Effect of replacing fish meal by sweet lupin meal on growth performance of African catfish fingerlings, *Clarias gariepinus* (Burchell, 1822)

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ABSTRACT

Scarcity of fish meal remains one of the major challenges in the development of aquaculture in Ethiopia. A growth performance experiment was conducted to investigate the best inclusion level of sweet lupin meal (SLM) in replacing fish meal (FM) to grow African catfish fingerlings, Clarias gariepinus. A total of 120 juveniles weighing an average of 6.01 to 6.09 g were stocked at a rate of 100 fishes m⁻³. The control group received 100% FM and 3 treatments with different proportions of SLM replacement level (50%, 75% and 100%) were tested and feed was provided at a rate of 5% of their live body weight. The mean live weight varied from 8.5 g to 30 g and condition factor from 0.44 to 0.68 after 9 weeks of feeding. The fingerlings grown in 50% SLM and 50% FM formulated feed showed better condition and growth than 75% and 100%. The feed conversion ratio was higher for all treatments but the FCR at 100% replacement level was significantly different from others. Cost of production was higher on the control group, while the profit index was better for treatment I. The study revealed the possibility of growing C. gariepinus juveniles to 30 g sized fingerlings in 9 weeks by replacing 50% of FM with cheaper and locally available SLM.

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INTRODUCTION

The major production cost in aquaculture is feed and the role locally available feed resources play is largely overlooked. Expensive and imported feeds are always considered for fish farming in Ethiopia (personal experience). In order to take the advantage of incorporating cheap protein sources in fish feed formulations, newly introduced and adapted (Likawent Yeheyis *et al.*, 2012a) yellow sweet lupin seeds (*Lupinus luteus*) were investigated.

The nutrient composition of fish meal (FM) from tilapia offal is 54.8% crude protein (CP) and 23.7% digestible energy (DE) (Dale *et al.*, 2004) and yellow sweet lupin *L. luteus* has 44.9% CP and 18.6% DE (Sipsas, 2003; Kim *et al.*, 2009; Likawent Yeheyis *et al.*, 2012b). But FM from tilapia offal is not always available and its price in Bahir Dar is expensive (25 Birr kg⁻¹) compared to SLM (5 birr kg⁻¹).

SLM is cheaper and can be incorporated in fish feeds with different inclusion levels depending on the species (Robaina *et al.*, 1995; Smith, 1997; Burel *et al.*, 1998; Williams, 1998). But yellow sweet lupin (*L. luteus*) has never been tested in *C. gariepinus* feed formulations. Niger seed (*Guizotia abyssinica*) cake contains 32.7% CP and 27% carbohydrate (Tadele Dessie and Ogle, 1997). The CP content of wheat (*Triticum aestivum*) bran is 16.4% and gross energy (GE) is 17 KJ g⁻¹ (Tekeba Eshete, 2005).

Considering the newly introduced sweet lupin, *L. luteus*, feed experiment using SLM as replacement to FM was conducted with the objective of evaluating the effect of different replacement levels of SLM on fish growth and to determine the optimum inclusion level at which *C. gariepinus* juveniles can perform better at less cost.

MATERIALS AND METHODS

Materials

The research was conducted in the laboratory of Bahir Dar Fishery and Aquatic Life Research Center (BFALRC) using twelve rectangular glass aquariums having a total volume of 0.18 m³ (45 cm x 45 cm x 90 cm) and actual water level of 0.1 m³ (25 cm x 45 cm x 90 cm).

Feed ingredients available in the area were collected from different sources. Nile tilapia (*Oreochromis niloticus*) leftover from Bahir Dar fish processing units and *L. luteus* from the local market were purchased. Different locally available feed sources were also incorporated to formulate fish feed. A premix of vitamins (from Alema koudijs-Debrezeit) and salt was also prepared and mixed with the other ingredients. Adding minerals in the premix in the diets containing fishmeal has no beneficial effect (Ng *et al.*, 2001). A batch of 120 healthy *C. gariepinus* juveniles reared by BFALRC from the same parent were selected and used. The juveniles used for the experiment had an average weight of 6.04 to 6.09 g and a standard length of 9.2 to 9.6 cm while stocking.

Aerators and thermostats were used and fixed to each aquarium for a regular supply of oxygen and for the maintenance of optimum temperature. Mortar, meat mincer, solar tent drier and different sized plastic buckets were used for the processing of feed ingredients. Electrical weighing scale (0.01 g precision), measuring board and hand held multi-parameter (Model 556 MPS) were used for measuring the temperature, dissolved oxygen and pH.

Methods

Prior to the formulation (processing) of the feed, the experimental feeds (FM and SLM) and other locally available ingredients were grounded to a fine powder using mortar and hammer mill. Locally available ingredients incorporated with the experimental feed included 12% wheat bran (*T. aestivum*), 5.5% niger seed cake

(*Guizotia abyssinica*) and 2% bone meal. The proportion of the experimental ingredients was 80% and the rest was 19.5% from other local ingredients and 0.5% from premixes. Then, the flour of FM, SLM, wheat bran, bone meal and oil cake were individually weighed and properly mixed together with a premix and adequate water added to ensure smooth pelleting.

The proportion of major ingredients (FM and SLM) in all the diets was 80% and the rest from other locally available ingredients and a premix. The replacement level of SLM treatments was 50%, 75% and 100% based on the acceptable minimum replacement level from other reports (Smith, 2002). The control group was provided with formulated feed without SLM, normally given in fish culture at DE content of 22%. Moist formulated feed was rolled using meat mincer and the strands were cut into short pieces. To remove the moisture, the feed was kept outside a room in a solar tent drier for a day. The pelleted diets, 2 mm in size, were then packed in water impermeable polyethylene bags and stored in a freezer until use. The feed was formulated based on equal proportions of CP (48%) but the DE content was 20% for T1, 19% for T2 and 18% for T3 (Table 1).

Nutrients	Without SLM (%)	50% SLM inclusion (%)	75% SLM inclusion (%)	100% SLM replacement (%)	
Dry matter	95	97.4	98.0	98.4	
Protein*	48	48.0	48.0	48.0	
Energy**	22	20.0	19.0	18.0	
Crude fat	13	13.4	13.8	14.5	
Ash	12	16.0	17.2	17.9	

Table 1. Chemical composition of experimental diets

*"Protein" stands for Crude protein, **"Energy" stands for Digestible energy.

The aquariums were filled with UV light treated water, pumped direct from Lake Tana. For the supply of oxygen and the maintenance of optimum temperature, thermostats and aerators were installed in each aquarium. Electric power was obtained from the grid and upon power interruption by a stand-by electric generator. *C. gariepinus* juveniles were stocked at a density of 10 fishes per aquarium (100 fishes m⁻³) in an indoor clear water aquarium.

First, the fish were provided with similar feed for three days of acclimatization and then, starved for the next 24 h in order to empty their stomach and ready them for new feed and feeding. After acclimatization, the fish were fed with different diets at a rate of 5% of their body weight twice a day (at 10:00 and 17:00) for 9 weeks. In this condition, three diets (different replacement levels of SLM) were tested. All glass aquariums were arranged in a random block of three treatments and a control group and each treatment was replicated three times. All the replicated aquaria were distributed equally to each treatment in a Completely Randomized Design (CRD). The aquariums were cleaned every day early in the morning, before feeding, using a long pipette without disturbing the fishes.

The weight and length of all fishes were measured every week early in the morning before feeding and then the feed amount was adjusted accordingly. At the end of 9 weeks of feeding, the average survival and growth performance of the fish fed with different replacement level of SLM were compared. The weight gain (%), actual growth rate (AGR) and specific growth rate (SGR), food conversion ratio (FCR) of the fish and the profit index were computed using the following formulas:

Condition factor (K)= $(b/L^3)*100$; Where b = body weight (g); L= standard length (cm).

Survival rate (%) = $\left(\frac{Number of fry that survived}{Total Number of fry at the start}\right)$ *100.

Weight gain = W_f - W_{in} ; Where, W_f = Final weight; W_{in} = Initial weight.

Actual growth rate (AGR, g day⁻¹) = $\left(\frac{Wf^{1/3} - Win^{1/3}}{t}\right)$; t is time in days.

Specific growth rate (g day⁻¹) = $\left(\frac{\ln Wf - \ln Win}{t}\right) * 100$ (Brown, 1957). Food conversion ratio (FCR) = $\left(\frac{Weight of feed used(g)}{Weight of fish produced (g)}\right)$ (Broody, 1945).

Profit index (PI) = $\left(\frac{\text{Value of fish (Birr)}}{\text{Cost of feed (Birr)}}\right)$.

Statistical analysis

The mean value of each water quality parameter was taken and compared between treatments. Experimental fish size increment was computed based on the measurement taken. The SGR, FCR and PI values of the different feeding regimes were compared to select the feeding level with better growth and low cost. The data obtained from this experiment was subjected to one-way analysis of variance test, and the means compared using SPSS version 20 software.

RESULTS AND DISCUSSION

Water quality

The mean daily temperature of the control group and the treatments varied between 25.1 to 26.3 °C with the lowest temperature on the control group. The dissolved oxygen level was $5.6\pm0.2 \text{ mgL}^{-1}$ for the control group, 5.4 ± 0.3 for T1, 5.1 ± 0.2 for T2 and $5.4\pm0.3 \text{ mgL}^{-1}$ for T3. The pH value was in a range of 7.6 and 8.01 (Table 1), with T3 with the highest reading (Table 2). The thermostat gauge failed for a few days in the 5th week where the temperature of T3 rose beyond 30 °C, and hence, the level of dissolved oxygen dropped to 4.7 mg L⁻¹. In this experiment, at high temperatures, dissolved oxygen was found to be low. It is natural that with rising temperatures, water holds less oxygen, metabolic rates of fish and their physiological demand for oxygen increase (Kelly and Linda, 1997).

Treatments	Wa	er		
(FM:SLM inclusion level in %)	Temperature (°C±SE) ^{NS}	Oxygen (mg L ⁻¹ ±SE) ^{NS}	$pH \pm SE^{NS}$	
Control (100:0)	25.2±1.0	5.6±0.2	7.6±0.2	
T1 (50:50)	25.1±1.3	5.4±0.3	7.8 ± 0.2	
T2 (25:75)	26.3±0.7	5.1±0.2	7.9 ± 0.3	
T3 (0:100)	25.1±0.8	5.4±0.3	$8.0{\pm}0.1$	

Table 2. Physical param	eters of the culture water
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Note:- Each value is the mean of three replicates; NS stands for no statistically significant difference; SE stands for standard error

Temperatures, dissolved oxygen levels and pH varied between treatments and the control group, though not significant. The mean levels were found within the optimum demand for the growth of *C*. *gariepinus* juveniles, i.e., 22 - 30 °C, $\ge 4 \text{ mgL}^{-1}$ dissolved oxygen and pH range of 6.5 to 9 (Schram *et al.*, 2010).

Fish adaptation and condition

As the intention is to grow fish fries into fingerlings, the average stocking size of a fish was 6.05 for the control group, 6.08 g for T1, 6.04 g for T2 and 5.88 g for T3, and the condition factor (K value) varied between 0.69 and 0.71. There was no significant difference among treatments. At the end of the experiment, the K values were significantly different, i.e., 0.68 for the control group, 0.67 for T1, 0.60 for T2 and 0.44 for T3 (Table 3). Some 90% of experimental fishes adapted well to the condition; adaptation levels significantly varied between treatments (40% of the fishes in T3 died during the experiment).

Variable	Mean for treatments (diets)						
	Control	T1	T2	Т3			
Survival (%)*	$100\pm0.0b$	100.0±0.0b	99.7±0.10b	60.0±1.3a			
Mean weight (g)							
Initial	6.1±0.2a	6.1±0.22a	6.0±0.20a	5.88±0.2a			
Final	30.7±0.4c	29.8±0.45c	19.7±0.30b	3.47±0.2a			
Condition factor (k)							
Initial	0.7±0.1a	0.71±0.03a	0.7±0.03a	0.71±0.0a			
Final	$0.7{\pm}0.0c$	0.67±0.01c	0.6±0.04bc	0.44±0.1a			
Weight gain (g)	23.2±1.1c	$22.6\pm0.8c$	13.5±0.07b	-3.75±0.6a			
AGR (g day-1)	0.1±0.0c	$0.044{\pm}0.0c$	$0.03 {\pm} 0.00 b$	0.02±0.0a			
SGR (g day-1)	2.6±0.1c	2.53±0.2c	$2.02 \pm 0.08 b$	-1.68±0.1a			
FCR	2.7±0.1c	2.73±0.1c	3.55±0.12b	-8.92±2.3a			

Table 3. Effect of sweet lupin replacement on the performance of C. gariepinus juveniles (mean \pm SD).

*Survival (%) stands for "Fish survival (%)"; means followed by different letters within a row are not significantly different from each other at α =0.05; all values are means of three replicates.

The mean adaptation of the fishes was generally high with no cannibalism observed during the experiment. This might be due to the

minimum stocking density (FAO, 2010) of the fingerlings, uniformity of the fingerlings and the management of the fingerlings in the same environment in which they have been reared and grown till they are recruited for the experiment. Juveniles at T3 did not perform well indicating that replacing FM 100% with SLM in feed formulation had a negative effect on the performance and condition of fish. Apart from incorporating SLM 100% in fish diet, death and weight loss of experimental fish increased when the dissolved oxygen level dropped to 3 mg L⁻¹.

Studies show that more than 90% of dissolved oxygen consumption of juvenile *C. gariepinus* is from tank water (Hecht and Uys, 1997) and at a temperature of 30 °C, oxygen level of the water dropped and the body condition of small *C. gariepinus* fishes decreased by 11% day⁻¹ (Degani *et al.*, 1988). The condition of the fish was good at the beginning compared to the end of the experiment due to the change in feeding regime whereby the experimental fishes become completely dependent on formulated feed.

Fish weight gain and growth performances

The weekly percentage weight gain showed a decreasing trend in time for all the treatments and the control group. The gain was very low for T3 and better for the control and T1 (Figure 1). The growth of the fingerlings (weight) for the control group and T1 was higher (30.6 and 29.8 g) but that of T2 and T3 was lower (19.7 and 3.5 g) and the difference was significant (Figure 2).

The average weight gained by *C. gariepinus* fingerlings was 23.17 g for T1, 22.64 g for T2, and 13.47 g for T3 and (-)3.75 g for the control (Table 3). The control group and T1 had better mean weight gain compared to the other treatments and the difference was significant. The decline in average mean weight gain for T3 was extreme and went below zero indicating that the fish failed to grow and dropped from their original size. This might be due to the presence of insoluble molecules (Carter and Hauler, 1999), protease (mainly chymotrypsin) inhibitors (Petterson *et al.*, 1997; Alonso *et al.*, 2000) and saponins in *L. luteus* (Cuadrado *et al.*, 1995). At the 8th week, all the experimental

fishes showed 90% and more reduction in weight. Fingerlings fed with 100% replacement of FM by SLM decreased from their original size and they were stunted. Fingerlings that were fed on 75% SLM achieved their maximum weight at the 9th week on which those on the control group and 50% inclusion of SLM attained earlier at their 5th week (Figure 2). Feed formulations with SLM inclusion level of 75% and more had significant effect on the growth of fish.

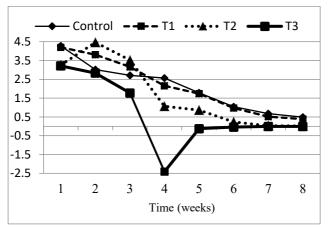
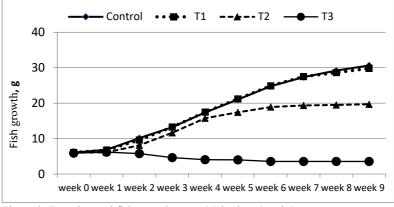


Figure 1. Weekly percentage weight gain of experimental fishes

The control group and T1 showed similar growth patterns in the growth curve but fingerlings in T2 had been growing so slowly and those in T3 have a negative growth. An overlapped error bars in the figure indicated no significance difference.

Growth rates and food conversion ratio

The AGR of fishes on the control and T1 was better than T2 and T3, which received higher proportion (75% and 100%) of SLM. The AGR was 0.045 g day⁻¹ for fishes that grow in 0 and 0.44 g day⁻¹ in 50% SLM replacement. Fishes that fed with higher proportion of SLM or lower proportion (25 and 0%) of FM had lower AGR (0.032 and 0.02 g day⁻¹). Over the duration of the study period, SGR was



2.57 for the control, 2.53 for T1, 2.02 for T2 and (-)1.68 for T3 (Table3). These values significantly varied.

Figure 2. Experimental fish growth curve (g) in time (weeks).

SGR and FCR values were the same for treatments that received equal or higher proportion of FM and SLM (Figure 3). But the FCR value was significantly higher for T3 which received 100% inclusion of SLM compared to the others. The FCR was very high for T3 with 100% replacement of SLM to that of FM, indicating lower efficiency of SLM in the production of fingerlings (Table 3).

The FCR of the control group and T1 was higher and hence the fish have converted feeds into biomass. Diets that contain a high level of animal protein and those composed principally of plant-based ingredients are digestible due to reduced trypsin and chymotrypsin inhibitory activities (Petterson *et al.*, 1997; Alonso *et al.*, 2000). In this study, feeds formulated with high level of SLM (75 and 100%) had poor growth performance of *C. gariepinus* fingerlings than those fed in 0% and 50% SLM inclusion levels. This poor performance and stunted growth in T3 might be due to the anti-nutritional factor found in small concentrations in sweet lupin seeds (Petterson *et al.*, 1997; Barneveld, 1999; Alonso *et al.*, 2000; Glencross, 2001) and the comparatively low concentration of methionine and cysteine, which

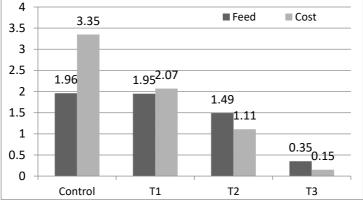
are important for the growth of fingerlings (Glencross, 2001; Likawent Yeheyis *et al.*, 2012b).

Better growth rates in the control group and T1 might be due to better digestibility and availability of amino acids and fish oil in FM in concentrations that satisfy what is required for *C. gariepinus* fingerlings. Studies also confirmed that feeds with higher proportion of dietary carbohydrate levels increased intestinal alpha-amylase activity of fishes (Ali and Jauncey, 2005).

The food conversion efficiency of fishes that received higher proportion of SLM was low, which might be because of high levels of saturated fats that reduce palatability (Goda *et al.*, 2007). Reduced FCR might also be caused by the quality of the feed, i.e., indigestibility of SLM and deficiency of amino acids methionine and cysteine on *L. luteus* (Petterson *et al.*, 1997). Juveniles of *C. gariepinus* fish require 50% CP (Ali, 2001). As the proportion of SLM increases, the strength of the pellets gets harder and stiffer for the juveniles of *C. gariepinus* and cannot eat it immediately.

Feasibility of sweet lupin meal replacement

All other costs were the same for the control group and treatments 1, 2 and 3 till the end of the experiment. Feed provided for experimental fishes was 1.96 kg for the control group and 1.95 kg for T1, 1.49 for T2 and 0.35 kg for T3. The feed consumed by T3 was significantly lower compared to the other treatments (Figure 3). This was due to the smaller number and size of the experimental fishes in this treatment group. Treatments with higher proportion of FM (50 and 100%) incurred significantly more cost than treatments with lower level; cost was higher for the control group (Table 4). The amount of feed consumed by T1 was the same with the control group but the cost was still higher for the control. This was due to the higher cost of FM which continues to increase with time in Ethiopia. Each treatment received equal number (n = 30) of juveniles at the start. The estimated market value of a fingerling of *C. gariepinus* was 0.6 Birr for 25 to 35 g weighing ones, 0.20 Birr for 15 to 24 g, 0.10 Birr for 5



to 14 g and 0.04 Birr for less than 5 g. This was estimated based on the actual price of tilapia fingerlings around Bahir Dar area.

Figure 3. Feed provided (kg) and cost expended for feed (Birr)

Ingredients & expenses	Unit		t of diets		
	price	Control	T1	T2	Т3
Fish meal (Tilapia offal)	Fish meal (<i>Tilapia offal</i>) 20.0		1.556	0.596	0.000
Sweet lupin meal‡	0.4	0.000	0.312	0.358	0.112
Niger seed cake	0.4	0.094	0.094	0.072	0.016
Wheat bran	0.8	0.086	0.086	0.066	0.016
Bone meal	0.6	0.024	0.024	0.018	0.0104
Premix	0.002	0.002	0.002	0.001	0.0004
Total feed cost (Birr)		3.35	2.07	1.11	0.15
Number of fingerlings grown	30	30	29	18	
Average weight of a fingerlin	30.67	29.82	19.68	3.47	
Market value of fingerlings (H	18	18	5.8	0.72	
Profit Index (PI)	5.4a	8.7b	5a	4.8a	

Table 4. Profit index of the different replacement level of sweet lupin.

Note:-Each PI value is a mean calculated from three replicates; (%)"; means followed by different letters within a row are not significantly different from each other at α =0.05 (F=46.56); \ddagger Sweet lupin (*L. luteus*) meal; wheat (*T. aestivum*) bran; niger seed (*G. abyssinica*) cake.

The total feed cost to grow 30.7 g sized fingerling for the control group was 3.4 Birr, to grow 29.8 g for T1 was Birr 2.1, to grow 19.7 g for T2 was Birr 1.1 and to grow 3.5 g for T3 was 0.2 Birr (Table 4; Figure 3).

Bigger *C. gariepinus* fingerlings with similar SGR and FCR were produced from T1 at a reduced cost than the control group (Table 3; Figure 4). This shows the possibility of growing *C. gariepinus* fingerlings to a grow-out sized ones with cheaper and locally available feed sources. All the fingerlings grown in the experiment were harvested for grow out ponds.

As indicated on Figure 4, the food conversion efficiency and growth rate of fish was higher at no replacement of FM by SLM but the feed cost was higher which might not be feasible for the producer. The growth performance and food conversion efficiency were reduced at higher inclusion level of SLM. Clarias gariepinus fingerlings with better food conversion efficiency, better growth rate and with lower feed cost were produced at 50% replacement level of SLM (T1). The arrow indicates the point where better fingerlings were produced at best cost. The PI value of T1 was significantly higher compared to the other treatments and the control group. The PI value of T1 was 8.7 but 5.4 for the control group, 5 for T2 and 4.8 for T3 (Table 4). The 50% replacement had better PI value, indicating that the cost expended to grow fingerlings was smaller than the other replacement levels. The PI was higher when large proportion of FM is replaced by SLM as plant proteins from locally available sources are cheaper. The optimum production cost with better FCR and SGR have been found at SLM to FM proportion of 50:50 (T1) as the fishes in this group grew better in a lower feed cost than the control and their market value was equal to the control group but more than T2 and T3.

CONCLUSION

For better growth and adaptation of the juveniles of African catfish (*C. gariepinus*), it is critically important to keep the water clean at all times and fix culture water temperature between 23 and 25 °C. In this study, feeds were formulated and tested for the juveniles of African catfish.

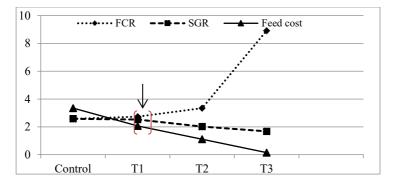


Figure 4. Feed conversion efficiency, growth rate and feed cost

It was confirmed that sweet lupin meal could effectively replace the standard fish meal but the inclusion level should not go beyond 50%. To increase the replacement level and reduce production cost, yellow sweet lupin (*L. luteus*) seed quality might have to be improved.

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Treatment	Ν	Mean	Standard deviation	Standard error	95% confidence interval		Min	Max
					Lower	Upper		
Ctrl	3	23.2	1.12	0.64	20.40	25.94	22.2	24.4
T1	3	22.6	0.75	0.43	20.78	24.50	21.8	23.2
T2	3	13.5	0.07	0.04	13.28	13.65	13.4	13.5
T3	3	3.7	0.56	0.33	5.19	2.32	4.3	3.1

Annex 1. Weight gained by experimental fishes (descriptive statistics)