

**IMPROVING THE REPRODUCTIVE PERFORMANCE OF
SNAKEHEAD FEMALE BROODSTOCK (*Channa striata*) USING A
COMBINATION OF DIET WITH SUPPLEMENT PROBIOTIC
EM4 (EFFECTIVE MICROORGANISM-4) AND
NONI FRUIT (*Morinda citrifolia*)**

Peningkatan Kinerja Reproduksi Induk Betina Gabus (*Channa striata*) Melalui
Pemberian Pakan Bersuplemen Produk Fermentasi Kombinasi Probiotik EM4
(Effective Microorganism-4) dan Buah Mengkudu (*Morinda citrifolia*)

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ABSTRACT

One of the main limiting factors in the reproduction of snakehead fish (*Channa striata*) is that the female broodstock fails to spawn, because they cannot complete the final stages of vitellogenesis and ovulation naturally. Therefore, a breakthrough is needed in snakehead fish hatchery to increase the percentage of female broodstock successfully spawning. The aim of this study was to evaluate the effect of providing fermented products combined with probiotic EM4 (Effective Microorganism-4) and noni fruit in feed on the reproductive performance of female broodstock snakehead fish. This research uses non-factorial experimental methods. The design used was a Completely Randomized Design (CRD) with three treatments and three replications, namely treatment A (feed without supplementation of the fermented product with a combination of EM4 probiotics and noni fruit), treatment B (feed with supplementation of the fermented product with a combination of EM4 probiotics and noni fruit at a dose 10%), and treatment C (feed supplemented with fermented products combined with EM4 probiotics and noni fruit at a dose 20%). The results of the research showed that the addition of snakehead female broodstock feed with a fermented product combining EM4 probiotics and noni fruit at a dose of 10% gave the best results, namely an average gonad maturity index (IKG) value of 2.56%, relative fecundity of 3562 eggs per female broodstock, fertilization rate 87.33%, hatching rate 91.67%, and egg yolk absorption time 78 hours, and prolarva survival rate 98.67%.

Keywords: female broodstock of *Channa striata*, noni fruit, reproduction performance, probiotic EM4.

ABSTRAK

Salah satu faktor pembatas utama dalam reproduksi ikan gabus (*Channa striata*) adalah induk betina gagal memijah, karena tidak dapat menyelesaikan tahap akhir vitelogenesis dan ovulasi secara alamiah. Oleh karena itu, diperlukan suatu terobosan dalam pembenihan ikan gabus guna meningkatkan persentase induk betina berhasil memijah. Tujuan penelitian ini untuk mengevaluasi pengaruh pemberian produk fermentasi kombinasi probiotik EM₄ (*Effective Microorganism-4*) dan buah mengkudu pada pakan terhadap kinerja reproduksi induk betina ikan gabus. Penelitian ini menggunakan metode eksperimen non-faktorial. Rancangan yang digunakan adalah Rancangan Acak Lengkap (RAL) dengan tiga perlakuan dan ulangan tiga kali, yaitu perlakuan A (pakan tanpa suplementasi produk fermentasi kombinasi probiotik EM₄ dan buah mengkudu), perlakuan B (pakan dengan suplementasi produk fermentasi kombinasi probiotik EM₄ dan buah mengkudu dosis 10%), dan perlakuan C (pakan dengan suplementasi produk fermentasi kombinasi probiotik EM₄ dan buah mengkudu dosis 20%). Hasil penelitian menunjukkan bahwa penambahan pakan induk betina gabus dengan produk fermentasi kombinasi probiotik EM₄ dan buah mengkudu dosis 10% memberikan hasil terbaik, yaitu rerata nilai indeks kematangan gonad (IKG) 2,56%, fekunditas relatif 3562 butir telur/induk, derajat pembuahan telur 87,33%, derajat penetasan telur 91,67%, dan waktu penyerapan kuning telur 78 jam, serta tingkat kelangsungan hidup prolarva 98,67 %.

Kata kunci: buah mengkudu, induk gabus betina, kinerja reproduksi, probiotik EM₄.

INTRODUCTION

Snakehead fish is one of the important economic in freshwater ecosystems in various Southeast Asian countries, including Indonesia, especially in the Central Kalimantan region which is characterized by swamps and peat. Gustiano *et al.* (2015) and Kusmini *et al.* (2016) stated that snakehead fish is one of the expensive aquaculture genetic resources and is currently experiencing fishing pressure and pollutants so that the population of superior broodstock in public waters is also continuing to decline. Ecological changes and social activities are crucial factors that greatly determine the succession of the reproductive performance of snakehead broodstock, both in their natural habitat and in the aquaculture environment.

The quality of gonad-matured snakehead fish (*Channa striata*) broodstock for the first time is greatly influenced by the conditions of their living environment (Maulana *et al.*, 2021), generally the highest spawning frequency occurs in the transition season from dry to rainy and vice versa (Anwar *et al.*, 2018; Utomo *et al.*, 1992). The reproductive cycle in this fish is influenced by internal and external factors. Internal factors are the hormonal system while external factors include: nutrition and environment (season). Reproductive constraints in snakehead fish include the parent failing to spawn, which is caused by the parent not being able to complete the vitellogenesis phase and the parent not being able to ovulate naturally. Various efforts to overcome these constraints include: hormonal induction, improving parent nutrition or a combination of hormonal and parent nutritional engineering. Nutritional engineering in parent feed is intended to improve the quality of eggs and larvae produced.

The success of the reproductive performance of female fish broodstock in general, including female snakehead broodstock is greatly influenced by a balanced hormonal and nutritional approach. Nainggolan *et al.* (2015) the addition of *Spirulina platensis* to the feed of catfish broodstock (*Clarias* sp.) at a dose of 3% combined with the injection of Oodev hormone at a dose of 15 IU can increase the acceleration of gonad maturity, egg and larval quality. Therefore, this study was conducted to evaluate the reproductive performance of female

snakehead broodstock given supplementation of fermented feed products combined with EM4 and noni fruit.

RESEARCH METHODS

Time and Place

The research was conducted for 2 months in December 2024-January 2025. The research was conducted at the Freshwater Aquaculture Center (BPBAT).

Tools and Materials

The tools used in this study include a digital scale with a capacity of 500 g x 0.02 g, a 1 x 1 x 1 m³ hapa, a basin, a spoon, a digital microscope, a thermometer, a Lutron DO-5510 DO meter, and an ATC digital pH meter. The materials used in this study were 18 snakehead broodstock (9 males and 9 females) with a weight range of 169.8-212.2 g, LP3 fish feed, EM4 probiotics, noni fruit, coconut water, brown sugar, white sugar, bran, powdered milk and tape yeast.

Research Design

The study was conducted using a completely randomized design (CRD) with three treatments and three replications. The treatments given in this study were feed without supplementation of a combination of probiotic fermentation products EM4 and noni fruit (treatment A), feed supplemented with a combination of probiotic fermentation products EM4 and noni fruit at a dose of 10% (treatment B) and feed supplemented with a combination of probiotic fermentation products EM4 and noni fruit at a dose of 20% (treatment C).

Mixing Feed with Fermentation of a combination of EM4 probiotics and noni fruit

Ripe noni fruit (3 kg) with a soft texture is blended into juice, then mixed with coconut water (3 L), brown sugar (300 g) and white sugar (300 g) diluted, bran (300 g), liquid skim milk (30 mL), tape yeast (15 g) and EM4 (40 mL). After all the ingredients are mixed evenly and put into a jerry can and tightly closed, then stored in a room with room temperature. After 2 weeks of fermentation, the jerry can lid is opened to release gas, then every 1 week the jerry can lid is opened until the fermentation time reaches 1 month. The fermentation results are filtered and ready to be used for mixing with brood fish feed. The pellet feed used is LP3 with a protein content of 31-33%. After the feed is evenly mixed with the treatment ingredients, it is then left for 4 hours before being ready to be given to the fish.

Preparation and Maintenance Container for Broodstock

The broodstock of snakehead fish were sourced from the Mandiangan Freshwater Aquaculture Center, consisting of 9 male broodstock and 9 female broodstock. The broodstock that were treated were female broodstock with a body weight ranging from 169.8-212.2 g.

The container for maintaining the female broodstock used was a circular tarpaulin pool with a diameter of 1.2 m and a height of 1 m, totaling 3 pools. The male broodstock were kept separately in the male broodstock maintenance pool. The broodstock were kept for 28 days before spawning. The female broodstock were kept at a density of 3 per pool according to the treatment. The broodstock were fed 3% of their body weight with a frequency of 2 times a day in the morning and evening.

The spawning pool was a circular tarpaulin pool with a diameter of 1.2 m and a height of 1 m, totaling 9 pools. The hatching and larval maintenance container was a plastic basin with a diameter of 20 cm and a height of 15 cm, totaling 9 pieces.

Parent Spawning, Egg Hatching and Prolarva Maintenance

Spawning of snakehead broodstock was carried out using natural techniques after the previous day of defloration. The snakehead broodstock was transferred to a spawning pond with a ratio of 1:1 (one male and one female) in one pond. Snakehead fish eggs were seen floating on water hyacinth plants (Saputra *et al.*, 2021). Snakehead fish eggs were taken and counted as many as 200 eggs and then placed in a hatching basin. Observation and counting of fertilized and unfertilized eggs were carried out after 8 hours. Fertilized eggs were clear in color while unfertilized eggs were white. Fertilized eggs hatched in the range of 24-40 hours after fertilization. Observation of prolarvae was carried out for 4 days after the eggs hatched.

Research Parameters

The research parameters evaluated were reproductive performance parameters, including gonad maturity index, fecundity, degree of egg fertilization, degree of egg hatching, yolk absorption time and prolarva survival.

Data Analysis

The data obtained in this study were tabulated using Microsoft Excel 2019. Statistical analysis was performed using SPSS software version 25.0 and the data were displayed in averages.

RESULT

The results showed that the gonad maturity index (IKG), number of eggs (fecundity), degree of egg hatching, yolk absorption time and survival of female catfish prolarvae supplemented with a combination of EM4 probiotic fermentation and noni fruit were significantly different compared to the control. Only the egg fertilization degree parameter did not differ significantly between treatments. The administration of treatment materials at a dose of 100 ml/kg feed was significantly different from the control in the parameters of gonad maturity index and number of eggs (fecundity). The administration of treatment materials at doses of 100 and 200 ml/kg feed was not significantly different for the parameters of egg hatching degree, yolk absorption time and prolarvae survival. All research parameters evaluated are presented in Figure 1, Figure 2, Figure 3, Figure 4, Table 1, Figure 5, Figure 6 and Figure 7.

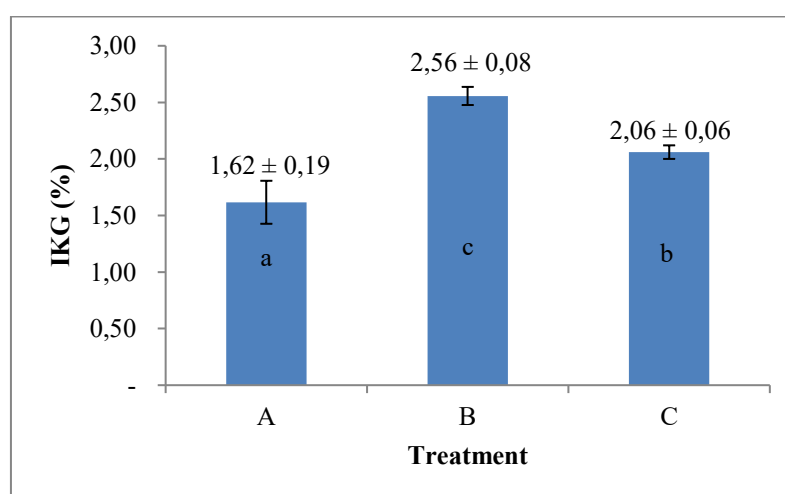


Figure 1. Gonad Maturity Index (GMI) of female snakehead broodstock given supplementation of combined fermentation of EM4 probiotics and noni fruit. Different letters in

different treatments indicate significant differences ($P < 0.05$). A=feed without supplementation of combined fermentation product of EM4 probiotics and noni fruit, B=feed with supplementation of combined fermentation product of EM4 probiotics and noni fruit at a dose of 10%, C=feed with supplementation of combined fermentation product of EM4 probiotics and noni fruit at a dose of 20%.

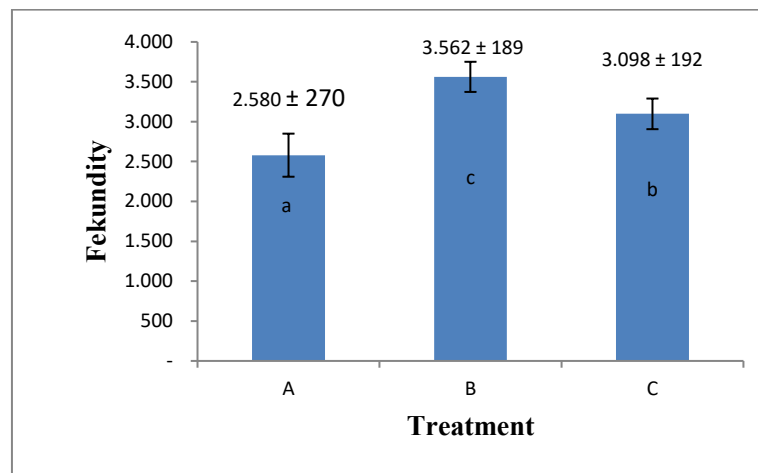


Figure 2. Number of Eggs (Fecundity) of female snakehead broodstock given supplementation of fermentation combination of EM4 probiotics and noni fruit. Different letters in different treatments indicate significant differences ($P < 0.05$). A=feed without supplementation of fermentation product combination of EM4 probiotics and noni fruit, B=feed with supplementation of fermentation product combination of EM4 probiotics and noni fruit at a dose of 10%, C=feed with supplementation of fermentation product combination of EM4 probiotics and noni fruit at a dose of 20%.

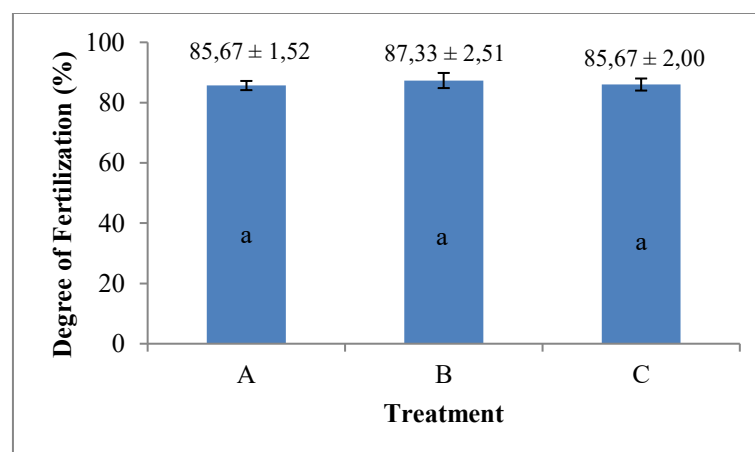


Figure 3. Fertilization Rate of female snakehead broodstock given supplementation of fermentation combination of EM4 probiotics and noni fruit. The same letter in different treatments indicates no significant difference ($P > 0.05$). A=feed without supplementation of fermentation product combination of EM4 probiotics and noni fruit, B=feed with supplementation of fermentation product combination of EM4 probiotics and noni fruit at a dose of 10%, C=feed with supplementation of fermentation product combination of EM4 probiotics and noni fruit at a dose of 20%.

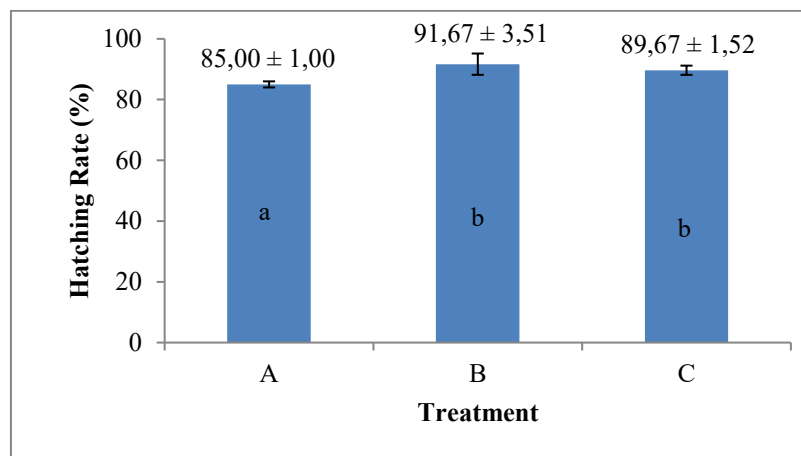


Figure 4. Hatching Rate of female snakehead broodstock given supplementation of fermentation combination of EM4 probiotics and noni fruit. Different letters in different treatments indicate significant differences ($P < 0.05$). A=feed without supplementation of fermentation product combination of EM4 probiotics and noni fruit, B=feed with supplementation of fermentation product combination of EM4 probiotics and noni fruit at a dose of 10%, C=feed with supplementation of fermentation product combination of EM4 probiotics and noni fruit at a dose of 20%.

Table 1. Egg Yolk Absorption Time

PERLAKUAN	EGG YOLK ABSORPTION TIME (HOUR)
A	75
B	78
C	78

Source: Personal Data Research Results 2024.

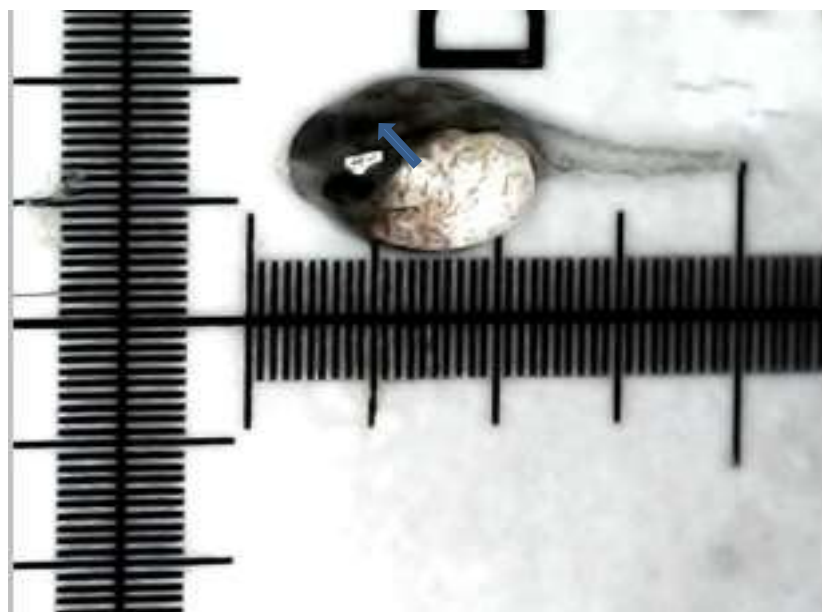


Figure 5. Prolarva Egg Yolk

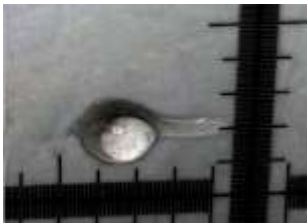
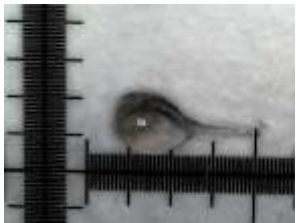
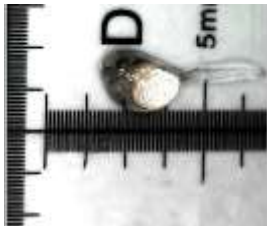
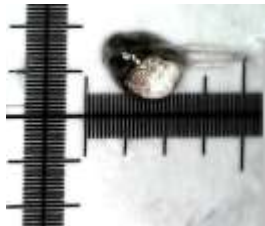
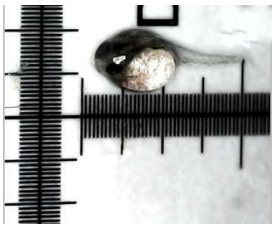
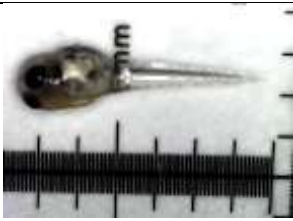
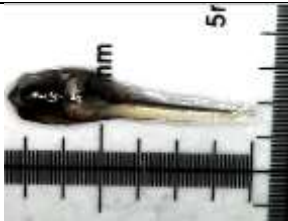
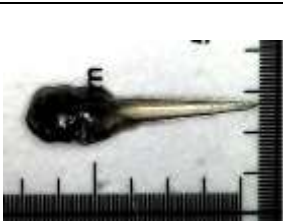
Waktu (Jam)	Perlakuan		
	A	B	C
1			
15			
42			
72			

Figure 6. Prolarva Egg Yolk Absorption Time
Source: Personal Data Research Results 2024.

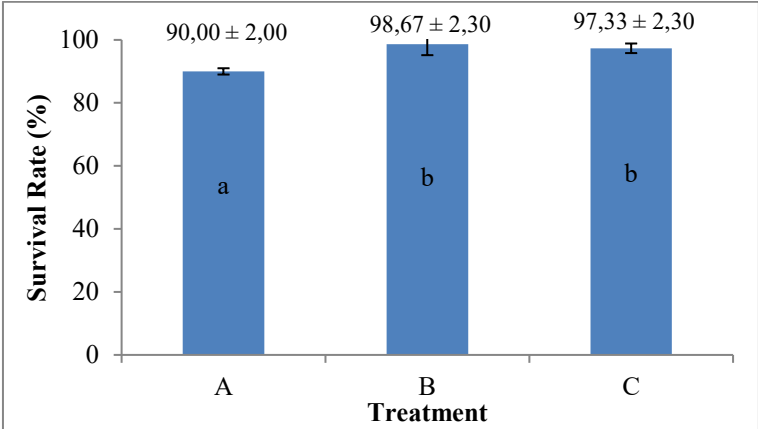


Figure 7. Survival Rate of female catfish prolarvae supplemented with a combination of EM4 probiotic fermentation and noni fruit. Different letters in different treatments

indicate significant differences ($P < 0.05$). A=feed without supplementation of a combination of EM4 probiotic fermentation product and noni fruit, B=feed supplemented with a combination of EM4 probiotic fermentation product and noni fruit at a dose of 10%, C=feed supplemented with a combination of EM4 probiotic fermentation product and noni fruit at a dose of 20%.

DISCUSSION

EM4 is basically a consortium product of beneficial microorganisms that are often used as feed additives (Dewi *et al.*, 2023) to increase the productivity of aquaculture biota and improve water quality (Khartiono 2020), which consists of *Lactobacillus* sp., Streptomyces, cellulose decomposing fungi, lactic acid bacteria, phosphorus-solubilizing bacteria, and photosynthetic bacteria (Walandow *et al.*, 2020). The use of commercial probiotic products EM4 is one effort to improve the quality of noni fruit during the fermentation process. The results of the study by Lee *et al.* (2015), noni fruit fermented with the probiotic *Pediococcus pentosaceus* and the addition of cellulase enzymes can significantly increase the levels of oligosaccharide, phenol and antioxidant extracts of noni fruit followed by an increase in the total number of probiotic bacteria. Specific important polyphenol compounds found in ripe noni fruit include rutin and kaempferol-3-O-rutinoside (Chen *et al.*, 2023) which have high antioxidant capacity. Although polyphenols are less stable during the digestive process in the intestine, phenols bound to fiber still have high antioxidant capacity as phenol bioavailability. The combined fermentation of EM4 products and noni fruit is thought to increase the number and capacity of beneficial bacteria that have prebiotic characteristics along with the increasing capacity of antioxidant compounds, this indicates a balance of probiotic and prebiotic bacterial biodiversity in the intestinal microbiome structure and a real contribution to increasing vitality and reproductive performance obtained by female snakehead broodstock that consume combined fermented feed supplementation of EM4 and noni fruit.

Supplementation of fermented product combination of probiotics and noni fruit is thought to play a role in producing exogenous protease. Furthermore, exogenous protease has the ability to hydrolyze macronutrients, namely protein into essential amino acids, one of which is tryptophan which is a precursor of serotonin, plays a key role in the development of follicles and vitellogenesis, as well as oocyte maturation, which are each induced by the hormones GTHI and GTHII, then ending in ovulation. Amino acids and fatty acids are thought to accelerate the process of vitellogenesis or vitellogenin synthesis in egg yolk. The gonad maturity index value of the female snakehead broodstock observed in this study ranged from 1.62-2.56%. The low IKG value is thought to be caused by the female snakehead parent undergoing a partial gonad maturity and spawning process, when compared to the IKG value of female snakehead parents living in their natural habitat, namely in the Sambujur River, Barito Watershed, South Kalimantan and the Sebangau River flood swamp, Palangka Raya, Central Kalimantan, ranging from 2.51-3.54% (Makmur *et al.*, 2006; Selviana *et al.*, 2020). The level of gonad maturity of female snakehead parents in the Rawa Pening waters is dominated by TKG II or the developing virgin phase with a female IKG ranging from 0.043%-4.324% (Puspaningdiah *et al.*, 2014). The highest number of eggs (fecundity) of snakehead fish was 20,035 eggs with a body length of 480 mm and a body weight of 875.6 grams, while the lowest fecundity was 1,282 eggs with a length of 318 mm and a body weight of 250.2 grams. Based on the research data, the average number of eggs (fecundity) of female snakehead fish ranges from 2580-3562 eggs per female snakehead fish, this number is in accordance with the research results of Makmur (2006) that in one of the natural habitats of snakehead fish in the flood swamp area of the Musi River, the number of eggs of mature female snakehead fish ready to spawn was found to be around 1062-57200 eggs per parent.

The optimum reproductive performance of the female snakehead broodstock was seen in November of the 2016 and 2018 observation periods (Bijaksana 2010) which was characterized by the highest egg diameter, fecundity and somatic gonado index, with values of 1.5 and 1.5 mm, 3070 and 4600 eggs, 3.2% and 2.98%, respectively. The magnitude of the somatic gonado index value has a close relationship with the vitellogenesis process that occurs in the liver. The increase in the somatic gonado index value is in line with the accumulation of vitellogenin in the oocyte development process. Vitellogenin is the core material of developing oocytes. During vitellogenesis, there is a multiplication of yolk granules which contributes to the increase in oocyte volume. The gonad weight of the female snakehead broodstock is directly proportional to its somatic gonado index, meaning that the greater the gonad weight, the greater the somatic gonado index value, then decreases after spawning.

Vitellogenin is essentially a polypeptide or plasma protein formed in the liver with the active role of estrogen hormone which will continue to increase during the reproductive period of the female cork parent, namely the hormone 17β -estradiol plays an active role in encouraging the final stage of oocyte maturation in the ovaries. The liver is a very vital organ in its role as a place for storing, mobilizing and distributing energy to all cells and tissues of the body. Energy stocks are clearly visible in the conversion of glucose into glycogen and fat, in addition to being able to produce and secrete glucose from the gluconeogenesis process mediated by beneficial microorganisms and glycogenolysis. Regulation of glucose metabolism, essential fatty acids and essential amino acids is largely determined by a cooperative combination of nutrient, hormonal and neuron signals (Rui 2014) all of which are available in the content of the fermented treatment ingredients of the combination of EM4 and noni fruit to support the fitness of the reproductive performance of the female cork parent. It is suspected that the female catfish broodstock that is given a combination of EM4 and noni fruit extract at a dose of 10% has sufficient biological nutrients in a very efficient dynamic balance that is very necessary for the vitellogenesis process to run optimally, such as essential amino acids, phosphovitin, vitelin, essential fatty acids, lipovitelin, vitamins and minerals.

Estrogen plays a key role in regulating serum GTH levels, oogenesis, vitellogenesis, ovulation and the behavior of female snakehead broodstock in conditions ready to spawn. Supplementation of a combination of EM4 and noni fruit extract in the feed of female snakehead broodstock is also thought to play an important role in protecting the structure and function of estrogen from damage by free radicals and other chemicals that are likely to be found in the aquatic environment. All of these processes have a significant impact on the sharp decrease in energy storage such as ATP, phosphocreatine, and glycogen, as well as rapid accumulation in the blood, which leads to a decrease in the reproductive performance of female snakehead broodstock. In other words, the supplementation treatment in this study is thought to be able to reduce oxidative stress that often occurs in broodstock fish from the time of broodstock selection to the preparation of broodstock for spawning.

Serotonin synthesis in the brain can be increased by supplementation of tryptophan in feed. Supplementation of a combination of fermented products of probiotics EM4 and noni fruit at a dose of 10% is thought to be able to increase the biological availability of tryptophan which will have a significant effect on follicle development and the vitellogenesis process, oocyte maturity and ovulation. The feed of female snakehead broodstock enriched with the addition of a combination of probiotic fermentation products EM4 and noni fruit in this study is one of the most crucial factors because it acts as the only source of essential nutrients that are determining factors for accelerating the maturation process, fecundity and survival rate of larvae. *Fernandez-Palacios et al.* (1997), put forward several sources of functional feed additives that are generally added to broodstock feed, including: probiotics, prebiotics, fatty acids, amino acids and vitamins. Noni fruit contains various vitamins and minerals, including:

vitamin C, iron, calcium, potassium, copper, magnesium, sodium, zinc, phosphorus and selenium. In addition, there are also antioxidant contents, such as flavonoids, lignans, iridoids, anthraquinones, coumarins, terpenoids, sterols, polysaccharides and fatty acids.

Essential fatty acids are useful as a source of energy, embryo development, cell membrane synthesis and prostaglandin precursors. Therefore, if the brood fish consumes essential fatty acid malnutrition feed, it will result in a low egg hatching rate and even a high percentage of prolarva abnormalities. Improving the quality of broodstock feed (nutrient content: essential fatty acids) is crucial for improving the quality of gonads and eggs produced by broodstock to be better. More efficient digestion metabolism and nutrient absorption in the treatment group causes a surplus of biological availability of nutrients and energy for hormone synthesis and release (GTH, estrogen), vitellogenesis process, gonad maturity and increased fecundity. The efficiency of egg yolk use related to the quantity and quality of body tissue synthesis is lower in the control because the efficiency of energy accumulation is lower and energy mobilization is less effective. The survival rate of prolarvae is lower than the treatment because the transition period of energy use from endogenous feeding (egg yolk) is shorter than exogenous feeding (natural feed from outside). The development of organogenesis related to mouth opening in the treatment group is more perfect because the efficiency of energy accumulation is higher and energy mobilization is more effective. Alkaloid compounds have the potential to inhibit the activity of the α -glucosidase enzyme, so that the process of glucose oxidation, glycogen synthesis, fat and protein increases. On the other hand, in the control there was an increase in the process of glycogenolysis, gluconeogenesis and ketogenesis, this causes the absorption time of egg yolk to be faster.

Vitellogenin synthesis in the liver is a very crucial stage in determining the success of spawning and natural ovulation of female snakehead broodstock. Providing supplementation with a combination of probiotic fermentation products EM4 and noni fruit is thought to be able to improve and/or increase the function of hepatocytes of female snakehead broodstock in terms of the production of digestive enzymes and antioxidant enzymes. In general, female snakehead broodstock that live in their natural environment have lower stress levels than in the cultivation environment. The results of this study are in line with the findings of Moh *et al.* (2021), which stated that there was a significant increase in amylase and lipase production by hepatopancreas cells of vaname shrimp due to the administration of noni fruit extract in feed, resulting in increased growth performance and stressor overwhelming. Furthermore, it is said that the concentration of noni fruit extract significantly increases the production of antioxidant enzymes, which are then very clearly responsible for the growth performance of whiteleg shrimp. Moh *et al.* (2021), also emphasized that the optimal concentration of noni fruit extract can significantly increase the number of hepatopancreas cells. Supplementation of noni fruit extract at a dose of 50 mg/g feed resulted in a decrease in the degeneration of hepatopancreas cells of whiteleg shrimp after a challenge test with the pathogen *Vibrio parahaemolyticus* compared to the control (Moh *et al.*, 2024). Vitellogenin is a precursor to egg yolk. Vitellogenin is synthesized in the liver, secreted into the blood, and then absorbed by oocytes selectively through the endocytosis process (Nagahama 1983). It is suspected that the results of this study indicate that supplementation of a combination of probiotic fermentation products EM4 and noni fruit can increase the concentration of FSH which induces the theca layer to convert testosterone into estradiol-17 β by the aromatase enzyme, thereby stimulating the acceleration of the final stage of the vitellogenesis process. In addition, supplementation of these ingredients is thought to be able to improve the performance of hepatocytes in synthesizing proteins and essential amino acids, such as albumin, glutamine, cysteine and glycine which are associated with the acceleration of the final stage of vitellogenesis (Ningrum & Abdulgani 2014).

CONCLUSION

Supplementation of feed for female snakehead broodstock that are spawned naturally with a combination of EM4 and noni fruit fermentation products at a dose of 100 ml/kg of feed can significantly increase the value of the gonad maturity index, fecundity, egg hatching rate, yolk absorption time and survival of prolarvae.

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