Kuroshio Science 11-1, 8-13, 2017

Symposium Proceedings

On-farm trials of phytoandrogen for sex inversion of tilapia

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ABSTRACT

The on-farm trials to test the feasibility of 100% Benguet pine pollen raw powder (T-1) and 50-50% pine pollen and 17 α-methyltestosterone was tested in six small-scale (100-500 m^2) tilapia ponds. Newly hatched fry was fed with hormone-treated feeds for 28 days and then nursery-reared for 30 days before stocked in the grow-out ponds for 3-4 months. Results obtained showed 88% and 83% male inversion under framers condition for 100% pine pollen (T-1) and 50-50% ratio of pine pollen and 17 α-methyltestosterone (T-2). But, on-station trial in concrete tanks reveals 100% sex inversion and survival in both treatments. Although statistical analysis yielded no significant differences, this result demonstrated the effectiveness of Benguet pine pollen (*Pinus kesiya*) phytoandrogen as an alternative source for synthetic hormone used in sex reversal of tilapias. Their use also eliminates possible health risks and pollution of the environment. Even as raw powder, pine pollen had a relative advantage in terms of cost, effectiveness and locally available alternative source hormone; hence, environment friendly. From the economic point of view, even with the raw pine pollen powder plus an additional input cost of PhP 206.65, a net income of PhP 9,231.60 can be realized. Using 50-50 PP-MT resulted to a net income of PhP 7,323.50. The gross margin of using 100% pine pollen is relatively higher (PhP 5,548.20) compared to those using 50-50% MT (PhP 206. 65). The use of 100% PP in sex inversion of tilapias is recommended, however, further studies on the development of pure extract product to solve the problem of quality, storage and shelf life should likewise be carried out.

Key words: On-farm trial, phytoandrogen, pine pollen, sex inversion

INTRODUCTION

Grow-out of monosex male populations is known to prevent or minimizes recruitment and thereby competition between recruits and stocked fish which, in mixed sex populations, can significantly reduce harvested yields. For this reason the use of all male tilapia stock is now well established. There are several approaches have been developed to achieve monosex male populations, direct hormonal sex reversal being the most commonly applied in the industry today, although monosex hybrids and even manual sexing, are also produced in many hatcheries (Mair and Little 1991, Mair *et al*. 1995).

Despite widespread use of the androgen 17 α-Methyl Testosterone (MT) in tilapia farming, the implications of tilapia hormone treatment in relation to human health and the environment is always an issue which has not been well articulated to the general public (Macintosh 2015). As such, alternative sources of hormone become a promising area of research.

The use of different plan-based natural products like phytoandrogen for re-directing the sex of undifferentiated tilapia fry and one of the innovative approaches in the production of all male tilapia stock is the us Benguet pine (*Pinus kesiya*) pollen. Pine pollen is the reproductive part of pine tree which is the richest seedbed of testosterone derived from plants. It is the male sperm of the pine tree, hence, its potential use for sex inversion. A research program entitled "Increasing Productivity of Tilapia Farms Using Product and Technology Innovation under the leadership of Central Luzon State University (CLSU) carried out on-stations studies on various sources of phytoandrogentic plants which showed that pollen from Benguet Pine (*Pinus kesiya*) produced male

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conversion percentages ranging from 87.3-95.1%. This paper reports the result of the on-farm trials conducted by BUTC to validate and confirm the results of the on-station trials done at CLSU using raw pine pollen from Benguet Pine (*Pinus kesiya*) at farmer's level.

MATERIALS AND METHODS

This project is a pilot project designed to validate the studies carried out on-stations against the performance under farmers' level using the same hormone-feed preparation technique.

On-farm trial procedures

A total of six small-scale tilapia farmer-cooperators participated in the on-farm testing using the following on-farm trials namely: Treatment 1 (T-1) − 100% Pine Pollen and Treatment 2 (T-2) − with 50% pine pollen and 50% 17 α -methyltestosterone.

The criteria used in the selection of farmer-cooperator include (1) willingness to participate in the validation trials; (2) availability of two (2) ponds with the size ranging from 100-500 m^2; and (3) with at least 2-3 years of experience in tilapia farming. A lecture-demonstration of the hormone-mix feed preparation and a review of tilapia farming were conducted prior to on-farm testing.

Hormone-mix feed preparation

Preparation of 100% Pine Pollen (T- I). The 100% pine pollen (PP) was prepared by measuring 10 g or 10,000 mg of PP and then transferring it in a test tube for immersion to boiling water for 15 minutes. After which, the 10 g PP is mixed with 500ml 95% ethanol and stirred to mix. The mixture is then covered Cover with aluminum foils for 7 days, and shake lightly to mix on a daily basis. After, the 7th day, a 1,250 grams fry mash was spray with the mixture evenly. Then air-dried for one day and commence feeding the following day. The hormone-mix feed (T-1) was kept covered and placed in an area not exposed to direct sunlight. Guide for feed and PP preparation (Fig. 1).

Preparation of 50% PP and 50% MT (T-2). The same procedure is followed except that 0.5 g of 17 α -methyltestosterone (MT) and 1g of PP powder is used. The 0.5g MT is dissolved in 50ml 95% ethanol and 1 g PP dissolved in 100 ml 95% ethyl alcohol. The mixture is placed in a container, mixed, covered with aluminum foil for 7 days and shake lightly on daily basis. On the 7th day, a 1,250 g fry mash is sprayed with the mixture evenly; air-dried for one day and commence feeding the following day, the remaining feed is stored.

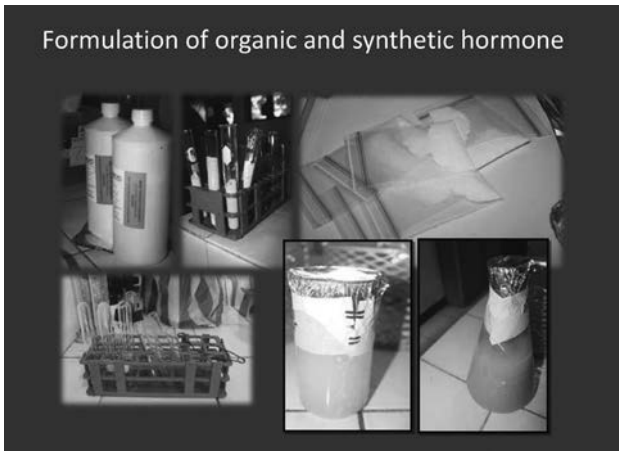

Fig. 1. Materials used in feed preparation.

Product Application

The locally produced hatchery-bred undifferentiated fry was treated (fed) for 28 days in concrete tanks in BUTC hatchery. A 1 m × 1 m × 1.5 m fine mesh net cage was installed in the concrete tank with aeration. The fry was stocked at a density of 500 pcs/net. The hormone treated feed was introduced on the following day to enable the fish to adjust in the new holding environment. A 28-days *ad libitum* feeding was employed. The inclusion of positive and negative control is not included.

Evaluation

After the 28 days sex inversion treatment, the fingerings was transferred in nursery area for one month. After which, the fingerlings were distributed to the project cooperators for grow-out another three months rearing in ponds at 5 pcs/m^2 in 200-1,000 m^2 pond. The grow-out phase makes use of farmers' practice in terms of pond preparation and management.

The evaluation under grow-out was done on-site by getting a random sample of 30 fish and carefully examining their external papillae for the opening of an oviduct and based on the appearance of the external genital papilla for the male, and internally by dissection. Examination of gonad: fish with gonads that have testicular tissue or fish with female papilla but have gonads comprised only of testicular tissue are males. The presence of recruits is deemed an indication that the treated population had not achieved all male population.

RESULTS

Effectiveness of the phytoandrogen under farmers' condition

Results from on-farm trials involving 6 farmer-

cooperators are shown in Table 1. The grow-out trials obtained an 88% and 83% male inversion, respectively from 100% PP (T-1) and 50-50% ratio of PP and 17 α -methyltestosterone (T-2). However, statistical analysis (*t*-test at 5% level of significance) showed no significant differences in terms of sex inversion, total harvest, ABW and survival under farmer's condition. Both produced recruit, indicating the occurrence of reproduction among the stock. This could be attributed to several factors which include among others the quality of PP powder and storage, feed processing, reliability of sexing and differences in farming practices.

To further validate its effectiveness, an on-station trail using 3 m × 3 m × 1.5 m concrete tanks concrete tanks using similar treatments was carried out. Result obtained showed a 100% sex inversion and survival for those fed with 100% PP and 50-50% PP and 17 α-methyltestosterone combination (Table 2). No recruits were observed in both treatments.

The success in sex inversion and higher survival obtained from the on-station trial could be attributed to the complete control of the stock compared to the farmers condition. However, in terms of ABW, those reared under farmers condition is realtively bigger.

Feasibility and economic viability of technology applications

To measure the feasibility and economic viability of using PP from Benguet Pine (*Pinus kesiya*), a simple comparative cost and return analysis (Table 3). Using fingerling treated with 100% PP and 50-50 PP-MT resulted to a net income of PhP 9,231.60 and PhP 7,323.50, respectively. This translates to a difference of some PhP 1,908.10 in the net income.

Similarly, although an additional input cost of PhP 206.65 is incurred in the farm using 100% PP, the proportion of revenue converted into gross profit (gross margin) is relatively higher at PhP 5,548.20 compared to those using the 50-50% MT with PhP 206.65 gross margin.

From the results obtained, the use of phytoandrogentic

Table 1. Overall performance in terms of sex inversion, total harvest, ABW and survival under farmer's condition.

Treatment	Harvest (kg)	ABW (g)	Survival (%)	Sex Inversion	
				M	F
100% Pine Pollen	143.5	244.4	82.8	88	13
50-50% PP-MET combination	120.0	188.8	68.5	83	17

Table 2. On-station performance in terms of sex inversion, total harvest, ABW and survival.

Treatment	Harvest (kg)	ABW (g)	Survival (%)	Sex Inversion	
				M	F
100% Pine Pollen	12	118	100	100	0
50-50% PP-MET combination	10	114	100	100	0

Table 3. Costs and returns analysis.

Items	Treatment	
	100% PP	50-50 PP-MET
1. Return		
Cash		
Tilapia Sold (kg)	143.50	120.00
Price (PhP)	90.00	90.00
Total Returns	12,915.00	10,800
2.Cost		
Cash costs		
Variable Cash Costs		
Fingerlings	508.75	715.00
Feeds	2,825.90	2,321.75
Labor	225.00	300.00
Miscellaneous	123.75	140.00
Total Costs	3683.40	3476.75
Net Income	9,231.60	7,323.25
Gross Margin	5,548.20	3,846.50

Table 4. Comparative advantage of the use of phytoandrogen.

Comparison	Treatment	
	100% pine pollen[1]	50-50 pine pollen-MT[2]
Percentage Conversion (%)	88	83
Cost per gram of hormone used (PhP)	400.00	125.00 (40 PP-85.00MT)
Availability	Locally available in crude extract	17 α-methyltestosterone is imported synthetic hormone
Environmental impact	Organic, without possible health risks; environment friendly	50% synthetic with possible health risks; may alter the dynamics of natural population and pollution of the environment

[1] Pine pollen @PhP 40.00/g

[2] 17 α-methyltestosterone @PhP 170.00/g

plants from PP Benguet Pine (*Pinus kesiya*) demonstrated the feasibility of its utilization in the sex inversion of tilapia fry. As an alternative source of male hormone in the masculinization of tilapia, it is comparable to 17 α-methyltestosterone in terms of male conversion success. The use of PP also offers other advantages worthnoting as shown in Table 4.

From the economic aspect, raw PP powder provide by CLSU only cost about PhP 40.00/g compared to 17 α-methyltestosterone which cost PhP 170.00/g, a difference of PhP 130.00/g with comparable male conversion success of 88% and 83%, respectively for T-1 and T-2.

The use of PP powder is also environmental-friendly being an organic-based plant hormone compared to 17 α-methyltestosterone which is synthetic or chemically prepared, hence, the usual danger of possible health hazards although its use to induce sex reversal in farmed tilapias has become a common practice in many parts of the world. In terms of availability, PP powder from Benguet Pine (*Pinus kesiya*) can be locally manufactured unlike the 17 α-methyltestosterone which is an imported synthetic hormone. *Pinus kesiya* is part of the vegetative cover in Baguio, Mountain Province in Luzon and Bukidnon in Mindanao.

Factors affecting the efficacy and acceptability

There are a number of factors observed to have influenced the efficacy and quality of the phytoandrogen and this include (1) storage, (2) the hormone-feed mixing, (3) possible error in sex examination, and (4) differences in farming techniques practiced.

The Benguet pine pollen (*Pinus kesiya*) contains a natural substance called phytoandrogen which has similar effects as androgen in animals. Although proven to have similar effect as 17 α-methyltestosterone, sex inversion efficacy maybe affected storage. Prolong and improper storage can affect quality (hormone) of the pine pollen under storage.

Sex inversion effectiveness also depends on the quantity of hormone-treated feed ingested or consumed while the quantity of hormone in the feed mix depends on the hormone-feed-mixing process. In the case of sex determination, physical examination is not a guarantee or reliable means of sexing because treated tilapias appear physically and functionally as male but possess the female genotype (XX). Thus, dissecting the fish for gonad examination becomes necessary.

The differences in farming techniques practiced by cooperating farmers were deemed as source of variation. This occurred because the farmer-cooperators simply followed their own farming practices; hence, pond preparation varies which can influence the presence or absence of natural fish food organism, predators and recruits from the previous farming season.

DISCUSSION

The on-farm trials demonstrated the effectiveness of Benguet pine pollen (*Pinus kesiya*) phytoandrogen as an alternative hormone source for the usual synthetic hormone used in sex reversal of tilapias as evidenced by the comparable percentages of male conversion of 88%. The success in sex inversion and higher survival obtained from the on-station trial further suggests its effectivenes pine pollen as alternative organic hormone source for sex invesion of tilapias. This findings clearly points out to the feasibility Benguet pine pollen (*Pinus kesiya*) for sex inversion. In Thailand, Red Kwao Krua is used as an effective replacement for synthetic hormone in tilapia sex reversal (Anocha Kiriyakit 2014). However, there are several areas that need further studies particularly on the developing pine pollen (PP) pure extract product to solve the problem of quality, storage and shelf life.

From economic perspectives, the raw PP powder provided by CLSU cost PhP 40.00/g but since the amount of PP used in 100% pine pollen (T-1) is 10 g, the total cost of hormone alone is about PhP 400.00/g. Comparatively, in 50-50% PP-MT combination (T-2), the amount of hormone used is 1 g PP + 0.5 g MT, at PhP 40.00/g of PP and PhP 170.00/g of MT, the total cost of hormone is only PhP 125.00/g.

Assuming that both made use the same amount of feed (fry mash) and silvent (ethanol), T-2 turned out to be cheaper in as much as there is not significant differences in terms of male inversion percentages obtained.

On the other hand, the use of PP also offers several advantages and the application of PP for masculinization of tialpias at the farm level showed several advantages and economic promise aside from male sex inversion effectiveness at on-farm trials although not statistically signifcant. Similar male conversion pattern was also obtained in Nueva Ecija trials with a 100% and 98-100% male conversion for 100% pine pollen fed and 50-50% pine pollen 17 α -methyltestosterone combination (Velasco *et al*. 2016). Even with the oral administration of 17 α -methyltestosterone treated feed (30 to 60 mg MT/kg feed) to tilapia fry for 21-28 d has yielded populations comprising no more than 5% females under a variety of protocols (Phelps and Popma 2000).

Simple cost and return analysis from the on-farm trails showed that using fingerling treated with100% PP (T-1) and 50-50 PP-MT (T-2) resulted to a net income of PhP 9,231.60 and PhP 7,323.50, respectively. This translates to a difference of some PhP 1,908.10 in the net income despite the additional input cost of PhP 206.65 incurred. It should be worth noting that the proportion of revenue converted into gross profit (gross margin) is relatively higher at PhP 5,548.20 compared to those using the 50-50% MT with PhP 206.65 gross margin.

The use of 17 α -methyltestosterone to induce sex reversal in farmed tilapias has become a common practice in many parts of the world. Interestingly, the implications of tilapia hormone treatment in relation to human health and the environment have not been well articulated to the fish trade, or the general public (Macintosh 2015). However, increasing concern about its implications on human health risk and environment is of paramount importance to the general public. Therefore, an effective alternative is a welcome development.

Benguet pine pollen (*Pinus kesiya*) contain high amount of phytoandrogens which has similar effects as androgen in animals. Being a natural substance, proper storage needs to be managed to maintain quality. In the present study, the PP powder used was obtained from the Freshwater Aquaculture Center − Central Luzon State University (FAC-CLSU) which was stored for sometimes as evidence by its yellow-orange color. As such, the prolonged storage may have caused changes in the chemical composition of the powder, hence, its hormone quality and efficacy. In this context, studies on the purified extract shelf life and efficacy is necessary.

The effective dose for sex inversion is dependent on the daily quantity of feed ingested or consumed as well as the quantity of hormone in the feed mix. The exact daily hormone intakes are difficult to accomplish. Moreover, knowing the exact weight and number of fry on a given day is difficult to determine. Besides, fish appetite may vary from day to day. In addition, in an outdoor setting where natural food is available, the efficacy of phytoandrogen depends largely on the dosage and feed intake. In controlled experiment, male conversion percentage of 95 and 90% for 160 and 320 mg/kg dosages respectively was noted (Velasco *et al*. 2016). In semi-controlled test, 80 (160 mg/kg), 85 (320 mg/kg) and 86 (640 mg/kg) phenotypic males was obtained (Velasco *et al*. 2016). The presence of natural food available in the culture environment is also a factor that may influence the efficacy due to food intake. Hormone-feed mixing may also result to uneven distribution of hormone in feed mix which in turn affects sex inversion success.

The sex inversion performance may also be influenced by the preparation of feed-hormone mixture. If not thoroughly mixed, the distribution of phytoandrogen in the feed mix may have been unevenly distributed which in turn affects hormonal intake during feeding resulting to poor hormone reaction and sex re-direction in the process. This inadequacy in the mixing process could be due to the manual mixing process employed; thus, the possibility that some fry may have ingested feed with very minimal hormone that could not influence the undifferentiated fry to be *phenotypic* males.

Although tilapia can be easily sorted into males and females which is distinguishable by visual inspection of the urogenital pores (anal papilla or the oviduct), errors can easily be made possible. It should be noted that tilapia larvae fed with mixed with male hormone will develop into phenotypic male which appears physically and functionally males but possesses the female genotype (XX) (Phelps and Popma 2000). As a matter of fact, observation showed that hormone-treated tilapia gonad does not always correspond with the shape of the papilla. In addition, the small number of sample size taken can also contribute to misinterpretation of efficacy. To insure sex determination, the sample fish was dissected and entire gonad located on the dorsal portion of the peritoneal lining was taken and gonads carefully examined ovary with apparent eggs that may be seen in the body cavity or trace of testicular tissue in the case of males.

The differences in the farming techniques of farm cooperators resulted to the variation in the average body weight because the trials were conducted where farmer applied his normal management procedures, harvesting the fish at a time of his choice. In most cases, farmers providing supplemental feeds produced relatively bigger fish. Tilapia recruits' was noted in almost all of the farmer cooperators. Observation also showed that since they are mostly engaged in backyard farming, pond preparation may have not been so extensively done; hence, recruits could possibly come from the remaining stock from the previous farming cycle. In a similar study done by Bolivar *et al*. (2008) noted that the availability of

phytoplankton as an alternative source of food for fry may have a slightly adverse effect on the efficacy of the hormone treatment in hapas, hence, small proportion of females are still observed. To verify this, an on-station trial using 3m x 3m x 1.5m concrete tanks was conducted. Results obtained showed 100% survival and sex inversion for those fed with 100% PP (T-1) and 50-50% % PP-MT (T-2). It is interesting to note that indeed, farmers practice may have something to do with the occurrence of recruits and therefore success in sex inversion.

On the aspect of farmer's acceptability, observation showed that a lot of tilapia farmers are interested to try sex reversed tilapia fingerlings for their backyard farms. In fact, some had the opportunity to stock with promising feedbacks. Others are interested with the hormone-mixed feed.

CONCLUSION AND RECOMMENDATION

The on-farm trials demonstrated the effectiveness of Benguet pine pollen (*Pinus kesiya*) phytoandrogen as an alternative hormone source for the usual synthetic hormone as evidenced by the comparable percentages of male conversion of 88%. However, there are several areas that need further studies particularly on the developing pine pollen pure extract product to solve the problem of quality, storage and shelf life.

The use of natural phytoandrogen also eliminates the apprehension of possible health risks and pollution of the environment without preempting the conduct of scientific studies for a more conclusive disclosure. From the economic standpoint, even with the raw pine pollen, a net income of PhP 9,231.60 can be realized despite the additional input cost of PhP 206.65. Moreover, the gross margin of using 100% pine pollen is relatively higher (PhP 5,548.20) compared to those using 50-50% MT (PhP 206.65). On this basis, the use of 100% PP is therefore recommended in the sex inversion of tilapia. Further studies are also recommended on the development of pure extract product to solve the problem of quality, storage and shelf life.

ACKNOWLEDGMENTS

This project is funded by the Philippine Council Agriculture Aquatic and Natural Resources Research and Development (PCAARRD) under the program "Increasing Productivity of Tilapia Farms Using Product and Technology Innovation" of Central Luzon State University (CLSU), Muñoz, Nueva Ecija. The authors would like to extend their warmest gratitude to Dr. Tereso A. Abella, Dr. Ravelina R. Velasco and Project Staff for their support and assistance.

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