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Haematological profile of hormonal sex-reversed *Sarotherodon melanotheron*

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Abstract

Sarotherodon melanotheron fry were reared in six (6) plastic tanks for three (3) months, of which three (3) tanks served as treatment tanks while the other three (3) served as the control. The fry were fed with 17α-methyl testosterone enzyme, which functions as a sex reversal hormone. The fry were administered this hormone for 30 days, to ensure complete sex reversal. All the S. melanotheron fry were reared to table size for duration of three (3) months, after which, blood samples were taken from both the control and treatment fishes. It was observed, that the sex reversed S. melanotheron individuals grew to a larger size than the control specimens at the end of the duration of the experiment. Wild specimens of S. melanotheron were also captured from the Lagos lagoon, and their blood samples were also taken and analyzed. The results showed that blood parameters except White Blood Cell count (WBC), were highest in sex reversed tilapia {Red Blood Cell count (RBC), 0.67 × 10^6, Haemoglobin concentration (HGB), 2.7g/dL, Mean Corpuscular Value (MCV), 14.7fL} and lowest in the wild specimens (RBC, 0.14 × 10^6, HGB, 1.7g/dL, MCV, 10.2fL). WBC was highest however, in the wild specimen with values of 2.0 × 10^6. The WBC, RBC, HGB, and MCV values for the control specimen were 1.0 × 10^6/L, 0.33 × 10^6/L, 2.1 g/dl. and 14.7fL. A total protein value for sex reversed specimens was 3.99g/dL, while urea and creatinine values were 0.2g/dl.. Alkaline Phosphatase, Aspartate transaminase and Alanine transaminase for the treatment specimen were 183nm/mg protein/min, 98nm/mg protein/min and 105nm/mg protein/min respectively. A total protein value for control specimens was 2.81g/dL, while urea and creatinine values were 0.2g/dL. Alkaline Phosphatase, Aspartate transaminase and Alanine transaminase for the control species were 174nm/mg protein/min, 93nm/mg protein/min and 106nm/mg protein/min respectively. Total protein values for the wild specimens were 2,75g/dL, while urea and creatinine values were 0.87g/dl.. Alkaline phosphatase, Aspartate transaminase and Alanine transaminase for the wild specimen were 390nm/mg protein/min, 217nm/mg protein/min and 243nm/mg protein/min respectively. In this study, the results showed that sex reversal in tilapia culture is not harmful to the metabolism of the fishes, and in fact, results in larger healthier fishes, than those caught from the heavily polluted waters of the Lagos lagoon. Growth and nutrient utilization analysis measurements were taken weekly for the sex reversed and control fish specimen. At the end of the duration of the experiment, the sex-reversed fishes had a specific growth rate (SGR) of 17.59, and the control specimen had a SGR value of 10.31. The results highlighted the suitability of freshwater culture systems for the rearing of S. melanotheron.

Keywords: Sand dredging, Ikpa River, plankton, water quality.

Introduction

Aquaculture is an important means of decreasing fish supply from the ocean, thus preventing depletion of capture fisheries due to overfishing. It also contributes to food security, poverty alleviation, employment, trade and income generation (Omotosho and Fagbenro, 2005b).The ever increasing demand for fish protein in Nigeria has motivated speedy development of aquaculture sector.

Tilapia is widely recognized as one of the most popular species for culture in a wide range of aquaculture systems worldwide (Beardemore et al., 2001). It is one of the most productive and internationally traded fish in the world (Fitzsimmons, 2008). Tilapia possesses qualities, which are very beneficial for aquaculture. These qualities include high market acceptance, ease of propagation, efficient utilization of diets which are high in plant protein, and they are very resistant to diseases (Jamu, 2001). Culturable tilapia in Nigeria include: *Sarotherodon melanotheron, Sarotherodon galileaus, Tilapia zillii, Tilapia guineensis,* and *Oreochromis niloticus.*

The members of the genus Tilapia and other related species have met the standards for culturability (FAO, 2006a). With adequate care and appropriate rearing techniques, tilapia fishes can also grow to a marketable size in a short time, and as an added bonus, they can also spawn without forceful and strenuous inducement, creating more fish that will also be grown for further sale, generating income for the seller, and increasing the protein intake of the buyer (FAO, 2006b).

One of the basic phenomenon of tilapia aquaculture is that males grow bigger and faster than females. In order to avoid unwanted spawning in a production unit, all-male populations are preferred (Guerrero, 2008). Several methods are used to skew sex ratios and increase the percentage of males in a population. The first method was culling through a population, discarding the females and keeping the males. The more common method of generating mostly male populations is through the use of steroid hormones fed to sexually undifferentiated fry (Phelps, et al., 1995). Exposing the fish to different forms of testosterone or estrogen may lead to sex-reversal. Hormones are generally included in the diets for several weeks when the fish start eating. Other hormones have been tested and sex-reversal has also been achieved by immersion in a solution (Obi and Shelton, 1993). Using this technique farms can produce populations of greater than 90% male fish. These populations grow faster than equivalent populations of mixed sex fish and have significantly less reproduction in the grow-out systems (Rothbard, et al., 1993). The only way of detecting the extent of the harmful substances present in this tilapia is by haematological tests. Blood haematology and biochemical parameters are good indicators to examine the health status of fish. Haematology has been developed and well utilized in the assessment of the health and well being of man and livestock. Svobodova et al. (1991) opined that ichthyo-haematology would be useful in the assessment of suitability of feeds and feed mixtures, evaluation of fish conditions, determination of toxic effects of substances, as well as diagnosis of disease. This study on the haematological comparison between cultured *Sarotherodon melanotheron* and those from the wild at the Lagos lagoon was carried out in order to determine the culture potential of *Sarotherodon melanotheron* (black jaw tilapia) in fresh water system and to investigate haematological profile of the species.This study also intend to determine the haematological changes in hormonal sex reversed *S. melanotheron* individuals.

Materials and Methods

Eighty adult individuals of *Sarotherodon melanotheron* were collected from the floating cages of the Department of Marine Sciences mariculture site in the Lagos lagoon on the 18th of August 2010 when salinity was about 20parts per thousand. The fishes were transported to the Department of Marine Sciences research laboratory of the University of Lagos, for research purposes. They were kept after collection in water of approximately 20 parts per thousand before acclimatization, in order to prevent osmoregulatory shock.

The brood stocks were transferred into a concrete pond with a dimension of 1×1×5m, using a scoop net and acclimatized for 31days. The water was gradually diluted with fresh water, and it took a period of four weeks for the salinity to reduce from 20‰ to 0‰. Physico-chemical parameter was taken daily for four weeks.

After the acclimatization period of 31 days, and the fishes had fully adapted to freshwater conditions, the water was changed once in two weeks to allow production of natural food (production of phytoplankton and zooplankton) in the pond (*Scenedesmos spp,* and mosquito larva) which was supplemented with artificial feed. The water was left to be turbid in order to allow the environment suitable for breeding. Spawning occurred after 41 days.

Sarotherodon melanotheron fry were obtained from concrete pond which was drained, and they were removed with a scoop net. After this, the fry were reared for three (3) months in six (6) 1×1×1.2 m plastic (High density polyethelene) tanks. The fishes in three (3) of the tanks were fed with 17α – methyl testosterone mixed with 0.3 – 0.5 mm coppens fish feed for one month, in order for sex reversal to occur. These served as our experimental tanks, while the other three (3) tanks contained *S. melanotheron* fry that were not fed with this diet of androgen hormone, but were instead fed with ordinary 0.3 – 0.5 mm coppens feed for the total three (3) month duration of this study. These served as our control.

At the end of the three (3) month duration, several individuals were selected from all the experimental and control tanks for collection of blood samples. These blood samples were sent to a laboratory for further analysis. Wild *S. melanotheron* individuals were also captured from the Lagos lagoon, and blood samples were also collected from these individuals and sent to the laboratory for further analysis. The black jaw tilapias that were reared for the duration of this study were fed with commercial feed (0.3 – 0.5mm coppens feed) 3 5 times every day. The tanks were siphoned in order to remove the fish droppings and unutilized feed at the bottom of the tank and suspended in the water column. Heavy aeration was maintained continually in all the plastic tanks in order to ensure there were optimum oxygen levels in the tanks for enhanced fish growth and to prevent stress of the fishes. Water was changed in all the tanks every 24 hours.

- **Application of the sex-reversal hormone.** The sex reversal hormone which is 17α – methyl testosterone, is mixed with the fish feed in the ratio of 60mg of hormone to 1kg of fish feed, and was administered to the fishes in the experiment tanks for a one (1) month period. 60mg methyl testosterone is dissolved in 40cl ethanol, and diluted with water until there is no precipitate, it is mixed intensively with one kg of coppens 0.3–0.5mm, and it is then dried at 60 – 80°C
- **Capture of wild *S. melanotheron*.** Cast nets were thrown into the lagoon at about 200 – 300m from the shore. The net was allowed to stay in place for approximately 25 minutes, and the net was retrieved and landed in a small boat. Amongst the fishes caught, *Sarotherodon melanotheron* individuals were quickly identified and kept separately from the by – catch, which included Bonga fishes, (*Ethmalosa*) and *Tilapia guineensis*. On reaching the shore, extraction of blood from the *S. melanotheron* individuals that were captured, occurred immediately so as to prevent

the blood of the fishes congealing in their blood vessels.

- **Collection of blood samples.** Blood samples were obtained from the gills of the cultured tilapia, both the control and experimental fishes (sex reversed fishes), and the wild tilapia caught in the Lagos lagoon, with the aid of a syringe and hypodermic needle. The needle which was attached to the syringe was inserted into the gills and blood flowed into the syringe. The blood in the syringe was immediately decanted into a vial containing an anti – coagulant, specifically EDTA (ethylene – diamine – tetracetic acid). This was done to prevent the blood from coagulating before reaching the laboratory. The use of plastic syringe was necessary, because contact with glass results in reduced coagulation time. After all the required blood samples had been taken, the samples were sent to the diagnostic laboratory of the Nigerian Institute of Medical Research, Yaba, Lagos, for analysis.
- **Haematological analysis.** The haematological tests carried out in this study include: Packed cell volume (PCV), Haemoglobin concentration (Hb), Urea and creatinine, Plasma enzyme activities (alkaline phosphate, alanine transaminase, and aspartate transaminase), Plasma protein (total protein albumen and triglyceride), Mean corpuscular value (MCV), Mean corpuscular haemoglobin (MCHC), Red blood cell (RBC), White blood cell (WBC).

Parameters analysis

- **Weight measurement.** The mean standard weight of the fish in each tank was determined at the beginning of the experiment and at every 1 week. The weight of all the fish in each tank was measured using scale (OHAUS MODEL Cs 5000, CAPACITY 5000×2g) and mean value was calculated.
- **Physico-chemical parameters measurement**. DO was measured using (Hanna Model III 1946, Microprocessor dissolved-oxygen meter), pH was measured using (Luttron pH 241, pH meter); Temperature was measured using a mercury in glass thermometer.
- **Growth and nutrient utilization parameters.** The following indices were used to determine the biological evaluation of growth performance and nutrient utilization of the experimental fish.
- **Mean weight gain.** The weight gain per week was calculated using the formula below.
- Final weight (g) – initial weight (g)
- **Percentage mean weight gain.** This was calculated using the formula below.

$$\frac{Mean\ weight\ gain\ (g)}{Initial\ mean\ weight\ (g)}\ x\ 100$$

- Percentages mean weight gain per week

$$\frac{Mean\ weight\ gain\ per\ week\ (g)}{Initial\ mean\ weight\ (g)}\ x\ 100$$

- Specific growth rate (SGR). This is the percentage rate of change in the logarithmic body weight. The SGR was calculated using the formula below.

$$SGR = \frac{Log_e W_f - Log_e W_i}{Time\ (in\ days)}\ x\ 100$$

Where W_f = Final body weight and W_i = initial body weight

- **Feed conversion ratio (FCR).** This is the amount of unit weight of food that the fish were able to convert into unit muscle.

$$FCR = \frac{Feed\ intake\ (g)}{Total\ weight\ gain\ (g)}$$

- **Daily rate of feeding (DRF).** This is determined by subtracting the remaining feed after feeding from the initial weight of the feed.
- **Weekly Rate of Feeding.** This is calculated by multiplying DRF by seven.

$$GEFC = \frac{Gross\ efficiency\ of\ food\ conversion\ (GEFC)}{Daily\ rate\ of\ feeding}$$

- **Protein Efficiency Ratio (PER).** This was calculated from the relationship between the increment in the weight of (i.e. weight gain of fish) and protein consumed.

$$GEFC = \frac{Gross\ efficiency\ of\ food\ conversion\ (GEFC)}{Daily\ rate\ of\ feeding}$$

Statistical Analysis

The data and readings obtained from the physicochemical parameters, weight measurements and hematological analysis were subjected to various statistical measures which include mean, standard deviation and range. The mean values were compared for significant differences using Duncan's multiple range tests using Statistical Package for Social Sciences (SPSS). This served as a means of inferential statistics.

Results

Table 1 shows the water quality parameters of the fresh water culture system in which the *S. melanotheron* individuals which served as the control were reared for 90 days, while Table 2 shows the water quality obtained in the fresh water culture system, in which the fishes were treated with methyl testosterone hormone. The readings obtained span the 90 day study period. The values shown in these tables indicate that the culture environment is suitable for the optimal growth and well being of the fish specimen.

Table 3 shows the initial, final and mean weights of the *S. melanotheron* individuals used in this study, and Table 4 shows the nutrient parameters and growth rates of the *S. melanotheron* specimen. These results can be used as indicators of the well being of the fishes, and thus the condition of the environment in which they were reared. Table 5 shows the results of the haematological analysis carried out on samples of the blood collected from the experimental fishes, the control fishes and the wild fishes caught in the Lagos lagoon.

Table 1: Water quality parameters of *Sarotherodon melanotheron* fresh water culture system for the control tanks.

Parameters	T01		T02		T03	
	Range	Mean	Range	Mean	Range	Mean
T(°C)	27-29	27.833 ± 0.717[a]	27-29	27.833 ± 0.717[a]	27-29	27.833 ± 0.717[a]
DO(mg/L)	6.2-10.1	8.416 ± 1.113[a]	6.2-10.1	8.416 ± 1.113[a]	6.2-10.1	8.416 ± 1.113[a]
pH	6.3-7.5	6.833 ± 0.322[a]	6.3-7.5	6.833 ± 0.322[a]	6.3-7.5	6.833 ± 0.322[a]

T0 = Control tanks, a = Mean values with the same superscript are not significanily different (p > 0.05).

Table 2: Water quality parameters of *Sarotherodon melanotheron* fresh water culture system for the treatment tanks.

Parameters	TI1		TI2		T13	
	Range	Mean	Range	Mean	Range	Mean
T(°C)	27-29	27.833±0.717[a]	27-29	27.833 ± 0.717[a]	27-29	27.833 ± 0.717[a]
DO(mg/L)	6.7-10.0	8.516 ± 1.075[a]	6.8-10.2	8.541 ± 1.027[a]	6.5-9.8	8.475 ± 1.145[a]
pH	6.4-7.0	6.238±0.197[a]	6.3-7.1	6.791 ± 0.231[a]	6.3-7.1	6.716 ± 0.224[a]

T1 = Tanks treated with methyl testosterone (Treatment tanks)
a = Mean values with the same superscript are not significantly different (p > 0.05)

Table 3: Mean weight, percentage mean weight gain per week and percentage mean weight gained in the various tanks.

Tanks	Initial mean weight(g)	Final mean weight(g)	Mean weight gained(g)	% mean weight gained(g)	% mean weight gain per week(g)
Control (1)	0.62[a]	6.41[a]	5.79[a]	933.90[a]	77.83[a]
Control (2)	0.88[a]	6.26[a]	5.38[a]	611.43[a]	50.95[a]
Control (3)	1.32[a]	6.83[a]	5.51[a]	417.42[a]	34.85[a]
Treatment (1)	0.56[b]	11.56[b]	11.00[b]	1964.35[b]	163.70[b]
Treatment (2)	0.90[b]	11.55[b]	10.65[b]	1183.33[b]	98.61[b]
Treatment (3)	0.62[b]	11.57[b]	10.95[b]	1766.13[b]	147.18[b]

a = Mean values with the same superscript are not significantly different (p > 0.05).
b = Mean values with the same superscript are not significantly different (p > 0.05).
Mean values with different superscript show significant difference of p < 0.005.

Table 4: Growth and nutrient parameters of the fish in control and treatment tanks.

Parameter	$T0_1$	$T0_2$	$T0_3$	$T1_1$	$T1_2$	$T1_3$
MWG	5.79[a]	5.38[a]	5.51[a]	11.00[b]	10.65[b]	10.95[b]
% MWG	933.90[a]	611.43[a]	417.42[a]	1964.35[b]	1183.33[b]	1766.13[b]
%MWG/WK	77.83[a]	50.95[a]	34.85[a]	163.70[b]	98.61[b]	147.18[b]
SGR	8.56[a]	12.17[a]	10.19[a]	18.78[b]	15.83[b]	18.15[b]
FCR	28.24[a]	30.41[a]	29.67[a]	14.85[b]	15.35[b]	14.93[b]
DRF	10.895[a]	10.895[a]	10.895[a]	10.895[b]	10.895[b]	10.895[b]
WRF	76.895[a]	76.895[a]	76.895[a]	76.895[b]	76.895[b]	76.895[b]
GEFC	0.0354[a]	0.0328[a]	0.0337[a]	0.0672[b]	0.0651[b]	0.0669[b]
PER	0.1033[a]	0.0960[a]	0.0983[a]	0.1964[b]	0.1901[b]	0.1955[b]

T0 = Control tanks, T1 = Treatment tanks, a = Mean values with the same superscript are not significantly different (p > 0.05), b = Mean values with the same superscript are not significantly different (p > 0.05), Mean values with different superscript show significant difference of p < 0.005.

Table 5: Results of the haematological analysis

Parameters	Control	Experiment	Wild
PCV	5.1%	5.3%	2.5%
HGB	2.1 g / dL	2.7 g / dL	1.7 g / Dl
RBC	0.33×10^9 / L	0.67×10^9 / L	0.14×10^9 / L
WBC	1.0×10^6 / L	1.0×10^6 / L	2.0×10^6 / L
MCV	14.7 fL	17.2 fL	10.2 fL
McHc	1.08 g / L	1.22 g / L	0.27 g / L
Total protein	2.81 g / dL	3.99 g / dL	2.75 g / Dl
Urea&Creatinine	0.2 g / dL	0.2 g / dL	0.87 g / dL
Alkaline Phosphatase	174nm / mg protein / min	183nm / mg protein / min	390nm/ mg protein / min
Aspartate transaminase	93nm / mg protein / min	98nm / mg protein / min	217nm/ mg protein / min
Alanine transaminase	106nm/ mg protein / min	105nm/ mg protein / min	243nm/ mg protein / min

Discussion

Sarotherodon melanotheron (black jaw tilapia) was successfully cultured in fresh water system. The physico-chemical parameters of fresh water did not alter the reproductive ability of the brood stocks and the fry were successfully cultured in fresh water system. There was obvious difference in the growth of fry fed with hormone treated feed and fry fed with ordinary feed. In this study, it was observed from the haematological analysis that, the blood parameters of the cultured *Sarotherodon melanotheron* and the wild specimens differed greatly in the following respects: The PCV, RBC, MCV, and MCHC values were higher in the cultured *Sarotherodon melanotheron* species, than in the wild ones caught in the lagoon. WBC values however, were higher in the wild *S. melanotheron* species, with values higher than 1.0×10^6/ L. PCV values for the *S. melanotheron* individuals caught in the Lagos lagoon was 2.5%, and the RBC value was less than the average values of 0.25×10^9/ L. The wild *S. melanotheron* individuals also had low values of MCV and MCHC values. It is apparent from this observation, that stress levels in the Lagos lagoon must be responsible for the lower blood parameter values, and the higher WBC values can be attributed to external infections which are numerous in wild culture conditions. This is in accordance with Akinrotimi et al. (2009), who worked on the haematological responses of *Tilapia guineensis* to acute stress. However, even amongst the cultured *S. melanotheron* individuals, there was a slight disparity in the blood parameter values, with the experimental fishes (sex reversed) having higher values of PCV, RBC, MCV, and MCHC than the control fishes. PCV of the experimental fishes was 5.3%, while the control specimen had PCV values of 5.1%. The RBC values in the sex reversed fishes was far above the average values 0f 0.25×10^9/ L, while the control specimen had slightly lower RBC values of 0.33×10^9/ L, but was still slightly above average. MCV and MCHC values in the sex reversed fishes were all indicative of a healthy environment and diet, as they greatly superceded the average values of haematological parameters recorded by Svoboda et al. (1991) during his work on the Unified methods of haematological examination of fishes. The WBC, MCV and MCHC of the control fishes however, were found to be around the average values. The reason for the higher values of haematological parameters except WBC values in the sex reversed *S. melanotheron*, can be attributed to the higher protein utilization of the sex reversed fishes, which agrees with Guerrero, (2008), who worked on Tilapia sex reversal. The fact that the difference in all these parameters between the experimental and control fishes was small and hardly noticeable, shows that sex reversal of *S. melanotheron* has no negative impact on its life functions, as Guerrero (2008) also stated in his work.

The urea and creatinine levels were higher in the wild specimen of *S. melanotheron*; with values of 0.87 g/ L, compared to the 0.2g/ L of the control and experimental fishes. This can be attributed to the fact that the environment in which they were caught was brackish, with salt contents of about 22 parts per thousand. Fishes inhabiting marine environments tend to concentrate urea in their blood for efficient osmoregulation (Trewavas, 1983).

However, urea is toxic to all living creatures, and high levels of this substance in the blood of *S. melanotheron* can cause harmful effects to the organisms that feed on them, including man (Basaglia, 2000).

Enzyme reactivity was higher in the *S. melanotheron* species caught in the Lagos lagoon, with high values of Alkaline Phosphatase, Aspartate transaminase, and Alanine transaminase, and this indicates stressful conditions in the Lagos lagoon, brought about by pollution. These results are in accordance with Alkahem, et al, (1998), who stated in their work on Toxicity bioassays and changes in haematological parameters of *Oreochromis niloticus* induced by trichloroform, that increased environmental stress on fishes increases the reactivity of liver enzymes.

The values for enzyme reactivity in the experimental fishes was slightly higher than that of the control, for Alkaline Phosphatase, Aspartate transaminase, and Alanine transaminase respectively, and this also agrees with the work of Guerrero, (2008), who stated in his work on Tilapia sex reversal, that the higher levels of liver enzyme reactivity in the sex reversed fishes, is due to the higher protein conversion ratio of sex reversed tilapia fishes. The values of Alkaline Phosphatase, Aspartate transaminase, and Alanine transaminase for the control fishes were found to be within the normal ranges outlined in the work of Gabriel et al. (2007a,2007b), who studied the blood of *S. melanotheron* fishes in freshwater culture systems.

The feeding trial revealed that *S. melanotheron* responded to commercial feeds with crude protein of 56 percent, *S. melanotheron* was able to utilize commercial feed for growth which was also successfully used to rear *S. melanotheron by* Tave, (1990). The rate of feeding was the same in each tank and a daily feed of 5 percent body weight was given three times to each tank daily. The highest mean weight was obtained in treatment tanks as compared to the control tanks were the lowest mean weight gain was recorded, Specific growth rate (SGR) was better in feed treated with hormone having the best SGR in treatment tank and control tank having the lowest SGR, FCR value is lowest and best in treatment tanks. While control tanks

have the highest and worst FCR. The significant difference in growth rate can be ascribed to the inclusion of 17 alpha-methyl testosterone in the feed of the fish in treatment tanks. Compared to the control tanks which were also fed with imported feed but not treated with 17 alpha-methyl testosterone this result is in line with Marjani, et al. (2009) which recorded a maximum gain in body weight i.e., 11.8 g, of *Sarotherodon melanotheron* fed with 17 alpha-methyl testosterone in 90 days.

At the end of 90 days the fry treated with methyl-testosterone shows better growth than fry that are not treated with methyl-testosterone, sex of fry treated with MT was completely reversed from female to male this result helped in cutting the prolific spawning ability of Tilapia which normally results in stunted growth and over population. Hanson et al. (1984) reported that 10-60 ppm MT-treatment showed a better growth than control, these are also in accordance with Dan and Little (2000), who compared the culture performance of different strains of *Sarotherodon melanotheron* and found that considering all strains, MT treatment resulted in a final size of fish 10.7% larger than mixed-sex fish. This result will definitely promote the use of Methyl-testosterone in farming of *S. melanotheron* brackish water specie) as more weight, length and hence growth is achieved in a lesser time compared to normal feed that is not treated with the growth hormone. The grow-out period of *S. melanotheron* is reduced and less feed is consumed thereby increasing income for tilapia producers.

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