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Research Article

Sex Reversal of Tilapia (*Oreochromis niloticus*) Fry by using Methyltestosterone (Mt) Treated Soybean Meal

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ABSTRACT

Experiment was conducted at Centre for Aquaculture-Agriculture Research and Production Pvt. Ltd. (CAARP) Chitwan from August-3, 2021 to December-5, 2021. For the experiment, Completely Randomized Design (CRD) was used having 5 treatments and 3 replications. Methyl testosterone hormone stock solution and hormone treated feed was prepared. Hapa (1 m^3) was set in the pond and 100 fry were stocked in each hapa. Fry are fed with the Methyltestosterone (MT) mixed feed 5 times daily. At final stage individual fish were dissected for sex identification. Economic analysis was carried by calculating B:C ratio of each treatment. It was found that percentage of male and female are same by providing soybean meal instead of fish meal. The higher B:C ratio (1.43 ± 0.03) of feed was recorded in treatment four i.e., 25% FM ± 75% SM. The lowest B:C (1.21 ± 0.04) ratio was recorded in treatment one i.e., 100% fish meal. This study results clearly demonstrate that 75% soybean meal can replace 100% fish meal in feed for sex reversal of Tilapia without decreasing the growth performance and survivability at low cost.
Keywords: Methyltestosterone, Sex reversal, Tilapia, Soybean meal

INTRODUCTION

Tilapia are currently known as “Aquatic chicken” due to their fast growth, adaptability to a wide range of environmental conditions, disease resistance, high flesh quality, ability to grow and reproduce in captivity and feed on low trophic levels [1-3]. Thus, they have become excellent species for aquaculture, especially in tropical and subtropical regions. This species has a huge local demand in Asian domestic markets and also for export [4].

The excessive reproduction of tilapia species leads to overcrowding, competition for food and tilapia stunted the growth in aquaculture system, which resulted in low yields of harvestable size of fish [5,6]. Benefits of mono-sex tilapia culture include faster growth that is about 50% faster than females, fetch higher prices for bigger and uniform fish, and avoid breeding in the culture system diverting reproductive energy to somatic growth [7]. Hormonal sex reversal is a technique of changing of sexes from one sex to another in fish by administering synthetic steroid hormones before and/or during the period of sexual differentiation. In this technique, the first feeding fry are treated with male hormones or androgens (i.e. 17 α-methyltestosterone), which develops testes and male sexual characteristics at maturity, while treatment with female hormones or estrogens (i.e.17-estradiol) produces individuals with ovaries and female characteristics in fish [8]. Monosex male is produced by feeding sexually undifferentiated newly hatched swim-up fry with a special diet containing 60 mg of 17-α methyl-testosterone (MT)/kg of feed in which fishmeal serves as the sole ingredient feeding for 21 days [8,9]. Fish meal is one of the most expensive ingredients in prepared fish diets and its availability as well as price fluctuations are threatening the aquaculture industry [10]. Therefore, in near future fish meal prices will increase and hence the costs of fish production will be higher than at present. Due to sustainability issues, the use of fish meal in aquaculture has been questioned and various attempts have been made to reduce or replace it [8].

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Soybean meal which constitutes higher plant protein feedstuffs will be one of the key ingredients of starter feed for hormone incorporation in sex reversal of Tilapia fry in the future. The aim of this study was to evaluate the suitability of commercially available soybean meal as a partial or complete substitute for fishmeal, with a view to use it as one of the potential dietary ingredients for developing a cost effective supplemental feed for the sex reversal of Tilapia fry [11].

MATERIAL AND METHODS

Location

The experiment was conducted at Centre for Aquaculture-Agriculture Research and Production Pvt.Ltd (CAARP), Khairahani-10, Kathar, Chitwan for 126 days. The site is located 30 km east from Narayanghat. The latitude is 27.5290°N and longitude is 83.35420°E. The research was conducted from August-3, 2021 to December-5, 2021.

Experiment details

The experiment was conducted in Completely Randomized Design (CRD). There were five treatments with three replications each.

Hapa preparation: A hapa is a cage like, rectangular or square net impoundment placed in a pond for holding fish for various purposes, made of fine mesh netting material. The size of hapa was 1 m^3 which was used in this research. They are set at 30 cm above the pond bottom.

Egg collection: Tilapia eggs are collected from the mouth of the female tilapia brood stock in the morning. Solid waste and dirt was removed followed by pre-washing with tap water. Deep cleaning, washing and sieving was carried out and then eggs are transfer to the incubation and hatching jars. Freshly laid eggs are normally yellow in color and it takes about four days to hatch, depending upon the temperature. Each subsequent stage takes approximately one day less to hatch. After hatching takes place, yolk-sac fry was transferred into shallow trays filled with re-circulated clear water. Three days after hatching, and immediately when the egg yolk sac had been absorbed, the swim-up fry was transferred out of the hatchery and stocked into Sex-Reversal Treatment (SRT) nursing hapa.

Methyl testosterone hormone stock solution preparation: To prepare the hormone stock solution, 0.5 g (or 500 mg) of methyltestosterone hormone was weighed out. One liter or 1,000 ml (or cc) of at least 95% ethyl alcohol into a suitable glass container was measured out. Then, 0.5 g (or 500 mg) of hormone into the 1 liter (1,000 cc or ml) of at least 95% ethyl alcohol was dissolved. The mixture was shacked for several minutes until all the hormone was fully dissolved in the ethyl alcohol. The standard stock solution bottles were labeled and put in a fridge at 4°C- 6°C. Kept cool and away from direct sunlight.

Hormone treated feed preparation for tilapia sex-reversal: Soybean grains were roasted, dehull and grinded to make fine powder also fish meal was prepared for experiment. One kg of fine powdered experimental feeds (fish meal and soybean meal) were taken into a clean plastic bowl [11]. Hormone was mixed evenly with the feed at the rate of 60 mg per kg feed either by machine or by hand manually. The treated hormone feed was dry at room temperature to reduce moisture and humidity levels. The hormone treated feed was kept in a labelled, sealed plastic bag or plastic box and stored in a refrigerator at 4°C -6°C for up to 21 days.

Fry stocking and feed application: Total 100 fry are fed with the MT mixed feed at 0.14 g, 0.30 g, 0.50 g and 0.84 g each day for the first five days, on days 6-10, days 11-15 and days 16-21 respectively, the feed was divided into five equal portions or meals for each day to feed 5 times daily. Then after 21 days, normal feeding was conducted for increasing their size to differentiate the sex reversal.

Weight taken: To get individual fry weight, electronic pocket scale was used by taking 10% sample of total population.

Sex determination: Gonadal examination and sex expression was performed according to Guerrero and Shelton as cited by Khanal [12]. Fish were dissected by making a cut near the anus to below the base of the pectoral fin. The entire gonad, located on the dorsal portion of the peritoneal lining was removed carefully beginning ventrally and going forward. All length of gonad was kept on slide and differentiated whether it is male or female. Female was differentiated by its ovary and male was differentiated by testis. Monosex male tilapia was differentiated by slight atrophy and had shorter testis as compared to others.

Economic analysis

Economic analysis was performed to determine the cost effectiveness of the prepared feed incorporated with MT. It was assumed that all other operating costs remained constant and only the cost of MT incorporated feeds used in calculations using price at which it was purchased. Total monosex fingerlings observed in each hapa and their market value was assumed to be the total income. The benefit cost ratios of the different treatments was calculated by using following formula;

$$Total\ feed\ cost = \sum Cost\ of\ feeds(NRs)$$

Where, Total Income=Total number of monosex fingerlings production × Price of monosex fingerlins (NRs/individuals)

B:C ratio with respect to feed only=(Total Income)/(Total feed cost for fish)

Statistical analysis

The data was collected during the course of time and on the basis of individual fish observations, the population means for each growth parameter was computed. The analysis of variance was used to compare different growth parameters using Gen-Stat 15^{th} edition. The mean and standard errors was calculated for each treatment. The data entry was done through MS Excel. The accepted level of significance was $p<0.05$.

RESULTS AND DISCUSSION

Male, female and survivability percentage

The number of fish examined for sex represents the total number of fish which survived in the pond at the end of the study. Analysis of variance on the percentage of male and female, survivability indicated no significant difference ($p>0.05$) among the different treatment means (Table 1). Which were similar to the findings of Cruz and Mair (1994). The androgen mixed with soybean and fish meal produced mean sex conversion rate of 95.4% in hapas. Macintosh et al., reported a same sex conversion of 95.7% in their experimental units stocked by providing Mt treated soybean meal and fish meal. The dose of hormone administrated in the feed was 60 mg/kg in accordance with the study of El-Greisy and El-Gamal, 2012; Singh, they have shown that 60 mg/kg of feed dose from the 7-28 days allows to obtain 95%-97% of males. The availability of phytoplankton as an alternative source of food for fry may have had a slightly adverse effect on the efficacy of the hormone treatment in hapa. Suspension of feed in water causing the possible dilution of hormone and the minimal feeding reaction of fry to the given feed due to the low temperature was also observed. Thus, it is apparent that a small proportion of females are still observed, emphasizing the need for optimizing sex-reversal to produce very high sex conversion rate, preferably in excess of 98% male. Cruz and Mair also mentioned that small portion of female and intersex were observed during sex reversal by hormonal method.

Table 1: Male (%), female (%) and survivability (%) of Tilapia as influenced by using MT treated soybean meal instead of fish meal, 2022.

Treatments	Male ± S.E (%)	Female ± S.E (%)	Survivability ± S.E (%)
T1	89.33 ± 2.19	0.67 ± 0.58	90.3 ± 2.73
T2	90.67 ± 0.58	0.34 ± 0.33	92.0 ± 1
T3	94.12 ± 2.52	0.65 ± 0.33	94.7 ± 2.67
T4	93.31 ± 2.85	0.56 ± 0.67	95.3 ± 2.33
T5	90.32 ± 2.03	0.38 ± 0.58	91.3 ± 1.76
Significance	ns	ns	ns
LSD	6.872	1.050	6.920
CV (%)	4.1	10.3	4.1

Note: CV, Coefficient of Variation; LSD, Least Significant Difference; S.E (±), Standard Error. Figures in the column with the same letter are not significantly different among each other according to DMRT (Duncan's Multiple Range Test) at 0.05 level of significance.

Final weight gain (g), DWG (g), SGR (%) of Tilapia

Final body weight, daily weight gain and specific growth rate were presented in Table 2. The result showed that analysis of variance indicated no significant difference ($p>0.05$) on final weight after 120 days, daily weight gains and specific growth rate among the different treatment means (Table 2).

Table 2: Final weight gain (g), DWG (g), SGR (%) of Tilapia as influenced by using MT treated soybean meal instead of fish meal, 2022.

Treatments	Initial weight(g) ± S.E	Final weight (g) ±	DWG(g) ± S.E	SGR(%) ± S.E
T1	0.047 0.89	23.41 ± 2	0.18 ± 0	2.59
T2	0.049 ± 0.45	23.86	0.19 ± 1	2.61 ± 0
T3	0.052 ± 0.63	17.53 1	0.17 ± 0.02	2.38
T4	0.042 ± 0.40	20.65	0.16 ± 1	2.51 ± 1
T5	0.046 ± 0.48	20.52 .40	0.15 ± 1	2.50
F-Probability	ns	ns	ns	ns
LSD	0.018	5.928	0.047	0.197
CV (%)	21.4	15.4	15.4	4.3

Note: CV: Coefficient of Variation; LSD: Least Significant Difference; S.E (±): Standard Error. Figures in the column with the same letter are not significantly different among each other according to DMRT (Duncan's Multiple Range Test) at 0.05 level of significance.

Economic analysis

Analysis of variance on benefit cost ratio indicated significant difference ($p<0.05$) among the different treatment means. The higher B:C ratio (1.43 ± 0.03) of feed was recorded in treatment four i.e., 25% FM+75% SM which is statistically at par with treatment five. Along with it, relatively lower B:C ratio was found in treatment three i.e., 50% FM+ 50% SM followed by treatment two. The lowest B:C ratio was recorded in treatment one i.e., 100% fish meal. Al-Kenawy clearly demonstrate that soybean meal could completely replace the fish meal in diets for Nile tilapia reared in earthen ponds without negative effects on growth, total production or net return(Table 3).

Table 3: Ratios with respect to feed as influenced by using MT treated soybean meal instead of fish meal, 2022.

Treatments	B:C ratio
T1	1.21 ± 0.04
T2	1.28 ± 0.01
T3	1.37 ± 0.04
T4	1.43 ± 0.03
T5	1.39 ± 0.03
Significance	*
LSD	0.09
CV (%)	4.1

Note: CV: Coefficient of Variation; LSD: Least Significant Difference; Sem (±): Standard error of mean. Figures in the column with the same letter are not significantly different among each other according to DMRT (Duncan's Multiple Range Test) at 0.05 level of significance. B: C ratio with respect to feed as influenced by using MT treated soybean meal instead of fish meal, 2022.

CONCLUSION

Methytestosterone incorporated with soybean meal resulted same sex reversal as produced by fish meal. Growth and survival of fry was also same by giving soybean meal instead of fish meal. The total feed cost was decreased by using soybean meal instead of fish meal. This study results clearly demonstrate that 75% soybean meal can replace 100% fish meal in feed for sex reversal of Tilapia without decreasing the growth performance and survivability at low cost.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

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