# International Fishmeal & Oil Manufacturers Association

EFFECT OF DIFFERENT DIETARY PROTEIN AND OIL LEVELS IN DIET ON GROWTH OF TILAPIA: COMPARISON OF AQUACULTURE GRADE (SOUTH AMERICAN) AND LOCALLY PRODUCED FISH MEALS

REPORT OF A TRIAL WITH TILAPIA IN TANKS AT THE BEIJING INSTITUTE OF FISHERIES

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**RESEARCH REPORT NUMBER: 1998-9**

**November 1998**

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by

Tian Jishun[1], Jiang Mingneng[1], Eric L. Miller[2], Ian H. Pike

## Extended Summary

A trial with tilapia (*Oreochromis niloticus*) was carried out at the Beijing Fisheries Research Institute to establish the optimum contents of protein and fat in the diet. Approximately half the protein and fat were supplied by aquaculture grade South American fish meal and fish oil. Additionally, a Chinese fish meal was included for comparison with the South American. The trial was carried out in tanks (0.13 $m^3$) with tilapia at start weighing approximately 35g over an eight week period, reaching approximately 90g.

A literature survey had indicated that the optimum protein and fat contents of the diet of tilapia are 30% and 8% respectively for juvenile/grower fish. The diets were designed to closely reflect typical practical tilapia diets used in China which normally have around 35% protein and 4-5% fat content. Consequently, the trial was designed as a 3 x 3 treatment comparison with three dietary protein levels (25, 30, 35%) and three different dietary fat levels (5, 8, 11%). 27% of the protein supplied by fish meal and half the added fat supplied by fish oil. An additional treatment (diet number 10) was included with Chinese fish meal and 30% protein and 8% fat content, similar to diet 5 which used aquaculture grade fish meal.

There was a linear effect of increasing dietary protein content improving growth (difference approaching significance) and feed conversion (significant, $P \leq 0.02$). As this response was linear, this trial did not establish the optimum protein level which may be above 35%. There was an improvement in both growth and feed conversion increasing fat content from 5% to 8% with both low and high protein diet.

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Comparing diets with 11% and 8% fat, growth and feed conversion were better with the former. These effects of dietary fat content were not significant. Nevertheless, the authors concluded 8% fat is optimum, along with 35% protein in the diet.

Comparing Chinese v aquaculture grade South America fish meals, growth was improved by 17% (118 v 138g) and feed conversion by 22% (2.38 v 1.86) with the latter. Only the feed conversion difference was significant.

In conclusion, this trial indicates that marked improvements in productivity may be achieved using an aquaculture grade South American fish meal rather than Chinese fish meal. Dietary protein content for fish from 40g to 100g liveweight should be at least 35% - the current content in practical diets. Increasing dietary fat to 8% using a mixed vegetable oil/fish oil may improve fish growth, using 1% to 2% fish oil addition. The trial did not indicate that increasing dietary fat has a sparing effect on dietary protein. Savings in dietary protein content may be possible for fish - this should be tested in larger facilities, e.g. ponds.

## Introduction

One of the main research areas in aquaculture feed production is the protein source for fish and shrimp. Currently, good quality commercial aquaculture feeds in China still use fish meal as a main protein source. With the rapid development in aquaculture, fish meal demand is increasing while the supply is limited. In research around the world, everyone is trying to utilise fish meal more effectively.

Aquaculture grade fish meals have been designed for marine carnivorous fish. They are superior in quality, achieved by using very fresh raw material (whole fish) and gentle drying, with antioxidant treatment to stabilise lipid material. Their content of biogenic amines (histamine cadaverine, putrescine and tyramine) is under 3,000 ppm. Because of their superior digestibility and quality of protein (amino acid profile) for fish, dietary nitrogen utilisation should be improved. This should allow less protein to be used in the diet to reduce nitrogen output and cost of diet.

The higher quality protein should permit a higher energy level diet to be used by including fish oil. Fish oil is normally the cheapest oil in international markets. The fatty acid composition of fish oil is characterised by its high content of long polyunsaturated n-3 fatty acids. Fish oil will be highly digestible when fed to fish if it is not oxidised.

Tilapia are a widely cultured finfish ranking second to carp in global production - 659 TT in 1995 of which around 25% were estimated to be reared on compound feed. For eating they are regarded as superior to carp. In consequence it has been predicted that the rate of increase in production will be greater than that for carp. The most important species is Nile tilapia ( some times referred to as red tilapia (*O. niloticus*)). This is an omnivorous freshwater fish consuming mainly aquatic plants, algae/bacteria, insect larvae and occasionally small crustacean. Intensively reared tilapia grow to marketable size (500g to 600g) in six months with a feed conversion of around 2.5:1. They are capable of rapid growth under a variety of conditions including in poor quality water and even in tanks, making them an easy species with which to carry out feeding trials. Being omnivorous, they can utilise a wide range of proteins, carbohydrates and fats(lipids).

As with all finfish, tilapia can effectively utilise protein as an energy source. However, in more intensive cultivation using compound feeds, these diets they should be formulated to spare expensive protein by using less expensive carbohydrate and, to a more limited extent, fat. Typically tilapia diets in China contain 10% to 15% fish meal and a mixture of vegetable proteins and oils, plus starchy root crop, for example, cassava/tapioca. Fish oil, if used, is added at low levels.

The objectives of this feeding trial were to demonstrate that: (i) a complete feed with appropriate levels of aquaculture grade fishmeal will be utilised efficiently by tilapia; optimising the content of protein and oil. (ii) diets with aquaculture grade fishmeal will give good growth and feed conversion compared with local fish meal. (iii) Good quality tilapia feed will increase growth rate thereby reducing the production time.

## Materials and Methods

### Dietary treatments - Ingredients and Formulation.

The trial was designed as a 3 x 3 latin square, with three protein and three oil levels (nine treatments). A 10th treatment was included - a local Chinese fish meal. The three protein levels 25, 30 and 35% were achieved by increasing aquaculture grade fish meal (Jack mackerel from Chile), soyabean meal and rapeseed meal, with fish meal providing half the protein (Table 1). More detailed analysis of the ingredients and diets are given in Appendix 1, Tables 1a to 4. The three oil levels, 5, 8 and 11% were achieved by adding a fish oil soyabean oil 50:50 by weight mixture. Dietary ingredients used were those typically used in fish feeds. Although it would have been desirable to keep the protein contribution from each ingredient the same, this was not possible using practical diets (see Appendix 2, Table A1). However, fish meal protein was always 27% of total dietary protein. The local Chinese fish meal was incorporated at the intermediate protein (30%) and oil (8%) levels.

## Fish

Tilapia (*Oreochromis niloticus*) were used from the Institutes breeding pond. The average initial weight was around 35g.

## Equipment

The tanks used were round fibreglass tanks with conical bottoms with a hole for discharging water. The volume of the tanks was about 0.13 $m^3$. Water was sourced from a thermal spring at a temperature of 50°c., held in a storage tank. This was square measuring 2m x 2m x 0.5m. The tank was separated into two parts by using a layer of ground zeolite 50cm thick and a layer of 0.5cm foamed plastic. The discharged water from the experimental tanks proceeded to the storage tank. The water was filtered and cleaned through the zeolite and foamed plastic layers, then pumped back to the experimental tanks. Complete water exchange in the experimental tanks was 3-4 hours. Water from the storage tank was used to maintain the temperature between 25°C-27°C in the fish tanks. An efflux pipe was fixed in the storage tank for aeration. Dissolved oxygen level in the storage tank was maintained above 5 ppm.

# Table 1. Feed Formulation

| | Dietary Treatments | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Ingredients | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Aquaculture grade fish meal(S.American) | 10.00 | 10.00 | 10.00 | 12.00 | 12.00 | 12.00 | 14.00 | 14.00 | 14.00 | |
| Chinese fish meal | | | | | | | | | | 13.51 |
| Rape seed meal | 6.50 | 6.50 | 6.50 | 7.80 | 7.80 | 7.80 | 9.10 | 9.10 | 9.10 | 7.80 |
| Soybean meal | 20.20 | 21.20 | 22.21 | 30.58 | 31.58 | 32.59 | 40.95 | 41.96 | 42.96 | 32.08 |
| Corn | 23.12 | 21.50 | 19.92 | 17.65 | 19.05 | 14.44 | 12.18 | 10.57 | 8.98 | 15.25 |
| Wheat shorts | 34.68 | 32.30 | 29.87 | 26.47 | 24.07 | 21.67 | 18.27 | 15.87 | 13.46 | 22.96 |
| $Ca(H_2PO_4)_2$ | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 | 1.50 |
| Mineral premix | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Vitamin premix* | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Choline | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Oil | 2.00 | 5.00 | 8.00 | 2.00 | 5.00 | 8.00 | 2.00 | 5.00 | 8.00 | 5.00 |
| **Calculated Analysis** | | | | | | | | | | |
| Protein | 25.0 | 25.0 | 25.0 | 30.0 | 30.0 | 30.0 | 35.0 | 35.0 | 35.0 | 30.0 |
| Fat | 5.0 | 8.0 | 11.0 | 5.0 | 8.0 | 11.0 | 5.0 | 8.0 | 11.0 | 8.0 |

*Vitamin premix includes compound antioxidants

## Method

Each diet was fed to 3 replicates of 12 tilapia each. The trial was conducted for 8 weeks. Tilapias were fed ad libitum 4 times a day at 08:00, 10:30, 13:00 and 16:00. Water temperature was monitored twice a day at 08:00 and 16:00 . The dissolved oxygen was determined once per day. The storage tank was cleaned daily and 0.5m$^3$ of freshwater was added. Ammonia, nitrite, and nitrate were analysed weekly and maintained within acceptable limits. pH values measured once a week are stable and slight alkaline.

Fish were weighed together every two weeks and the experimental tanks were cleaned.

## Results

The chemical analysis of the 10 diets is shown in Appendix 1 - Table 4. Proteins tended to be higher than calculated, but they agreed reasonably well within protein treatment e.g., diets 1,2 and 3. Fat values agreed reasonably with calculated values with the exception of treatment 10 where fat was over 1% unit below the calculated value 6.8 v 8%.

The weight gain of the fish and their feed conversion rates (FCR - feed required per unit weight gain) are given in Table 2.

Statistical analysis of the data was undertaken by Dr. E.L. Miller. Detailed notes are given in Appendix 2. Only the main points are reproduced here.

Growth increased with increasing dietary protein; the effect approached significance ($P<0.10$). FCR increased also; this effect was significant ($P<0.02$) (Appendix 2, Table A5). Since there was no deviation from a linear response to protein, this trial has not established the optimum - it could be above 35%.

There was no statistically significant effect of increasing dietary oil levels, although the first increment (5% to 8%) resulted in numerically better growth and FCR. Problems with the physical form of the high oil treatment feed pellets may have constrained growth on this treatment.

Because the trial was not originally designed to include local fish meal, this treatment (10) could only be compared against treatment (5) with South American aquaculture grade fish meal. Although the fish receiving the latter had a considerably higher growth rate - weight gain 138.4g v 118.17 over eight weeks, this difference was not significantly different probably because of the limited numbers of fish available for this comparison. However, the improvement in FCR with aquaculture grade fish meal (1.86 v 2.38, a 28% difference) was significant ($P<0.05$) when the pooled mean of the differences between replicates of all 10 treatments were used - possible because the variation in these two treatments did not differ from the others (see Appendix 2, Tables A6a, b and c). It is possible that low palatability of diet 10 with local Chinese fish meal reduced feed intake, increased wastage to raise FCR, and caused reduced growth.

## Table 2. Results of Trials

| DIETS | INITIAL WEIGHT (g) | FINAL WEIGHT (g) | WEIGHT GAIN (%) | FEED CONVERSION RATIO |
|---|---|---|---|---|
| 1 | 36.14 | 85.25 | 135.88 | 2.02 |
| 2 | 35.09 | 86.81 | 140.50 | 1.95 |
| 3 | 35.93 | 83.49 | 132.37 | 2.03 |
| 4 | 36.60 | 87.77 | 146.54 | 1.80 |
| 5 | 36.33 | 86.61 | 138.40 | 1.86 |
| 6 | 36.18 | 88.97 | 145.38 | 1.91 |
| 7 | 36.28 | 88.38 | 143.59 | 1.83 |
| 8 | 35.33 | 92.39 | 161.54 | 1.68 |
| 9 | 35.82 | 90.06 | 151.45 | 1.74 |
| 10 | 35.95 | 78.43 | 118.17 | 2.38 |

Survival rate 100% on all treatments.

## Discussion

The fish used in this trial were small; larger fish may have a lower requirement for protein. It must be emphasised that whilst the highest protein level gave the best results, larger fish may require less protein. This can only be tested by using larger tanks or ponds. A follow-up trial with cages in ponds is planned.

Whilst the smaller fish used in this trial have an optimum dietary protein requirement when in tanks of over 35%, the economic optimum is likely to be lower. Furthermore, fish of this size in ponds are likely to get significant amounts of biomass, reducing their dependence on formulated feeds; as they get larger they become fully dependent.
The comparison of local Chinese fish meal with South American fish meal did show a marked difference. A part of this difference could have been due to the lower than

expected fat content of the diet containing Chinese fish meal. This part of the difference, however, is likely to be small as fish gave only a small response to increases in dietary fat. If this difference is seen in commercial ponds, the effect on the economic return is likely to be a considerable improvement.

In the experimental tank systems, the growth of tilapia is slow. By using this system, the pellets fed which had not been eaten would be removed in the discharged water. To prevent excessive feed losses, the amount fed was relatively small. Therefore, low feed intake was observed. This could possibly explain the slow growth rate of tilapia in this experiment. Generally, these fish would grow slower than pond raised fish. The purpose was to compare the effects of different diets under the same experimental conditions. Therefore, given the slow growth rates, the results can be considered as the effect of different diet treatments.

The growth rate and feed utilisation improved when the lipid content was increased to 8%. This phenomenon is worthy of further research to determine whether it is related to inappropriate Protein:Energy ratio in the diets. According to Tian Jishun's previous study, tilapias are not able to utilise high levels of dietary lipid as effectively as common carp. The efficacy of various sources of dietary lipid for tilapias is rather different. Tilapia has demonstrated a requirement for EFA (essential fatty acids) of the n-3 series. Prof. Yong Wenyue, a famous nutrition researcher in Changjiang Fisheries Research Institute, examined the growth responses of *Oreochromis niloticus* fed various oils. He concluded that lipid content should consist of plant oil and fish oil, and plant oil should have a larger percentage of the lipid content than fish oil. To maximise protein utilisation, lipid level in the diet which is no more than 10% would appear optimal. This conclusion is similar to that of this experiment.

Fish oil in diets can be used as an important energy source for tilapia.

## Conclusion

The optimum dietary protein requirement of tilapia above 40 g is at least 35%. The optimum dietary lipid requirement of tilapia of above 40 g is 8%.
The quality of fish meal is major factor determining the quality of feed and feeding utilisation. Aquaculture grade fish meal is better than local fish meal.

Increasing protein rather than energy was of greater significance in tilapia feeds.

Fish oil in diets can be used as an important energy source for tilapia. It may also spare protein and improve protein efficiency.

## Acknowledgement

This experiment was financially supported by the International Fishmeal & Oil Manufacturers Association, supervised by Dr. Ian H. Pike, and co-ordinated by Mr. Chen Delong, Bureau of Fisheries, Ministry of Agriculture, PRC.

# APPENDIX 1

**Main Dietary Ingredients:**

Aquaculture grade fish meal and fish oil: Provided by the International Fishmeal & Oil Manufacturers Association and imported from Chile. Meal - crude protein 71.20%, moisture 6.66% (see Table 1a and 1b below).

Local fish meal: Made from Eastern China, crude protein 63.46%, moisture 6.09% (see below).

Rape seed meal: crude protein 41.05%, moisture 7.12%.

Corn: crude protein 9.34%, moisture 9.54%.

Soybean cake: Expeller soybean cake from North-eastern China, crude protein 47.75%, moisture 7.44%.

Wheat shorts: crude protein 13.59%, moisture 7.56%.

The diet composition and their nutritive values are shown in Tables 2, 3 and 4 below:-

## Appendix 1 - Table 1a

**Analysis of the fish meals by a European Laboratory**

| | AQUAGRADE CHILEAN % | LOCAL % |
|---|---|---|
| Crude Protein | 71.1 | 63.5 |
| Oil Content | 7.5 | 6.5 |
| Moisture Content | 8.0 | 11.5 |
| Ash Content | 12.9 | 22.6 |
| Pepsin Digestibility | 94.7 | 52.5 |
| *Biogenic Amines:-* | | |
| Histamine | under 1 | 146 |
| Cadaverine | 723 | 113 |
| Putrescine | 213 | 300 |
| Tyramine | 137 | 29 |

## Appendix 1 - Table 1b

**Analysis of fish oils by a European Laboratory**

| | AQUAGRADE CHILEAN % | LOCAL % |
|---|---|---|
| Iodine Value | 131.5 | 140.7 |
| Peroxide Value | 21.8 | 5.4 |
| Anisidine Value | 10.8 | 11.1 |
| Totox Value | 54.3 | 22 |

## Appendix 1 - Table 2

| Vitamin supplementation levels | |
|---|---|
| Vitamin | Supplementation levels per kg feed |
| Thiamine | 10 mg |
| Riboflavin | 10 mg |
| Pyridoxine | 10 mg |
| Niacin | 50 mg |
| Pantothenic acid | 30 mg |
| Ascorbic acid | 300 mg |
| Choline chloride | 800 mg |
| Folic acid | 5 mg |
| Vitamin $B_{12}$ | 0.02 mg |
| Biotin | 0.05 mg |
| Inositol | 200 mg |
| A | 5000 IU |
| D | 1600 IU |
| E | 100 IU |
| K | 10 mg |

## Appendix 1 - Table 3

| Composition of mineral premix. | |
|---|---|
| Ferric sulphate($FeSO_4$) | 2.0g |
| Magnesium carbonate($MgCO_3$) | 10.0g |
| Manganese carbonate($MnCO_3$) | 0.4g |
| Potassium iodide(KI) | 1.0g |
| Zinc sulphate($ZnSO_4$) | 0.4g |
| Sodium fluoride(NaF) | 0.4g |
| Cobalt sulphate($CoSO_4$) | 0.3mg |
| Sodium selinite($Na_2SeO_3$) | 0.1mg |

## Appendix 1 - Table 4 Diet Analysis[1]

| | Protein | Fat | Moisture | Ash | Fibre | Ca | P |
|---|---|---|---|---|---|---|---|
| **1** | 27.15 | 4.68 | 6.65 | 6.10 | 5.80 | 1.82 | 1.08 |
| **2** | 26.77 | 8.17 | 5.29 | 5.92 | 5.83 | 1.53 | 1.08 |
| **3** | 26.63 | 10.97 | 6.57 | 6.42 | 6.61 | 1.32 | 1.08 |
| **4** | 32.39 | 4.83 | 4.53 | 7.00 | 5.63 | 1.53 | 1.06 |
| **5** | 32.74 | 7.74 | 6.43 | 7.12 | 6.23 | 2.25 | 1.19 |
| **6** | 32.73 | 10.50 | 4.29 | 6.88 | 6.06 | 1.54 | 1.05 |
| **7** | 37.74 | 4.22 | 4.66 | 7.72 | 4.80 | 1.92 | 1.25 |
| **8** | 38.29 | 7.46 | 3.68 | 7.37 | 6.60 | 2.12 | 1.04 |
| **9** | 37.95 | 10.17 | 4.38 | 7.27 | 6.48 | 1.43 | 1.21 |
| **10** | 33.49 | 6.76 | 5.13 | 9.88 | 5.38 | 1.74 | 1.15 |

[1]Values are %

# APPENDIX 2

## Statistical Analysis

The original report implied that treatment 5 gave more variable responses than some other treatments. The standard deviation and Coefficient of Variation for each treatment in Table A2 has been calculated. Treatment 5 is not different in this respect, but it is noticeable that diet 10 is variable with respect to growth rate but not so variable with respect to FCR. For other diets variation in growth rate is also reflected in variation in FCR.

The inclusion of a 10th diet outside the 3 x 3 factorial causes some complication in how to analyse the data and also complicates the use of some statistical packages. The ANOVA is shown in Table A3 and A4 and the results are summarised in Table A5. The protein x oil interaction is not significant. There is a linear effect of increasing protein level improving growth ($P<0.10$) and FCR ($P<0.02$). There is no effect at all of oil level. Since there is no sign of deviation from a linear response to protein this trial has not established an optimum level. Further trials will need to explore even higher protein levels; they should also explore the use of increasing fish meal v increasing soya bean meal.

A t-test of diet 5 v diet 10 using only the 6 values for these two diets shows only a trend to better values for the aquaculture grade ($P = 0.08$ for both growth and FCR). However, with only 3 replicates for each treatment and 4 df for the error term this is a very poor test (Table A6a,b,c). Since the variation of these two treatments does not appear to differ from the other treatments a better estimate of the error of the experiment is obtained from the pooled error of the differences between the replicates of all 10 treatments. This has the advantage of increasing the df for the error term to 20 and consequently the critical t value is lower. However, the pooled error mean square is greater for weight gain but smaller for FCR than using that of treatments 5 and 10 only. Consequently the difference in weight gain is not significant even at $P = 0.10$ but the difference in FCR is now significant at $P<0.05$ (Table A6d).

The difference in variance of weight gain and FCR for diet 10 v all the rest prompted a closer examination. From the data supplied it is not possible to back calculate the food consumption. It may prove instructive to plot food consumption v weight gain and to look for evidence of poor intake as opposed to poor feed utilisation. I plotted FCR against weight gain %. See Figures A1 and A2. Since FCR is presumably food consumption /actual weight gain there is an element of weight gain in both axes. The slope of the relationship is the improvement in FCR with each increment in weight gain %. The relationship with diet 10 is strikingly different from all others. Whereas the other 9 diets all fit to a single relationship:

$$FCR = -0.0118 \text{ (protein gain \%)} + 3.5664 \qquad R2 = 0.9164$$

the relationship for diet 10 was :

$$FCR = -0.0372 \text{ (protein gain \%)} + 6.7669 \qquad R2 = 0.9989.$$

The reason for this difference seems worth exploring. Clearly if a fish does not grow at all the food consumed would be used for maintenance and FCR would be infinity. So a linear relationship cannot be expected to hold down to low growth rates. But the growth rates of diet 10 do overlap with other diets so this does not seem to be the reason. If food is fed but is not palatable and is wasted but treated as consumed then the FCR will be artificially high. The reason could be the steep improvement in FCR with increase in weight gain % for diet 10 reflecting differences in feed wastage between these three replicates rather than differences due to efficiency of use of consumed feed.

## Appendix 2 - Table A1. Contribution of fish meal, rapeseed meal and soya bean meal to dietary protein

| Diet | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| FM % | 10 | 10 | 10 | 12 | 12 | 12 | 14 | 14 | 14 | 13.51 |
| FM CP % | 71.2 | 71.2 | 71.2 | 71.2 | 71.2 | 71.2 | 71.2 | 71.2 | 71.2 | 63.46 |
| Determined diet CP | 27.15 | 26.77 | 26.63 | 32.39 | 32.74 | 32.73 | 37.74 | 38.29 | 37.95 | 33.49 |
| FM proportion | 0.26 | 0.27 | 0.27 | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 | 0.26 |
| Planned diet CP % | 25 | 25 | 25 | 30 | 30 | 30 | 35 | 35 | 35 | 30 |
| FM proportion | 0.28 | 0.28 | 0.28 | 0.28 | 0.28 | 0.28 | 0.28 | 0.28 | 0.28 | 0.29 |
| | | | | | | | | | | |
| RSM % | 6.5 | 6.5 | 6.5 | 7.8 | 7.8 | 7.8 | 9.1 | 9.1 | 9.1 | 7.8 |
| RSM CP % | 41.05 | 41.05 | 41.05 | 41.05 | 41.05 | 41.05 | 41.05 | 41.05 | 41.05 | 41.05 |
| RSM CP proportion | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| | | | | | | | | | | |
| SBM % | 20.2 | 21.2 | 22.21 | 30.58 | 31.58 | 32.59 | 40.95 | 41.96 | 42.96 | 32.08 |
| SBM CP % | 47.75 | 47.75 | 47.75 | 47.75 | 47.75 | 47.75 | 47.75 | 47.75 | 47.75 | 47.75 |
| SBM CP proportion | 0.36 | 0.38 | 0.40 | 0.45 | 0.46 | 0.48 | 0.52 | 0.52 | 0.54 | 0.46 |

FM - fish meal; RSM - rapeseed meal; SBM - soyabean meal.

## Appendix 2 - Table A2. Examination of variation within each treatment

Wt Gain %

| Diet | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Rep1 | 122.5 | 126.3 | 157 | 137.2 | 139 | 138.9 | 137.3 | 122.1 | 153.2 | 119.3 |
| Rep2 | 140.5 | 173.8 | 122.6 | 152.2 | 150.7 | 149.1 | 128.1 | 133.4 | 147 | 126.8 |
| Rep3 | 144.3 | 139.6 | 151.8 | 131.2 | 126.2 | 199.4 | 131.8 | 181.7 | 154.3 | 108.3 |
| Mean | 135.77 | 146.7 | 143.8 | 140.2 | 138.63 | 162.47 | 132.4 | 145.7 | 151.5 | 118.1 |
| SD | 11.64 | 24.50 | 18.54 | 10.82 | 12.25 | 32.39 | 4.63 | 31.66 | 3.94 | 9.31 |
| CV | 8.58 | 16.72 | 12.89 | 7.72 | 8.84 | 19.94 | 3.50 | 21.72 | 2.60 | 7.88 |

Feed Conversion Ratio (FCR)

| Diet | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Rep1 | 2.25 | 2.07 | 1.7 | 2.05 | 1.86 | 1.83 | 1.98 | 2.17 | 1.74 | 2.35 |
| Rep2 | 1.98 | 1.6 | 2.03 | 1.76 | 1.74 | 1.74 | 2.12 | 2.08 | 1.75 | 2.05 |
| Rep3 | 1.85 | 1.82 | 1.78 | 2.04 | 2.01 | 1.19 | 1.98 | 1.49 | 1.72 | 2.74 |
| Mean | 2.027 | 1.830 | 1.837 | 1.950 | 1.870 | 1.587 | 2.027 | 1.913 | 1.737 | 2.380 |
| SD | 0.204 | 0.235 | 0.172 | 0.165 | 0.135 | 0.346 | 0.081 | 0.369 | 0.015 | 0.346 |
| CV | 10.07 | 12.85 | 9.37 | 8.44 | 7.23 | 21.84 | 3.99 | 19.30 | 0.88 | 14.54 |

SD - standard deviation; CV - coefficient of variation.

## Appendix 2 - Table A3 ANOVA of Weight gain %

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Sample | 1223.214 | 2 | 611.607 | 1.602 | 0.229 | 3.555 |
| Columns | 126.130 | 2 | 63.065 | 0.165 | 0.849 | 3.555 |
| Interaction | 607.744 | 4 | 151.936 | 0.398 | 0.807 | 2.928 |
| Within | 6870.373 | 18 | 381.687 | | | |
| Total | 8827.461 | 26 | | | | |

| | | | | | |
|---|---|---|---|---|---|
| SED | 9.210 | t 18 df P=0.1 | 1.734 | LSD | 15.970 |
| Linear effect (a) | 16.467 P<0.1 | t 18 df P=0.05 | 2.101 | LSD | 19.350 |

(a) linear effect is the difference 35% CP mean - 25% CP mean = 17.0

## Appendix 2 - Table A4. ANOVA of FCR

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Sample | 0.3563 | 2 | 0.1781 | 3.6839 | 0.0456 | 3.5546 |
| Columns | 0.0518 | 2 | 0.0259 | 0.5355 | 0.5944 | 3.5546 |
| Interaction | 0.0654 | 4 | 0.0163 | 0.3381 | 0.8487 | 2.9277 |
| Within | 0.8704 | 18 | 0.0484 | | | |
| Total | 1.3439 | 26 | | | | |

| | | | | | |
|---|---|---|---|---|---|
| SED | 0.1037 | t 18 df P=0.1 | 1.734 | LSD | 0.1797 |
| Linear | -0.2811 P<0.02 | t 18df P=0.05 | 2.101 | LSD | 0.2178 |
| | | t 18df P=0.02 | 2.552 | LSD | 0.2645 |
| | | 18 df P=0.01 | 2.878 | LSD | 0.2983 |

## Appendix 2 - Table A5. Effect of protein and oil level on weight gain % and Feed Conversion Ratio (FCR)

| | Protein % | | | | |
|---|---|---|---|---|---|
| | 25 | 30 | 35 | SED | Linear effect |
| Wt gain % | 136 | 144 | 153 | 9.2 | P <0.1 |
| FCR | 2.00 | 1.87 | 1.72 | 0.104 | P<0.02 |

| | Oil % | | | | |
|---|---|---|---|---|---|
| | 5 | 8 | 11 | SED | |
| Wt gain % | 142 | 147 | 143 | 9.2 | NS |
| FCR | 1.90 | 1.80 | 1.89 | 0.104 | NS |

No Protein x Fat interaction

## Appendix 2 - Table A6 Comparison of diets 5 and 10
### (a) Weight gain %

ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 630.375 | 1 | 630.375 | 5.325 | 0.082 | 7.709 |
| Within Groups | 473.493 | 4 | 118.373 | | | |
| | | | | | | |
| Total | 1103.868 | 5 | | | | |

## Appendix 2 - Table A6
### (b) FCR

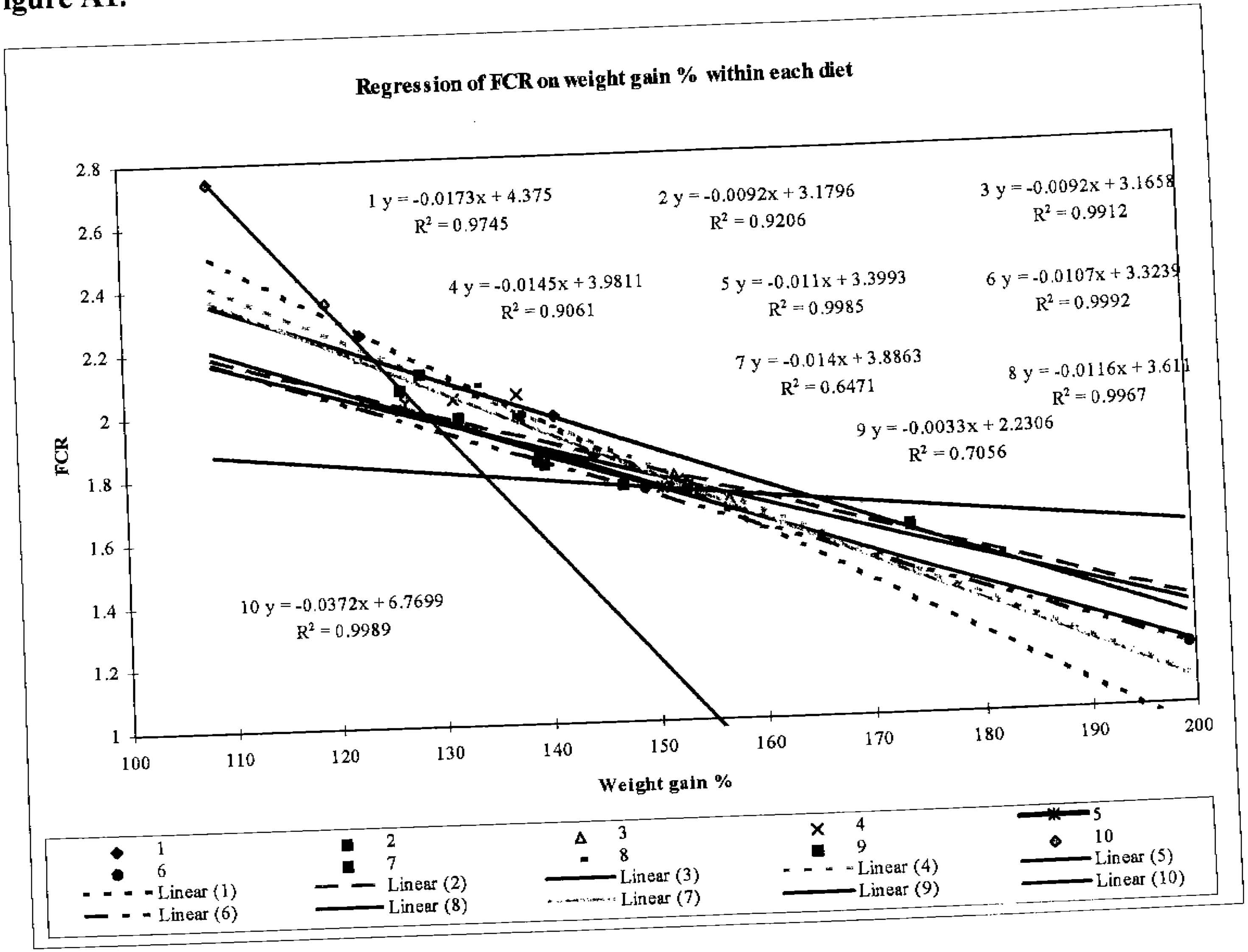
ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Between Groups | 0.390 | 1 | 0.390 | 5.654 | 0.076 | 7.709 |
| Within Groups | 0.276 | 4 | 0.069 | | | |
| | | | | | | |
| Total | 0.666 | 5 | | | | |

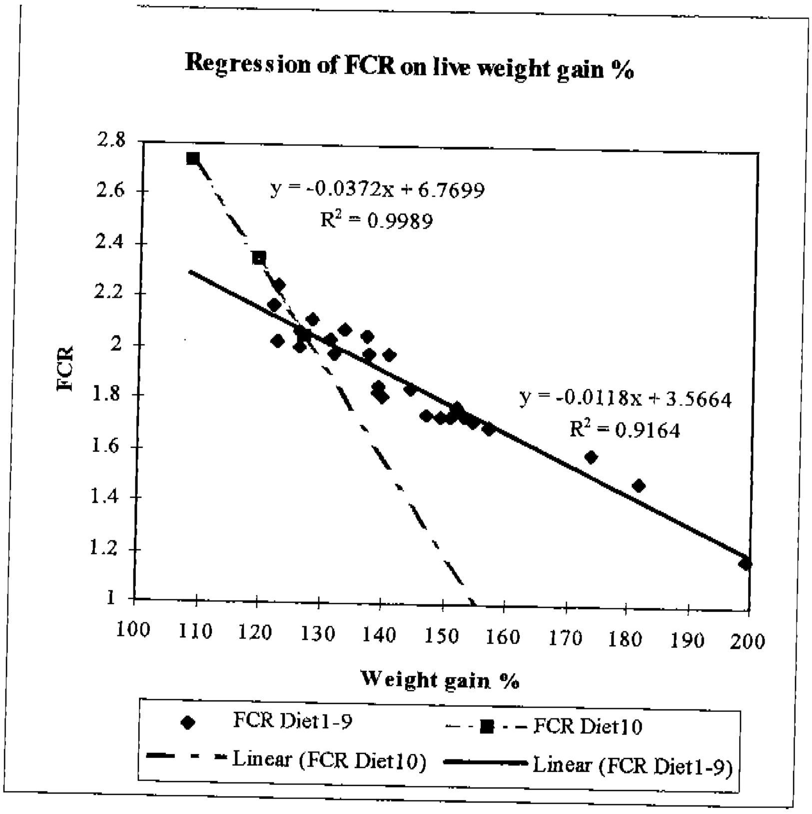
## Appendix 2 - Table A6
### (c) Summary

| | Aquaculture grade | Regular fish meal | SED | P |
|---|---|---|---|---|
| Wt gain % | 139 | 118 | 8.9 | 0.082 |
| FCR | 1.87 | 2.38 | 0.214 | 0.076 |

## Appendix 2 -Figure A1.



**Appendix 2 - Figure A2.**

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