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Supplement Effect of *Tribulus Terrestris* Seeds Extract in Feed of Nile Tilapia Fries *Oreochromis niloticus*, on Growth Performance and Feed Efficiencies

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Abstract
The current work was conducted to study the effect of Supplemented Seeds Extract of *Tribulus terrestris* in Feed on Growth Performance and Feed Efficiencies of Nile Tilapia Fries Oreochromis niloticus. The freeze-dried dietary extract of *Tribulus terrestris* seeds was incorporated into feeds for *O. niloticus* fry. Four experimental diets were prepared using a commercial feed containing 0.00 g/kg, 2.00 g/kg, 2.50 g/kg, and 3.00 g/kg of the extract. These diets were designated control feed, feed1, feed2, and feed3, respectively, and were tested in triplicate. Fries, averaging 0.012 ± 0.002 g, were randomly distributed into aquaria at a density of 180 fries per aquarium. The experimental feeds were provided to the fries immediately after they had completely absorbed their yolk sacs. The fries were fed four times a day (at 7:00 am, 11:00 am, 4:00 pm, and 7:00 pm) for 70 days. The experiment was conducted indoors to prevent the algal growth in the aquaria, and the water conditions were regularly monitored. Growth performance and feed efficiency parameters were calculated. The effects of the dietary treatments on different parameters were analyzed using one-way analysis of variance (ANOVA). Significant differences were found, and Duncan's multiple comparison test was used to determine mean differences. Statistical analysis was conducted using SPSS version 16.0, and data were presented as mean ± standard deviation (SD). The weight gain (WG) of the fish group fed feed3 (3.00 g/kg) was significantly the highest at 0.086 ± 0.024 g, and the highest absolute growth rate (AGR) was also registered for the same group. Conversely, the least growth performance (both WG and AGR) was observed in the fish group fed with feed2 (2.00 g/kg), although they exhibited the best feed conversion ratio (FCR). These findings indicate that incorporating the dietary extract of *T. terrestris* seeds into the feed improved the growth and feed efficiency of *O. niloticus* fry, with the improvements correlating with increased extract dosage. The extracted product is cost-effective and can potentially substitute other expensive fish additives used in fish feed formulation.

Keywords: *Tribulus terrestris*, seed extraction, *O. niloticus* fry feed, growth performance, feed efficiency.

Introduction

Medicinal herbs have garnered considerable attention for their ability to stimulate digestion, particularly in their effects on bile secretion and pancreatic enzyme activity. Essentially, olfactory feed ingredients serve as powerful feeding enhancers, encouraging fish to consume more feed than they typically would under normal conditions (Adams, 2005). As the aquaculture industry shifts away from reliance on synthetic drugs, medicinal herbs are gaining acceptance as viable alternatives to antibiotic growth promoters in fish farming (Adedeji *et al*., 2008). Among the array of phytoproducts available, the extract of the herb Tribulus

terrestris has emerged as one of the most prominent. Rich in furostanol-type saponins, particularly the additive known as protodioscin, this herb has captured the interest of researchers and aquaculturists alike. Recent studies involving various farm animals, including rats, pigs, cocks, rabbits, and fish, have documented the remarkable benefits of *Tribulus terrestris* extract on reproductive parameters as sperm quality, sexual behaviour, and fertilization ability. Despite these promising findings, there remains a notable scarcity of literature addressing the influence of *Tribulus terrestris* extract on the growth performance and feed efficiency of fish, especially when compared with the conventional use of antibiotics and synthetic chemicals. As such, the exploration of dietary supplements and ingredients that bolster fish health, enhance growth rates, improve feed efficiency, and ensure the production of safe, high-quality aquaculture products has become increasingly crucial. Over the past two decades, an upsurge in research has yielded conclusions affirming that Tribulus terrestris can indeed serve as a suitable alternative to conventional antibiotics, chemotherapeutics, and synthetic hormones in aquaculture (El-Sayed *et al*., 2004). Furthermore, the integration of plant-based additives into fish diets is an established strategy for improving weight gain and feed efficiency in cultivated fish species, improving the quality of fish seed, fry, and fingerling production. However, studies specifically focusing on the effects of *Tribulus terrestris* seed extract on fish performance and feed utilization during the early growth stages of fish are remarkably limited. Consequently, there is a pressing need for information on the performance and feed utilization of Nile Tilapia, *O. niloticus.* The importance of the quality and artificial feeds, even for species such as omnivorous tilapia, makes perfect sense at critical stages (juveniles or brood stock) when fish are maintained under intensive clear-water farming conditions and dependent entirely on nutritionally complete diets (Tacon, 1988). Global research for the identification of cost-effective substitutes to conventional materials continues (Ayoola, 2010; Elsayed, 2004; El-Sayed and Tacon, 1997; Hasan *et al*., 2007; Karalazos, 2007; Obirikorang *et al*., 2015). A wide range of feed additives is available to improve fish growth performance. Some additives used in feed mills are chemical products, especially hormones and antibiotics, which may cause adverse side effects. The use of antibiotic growth promoters (AGPs) as feed additives in the aquaculture industry has been criticized by government policies and consumers because of possible development of microbial resistance to these products and their potential harmful effects on human health (Baruah *et al*., 2008). The present study aims to evaluate the effects of varying supplementation levels of *Tribulus terrestris* on the growth performance and feed efficiency of *O. niloticus* fry. However, the specific objectives of this research were twofold: first, to prepare fish feed incorporating different percentages of *Tribulus terrestris* seed extract, and evaluate the effects of these inclusion levels on the growth performance and feed efficiency of O. niloticus fry.

Objective of this study is to determine the effect of Supplemented Seeds Extract of *Tribulus terrestris* in Feed on Growth Performance and Feed Efficiencies of Nile Tilapia Fries Oreochromis niloticus.

Materials and Methods

Experimental Sites

The extraction of *Tribulus terrestris* was done in the Institution of Aromatic and Medicinal plants (IAMP) belongs to the Ministry of Agriculture and Animal Resources, Khartoum, Sudan. The experiment on growth performance was done in the Department of Fisheries Science Hatchery, College of Natural Resources and Environmental studies, University of Bahri, Khartoum, Sudan.

Materials

Chemicals and Apparatus Used

Solutions: Ethanol, Formalin were used in the extraction method of *Tribulus trrestris*, and nitrogen gas were used in the process of extraction. Freeze-dryers were used to dry the liquid extract of Tribulus terrestris seeds. Aquaria were used for the acclimatization of hatched O. niloticus eggs and fry. Dragonstar grinder (Development Industrial Ltd, Hong Kong, China), rotary evaporator (Scientific Industrial and Trade Ltd, Greatwall, China), Incubator (Nanchang Huibing Electronics Co., Ltd., China) Sensitive Balance (Super Accomplish Health Technical Co. Ltd, China), centrifuge (Changsha Yingtai Instrument Co., Ltd., China) Glass wares, Refrigerator and Petri dish using in extraction method, oxygen meter, pH meter, ammonia test kits, thermometer and where apparatus were used during growth experiment. Freeze dryer (Changzhou Yang Chuan Precision Machinery Co., Ltd., China) was used to dry the extracted substance.

Tribulus Trrestris Seed Collection and Preparation

The wild herbal plant *Tribulus terrestris* seeds were collected from Alkadro area, North East University of Bahri campus, and were washed by distilled water, air dried under an open-air shaded area, and then ground by Dragonstar grinder. The finely ground seed powder was kept in a clean, dry box for extraction

Extraction Method of Tribulus Terrestris

The finely ground seed powder sample was extracted in the Institution of Aromatic and Medicinal Plants (IAMP) at Khartoum Bahri. Two hundred fifty grams of sub-sample was soaked in alcohol (ethanol, 70%) for five days, daily filtration and evaporation using (Soxhlet apparatus was performed. The extracted substance was freeze-dried (Image 1)

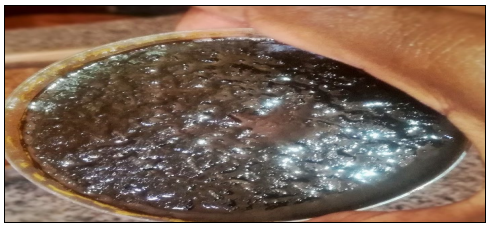


Image 1: The resultant extract of *Tribulus terrestris* seeds

Experimental Feeds Preparation Using Tilapia Commercial Feed

A local commercial tilapia feed (powdered basal) purchased from Altkhasosi fish feeds Factor in Khartoum Bahri, with a proximate chemical composition as shown in Table 1 below, was used. Four experimental feeds were prepared containing different levels of Tribulus terrestris seed extract. Four feeds were prepared to include 0.00g/kg, 2.00g/kg, 2.50g/kg, and 3.00g/kg of the *Tribulus terrestris* seed extract, which were referred to as control, feed1, feed2, and feed3, respectively. Two kg of each feed were prepared. Using Guerrero (1975), the extracts were dissolved in 100 ml of ethanol 95% and added to the feed in spray form, then left for 24 hours to dry in an open shade place (Image 2).

Table 1: Ingredients and proximate composition of the commercial feed (in dry powdered form) used in preparation of the four experimental feeds

Ingredient	%
Fish meal	18.00
Soy bean meal	20.52
Wheat bran	25.70
Yellow corn	25.68
Bean nut oil	4.00
Mineral premix	2.50
Vitamin premix	2.50
Chemical composition	
Moisture	2,39
Crude Protein	37.16
Crude Fat	13,11
Crude Fiber	4,76
Ash	12,03
NFE	3055
Total	100

Experimental Fish
A number of 4000 fertilized eggs of Nile tilapia, *O. niloticus* were brought from Arab organization for Agriculture development (AOAD). The fertilized eggs were brought to the hatchery and artificially allowed to hatch in a three-liter incubator. The hatched fries were fed with the commercial feed after total absorption of yolk sac and when fries start search for feed. Fry fish were acclimatized for one week prior starting the experiment.

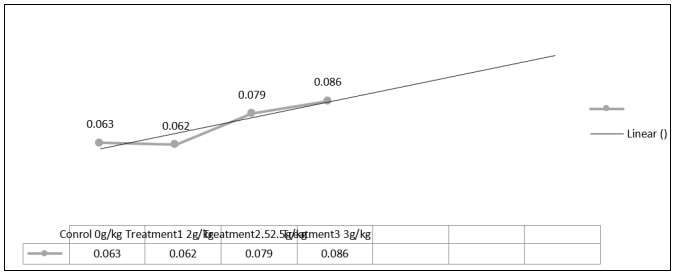
Experimental Design
A completely randomizing design was used to conduct the experiment. One hundred eighty *O. niloticus* fry with average body weight of 0.012g were randomly distributed for each of the twelve plastic Aquaria (R=3) and fed with experimental feeds of different dosage of 0.00g/kg, 2.00g/kg, 2.50g/kg and 3.00g/kg of the extract. 2700 fries of and total length 10mm equally distributed in 12 aquaria (180 fries) in each aquarium (41x32x30cm) with 26 cm water column feed with experimental feed for 70 days.

Water Mentoring
The water quality parameters such dissolved oxygen, pH, ammonia concentration and water temperature were regularly mentored throughout the experimental period using oxygen meter, pH meter, ammonia test kid and thermometer respectively.

Growth Performance and Feed Conversion Ratio Determination
The growth performance was evaluated by applying the following parameters: the Weight Gain determination of fish was calculated by formula: (WG) = 100 × [(final fish weight – initial fish weight)/initial fish weight]. Absolute growth rate determination was calculated by formula: (AGR) =100× [(final fish weight-initial fish weight/period of time. Survival Rate determination was calculated by formula (SR) = [(total number of fish at the end of trial/total number of fish at the start of trial) × 100]. The feed conversion ratio was calculated by formula (FCR) = feed intake/ weight gain.

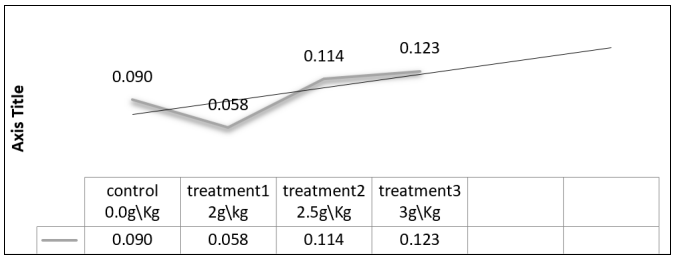
Statistical Analysis
The data were subjected to statistical analysis including analysis of variance (one-way ANOVA) using 17.0 (SPSS Inc.,) software program. The levels of significance between the means of groups were assessed using Tukey's honest significance difference (HSD) and the significance level was assessed at $P < 0.05$. The data were presented as mean± standard deviation (mean ± S.D, n =3). The graphs were plotted using simple excel software of Microsoft office 2010 version.

Results
Growth Performance Determination
Graph 4.1 below showed total weight gain of fish. The results shows significant difference ($p \leq 0.05$). The best weight gain (0.086 ± 0.024^{a}) was recorded for experimental fish group fed diets with 3g/Kg, and (0.079 ± 0.010^{b}) experimental fish group fed diets with 2.5g\kg of *Tribulus terrestris* extract, while there are no significant difference in experimental fish group fed diets with treatment (2g/Kg), (0.062 ± 0.008^{c}) compering with control groups 0.0g/Kg, ($0.063 \pm 0.006^{c)}$ ^{of} *Tribulus terrestris* extract.



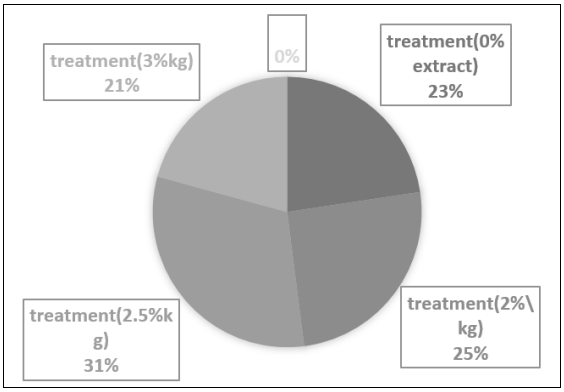
Graph 1: Total weight gain for the four groups of the experimental *Nile tilapia* fries with different dosage of *Tribulus terrestris* extract (0.00 g/kg, 2.00g/kg, 2.50g/kg and 3.00g/kg)

Absolute Growth Rate of Fish Fries
Graph 4.2 below, Absolute growth rate result. shows significant difference ($p<0.05$) in the (AGR) (0.123 ± 0.034^{a}) was recorded in experimental fish group fed with 3.00g/Kg and (0.114 ± 0.014^{b}) of experimental fish groups fed diets with 2.50g/Kg, *Tribulus terrestris* extract, there are no significant difference in experimental fish group fed diets with treatment (2.00g/Kg), (0.058 ± 0.041^{c}) compering with control groups (0.00g/Kg). (0.090±0.023) *of Tribulus terrestris* extract.



Graph 2: The absolute growth rate for the four groups of the experimental Nile tilapia fries with different dosage of *Tribulus terrestris* extract (0.00 g/kg, 2.00g/kg, 2.50g/kg and 3.00g/kg).

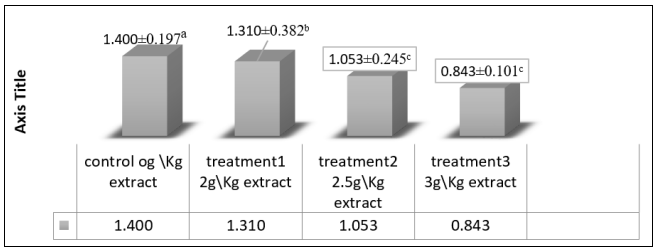
Survival Rate
The Highest survival rate percentage was recorded for fish group fed with feed2 (2.50g\kg) with (43.13 ± 6.31^{a}), while lowest survival rate was noticed for fish group treatment3 (3.00g\kg) with (28.67 ± 8.32^{c}). The control fish group which fed with 0.00g\kg with and treatment1 (2.00g\kg) are given (31.23 ± 3.37^{c}) and (34.97 ± 6.2^{b}), respectively. Graph 3, below shows the survival rate for the four *O. niloticus* fry groups fed with different dosage of *Tribulus terrestris* extracts (0.00 g/kg, 2.00g/kg, 2.50g/kg and 3.00g/kg).



Graph 3: The survival rate for the four groups of *O. niloticus* fries fed diets with different concentration of *Tribulus terrestris* extract (0.00g/kg, 2.00g/kg, 2.50g/kg and 3.00g/kg).

Feed Conversion Ratio by Fish Fries

The highest feed conversion ratio among the four experimental *O. niloticus* fry's groups was recorded for fry group treatment1 2g/k (4.02±0.37) and lowest FCR in treatment3 3g/k (3.06± 0.98) while the result of treatment 2 and control.



Graph 4: The feed conversion ratio for the four groups of the experimental *O. niloticus* fries fed diets with different concentration of *Tribulus terrestris* extract (0 g/kg, 2g/kg, 2.5g/kg and 3g/kg).

Discussion

Growth Performance of *O. Niloticus* Fry

The obtained results, showed the positive adding effects of the dietary Tribulus terrestris extract on performance and efficiency of fry feeding when compared with the control feed (0.063±0.006c). The treatment3 which was recorded giving the highest performance and efficiency as shown in Graphs 1, 2 and 4, compared to feed treatment2 and treatment1 (0.079±0.010b) is higher compering with control (Treatment1 and treatment2 is significantly different (0.05) effect compared with the control. The performance and feed conversion ratio of *O. niloticus* fry group fed with treatment1 were insignificantly different when the control compering with control group of *O. niloticus* fry. From the obtained results, there was positive increase in fry weight gain with increased in the Tribulus terrestris extract inclusion levels, when the inclusion level was 2.50g/kg of the extract. In other words, the 0.20g/kg of the extract showed insignificant difference on the total weight gain of fry compared the control fry group. These results agree with Gültepe *et al*. (2014) on effects of dietary extract supplementation on growth, feed utilization, hematological, immunological and biochemical variables of *O. niloticus*, used 40% Saponins. Similarly, the absolute growth rate of O. niloticus fry was confirm to direction of the positive effect of *Tribulus terrestris* seed extract when its level increased in fry feed up to 2.5g/kg in fry feed (Graph 2). This finding agrees with that reported by Gültepe. *et al*. (2014) on effects of dietary *Tribulus terrestris* extract on growth performance of and feed utilization for fingerling of *O. niloticu*s weighed 2.61±0.35g and there is no significance difference in treatmen1 (0.062±0.008c) and disagree in general factors of treatment, dosage uses and fish size. The obtained results of survival rates were significantly different (0.05) among the four groups of *O. niloticus* fry fed with different levels of *Tribulus terrestris* extract, which can be referred to interruption of electricity power during the experiment period specially at the second and tenth weeks of the experiment. However, the highest mortality was recorded to fry group fed with 3.00g/kg inclusion level of the *Tribulus terrestris* extract (Graph 3). In general, high mortality was recorded among fry groups during throughout the experiment time.

Feed Conversion Ratio of Fish Fries

Significant differences were found among the *O. niloticus* groups fed with the different levels of the *Tribulus terrestris* extract (Graph 4). It was observed that the improve in feed conversation ratio of *O. niloticus* fry groups was parallel with increase of extract levels on fry feeds. Similarly, the same finding was reported by Sakineh *et al*. (2017) in their study on effects extract on growth and reproductive performance of male convict cichlid, *Cichlasoma nigrofasciatum*.

Conclusion

Dietary inclusion of low cost *Tribulus terrestris* extract fed to *O. niloticus* fry produced consistent benefits in growth, feed efficiency, and can be a practical, cost effective strategy for small scale and commercial hatcheries when properly formulated and dosed.

Acknowledgment

The authors thanks forwarded to the Deputy Administrator of Tilapia Fish farm in Elshejera locality, Khartoum/Sudan belongs to Arab organization for Agriculture development (AOAD) for providing the fertilized eggs of Nile tilapia, *O. niloticus*.

Ethical Approval and Consent to Publish

This research is original and conducted by the authors.

Recommendation

According to the findings; the study recommended that; more research are needed to study deeply the effect of Supplemented Seeds Extract of *Tribulus terrestris* in Feed on Growth Performance and Feed Efficiencies of Nile Tilapia Fries Oreochromis niloticus and to increase the Extract of *Tribulus terrestris* in Feed in order to check the additional impacts on Growth Performance and Feed Efficiencies of Nile Tilapia Fries Oreochromis niloticus.

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