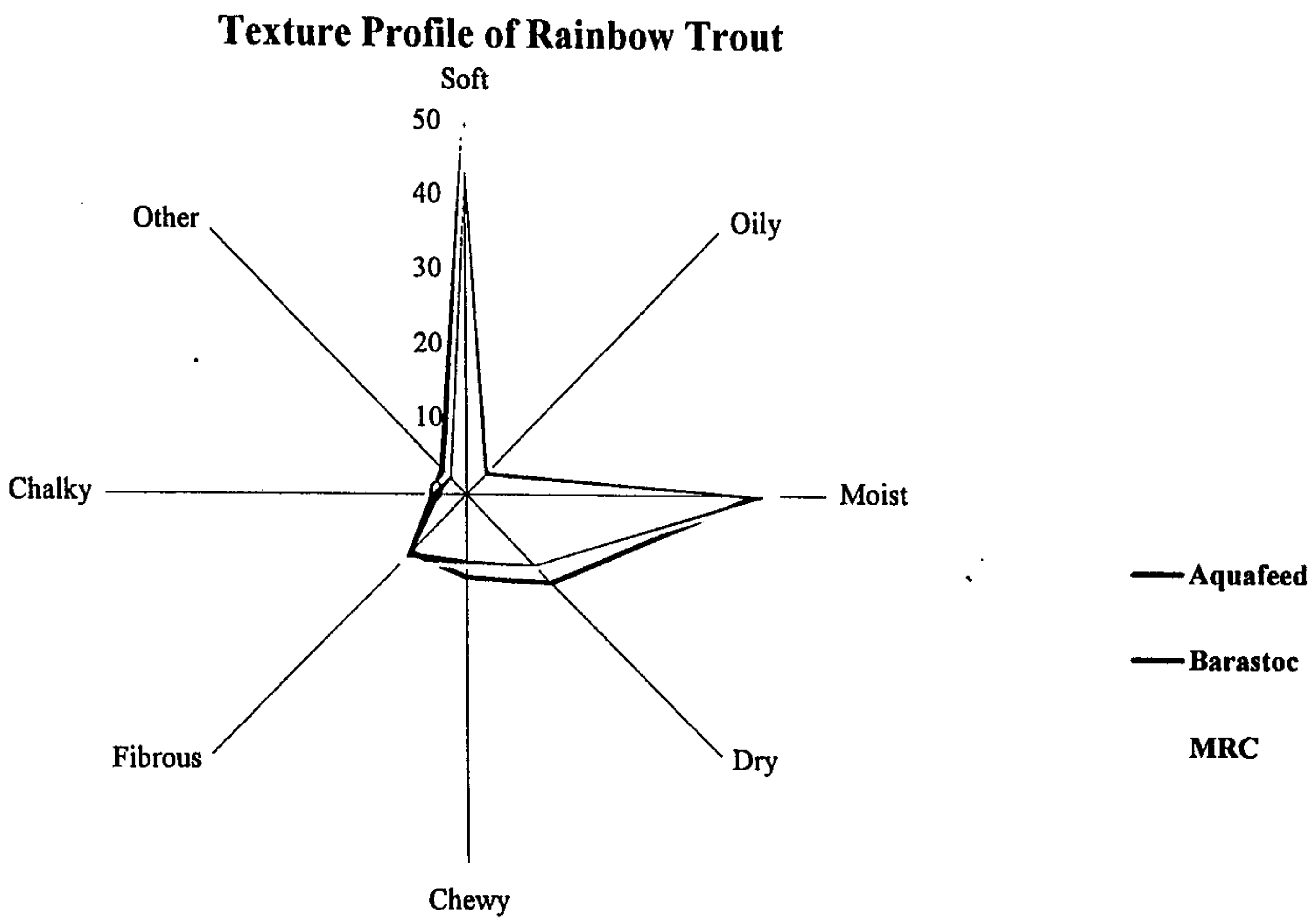
Texture Profile of Rainbow Trout
Soft
50
40
30
20
10
Oily
Moist
Dry
Chewy
Fibrous
Chalky
Other
Aquafeed
Barastoc
MRC

Figure 4

Flavour comments on "Other" flavours

Aquafeed

- Slightly muddy.
- Acidic - lemon type of acidity.
- Muddy.
- Weedy oily taste.
- Sweaty.
- No off or odd flavours, slight astringent aftertaste.
- Very nice meaty flavour with little muddiness.
- Oily flavour, otherwise clean taste.

Barastoc

- Slight muddy taste.
- Weedy.
- Sawdusty.
- Muddy taste with acidic aftertaste.
- Metallic.
- Acidic - lemon like.
- Fishy, very volatile.
- "Fruity", weedy flavour.
- No flavour, tad oily.
- Slight astringent aftertaste.
- Fairly bland.

MRC

- Buttery.
- Weedy taste - typical freshwater fish.
- Metallic.
- Dusty aftertaste.
- Slight acidic aftertaste.
- Slight chemical taste.
- Muddy.
- Sweaty.
- Bitter, weed taste.
- No real lingering taste.
- Best flavour of three samples.
- Cod liver oil flavour.

General comments on fish from tasters

Aquafeed

- Very agreeable.
- Very nice!
- Very attractive pink colour flesh, good flavour.
- Slightly drier flesh.
- No mustiness, oil around belly area.
- Texture solid.
- Flesh looks good with some colour.
- Beautiful!
- Needs sweetness, otherwise good.
- Texture sticky.

Barastoc

- Quite acceptable sample.
- Again, nice!
- Similar to other samples but a little softer.
- Flavour and texture not bad, no sweetness.
- Firm flesh, bland but quite good.
- Poor texture.

MRC

- Chewy, metallic, not the best.
- Texture tended to vary a lot through the sample. The majority was soft.
- I don't find a big difference between samples except in colour. This sample has some oiliness, especially round belly area.
- Not tasty and mouthfeel like wet sawdust.

Effect of feed on the sensory properties of aquacultured barramundi, rainbow trout, and prawns.

Anne Ford and Rob Roberts
Centre for Food Technology, Queensland Department of Primary Industries.

The range of artificial feeds currently available for seafood aquaculture is limited and feeds are expensive. Research into specific feeds for individual species, which provide the required nutrition for fast growth, incorporate less expensive raw materials and enhance desirable characteristics, is necessary in order to allow the industry to expand and become more cost effective. It is also important to ensure that these feeds do not cause any major changes in the sensory properties of the flesh which may affect consumer acceptance of the final product.

This paper reports briefly on some of the results from sensory assessments conducted at the Centre for Food Technology on fish and prawns from three separate feed trials.

METHODOLOGY

The fish and prawns tested had all been cultured in commercial grow out conditions as part of research trials.

Sensory Evaluation

The questionnaires and experimental tasting designs were customised for each type of seafood. Profiling questionnaires based on the Australian Standard Rating Test (AS2542.2.3), with unstructured graphic line scales were used. Attributes measured included appearance, odour, flavour and texture characteristics, and scales were labelled "none" at one end and "very" at the other end.

The fish and prawns were assessed by experienced panels of seafood tasters selected from staff at the centre. The panels identified the individual characteristics to be scaled during preliminary tasting sessions.

All the samples were served in individual booths under white light (daylight equivalent) and purified water was provided for palate cleansing. Order of tasting samples was balanced over the panel to minimise bias from tasting order Data was collected directly into computers using an integrated software package, CSA (Compusense Inc., Canada).

Barramundi

The fish tested were from four replicates of each of four diets:

- Control including fish meal
- Including meat meal 1
- Including meat meal 2
- Commercial

They were received frozen whole and were held at -18°c until required for testing. The fish were then weight ranked, heaviest to lightest, within each replicate of each dietary treatment.

Five fish from one weight matched replicate of each diet were defrosted overnight at 5°C and filleted. Fillets were rinsed under cold tap water and two samples (average weight 22.5 g) were cut from the central portion of each fillet. Samples were placed in individual foil dishes and covered with a foil lid. They were placed in a 5°C refrigerator and removed to room temperature (24°C) to equilibrate for one hour prior to cooking. Samples were then cooked in a fan forced electric oven at 200°C for six minutes and were transferred to a holding oven at 75°C for up to 30 minutes prior to tasting.

A total of sixteen tasters (thirteen male, three female) assessed four samples (one from each dietary treatment) at each of four sessions. A list of descriptors used to identify eating characteristics on the scoresheet is shown in table 1 on page 3.

Rainbow Trout

Rainbow trout were grown out under commercial conditions at Snob's Creek Freshwater Research Station, Victoria and fed one of three diets:
- Commercial diet 1
- Commercial diet 2
- Experimental diet including meat meal

The trout had been cleaned and gutted prior to packing in plastic bags in ice and airfreighting to Brisbane. When received, they were individually weighed and ranked within feed groups according to weight. They were rinsed in tap water, placed in rank order in clean plastic bags, immersed in ice, and stored in a refrigerated room at 1-2°C, until they were tested during the following two days.

After removing the head, fish were cut into two portions providing a "head end" and "tail end", individually wrapped in aluminium foil, and oven-baked at 200°c for eleven minutes. Samples were then held in a warming oven at 75°C. Throughout the cooking and testing procedure individual fish were identified by weight rank order so that fish of similar weights were compared from each diet.

Thirteen panellists tasted fish from all three diets at each session. Three replicate sessions were held based on fish size within dietary treatments. Lists of descriptors used to profile the eating characteristics are in table 1.

Kuruma Prawns

The Kuruma prawns (Penaeus japonicus) had been fed on one of four diets A,B,C,D at a prawn farm in Northern New South Wales. They were airfreighted live in sawdust and held at 13 to 15°C until tested (within 3 days).

Raw prawns were assessed after they had been rinsed under cold tap water while still alive, decapitated, then served immediately together with the head.

Cooked prawns were rinsed to remove sawdust, and covered and cooked in a 600 watt microwave oven to a "just done" level (70-75°C), and served warm to panellists.

Twelve panellists assessed prawns from all four diets at each session. A total of six sessions were held, three each for raw and cooked prawns.

Table 1

Descriptors used on scales to describe characteristics of seafood tested:

	Barramundi	Rainbow trout	Raw prawns	Cooked prawns
Odour	Fishy Weedy/herbaceous Muddy/earthy Meaty/baked Milky Other	Clean Fishy Chicken/meaty Buttery Mouldy/musty Muddy/earthy Weedy Other	Not used	Not used
Appear-ance	Colour of internal flesh: Greyness Yellowness Other	Flesh colour: White Grey Cream Beige Orange Pink	*Tail:* Brightness Blue Maroon White Yellow *Abdomen:* White Maroon Black *Head:* White Maroon Black	*Stripes:* Orange Red
Texture	Firm Moist Fibrous Sticky Other	Soft Oily Moist Dry Chewy Fibrous Chalky Other	Firm Moist Fibrous Sticky Dry Springy Soft Rubbery Watery Other	Firm Moist Fibrous Sticky Dry Springy Soft Rubbery Watery Other
Flavour	Metallic Meaty Sweet Fishy Muddy/earthy Stale Weedy/herbaceous Other	Clean Sweet Fishy Chicken/meaty Oily Buttery Salty Mouldy/musty Weedy Bitter Aftertaste Other	Metallic Meaty Sweet Seafoody Muddy/earth Fresh Salty Weedy/herbaceous Other	Metallic Meaty Sweet Seafoody Muddy/earthy Fresh Salty Weedy/herbaceo us Other

RESULTS AND DISCUSSION

Numerical scores of 0 and 100 were assigned to all scales on the questionnaire, with 0 representing the left hand end (=Not at all) and 100 the right hand and (=Very much) of the attribute labelled on the profiling scales.

The data was averaged over all tasters and all sessions for each treatment. Where no rating was given for a particular characteristic, a score of zero was assigned. All data was subjected to analysis of variance, and pairwise comparison of means for those attributes which showed a significant difference ($p<0.05$) between the diets.

Barramundi

Fish from all dietary treatments were considered equally acceptable although some differences were noted in flavour and texture. Odour profiles were very similar for all diets, with odours described mainly as fishy and meaty/baked.

Sweet and fishy flavour showed significant ($p<0.01$, $p<0.05$ respectively) differences between dietary treatments (figure 1). Inclusion of meat meal appeared to increase the sweetness of the flesh. The commercial diet produced fish with less fishy flavour than the other diets. There were no significant differences in other flavour characteristics.

Fish fed the control diet, had flesh which was rated significantly less firm than that from the other diets.

Rainbow Trout

In general the fish from the different diets were fairly similar. There were obvious pink markings on the skin of the uncooked fish fed Commercial Diet 1, and the flesh colour of 87% of samples tested was described as pink or orange. The flesh colour from other feeds was mainly described as white/grey or cream/beige. The only odour attribute which showed any obvious difference was "fishy". The meat meal diet produced slightly higher (but not significantly so) scores than either of the others as can be seen in the odour profiles in figure 2.

In general the fish were considered to have a predominantly clean, sweet, chicken/meaty and fishy taste (see figure 2). The only flavour characteristics showing a significant ($p<0.05$) overall difference between diets were "clean" and "weedy", but pairwise comparisons of treatment means using Turkey's LSD at the 5% level were all non significant. Flavour scores for "fishiness" showed the same rank order as those for fishy odour, but were not significantly different.

The flesh texture of all fish was rated soft, moist and slightly fibrous. No significant differences ocurred between feed treatments.

Kuruma Prawns

The flavour and texture of the prawns were fairly similar. However, some significant differences in colours and flavours were detected between diet D (control) and the three artificial feeds.

Colours in the tail fan of raw prawns were judged significantly brighter in prawns fed diets A and C than those fed diet D. Diet D produced stripes with a significantly lower maroon intensity than any other diet, and diet C provided the best maroon colour in both bodies and head of the prawns. Black colouration was significantly darker in the bodies and heads of prawns fed diet D, and lowest in those fed diet C.

In general the raw prawns were considered to have predominantly fresh, sweet, meaty and seafoody taste (see figure 4). Prawns fed diet D received significantly lower scores for fresh and sweet tastes, and significantly higher scores for muddy/earthy and weedy/herbaceous tastes. There were no significant differences between any of the other diets in any flavour characteristic. All diets produced prawns with very similar textural qualities. Prawns fed diet D were rated significantly "stickier" in texture, but the difference was small.

Colour and flavour differences were not apparent once cooked, and the only attribute showing significant diet effects in cooked prawns was "rubbery" texture. This was significantly higher for diet D than for diet A (table 2).

Table 2 Texture scores for cooked prawns (mean of three replicates)

	Firm	Moist	Fibrous	Dry	Springy	Soft	Rubbery*	Watery
Diet A	56.7	46.8	11.7	4.2	20.9	13.0	11.1[a]	4.9
Diet B	60.7	43.2	10.8	7.2	21.4	10.5	14.7[b]	3.9
Diet C	62.2	49.2	11.3	6.7	20.9	6.0	13.0[ab]	4.9
Diet D	62.2	45.4	12.2	7.1	21.7	7.1	16.1[b]	3.3

0 = Not at all 100 = Very much

* Significant difference between diets ($P<0.05$)

CONCLUSION

Trials with rainbow trout and kuruma prawns have shown that it is possible to alter both skin and flesh colour in fin fish, and to increase the intensity and brightness of pigmentation in the shells of live Kuruma prawns by inclusion of pigments in the feed. This could increase the market value of the products provided it does not cause any decrease in the eating quality.

Substitution of fish meal with meat meal in aquaculture diets was shown to modify the flavour profile in both barramundi and rainbow trout. Surprisingly one of the main effects was an increase in "fishy" odour and flavour in fish fed the diets containing meat meal. Texture was also altered slightly by diet, probably due to the difference in saturation level of the fatty acids in the respective diets.

The next step for this research is to ascertain that these changes do not in anyway lower the acceptance of the products to consumers.

The difference in results obtained for raw and cooked prawns also demonstrates the importance for the product to be assessed in the form in which it will be consumed.

The trials reported here have confirmed that aquaculture feeds do have an impact on the final product quality, and it would be wise to incorporate this type of assessment into all research on potential feeds.

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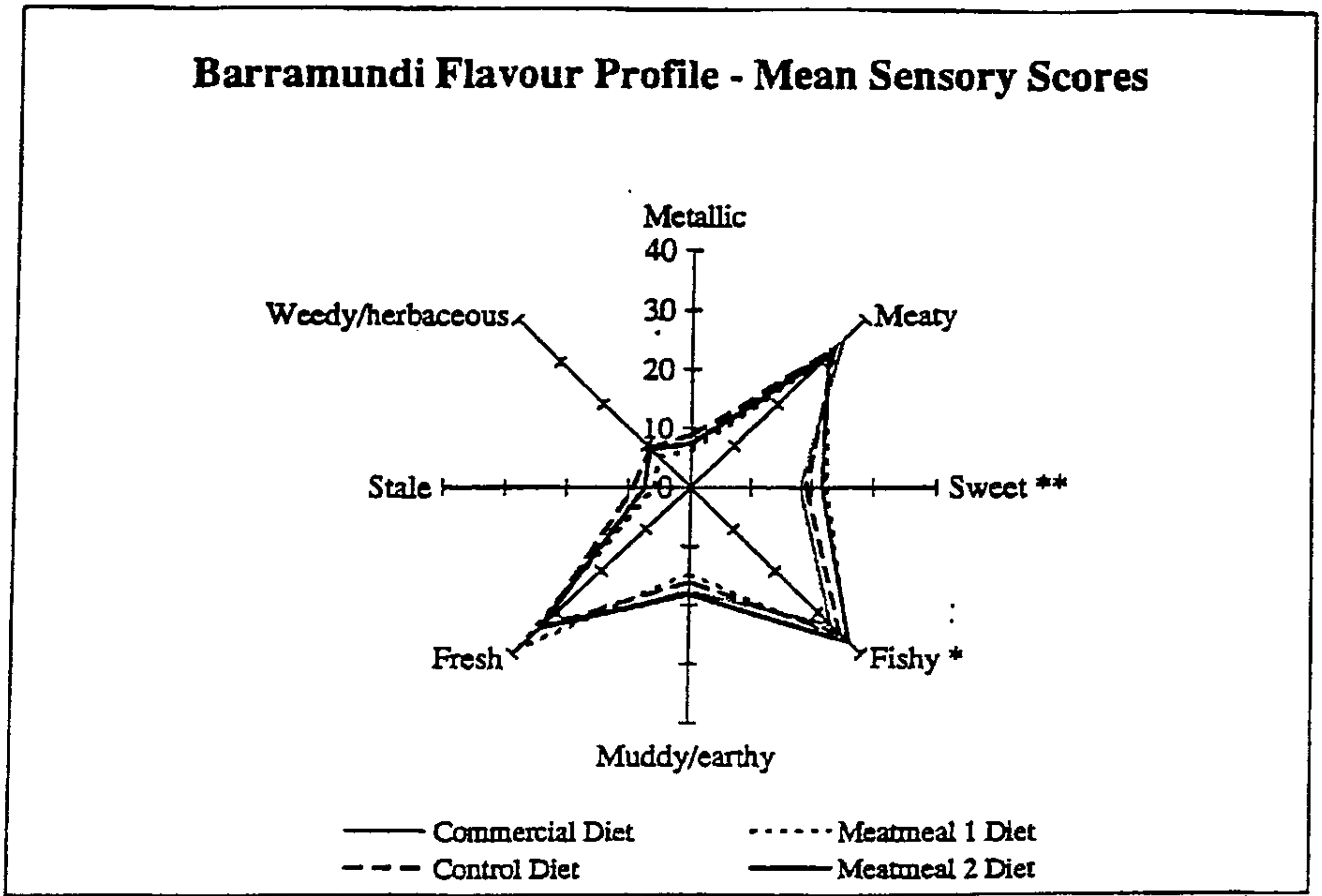
- Ken Hansen, Technical Assistant, Sensory Evaluation, Centre for Food Technology
- Riantong Wansri student University of QLD, Gatton College
- Seafood Taste Panel, Centre for Food Technology
- Steve Nottingham, Biometrician, Centre for Food Technology.


Barramundi Flavour Profile - Mean Sensory Scores
Metallic
40
30
20
10
0
Meaty
Sweet **
Fishy *
Muddy/earthy
Fresh
Stale
Weedy/herbaceous
Commercial Diet
Control Diet
Meatmeal 1 Diet
Meatmeal 2 Diet

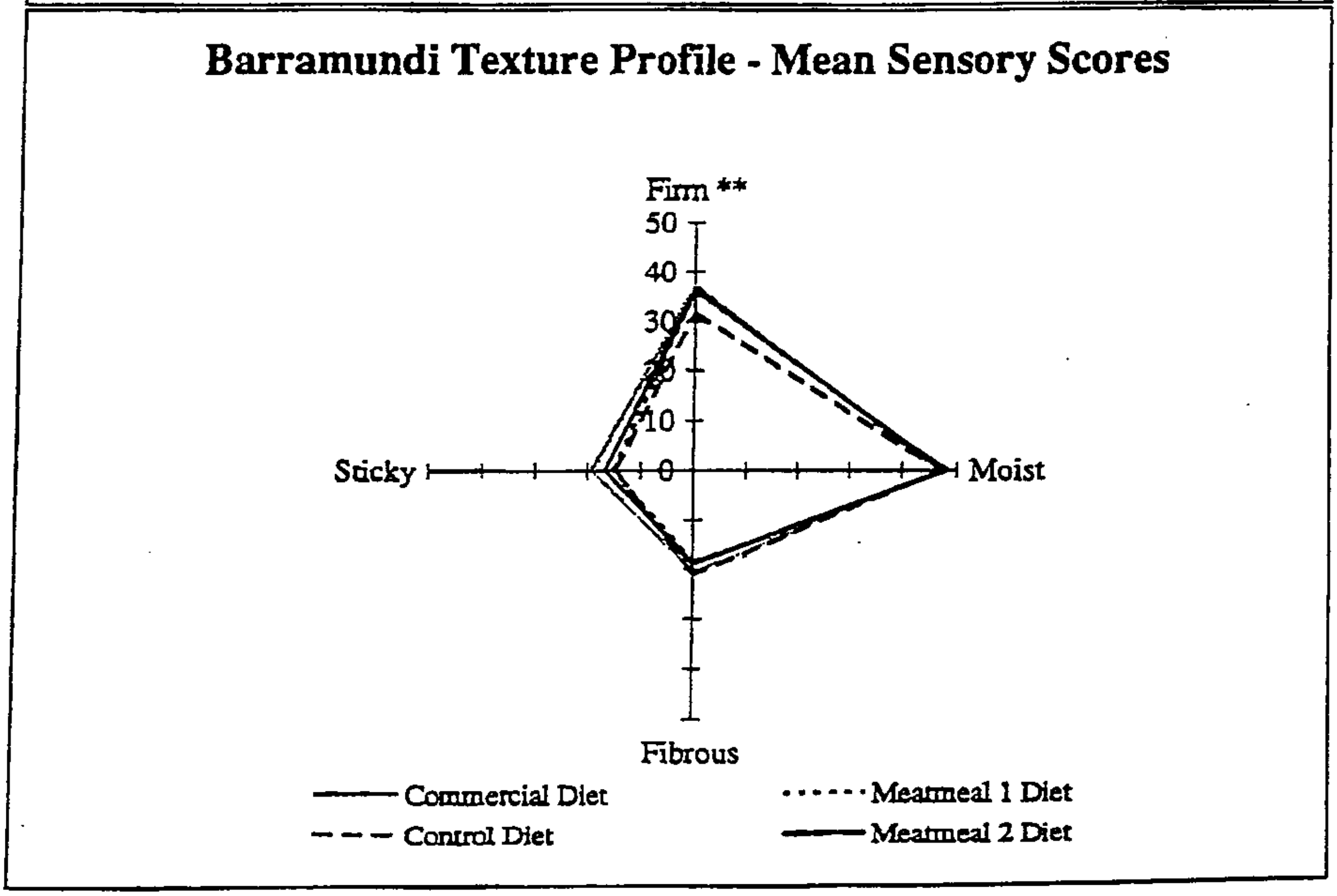
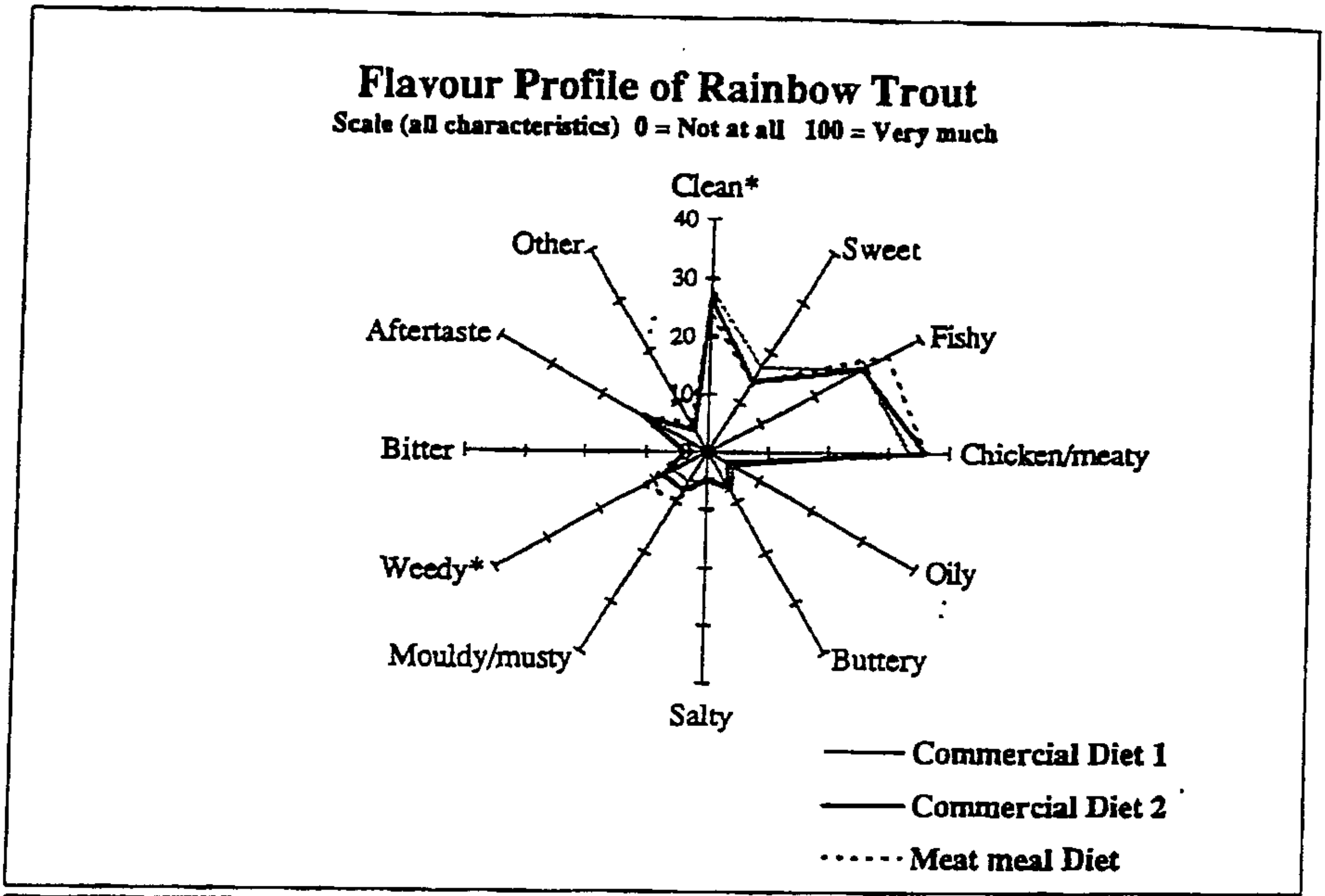
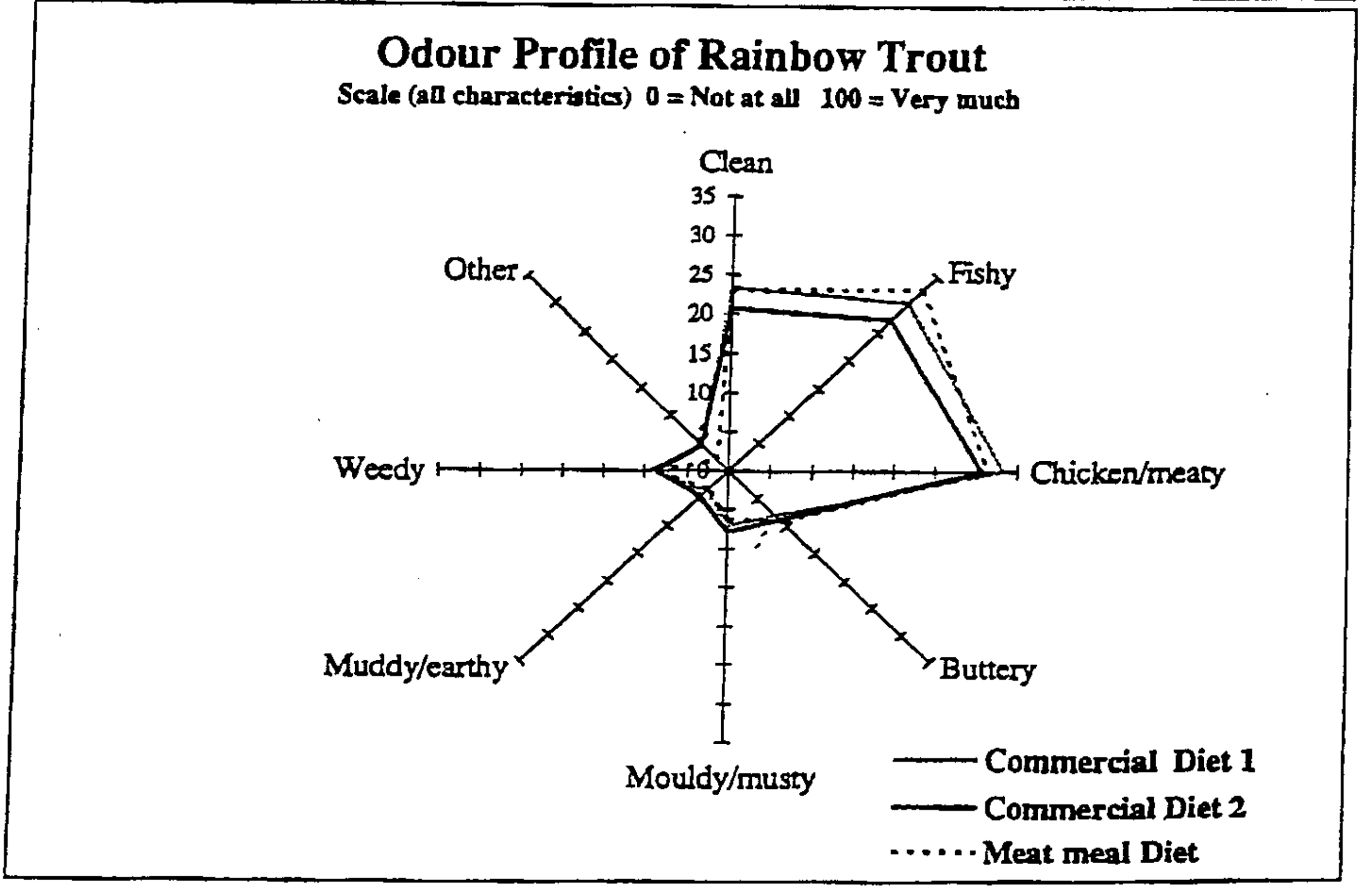

Barramundi Texture Profile - Mean Sensory Scores
Firm **
50
40
30
20
10
0
Moist
Fibrous
Sticky
Commercial Diet
Control Diet
Meatmeal 1 Diet
Meatmeal 2 Diet

Figure 1



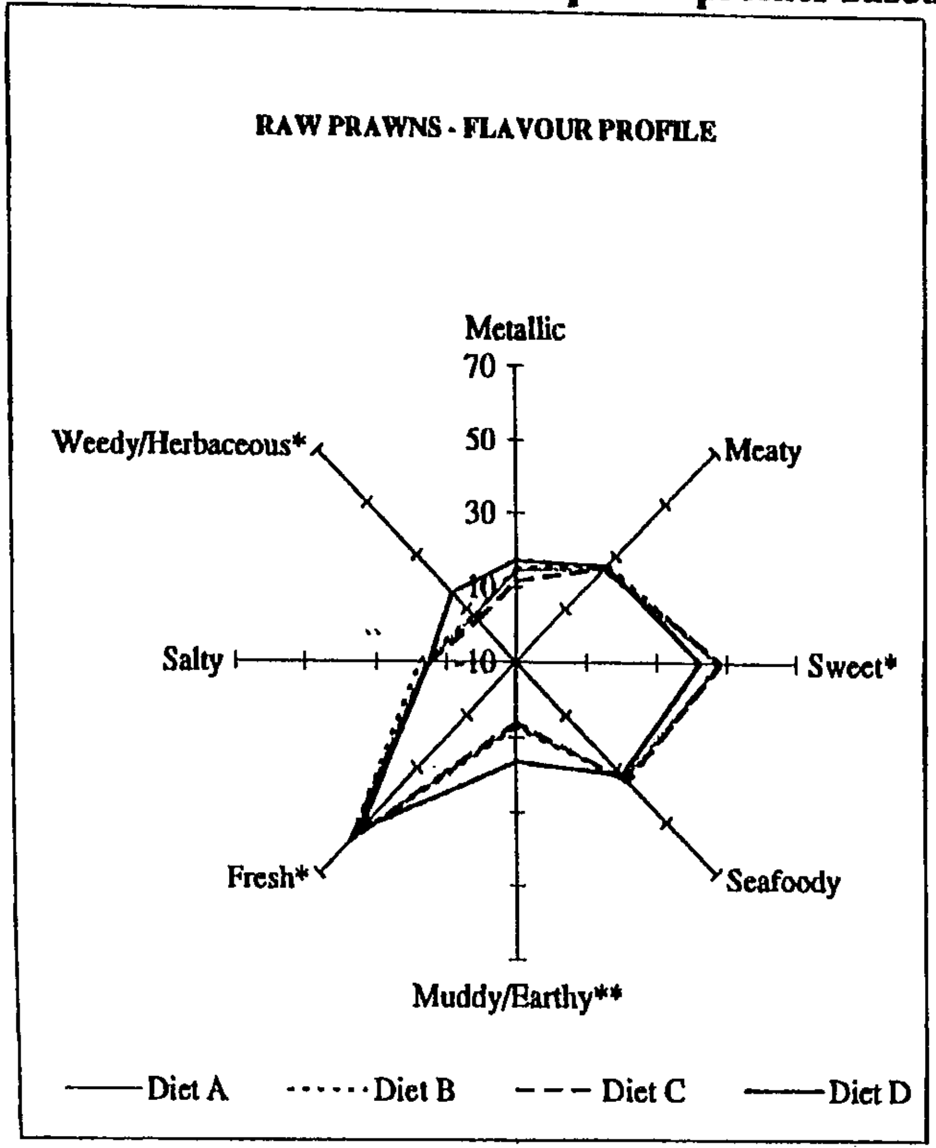
Flavour Profile of Rainbow Trout
Scale (all characteristics) 0 = Not at all 100 = Very much
Clean*
40
30
20
10
Sweet
Fishy
Chicken/meaty
Oily
Buttery
Salty
Mouldy/musty
Weedy*
Bitter
Aftertaste
Other
Commercial Diet 1
Commercial Diet 2
Meat meal Diet

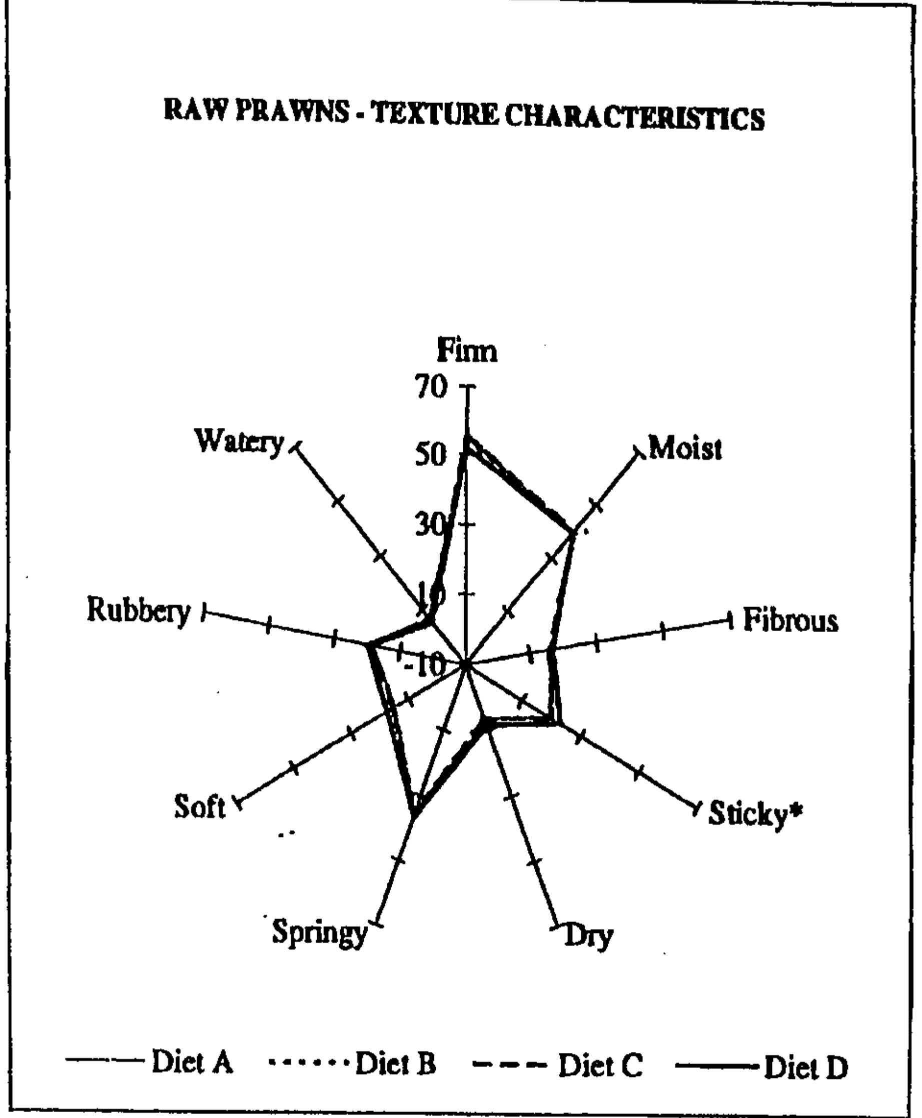


Odour Profile of Rainbow Trout
Scale (all characteristics) 0 = Not at all 100 = Very much
Clean
35
30
25
20
15
10
Fishy
Chicken/meaty
Buttery
Mouldy/musty
Muddy/earthy
Weedy
Other
Commercial Diet 1
Commercial Diet 2
Meat meal Diet

Figure 2

Raw prawn profiles based on mean sensory scores





Scale (all characteristics) 0 = Not at all 100 = Very much

Figure 4

Effect of including pigments in the diet on the colour of the stripes in Kuruma prawns

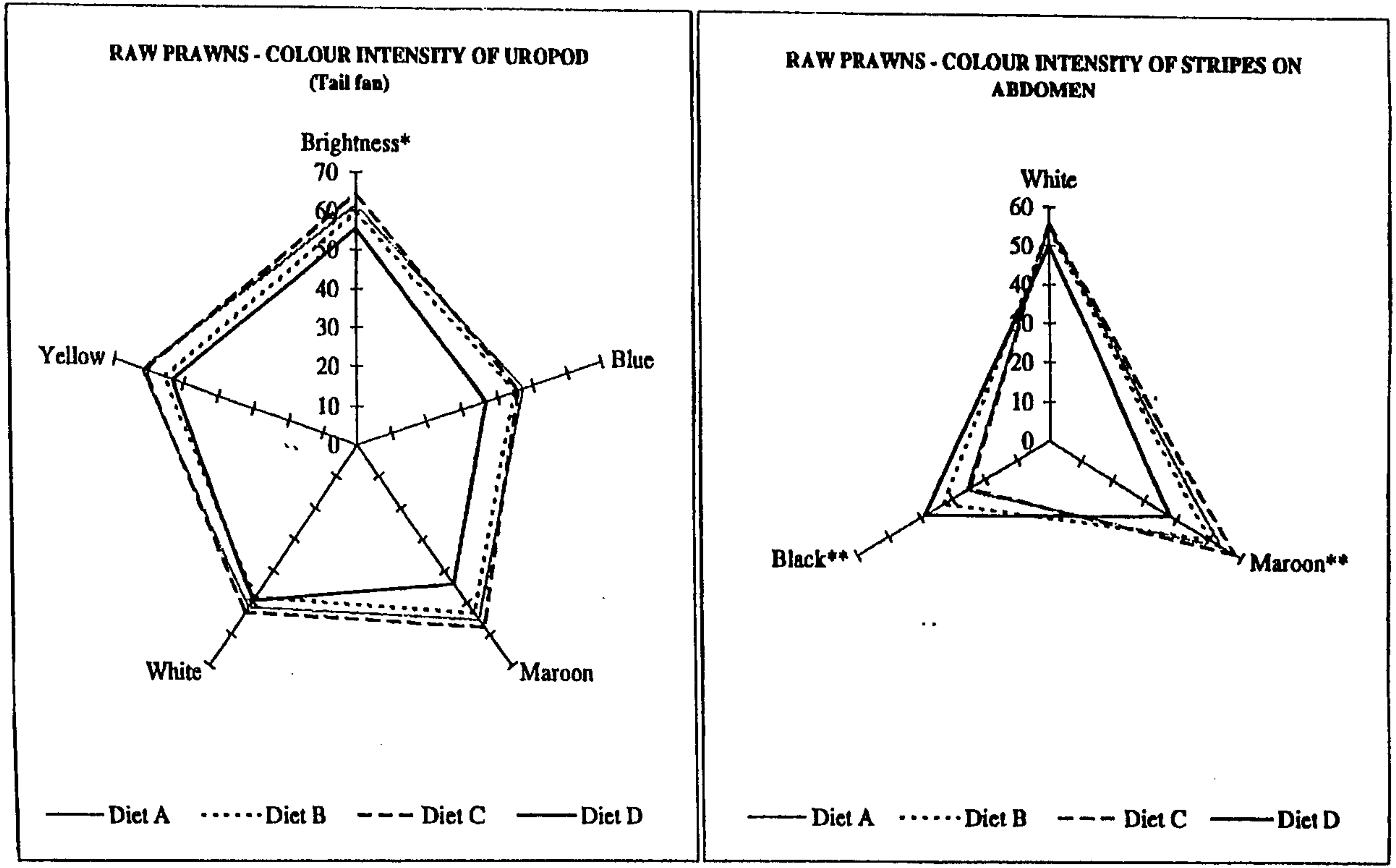


Figure 3