THE EFFECT OF FEEDING HEAT TREATED *MORINGA OLEIFERA (LAM*) LEAF MEAL ON THE GROWTH PERFORMANCE OF *OREOCHROMIS NILOTICUS* (*LAM*) FRY.

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ABSTRACT
In aquaculture the increasing price of feed is one of the most important factors that limit profitability. The high costs have led to the need to identify alternative protein sources for use in fish feed formulations. The utilization of non-conventional and lesser–utilized plant protein sources to replace fishmeal in fry diets has been an area of focus in aquaculture nutrition. The objective of the study was to determine the effects of feeding heat-treated moringa (Moringa oleifera) supplemented diets on the growth performance of the Oreochromis niloticus fry. Four iso-nitrogenous diets were used; 5 % boiled moringa and 95 % fry meal (Diet A); 10 % boiled moringa and 90 % fry meal (Diet B); 5 % steamed moringa and 95 % fry meal (Diet C) and 10 % steamed moringa and 90 % fry meal (Diet D),and Diet E was the control diet containing fishmeal as protein source. A standard 24-day fry feeding trial was carried out in fry tanks with each tank stocked with 15 000 fry. The growth rate, feed conversion ratio and protein efficiency ratio of fry fed for the five diets were similar. The body weight gain were not different) and ranged from 0.012 to 0.014 g/d for fry fed boiled moringa and the control diets. Fry fed steamed moringa and control diets had higher FCR values compared to those on boiled moringa diets but the differences were not significant. Fry fed steamed diets had better growth performance than those on boiled diets although the differences were not significant. It was concluded that steam-heated moringa leaf meal could be used to substitute 10 % of dietary protein in Nile tilapia fry diets without significant reduction in growth performance.

Key words: *Moringa oleifera*, heat treatment, *Oreochromis niloticus,* growth performance, protein efficiency ratio.

INTRODUCTION
The Nile tilapia (*Oreochromis niloticus*) was one of the first fish species cultured and is still the most widely cultured species of tilapia in Africa. Positive aquacultural characteristics of tilapia species include their tolerance to poor water quality and the fact that they eat a wide range of natural food. Out of the total world production of fish, which amounted to 112.30 million tonnes, 18.97 % came from the aquaculture sector and the rest came from the captured fishery (FAO, 2000). Most of the increase in fish production is expected to come from aquaculture, which is currently the fastest growing food production sector in the world.

In aquaculture systems the increasing price of feed is considered one of the most important factors that limit profitability, caused mainly by the cost of fishmeal used as a primary source of protein (Usmani *et al*., 1997). As a result, there is a need to search for alternative protein sources for aquaculture diets. The high cost and fluctuating quality of imported fish meal have led to the need to identify alternative protein sources for use in fish feed formulations (Olvera *et al*., 1990). The identification and utilization of non-conventional and lesser–utilized plant protein sources to replace fishmeal, either partially or totally in practical fry diets has been an area of focus in aquaculture nutrition (Hossain *et al*., 2003). Earlier studies have shown that, *Moringa oleifera* is a promising protein source for inclusion in fish diets at low levels (Chiseva, 2006). Plant proteins are cheap and readily available, but have some antinutritional factors that limit their use as aquaculture feeds. These limitations could be successfully overcome by different methods of heat treatment (Olvera *et al*., 1990; Afuang *et al*., 2003). The objective of the study was to determine the effects of feeding heat-treated moringa supplemented diets on the growth performance of the Nile tilapia (*Oreochromis niloticus*) fry.

MATERIALS AND METHODS

Experimental animals

O. niloticus fry with average body weight (ABW) of 0.01 g were taken from Lake Harvest hatchery, Kariba, Zimbabwe. The collection and transportation of the fry was done as recommended (Hossain *et al*., 2003). They were taken to the experimental tanks in the early hours of the day from 0500 to 0700 hr in order to reduce stress due to high day temperatures.

Fry tanks and fry stocking

A total of ten fry tanks were used and each treatment diet was randomly allocated to two fry tanks. Water in the fry tanks was continuously exchanged throughout the experiment that lasted for 24 days. A compressor was used to supply oxygen into fry tanks via air stones and this ensured adequate dissolved oxygen to be above 80 % saturation. Each individual experimental tank with a volume of 3.16 m^3 was stocked with 15 000 fry. The fry were weighed at the beginning and progressively at weekly intervals. No feed was given on the weighing days to prevent stress.

Processing of moringa leaves and diet preparation

M. oleifera leaves were taken from Lake Harvest forestry unit and were dried under shed. After drying, some of the leaves were either heat treated by boiling or steam heating at a temperature of between 60 ˚C – 80 ˚C for 15 minutes. Steam heating and boiling was meant to minimize or deactivate the anti-nutritive factors such as tannins, phytic acid and saponins that inhibit the digestion of plant proteins in Nile tilapia. After the heat treatments the leaves were allowed to dry under shed before being milled through a 0.01 mm screen.

Four isonitrogenous diets were formulated to have 450 g/kg DM of crude protein (CP). Diets A and C were composed of 5 % boiled and 5 % steamed moringa leaves, respectively, whilst 95 % by mass was fry meal. Diets B and D were composed of 10 % boiled and 10 % steamed moringa leaf meals, respectively, whilst 90 % by mass was the fry meal. The standard fry meal, Diet E, which contained no moringa leaf meal, served as a control and had fishmeal as a protein source.

Feeding

The fry were fed a daily ration at a rate of 15 % bodyweight. The daily ration was divided into eight feedings per day at an hourly interval from 0800 hours to 1500 hours.

Data collection

The fry in each tank were weighed weekly in order to assess their growth performance. A Tefal electronic digital scale was used to measure the mass of fry per week. The fish fry were weighed and returned into their respective fry tanks. No feed was offered during sampling days. Salt was added to fry tanks at a rate of 5 mg/l after sampling to prevent stress, which would have caused high mortalities.

Growth performance were analyzed in terms of total body weight gain (BWG), average daily gain (ADG), feed offered (FO), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival percentages. The following formulae as described (Sidduraju and Becker, 2003):

BWG (g) = Final body weight – Initial body weight
ADG (g/d) = BWG/21 days
FO = Total dry feed offered (g)
FCR = Total dry feed offered (g)/ Live body weight gain (g)
PER = Wet body weight gain (g)/Crude protein fed (g)

Laboratory analysis

The diets used were analyzed for dry matter (DM), crude protein (CP), crude fibre (CF), Ash, Ca, P and energy content using standard procedures (AOAC, 1990).

Statistical analysis

The growth performance was analysed using the one-way analysis of variance (ANOVA) using Statistical Analysis System (SAS, 1990) with the following model:

$Y_{ij} = \mu + P_i + e_{ij}$

Where:

Y_{ij} is the dependent variable (e.g. average daily gain, feed conversion ratio, etc.), μ is the overall mean, P_i = is the effect of the i^{th} diet (i=1, 2, 3, 4, 5) and e_{ij}= the random residual error.

Statistical differences between treatment means were tested using the Tukey Studentised Range test.

RESULTS

Chemical composition of diets

The chemical composition of the diets is presented in Table 1. The diets had CP content that ranged from 46.4 to 46.9 %. The crude fibre of the diets that contained moringa leaves was high, ranging from 2.95 to 4.17 % compared to that of fry meal which was 1.97 %. The ash content of diet A and C was higher (17.27% and 18.57%) as compared with other diets as shown in Table 1. The calcium and phosphorus concentration in the diets was not different. The energy content of the five diets ranged from 8.2 to 12.5MJ/kg.

Table 1: Proximate composition of experimental diets 1 (% on DM basis)

Constituent	[1]Diet A	Diet B	Diet C	Diet D	Diet E
Dry matter	87.9	89.9	88.1	89.6	90.00
Crude protein	46.5	46.4	46.7	46.4	46.9
Crude fibre	3.44	4.17	2.95	3.32	1.97
Ash content	17.27	13.37	18.57	11.03	11.12
Calcium	2.42	2.68	2.48	2.49	2.41
Phosphorus	1.42	1.5	1.76	1.14	1.07
M.E (MJ/Kg)	10.7	9.8	8.2	12.3	12.5

[1]Diet A contains 5% boiled moringa leaves and 95% fry meal
Diet B contains 10% boiled moringa leaves and 90% fry meal
Diet C contains 5% steamed moringa leaves and 95% fry meal
Diet D contains 10% steamed moringa leaves and 90% fry meal
Diet E contains fry meal only

Feed intake, growth performance and feed utilization

The growth performance and feed utilization in terms of body weight gain (BWG), average daily gain (ADG), feed conversion ratio (FCR) and protein efficiency ratio (PER) are presented in Table 2. There was no rejection of feed until the end of the experiment and the acceptability of the diets was similar. No mortality or any signs of disease were observed in any of the dietary groups during the study period.

There was no significant difference ($P > 0.05$) on total body weight gain and average daily gain of the fry fed in the five diets. Fry on diets C, D and E produced the best FCR and PER as compared to all other diets, but this did not differ significantly ($P > 0.05$). In general, among the five diets, fry fed diets containing steamed moringa leaves showed better growth performance in terms of final body weight, gain in body weight, FCR and PER than those fed boiled moringa leaves.

Table 2: Growth performance and nutrient utilization of tilapia fed different experimental diets[1].

Parameters	Diet A	Diet B	Diet C	Diet D	Diet E
IBW (g)	0.01	0.01	0.01	0.01	0.01
FBW (g)	0.261	0.253	0.279	0.288	0.298
BWG (g)	0.251	0.243	0.269	0.278	0.288
ADG (g/d)	0.012	0.012	0.013	0.013	0.014
FCR	1.2	1.3	1.1	1.1	1.0
PER	1.8	1.7	1.9	2.0	2.0

[1]Diet A contains 5% boiled moringa leaves and 95% fry meal
Diet B contains 10% boiled moringa leaves and 90% fry meal
Diet C contains 5% steamed moringa leaves and 95% fry meal
Diet D contains 10% steamed moringa leaves and 90% fry meal
Diet E contains fry meal only
IBW = initial body weight, FBW = final body weight, BWG = body weight gain, ADG = Average daily gain, FCR = Feed conversion ration, PER = Protein efficiency ratio

DISCUSSION

The crude protein content of the experimental diets used in this study was within the range used in a previous study (Usmani *et al.*, 1997). Protein is very important in fish growth and thus crucial ingredient in fish diets. The proximate analysis of the experimental diets showed that the crude protein was ranging from 45.4 % to 46.9 % well within the range recommended for fry feed (McCoy, 1998). This range is within Lake Harvest requirements for the growth of fry which ranges from 45 % to 47 % CP.

The diet which contained 10% steamed moringa leaves (Diet D) showed the highest growth performance compared to all other formulated diets, except for the fry meal (Control diet) although the differences were not statistically different. In terms of growth rate, fish which received the diet which contained 5 % steamed moringa (Diet C) had low growth rate compared to diet D. This is contrary to the previous study (Richter *et al.*, 2003) which showed that higher inclusion levels of moringa leaves in fish meal had an impact on lowering the growth performance because of the presence of anti-nutrients such as phenols, tannins, phytates and saponins. This present study indicate that a 10 % inclusion level of moringa in fry meal yielded good growth performance possibly because the anti-nutrients such as phenols, tannins, phytates and saponins were could have been inactivated by steam heating (Sidduraju and Becker, 2003). This could have resulted in the reduction of palatability-reducing factors.

Heat treatment methods employed might have increased the digestibility of proteins and other dietary components such as starch related compounds leading to high FCR and PER in fish fed with diets C and D. The reduction in anti-nutrients by processing techniques such as soaking, drying and heat treatment on plant-based fish ingredients have resulted in better palatability, increased feed digestibility and growth in fish (Richter *et al.*, 2003).

Generally steam heating reduces loss of soluble nutrients from moringa leaves since that process does not involve a solvent media to dissolve the nutrients. Apart from that, steaming employed in this study might have resulted in little protein being denatured thus making more quality protein being made available in steamed leaves than boiled leaves.

Boiling breaks cell components like cell walls and cell membranes of plants cells. Some of the nutrients within the cells of boiled moringa leaves were lost to boiling water during the heat treatment process. The soluble cell components such as soluble proteins and glucose molecules might have dissolved in water during boiling. This could have caused the reduction of essential amino acids (EAA) in diet A and diet B. Boiling might have caused the inactivation of anti-nutrients such as saponins, phytates, phenols and tannins that bind some quality proteins and inhibit digestion in fish. Apart from breaking the cell components; boiling induces the precipitation of polyphenolic and other phytochemical compounds which might have depressed the growth of fish receiving feed with boiled moringa leaves. Boiling also induces the formation of colloidal starches as a result this reduces the amount of available glycoproteins to fish (Sidduraju *et al*., 2003).

Boiling and steaming showed no significant effect on the crude fibre content but it was within Lake Harvest requirements for the growth of fish; except for diet B that had a higher crude fibre content of 4.17 %. This might have contributed to the lowest growth rate of fish fed with diet B. It has been shown that fibre can bind nutrients like fats, proteins and essential minerals, and reduce their bioavailability (Afung *et al*., 2003; Richter *et al*., 2003). Dietary fibres apparently influence the movement of nutrients along the gastrointestinal tract and significantly affect nutrient absorption.

CONCLUSION

The results of this study indicated that up to 10% inclusion of steam heated moringa leaves can be recommended for *Nile tilapia*. In view of the favorable amino acid profile of moringa leaves and their wide and ready availability throughout the tropics and subtropics, moringa can be considered as a potential feed component with high nutritive value for *Nile tilapia*.

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REFERENCES

Afuang, W., Siddhuraji, P. and Becker, K. (2003). Comparative nutritional evaluation of raw, methanol extracted residue and methanol extracts of Moringa (*Moringa ol*eifera Lam.) leaves on growth performance and feed utilization in Nile tilapia (*Oreochromis niloticus* L.). *Aquaculture* 34: 1147-1159

AOAC (1990). *Official Methods of Analysis,* 15th Ed. Association of Official Analytical Chemists, Arlington,, VA, USA.

Chiseva, S. (2006). The growth rates and feed conversion ratios of fry fed conventional fry diets and *Moringa oleifera* supplemented diets. Unpublished B. Sc. Dissertation, Bindura University of Science Education, Bindura, Zimbabwe.

Food and Agricultural Organisation (2000). Yearbook of Fishery statistics 1998. Vol. 86/2. Aquaculture production. FAO statistics series No.154 and Fisheries series No.56, Rome, Italy. 182p.

Hossain, M.A., Focken, U. and Becker, K. (2003). Anti-nutritive effects of galactomannan-rich endosperm of Sesbania (*Sesbania aculeata*) seeds on growth and feed utilisation in tilapia, *Oreochromis niloticus*. *Aquaculture Research* 34: 1171 – 1179.

McCoy, H.D. (1998). Fishmeal: The critical ingredient in aquaculture feeds. *Aquaculture Magazine* 16 (2): 43-50.

Olvera, N.M.A., Campus, G.S., Sabido, G.M. and Martinez, P.C.A. (1990). The use of alfalfa leaf protein concentrates as a protein source in diets for tilapia (*Oreochromis mossambicus). Aquaculture* 90: 291-302.

Richter, N., Siddhuraju, P. and Becker, K. (2003). Evaluation of nutritional quality of Moringa (*Moringa oleifera* Lam.) leaves as alternative protein source for tilapia (*Oreochromis niloticus* L.). *Aquaculture* 217: 599-611.

Sidduraju, P. and Becker, K. (2003). Comparative nutritional evaluation of differentially processed mucuna seeds [*Mucuna pruriens* (L.) DC. var *utilis* (Wall ex Wight) Baker ex Burck] on growth performance, feed utilisation and body composition in Nile tilapia (*Orechromis niloticus* L.). *Aquaculture Research* 34: 487 – 500.

SAS (1990). Statistical Analysis System Institute Inc., SAS users guide: Statistics, Version 6, 3rd edition, SAS Institute Inc., Cary, NC, USA.

Usmani, N., Jafri, A.K., Alvi, A.S. (1997). Effects of feeding glanded cotton seed meal on the growth, conversion efficiency and carcass composition of *Labeo rohita* fry. *Journal of Aquaculture in Tropics* 12:73-78.