3. MATERIALS AND METHOD

3.1. PROXIMATE COMPOSITION OF DIET AND FEED PREPARATION

The control diet in this experiment is a commercial diet manufactured by Dinding Soya and Multifeeds Sdn. Bhd. The feed ingredients for the control diet are not disclosed due to company policy but the proximate composition of diet in terms of 20% crude protein, 11% moisture, 4% crude fat, 8% crude ash and ME Kcal/\% protein (calorie/protein) ratio (150:1) is given.

Feed ingredients for the experimental diet (UF) included rice bran, corn, palm kernel cake (PKC), fishmeal and molasses. These ingredients are easily available from local sources and therefore are cost effective.

The experimental diet (UF) were analyzed for moisture, protein, fat, energy, ash, fiber, and NFE (Nitrogen-Free-Extract) by standard AOAC methods (AOAC, 1984). The diets were prepared and handled as described by Cho, Cowey and Watanabe, (1985) and De Silva and Perera, (1995) shown in Table 3.1. The proximate composition of the experimental feed was analysed in the laboratories of the University of Malaya and the Department of Veterinary Services (JPH), Petaling Jaya.
Table 3.1: Proximate feed composition for the experimental diet (UM diet)

<table>
<thead>
<tr>
<th>Feed Ingredients</th>
<th>Crude Protein (%)</th>
<th>Crude Fat (%)</th>
<th>Crude Ash (%)</th>
<th>Moisture (%)</th>
<th>Fiber (%)</th>
<th>Metabolizable Energy (kcal/kg) (Calculated)</th>
<th>Nitrogen-Free Extract (NFE) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fishmeal</td>
<td>57.8</td>
<td>5.7</td>
<td>22.0</td>
<td>11.8</td>
<td>1.4</td>
<td>2425</td>
<td>1.3</td>
</tr>
<tr>
<td>Rice bran</td>
<td>13.1</td>
<td>15.7</td>
<td>7.9</td>
<td>9.6</td>
<td>6.1</td>
<td>3506</td>
<td>47.4</td>
</tr>
<tr>
<td>Corn</td>
<td>8.5</td>
<td>2.8</td>
<td>1.8</td>
<td>11.1</td>
<td>3.1</td>
<td>3118</td>
<td>72.7</td>
</tr>
<tr>
<td>PKC</td>
<td>13.9</td>
<td>11.2</td>
<td>4.5</td>
<td>6.9</td>
<td>14.7</td>
<td>2523</td>
<td>48.8</td>
</tr>
<tr>
<td>Molasses</td>
<td>2.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3395</td>
<td>-</td>
</tr>
</tbody>
</table>
3.1.1. EXPERIMENTAL DIETS

The experimental diet was formulated to contain 20.0% crude protein, 7.7% fat, 7.6% ash, 9.6% moisture and 150% ME kcal/% protein (calorie: protein) (Plate 1). The energy/protein of the experimental diet (UF) was made almost similar to that of commercial diet (CF), so as to make proper comparisons.

Plate 1: UF pellets

Plate 2: CF pellets
Table 3.2 shows the digestible energy and digestible protein content of various feed ingredients in the experimental diet.

**Table 3.2**: Diet composition of fish feed ingredients and metabolizable energy

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>% CP</th>
<th>Percentage in Diet (Kg)</th>
<th>Available ME (Kcal)</th>
<th>Amount Crude Protein Available (Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fishmeal</td>
<td>57.8</td>
<td>20</td>
<td>48500</td>
<td>11.56</td>
</tr>
<tr>
<td>Rice bran</td>
<td>13.1</td>
<td>25</td>
<td>87650</td>
<td>3.28</td>
</tr>
<tr>
<td>Corn</td>
<td>8.5</td>
<td>35</td>
<td>109130</td>
<td>2.98</td>
</tr>
<tr>
<td>PKC</td>
<td>13.9</td>
<td>15</td>
<td>37845</td>
<td>2.09</td>
</tr>
<tr>
<td>Molasses</td>
<td>2.6</td>
<td>5.0</td>
<td>16975</td>
<td>0.13</td>
</tr>
<tr>
<td>Total (Kg)</td>
<td>100 Kg</td>
<td>300100 Kcal</td>
<td>20.04%</td>
<td></td>
</tr>
</tbody>
</table>

\[ \text{ME Kcal/kg} = \frac{\% \text{ Crude Protein} = 20.04}{3001} \]

\[ \text{ME Kcal/kg} = 150:1 \]

% Protein
3.1.1.1. Experimental populations

The three populations of *Oreochromis* used in this study are bred and maintained in the University of Malaya Farm. The original source of these three strains are listed in Table 3.3. The populations were obtained as 8 weeks old fry at the time of commencement of the study and were of similar size. However the precise ancestry of the populations is not known.

**Table 3.3:** Experimental populations of this study

<table>
<thead>
<tr>
<th>STRAIN AND SOURCE</th>
<th>DESIGNATION</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O.mossambicus</em> from Thailand via Department of Fisheries, Malaysia</td>
<td><em>O.m</em></td>
</tr>
<tr>
<td><em>O.niloticus</em> (An Egyptian strain from Aquatic Biosystems, Philippines)</td>
<td><em>O.np</em></td>
</tr>
<tr>
<td><em>O.niloticus</em> from a farm in Malaysia (Local)</td>
<td><em>O.nl</em></td>
</tr>
</tbody>
</table>

3.1.1.2. Management of diet

3.1.1.2.1. Initial stage

Swim-up fry which were obtained from the hatchery unit of University of Malaya farm were counted and placed in the glass aquariums (90 cm x 45 cm x 45 cm). They were fed 4 times daily, the feed used were floating pellets produced by a commercial
farm - Dinding Soya and Multi feed Sdn Bhd. As these were stocking fry in aquariums, no data was collected during this period.

Subsequently eighteen tanks (2 x 2 x 1 m) were used for stocking fry. Of these 18 tanks, 8 tanks were used for maintaining each of *O.mossambicus* and *O.niloticus* strains and two tanks were used for *O.niloticus* local. One half of the tanks for each strain was used for CF, the other half was for UF. Only two tanks were used for *O.niloticus* local as the number of contemporary *O.niloticus* philippines fries were very small during the beginning of this study.

3.1.1.2.2. **Experimental phase**

When the fingerlings were 8 weeks old inside the tanks, the fingerlings were weighed and transferred from the growing tanks to cages in the ponds. A total of 8 cages (plate 3) were used for each of *O.mossambicus* and *O.niloticus* philippines. For *O.niloticus* local, only 2 cages were used due to insufficient fingerlings. A random sample of 50 fingerlings were stocked in each of the above mentioned cages
From this stage onwards the amount of feed given to the fish were 5% of average body weight and were split in two feedings (9.00 AM and 13.00 PM).

Commercial feed used was floating pellets, but as UF pellets were not of floating type, the pellets were placed in the hanging containers and immersed in the water for the fingerling to obtain their feed.

3.1.1.3. **Traits Studied**

The following parameters; body conformation, growth and feed conversion traits were studied at bimonthly intervals starting from the age of 10 weeks to 32 weeks. Both meristic counts and morphometric measurements were made (Fig. 1),
Fig 1: Morphometric measures and meristic counts

Total Length (TL): The length from tip of snout to end of caudal fin.

Standard Length (SL): The total length from tip of snout to end of body (i.e. base of caudal fin).

Head Length (HL): This is measured from the anterior of the upper lip to the end of the opercular membrane.

Body weight (BW): Body weight (to nearest gram) was measured using a pan balance.

Feed consumption (FC): Feed consumption in each of the cages was measured daily by deducting the amount of feed not consumed from the total feed given. Individual daily weight gain (DWG), percent specific growth rate (SGR), and feed conversion ratio (FCR) were measured as follows:
Daily weight gain (DWG) = \( \frac{(W2(g) - W1(g))}{(T2 - T1)} \)

Where \( W2 \) is the weight in grams at time \( T2 \); \( W1 \) is the weight in grams at time \( T1 \); and \( T2 \) is later than \( T1 \).

Percent Specific growth rate (SGR) = \( \left[ \frac{\ln W2 - \ln W1}{(T2 - T1)} \right] \times 100 \)

Where \( W2 \) is the weight in grams at time \( T2 \); \( W1 \) is the weight in grams at time \( T1 \); and \( T2 \) is later than \( T1 \).

Feed conversion ratio (FCR) = \( \frac{\text{Weight of feed given(g)}}{\text{Body weight gain (g)}} \)

3.2. METHODS FOR MEASUREMENT OF CARCASS TRAITS

The amount of moisture, protein, amino acids, fat and ash in the carcass was analysed. Methods used for chemical composition are given below.

3.2.1. Carcass Composition

Samples were taken randomly at harvest (32 weeks), from 18 cages, 10 males and 10 females were taken from each cage.

Individual fish were killed by severing the neck. Head, fin, tail, and bone were removed. The meat was removed from the bone and weighed. The meat samples were
individually wrapped in an aluminum foil and weighed. The samples were then dried in the oven at 100°C for 1 to 2 days.

The dried samples were reweighed to obtain the moisture content of the meat, and calculated as:

\[
\% \text{ Water} = \frac{\text{Weight of fresh sample} - \text{Weight of dry sample}}{\text{Weight of fresh sample}}
\]

The dried samples were grounded using the mortar and pounded to mash for fat, protein, ash, and amino acids analysis.

3.2.1.1. Crude Fat Analysis

Analysis was carried out by using soxtherm-Automatic system machine. Duplicate trials for the individual crude fat analysis were done on individual samples. 0.2 gram samples were placed in the whatman filter paper, weighed (W1) and then inserted into the extraction thimble. The extraction took about 70 minutes after which the remaining solvent was recovered and the glass extraction beakers were removed from the apparatus, the whatman filter paper with sample without fat were taken out from the extraction thimbles, dried and weighed (W2):

\[
\% \text{ Crude fat (Dried meat)} = \frac{W_1 - W_2}{W_1} \times 100
\]
3.2.1.2. Crude Protein Analysis

0.2 gram samples were weighed and put into digestion tubes. 125 ml concentrate H₂SO₄ (digestion acid) and 0.2 gram of catalyst (K₂SO₄, CUSO₄ and Selenium in the ratio 20:20:1) were added. The digestion tubes with the samples were placed into kjeldahl digestion block at 25°C for 10 minutes, the temperature was raised to 280°C. After two hours the digestion tubes were removed and let to cool. The samples were then diluted with 75ml of distilled water. Vapodest-3-automatic system were used for distillation. At the beginning of the distillation, 30-50% NaOH solution is added to the previously water diluted sulfuric acid digestion solution.

\[
\text{% Crude protein} = \frac{(V_a - V_b) \times N \times 14/1000 \times 100 \times N_p}{\text{Sample Weight}}
\]

Where:

- \(V_a\) = Titrating acid for sample (ml)
- \(V_b\) = Titrating acid for control value (HCL)
- \(N\) = Normality of the titration acid = 0.1 N
- \(N_p\) = Protein factor = 6.25
3.2.1.3. Crude Ash Analysis

Porcelain crucibles were oven dried at 105°C overnight to remove all moisture. The crucibles were cooled in a dessicator for 30 minutes. Then the crucibles containing the samples were weighed (C2). The samples were then ashed in a muffle furnace at 550°C for 8 hours. After ashing the samples were cooled and weighed (C3)

\[
\text{% Crude Ash} = \frac{C2 - C3}{C2} \times 100
\]

Where:

\[C2 = \text{Sample and crucible weight},\]
\[C3 = \text{Crucible and sample after ashing}\]

3.2.1.4. Amino Acids Analysis

Amino acid analysis (lysine, leucine and methionine) was made for the carcass meat using the AOAC standard method (1984) with auto-analyzer at the laboratory of the Department of Veterinary Services, Petaling Jaya.
3.3. STATISTICAL ANALYSIS

An analysis of variance (ANOVA) was done to estimate differences between strains, diets and strains x diet interaction. With correction for co-variables when necessary, differences between individual groups within the significant sources of variation were then tested. Means and coefficients of variation were also calculated. Statistical analysis involved the use of SPSS (Statistical Package for the Social Science) (Norman, 1975). Differences between the mean values of particular diet effect for weight, length, standard length and head length level were determined by using Duncan’s Multiple Range test or t-test.