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Formulation of Bovine Testes Meal and Karamunting Leaves Extract (*Melastoma malabathricum*) on the Masculinization of the Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

Bovine testes meal contains natural testosterone hormone which plays a role in the process of male sex differentiation. *Melastoma malabathricum* L. or rose mrytle, karamunting (Indonesian) plant has bioactive compounds that can increase testosterone levels in male rats. This study was conducted to examine the use of bovine testes meal and karamunting leaves extract as an alternative source of hormones in the masculinization process in the Nile tilapia. This study used tilapia aged 5-10 days, reared in tanks with a stocking density of 1 fish/L. This study used a completely randomized design with 4 treatments and 3 replicates. The treatments consisted of a control (commercial feed only), P1 (70% bovine testes meal + 30% commercial feed with 1.5% karamunting leaf extract), P2 (50% bovine testes meal + 50% commercial feed with 1.5% karamunting leaf extract), and P3 (30% bovine testes meal + 70% commercial feed with 1.5% karamunting leaf extract). As a result, the percentage of males and absolute weight growth were observed with significantly different values, while the survival rate showed values not significantly different between treatments. The water quality parameters were maintained within the optimal range for tilapia fry: temperature at 27–28°C, pH 7.0, ammonia at 0.25mg/ L, and dissolved oxygen at 8.0mg/ L. The administration of bovine testicular meal and karamunting leaf extract can be used for the masculinization of tilapia.

INTRODUCTION

The Nile tilapia (*Oreochromis niloticus*) is a species of fish that has economic value and is an important commodity in the freshwater fish business **(Simanjuntak *et al.*, 2018; Simanjuntak *et al.*, 2022a, 2022b)**. This fish is also one type of cultured fish that is growing rapidly in Indonesia since it serves as an important source of animal protein for the community **(Rachmawati *et al.*, 2010)**. Tilapia has high economic value and has become one of the commodities that provide large role in fisheries production. The great potential for tilapia aquaculture activities is evidently promising in the future, especially

for monosex tilapia farming. Biologically, the growth rate of male tilapia is faster than female tilapia (sex dimorphism) **(Zairin, 2002: Ayuningtyas, 2014)**. Empirical data show that the use of monosex males in tilapia farming will provide better production than mixed-sex populations **(Ariyanto *et al.*, 2010)**.

One of the obstacles that often arises in tilapia farming is the low growth of cultured fish **(Simanjuntak *et al.*, 2018)**. Female tilapia have lower productivity compared to male tilapia, because in females the energy allocation will be greater for reproduction than growth **(Angienda *et al.*, 2010)**. As a result, the size of the yield is very diverse and the harvest weight obtained is not maximized, causing high production costs. The solution to overcome these problems is to develop an alternative cultivation method for single-sex fish rearing, only raising male tilapia seeds. This is because male tilapia grows faster and is larger in size than female tilapia **(Rachmat *et al.*, 2018)**.

The production of male tilapia (masculinization) is absolutely necessary to maximize the growth out of tilapia. The method used in masculinization in this study is the oral method where the test treatment is carried out by giving bovine testes meal in the feed. Bovine testes meal contains the natural hormone testosterone which is needed to stimulate the male sex differentiation process **(Rachmat *et al.*, 2018)**. The bovine testes originally was reported for the increase masculinization in fish. However, the research study using bovine testes to masculinization have been widely reported. However, the formulas are still not optimal to increase masculinization. Current studies report that the local and natural plant may potentially contain steroid, which can be clearly increasing reproduction factor, including sex reversal. However, the combination of the testes ingredients with these natural plants to optimize masculinization has not yet been reported.

Melastoma malabathricum or karamunting (karamunting, Indonesian name) is a plant which originates from Asia and the Pacific Islands. The plant is also known as medicinal plant by local people **(Joffry *et al.*, 2012)**. The plant has demonstrated efficacy in enhancing reproductive parameters, including improved fertility and uterine strengthening **(Koay, 2008)**. Additionally, it has been shown to increase spermatozoa concentration and motility while elevating testosterone levels in male rats **(Balamurugan *et al.*, 2013)**. The potential of karamunting leaves as a maturation stimulator in the vannamei shrimp has been reported by **Ridwan *et al.* (2015)**. Furthermore, **Farizah *et al.* (2017)** reported that karamunting leaves extract can affect the process of ovarian maturation in *Scylla olivacea*. Based on the literature study above, research was conducted to examine the use of bovine testes meal and karamunting leaves extract as an alternative source of hormones in the masculinization process in the Nile tilapia.

MATERIALS AND METHODS

The research was conducted at Babakanjawa Fish Seed Center, Majalengka, West Java, Indonesia. Materials used included one hundred and twenty tilapia larvae (10 days after hatching), bovine testes meal, karamunting leaves extract and commercial feed. The experiment was conducted in rectangular concrete tanks (60 × 50 × 40cm) under controlled conditions: water temperature maintained at 25-30°C, continuous 24-hour aeration (maintaining dissolved oxygen at 5mg/ L), and twice-daily feeding (at 09:00 and 16:00h) at 5% of body weight. The treatment consisted of four treatments with three replicates, including:

- Control : 0% Bovine testes meal with 0% rose myrtle extract
- P1 : 70% Bovine testes meal + 30% Commercial feed with 1,5% rose myrtle extract
- P2 : 50% Bovine testes meal + 50% Commercial feed with 1,5% rose myrtle extract
- P3 : 30% Bovine testes meal + 70% Commercial feed with 1,5% rose myrtle extract

The parameters that were observed include:

1. **Percentage of masculization (Zairin, 2002)**
   The percentage of male fish represents the chance of masculinization occurring after treatment.

$$\text{Percentage of male sex (\%)} = \frac{\text{£ Fish Male } (\textit{sampling})}{\text{£ } \textit{total} \text{ fish sampling}} \; x\, 100\%$$

2. **Survival rate (Simanjuntak *et al.*, 2018)**
   Fish survival rate describes the chance of fish survival that occurs after treatment

$$SR\ (\%) = \frac{Nt}{No}\ x\ 100\%$$

   Where:
   SR = Survival rate
   Nt = Amount of fish in end of treatment
   No = Amount of fish in early treatment

3. **The growth out of tilapia (Simanjuntak *et al.*, 2022)**
   The growth out was measured as the delta of body weight from the initial to the final treatment:

$$W = Wt - Wo$$

Where :
W = The Growth Out
Wt = Average of final body weight.
W0 = Average of initial body weigth.

4. Water quality parameters

Water quality monitoring included four key parameters: (1) temperature, (2) pH, (3) dissolved oxygen (DO), and (4) ammonia concentration, all analyzed in compliance with Indonesian National Standard SNI 6141:2009.

5. Data analysis

All collected parameters were analyzed using one-way ANOVA, with post-hoc Duncan's Multiple Range Test (DMRT) performed using SPSS version 23 for further comparison of means.

RESULTS

The provision of bovine testes meal and karamunting leaves extract in the process of masculinization in tilapia fingerlings has a significant effect. The percentage of male tilapia observed is presented in Fig. (1).

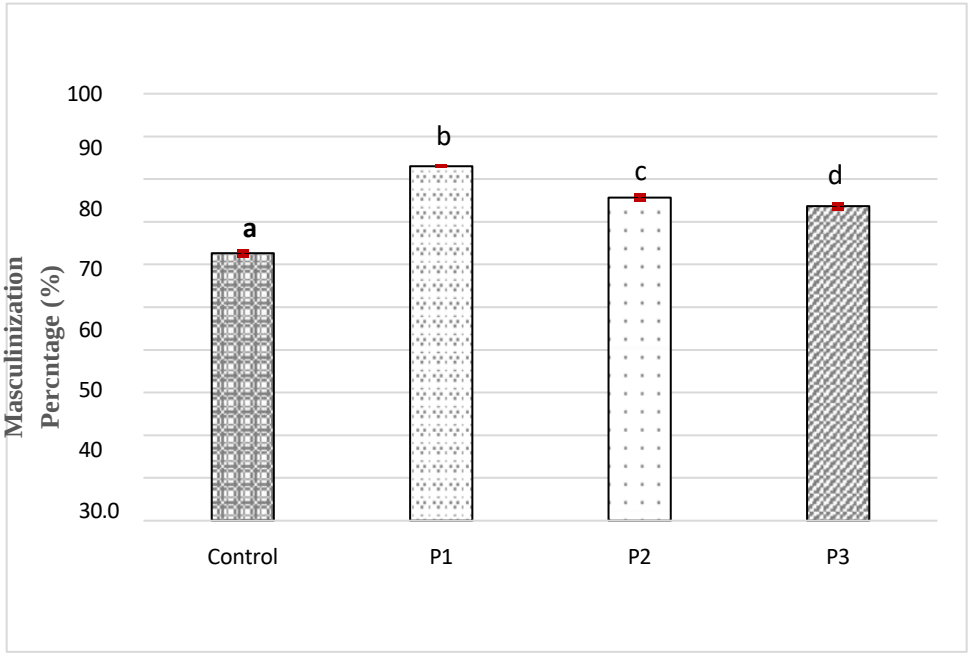

Fig. 1. Rate of masculinization in tilapia. The numbers followed by superscript (^{a,b,c,d}) indicate significant differences between columns at each treatment level with 95% confidence level

Based on Fig. (1), the rate of masculinization in treatment P1 reached 80% compared to the other treatments. The success rate of the masculinization process obtained; control at 62.7±0.5%, P1 at 83.0±0%, P2 at 75.7±0.5%, P3 at 73.7±0.5%. The success rate of the masculinization process was higher in all treatments compared to the control. Based on the statistical results, the provision of formulation bovine testes meal with karamunting gives a significant effect when compared to the control. Following significant ANOVA results, post-hoc Duncan's Multiple Range Test (DMRT) revealed that treatment P1 showed statistically significant differences ($P< 0.05$) compared to all other treatments. These results indicate that the formulation of bovine testes meal with karamunting meal has a significant effect on the masculinization process in tilapia.

The enhanced masculinization treatment significantly influenced absolute weight gain in tilapia. The growth performance results throughout the study period are presented in Fig. (2).

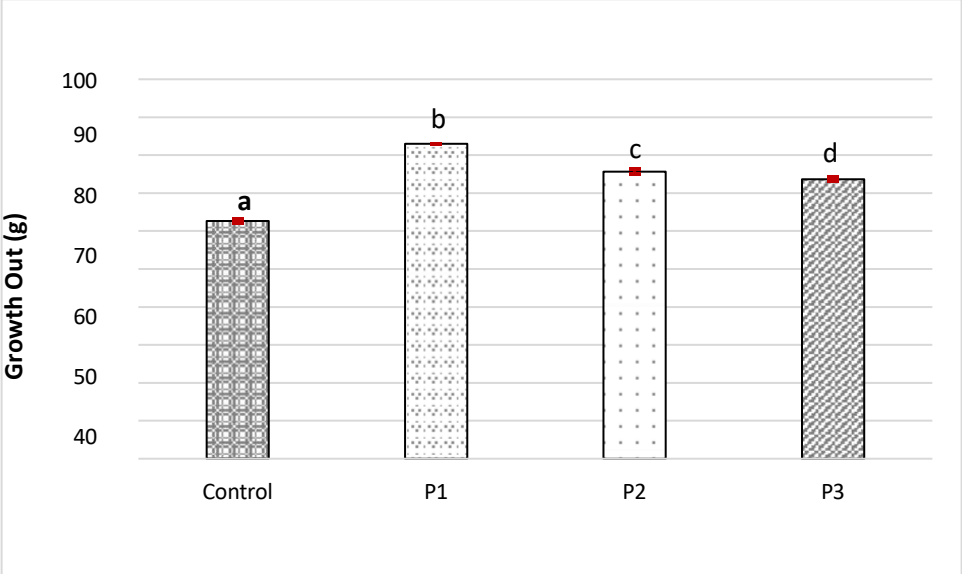

Fig. 2. Growth out of tilapia. The numbers followed by superscript (^{a,b,c,d}) indicate significant differences between columns at each treatment level with 95% confidence level

Tilapia rearing during the study period showed an increase in the average weight of individuals in each treatment. Absolute weight gain varied significantly across treatments ($P< 0.05$). While controls reached 1.20 ± 0.00g, supplemented treatments showed progressive increases: P1 (1.60 ± 0.05g) > P2 (1.40 ± 0.03g) > P3 (1.30 ± 0.06g).

The formulation of bovine testes meal and karamunting produced better growth out than the control. Based on Fig. (2), the results show that treatment 1 has more optimal

weight growth compared with other treatments. The absolute weight growth of tilapia showed that the control treatment was significantly different from the P1 and P2 treatments, while the P3 treatment was not significantly different. The highest absolute weight increase was obtained in P1 with 1.6g compared to other treatments.

The increase in tilapia growth was also supported by the survival rate of tilapia during the study. Survival is an important parameter in the maintenance of test fish, so that the final results of the treatment of test fish can be known. The results of observations of tilapia survival during the study are presented in Fig. (3).

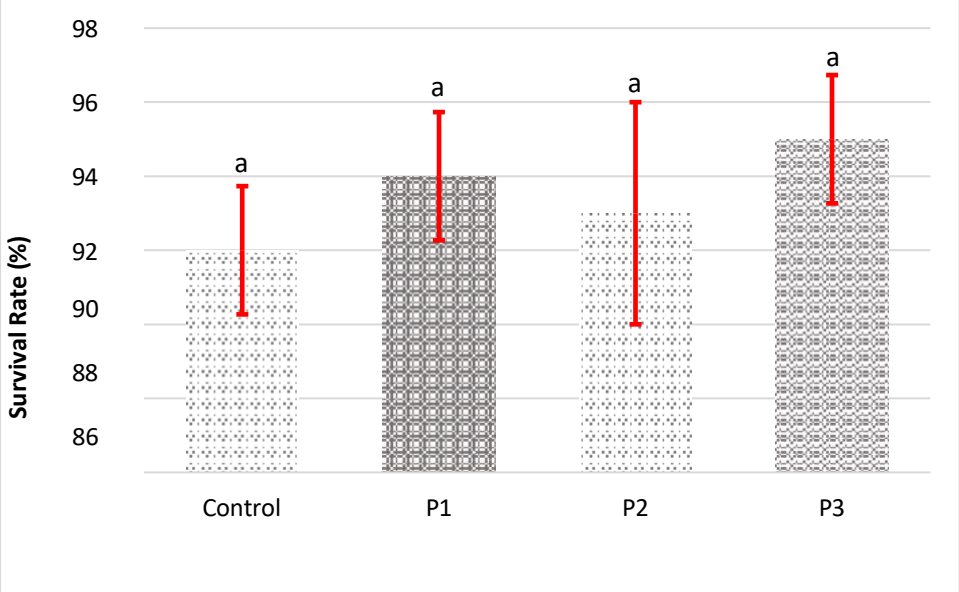

Fig. 3. Survival rate of tilapia. The numbers followed by superscript ([a]) indicate no statistical significant differences between columns at each treatment level with 95% confidence level

Survival rate of the tilapia at the end of the study was still high ranging from 92 to 95%. The survival rate of fish given bovine testes meal and karamunting leaves extract (P1, P2 and P3) was statistically not significantly different from the control. Fig. (8) presents the survival rates observed during the study period: control at 92.0 ± 1.7%, P1 at 94.0 ± 1.7%, P2 at 93.0 ± 3.0%, and P3 at 95.0 ± 1.7%.

Water quality is very influential on fish survival and growth. Its also affects the formation of male sex when tilapia is still in a labile period when sex differentiation takes place. Therefore its important to manage water quality parameters during the study. During tilapia rearing, the water quality of the fish rearing media both during treatment

and maintenance was still within the range that did not interfere with the growth and survival of tilapia. The temperature range was 27-28°C, the pH value of the water obtained during the study was 7, the dissolved oxygen value was around 8.0mg/ L, and the ammonia value was 0.25mg/ L. These values meet the requirements for tilapia rearing according to SNI 6141-2009.

DISCUSSION

Based on Fig. (1), the success rate of sex reversal in P1 reached 80% compared to the other treatments. This is presumably because the fed formulation that has been made can be utilized well by the cultivan. The success of supplementation is highly dependent on the time interval of gonad development, which is when the gonads are in a labile state, so they are easily influenced by these additional ingredients **(Hunter & Donaldson, 1983; Yamazaki, 1983)**. An attempt to change the sex ratio of each species must be carried out at the right time and for the right period, because this is related to the differentiation sex that is unique to each species.

The success rate of the masculinization process was higher for all treatments compared to the control (Fig. 1), presumably related to the levels of the testosterone contained in the bovine testes meal and karamunting leaves extract added to the commercial fed. Bovine testes have testosterone levels of 10.01mcg/ g **(Muslim, 2010)**. In addition, karamunting leaves are able to increase the level of testosterone which plays a role in the process of sex differentiation, in accordance with what was found by **Balamuragan *et al.* (2013)**, the administration of karamunting leaves extract to male rats increased testosterone levels and spermatozoa motility.

Factors that affected the success of sex reversal/masculinization or monosex aquaculture are size and age, environment, length of treatment **(Phelps & Popma, 2000)**, fish species, genetics, hormone type and dose **(Dunham, 2004)**. In this study, the higher the percentage of bovine testies meal used, then the higher the success of becoming a male. In addition, rose mrytle leaves are able to increase the level of testoterone which plays a role in the process of sex differentiation or masculinization.

Tilapia rearing during the study showed an increase in the growth out in each treatment. Energy allocation from feed is used to support growth after energy needs for maintenance are met **(Lestari *et al.*, 2013)**. Biologically, the growth rate of male tilapia is faster than female tilapia (sex dimorphism) **(Zairin, 2002; Ayuningtyas, 2014)**. Female tilapia have lower productivity compared to male tilapia, because in females the energy allocation will be greater for reproduction than growth **(Angienda *et al.*, 2010)**. The percentage of male fish and growth is one of the important indicators of aquaculture

performance that can be used to measure the success of aquaculture itself. The percentage of males greatly affects the level of population growth of cultured fish, this is because the growth rate of male fish is faster than female fish **(Popma & Masser, 1999; Phelps & Popma, 2000; Dunham, 2004; Manosroi *et al.*, 2004; Shalaby *et al.*, 2007; Simanjuntak *et al.*, 2022b)**.

The absolute weight gain results showed significant differences among treatments (Fig. 2). This suggests that: (1) the mixed feed formulation was effectively utilized by the cultivar, and (2) varying doses of testicular meal influenced masculinization success. Changes in male sex are directly proportional to the growth of the cultivar. In the P1 treatment, the additional dose of bovine meal was greater than the other treatments (P2 and P3). According to **Nakamura *et al.* (1998)**, the administration of steroid hormones with low doses will not be able to form a maximum male population, and cause the formation of intersex individuals. This is supported by the levels of proximate results of bovine testes meal in dry weight: protein at 76.26%, fat at 13.40%, ash content at 7.41%, crude fiber at 0.02% and BETN at 2.91% **(Muslim, 2010)**.

The high protein content in bovine testes meal also affects the growth of the test fish. Fish growth tends to increase with increasing percentage of bovine testes meal. In addition to bovine testes meal being able to change the sex toward male individuals in tilapia, it also plays a role in growth. Bovine testes meal serves as a source of hormones for masculinization (genetic function), as well as having a somatic function (growth) **(Muslim, 2010)**. The masculinization process using hormones or synthetic chemicals only functions genetically **(Muslim, 2010)**.

The increase in masculinization results and growth out is also followed by an increase in the survival rate value (Fig. 3). Survival rate value indicates that the provision of bovine testes meal formulations and karamunting leaves extracts containing testosterone and steroids can increase the body resistance of tilapia. Increasing endurance will affect the process of masculinization and growth of tilapia **(Simanjuntak *et al.*, 2018; Simanjuntak *et al.*, 2022a; Simanjuntak *et al.*, 2022b)**.

CONCLUSION

The study demonstrates that a feed formulation containing 70% bovine testes meal supplemented with 1.5% karamunting leaf extract optimally enhances tilapia masculinization. This natural combination proves effective as an alternative to synthetic testosterone for monosex seed production, offering a sustainable solution for aquaculture operations.

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