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Effect of varying levels of selected plant leaf meal diet on growth performance, haematological indices and biochemical parameters of Tilapia fish (Oreochromis mossambicus)

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ABSTRACT

The high cost and fluctuating quality as well as the uncertain availability of fish meal have led to the need to identify alternative protein sources for fish feed formulation. The fish *Oreochromis mossambicus* were collected from Aliyar dam near Pollachi and they were acclimatized. The selected plants (Moringa oleifera and Amaranthus crutenus) were collected, washed, shade dried and powdered using electric pulverizer. The feed was prepared by using basal ingredients like rice bran, coconut oil cake, chicken waste and corn flour as a binder. The feed was dried and stored in refrigerator. The fishes were fed with these supplemented feed for 120 days and the results were obtained for every15days once for growth parameter. The haematological parameters were done on the end of the experimental day (120DAT). The biochemical parameters on the muscle tissue of fishes were done on initial and final day of the experiment. The data were subjected to one way ANOVA and the means were separated using least significant differences (LSD). From the 15th day after treatment (DAT) to 120th day after treatment (DAT) the Fishes fed with 5% Moringa oleifera supplemented feed showed maximum increase in length. From the15th day after treatment (DAT) to 120th day after treatment (DAT) the Fishes fed with 5% Amaranthus crutenus supplemented feed showed maximum increase in weight, specific growth rate, total feed consumed, condition factor, survival rate and feed conversion ratio. At the end of the experiment haematological parameters on RBC,WBC,HB, Platelets, PCV, MCV, MCH and MCHC was found to be maximum in 5% Amaranthus crutenus supplemented fishes. The biochemical parameter on protein, carbohydrate, lipid and amino acid was found to be maximum in 5% Amaranthus crutenus supplemented fishes. The above result indicates that there is possibilities of using selected plant powders supplemented feed for *Oreochromis mossambicus*.

(Key Words: The Fish Oreochromis mossambicus, Moringa oleifera and Amaranthus crutenus, growth performance, haematological and biochemical parameters).

1. INTRODUCTION

Success in aquaculture depends to a great extent on sound nutritional practices based on the knowledge of nutrients required by the species cultured. In India 60 to 70% of people are living in rural areas. Tilapia has admirable feature in aquaculture (Poma and Masser, 1999; Ekanem and Okoronkwo, 2003) and is an important fish all over the world as fishery resources. It is the most widely cultured fish in the tropics and subtropics and second to Carp among the fresh water fish in the world (Offem et al., 2010).

Tilapia culture is widely practiced in many tropical and sub tropical region of the world. It constitutes the world 3rd largest group of farmed fin right after Carp and Salmonids with an annual growth rate around 11.5% much of rapid increase in aquaculture production has come from the increasing of extending system (Court etal., 2003).

Tilapia is an unique fish in the inland fish production. There are possibilities to enhance Tilapia culture further through other systems of aquaculture practices. However the feed cost is the highest operating cost in the aquaculture practices and an economically efficient feed would play a major role in stimulating feed based aquaculture of *Tilapia*.

Attempts to use the natural materials such as medicinal plants could be widely accepted as feed additives to enhance efficiency of feed utilization and animal productive performance (Levic et al., 2008). The Moringa (Moringa oleifera) is a fast growing plant widely available in tropics and subtropics with several economic-importance, industrial and medicinal uses and is a native food in Southeast Asia. The Moringa leaves have been known to be effective for certain medicinal purposes.

Amaranthus cruentus is a popularly grown leafy vegetable in tropical regions of the world. The economic and nutritional advantage of the A. cruentus leafy vegetable is accentuated by its agronomic superiority over many plant protein sources.

The acute shortage of protein has been attributed to the phenomenal rise in the prices of animal feeds which account for about 75-85% of the recurrent production inputs in intensive monogastric animal production (Fetuga, 1977). A growing interest in the use of unconventional sources of protein and energy has gained prominence. So, the aim of the present study is to evaluate the effect of Moringa oleifera and Amaranthus cruentus leaf meal as enhancers to Tilapia fish (Oreochromis).

OBJECTIVES

- To study the response of *Oreochromis mossambicus* to the formulated feed.
- To evaluate the efficient feed as a growth promoters.
- To evaluate the efficient feed as good muscleproducer.
- To evaluate the efficient feed as an immune stimulant.

To recommend the efficient feed at farm level.

2. MATERIALS AND METHODS

An investigation was carried out in our laboratory to evaluate the effect of formulated feed on the growth, biochemical and haematological parameters of *Tilapia* fish *Oreochromis mossambicus*. The materials and methods used for the present study are described under the following headings:

2.1. Experimental Animal

Oreochromis mossambicus is a riverine fish endemic to Africa, but now is found in many tropical countries. It is commonly called as the African mouth breeders. Earlier, *Tilapia* belongs to the species *Tilapia mossambica*. Later, the nomenclature is revised as *Sarotherodon mossambicus* now at present it is called as *Oreochromis mossambicus* which belongs to the dominant group of breech like fishes of the family Cichilidae. *Tilapia* is commonly known as java *Tilapia*.

2.2. Collection and Acclimation:

The fish *Oreochromis mossambicus* were collected from Aliyar dam near Pollachi, Coimbatore District, Tamil Nadu. They were acclimatized to fresh water condition for 2-3 weeks in the laboratory and transferred to experimental tanks. Aerators were used to raise the dissolved oxygen level. The tanks were maintained neat and clean. Care was taken to avoid contamination. Fishes were fed initially for a week with control feed. The excess of food and feacal matter were cleaned daily in order to prevent fungal and other microbial growth.

2.3. Collection of plants:

Fresh leaves of selected plants were collected for feed preparation in and around Coimbatore.

2.4. Preparation of plantpowder:

The fresh leaves of selected plants were collected washed and shade dried. The dried leaves were ground in to fine powder using an electric pulverizer, passed through a sieve and retained in packets. The powders thus obtained were used for the preparation of the feed.

2.5 Preparation of fish-feed:

Fish feed was prepared by adding equal proportions of coconut oil cake, rice bran and chicken waste in the ratio of 2:2:1 and corn flour as a binder. Seven experimental diets were prepared, by mixing these substances thoroughly with hot water and it was boiled for 25-30 minutes and then cooled at room temperature for 30 minutes and pellets were prepared by using domestic appliances with 0.5m m diameter. The feed without plant powder (T1–Control) and the feed with plant powders (T2–Moringa oleifera 2.5%, T3– M.oleifera 5%, T4- M.oleifera 10%, T5-Amaranthus cruentus 2.5%, T6– A. cruentus 5%, T7– A. cruentus 10%). The small pellets were dried and stored in refrigerator.

2.6.Experimental design:

The laboratory experiment was laid incompletely randomized design (CRD). Three replications from each concentration and control were maintained simultaneously. The experiment was conducted using 15 liter plastic troughs. The troughs were stocked with 5 fishes with mean initial body weight of 5 ± 0.5 grams and uniform size. The fishes were starved for a night prior to the experiment. The experiment was conducted for 120 days and the fishes

were fed with experimental diets. The medium was changed daily in order to remove the faecal and unconsumed wastes.

2.7.Growth parameters:

After the feeding trail the following observations were recorded for each treatment and control every15 days once.

1.Length growth rate: It was calculated by using the formula.

Length growth rate (cm/day) = Final length of fish-Initial length of fish

No.of days

2. Weight growth rate: It was calculated by using the formula,

Weight growth rate (gm/day) = Final weight of the fish-Initial weight of the fish

3. Specific growth rate: It was calculated by using the formula,

Specific growth rate (%/day) = Final weight of the fish-Initial weight of the fish x 100

No.of.Days

4.Normalized biomass index: It was calculated by using the formula,

Normalized biomass index (%/day) = Final weight of the fish x No.of fish-Initial

weight of the fish x No.of fish

100

5.Total feed consumed: It was calculated by using the formula,

Total feed consumed (gm/day) = Weight of the dry feed given – Dry weight of the

unconsumed feed x100

6. Feed conversional ratio: It was calculated by using the formula,

Feed conversional ratio (gm/day) = Feed consumed (gm) x No.of fishes Weight gain in grams

7. Protein efficiency ratio: It was calculated by using the formula,

Protein efficiency ratio (gm/day) = Weight.gain (gm) x No.of fish Protein intake

8.Survival rate: It was calculated by using the formula,

Survival rate (%/day) = Final No.offish-Initial number of Fish

100

9.Condition factor: It was calculated by using the formula,

Condition factor (g/cm³) =Average final weight x100 (Average initial length³)

2.8. Haematological parameters

- 1. **Total Erythrocyte count -** by using Haemocytometer.
- 2. **Total Leucocyte count -** by using Haemocytometer.
- 3. **Haemoglobin estimation -** determined by Sahli's haematin method using Sahli's haemoglobinometer.
- 4. Red blood cell indices assessed RBC indices like Mean Corpuscular Volume (MCV), Mean Corpuscular haemoglobin (MCH) and Mean Corpuscular haemoglobin Concentration(MCHC).

2.9. Biochemical parameters

1. Estimation of Protein - The protein content of the sample was determined using the method developed by Lowry et al., (1951).

2. Estimation of Carbohydrate

- **3.Estimation of Amino acid -** The amino acid content of the sample was determined by using Ninhydrin method.
- **4.Estimation of lipid content**
- 5. Estimation of Ash

3.10.Statistical analysis:

The data on growth parameters were transformed into arc sine values before subjecting into ANOVA. To find out the significance of comparative efficacy of different leaf extraction length, weight, specific growth rate, Normalized biomass index,total feed consumed, feed conversion ratio, feed Efficiency ratio,survival rate,condition factor and protein efficiency ratio one way Analysis of variance was used. Mean differences of each variable were further Analyzed using Least Significant Differences using SPSS package.

3.RESULT

The impact of selected plant leaf meal on growth performance, 32 haematological indices and biochemical parameters of *Oreochromis mossambicus* are described below. The statistical analysis of the data revealed highly significant between the control and different treatments.

3.1. Effect of selected plant leaf meal on length parameter of *Tilapia* fish (Oreochromis mossambicus):

Observation on the length gain was found to be maximum in 5% Moringa oliefera supplemented feed of 0.90% (15DAT),1.20% (30DAT), 1.54% (45DAT), 2.01% (60DAT), 2.63%(75DAT), 3.10% (90DAT), 3.26% (105DAT) and 3.70% 120DAT) when compared to control of 0.36% (15DAT), 0.60% (30DAT), 1.00% (45DAT), 1.20% (60DAT),1.33% (75DAT),1.70% (90DAT),1.90% (105DAT) and 2.06% (120DAT) respectively.(Figure-1).

3.2. Effect of selected plant leaf meal on weight parameters of *Tilapia* fish (*Oreochromis mossambicus*):

After feeding trail,5% Amaranthus cruentus supplemented feed showed increased weight of 3.03%(15DAT),4.43%(30DAT),6.63%(45DAT),7.76%(60 DAT),9.53% (75DAT),12.63%(90DAT),20.03%(105DAT)and 26.63%(120 DAT), when compared to control of 1.23% (15DAT), 2.46% (30DAT), 3.00% (45 DAT), 3.46% (60DAT), 4.26% (75DAT), 5.00% (90DAT), 5.40% (105DAT) and 7.23%(120DAT). (Figure-2).

3.3. Effect of selected plant leaf meal on specific growth rate parameter of Tilapia fish (Oreochromis mossambicus):

Observation on specific growth rate showed that 5% Amaranthus cruentus Supplemented feed exhibited maximum SGRof20.28% (15DAT),17.66% (30 DAT), 19.33% (45DAT), 5.33% (60DAT),13.90% (75DAT),14.57% (90DAT), 20.19% (105DAT) and 23.03% (120DAT) respectively, whereas control recorded 8.20%(15DAT),9.10%(30DAT),7.30%(45DAT),6.13%(60DAT),6.30% (75DAT). (105DAT) and 6.36% (120DAT). (Figure-3).

3.4.Effect of selected plant leaf meal on normalized biomass index of Tilapia fish (Oreochromis mossambicus):

Observation on normalized biomass index showed that in 5% Amaranthus supplemented feed Cruentus there maximum value of 0.12% (15DAT), was 0.19%(30DAT),0.29%(45DAT),0.33%(60DAT),0.41%(75DAT),0.52%(90DAT),0.84%(105DAT)and1.12%(120 DAT)thanthecontrolof0.04%(15DAT),0.10%(30DAT),0.12%(45DAT),0.14%(60DAT),0.18%(75DAT),0.20%(90 DAT), 0.01% (105DAT) and 0.30% (120DAT), respectively.

3.5. Effect of selected plant leaf meal on total feed consumed of *Tilapia* fish (Oreochromis mossambicus):

Results on total feed consumed showed that 5% Moringa oliefera and 5% Amaranthus cruentus supplemented feed was on par with each other with maximum value than the other treatments. Whileincontrolrecorded 1.03% (15 DAT), 1.25% (30DAT),1.53%(45DAT),1.77%(60DAT),1.97%(75DAT),2.20%(90DAT),2.60%(105DAT)and2.76%(120DAT)re spectively.

3.6.Effect of selected plant leaf meal on feed conversion ratio of Tilapia fish (*Oreochromis mossambicus*):

The results obtained shows that there is significant value in 5% Amaranthus cruentus supplemented feed 0.83% (15DAT),1.27% (30DAT),1.42% (45DAT),1.48% (60DAT),1.51% (75DAT),1.55% (90DAT),1.60% (105DAT) and 1.82% (120DAT) respectively. while, control recorded 0.80% (15DAT), 0.47% (30DAT), 0.47% (45DAT), 0.43% (60DAT), 0.42% (75DAT), 0.44% (90DAT), 0.46% (105DAT) and 0.31% (120DAT).

3.7. Effect of selected plant leaf meal on protein efficiency ratio of *Tilapia* fish (Oreochromis mossambicus):

Significant increase in protein efficiency ratio was observed for 5% Amaranthus cruentus supplemented feed of 14.33% (15DAT),18.13% (30DAT),22.65% (45DAT), 22.44% (60DAT), 24.26% (75DAT), 27.64% (90DAT), 35.85% (105DAT) and 44.41% (120DAT), when compared to control of 2.93% (15DAT), 4.53% (30.34DAT), 4.21% (45DAT), 4.62% (60DAT), 5.35% (75DAT), 5.24% (90DAT), 5.55% (105DAT), 7.19% (120DAT) respectively. (**Figure-4.**)

3.8. Effect of selected plant leaf meal on survival rate of *Tilapia* fish

(Oreochromis mossambicus):

Observation on 120DAT, shows that all the treatments exhibited better survival rate of 99.99%.

3.9. Effect of selected plant leaf meal on condition factor of *Tilapia* fish

(Oreochromis mossambicus):

The results obtained after 120DAT shows that maximum feed efficiency ratio was recorded for 5% Amaranthus cruentus supplemented feed of 1.89% (15DAT), 2.25% (30DAT), 2.74% (45DAT), 2.92% (60DAT), 3.33% (75DAT), 4.06% (90DAT), 5.45% (105DAT) and 6.82% (120DAT) than the control of about 1.87%(15DAT), 2.29% (30DAT), 2.47% (45DAT), 2.65% (60DAT), 2.96% (75DAT), 3.08%(90DAT),3.24%(105DAT) and 3.77%(120DAT) respectively. (Figure-5).

3.10. Effect of selected plant leaf meal on Erythrocyte count of *Tilapia* fish

(Oreochromis mossambicus):

A maximum increase of erythrocyte count was noticed in 5% Amaranthus cruentus supplemented feed (0.52 million/cu mm %), this was followed by 10% Moringa oleifera leaves supplemented feed (0.26million/cu mm %) while control recorded (0.11 million/cu mm%) respectively.

3.11.Effect of selected plant leaf meal on Leucocyte count of *Tilapia* fish (Oreochromis mossambicus):

Significant increase in leukocyte count was observed in 5% Amaranthus cruentus supplemented feed (4510cells/cu.mm)whereas other treatments were on par

With each other while control recorded (97.9cells/cu.mm) respectively.

3.12.Effect of selected plant leaf meal on haemoglobin content of *Tilapia* fish (Oreochromis mossambicus):

The observation on haemoglobin content of fish after treatment was observed that 5% Amaranthus cruentus supplemented feed showed maximum Haemoglobin content (2.66gm%) while control recorded (0.66gm%).

3.13.Effect of selected plant leaf meal on PCV content of Tilapia fish (Oreochromis mossambicus):

Significant increase of PCV was observed in 5% Amaranthus cruentus supplemented feed (4.20%) where control recorded (0.73%).

3.14.Effect of selected plant leaf meal on MCV content of *Tilapia* fish (Oreochromis mossambicus):

A maximum increase in MCV content was observed in 5% Amaranthus cruentus Supplemented feed (108.3fL) while control recorded (97.3fL) respectively.

3.15.Effect of selected plant leaf meal on MCH content of *Tilapia* fish (Oreochromis mossambicus):

MCH was observed to be high in 5% Amaranthus cruentus supplemented feed (60.99pg) followed by 10% Amaranthus cruentus supplemented feed(55.61pg) while control recorded (55.0pg) respectively.

3.16. Effect of selected plant leaf meal on MCHC content of Tilapia fish (Oreochromis mossambicus):

The 5% Amaranthus cruentus supplemented feed showed maximum MCHC level (85.0g/L) followed by 10% Moringa oliefera (70.0g/L) while control recorded (57.14g/L) respectively.

3.17. Effect of selected plant leaf meal on protein content of muscle of *Tilapia* Fish (Oreochromis mossambicus):

The maximum increase in protein content was observed in the fishes supplemented with 5% Amaranthus cruentus (8.5mg %) followed by10% Amaranthus cruentus (8.4mg%) while control recorded (7.34mg%) respectively. (Figure-6).

3.18. Effect of selected plant leaf meal on carbohydrate content of muscle of

Tilapia fish(*Oreochromis mossambicus*):

The carbohydrate content was found to be maximum in the fishes supplemented with 5% Amaranthus cruentus (23.8mg%) followed by10% **Amaranthus** cruentus (22.0mg%) while control recorded (10.0mg%) respectively. (Figure-7).

3.19.Effect of selected plant leaf meal on lipid content of muscle of *Tilapia* fish (Oreochromis mossambicus):

The lipid content in the muscle of the fishes supplemented with 5% Amaranthus cruentus showed the significant increase (78.0mg %) followed by 10% Amaranthus cruentus (65.8mg%) while control recorded (30.3mg%)respectively. (Figure-8).

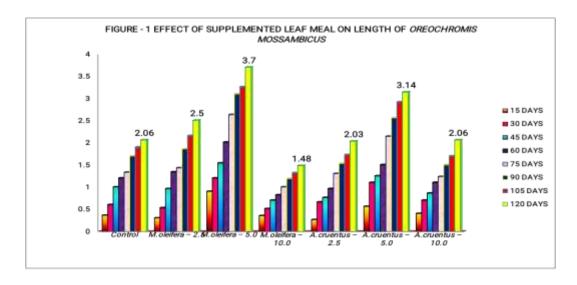
3.20. Effect of selected plant leaf meal on amino acid content of muscle of Tilapia fish (Oreochromis mossambicus):

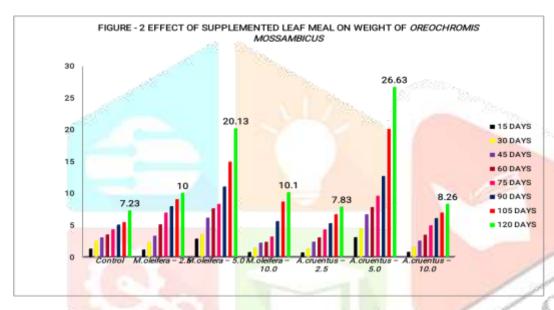
The higher percentage of lipid content was noticed in the fishes fed with 5% Amaranthus cruentus (12.5mg %) followed by 10% Amaranthus cruentus (11.8mg%) while control recorded (5.6mg%) respectively. (Figure-9).

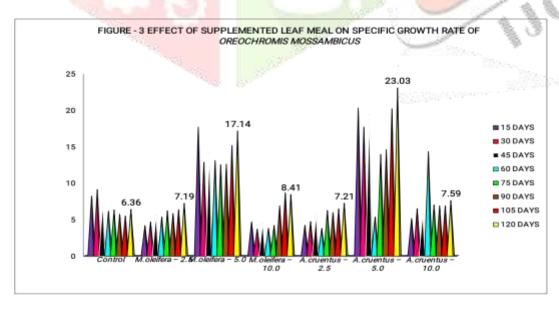
3.21.Effect of selected plant leaf meal on ash content of muscle of tilapia fish (Oreochromis mossambicus):

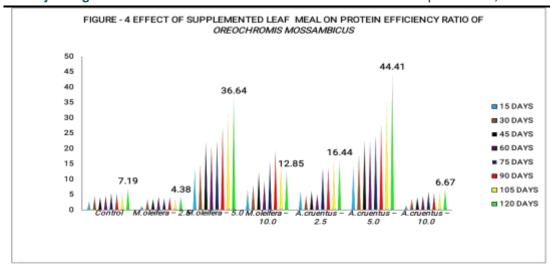
The amount of ash content was found to be maximum in the muscle of fishes Fed with 5% Amaranthus cruentus (88.80mg%) followed by 2.5% Amaranthus Cruentus (75.8mg%) while control recorded (30.2mg%) respectively. (Figure-10).

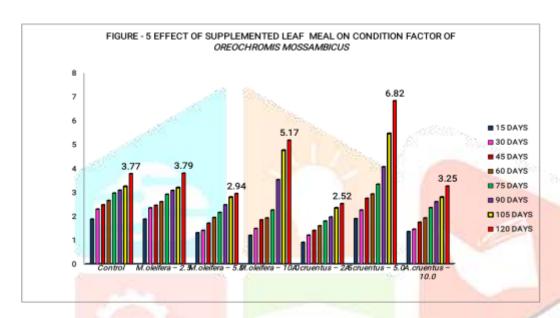
FIGURES

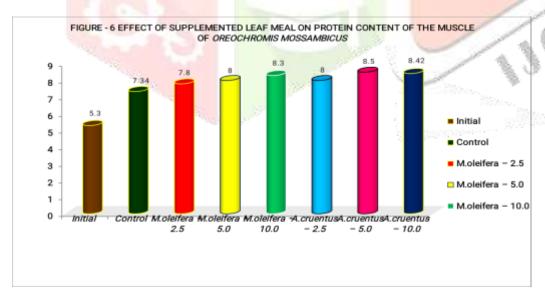


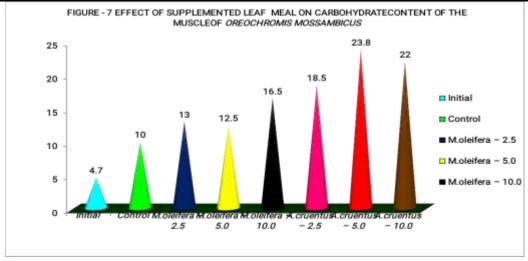


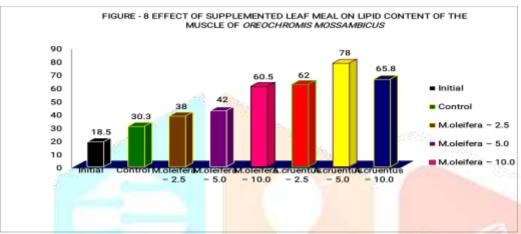


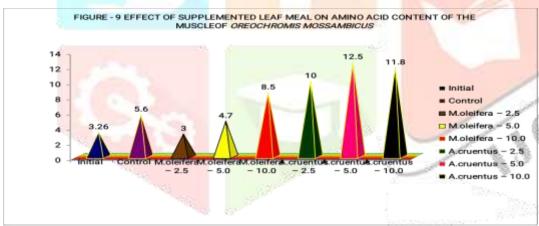


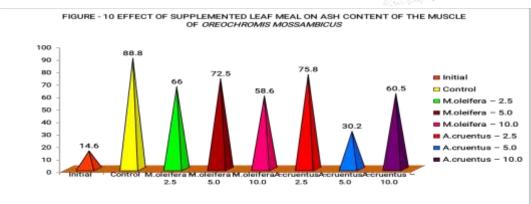












4. DISCUSSION

Tilapia (Oreochromis mossambicus) is one of the main economical fresh water fish that are cultured world wide and the 3rd most commonly farmed fish after carp and salmon with global population of 1.49 million metric tonnes (m mt) in 2002, and farmed *Tilapia* is exceeded two million metric tons in 2004 world wide (Fitzimmons, 2003). Practical fish feed has been an area of focusing aquaculture nutrition research recently (Gomes et al., 1995; Hossain et al., 2001; Siddhuraju and Becker, 2003).

India is a large depository of medicinal plant but studies on the effects of number of medicinal plant extracts as immunostimulants and growth promoters of cultured fish are fragmentary. So in the present study the selected plant leaves like Amaranthus cruentus and Moringa oliefera were used as supplemented leaf meal for Tilapia fish Oreochromis mossambicus.

M.oliefera is well documented world renowned plant herb for its extraordinary nutritional and medicinal properties. The leaves of these plants are rich in 60 proteins, carotenoids, ascorbic acids and iron (Shanchez et al.,2006). The leaves are also used as nutritional supplemented growth promoters due to the significant presence of protein, β-carotene and α-tocopherol (Foidl et al., 2001 and Sanchez., 2006).

In the present investigation all the experimental diets were accepted by *Tilapia* fish (Oreochromis mossambicus) till the end of the experiment, where fish fed with different concentration of leaf meal consumed their feed reluctantly. This showed that the levels of incorporation of leaf meal in the diets were not likely to effect the acceptability of the feed by the fish thus supporting the work of Francis et al., (2001), Siddhuraj and Becker, (2003) and Adeniji et al., (2007).

In the present study also the extract supplemented diet induce significant increase in total WBC count. Similar results are obtained by (Sahu et al., 2007) when Labeo rohita has been fed with Mangifera indica kernel and by (Dada and Ikuerowo, 2009) when studied the effect of ethanolic extract of G. cambogia in catfish brood stock. The proliferation WBC dose dependent manner in experimental group may be because of leucopoesis particularly lymphopoesis as response to immunity.

The MCV, MCH values were found to be decreased in most of the plant extract treated groups. MCHC has maintained a steady state in experimental group. The maintenance of constant level or increase of MCHC of Tilapia fish fed on Amaranthus cruentus plant powder may be attributed to the improvement of fish health as suggested by (Suresh and Amolkumar, 2009).

From the present study it was concluded that 5% Amaranthus cruentus followed by 10% Amaranthus cruentus showed significant increase in growth, Haematology and biochemical parameters of Tilapia fish Oreochromis mossambicus. Thus it can be used as a fish feed formulations.

5. SUMMARY AND CONCLUSION

The detail of the study is summarized below:

- The fish Oreochromis mossambicus were collected from Aliyar dam near Pollachi and they were acclimatized to fresh water condition for 2–3weeks in the laboratory and transferred to experimental tanks.
- Aerators were used to raise the dissolved oxygen level and the tanks were maintained neat and clean.
- During acclimation period fishes were fed initially for a week with control feed.
- Excess of food and faecal matter were cleaned daily in order to prevent fungal and other microbial growth
- The selected plants (Moringa oleifera and Amaranthus crutenus) were collected, washed, shade dried and powdered using electric pulverizer.
- The feed was prepared by using basal ingredients like rice bran, coconut oil cake, chicken waste and corn flour as a binder.
- The basal ingredients were mixed using water and made into dough, steamed, cooled and selected plant powders were mixed and made in to noodles using domestic appliances in desired concentrations.
- The feed was dried and stored in refrigerator.
- The fishes were fed with these supplemented feed for 120 days and the results were obtained for every15days once for growth parameter.
- The haematological parameters were done on the end of the experimental day (120DAT).
- The biochemical parameters on the muscle tissue of fishes were done on initial and final day of the experiment.
- The data were subjected to one way ANOVA and the means were separated Using least significant differences (LSD).
- From the 15th day after treatment (DAT) to 120th day after treatment (DAT) the Fishes fed with 5% Moringa oleifera supplemented feed showed maximum increase in length.
- From the 15th day after treatment (DAT) to 120th day after treatment (DAT) the Fishes fed with 5% Amaranthus crutenus supplemented feed showed maximum increase in weight, specific growth rate, total feed consumed, condition factor, survival rate and feed conversion ratio.
- At the end of the experiment haematological parameters on RBC,WBC,HB, Platelets, PCV, MCV, MCH and MCHC was found to be maximum in 5% Amaranthus crutenus supplemented fishes.
- The biochemical parameter on protein, carbohydrate, lipid and amino acid was found to be maximum in 5% Amaranthus crutenus supplemented fishes.
- The above result indicates that there is possibilities of using selected plant powders supplemented feed for Oreochromis mossambicus.

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