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Original Article

Morphometric and gut microbial evaluation of Tilapia (*Oreochromis niloticus*) fed on different levels of *Moringa oleifera*

Avaliação morfométrica e microbiana intestinal de tilápia (*Oreochromis niloticus*) alimentada com diferentes níveis de *Moringa oleifera*

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Abstract

In current study, different feeding levels of *Moringa oleifera* formulated diet was compared to analyze the growth performance, feed conversion ratio, feed conversion efficiency and gut microbiology of *Oreochromis niloticus*. The study was comprised of four treatment groups including 4%, 8% and 12% *Moringa oleifera* and one control group which was devoid of *Moringa* leaves. The experimental trial was conducted at the Zoology laboratory of Pakistan Institute of Applied and Social Sciences, (PIASS) Kasur. The physicochemical parameters of water such as temperature, dissolve oxygen, pH, total dissolved solids and salinity in all aquaria were found non-significantly different from each other. In control condition T1, the average weight gain was 14.89±16.90a grams, while average length gain was 11.52±7.444a cm. However, the total viable count on Eosin methylene blue was 7.4×10^7, 5.8×10^7 on Tryptic soy agar and 5.8×10^7on Nutrient agar. In T2, the average weight gain was 16.22±16.09b grams and average length gain was 12.97±7.79b cm. The total viable count on Eosin methylene blue was 7×10^7, 5.5×10^7 on Tryptic soy agar and 5.8×10^7on Nutrient agar. In T3, the average weight gain was 37.88±27.43c grams, while the average length gain was recorded as 16.48±12.56c cm. However, the total viable count for treatment 3 was 6.4×10 on Eosin methylene blue, 4.8×10^7 on Tryptic soy agar and 5.2×10^7on Nutrient agar. In T4, the average weight gain was 44.22±31.67d grams, while the average length gain was 15.25±10.49d cm. The total viable count was 4.3×10^7on Eosin methylene blue, 3.1×10^7 on Tryptic soy agar and 3.8×10^7 on Nutrient agar. The effect of *Moringa oleifera* on the growth of *Oreochromis niloticus* was found to be significant and 12% *Moringa* extract showed maximum length and weight gain and minimum feed conversion ratio with the least microbial count in fish intestine.

Keywords: growth, microbiology, *Moringa oleifera,* Tilapia.

Resumo

No presente estudo, diferentes níveis de alimentação da dieta formulada com *Moringa oleifera* foram comparados para analisar o desempenho de crescimento, taxa de conversão alimentar, eficiência de conversão alimentar e microbiologia intestinal de *Oreochromis niloticus*. O estudo foi composto por quatro grupos de tratamento, incluindo 4%, 8% e 12% de *Moringa oleifera* e um grupo de controle sem folhas de *Moringa*. O ensaio experimental foi realizado no laboratório de Zoologia do Instituto de Ciências Aplicadas e Sociais do Paquistão (PIASS), Kasur. Os parâmetros físico-químicos da água como temperatura, oxigênio dissolvido, pH, sólidos totais dissolvidos e salinidade em todos os aquários foram encontrados não significativamente diferentes entre si. Na condição controle T1, o ganho médio de peso foi de 14,89±16,90a gramas, enquanto o ganho médio de comprimento foi de 11,52±7,444a cm. No entanto, a contagem total viável em azul de metileno de eosina foi de 7,4×107, 5,8×107 em ágar de soja Tryptic e 5,8×107 em ágar Nutriente. Em T2, o ganho médio de peso foi de 16,22±16,09b gramas e o ganho médio de comprimento foi de 12,97±7,79b cm. A contagem total viável em azul de metileno de eosina foi 7×107, 5,5×107 em ágar de soja Tryptic

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e 5,8×107 em ágar Nutriente. Em T3, o ganho médio de peso foi de 37,88±27,43c gramas, enquanto o ganho médio de comprimento foi registrado como 16,48±12,56c cm. No entanto, a contagem total viável para o tratamento 3 foi de 6,4×10 em azul de metileno de eosina, 4,8×107 em ágar soja Tryptic e 5,2×107 em ágar Nutriente. Em T4, o ganho médio de peso foi de 44,22±31,67d gramas, enquanto o ganho médio de comprimento foi de 15,25±10,49d cm. A contagem total viável foi de 4,3×107 em Eosin metileno blue, 3,1×107 em Tryptic soy agar e 3,8×107 em Nutrient agar. O efeito da *Moringa oleifera* no crescimento de *Oreochromis niloticus* foi significativo e o extrato de *Moringa* a 12% apresentou ganho máximo de comprimento e peso e conversão alimentar mínima com a menor contagem microbiana no intestino dos peixes.

Palavras-chave: crescimento, microbiologia, *Moringa oleifera*, *Oreochromis niloticus*.

1. Introduction

Moringa oleifera is a member of the Moringaceae family. It is a tropical and subtropical plant that is native to Pakistan, Africa, India, Saudi Arabia, the sub-Himalayan area and Asia (Mughal et al., 2019). *Moringa* leaves are utilized for a variety of industrial and medical applications (Yuangsoi and Masumoto, 2012). In aquaculture, *Moringa* has appeared as a viable alternative plant protein source having a protein content around 260 g/kg. Furthermore, methionine, cysteine, tryptophan and lysine are among the important amino acids found in leaves (Ahmed et al., 2014). *M. oleifera* is an angiosperm plant with various useful effects depending on the plant parts and origin (Leone et al., 2015). The seeds of this plant have antibacterial properties against some bacterial diseases and used to purify water due to coagulation characteristics (Suarez et al., 2003). *M. oleifera* have different ethnobotanical prosperities such as diuretic nature, hepatoprotective, antiulcer effects, reduces cholesterol levels and is used to treat a variety of human health problems including malnutrition and cardiovascular disease (Luqman et al., 2012). The leaves of *M. oleifera* contain a high amount of crude protein, ranging from 25% to 32% (Soliva et al., 2005) and have antimicrobial properties (Usama et al., 2022).

The Nile tilapia (*Oreochromis niloticus*) is a surface-feeding omnivore fish of the Cichlidae family (Jahn, 1989). Tilapia is a common fish in the world of aquaculture. Tilapia can grow very fast and it can undertake a bigger mass within a very short period. It relies mainly on small invertebrates, algae, detritus, aquatic plants and planktons for its nutritional requirements (Diana et al., 1991). For the formation and preservation of food items, organic antioxidants and antimicrobial effects plant resources are suitable (Sadiq et al., 2017).

Plant extracts have lately been recommended for preserving the storage quality of frozen chopped and filleted fish products (Lugasi et al., 2007). The effective culturing of Tilapia may be exaggerated via frequencies of mass mortality due to the occurrence of bacterial infections (Ahmed and Abdalla, 2005). The most occurring bacterial infections in Tilapia are triggered by *Streptococcus* and *Pseudomonas* species (Miyazaki et al., 1984), *Edwardsiella* species (Kaige et al., 1986) and *Vibrio vulnificus* (Sakata and Hattori, 1988).

Antibiotic use is a widespread practice in aquaculture but it has also been complained about due to its amplified resistance against microbes and there are also chances of antibiotic accumulation in the fish tissues (Chevassus and Dorson, 1990). The medicinal industries are giving their best in the production of new and improved antibiotics and as a result, the microbial population is improving their metabolism and genetic makeups to become adapted to the anti-microbial drugs (Tenover, 2006). Therefore, the production of improved drugs against drug-resistant microbes is of major concern to the pharmaceutical community. For this purpose, plants, algae, and natural animal extracts should be considered. For a long time, traditional medicinal plants extract is being used for the treatment of infectious diseases. Recently, medicinal plants are being explored heavily for drug discovery and development (Cragg and Newman, 2005). The proximate analysis of the *Moringa* tree showed that it can be included in the fish feed formulation (Yuangsoi and Masumoto, 2012). Feed additives are palatable ingredients which are included in fish feed in minute quantities to reduce fish mortality and to increase feed consumption rate and growth efficiency (Dada, 2015). There are many studies on fish growth in the existing literature which were aimed to calculate the efficiency of plant-based feed additives including *Moringa* leaves (Afuang et al., 2003).

The emphasis of the current study was to analyze the effects of *Moringa* leaves on the growth and gut microflora of Tilapia. The aims of this study were the comparison of feeding levels by analyzing growth performance, Feed conversion ratio (FCR), Feed conversion efficiency (FCE), and microbiology of *O. niloticus* fed with *M. oleifera*, and to evaluate the digestibility of *M. oleifera* by *O. niloticus.* In addition, to assess the microbial count in the flesh of *O. niloticus* on the different growth media as Nutrient Agar (NA), Tryptic soy agar (TSA) and Eosin methylene blue (EMB).

2. Materials and Methods

2.1. Study area

The Experimental trial was performed at the Department of Zoology, Pakistan Institute of Applied and Social Sciences (PIASS) Kasur using glass aquariums with experimental species *O. niloticus* (Nile tilapia). The fish were bought from a commercial nursery pond.

2.2. Experimental design

A total number of sixty fish samples of different sizes from 20 to 30 grams were delivered in the laboratory. Then the samples were divided equally into four different treatment groups. Fish were fed for three months according to the formulated diets. The feeding was done at dawn and dusk with a relative amount of 3% of the bodyweight throughout the trial. There was one control group and three treatment groups. The control group was fed with a normal fish diet without *M. oleifera* and was indicated as treatment 1 (control) T1, whereas the remaining three groups were fed with different feed formulations.

2.3. Diet preparation

A supplemented feed with 30% crude protein (CP) level was set by 4%, 8% and 12% *Moringa* leaves inclusion having different feed ingredients as T2, T3 and T4 respectively (Table 1). This special feed formulation method was used as a trial to analyze the maximum growth, FCR, and gut microbiology of *O. niloticus.*

2.4. Fish measurement

Fish measurement refers to the measuring of length and weight. The fish weights in grams were recorded using an electric balance and for precise body length, a wooden measuring tray integrated with a millimeter-scale was used (Khalid and Naeem, 2017).

2.5. Gut microbial estimation

To determine the effect of *M. oliefira* on total viable count in the intestine of *O. niloticus,* the samples were taken from the aquaria. Fish were kept in sterilized polythene bags and brought to the microbiological laboratory. The colonies formed by bacteria were cultured by pouring and spreading 10 µl of the suspension from the respective dilution on the surface of the relevant solidified media with the help of a micropipette. Then the plates were kept in an incubator at 37°C after inoculation. The counting of bacterial colonies was done after 24- and 48-hours following incubation. During whole microbiological procedure dry, moist sterilization and sterilization by radiation were observed.

2.6. Total viable count

The pour plate method was used for the estimation of total viable counts. 1ml each of the relevant dilutions was transferred to sterile and triplicated petri plates. Then 15-20 ml of Tryptic soy agar was transferred onto the petri plates, which melted at about 45ºC and cooled. After the solidification, the inversion of the plates was performed to prevent the moisture condensation on the agar surface. All the dilutions of microbial cells were spread thoroughly and uniformly by using a glass rod on the solidified plates. The colonies of microbes on Nutrient agar and Eosin methylene blue (EMB) were counted after incubation by using the same method. The precautionary measures were taken to avoid the contamination. The total viable count per ml of sample was calculated by using the following Formula 1:

$$\text{Total} \cdot \text{ Viable} - \text{ Count} = \text{ Average number} \cdot \text{ of} - \text{colonies} \times \text{-Dilution} \cdot \text{ factor} \tag{1}$$

2.7. Cell morphology and staining characteristics

Among the various characteristics, cell morphology like shape (bacilli, spiral, filamentous) and arrangement of bacterial cells (chains and clusters) were examined under a compound microscope after staining. Gram staining was performed to classify either bacterium was gram-positive or gram-negative.

2.8. Statistical analysis

The obtained data were analyzed by using SPSS software (SPSS 19.0, IBM software, inc., Chicago, IL, USA). The parameters calculation was studied by using Analysis of Variance (ANOVA) and for comparing means, Duncan's Multiple Range Test (DMRT) was applied (Duncan, 1956).

3. Results

3.1. Physicochemical parameters

The physicochemical parameters of all aquariums were recorded on daily basis and analyzed by using one-way ANOVA. The water quality parameters were recorded for T1 (control), T2, T3 and T4 simultaneously. The temperature of T1, T2, T3 and T4 was not significantly different (P>0.05). It ranged from 21.2 to 23.5ºC with an average of 22.26 ± 0.93 for T1 (22.6 ±1.47), for T2 (21.96 ± 0.725) for T3 and (22.09 ± 0.866) for T4. Dissolved oxygen (DO) level of T1, T2, T3 and T4 was not significantly different (P>0.05). It ranged from 4.8 to 5.5 with an average of (5.62 ± 0.304) for T1, (4.97 ± 1.02) for T2, (5.27 ± 0.612) for T3 and (4.96 ± 0.547) for T4. The pH of all the treatment groups was not

Table 1. Fish feed ingredients with different percentages of *Moringa* leaves for different treatments.

Ingredients	Treatment 1 (Control)	Treatment 2	Treatment 3	Treatment 4
Vitamins	1	1	1	1
Molasses	3	3	3	3
Maize Glutton	15	15	15	15
Soybean Meal	15	15	15	15
Fish Meal	30	30	30	30
Rice Polish	40	40	40	40
Moringa Leaves	0	4	8	12

The ingredients used in fish feed were as upper described concentrations. All treatment groups were fed with 4%, 8% and 12% *Moringa* leaves. While, control diet was free from *Moringa* leaves.

significantly different (P>0.05). The average pH range of T1, T2, T3 and T4 was (7.7 ± 0.32), (7.9 ± 0.27), (7.6 ± 0.39) and (7.4 ± 0.37) respectively. The concentration of total dissolved solids was not significantly different (P>0.05), for all the treatment groups. The average TDS range of T1, T2, T3 and T4 was (1084.1 ± 332.068), (1599.1 ± 474.825), (1196.7 ± 311.49) and (1043.24 ± 267.11) respectively. The salinity of all the treatment groups was significantly not different (P<0.05).

3.2. Morphometric measurements

The morphometric analysis by mean values of final body weight, body weight gain, final body length, body length gain, feed conversion efficiency and feed conversion ratio of *O. niloticus* fed with graded levels of *M. oleifera* were recorded simultaneously and their results were analyzed by using one way ANOVA and their means were compared accordingly. The results indicated that after 14 weeks of feeding trial, a significant increase (P>0.05) in the growth of fish in all the treatments (T2, T3, T4).

In the control condition (T1), the fish feed was devoid of *Moringa* leaves and feed conversion efficiency and feed conversion ratio were decreased over time. In treatment 2, the fish were supplemented with 4% *Moringa* extract. The Feed conversion efficiency increases and the feed conversion ratio decreases with an increase in weight and length as compared to treatment 1. In treatment 3, the fish were supplemented with 8% *Moringa* extract, The Feed conversion efficiency increases and the feed conversion ratio decreases with an increase in weight and length as compared to treatment 2. In treatment 4, the fish were supplemented with 12% *Moringa* extract, the feed conversion efficiency increases and feed conversion ratio decreases with an increase in weight and length of fish as compared to treatment 1, 2 and 3. These results indicate overall significant differences in the morphometric parameters, feed conversion efficiency and feed conversion ratio with an increase in the concentration of *Moringa* extract supplemented feed as given in Table 2 and Figure 1.

3.3. Microbial estimation by total viable counts

Microbial estimation in the intestine of Tilapia from each treatment were studied and the results were compared with the treatment 1 (control group). The microbiota of the control group was different from all treatments. There was approximately 80% growth of bacteria in the intestines of fish under control group with maximum number of disease-causing bacteria as compared to the remaining treatments. During present study, *Escherichia coli, Pseudomonas aeruginosa and Salmonellae enteritidis* were recognized in large amounts in the control group while the remaining treatments with different levels of *Moringa* leaves contained a smaller number of disease-causing bacteria comparatively.

During treatment 1, the total viable count on Eosin methylene blue was 7.4×10^7, for T2, T3 and T4 it was recorded 7×10^7, 6.4×10^7 and 4.3×10^7 respectively. The total viable count on Tryptic soy broth in T1 (control), T2, T3 and T4 was recorded 5.8×10^7, 5.5×10^7, 4.8×10^7 and 3.1×10^7 respectively. While the total viable count on Nutrient agar for T1 (control), T2, T3 and T4 was recorded 5.8×10^7, 5.8×10^7, 5.2×10^7 and 3.8×10^7 respectively (Tables 3, 4).

4. Discussion

In the current study, the growth performance and feed efficiency were found non-significant (P>0.05) among all the treatment groups. As, the nutrient digestibility is reduced by the consumption of fiber contents of plant-based diets which ultimately results in growth depression. These results are agreed with Afuang et al. (2003) who described that the diets supplemented with methanol-extracted *Moringa* leaf in variable concentration remained unaffected for the growth of *O. niloticus*. Richter et al. (2003) observed that 10% use of raw *Moringa* leaf in the diets for Tilapia was effective for the growth performance. Ebtehal (2017) also found a significant increase in the growth rate of Tilapia, fed with 12% concentration of *Moringa* leaves as a growth promoter. With the increase in the concentration of *Moringa* leaf extract, there is a significant increase in the growth of fish (Figure 1).

Moreover, Ozovehe (2013) reported that the decrease in feed consumption results in depression in growth performance. Similar findings were observed for the growth rate in Tilapia when fish feed was supplemented with plant-based proteins (Afuang et al., 2003). The addition of *Moringa* leaf extract in the feed during the experimental trial resulted in the improvement of specific growth rate

Table 2. Statistical analysis of growth performance and feed utilization of Tilapia.

Parameters	Treatment 1 (Control)	Treatment 2	Treatment 3	Treatment 4	*P* value
Final body weight	24.49 ± 15.34a	28.73 ± 16.66b	39.34 ± 25.79c	44.69 ± 31.11d	0.001
Final body length	11.445 ± 7.56a	12.38 ± 8.12b	15.76 ± 12.87c	15.28 ± 8.89d	0.002
Body weight gain	14.89 ± 16.90a	16.22 ± 16.09b	37.88 ± 27.43c	44.22 ± 31.67d	0.000
Body length gain	11.52 ± 7.444a	12.97 ± 7.79b	16.48 ± 12.56c	15.25 ± 10.49d	0.002
FCE	0.44 ± 0.13a	0.84 ± 0.39b	0.83 ± 0.39b	0.96 ± 0.40d	0.157
FCR	2.87 ± 1.43a	1.52 ± 0.69b	1.89 ± 0.29c	1.87 ± 0.25d	0.094

F.C.E.= Feed conversion efficiency; F.C.R.= Feed conversion ratio; [a,b,c,d] the mean values with different case letters shows significantly different from each other (DMRT).

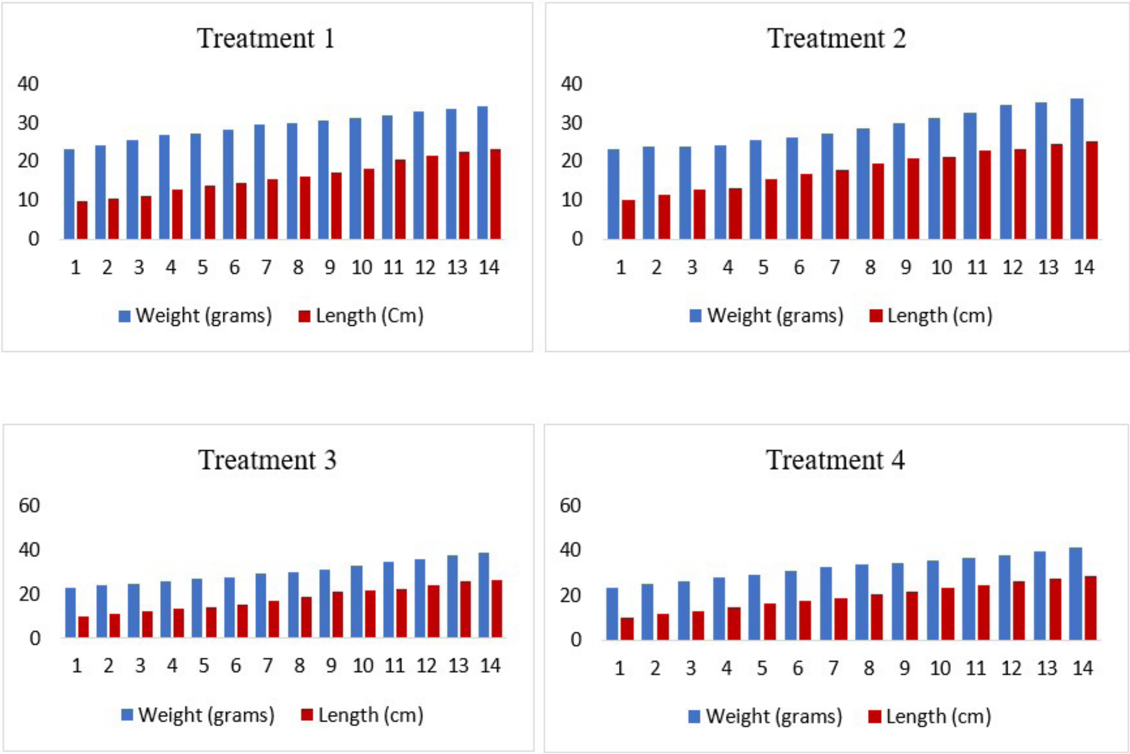

Figure 1. Average weight (grams) and length (cm) gain of T1-T4.

Table 3. Microbial load culture by using different stains.

Growth media	Microbial parameters	Treatment 1 (Control)	Treatment 2	Treatment 3	Treatment 4
Eosin methylene blue	No. of colonies	9	8	7	5
	Colony forming units	90000000	80000000	70000000	50000000
	Total viable count	7.4×10^7	7×10^7	6.4×10^7	4.3×10^7
Tryptic soy agar	No. of colonies	7	7	6	4
	Colony forming units	70000000	70000000	60000000	40000000
	Total viable count	5.8×10^7	5.5×10^7	4.8×10^7	3.1×10^7
Nutrient agar	No. of colonies	8	7	6	5
	Colony forming units	80000000	70000000	60000000	50000000
	Total viable count	5.8×10^7	5.8×10^7	5.2×10^7	3.8×10^7

including body weight and length (Figure 1). The present outcomes suggested that Tilapia behave as chemo reactive as well as olfactory oriented in the detection and selection of feed. According to present study, the concentration of feed attractants triggers the appetite level of Tilapia accordingly as noted by Abou-Zied (1998). He observed fish appetite level, a good measure for testing the effectiveness of feed attractants. The existing literature indicates that the potential of *Moringa* leaves has been widely observed for the growth performance of Tilapia and other fishes whereas the majority of results are promising towards its addition in supplemented fish diet. *Moringa* leaf has the potential to be replaced partially to other widely used diets without any growth depression. According to Richter et al. (2003), *Moringa* leaf extract has no adverse effects on the growth performance of Tilapia upto 12% replacement with fishmeal-based dietary protein which is in accordance to the current findings. The nutritional quality of *Moringa* leaf meal in Tilapia showed that 12% moringa extract in diets did not cause any adverse effect on growth performance. This feeding level shows maximum growth performance in terms of body length and weight. And it is noted that a

Table 4. Bacterial susceptibility in Tilapia at concentration of 20 mL/100µl.

Bacterial species	Treatment 1 (Control)	Treatment 2	Treatment 3	Treatment 4
Escherichia coli	1020	23	14	9
	787	56	13	3
	543	49	4	0
	545	45	3	0
	445	9	0	0
Pseudomonas aeruginosa	5500	8	11	7
	1050	3	7	6
	576	2	0	4
	454	1	0	0
	223	0	0	0
Salmonella enteritidis	4200	59	43	7
	3453	49	41	5
	723	29	24	1
	676	8	22	1
	589	0	9	0

severe growth depression was observed at 15% with higher inclusion levels of *Moringa* leaves in supplemented diet Yuangsoi and Masumoto, 2012). The current outcomes showed that the average growth for T1, T2, T3 and T4 was 0.92±.38, 0.77±.97, 1.48±1.79 and 1.31±.68 respectively.

The intestine of Tilapia was examined both quantitatively and qualitatively for the observation of bacterial flora of fish intestine. During present study, it was observed that by using different stains, the total viable counts of bacteria in fish intestines become decreased with increase in *Moringa* leaves in the fish supplemented diets. The presence of *Escherichia coli, Pseudomonas aeruginosa and Salmonellae enteritidis* in abundance in control group rather than other treatments shows that *Moringa* supplemented have antimicrobial activity (Usama et al., 2022). The inclusion of *Moringa* leaves in the fish feed repressed the growth of bacteria in the fish intestines. The present experiment highlights the total viable count in the intestine of the samples of *O. niloticus*. This was recorded to be 7.4×10^7 on EMB, 5.8×10^7on TSA and 5.8×10^7 on Nutrient agar for T1. The investigation on leaf extract *of M. oleifera* on certain pathogenic and orthopedic wounds shows its antimicrobial activities (Chuang et al., 2007; Mahajan et al., 2009). A recent study on *M. oleifera* extract displays antimicrobial activities against common poultry pathogens that includes *E. coli* and *C. perfringens* (Usama et al., 2022). These studies are in correlation with our results.

5. Conclusions

The present study was conducted with a focus on monitoring the effects of *M. olifera* on the growth and gut microbial load of Tilapia (*O. niloticus*). It was concluded that feeding supplemented *M. oleifera* has a significant effect on the growth of *O. niloticus*. The results indicated that 12% extract of *M. oleifera* (T4) contributed to maximum weight gain, length gain with minimum feed conversion ratio as compared to other treatment levels. The bacterial species (*Escherichia coli, Pseudomonas aeruginosa* and *Salmonellae enteritidis*) sampled from fish intestine were observed maximum in number in control group while gradually decreasing in number from T2, T3 and T4. It is found that *Moringa* leaves have antimicrobial activity that are responsible for better growth of fish.

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