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The addition of moringa flour (*Moringa oleifera*) to the diet of nile tilapia (*Oreochromis niloticus*) and its effect on productive parameters

La adición de harina de moringa (*Moringa oleifera*) a la dieta de la tilapia del nilo (*Oreochromis niloticus*) y su efecto sobre los parámetros productivos

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RESUMEN

Debido a la importancia que tiene el encontrar fuentes de proteína sostenibles, que puedan reemplazar parcial o totalmente a la harina de pescado, varios estudios han propuesto la adición de harina de *Moringa oleifera* en dietas de *Oreochromis niloticus*. El objetivo del presente estudio fue evaluar la adición de cuatro porcentajes diferentes de harina de *M. oleifera* en dietas de *O.niloticus* sobre parámetros productivos. La calidad del agua se monitoreó diariamente y la biometría se realizó cada 15 días. Se formularon cuatro dietas isoproteicas que incluían 30% de proteína total e isoenergética (3900 kcal / kg). La harina de moringa se incluyó en porcentajes de 0%, 10%, 15% y 20% (tratamientos T0, T10, T15 y T20, respectivamente). El desarrollo del crecimiento se evaluó durante 60 días con base en: tasa de crecimiento específico (SGR), ganancia de peso (WG), factor de condición de Fulton (FC), tasa de crecimiento absoluto (AGR), tasa de conversión alimenticia (FCR) y tasa de supervivencia (SR). Se observaron diferencias significativas ($p < 0,05$) entre T0 y T15 con los tratamientos T30 y T45 a los 30 días, mostrando los dos primeros los valores más altos. A partir de los 60 días, hubo una relación decreciente entre el nivel de inclusión y el peso total. Hubo una relación inversa entre la inclusión de harina de *M. oleifera* y los principales parámetros de crecimiento de *O. niloticus*, mientras que no hubo diferencias en cuanto a SR y FCR.

Palabras clave: acuicultura; alevines; crecimiento; nutrición; moringa.

ABSTRACT

Due to the importance of finding sustainable protein sources, that could partially or totally replace fish meal, several studies have demonstrated the potential of *Moringa oleifera* in *Oreochromis niloticus* diets. The aim of the present study was to evaluate the addition of four different percentages of *M. oleifera* flour in *O. niloticus* diets on productive parameters. Water quality was monitored daily, and biometrics were carried out every 15 days. Four isoproteic diets were formulated that included 30% total protein, and isoenergetic (3900 kcal/kg). Moringa flour was included at percentages of 0%, 10%,15% and 20% (T0, T10, T15 and T20 treatments, respectively). Growth development was evaluated for 60 days based on: specific growth rate (SGR), weight gain (WG), Fulton's condition factor (FC), absolute growth rate (AGR), food conversion rate (FCR) and survival rate (SR). Significant differences ($p < 0.05$) were observed between T0 and T15 with T30 and T45 treatments at 30 days, the first two showing the highest values. From 60 days onwards, there was a decreasing relationship between the inclusion level and the total weight. There was an inverse relationship between the inclusion of *M. oleifera* flour and the main growth parameters of *O. niloticus*, while there were no differences in terms of SR and FCR.

Keywords: aquaculture; fingerlings; growth; nutrition; moringa.

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1. Introduction

The diet of fish must have an adequate balance of nutrients, mainly protein, to achieve optimal growth and development of their metabolism (Ogunji, 2004). The main component of diets formulated for this purpose is fishmeal, because of its high nutritional benefits in macronutrients, vitamins, and minerals (Auchterlonie, 2018). However, the cost of fishmeal production has increased over time considering the fluctuation in current anchovy populations and high demand and that production has not increased in recent years, affecting both the quality and the availability of fishmeal (FAO, 2018; Naylor et al., 2009). This fact makes the search for other alternative and sustainable protein sources necessary. Therefore, looking for the replacement of fishmeal by plant-based protein such as soybeans, cottonseed meal, sunflower seeds, sesame, and others is ongoing (Ogunji, 2004). However, since some of these supplies are also in demand for direct human consumption and do not have the needed nutritional profile, it has not been possible to completely replace fishmeal (Martinez-Palacios et al., 1999) and the search for a plant source that meets the requirements of good palatability and digestibility, few antinutritional factors and that is both economical and sustainable, continues (Naylor et al., 2009).

Moringa oleifera (Moringaceae) is an oleaginous plant that has been considered as a possible substitute for fishmeal. This vegetable protein source has crude protein levels of close to 30%, a low amount of anti-nutritional compounds and a high vitamin and mineral profile (Alegbeleye, 2018), in addition to presenting rapid growth and adaptability to tropical and subtropical environments, with few cultivation requirements.

Several studies have demonstrated the potential of *M. oleífera* to improve the productive parameters of *O. niloticus* (Abd El-Gawad et al., 2019; Djissou et al., 2019; Shourbela et al., 2019; Billah et al., 2020; El-Kassas et al., 2020), antioxidant activity (Ahmed et al., 2020) and their autoimmune response to pathogenic and toxicological agents (Elabd et al., 2019; Ahmed et al., 2019; Ibrahim et al., 2019; Monir et al., 2020; Soror et al., 2021). However, there are differences in the results obtained due to the different quantities of *M. oleifera* in the diets, length of diet exposure, type of product processing and initial stages of *O. niloticus* growth in the different studies. Nonetheless, given the encouraging results reported in several studies, further studies are needed to determine the effects of these different factors in *O. niloticus* production.

Therefore, the aim of the present study was to evaluate the effects of the inclusion of four different amounts of *M. oleifera* flour on the production of *O. niloticus*.

2. Materials and methods

Experimental design

Nile tilapia (*O. niloticus*) were purchased from the FONDEPES Piura Aquaculture Center and then transferred to the Experimental Larviculture Laboratory, where they went through an acclimatization period of one week in a 500 m^3 tank. Subsequently, 180 fish were randomly selected and placed in 12 fish tanks (45x30x30 cm) with an effective capacity of 40 liters, in which they randomly received one of the four treatments with three replicates including 15 fish (1 ± 0.5 g) per aquarium. The study was carried out in separate aquarium systems, maintaining constant aeration throughout the experimental phase. Prior to feeding, the fish tanks were cleaned daily, removing feces, and replacing the water by 15% dechlorinated water. Water quality parameters including temperature (°C), pH and dissolved oxygen (mg L^{-1}) were monitored daily, while total ammonia nitrogen (mg L^{-1}) and nitrites (mg L^{-1}) were measured weekly. An initial feeding rate of 10% was used, and then adjusted every biometric date, ending with a rate of 5%. The food was supplied six days a week, in four daily rations. Biometric data collection was carried out every 15 days, with the fish having undergone 24 h fasting. Following the biometrics assessment, total water exchange was carried out.

Table 1
Formulation and composition of ingredients of experimental diets (0, 10, 15 and 20%) on dry matter basis

Ingredients	T0	T10	T15	T20
Fishmeal flour	21.6	19.0	18.0	17.0
Soybean flour	20.0	20.0	18.6	19.4
Moringa flour	0.0	10.0	15.0	20.0
Corn gluten	11.5	12.5	13.3	14.5
Corn flour	21.8	23.4	20.0	14.7
Wheat flour	20.0	10.0	10.0	9.5
L-Lisina	0.5	0.5	0.5	0.5
DL-Methionine	0.4	0.4	0.4	0.4
Soybean oil	1.8	1.8	1.8	1.8
Dicalcium phosphate	0.7	0.7	0.7	0.7
Vitamin C	0.5	0.5	0.5	0.5
Salt	1.0	1.0	1.0	1.0
Premixed vitamins	0.8	0.8	0.8	0.8

Diet formulation

The *M. oleifera* leaf meal used for diet formulation was purchased from Moringa Jochegu E.I.R. and was added to the experimental diets as shown in Table 1.

Four isoproteic diets were formulated at 30% of total dietary protein, and isoenergetic intake (3900 kcal kg^{-1}). Moringa leaf meal was included at percentages of 0%, 10%, 15% and 20%, with treatments T0, T10, T15 and T20, respectively, to achieve a similar composition among the diets (Table 2).

Table 2
Composition of the diet per treatment on dry matter basis (%DM)

Composition (%DM)	T0	T10	T15	T20
Protein	29.95	29.62	29.72	29.37
Lipids	4.34	4.32	4.42	4.51
Ash	8.58	9.63	9.82	10.25
Fiber	3.25	3.59	4.11	3.77
Energy (kcal/ kg)	3906	3907	3915	3909

Evaluation of productive parameters

Growth development was evaluated over 60 days based on the following parameters: absolute growth rate (g day^{-1}) (AGR), specific growth rate (% day^{-1}) (SGR), weight gain (%) (WG), Fulton's condition factor (CF), food conversion rate (FCR), and survival rate (SR).

AGR (g day^{-1}) = (Wf-Wi/Tf-Ti)* 100 (1)
SGR (% day^{-1}) = (LnWf-LnWi/Tf-Ti)*100 (2)
WG(%) = (Wf-Wi/Wi)*100 (3)
CF = (Weight/$Length^3$) *100 (4)
CFR = Amount of food supplied/weigh increase (5)
SR = (Final number fish/initial number fish)*100 (6)

Whereas Wi: Initial weight; Wf: Final weight; t2 to t1: time between Wf and Wi.

Statistical analysis

Data analysis was performed using the Minitab 19.0 program. The distribution of normality was assessed using the Shapiro-Wilk test. The Levene's test was carried out to determine normality and homogeneity, respectively, and ANOVA and post hoc Tukey were used to determine significant differences among treatments ($p < 0.05$). The values expressed as means ± standard deviation.

3. Results and discussion

Physico-chemical parameters

The parameters of water quality were within the recommended range for the development of this species (Table 3). Dissolved oxygen (mg L^{-1}), temperature (°C), pH, total ammonia nitrogen (TAN) and nitrite (NO_2-) values did not show significant differences along the experimental period. During the test, all water quality parameters were kept within the range appropriate for *O. niloticus* (Balarin & Hatton, 1979).

Productive parameters

The mean weight of the tilapia fingerlings was 1.15 ± 0.13 g with no significant differences among treatment groups. Significant differences ($p < 0.05$) were observed at 30 days, T0 and T15 with T30 and T45, with the first two treatments showing the highest growth values. At 45 days, there were still no significant differences between T0 and T15, while at 60 days there was a decreasing relationship between the level of moringa in the diet and total weight (Figure 1).

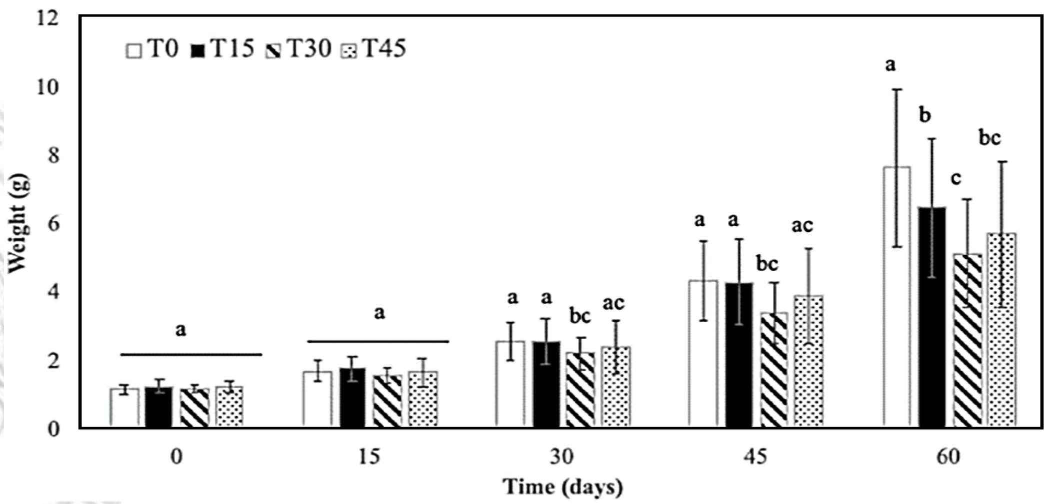

Figure 1. Mean values of total weight (g) in *Oreochromis niloticus* fed different amounts of *Moringa oleifera* flour over 60 days. The bars indicate the standard error. Bars with different letters indicate significant differences ($p < 0.05$).

Table 3
Average values and standard deviation of the physical-chemical parameters of the water in the experimental tanks, according to the treatment

Physico-chemical parameters	Experimental Diets			
	T0	T10	T15	T20
Temperature (°C)	29.45 ± 1.33	29.38 ± 1.05	28.90 ± 1.06	29.32 ± 1.06
Dissolved oxygen (mg L^{-1})	4.58 ± 0.40	4.56 ± 0.49	4.99 ± 0.57	4.50 ± 0.43
TAN (mg L^{-1})	0.25 ± 0.11	0.45 ± 0.18	0.64 ± 0.30	0.53 ± 0.32
Nitrites (mg L^{-1})	0.62 ± 0.26	0.62 ± 0.24	0.56 ± 0.25	0.63 ± 0.24
pH	7.84 ± 0.05	7.86 ± 0.00	7.84 ± 0.03	7.86 ± 0.05

* TAN: total ammonia nitrogen.

Table 4
Mean values and standard deviation of the productive parameters of *Oreochromis niloticus* for the four treatments over the 60-day study period

Productive parameters	Experimental diets			
	T0	T10	T15	T20
Initial weight (g)	1.15 ± 0.13[a]	1.26 ± 0.19[a]	1.20 ± 0.12[a]	1.25 ± 0.18[a]
Final weight (g)	7.63 ± 2.32[a]	6.47 ± 2.01[b]	5.14 ± 1.55[c]	5.69 ± 2.13[bc]
AGR (g day^{-1})	0.11 ± 0.00[a]	0.09 ± 0.00[b]	0.07 ± 0.00b[c]	0.07 ± 0.01[c]
SGR (% day^{-1})	3.15 ± 0.02 [a]	2.73 ± 0.10[b]	2.43 ± 0.03[c]	2.52 ± 0.10[c]
WG (%)	561.82 ± 8.56[a]	414.32 ± 29.92[b]	329.27 ± 7.67[c]	355.37 ± 26.12[c]
FC	1.55 ± 0.04[a]	1.55 ± 0.03[a]	1.54 ± 0.06[a]	1.55 ± 0.03[a]
FCR	0.96 ± 0.21[a]	0.97 ± 0.22[a]	0.83 ± 0.16[a]	0.90 ± 0.26[a]
SR (%)	97.7[a]	97.7[a]	97.7[a]	95.5[a]

* Different letters in the same column indicate differences in the level of $p < 0.05$.
AGR: Absolute growth rate (g day^{-1}), SGR: Specific growth rate (% day^{-1}), WG: Weight gain (%), CF: Condition factor, FCR: Feed conversion rate and TS: Survival rate (%).

There were significant differences in SGR, AGR and WG in *O. niloticus* fingerlings according to the percentage of *M. oleifera* flour included in the study diets (Table 4), with higher values in the T0 diet (0% inclusion) and with reductions in these values based on the percentage of *M. oleifera* added to the diet. There were no significant differences in CF, FCR and SR among the different treatments ($p < 0.05$). The results obtained show that after 30 days of feeding with a diet including more than 15% of *M. oleifera* flour, weight gain was lower compared to the diet without this flour. Furthermore, the higher the percentage of moringa added, the lower the growth parameters of AGR, SGR and WG. Similar results were found in the work of Djissou et al. (2019), regarding the inverse relationship between the level of inclusion of *M. oleifera* and the growth performance of *O. niloticus*. These authors found that the control diet (excluding *M. oleifera*) presented the best productive performance with a SGR of 2.57%. However, in the present study, a higher SGR was obtained with 3.15%, while the T20 (20% inclusion) presented similar results. This could be since these authors worked with specimens of 4.6 g on average, in which the SGR could be reduced compared to fish of 1.15 g, as in the present study. The time of exposure to the diet appears to be relevant to the effect of *M. oleifera* in tilapia growth. This was observed in the work of Billah et al. (2020), who found that on day 40 of the experiment there was a greater increase in weight of the tilapia fed with an inclusion level of 10% and 20% of moringa in the diet, compared to the commercial food without the inclusion of moringa, while at 60 days of experiment, the diet with 10% inclusion had better results than the commercial diet and that with 20% inclusion.

Elabd et al. (2019) found that the inclusion of 1.5% of *M. oleifera* flour in tilapia diets of 2 g, improved the productive performance and reduced stress. The mean SGR achieved in this study was 3.4% day^{-1} for this percentage of moringa, being higher than that found in the present study carried out over three months.

Abozaid et al. (2018) also found favorable results with the addition of 5% of *M. oleifera* flour, describing not only significant differences in performance in relation to the control diet, but also reducing costs thanks to the replacement of fishmeal.

These results are complemented by those of El-Kassas et al. (2020), who found that an addition of 5% and 10% of *M. oleifera* leaf meal generated increases in the length and width of the intestinal villi. This, as mentioned, probably explains the improvements in the productive parameters of the species.

Karina et al. (2015) reported favorable results in the SGR with the addition of 32% of *M. oleifera* flour, however, the specimens studied had initial mean weights ranging from 5 to 12 g. Thus, the SGR achieved was lower than that of the present study (1.45% day^{-1}), but the tilapias studied were larger.

Knowledge that there are no significant differences in *O. niloticus* production in relation to the replacement of fishmeal during the first 30 to 45 days of culture is encouraging since food management strategies could be established in this regard. These strategies can include not only a reduction in costs but can also promote sustainable aquaculture (Naylor et al., 2009) as well as increase immunocompetence and resistance to diseases. Indeed, the latter was demonstrated in a study by Abd El-Gawad et al. (2019) who found that tilapias fed with a 5% supplement of *M. oleifera* flour showed better immune response in phagocytic and lysozymatic activity when exposed to *Aeromonas hydrophila*. Furthermore, survival was 100% in fish receiving the supplement compared to 80% in those which did not. At the mentioned supplementation and an experimental period of two weeks, they found no significant differences in relation to growth.

Additionally, in the study by Shourbela et al. (2019) it was found that diets supplemented *with M. oleifera* flour reduced stress related to hypoxia, and the authors proposed its use as a growth, anti-stress and antioxidant promoter. However, this study used only the aqueous extract to obtain the bioactive principles. In addition, using 400 mg kg^{-1} of this extract, a significantly higher growth was achieved compared to the control treatment. Moringa flour has also been described as having a positive effect on the growth of *O. niloticus* when these fish were exposed to toxic agents such as chlorpyrifos, one of the most common insecticide compounds found in fresh water (Ibrahim et al., 2019). Further studies are needed to evaluate the most effective supplementation values and exposure time.

All the diets evaluated in the present study had a similar protein level in compliance with the 30% suggested for this species at this stage (Méndale et al., 2013), and therefore, the percentage of this macronutrient does not explain the differences between the productive growth parameters found in this study.

On the other hand, reduced production performance could be due to the lack of attractants capable of increasing intake (Espe et al., 2006) or the presence of anti-nutritional factors (Dias et al., 2005). In the present study, the FCR (parameter related to the food ingested) did not present significant differences, therefore, the first factor might not be as associated with production as the second. The latter is supported by the work of Richter et al. (2003), who suggested that the presence of phenols, tannins, saponins and phytic acid in the leaves of *M. oleífera* might affect the growth of *O. niloticus*, since these phytochemicals can inhibit digestive enzymes, making it impossible to take advantage of the proteins provided by this product (Francis et al., 2001).

However, production measures can be used to reduce the antinutritional effects of this type of product, such as heat treatment, germination and fermentation, taking into account that these should not affect the nutritional quality of the product if it is to be incorporated as a source of protein (Francis et al., 2001).

4. Conclusions

There is an inverse relationship between the inclusion of *M. oleifera* flour and the main growth parameters (AGR, SGR, WG) of *O. niloticus* production, while there are no differences in terms of the production parameters of CF, FCR and SR. Further studies are needed to determine the cost benefits of adding *M. oleifera* flour at 15% during the first 30 or 45 days of cultivation given that there were no implications for growth and this product could support the immune system of *O. niloticus*.

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