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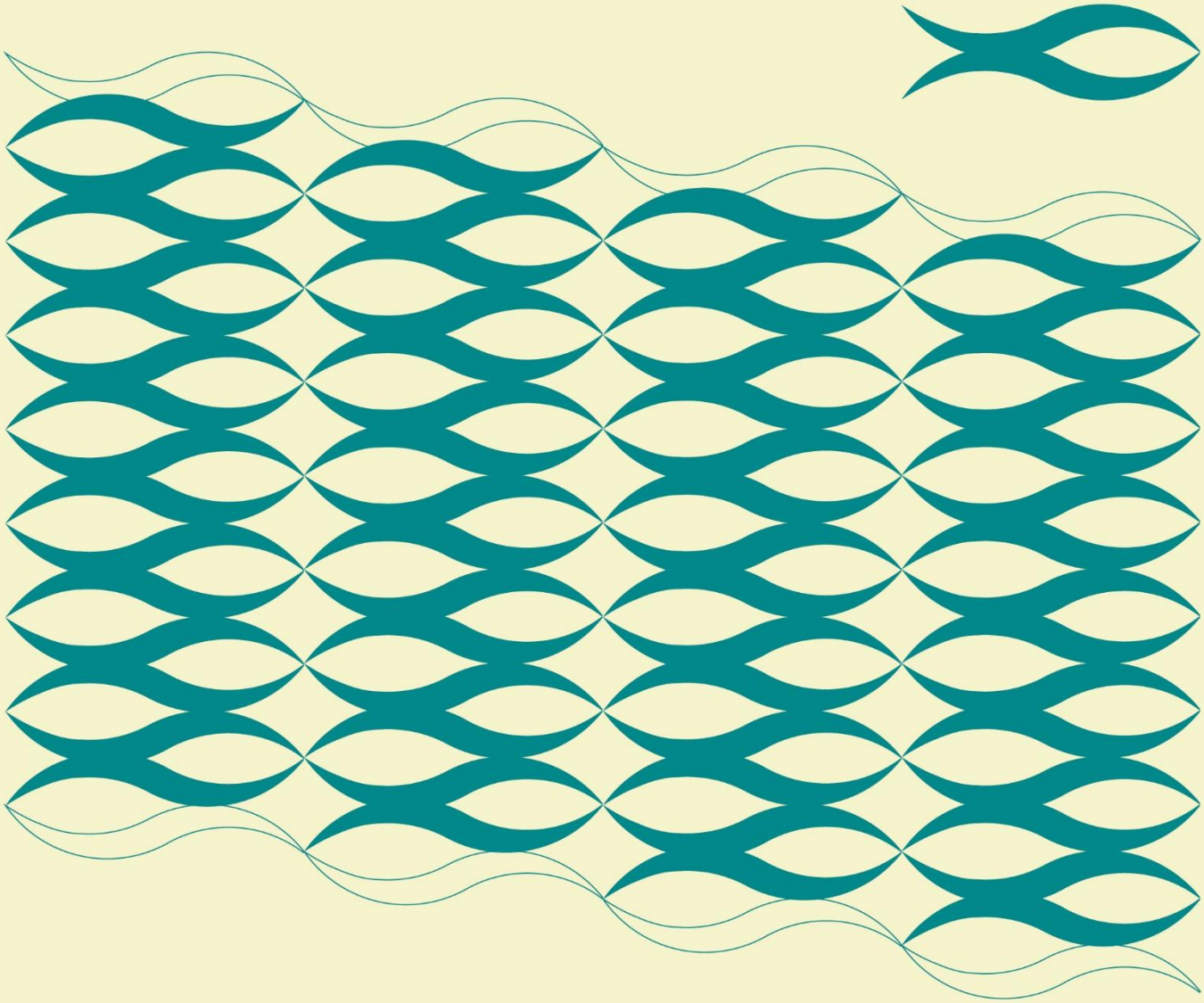

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TAGEM JOURNALS

KORESPONDENSI

Nama Jurnal : Turkish Journal of Fisheries and Aquatic Sciences

Judul Artikel : Evaluation of probiotic-fermented feed addition and laser firing to accelerate mature broodstocks and seed productions of African catfish (*Clarias gariepinus*)

No.	**Proses**	**Waktu**
1.	Submit manuskrip	21 Februari 2021
2.	Under review oleh reviewer jurnal	21 Februari 2021
3.	Revisi dari reviewer jurnal	30 Juni 2021
4.	Reminder dari editor jurnal	14 Juli 2021
5.	Reminder dari editor jurnal	23 Juli 2021
6.	Revisi manuskrip dan re-submit	29 Juli 2021
7.	Revisi manuskrip dari editor jurnal	6 September 2021
8.	Respon balik author pada editor	10 September 2021
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Suggestions

Editor Comments
1. Reviewer Comments
English and grammar need to be improved.

2. Reviewer Comments
your study is interesting, best practical, and will be even more interesting if you do a bioprocess evaluation (gonad histology / liver, before and after laser firing, hormone profile of each laser firing)

3. Reviewer Comments
Please check paper file

Reviewer 1

Comment File :	Show File (https://www.trjfas.org/submit/uploads/rev_com/TRJFAS-19303-rev-file-comments-to-the-authors-tjfas.docx)
Date Invited:	Mar 29, 2021
Date Returned:	Apr 15, 2021
Check Revision:	Yes
Newness, currency and originality in the manuscript:	Middle
Straightness and validity of material - method:	Poor
Reliability, consistence of findings and power of discussion:	Good
Coordination of statement and wording and fluency of language:	Poor
Success in pursuing, selecting and presentation of references:	Good
Manuscript category:	Research Paper
Note/Commend/Suggest About Abstract:	The section needs to be revised.
Note/Commend/Suggest About Introduction:	Almost well written.
Note/Commend/Suggest About Material and Methods:	Poor.
Note/Commend/Suggest About Results:	Not up to the mark.

Reviewer 2

Comment File :	Show File (https://www.trjfas.org/submit/uploads/rev_com/TRJFAS-19303-rev-file-19303-trjfas-19303-8-manuscript-to-tjfas.pdf)
Date Invited:	May 23, 2021
Date Returned:	May 24, 2021
Check Revision:	No
Newness, currency and originality in the manuscript:	Good
Straightness and validity of material - method:	Good
Reliability, consistence of findings and power of discussion:	Middle
Coordination of statement and wording and fluency of language:	Good
Success in pursuing, selecting and presentation of references:	Good
Manuscript category:	Research Paper
Note/Commend/Suggest About Abstract:	all is good, but check grammarly !
Note/Commend/Suggest About Introduction:	good
Note/Commend/Suggest About Material and Methods:	need improvement
Note/Commend/Suggest About Results:	good, Discussion: need improvement !

Reviewer 3

Comment File :	Show File (https://www.trjfas.org/submit/uploads/rev_com/TRJFAS-19303-rev-file-19303-trjfas-19303-8-manuscript-to-tjfas.docx)
Date Invited:	May 23, 2021
Date Returned:	Jun 14, 2021
Check Revision:	Yes
Newness, currency and originality in the manuscript:	Good
Straightness and validity of material - method:	Middle
Reliability, consistence of findings and power of discussion:	Poor
Coordination of statement and wording and fluency of language:	Poor
Success in pursuing, selecting and presentation of references:	To Poor
Manuscript category:	Research Paper
Note/Commend/Suggest About Abstract:	Need corrections
Note/Commend/Suggest About Introduction:	Need corrections and weak literature review
Note/Commend/Suggest About Material and Methods:	Need correction
Note/Commend/Suggest About Results:	No comments

Manuscript Information

Manuscript ID: **TRJFAS-19303**

Title: Addition of probiotic-fermented feed and laser induction to accelerate mature broodstocks and seed productions of African catfish (Clarias gariepinus)

Small Title: Effects of fermented feed and laser induction

Authors: Pungky Slamet Wisnu Kusuma[1], Dyah Hariani[2], Akhmad Taufiq Mukti[3]

Institutions: [1]Faculty of Technology Science, Universitas PGRI Adi Buana, Study Program of Biology, Surabaya, Indonesia
[2]Faculty of Mathematic and Natural Science, Universitas Negeri Surabaya, Study Program of Biology, Surabaya, Indonesia
[3]Faculty of Fisheries and Marine, Universitas Airlangga, Department of Aquaculture, Surabaya, Indonesia

Keywords: Fermented feed, Probiotic, Soft-laser, Gonadal maturity, African catfish

Manuscript Type: Research Paper

Manuscript Category: Aquaculture

Processing Status: Major Revision

Abstract

The decreasing availability of mature broodstocks and seed productions can hinder the sustainability of catfish cultivation. An alternative solution is intensive hatchery by the addition of fermented feed and laser induction so that the availability of mature broodstocks and seeds of catfish, both quantity and continuity are guaranteed. This study aimed to determine the effects of addition of fermented feed using probiotic and laser induction on accelerate the mature broodstocks and seed productions of African catfish. Fish were used male and female broodstocks of African catfish. The method was used a completely randomized design with three treatments, namely unfermented feed as control, probiotic-fermented feed (PFF), and probiotic-fermented feed+laser induction (PFF+Li) and three times replication, respectively. Laser induction was performed every 15 days. The gonadal maturity time of male and female broodstocks, fertilization rate (FR), hatching rate (HR), and seed production performances include survival rate (SR) were observed. The results showed that the addition of PFF+Li reach fastest time to mature of the female gonad (31-41 days) and the male gonad (32-37 days), and produce highest FR, HR, and SR more than 90%, respectively, and catfish seed yield of 2.1-3.0 cm size compared to other treatments ($P<0.05$).

Manuscript Files

File Name	File Size	Date Created	Category	Description
TRJFAS-19303-8-manuscript-to-tjfas.pdf (../pdf-files/out/19303-TRJFAS-19303-8-manuscript-to-tjfas.pdf)	80 KB	Feb 21, 2021	Main Document	Main Manuscript
TRJFAS-19303-3-figure-1-6-to-tjfas.pdf (../pdf-files/out/19303-TRJFAS-19303-3-figure-1-6-to-tjfas.pdf)	42 KB	Feb 21, 2021	Figure	Figures
TRJFAS-19303-5-title-page-of-tjfas.pdf (../pdf-files/out/19303-TRJFAS-19303-5-title-page-of-tjfas.pdf)	14 KB	Feb 21, 2021	Title Page	Title page
TRJFAS-19303-4-copyright-release-form-tjfas.pdf (../pdf-files/in/19303-TRJFAS-19303-4-copyright-release-form-tjfas.pdf)	0 KB	Feb 21, 2021	Copyright Form	None
TRJFAS-19303-7-copyright-release-form-tjfas-.pdf (../pdf-files/in/19303-TRJFAS-19303-7-copyright-release-form-tjfas-.pdf)	219 KB	Feb 21, 2021	Copyright Form	Copyrights Release Form

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Suggestions

Editor Comments
1. Reviewer Comments
Dear authors
All corrections were checked and your paper can be investigated by the editor for publication in the TrJFAS:
Good lock

2. Reviewer Comments
Nice work

Reviewer 1

Date Invited:	Aug 21, 2021
Date Returned:	Aug 23, 2021
Check Revision:	No
Newness, currency and originality in the manuscript:	Good
Straightness and validity of material - method:	Good
Reliability, consistence of findings and power of discussion:	Good
Coordination of statement and wording and fluency of language:	Good
Success in pursuing, selecting and presentation of references:	Good
Manuscript category:	Research Paper
Note/Commend/Suggest About Abstract:	The authors added requested comments
Note/Commend/Suggest About Introduction:	Proper introduction
Note/Commend/Suggest About Material and Methods:	Proper introduction
Note/Commend/Suggest About Results:	Proper introduction

Reviewer 2

Date Invited:	Aug 21, 2021
Date Returned:	Aug 27, 2021
Check Revision:	No
Newness, currency and originality in the manuscript:	Good
Straightness and validity of material - method:	Middle
Reliability, consistence of findings and power of discussion:	Good
Coordination of statement and wording and fluency of language:	Good
Success in pursuing, selecting and presentation of references:	Good
Manuscript category:	Research Paper
Note/Commend/Suggest About Abstract:	Suggestions incorporated
Note/Commend/Suggest About Introduction:	Agreed with corrections
Note/Commend/Suggest About Material and Methods:	Nicely prepared
Note/Commend/Suggest About Results:	Satisfied

Manuscript Information

Manuscript ID: **TRJFAS-19303.REV-1**

Title: Evaluation of probiotic-fermented feed addition and laser firing to accelerate mature broodstocks and seed productions of African catfish (Clarias gariepinus)

Small Title: Effects of fermented feed and laser firing

Authors: Pungky Slamet Wisnu Kusuma[1], Dyah Hariani[2], Akhmad Taufiq Mukti[3]

Institutions: [1]Faculty of Technology Science, Universitas PGRI Adi Buana, Study Program of Biology, Surabaya, Indonesia
[2]Faculty of Mathematic and Natural Science, Universitas Negeri Surabaya, Study Program of Biology, Surabaya, Indonesia
[3]Faculty of Fisheries and Marine, Universitas Airlangga, Department of Aquaculture, Surabaya, Indonesia

Keywords: Fermented feed, Probiotic, Soft-laser, Gonadal maturity, African catfish

Manuscript Type: Research Paper

Manuscript Category: Aquaculture

Processing Status: Minor Revision

Manuscript Files

File Name	File Size	Date Created	Category	Description
TRJFAS-19303-8-manuscript-to-tjfas.pdf (../pdf-files/out/20344-TRJFAS-19303-8-manuscript-to-tjfas.pdf)	80 KB	Feb 21, 2021	Main Document	Main Manuscript
TRJFAS-19303-3-figure-1-6-to-tjfas.pdf (../pdf-files/out/20344-TRJFAS-19303-3-figure-1-6-to-tjfas.pdf)	42 KB	Feb 21, 2021	Figure	Figures
TRJFAS-19303-5-title-page-of-tjfas.pdf (../pdf-files/out/20344-TRJFAS-19303-5-title-page-of-tjfas.pdf)	14 KB	Feb 21, 2021	Title Page	Title page
TRJFAS-19303-4-copyright-release-form-tjfas.pdf (../pdf-files/in/20344-TRJFAS-19303-4-copyright-release-form-tjfas.pdf)	0 KB	Feb 21, 2021	Copyright Form	None
TRJFAS-19303-7-copyright-release-form-tjfas-.pdf (../pdf-files/in/20344-TRJFAS-19303-7-copyright-release-form-tjfas-.pdf)	219 KB	Feb 21, 2021	Copyright Form	Copyrights Release Form
TRJFAS-20344-4-revised-manuscript-to-tjfas.rev-1.pdf (../pdf-files/out/20344-TRJFAS-20344-4-revised-manuscript-to-tjfas.rev-1.pdf)	85 KB	Jul 29, 2021	Main Document	Main Manuscript
TRJFAS-20344-6-revised-figure-1-6-to-tjfas.rev-1.pdf (../pdf-files/out/20344-TRJFAS-20344-6-revised-figure-1-6-to-tjfas.rev-1.pdf)	42 KB	Jul 29, 2021	Figure	Figures
TRJFAS-20344-1-letter-of-responses-to-reviewers.rev-1.pdf (../pdf-files/out/20344-TRJFAS-20344-1-letter-of-responses-to-reviewers.rev-1.pdf)	73 KB	Jul 29, 2021	Response To Reviewer	Letter of Responses

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Suggestions

Editor Comments
The authors have addressed the suggestions of the reviewers. It is now in acceptable form

Accepted

Manuscript Information

Manuscript ID:	**TRJFAS-19303.REV-2**
Title:	Evaluation of probiotic-fermented feed addition and laser firing to accelerate mature broodstocks and seed productions of African catfish (Clarias gariepinus)
Small Title:	Effects of fermented feed and laser firing
Authors:	Pungky Slamet Wisnu Kusuma[1], Dyah Hariani[2], Akhmad Taufiq Mukti[3]
Institutions:	[1]Faculty of Technology Science, Universitas PGRI Adi Buana, Study Program of Biology, Surabaya, Indonesia [2]Faculty of Mathematic and Natural Science, Universitas Negeri Surabaya, Study Program of Biology, Surabaya, Indonesia [3]Faculty of Fisheries and Marine, Universitas Airlangga, Department of Aquaculture, Surabaya, Indonesia
Keywords:	Fermented feed, Probiotic, Soft-laser, Gonadal maturity, African catfish
Manuscript Type:	Research Paper
Manuscript Category:	Aquaculture
Processing Status:	Accepted

Abstract

This study aimed to determine the effects of fermented feed using probiotics and laser-firing to accelerate the mature broodstocks and seed productions of African catfish (Clarias gariepinus). Fish has used male and female broodstocks of catfish. The method was used a completely randomized design with three treatments: unfermented feed as control, probiotic-fermented feed (PFF), and probiotic-fermented feed+laser firing (PFF+Li). In the first study, a laser-firing dose of 1.125 Joule was performed on fish broodstocks every 15 days. The gonadal maturity time of male and female broodstocks was observed. The second study, treated female broodstocks, was mated with mature male broodstocks without any prior treatments (control). Fertilization rate, hatching rate, and seed production performances such as survival rate and total length were measured. The results showed that treatment of PFF+Li has a significant effect ($P<0.05$) on the gonadal maturity time of males and females. This treatment reaches the fastest time to mature of the female gonad (31-41 days) and the male gonad (32-37 days) than other treatments ($P<0.05$). This treatment also produced the highest fertilization, hatching, and survival rates of more than 90%, respectively, and the highest seed yield of 2.1-3.0 cm size compared to other treatments ($P<0.05$) in African catfish.

Manuscript Files

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TRJFAS-19303-8-manuscript-to-tjfas.pdf (../pdf-files/out/20677-TRJFAS-19303-8-manuscript-to-tjfas.pdf)	80 KB	Feb 21, 2021	Main Document	Main Manuscript
TRJFAS-19303-3-figure-1-6-to-tjfas.pdf (../pdf-files/out/20677-TRJFAS-19303-3-figure-1-6-to-tjfas.pdf)	42 KB	Feb 21, 2021	Figure	Figures
TRJFAS-19303-5-title-page-of-tjfas.pdf (../pdf-files/out/20677-TRJFAS-19303-5-title-page-of-tjfas.pdf)	14 KB	Feb 21, 2021	Title Page	Title page
TRJFAS-19303-4-copyright-release-form-tjfas.pdf (../pdf-files/in/20677-TRJFAS-19303-4-copyright-release-form-tjfas.pdf)	0 KB	Feb 21, 2021	Copyright Form	None
TRJFAS-19303-7-copyright-release-form-tjfas-.pdf (../pdf-files/in/20677-TRJFAS-19303-7-copyright-release-form-tjfas-.pdf)	219 KB	Feb 21, 2021	Copyright Form	Copyrights Release Form
TRJFAS-20344-4-revised-manuscript-to-tjfas.rev-1.pdf (../pdf-files/out/20677-TRJFAS-20344-4-revised-manuscript-to-tjfas.rev-1.pdf)	85 KB	Jul 29, 2021	Main Document	Main Manuscript
TRJFAS-20344-6-revised-figure-1-6-to-tjfas.rev-1.pdf (../pdf-files/out/20677-TRJFAS-20344-6-revised-figure-1-6-to-tjfas.rev-1.pdf)	42 KB	Jul 29, 2021	Figure	Figures
TRJFAS-20344-1-letter-of-responses-to-reviewers.rev-1.pdf (../pdf-files/out/20677-TRJFAS-20344-1-letter-of-responses-to-reviewers.rev-1.pdf)	73 KB	Jul 29, 2021	Response To Reviewer	Letter of Responses
TRJFAS-20677-1-revised-manuscript-to-tjfas.rev-2.pdf (../pdf-files/out/20677-TRJFAS-20677-1-revised-manuscript-to-tjfas.rev-2.pdf)	86 KB	Sep 21, 2021	Main Document	Main Manuscript
TRJFAS-20677-4-revised-figure-1-6-to-tjfas.rev-2.pdf (../pdf-files/out/20677-TRJFAS-20677-4-revised-figure-1-6-to-tjfas.rev-2.pdf)	42 KB	Sep 21, 2021	Figure	Figures
TRJFAS-20677-9-revised-title-page-of-tjfas.rev-2.pdf (../pdf-files/out/20677-TRJFAS-20677-9-revised-title-page-of-tjfas.rev-2.pdf)	15 KB	Sep 21, 2021	Title Page	Title page
TRJFAS-20677-8-letter-of-response-to-reviewer.rev-2.pdf (../pdf-files/out/20677-TRJFAS-20677-8-letter-of-response-to-reviewer.rev-2.pdf)	39 KB	Sep 21, 2021	Response To Reviewer	Letter of Responses

akhmad taufiq mukti <akhmad-t-m@fpk.unair.ac.id>

New Manuscript Submission

1 message

Turkish Journal of Fisheries and Aquatic Sciences <info@trjfas.org> Sun, Feb 21, 2021 at 5:54 PM
To: akhmad-t-m@fpk.unair.ac.id

ISSN: 1303 - 2712
E-ISSN: 2149 - 181X

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Dear Author,

Associated Prof.Akhmad Taufiq Mukti has uploaded the article "Addition of probiotic-fermented feed and laser induction to accelerate mature broodstocks and seed productions of African catfish (Clarias gariepinus)"; which you are also among its authors.

If you have any problem or question please send an e-mail to info@trjfas.org

Yours sincerely

Editorial Office
Turk. J. Fish & Aquat. Sci.
www.trjfas.org

akhmad taufiq mukti <akhmad-t-m@fpk.unair.ac.id>

Revision request for your manuscript

1 message

Turkish Journal of Fisheries and Aquatic Sciences <info@trjfas.org> Wed, Jun 30, 2021 at 10:09 PM
To: akhmad-t-m@fpk.unair.ac.id

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E-ISSN: 2149 - 181X

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Dear Associated Prof.Akhmad Taufiq Mukti,

The Editors have now assessed the reviewer response and have concluded that, in its present form, the manuscript (19303) is not yet ready for publication in the journal. You will find the relevant reviewer comments and editorial notes on the online system. Acceptance of the paper is contingent upon effectively revising the work by taking these comments into serious consideration, and by responding or rebutting them in detail within the 30 days.

We ask you to submit your revision through the online system.

Please upload the file containing your revised manuscript. The rebuttal letter should be placed in “cover letter” section. Please note that you should submit your revised letter by clicking on “Submit Revision” link, not as a new manuscript.

If you have any problem please send an e-mail to info@trjfas.org

Sincerely,

Esen ALP ERBAY
Manager Editor
Turk. J. Fish & Aquat. Sci.
info@trjfas.org
+(90) 462 341 1053

Comments to the Authors

Major Comments

Abstract

1. The section has not been written in a proper way.
2. Methodology is poor.
3. Results should be more numeric.
4. Conclusion of the section need to be revised.
5. First letter of keywords should be in Capital and design in an alphabet format.

Introduction

1. The section has been written in good manner.
2. The conclusion of the section need to be improved.
3. English and grammar are weak.

Materials and methods

1. The average weight of the experimental animals should be written as, 'weight + SE/SD'.
2. Units (ml or mL should be followed informally throughout the manuscript).
3. Feeding details are missed.
4. SPSS version used for the data analysis is very old, try at least SPSS Version 18 or above.
5. The sentence restructuring is required in several places of MM section.
6. English needs to be cross-checked from an expert.

Results

1. Authors did not mention the significance symbol in few places.
2. English need to be improved.

Discussion

1. I suggest to incorporate few more references in the First paragraph.
2. It may be more authentic if the discussion section will arrange in the sub-heading format.

Conclusion

1. The section need to be improvised.

Minor comments

The minor comments have been incorporated in the attached PDF file.

Addition of probiotic-fermented feed and laser induction to accelerate mature broodstocks and seed productions of African catfish (*Clarias gariepinus*)

Abstract

~~The decreasing availability of mature broodstocks and seed productions can hinder the sustainability of catfish cultivation. An alternative solution is intensive hatchery by the addition of fermented feed and laser induction so that the availability of mature broodstocks and seeds of catfish, both quantity and continuity are guaranteed.~~ This study aimed to determine the effects of addition of fermented feed using probiotic and laser induction on accelerate the mature broodstocks and seed productions of African catfish. Fish were used male and female broodstocks of African catfish. The method was used a completely randomized design with three treatments, namely unfermented feed as control, probiotic-fermented feed (PFF), and probiotic-fermented feed+laser induction (PFF+Li) ~~and three times~~with three replicate~~sion~~, respectively. Laser induction was performed every 15 days. The gonadal maturity time of male and female broodstocks, fertilization rate (FR), hatching rate (HR), and seed production performances include survival rate (SR) were observed. The results showed that the addition of PFF+Li reach fastest time to mature of the female gonad (31-41 days) and the male gonad (32-37 days), and produce highest FR, HR, and SR more than 90%, respectively, and catfish seed yield of 2.1-3.0 cm size compared to other treatments (P<0.05).

Key words: Fermented feed, Probiotic, Soft-laser, Gonadal maturity, African catfish

observed The results showed that addition of probiotic-fermented feed and laser induction (PFF+Li) in the broodstocks before spawned have a significant effect (P<0.05) on the gonadal maturity time of males and females Africant catfish. the addition of PFF+Li reach fastest time to mature of the female gonad (31-41 days) and the male gonad (32-37 days), and produce highest FR, HR, and SR more than 90%, respectively, and catfish seed yield of 2.1-3.0 cm size compared to other treatments (P<0.05).

Introduction

The main problem faced by catfish breeders in hatchery centres is the conventional cultivation method, catfish that have been spawned need recovery time to be able to spawn around three months later, thus the availability of broodstocks and seeds in the community

Commented [G1]: And laser alone!!!!!!

Commented [G2]: Please report in statistic form

Commented [G3]: This part need to rewrite and expansion

Commented [G5]: This part need to rewrite and expansion

Commented [G4]: Please report in statistic form

Commented [G6]: Weak literature reviw

is limited and not continuous, so it can hinder productivity catfish farming business. The market demand for this catfish commodity continues to increase, meanwhile the availability of gonadal mature and seeds of catfish are decreasing. It is necessary to find alternative solutions through more intensive farming of catfish, namely by increasing the quality of feed and the application of appropriate biostimulation laser technology so that the availability of mature broodstocks and seeds, especially the number and continuity can take place continuously.

In general, the protein content in catfish feed could affect several things, such as gonadal development, spermatozoa and eggs productions limit number and quality. Based on study according to Coldebella et al. (2011) showed that the protein content of the feed was proven to affect the survival of larvae, to very low levels of feed protein content (10 to 20%) resulting in low fertilization rate of eggs and a higher percentage of abnormal larvae. Hence, the quality of feed in the broodstocks needs to be improved. The results of the study according to Sakamole et al. (2014) showed that the addition of the probiotic in feed was proven to be able to break down feed complex compounds into simple ~~ones~~ ingredients so that they are ready for use by fish bodies because of the presence of enzymes such as amylase, protease, lipase and cellulose could increase nutritional value and digestibility feed given. Elumalai et al. (2013) stated that probiotics are live microorganisms that can be used to prevent disease in cultured fish, to increase production, and to reduce economic losses in fish cultivators. The addition of probiotics in feed also increase immunity which in turn affects the survival of the cultured fish. The results of the study according to Iribarren et al. (2012) and Agustin et al. (2014) indicated that the use of probiotics in feed can increase the survival rate and body resistance of fish against pathogenic infections.

The use of laser technology has been successfully developed by catfish farmers in East Java and Central Java. Catfish broodstocks that was induced by laser for 15 seconds at the reproductive point, precisely at 2/3 of the ventral body with an induction frequency of once in two weeks was proven within 15 days has optimal affected for biostimulation of the gonadal growth and development, indicated the increasing gonadal maturation level and ready to be spawned of catfish broodstocks. Kusuma, P. S. W., Ngadiani, Hariani, D. (2015). This proves that laser induction in the reproductive point of catfish is believed to have an optimal affect to increase neurotransmitter and neurohormone activities for the

Commented [WU7]: Bisa ditambahkan Ghaedi et al. (2019)

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Commented [WU8]: Bisa dilihat di artikel pdf probiotik . Leblanc et al., 2011; Ray et al., 2012; Oktavianawati et al.,2016).

Commented [WU9]: Krishna et al., 2015; Hosainfar 2018; Chowdhury and Roy, 2020; Ringo 2020.

Commented [G10]: The authors need to add references related probiotics and reproduction status not growth or immunity

1- Integrated control of fish metabolism, wellbeing and **reproduction**: the role of **probiotic**

2- .....

Formatted: Font: 12 pt, Font color: Blue, English (United Kingdom)

Commented [G11]: 1- Too long sentences
2- no reference ??!!

Bisa ditambahkan Hariani et al. (2020); **ditambah stranas p.pungky ya**

gonadotropin-releasing hormone (GnRH) synthesis and release in the hypothalamus and pituitary (Kusuma & Hariani, 2019). ~~What is the role of the laser and fermented feed on gonadal maturity and seeds production in the fish?~~ This study aimed to determine the effects of addition the fermented feed using probiotic and laser induction on accelerate the mature broodstocks and seed productions of African catfish.

Commented [G12]: Literature rewiev for laser and fish reproduction?

1-Application of low-intensity **laser** radiation in technology of sturgeon fish **reproduction**

Bisa diambilkan di Kusuma et al. 2015; Mukti et al 2020

Materials and Methods

This research was conducted at the Unit Pelaksana Teknis Pelatihan Teknis Perikanan Budidaya dan Pengolahan Produk Kelautan dan Perikanan (UPT PTPBP2KP), Kepanjen, Malang, East Java, Indonesia. This study was used experimental with a completely randomized design consisting of three treatments and three time replicates.

Commented [G13]: Summarize the name

Animals

The immature male and female broodstocks of African catfish with the average age of 1 to 1.5 years and the average body weight of 900 to 1,500 g and 1,140 to 1,750 g, respectively were used. In this study, the experimental protocols were approved by the Scientific Committee with Protocol Number 027/SP2H/AMD/LT/MULTI/L7/2020.

Feed and Probiotic

In this study, ~~the treatments were used~~ commercial feed contains of 38% crude protein was used. The commercial feed was fermented using probiotic (Probio-7) dose of 5 mL as fermenter produced by Tamasindo Veterinary with composition of *Saccharomyces cerevisiae* and *Aspergillus oryzae* fungus and *Lactobacillus acidophilus*, *Bacillus subtilis*, *Rhodopseudomonas, Actinomycetes*, and *Nitrobacter* bacteria contains more than 1×10^{11} CFU kg^{-1}, respectively.

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Perlu ditambahkan ini.....(Peneliti tidak memasuk strain karena hanya menggunakan probiotik yang ada dipasaran) The acclimatization of catfish broodstock was carried out separately for 2 weeks in concrete ponds (2m x 2m x 90 cm). Broodstocks were fed with commercial floating pellet (PF-128), with a protein content of 38% and probiotics added (Probio-7, Tamasindo Veterinary product) with the composition (according to the label): *Saccharomyces cerevisiae, Aspergillus oryzae, Lactobacillus acidophilus, Bacillus subtilis, Rhodopseudomonas, Actinomycetes* and *Nitrobacter* with density for each bacteria of > 1

x 1011 CFU/L. Feed was administered twice, in the morning and evening, as much as 5% BW until the catfish gonads were matured.

Rearing of Fish Broodstocks

The broodstocks were acclimatized separately and fed of commercial feed for 2 weeks in a controlled pond size of 2 m × 2 m × 0.9 m to prevent spawning before treatment. After the broodstocks were acclimatized, the broodstocks were treated using three treatments, namely unfermented feed as control, probiotic-fermented feed (PFF), and probiotic-fermented feed+laser induction (PFF+Li). The broodstocks were fed twice (morning and evening) as much as 5% of body weight.

Laser Induction

Laser was used diode soft-laser of 15 mW with wavelength of 532 nm equivalent with 1,125 Joule/cm square/point is still in the safe range for biostimulation of biological organs The female broodstocks were induced by laser at the reproductive acupoint, precisely in 2/3 of the body ventral part for 15 seconds whereas in male broodstock without laserpuncture induction. (Kusuma et al., 2015). Laser induction was performed every 15 days until the gonad mature (the IV maturity stage) reached and ready to be spawned.

Cara menghitung :
Panjang gelombang laserpunktur yang digunakan untuk biostimulasi 532 nm, Daya 5 mW, Luas luaran cahaya 0,2 cm persegi dengan lama paparan 15 detik., Watt = Joule/detik 15 mW = 15/1000Watt/detik = 0,015.....0,015/0,2 = 0,075 Joule/cm persegi, 1 titik reproduksi dipapar 15 detik 15 X 0,075 = 1,125 Joule/cm persegi/titik masih dalam kisaran aman untuk biostimulasi organ bioJogi.

Measurement of Gonadal Maturity

The gonadal maturity of broodstocks, both males and females were observed once every 2 to 5 days to determine the gonadal maturity time after treatment. The gonadal maturity was observed to spawning behavior signs and to genetalia visually of catfish. Then, the one pair mature broodstock was reared at spawning pond.

Spawning and Eggs Incubation

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Fish spawning was conducted to a spawning pond size of 2 m × 2 m × 0.9 m using a substrat size of 1.4 m × 0.4 m to the eggs attachment and aerations to the supply dissolved oxygen during eggs incubation, separated between treatments. After 8 hours, the broodstock completes the spawning process. Then, the substrat size 0.3 m × 0.3 m was cutted as sample to manually count the fertilized eggs number and incubated at the controlled aquarium size of 0.5 m × 0.5 m × 0.5 m with good aeration until hatching, separated between treatments. The eggs qualities of spawned catfish such as fertilization rate (FR) and hatching rate (HR) were measured. The formulas were used to calculate fertilization rate (FR) and hatching rate (HR), respectively, as follows:

$$\text{FR (\%)} = \frac{\text{Number of fertilized eggs}}{\text{Total number of eggs}} \times 100$$

$$\text{HR (\%)} = \frac{\text{Number of eggs hatched}}{\text{Total number of fertilized eggs}} \times 100$$

Rearing of Seed

The catfish fry of three days after hatching (dah) was reared at the controlled pond size 2 m × 2 m × 1 m, separated between treatments with density of 100,000 fish, respectively for three months. The fry was fed gradually. The first, fry was fed live feed of silkworms, at-satiation, three times a day for 1 month. Next, the seed was fed commercial feed contains of 35% crude protein for 1 month continued commercial feed contains of 35% crude protein until end rearing as much as 5% of the biomass, three times a day, respectively. The formulas were used to calculate survival rate (SR) according to Mukti et al. (2020a), as follows:

$$\text{SR (\%)} = \frac{\text{Life fish number at the final of rearing}}{\text{Life fish number at the initial of rearing}} \times 100$$

Measurement of Survival and Total Length

The mortality of seed was observed and counted every day. The survival rate (SR) and the total length of seed were measured at the end of fish rearing. The seed was sorted and grouped into two grades of total length, i.e. 1.0 to 2.0 cm and 2.1 to 3.0 cm, respectively.

Data Analysis

Data on gonadal maturity time of catfish broodstocks, FR, HR, SR, and total length grades of catfish seed were statistically analyzed using analysis of variant (ANOVA) with SPSS

ver. 10 software (SPSS Inc., Chicago, IL, USA) followed by Duncan's multiple range test with a confidence level of 95% according to Mukti et al. (2020a,b).

Analisis anava ini sudah benar karena yang di induksi laserpunktur hanya induk betina sedang hasil anak diperoleh dari hasil persilangan induk betina dengan jantan normal.

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Why you did not use two-way ANOVA??

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Ethical Statement

All authors declare that the present study was conducted in an ethical, professional, and responsible manner.

Results

Gonadal Maturity Time of Broodstocks

The results showed that addition of probiotic-fermented feed and laser induction (PFF+Li) in the broodstocks before spawned have a significant effect (P<0.05) on the gonadal maturity time of males and females Africant catfish. The gonadal maturity time of PFF+Li treatment was significantly fastest (32 to 37 days) compared to fermented feed (PFF) and control treatments (P<0.05) as shown in the Figures 1 and 2. The result showed that laser induction accelerates the gonadal maturity time of 1.68 and 1.26 times fastest compared to control and PFF treatments, respectively, while PFF treatment accelerates the gonadal maturity time of 1.34 times faster than control.

Seed Production

The results indicated that the FR, HR, SR, dan seed size production had significant differences between treatments (P<0.05). The PFF+Li treatment have FR, HR, and SR highest compared to other treatments as shown in Figures 3, 4, and 5, respectively. This study indicated that the laser induction increase FR and HR of eggs and SR of seed in African catfish. Meanwhile, the addition of fermented feed increase level of FR, HR, and SR and significant differences than control (P<0.05).

The results also showed that addition of probiotic-fermented feed and laser induction have a significant differences (P<0.05) on size grades of catfish seed after being reared for 3 months. This study indicated that the PFF+Li treatment produces the seed number of 2.1 to

3.0 cm size highest compared to other treatments with a ratio between 1.0 to 2.0 cm and 2.1 to 3.0 cm sizes was 1:2.64. In the control, a ratio between 1.0 to 2.0 cm and 2.1 to 3.0 cm sizes was 1:2.53, while PFF treatment has the same relative ratio between 1.0 to 2.0 cm and 2.1 to 3.0 cm sizes (Figure 6).

Discussion

This study indicated that the addition of probiotics as fermenter in commercial feed was proven to accelerate the gonadal maturity time of male dan and female African catfish. Male and female broodstocks of catfish that treated probiotic-fermented feed (PFF) shows faster maturity time in the gonad than unfermented feed (control). This indicated that addition of probiotics could increase the nutrition value of the feed. Probiotic can produce several digestive enzymes, such as amylase, protease, lipase, and cellulose. These enzymes were advantageous in hydrolyzing carbohydrates, proteins, and fats of commercial feed into simpler molecules, so facilitate the digestion and absorption processes in the digestive tract of catfish. Also, several studies shown that bacteria present in probiotics could increase the nutritional value of feed by synthesizing vitamins, proteins, essential fatty acids, amylase, protease, and lipase enzymes (Irianto & Austin, 2002; Ghosh et al., 2008; Putra, 2010).

The results of the nutritional content analysis in probiotic-fermented commercial feed using Probio-7 found that there contain several types of non-essential amino acids, such as alanine, asparagine, aspartate, glutamate, glycine, serine, tyrosine (Kusuma & Hariani, 2019). Glutamate is one of the amino acids that plays an influential role as a major transmitter in the fish brain, which functions as a mediator to transmit signals in postsynaptic. This glutamate also functions as a precursor to the neurotransmitter Gamma amino butyric acid (GABA). So, if probiotic-fermented feed is given and combined with laser induction on the catfish broodstocks, there will be a physiological reaction in the body of the main fish in the brain. This study indicated that laser induction have that effect. Where GABAergic neurons that were not active before become reactive due to the activity of the GAD-65 enzyme. GABAergic will synthesize GABA, then GABA will stimulate the synthesis of the release of gonadotropin hormone (GnRH) from the hypothalamus and pituitary. GnRH plays a role in stimulating the synthesis and release of the gonadotropin hormones, follicle stimulating hormone (FSH) and Luteinizing hormone (LH) (GtH-I and GtH-II). This molecular

activity can occur because there is a relationship between neurons in the catfish broodstocks brain that is induced by laser (Kusuma & Hariani, 2017).

The release of GtH-I in female catfish stimulates the gonads to produce steroid hormones such as estradiol-17β. Estradiol-17β produced by granulosa cells from ovarian follicles was then carried by the bloodstream to hepatic hepatocytes which will have the effect of stimulating vitellogenesis to synthesize and release vitellogenin. Then vitellogenin was carried by the bloodstream to developing oocytes to be absorbed and accumulated, and as a result, the oocytes will increase in diameter and mature (Kusuma & Hariani, 2019). Sabet et al. (2009) and Taghizadeh et al. (2013) stated that the same thing that changes in estradiol-17β levels in blood plasma were closely related to oocyte development. The release of GtH-II plays a role in stimulating the final maturation of gonads, ovulation and spawning of female catfish. Arukwe and Goksøyr (2003) stated that the synthesis process of yolk protein precursor was vitellogenin due to stimulation of estradiol-17β. Vitellogenin was secreted in the liver and then carried by the bloodstream to the developing ovary for absorption.

The development of large and mature oocytes was indicated by the value increasing of the hepatosomatic index (HSI) and ganado somatic index (GSI) (Cerda et al., 2007; Hariani & Kusuma, 2019). Furthermore, Araoye (2001), Laleye et al. (2006), and Shinkafi and Ipinjolu (2012) stated that the value of HSI and GSI reached a maximum before spawning and then decreased after spawning. Nutrition in brood fish feed was one of the most studied because biological mechanisms, such as gonad maturity were a complicated process. Gonadal development and fecundity are influenced by several nutrients, especially those associated with fish spawning (Izquierdo et al., 2001).

The effect of nutrition in fish feed was important in gonad maturation and egg development. Proteins and lipids were the main components of egg yolk which function as the main source of nutrition during embryogenesis so that their existence was needed to support the survival of embryos and larvae so that they are resistant to changes in the aquatic environment (Izquierdo et al., 2001). Brooks et al. (1997) stated that protein in feed affects the protein in fish eggs, such as lipoproteins, hormones, and enzymes, all of which determine egg quality. The quality of these eggs will determine seed production on a large scale. In general, the nutritional status of fish feed could influence gonad development and

limit the number and quality of eggs and sperm produced. Coldebella et al. (2011) mentioned that the protein content in broodstock feed was proven to affect the survival of larvae, if the protein content in the feed is very low (10 to 20%) it can result in a low egg fertilization rate and a greater percentage of abnormal larvae.

Egg yolk protein play a role in embryogenesis and food reserves before catfish seeds found their food according to Tang and Affandi (2000) and Salerno et al. (2002) who stated that vitellogenin is a glycophosphoprotein containing 20% lipid, especially phospholipids, triglycerides, lipoproteins and cholesterol. These protein molecules were crucial because they would be used as a source of energy in the growth and development of the embryo into larvae.

Vitellogenin synthesis was characterized by an increase in the volume of the cytoplasm originating from outside the cell, namely the yolk, therefore the quality of eggs was very much determined during vitellogenesis. Several factors such as feed quality, environment, and activity of gonadotropin hormones were very influential in supporting the success of this process. Feed quality, a supportive environment and homon gonadotropin activity were very influential in determining the quality of eggs produced. The eggs quality produced was determined by the yolk quality protein in the oocytes. The protein could increasing the percentage of the number of fertilized eggs and the high number of eggs that hatch into larvae.

The mechanism could be explained as follows: first, commercial feed containing carbohydrate, protein, fat, vitamins, minerals, fibre, and water when added with probiotics and brooded for a day, the feed will immediately be fermented into a fermented feed. The fermented feed had an acidic pH, if this fermented feed enters the digestive system of the fish, it will then stimulate the gastric epithelium to increase hormone synthesis and release of digestive enzymes. Digestive enzymes released in the digestive tract act to degrade fermented feed into amino acids which are easily absorbed by the intestinal epithelium by endocytosis. From the intestinal epithelium, the nutrients that were readily absorbed were carried by the bloodstream to the liver, brain and gonads. In the liver, nutrients will be used as a basis for the synthesis of vitellogenin, in the brain, nutrients are used to increase the activity of neurotransmitters and neurohormones which were advantageous in stimulating the synthesis of gonadotropin hormones in the hypothalamus and pituitary, while in the

ovaries, nutrients were used for the development and maturation of eggs and in the testis nutrients play a role in steroidogenesis and gametogenesis in the formation of spermatozoa.

The results showed that addition of probiotic-fermented feed and laser induction before spawning had been proven to accelerate gonadal maturity of catfish broodstock and ready to be spawned to produce higher seeds than the control. This shows that the feed nutrients that given in catfish broodstock before spawning were play important role because they were leading in accelerating the gonad mature of catfish broodstocks and the quality of seeds produced.

Penelitian yang dilakukan Herdis (2010), tentang aplikasi teknologi laserpunktur untuk gertak birahi pada domba Garut, hasil penelitian membuktikan bahwa induksi laserpunktur pada titik-titik reproduksi tepatnya pada: Titik Ming-Meng/estrus (tunggal) terletak di daerah dorsal dari persendian *vet. lumbal* II dengan *vet. lumbal* III (sebagai *generalpoint*). Rangsangan pada titik ini akan meningkatkan aktivitas kelenjar hypofisa. Titik Shen Yu/Ovarium (*dexter* dan *sinister*) terletak di daerah dorsal antara *processus transversus* dari *vet. lumbal* I- II - III. Rangsangan pada titik ini akan meningkatkan aktivitas ovarium dalam hal pembentukkan folikel dan pembentukan hormon reproduksi.Titik Oviduk (*dexter* dan *sinister*) terletak di daerah dorsal antara *processus transversus* dari *vet. lumbal* III-IV-V-VI. Rangsangan pada titik ini akan menghindari sistik ovari dan hipofungsi ovari. Titik cervik uteri (*dexter* dan *sinister*) terletak di daerah ujung atas os ileum. Rangsangan pada titik ini akan mengoptimalkan servik uteri pada kejadian estrus dimana akan membuka untuk keluarnya lendir berahi.Titik Hormonal (tunggal) terletak di daerah dorsal dari persendian *sacro-coccygea.* Rangsangan pada titik ini akan meningkatkan produksi hormon prostaglandin dari dalam tubuh dan GV-1 satu buah titik akupunktur terletak di atas vulva dan dua buah titik akupunktur terletak di sisi kiri dan kanan pertengahan vulva. Hasil penelitian menunjukkan hampir semua domba betina yang di induksi laserpunktur selama tiga kali selama 20 detik rata-rata timbul entrus 16 jam setelah akhir perlakuan menunjukkan respon estrus, ditandai 95% betina diam apabila dinaiki pejantan pemancing.

Pustaka:

Herdis, 2010. Aplikasi Teknologi Laserpunktur dalam Meningkatkan Libido Pejantan Domba Garut (Ovis aries). Pusat Teknologi Produksi Pertanian Bidang TAB BPPT. Jakarta. Vol. 12(1):25-30.

Penelitian Binawati (2008), induksi laserpunktur dengan daya 0,2 Joule/titik pada titik ova tepatnya di daerah dorsal persendian thorax akhir dengan lumbal pertama diikuti dengan induksi laserpunktur pada titik nafsu makan dan titik ketahanan tubuh, terbukti berpengaruh signifikan ($P<0.05$) pada produksi telur dan total berat lemak dalam rongga abdominal ayam Arab (Gallus turcicus). Perlakuan induksi laserpunktur dengan durasi 15, 30 dan 45 detik. Berturut-turut menghasilkan produksi telur yang semakin tinggi, sedangkan pada kontrol produksi telur kurang tinggi.

Pustaka:

Binawati, K. 2008. Pengaruh lanskeptur terhadap kualitas telur ayam Arab. Journal of Science. 1 (2) : 28-34.

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Conclusion

Overall study results could that addition fermented feed and laser induction to the catfish broodstocks before the spawn was accelerated the preparation time of gonadal maturity and ready to be spawned. Combination of probiotic-fermented feed and laser induction increase FR, HR, SR, and seed production levels.

Acknowledgments

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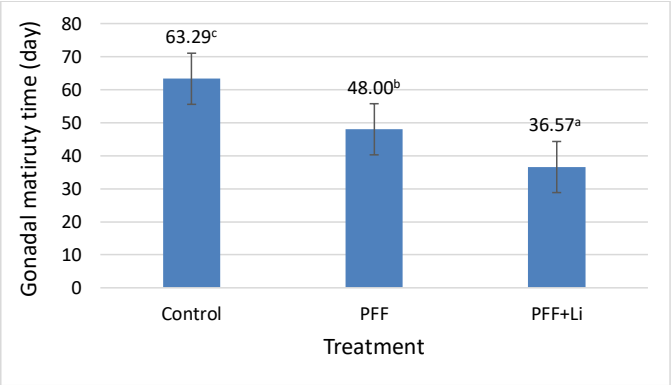

Figure 1. Gonadal maturity time of male catfish broodstocks between treatments. PFF = probiotic-fermented feed, PFF+Li = probiotic-fermented feed+laser induction. Mean values not sharing the same letter indicate a significant differences (P<0.05).

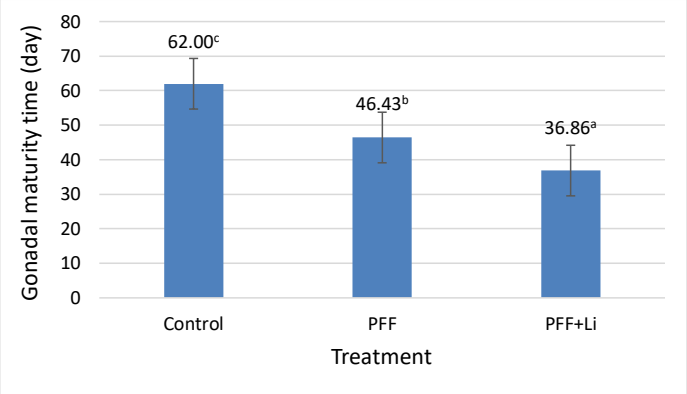

Figure 2. Gonadal maturity time of female catfish broodstocks between treatments. PFF = probiotic-fermented feed, PFF+Li = probiotic-fermented feed+laser induction. Mean values not sharing the same letter indicate a significant differences (P<0.05).

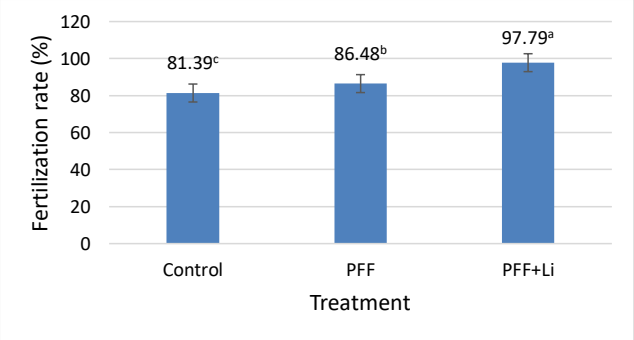

Figure 3. Fertilization rate of catfish eggs between treatments. PFF = probiotic-fermented feed, PFF+Li = probiotic-fermented feed+laser induction. Mean values not sharing the same letter indicate a significant differences (P<0.05).

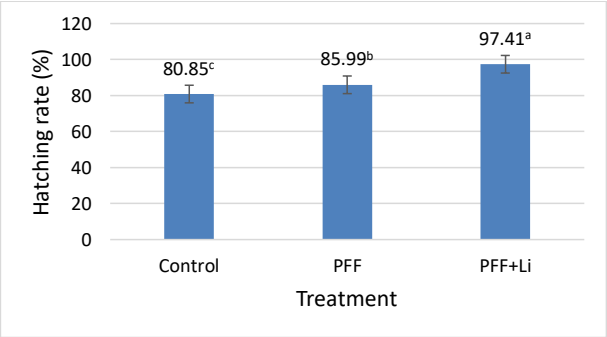

Figure 4. Hatching rate of catfish eggs between treatments. PFF = probiotic-fermented feed, PFF+Li = probiotic-fermented feed+laser induction. Mean values not sharing the same letter indicate a significant differences (P<0.05).

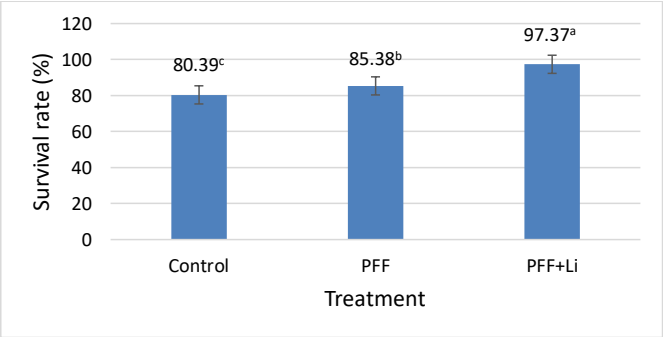

Figure 5. Survival rate of catfish seed between treatments. PFF = probiotic-fermented feed, PFF+Li = probiotic-fermented feed+laser induction. Mean values not sharing the same letter indicate a significant differences (P<0.05).

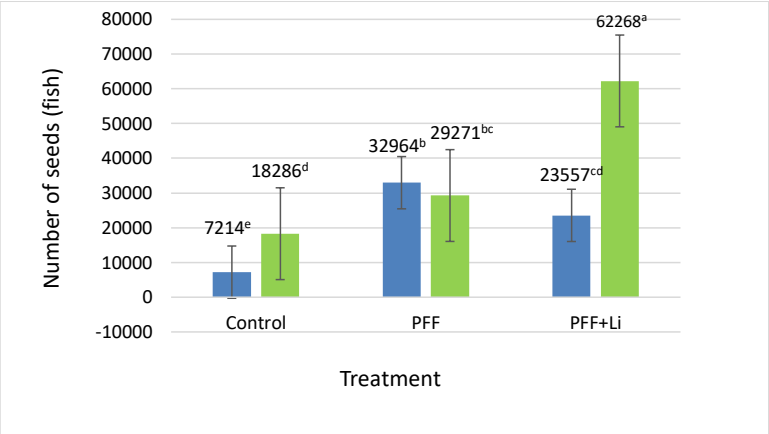

Figure 6. The number of catfish seeds with two grades of total length size produced by different treatments. PFF = probiotic-fermented feed, PFF+Li = probiotic-fermented feed+laser induction (blue bar = grade of 1.0 to 2.0 cm, green bar = grade of 2.1 to 3.0 cm). Mean values not sharing the same letter indicate a significant differences (P<0.05).

Highlights

- Availability of catfish broodstocks and seeds is limited and not continuous
- Probiotics can use as fermenter in commercial feed
- Probiotics have increased immunity, survival rate, and body resistance of fish
- Laser as bio stimulation to induce the gonadal development
- Combination fermented feed and laser induction accelerate the gonadal maturity and increase seed performances

Addition of probiotic-fermented feed and laser induction to accelerate mature broodstocks and seed productions of African catfish (*Clarias gariepinus*)

Abstract

The decreasing availability of mature broodstocks and seed productions can hinder the sustainability of catfish cultivation. An alternative solution is intensive hatchery by the addition of fermented feed and laser induction so that the availability of mature broodstocks and seeds of catfish, both quantity and continuity are guaranteed. This study aimed to determine the effects of addition of fermented feed using probiotic and laser induction on accelerate the mature broodstocks and seed productions of African catfish. Fish were used male and female broodstocks of African catfish. The method was used a completely randomized design with three treatments, namely unfermented feed as control, probiotic-fermented feed (PFF), and probiotic-fermented feed+laser induction (PFF+Li) and three times replication, respectively. Laser induction was performed every 15 days. The gonadal maturity time of male and female broodstocks, fertilization rate (FR), hatching rate (HR), and seed production performances include survival rate (SR) were observed. The results showed that the addition of PFF+Li reach fastest time to mature of the female gonad (31-41 days) and the male gonad (32-37 days), and produce highest FR, HR, and SR more than 90%, respectively, and catfish seed yield of 2.1-3.0 cm size compared to other treatments (P<0.05).

Key words: Fermented feed, Probiotic, Soft-laser, Gonadal maturity, African catfish

Introduction

The main problem faced by catfish breeders in hatchery centres is the conventional cultivation method, catfish that have been spawned need recovery time to be able to spawn around three months later, thus the availability of broodstocks and seeds in the community is limited and not continuous, so it can hinder productivity catfish farming business. The market demand for this catfish commodity continues to increase, meanwhile the availability of gonadal mature and seeds of catfish are decreasing. It is necessary to find alternative solutions through more intensive farming of catfish, namely by increasing the quality of feed and the application of appropriate biostimulation laser technology so that the availability of

mature broodstocks and seeds, especially the number and continuity can take place continuously.

In general, the protein content in catfish feed could affect several things, such as gonadal development, spermatozoa and eggs productions limit number and quality. Based on study according to Coldebella et al. (2011) showed that the protein content of the feed was proven to affect the survival of larvae, to very low levels of feed protein content (10 to 20%) resulting in low fertilization rate of eggs and a higher percentage of abnormal larvae. Hence, the quality of feed in the broodstocks needs to be improved. The results of the study according to Sakamole et al. (2014) showed that the addition of the probiotic in feed was proven to be able to break down feed complex compounds into simple ones so that they are ready for use by fish bodies because of the presence of enzymes such as amylase, protease, lipase and cellulose could increase nutritional value and digestibility feed given. Elumalai et al. (2013) stated that probiotics are live microorganisms that can be used to prevent disease in cultured fish, to increase production, and to reduce economic losses in fish cultivators. The addition of probiotics in feed also increase immunity which in turn affects the survival of the cultured fish. The results of the study according to Iribarren et al. (2012) and Agustin et al. (2014) indicated that the use of probiotics in feed can increase the survival rate and body resistance of fish against pathogenic infections.

The use of laser technology has been successfully developed by catfish farmers in East Java and Central Java. Catfish broodstocks that was induced by laser for 15 seconds at the reproductive point, precisely at 2/3 of the ventral body with an induction frequency of once in two weeks was proven within 15 days has optimal affected for biostimulation of the gonadal growth and development, indicated the increasing gonadal maturation level and ready to be spawned of catfish broodstocks. This proves that laser induction in the reproductive point of catfish is believed to have an optimal affect to increase neurotransmitter and neurohormone activities for the gonadotropin-releasing hormone (GnRH) synthesis and release in the hypothalamus and pituitary (Kusuma & Hariani, 2019). What is the role of the laser and fermented feed on gonadal maturity and seeds production in the fish? This study aimed to determine the effects of addition the fermented feed using probiotic and laser induction on accelerate the mature broodstocks and seed productions of African catfish.

Materials and Methods

This research was conducted at the Unit Pelaksana Teknis Pelatihan Teknis Perikanan Budidaya dan Pengolahan Produk Kelautan dan Perikanan (UPT PTPBP2KP), Kepanjen, Malang, East Java, Indonesia. This study was used experimental with a completely randomized design consisting of three treatments and three time replicates.

Animals

The immature male and female broodstocks of African catfish with the average age of 1 to 1.5 years and the average body weight of 900 to 1,500 g and 1,140 to 1,750 g, respectively were used. In this study, the experimental protocols were approved by the Scientific Committee with Protocol Number 027/SP2H/AMD/LT/MULTI/L7/2020.

Feed and Probiotic

In this study, the treatments were used commercial feed contains of 38% crude protein. The commercial feed was fermented using probiotic (Probio-7) dose of 5 mL as fermenter produced by Tamasindo Veterinary with composition of *Saccharomyces cerevisiae* and *Aspergillus oryzae* fungus and *Lactobacillus acidophilus*, *Bacillus subtilis*, *Rhodopseudomonas, Actinomycetes*, and *Nitrobacter* bacteria contains more than 1×10^{11} CFU kg^{-1}, respectively.

Rearing of Fish Broodstocks

The broodstocks were acclimatized separately and fed of commercial feed for 2 weeks in a controlled pond size of 2 m × 2 m × 0.9 m to prevent spawning before treatment. After the broodstocks were acclimatized, the broodstocks were treated using three treatments, namely unfermented feed as control, probiotic-fermented feed (PFF), and probiotic-fermented feed+laser induction (PFF+Li). The broodstocks were fed twice (morning and evening) as much as 5% of body weight.

Laser Induction

Laser was used diode soft-laser of 15 mW with wavelength of 532 nm. The broodstocks were induced by laser at the reproductive acupoint, precisely in 2/3 of the body ventral part for 15 seconds (Kusuma et al., 2015). Laser induction was performed every 15 days until the gonad mature (the IV maturity stage) reached and ready to be spawned.

Measurement of Gonadal Maturity

The gonadal maturity of broodstocks, both males and females were observed once every 2 to 5 days to determine the gonadal maturity time after treatment. The gonadal maturity was observed to spawning behavior signs and to genetalia visually of catfish. Then, the one pair mature broodstock was reared at spawning pond.

Spawning and Eggs Incubation

Fish spawning was conducted to a spawning pond size of 2 m × 2 m × 0.9 m using a substrat size of 1.4 m × 0.4 m to the eggs attachment and aerations to the supply dissolved oxygen during eggs incubation, separated between treatments. After 8 hours, the broodstock completes the spawning process. Then, the substrat size 0.3 m × 0.3 m was cutted as sample to manually count the fertilized eggs number and incubated at the controlled aquarium size of 0.5 m × 0.5 m × 0.5 m with good aeration until hatching, separated between treatments. The eggs qualities of spawned catfish such as fertilization rate (FR) and hatching rate (HR) were measured. The formulas were used to calculate fertilization rate (FR) and hatching rate (HR), respectively, as follows:

$$\text{FR (\%)} = \frac{\text{Number of fertilized eggs}}{\text{Total number of eggs}} \times 100$$

$$\text{HR (\%)} = \frac{\text{Number of eggs hatched}}{\text{Total number of fertilized eggs}} \times 100$$

Rearing of Seed

The catfish fry of three days after hatching (dah) was reared at the controlled pond size 2 m × 2 m × 1 m, separated between treatments with density of 100,000 fish, respectively for three months. The fry was fed gradually. The first, fry was fed live feed of silkworms, at-satiation, three times a day for 1 month. Next, the seed was fed commercial feed contains of 35% crude protein for 1 month continued commercial feed contains of 35% crude protein until end rearing as much as 5% of the biomass, three times a day, respectively. The formulas were used to calculate survival rate (SR) according to Mukti et al. (2020a), as follows:

$$\text{SR (\%)} = \frac{\text{Life fish number at the final of rearing}}{\text{Life fish number at the initial of rearing}} \times 100$$

Measurement of Survival and Total Length

The mortality of seed was observed and counted every day. The survival rate (SR) and the total length of seed were measured at the end of fish rearing. The seed was sorted and grouped into two grades of total length, i.e. 1.0 to 2.0 cm and 2.1 to 3.0 cm, respectively.

Data Analysis

Data on gonadal maturity time of catfish broodstocks, FR, HR, SR, and total length grades of catfish seed were statistically analyzed using analysis of variant (ANOVA) with SPSS ver. 10 software (SPSS Inc., Chicago, IL, USA) followed by Duncan's multiple range test with a confidence level of 95% according to Mukti et al. (2020a,b).

Ethical Statement

All authors declare that the present study was conducted in an ethical, professional, and responsible manner.

Results

Gonadal Maturity Time of Broodstocks

The results showed that addition of probiotic-fermented feed and laser induction (PFF+Li) in the broodstocks before spawned have a significant effect (P<0.05) on the gonadal maturity time of males and females Africant catfish. The gonadal maturity time of PFF+Li treatment was significantly fastest (32 to 37 days) compared to fermented feed (PFF) and control treatments (P<0.05) as shown in the Figures 1 and 2. The result showed that laser induction accelerates the gonadal maturity time of 1.68 and 1.26 times fastest compared to control and PFF treatments, respectively, while PFF treatment accelerates the gonadal maturity time of 1.34 times faster than control.

Seed Production

The results indicated that the FR, HR, SR, dan seed size production had significant differences between treatments (P<0.05). The PFF+Li treatment have FR, HR, and SR highest compared to other treatments as shown in Figures 3, 4, and 5, respectively. This study indicated that the laser induction increase FR and HR of eggs and SR of seed in African catfish. Meanwhile, the addition of fermented feed increase level of FR, HR, and SR and significant differences than control (P<0.05).

The results also showed that addition of probiotic-fermented feed and laser induction have a significant differences (P<0.05) on size grades of catfish seed after being reared for 3

months. This study indicated that the PFF+Li treatment produces the seed number of 2.1 to 3.0 cm size highest compared to other treatments with a ratio between 1.0 to 2.0 cm and 2.1 to 3.0 cm sizes was 1:2.64. In the control, a ratio between 1.0 to 2.0 cm and 2.1 to 3.0 cm sizes was 1:2.53, while PFF treatment has the same relative ratio between 1.0 to 2.0 cm and 2.1 to 3.0 cm sizes (Figure 6).

Discussion

This study indicated that the addition of probiotics as fermenter in commercial feed was proven to accelerate the gonadal maturity time of male dan and female African catfish. Male and female broodstocks of catfish that treated probiotic-fermented feed (PFF) shows faster maturity time in the gonad than unfermented feed (control). This indicated that addition of probiotics could increase the nutrition value of the feed. Probiotic can produce several digestive enzymes, such as amylase, protease, lipase, and cellulose. These enzymes were advantageous in hydrolyzing carbohydrates, proteins, and fats of commercial feed into simpler molecules, so facilitate the digestion and absorption processes in the digestive tract of catfish. Also, several studies shown that bacteria present in probiotics could increase the nutritional value of feed by synthesizing vitamins, proteins, essential fatty acids, amylase, protease, and lipase enzymes (Irianto & Austin, 2002; Ghosh et al., 2008; Putra, 2010).

The results of the nutritional content analysis in probiotic-fermented commercial feed using Probio-7 found that there contain several types of non-essential amino acids, such as alanine, asparagine, aspartate, glutamate, glycine, serine, tyrosine (Kusuma & Hariani, 2019). Glutamate is one of the amino acids that plays an influential role as a major transmitter in the fish brain, which functions as a mediator to transmit signals in postsynaptic. This glutamate also functions as a precursor to the neurotransmitter Gamma amino butyric acid (GABA). So, if probiotic-fermented feed is given and combined with laser induction on the catfish broodstocks, there will be a physiological reaction in the body of the main fish in the brain. This study indicated that laser induction have that effect. Where GABAergic neurons that were not active before become reactive due to the activity of the GAD-65 enzyme. GABAergic will synthesize GABA, then GABA will stimulate the synthesis of the release of gonadotropin hormone (GnRH) from the hypothalamus and pituitary. GnRH plays a role in stimulating the synthesis and release of the gonadotropin hormones, follicle

stimulating hormone (FSH) and Luteinizing hormone (LH) (GtH-I and GtH-II). This molecular activity can occur because there is a relationship between neurons in the catfish broodstocks brain that is induced by laser (Kusuma & Hariani, 2017).

The release of GtH-I in female catfish stimulates the gonads to produce steroid hormones such as estradiol-17β. Estradiol-17β produced by granulosa cells from ovarian follicles was then carried by the bloodstream to hepatic hepatocytes which will have the effect of stimulating vitellogenesis to synthesize and release vitellogenin. Then vitellogenin was carried by the bloodstream to developing oocytes to be absorbed and accumulated, and as a result, the oocytes will increase in diameter and mature (Kusuma & Hariani, 2019). Sabet et al. (2009) and Taghizadeh et al. (2013) stated that the same thing that changes in estradiol-17β levels in blood plasma were closely related to oocyte development. The release of GtH-II plays a role in stimulating the final maturation of gonads, ovulation and spawning of female catfish. Arukwe and Goksøyr (2003) stated that the synthesis process of yolk protein precursor was vitellogenin due to stimulation of estradiol-17β. Vitellogenin was secreted in the liver and then carried by the bloodstream to the developing ovary for absorption.

The development of large and mature oocytes was indicated by the value increasing of the hepatosomatic index (HSI) and ganado somatic index (GSI) (Cerda et al., 2007; Hariani & Kusuma, 2019). Furthermore, Araoye (2001), Laleye et al. (2006), and Shinkafi and Ipinjolu (2012) stated that the value of HSI and GSI reached a maximum before spawning and then decreased after spawning. Nutrition in brood fish feed was one of the most studied because biological mechanisms, such as gonad maturity were a complicated process. Gonadal development and fecundity are influenced by several nutrients, especially those associated with fish spawning (Izquierdo et al., 2001).

The effect of nutrition in fish feed was important in gonad maturation and egg development. Proteins and lipids were the main components of egg yolk which function as the main source of nutrition during embryogenesis so that their existence was needed to support the survival of embryos and larvae so that they are resistant to changes in the aquatic environment (Izquierdo et al., 2001). Brooks et al. (1997) stated that protein in feed affects the protein in fish eggs, such as lipoproteins, hormones, and enzymes, all of which determine egg quality. The quality of these eggs will determine seed production on a large

scale. In general, the nutritional status of fish feed could influence gonad development and limit the number and quality of eggs and sperm produced. Coldebella et al. (2011) mentioned that the protein content in broodstock feed was proven to affect the survival of larvae, if the protein content in the feed is very low (10 to 20%) it can result in a low egg fertilization rate and a greater percentage of abnormal larvae.

Egg yolk protein play a role in embryogenesis and food reserves before catfish seeds found their food according to Tang and Affandi (2000) and Salerno et al. (2002) who stated that vitellogenin is a glycophosphoprotein containing 20% lipid, especially phospholipids, triglycerides, lipoproteins and cholesterol. These protein molecules were crucial because they would be used as a source of energy in the growth and development of the embryo into larvae.

Vitellogenin synthesis was characterized by an increase in the volume of the cytoplasm originating from outside the cell, namely the yolk, therefore the quality of eggs was very much determined during vitellogenesis. Several factors such as feed quality, environment, and activity of gonadotropin hormones were very influential in supporting the success of this process. Feed quality, a supportive environment and homon gonadotropin activity were very influential in determining the quality of eggs produced. The eggs quality produced was determined by the yolk quality protein in the oocytes. The protein could increasing the percentage of the number of fertilized eggs and the high number of eggs that hatch into larvae.

The mechanism could be explained as follows: first, commercial feed containing carbohydrate, protein, fat, vitamins, minerals, fibre, and water when added with probiotics and brooded for a day, the feed will immediately be fermented into a fermented feed. The fermented feed had an acidic pH, if this fermented feed enters the digestive system of the fish, it will then stimulate the gastric epithelium to increase hormone synthesis and release of digestive enzymes. Digestive enzymes released in the digestive tract act to degrade fermented feed into amino acids which are easily absorbed by the intestinal epithelium by endocytosis. From the intestinal epithelium, the nutrients that were readily absorbed were carried by the bloodstream to the liver, brain and gonads. In the liver, nutrients will be used as a basis for the synthesis of vitellogenin, in the brain, nutrients are used to increase the activity of neurotransmitters and neurohormones which were advantageous in stimulating

the synthesis of gonadotropin hormones in the hypothalamus and pituitary, while in the ovaries, nutrients were used for the development and maturation of eggs and in the testis nutrients play a role in steroidogenesis and gametogenesis in the formation of spermatozoa.

The results showed that addition of probiotic-fermented feed and laser induction before spawning had been proven to accelerate gonadal maturity of catfish broodstock and ready to be spawned to produce higher seeds than the control. This shows that the feed nutrients that given in catfish broodstock before spawning were play important role because they were leading in accelerating the gonad mature of catfish broodstocks and the quality of seeds produced.

Conclusion

Overall study results could that addition fermented feed and laser induction to the catfish broodstocks before the spawn was accelerated the preparation time of gonadal maturity and ready to be spawned. Combination of probiotic-fermented feed and laser induction increase HR, SR, and seed production levels.

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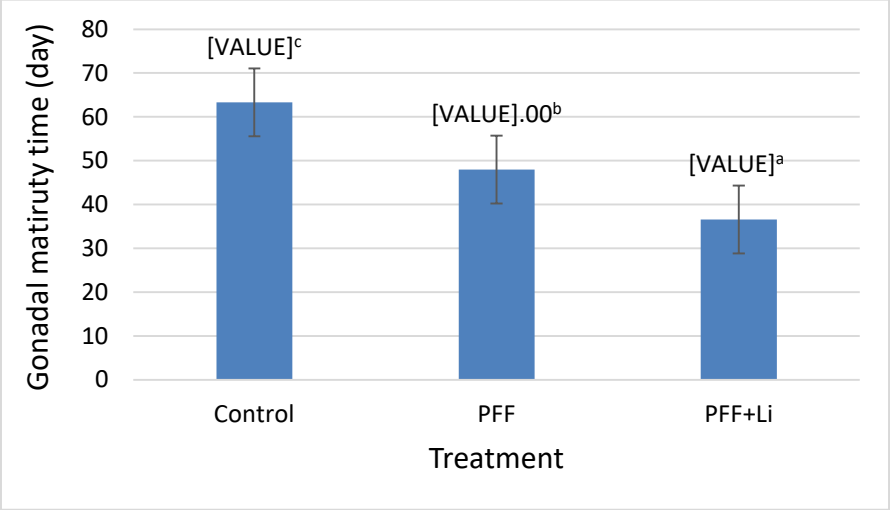

Figure 1. Gonadal maturity time of male catfish broodstocks between treatments. PFF = probiotic-fermented feed, PFF+Li = probiotic-fermented feed+laser induction. Mean values not sharing the same letter indicate a significant differences (P<0.05).

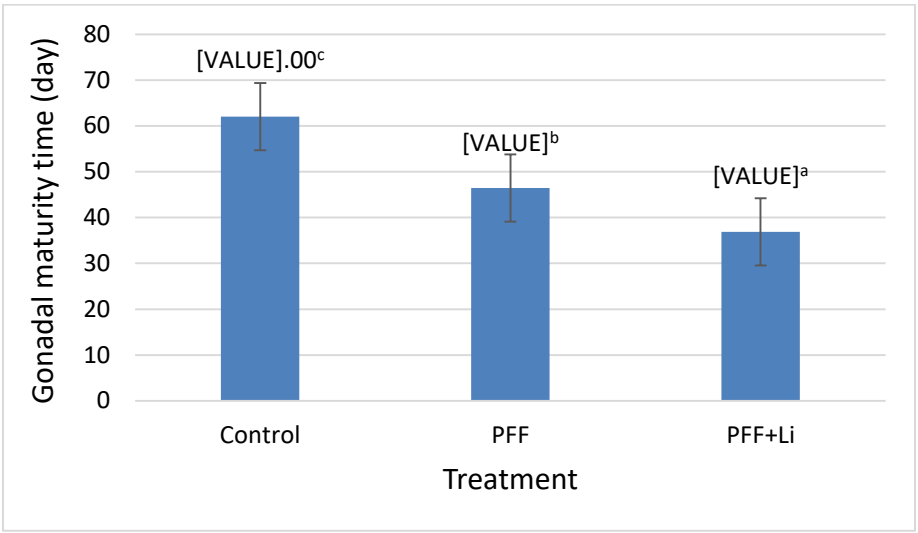

Figure 2. Gonadal maturity time of female catfish broodstocks between treatments. PFF = probiotic-fermented feed, PFF+Li = probiotic-fermented feed+laser induction. Mean values not sharing the same letter indicate a significant differences (P<0.05).

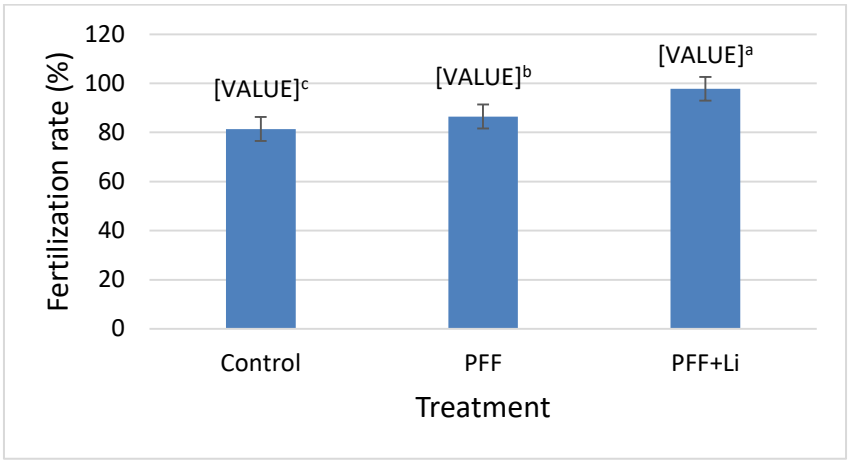

Figure 3. Fertilization rate of catfish eggs between treatments. PFF = probiotic-fermented feed, PFF+Li = probiotic-fermented feed+laser induction. Mean values not sharing the same letter indicate a significant differences (P<0.05).

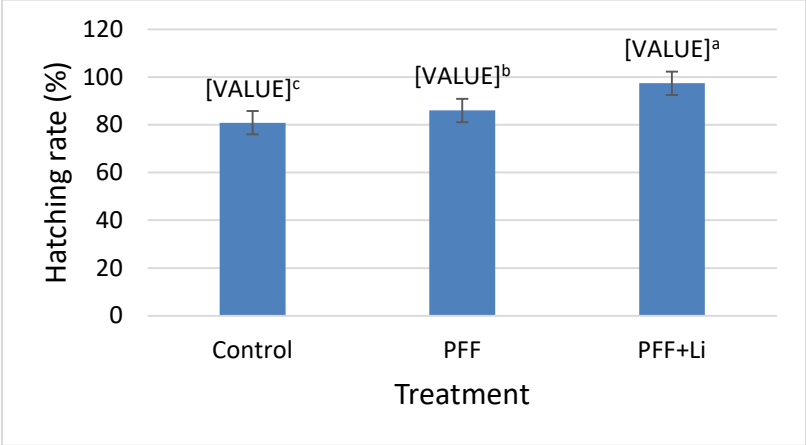

Figure 4. Hatching rate of catfish eggs between treatments. PFF = probiotic-fermented feed, PFF+Li = probiotic-fermented feed+laser induction. Mean values not sharing the same letter indicate a significant differences (P<0.05).

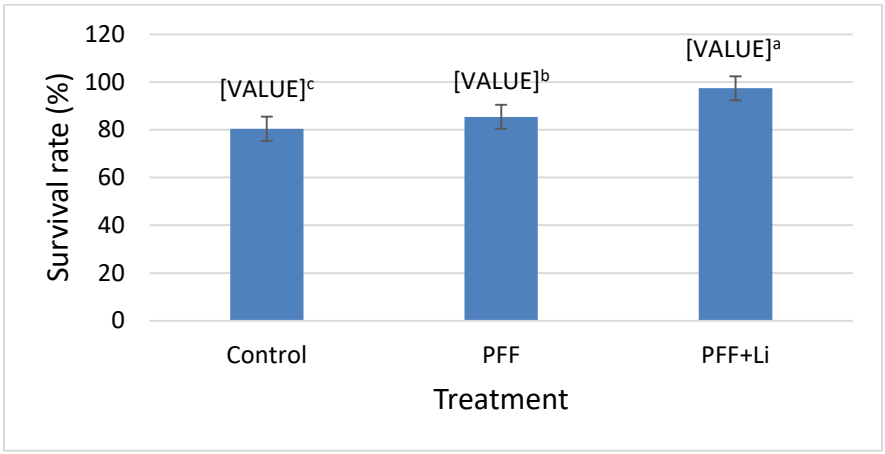

Figure 5. Survival rate of catfish seed between treatments. PFF = probiotic-fermented feed, PFF+Li = probiotic-fermented feed+laser induction. Mean values not sharing the same letter indicate a significant differences (P<0.05).

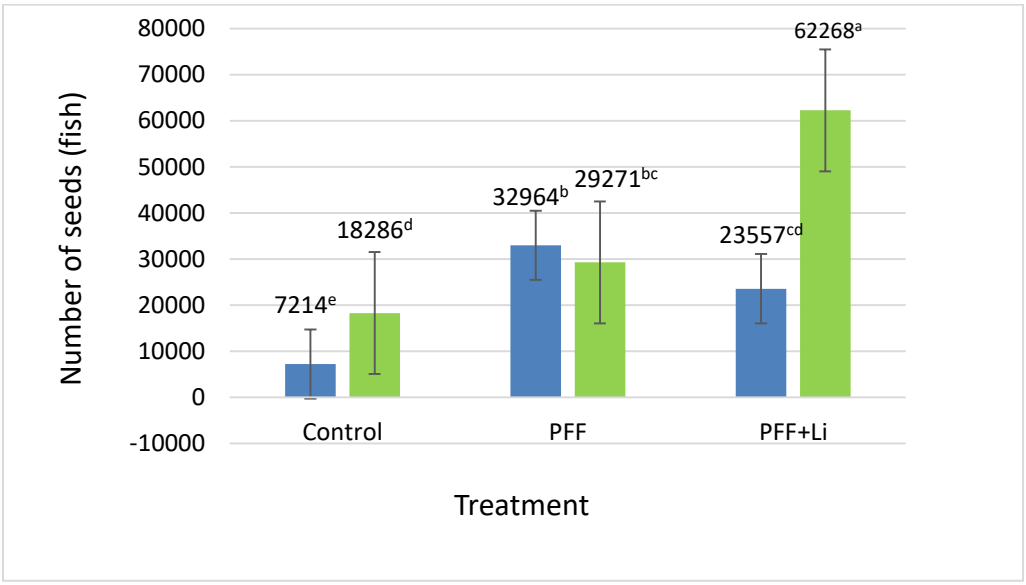

Figure 6. The number of catfish seeds with two grades of total length size produced by different treatments. PFF = probiotic-fermented feed, PFF+Li = probiotic-fermented feed+laser induction (blue bar = grade of 1.0 to 2.0 cm, green bar = grade of 2.1 to 3.0 cm). Mean values not sharing the same letter indicate a significant differences (P<0.05).

Highlights

- Availability of catfish broodstocks and seeds is limited and not continuous
- Probiotics can use as fermenter in commercial feed
- Probiotics have increased immunity, survival rate, and body resistance of fish
- Laser as bio stimulation to induce the gonadal development
- Combination fermented feed and laser induction accelerate the gonadal maturity and increase seed performances

Evaluation ~~Addition~~ of probiotic-fermented feed addition and laser induction to accelerate mature broodstocks and seed productions of African catfish (*Clarias gariepinus*)

Abstract

~~The decreasing availability of mature broodstocks and seed productions can hinder the sustainability of catfish cultivation. An alternative solution is intensive hatchery by the addition of fermented feed and laser induction so that the availability of mature broodstocks and seeds of catfish, both quantity and continuity are guaranteed.~~ This study aimed to determine the effects of fermented feed using probiotic and laser induction to~~addition of fermented feed using probiotic and laser induction on~~ accelerate the mature broodstocks and seed productions of African catfish. Fish ~~were~~ have used male and female broodstocks of African catfish. The method ~~was used a completely randomized design with three treatments, namely unfermented feed as control, probiotic-fermented feed (PFF), and probiotic-fermented feed+laser induction (PFF+Li) and three times~~with~~used a completely randomized design with three treatments: unfermented feed as control, probiotic-fermented feed (PFF), and probiotic-fermented feed+laser induction (PFF+Li)~~.~~ t. Three replicates~~ion~~, respectively. First study, l~~L~~aser firring~~induction~~ dose of 1.125 Joule cm^{-2} was performed on fish broodstocks every 15 days. The gonadal maturity time of male and female broodstocks was observed. Second study, female broodstocks of treatments was mated with mature male broodstocks without any prior treatments (control). Parameters of~~,~~ fertilization rate ~~(FR)~~, hatching rate ~~(HR)~~, and seed production performances such as~~include~~ survival rate and total lenght ~~(SR)~~ were counted and analyzed. The results showed that the addition of probiotic-fermented feed and laser induction (PFF+Li) in the broodstocks before spawned have a significant effect (P<0.05) on the gonadal maturity time of males and females African catfish. The ~~observed. The results showed that the~~ addition of PFF+Li reaches the fastest time to mature of the female gonad (31-41 days) and the male gonad (32-37 days)~~, and~~ produce the highest fertilization~~FR~~, hatching~~HR~~, and survival rates~~SR~~ more than 90%, respectively, and produce the highest ~~catfish~~ seed yield of 2.1-3.0 cm size compared to other treatments (P<0.05) in African catfish.

Key-words: Fermented feed, Probiotic, Soft-laser, Gonadal maturity, African catfish

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Introduction

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The main problem faced by catfish breeders in hatchery ~~centres~~ centers is the conventional cultivation method~~, catfish that have been spawned need recovery time to be abl~~. Catfish that have been spawned need recovery time to ~~spawn~~ generate around three months later~~, t~~. Thus the availability of broodstocks and seeds in the community is limited and not continuous~~, so~~ to ~~it can~~ hinder the productivity catfish farming business. The market demand for this catfish commodity continues to increase~~, ;~~ meanwhile, the availability of gonadal mature and ~~seeds of catfish~~catfish seeds ~~are~~ is decreasing. It is necessary to find alternative solutions through more intensive farming of catfish, namely by increasing the quality of feed and ~~the application of~~applying appropriate biostimulation laser technology ~~so that~~to ~~the availability of mature broodstocks and seeds, especially the number and continuity can take~~mature broodstocks and seeds, especially the number and continuity place continuously.

Generally, t~~In general, t~~he protein content in catfish feed could affect several things, such as gonadal development, spermatozoa, and eggs productions limit number and quality. Based on study according to Coldebella et al. (2011) showed that the protein content of the feed was proven to affect the survival of larvae, to ~~very low~~deficient levels of feed protein content (10 to 20%), resulting in low fertilization rate of eggs and a higher percentage of abnormal larvae. ~~Hence, the quality of feed in the broodstocks needs to be improved.~~ Watanabe (1995) and Bromage (1995) stated that nutrition have a considerable effect on gonadal development and reproductive performance in fish. The lacking lipids, proteins, fatty acids, vitamins C and E, and carotenoids in the fish diet produced poorer quality of fish eggs because these components influences various processes in reproduction such as fertility of eggs, hatching rate of embryo, and survival rate of larvae. Hence, the quality of feed in the broodstocks needs to be improved.

The results of the study according to Sakamole et al. (2014) showed that the addition of the probiotic in feed was proven to be able to break down feed complex compounds into simple ingredients so that they are ready for use by fish bodies because of the presence of enzymes such as amylase, protease, lipase and cellulose could increase nutritional value and digestibility feed given. Elumalai et al. (2013) stated that probiotics are live microorganisms that can be used to prevent disease in cultured fish, to increase production,

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and to reduce economic losses in fish cultivators. The addition of probiotics in feed also increase immunity which in turn affects the survival of the cultured fish. The results of the study according to Iribarren et al. (2012) and Agustin et al. (2014) indicated that the use of probiotics in feed can increase the survival rate and body resistance of fish against pathogenic infections.

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Peran probiotik yang tidak kalah penting lainnya adalah meningkatkan performa reproduksi. Beberapa penelitian menunjukkan pengaruh probiotik terhadap reproduksi ikan (Aydın and Çek-Yalnız 2019. Effect of Probiotics on Reproductive Performance of Fish. Natural and Engineering Sciences, 4(2): 153-162; Mehdinejad, N., Imanpour, M.R., & Jafari, V. (2018). Combined or individual effects of dietary probiotic, Pediococcus acidilactici and nucleotide on reproductive performance in goldfish (Carassius auratus). Probiotics and Antimicrobial Proteins, 1-8, http://doi.org/10.1007/s12602-017-9297-3.), baik ikan jantan maupun betina. Ghosh, S., Sinha, A., & Sahu, C. (2007). Effect of probiotic on reproductive performance in female livebearing ornamental fish. Aquaculture Research, 38, 518-526.), carried out the pioneer investigation on the effect of probiotic administration on reproduction of fish. Rahman, M.L., Akhter, S., Mallik, Md K.M., & Rashid, I. (2018). Probiotic enrich dietary effect on the reproduction of butter catfish, Ompok pabda (Hamilton, 1872). International Journal of Current Research in Life Sciences, 7(2), 866-873. ISSN: 2319-9490.) investigated the effects of probiotics on the reproduction of butter catfish, Ompok pabda. The results demonstrated the beneficial effects of probiotics on the reproductive performance; the GSI, fecundity, and larval survival were significantly enhanced by probiotic administration. Further studies have emphasized probiotics ability to stimulate gonadal development, maturation, gamete quality. Carnevali, O., Maradonna, F., & Gioacchini, G. (2017). Integrated control of fish metabolism, wellbeing and reproduction: the role of probiotic. Aquaculture, 472, 144–155. https://doi.org/ 10.1016/j.aquaculture.2016.03.037), reviewed the effects of probiotics on the integrated control of fish metabolism. In the last section of their assessment, condenses recent findings regrading to the positive effects of probiotic administration

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1- Integrated control of fish metabolism, wellbeing and **reproduction**: the role of **probiotic**

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on fish gonads, which deeply support their role towards reproduction and gamete quality mainly achieved on zebrafish.

Very few studies have been Natural and Engineering Sciences 155 performed on the effects of probiotics on fish reproduction (Abasali and Mohamad, 2010; Giorgini et al., 2010; Gioacchini et al., 2010a,b and c; Gioacchini et al.,2011; Gioacchini et al.,2012; Gioacchini et al.,2013; Miccoli et al., 2015; Ariole, 2012; Carnevali et al., 2017).

Di bagian yang lain, the effect of laser firring telah diteliti pada beberapa spesies ikan, such as African catfish (Kusuma …) and striped catfish, Pangasius hpth (Mukti et al. 2020b). T

~~T~~he use of laser technology has been successfully developed by catfish farmers in East Java and Central Java. Laser i~~Catfish broodstocks that~~nduction ~~was induced by laser~~ for 15 seconds at the reproductive point, precisely at 2/3 of the ventral body with ~~with an induction~~ the frequency of once in two weeks, ~~was proven within 15 days has~~ has optimal ~~affected for biostimulation of the~~accelerated gonadal ~~gonadal~~ growth, ~~and~~ development, anad ~~indicated the increasing gonadal~~ maturation of ~~level and ready to be spawned of~~ catfish broodstocks (……). Laser induction optimally affects the increase in the activity of neurotransmitters and neurohormones. The increased neurohormones are shown to synthesize g~~. This proves that laser induction in the reproductive point of catfish is believed to have an optimal affect to increase neurotransmitter and neurohormone activities for the g~~onadotropin-releasing hormone (GnRH)~~)~~ ~~synthesis and release~~ in the hypothalamus and~~-~~ released in the pituitary (Kusuma & Hariani, 2019). Studi oleh Kusuma (..) membuktikan bahwa ….. Berdasarkan pengalaman studi-studi tersebut, maka t~~What is the role of the laser and fermented feed on gonadal maturity and seeds production in the fish? T~~his study aimed to determine the effects of ~~addition~~ adding the fermented feed using probiotic and laser induction on ~~accelerate~~ accelerating the mature broodstocks and seed productions of African catfish.

Materials and Methods

This research was conducted at the ~~Unit Pelaksana Teknis Pelatihan Teknis Perikanan Budidaya dan Pengolahan Produk Kelautan dan Perikanan (~~UPT PTPBP2KP~~)~~, Kepanjen, Malang, East Java, Indonesia. This study was used experimentally with a completely randomized design consisting of three treatments and ~~three~~ three-time replicates. Studi ini dilakukan melalui 2 tahap uji, yaitu pengaruh perlakuan terhadap kematangan gonad induk

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ikan jantan dan betina (first study) dan evaluasi performa benih yang diproduksi dari ikan betina hasil perlakuan (second study)

Animals

The I gonadal maturity stage ~~immature~~ male and female broodstocks of African catfish with the average age of 1 to 1.5 years and the average body weight of 1500±10900 ~~to 1,500 g and 1,140 to 1,750~~ g~~, respectively~~ were used, respectively. In this study, the experimental protocols were approved by the Scientific Committee with Protocol Number 027/SP2H/AMD/LT/MULTI/L7/2020.

Feed and Probiotic

In this study, the ~~the treatments were used~~ commercial feed contains ~~of~~ 38% crude protein was used. The commercial feed was fermented using a commercial probiotic in the market (Probio-7) dose of 5 mL kg^{-1} as fermenter produced by Tamasindo Veterinary. Probio-7 consists of~~with composition~~ fungi (*Saccharomyces cerevisiae* ~~of *Saccharomyces cerevisiae* and~~ and *Aspergillus oryzae*), ~~fungus~~ and bacteria (*Lactobacillus acidophilus*, *Bacillus subtilis*, *Rhodopseudomonas, Actinomycetes*, and *Nitrobacter*) have contained ~~bacteria contains~~ more than 1~~×~~10^{11} CFU ~~kg~~L^{-1}, respectively.

First Study: Rearing of Fish Broodstocks

The broodstocks (males and females) were acclimatized separately and fed ~~of~~ commercial feed for ~~2~~ two weeks~~-~~ in a controlled pond size of 2 m × 2 m × 0.9 m to prevent spawning before treatment. Next~~After the broodstocks were acclimatized,~~, the broodstocks were treated using three treatments~~, namely~~: unfermented feed as control, probiotic-fermented feed (PFF), and probiotic-fermented feed+laser induction (PFF+Li). The broodstocks were fed twice (morning and evening) a~~s much as~~nd 5% of the body weight until the gonad of fish were matured.

Laser Firring~~Induction~~

~~Laser~~ The laser was used a diode soft-laser of 15 mW with a wavelength of 532 nm equivalent with 1.125 Joule cm^{-2} is still in the safe range for biostimulation of biological organs. The laser firring was conducted~~broodstocks were induced by laser~~ at the reproductive acupoint of fish broodstocks, precisely in 2/3 of the body ventral part for 15 seconds (Kusuma et al., 2015). Laser ~~induction~~ firring was performed every 15 days until the gonad mature ~~(the IV maturity stage) reached and~~s (the IV maturity stage) and is ready to be spawned.

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Measurement of Gonadal Maturity

The gonadal maturity of broodstocks, both males and females, were observed once every 2 to 5 days to determine the gonadal maturity time after treatment. The gonadal maturity was ~~observed~~ marked to spawning behavior signs and ~~to~~ genitalia visually of catfish. ~~Then, the one pair mature broodstock was reared at spawning pond.~~

Second Study: Spawning and Eggs Incubation

In second study, only female broodstocks from the treatments were used, while mature male broodstocks without any prior treatments (control) were used as parent couple. Then, the one pair of mature broodstocks was reared and mated at the spawning pond size of 2 m × 2 m × 0.9 m separately between treatments. Fish spawning was conducted to ~~a~~ spawning pond ~~size of 2 m × 2 m × 0.9 m~~ using a substrate size of 1.4 m × 0.4 m to the eggs attachment and aerations to the supply dissolved oxygen during eggs incubation, separated between treatments. Selama proses pemijahan, induk ikan dipuasakan. After 8 hours, the broodstock completes the spawning process. Then, the substrate size 0.3 m × 0.3 m was ~~cutted~~ quoted as a sample to ~~manually count the fertilized eggs number~~count the number of fertilized eggs manually and incubated at the controlled aquarium size of 0.5 m × 0.5 m × 0.5 m with good aeration until hatching, separated between treatments. The eggs qualities of spawned catfish, such as fertilization rate ~~(FR)~~ and hatching rate ~~(HR),~~ were measured. The formulas were used to calculate fertilization rate ~~(FR)~~ and hatching rate ~~(HR)~~, respectively, as follows:

$$\text{Fertilization rate}^{\sim\sim R\sim\sim}\ (\%) = \frac{\text{Number of fertilized eggs}}{\text{Total number of eggs}} \times 100$$

$$\text{Hatching rate}^{\sim\sim R\sim\sim}\ (\%) = \frac{\text{Number of eggs hatched}}{\text{Total number of fertilized eggs}} \times 100$$

Rearing of Seed

The catfish fry of three days after hatching (dah) was reared at the controlled pond size 2 m × 2 m × 1 m, separated between treatments with a density of 100,000 fish, respectively, for three months. The fry was fed gradually. The ~~first,~~ fry was fed live feed of silkworms, at-satiation, three times a day for ~~1~~ one month. Next, the seed was fed commercial feed contain~~s of 35% crude protein for 1 month continued commercial feed contains of 35% crude protein until end rearing as much as 5% of the biomass, three times a day, respectivel~~ing 35% crude protein for one month; continued commercial feed contains 35% crude protein until end

rearing as much as 5% of the biomass, three times a day. The formulas were used to calculate survival rate ~~(SR)~~ according to Mukti et al. (2020a), as follows:

$$\text{Survival rate}_{\text{R}}\ (\%) = \frac{\text{Life fish number at the final of rearing}}{\text{Life fish number at the initial of rearing}} \times 100$$

Measurement of Survival and Total Length

The mortality of seed was observed and counted every day. The survival rate ~~(SR)~~ and the total length of the seed were measured at the end of fish rearing. The seed was sorted and grouped into two grades of total length, i.e., 1.0 to 2.0 cm and 2.1 to 3.0 cm, respectively.

Data Analysis

Data on gonadal maturity time of ~~catfish~~ broodstocks (first study), fertilization rate, hatching rate, survival rate~~FR, HR, SR~~, and total length grades of ~~catfish~~ seed (second study) were statistically analyzed using ~~analysis of variant (ANOVA)~~variant (ANOVA) analysis with SPSS ver. ~~10~~ 18 software (SPSS Inc., Chicago, IL, USA). ~~followed~~ Followed by ~~Duncan's~~ Duncan's multiple range test with a confidence level of 95%, according to Mukti et al. (2020a,b).

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Why you did not use two-way ANOVA??

Ethical Statement

All authors declare that the present study was conducted in an ethical, professional, and responsible manner.

Results

Gonadal Maturity Time of Broodstocks

The results showed that the addition of probiotic-fermented feed and laser induction (PFF+Li) in the broodstocks before spawned have a significant effect (P<0.05) on the gonadal maturity time of males and females African~~t~~ catfish. The gonadal maturity time of PFF+Li treatment was significantly fastest (32 to 37 days) compared to fermented feed (PFF) and control treatments (P<0.05), as shown in ~~the~~ Figures 1 and 2. The result showed that laser induction accelerates the gonadal maturity time of 1.68 and 1.26 times faste~~st compared to control and PFF treatments, respectively~~r than control and PFF treatments~~, while~~. In contrast, PFF treatment accelerates the gonadal maturity time of 1.34 times faster than control.

Seed Production

The results indicated that the fertilization rate~~FR~~, hatching rate~~HR~~, survival rate~~SR~~, dan seed size production had significant differences between treatments (P<0.05). The PFF+Li treatment ~~have~~ has the fertilization rate, hatching rate, and survival rate ~~FR, HR, and SR~~ highest compared to other treatments, as shown in Figures 3, 4, and 5, respectively. This study indicated that induction of ~~the~~ laser ~~induction~~ increase fertilization rate and hatching rate ~~FR and HR~~ of eggs and SR of seed in African catfish. Meanwhile, the addition of fermented feed increase ~~level of~~ s the fertilization rate, hatching rate, survival rate, ~~FR, HR, and SR~~ and significant differences than control (P<0.05).

The results also showed that the addition of probiotic-fermented feed and laser induction have ~~a~~ significant differences (P<0.05) on size grades of catfish seed after being reared for ~~3~~ three months. This study indicated that the PFF+Li treatment produces the seed number of 2.1 to 3.0 cm size highest compared to other treatments with a ratio between 1.0 to 2.0 cm and 2.1 to 3.0 cm sizes was 1:2.64. In ~~the~~ control, a ratio between 1.0 to 2.0 cm and 2.1 to 3.0 cm sizes was 1:2.53, while PFF treatment has the same relative ratio between 1.0 to 2.0 cm and 2.1 to 3.0 cm sizes (Figure 6).

Discussion

This study indicated that the addition of probiotics as fermenters in commercial feed was proven to accelerate the gonadal maturity time of male dan and female African catfish. Male and female broodstocks of catfish that treated probiotic-fermented feed (PFF) show~~s~~ a faster maturity time in the gonad than unfermented feed (control). This result indicated that the addition of probiotics could increase the nutrition value of the feed. Probiotics can produce several digestive enzymes, such as amylase, protease, lipase, and cellulose. These enzymes were advantageous in hydrolyzing carbohydrates, proteins, and fats of commercial feed into simpler molecules, ~~so facilitate~~facilitating the digestion and absorption processes in the digestive tract of catfish. Also, several studies ~~shown~~ showed that bacteria present in probiotics could increase the nutritional value of feed by synthesizing vitamins, proteins, essential fatty acids, amylase, protease, and lipase enzymes (Irianto & Austin, 2002; Ghosh et al., 2008; Putra, 2010).

The ~~results of the nutritional content analysis in~~ probiotic-fermented commercial feed using Probio-7 found ~~that there contain~~ several types of non-essential amino acids, such as alanine, asparagine, aspartate, glutamate~~,~~ glycine, serine, tyrosine (Kusuma & Hariani, 2019). Glutamate is one of the amino acids that play~~s~~ an influential role as a ~~major~~ primary transmitter in the fish brain, which functions as a mediator to transmit signals in postsynaptic. This glutamate also ~~functions~~ works as a precursor to the neurotransmitter ~~Gamma~~ Gamma-amino-butyric acid (GABA). So, ~~if probiotic fermented feed is given and combined with laser induction on the catfish broodstocks~~suppose probiotic-fermented feed is given and combined with laser induction on the catfish broodstocks. In that case, there will be a physiological reaction in the body of the main fish in the brain. This study indicated that laser induction ~~have~~ has that effect. Where GABAergic neurons that were not active before become reactive due to the activity of the GAD-65 enzyme. GABAergic would ~~will~~ synthesize GABA. ~~, then~~ GABA ~~will~~ stimulate~~s~~ the ~~synthesis of the release of gonadotropin hormone (GnRH)~~gonadotropin-releasing hormone (GnRH) synthesized from the hypothalamus and pituitary. GnRH plays a role in ~~stimulating~~ facilitating the synthesis~~is~~ and release ~~of the gonadotropin hormones,~~of ~~follicle~~ follicle-stimulating hormone (FSH) and Luteinizing hormone (LH) (GtH-I and GtH-II). This molecular activity can occur because there is a relationship between neurons in the catfish broodstocks brain ~~that is~~ induced by laser (Kusuma & Hariani, 2017).

The release of GtH-I in female catfish stimulates the gonads to produce steroid hormones such as estradiol-17β. Then, t~~Estradiol-17β produced by granulosa cells from ovarian follicles was then carried by the bloodstream~~he bloodstream carried Estradiol-17β produced by granulose cells from ovarian follicles to hepatic hepatocytes. Estradiol-17β ~~which~~ will have the effect of stimulating vitellogenesis to synthesize and release vitellogenin. Then vitellogenin was carried by the bloodstream to developing oocytes to be absorbed and accumulated, and as a result, the oocytes will increase in diameter and mature (Kusuma & Hariani, 2019). Sabet et al. (2009) and Taghizadeh et al. (2013) stated that the same thing that changes in estradiol-17β levels in blood plasma were closely related to oocyte development. The release of GtH-II plays a role in stimulating the final maturation of gonads, ovulation, and spawning of female catfish. Arukwe and Goksøyr (2003) stated that the synthesis process of the yolk protein precursor was vitellogenin due to the stimulation of estradiol-17β.

Vitellogenin was secreted in the liver and then carried by the bloodstream to the developing ovary for absorption.

The development of large and mature oocytes was indicated by the value increasing of the hepatosomatic index (HSI) and ~~ganado~~ gonadosomatic index (GSI) (Cerda et al., 2007; Hariani & Kusuma, 2019). Furthermore, Araoye (2001), Laleye et al. (2006), and Shinkafi and Ipinjolu (2012) stated that the value of HSI and GSI reached a maximum before spawning and then decreased after spawning. Nutrition in brood fish feed was one of the most studied because biological mechanisms, such as gonad maturity, were ~~a complicated process~~complicated. Gonadal development and fecundity are influenced by several nutrients, especially those associated with fish spawning (Izquierdo et al., 2001).

The effect of nutrition in fish feed was ~~important~~ substantial in gonad maturation and egg development. Proteins and lipids were the main components of egg yolk, which function as the ~~main~~ primary source of nutrition during embryogenesis ~~so that t~~. Their existence was needed to support the survival of embryos and larvae so that they are resistant to changes in the aquatic environment (Izquierdo et al., 2001). Brooks et al. (1997) stated that protein in feed affects the protein in fish eggs, such as lipoproteins, hormones, and enzymes, all of which determine egg quality. The quality of these eggs will determine seed production on a large scale. In general, the nutritional status of fish feed could influence gonad development and limit the number and quality of eggs and sperm produced. Coldebella et al. (2011) mentioned that the protein content in broodstock feed was proven to affect the survival of larvae~~, i~~. If the protein content in the feed is very low (10 to 20%), it can result in a low egg fertilization rate and a greater percentage of abnormal larvae.

Egg yolk protein plays a role in embryogenesis and food reserves before catfish seeds found their food. ~~according to~~ Tang and Affandi (2000) and Salerno et al. (2002) ~~who~~ stated that vitellogenin is a glycophosphoprotein containing 20% lipid, especially phospholipids, triglycerides, lipoproteins, and cholesterol. These protein molecules were crucial because they would be used as a source of energy in the growth and development of the embryo into larvae.

Vitellogenin synthesis was characterized by an increase in the volume of the cytoplasm originating from outside the cell, namely the yolk~~, t~~. Therefore, ~~-~~the quality of eggs was very much determined during vitellogenesis. Several factors such as feed quality, environment,

and activity of gonadotropin hormones were very influential in supporting the success of this process. Feed quality, a supportive environment, and ~~homon~~ gonadotropin hormone activity were ~~very influential~~significant in determining the eggs' quality~~quality of eggs produced~~. The egg's quality produced was determined by the yolk quality protein in the oocytes. The protein could ~~increasing~~ increase ~~the percentage of~~ the number of fertilized eggs and the high number of eggs that hatch into larvae.

The mechanism could be explained as follows: first, commercial feed containing carbohydrate, protein, fat, vitamins, minerals, ~~fibre~~fiber, and water when added with probiotics and brooded for a day, the feed will immediately be fermented into a fermented feed. The fermented feed had an acidic pH~~, i~~. If this fermented feed enters the ~~digestive system of the fish~~fish's digestive system, it will ~~then~~ stimulate the gastric epithelium to increase hormone synthesis and release ~~of~~ digestive enzymes. Digestive enzymes released in the digestive tract function~~act~~ to degrade fermented ~~-~~feed into amino acids, so~~which are~~ easily absorbed by the intestinal epithelium by endocytosis. ~~From the intestinal epithelium,~~ The bloodstream carried the nutrients that were readily absorbed~~the nutrients that were readily absorbed were carried by the bloodstream~~ to the liver, brain, and gonads from the intestinal epithelium. In the liver, nutrients will be used as a basis for the synthesis of vitellogenin~~, i~~. In the brain, nutrients are ~~are~~ used to increase ~~the activity of~~ neurotransmitters and neurohormones ~~which were advantageous in stimulating the synthesis of gonadotropin hormones in the hypothalamus and pituitary, while~~. In contrast, in the ovaries, nutrients were used for the development and maturation of eggs. I~~ and i~~n the testis, nutrients play a role in ~~in~~ steroidogenesis and gametogenesis for ~~in the~~ spermatozoa formation ~~of spermatozoa~~.

The results showed that the addition of probiotic-fermented feed and laser induction before spawning had been proven to accelerate gonadal maturity of catfish broodstock and ready to be spawned to produce higher seeds than the control. This result also shows that the feed nutrients ~~that given in catfish broodstock before spawning were play important role~~given in catfish broodstock before spawning were vital because they ~~were leading in accelerating~~accelerated the gonad mature of catfish broodstocks and the quality of seeds produced.

Penelitian yang dilakukan Herdis (2010), tentang aplikasi teknologi laserpunktur untuk gertak birahi pada domba Garut, hasil penelitian membuktikan bahwa induksi laserpunktur pada titik-titik

Commented [P18]: Riset yang terkait stimulasi laserpunktur untuk meningkatkan performa reproduksi pada ternak

reproduksi tepatnya pada: Titik Ming-Meng/estrus (tunggal) terletak di daerah dorsal dari persendian *vet. lumbal* II dengan *vet. lumbal* III (sebagai *generalpoint*). Rangsangan pada titik ini akan meningkatkan aktivitas kelenjar hypofisa. Titik Shen Yu/Ovarium (*dexter* dan *sinister*) terletak di daerah dorsal antara *processus transversus* dari *vet. lumbal* I- II - III. Rangsangan pada titik ini akan meningkatkan aktivitas ovarium dalam hal pembentukkan folikel dan pembentukan hormon reproduksi.Titik Oviduk (*dexter* dan *sinister*) terletak di daerah dorsal antara *processus transversus* dari *vet. lumbal* III-IV-V-VI. Rangsangan pada titik ini akan menghindari sistik ovari dan hipofungsi ovari. Titik cervik uteri (*dexter* dan *sinister*) terletak di daerah ujung atas os ileum. Rangsangan pada titik ini akan mengoptimalkan servik uteri pada kejadian estrus dimana akan membuka untuk keluarnya lendir berahi.Titik Hormonal (tunggal) terletak di daerah dorsal dari persendian *sacro-coccygea*. Rangsangan pada titik ini akan meningkatkan produksi hormon prostaglandin dari dalam tubuh dan GV-1 satu buah titik akupunktur terletak di atas vulva dan dua buah titik akupunktur terletak di sisi kiri dan kanan pertengahan vulva. Hasil penelitian menunjukkan hampir semua domba betina yang di induksi laserpunktur selama tiga kali selama 20 detik rata-rata timbul entrus 16 jam setelah akhir perlakuan menunjukkan respon estrus, ditandai 95% betina diam apabila dinaiki pejantan pemancing.

Pustaka:

Herdis, 2010. Aplikasi Teknologi Laserpunktur dalam Meningkatkan Libido Pejantan Domba Garut (Ovis aries). Pusat Teknologi Produksi Pertanian Bidang TAB BPPT. Jakarta. Vol. 12(1):25-30.

Penelitian Binawati (2008), induksi laserpunktur dengan daya 0,2 Joule/titik pada titik ova tepatnya di daerah dorsal persendian thorax akhir dengan lumbal pertama diikuti dengan induksi laserpunktur pada titik nafsu makan dan titik ketahanan tubuh, terbukti berpengaruh signifikan ($P<0.05$) pada produksi telur dan total berat lemak dalam rongga abdominal ayam Arab (Gallus turcicus). Perlakuan induksi laserpunktur dengan durasi 15, 30 dan 45 detik. Berturut-turut menghasilkan produksi telur yang semakin tinggi, sedangkan pada kontrol produksi telur kurang tinggi.

Pustaka:

Binawati, K. 2008. Pengaruh lanskeptur terhadap kualitas telur ayam Arab. Journal of Science. 1 (2) : 28-34.

Conclusion

Overall study results could include ~~that addition~~ fermented feed and laser induction to the catfish broodstocks before the spawn was accelerated the preparation time of gonadal maturity and ready to be spawned. Combination of probiotic-fermented feed and induction of laser ~~induction~~ increase f~~F~~ertilization rate~~R~~, hatching rate~~HR~~, survival rate~~SR~~, and seed production levels.

Acknowledgments

The authors would like to thank the Directorate of Research and Community Service of the Directorate General of Research and Development Strengthening ~~of~~ the Ministry of Research, Technology, and Higher Education, Republic of Indonesia. The authors also would like to thank the Head and staff of the UPT PTPBP2KP, Kepanjen, Malang, East Java, Indonesia, for providing African catfish broodstocks and facilities for study. The authors would also like to acknowledge~~ment the comments, corrections, and suggestions given by the reviewers, editor, and proof readers~~ the comments, corrections, and suggestions given by the reviewers, editor, and proofreader to improve this article.

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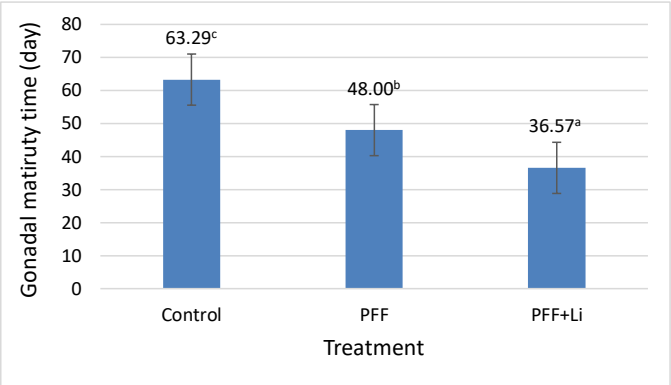

Figure 1. Gonadal maturity time of male catfish broodstocks between treatments. PFF = probiotic-fermented feed, PFF+Li = probiotic-fermented feed+laser induction. Mean values not sharing the same letter indicate a significant differences (P<0.05).

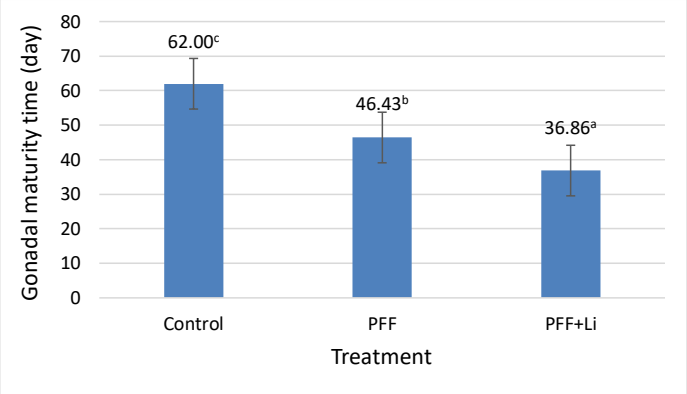

Figure 2. Gonadal maturity time of female catfish broodstocks between treatments. PFF = probiotic-fermented feed, PFF+Li = probiotic-fermented feed+laser induction. Mean values not sharing the same letter indicate a significant differences (P<0.05).

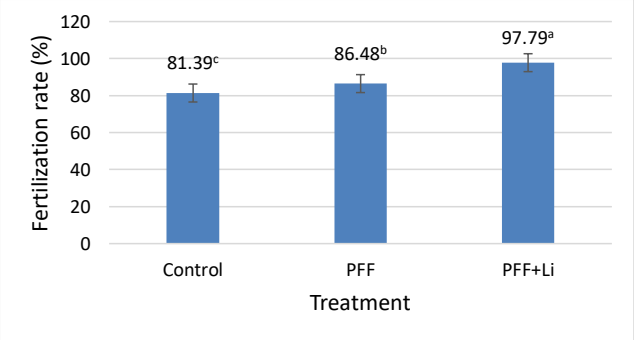

Figure 3. Fertilization rate of catfish eggs between treatments. PFF = probiotic-fermented feed, PFF+Li = probiotic-fermented feed+laser induction. Mean values not sharing the same letter indicate a significant differences (P<0.05).

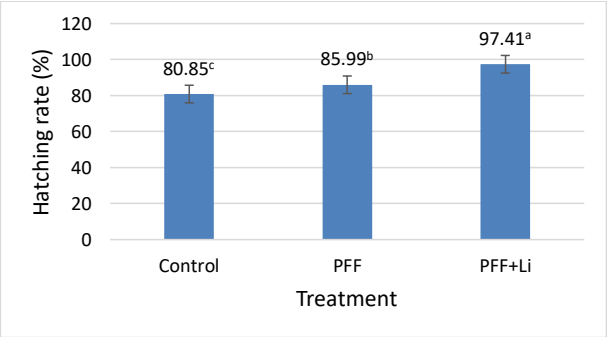

Figure 4. Hatching rate of catfish eggs between treatments. PFF = probiotic-fermented feed, PFF+Li = probiotic-fermented feed+laser induction. Mean values not sharing the same letter indicate a significant differences (P<0.05).

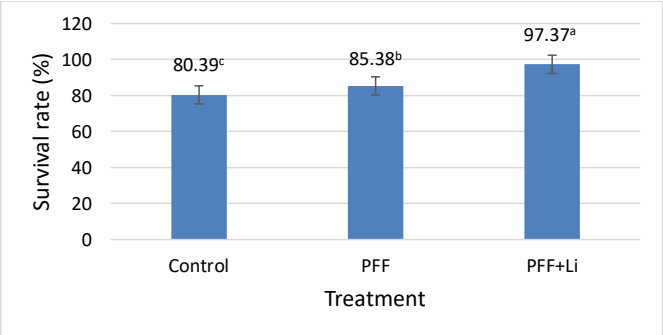

Figure 5. ~~Survival~~ The survival rate of catfish seed between treatments. PFF = probiotic-fermented feed, PFF+Li = probiotic-fermented feed+laser induction. Mean values not sharing the same letter indicate a significant difference~~s~~ (P<0.05).

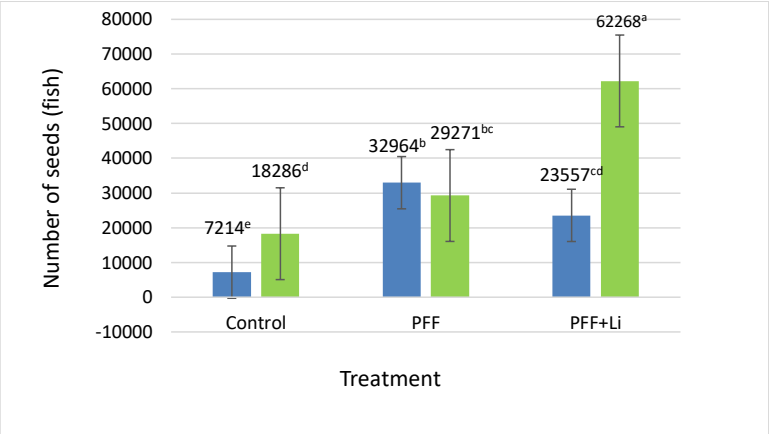

Figure 6. The number of catfish seeds with two grades of total length size produced by different treatments. PFF = probiotic-fermented feed, PFF+Li = probiotic-fermented feed+laser induction (blue bar = grade of 1.0 to 2.0 cm, green bar = grade of 2.1 to 3.0 cm). Mean values not sharing the same letter indicate a significant differences (P<0.05).

Highlights

- Availability of catfish broodstocks and seeds is limited and not continuous
- Probiotics can use as fermenters in commercial feed
- Probiotics have increased immunity, survival rate, ~~and~~ body resistance, and reproductive performance ~~-~~of fish
- ~~Laser~~ A laser as bio~~-~~stimulation to induce the gonadal development
- Combination fermented feed and laser induction accelerate the gonadal maturity and increase seed performances

akhmad taufiq mukti <akhmad-t-m@fpk.unair.ac.id>

First Reminder for Revision

1 message

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Dear Akhmad Taufiq Mukti,

This is an automatic system message. We would like to remind you that your revisions or rebutting on the manuscript entitled "Addition of probiotic-fermented feed and laser induction to accelerate mature broodstocks and seed productions of African catfish (Clarias gariepinus)" have not been received although it has been 14 days since the request was sent to you. Your manuscript will be rejected if we will not receive the revised manuscript within 16 days. You can submit your revision through the online system.

The site is located at http://www.trjfas.org/ Please upload the file containing your revised manuscript. The rebuttal letter should be placed in “cover letter” section. Please note that you should submit your revised letter by clicking on “revision requested” link, not as a new manuscript.
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We would like to remind you that the revised version of your manuscript entitled "Addition of probiotic-fermented feed and laser induction to accelerate mature broodstocks and seed productions of African catfish (Clarias gariepinus)" has not been yet received although 23 days passed since the request has been sent to you. You can submit your revision through the online system. The site is located at http://www.trjfas.org/ Please upload the file containing your revised manuscript. The rebuttal letter should be placed in “cover letter” section. Please note that you should submit your revised letter by clicking on “revision requested” link, not as a new manuscript.

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Dear Associated Prof.Akhmad Taufiq Mukti,

This is to acknowledge receipt of your revised manuscript entitled Evaluation of probiotic-fermented feed addition and laser firing to accelerate mature broodstocks and seed productions of African catfish (Clarias gariepinus). You can learn the stage of your manuscript in the review process through the author center. Thank you.

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LETTER OF RESPONSE TO REVIEWERS

MANUSCRIPT TITLE : Evaluation of probiotic-fermented feed addition and laser firing to accelerate mature broodstocks and seed productions of African catfish (*Clarias gariepinus*)

MANUSCRIPT ID : TRJFAS-19303

Reviewer 1

Abstract

1. The section has not been written in a proper way.
   Authors response: We have revised the Abstract of manuscript according to the suggestion (page 1, lines 5-20).

2. Methodology is poor.
   Authors response: We have revised the methodology section in the Abstract of manuscript (page 1, lines 7-14): "The method used …"

3. Results should be more numeric.
   Authors response: We have revised and mentioned numeric or statistic form from the results in the Abstract of manuscript (page 1, lines 15-19): "(P<0.05)"

4. Conclusion of the section need to be revised.
   Authors response: We have revised the conclusion section in the Abstract of manuscript (page 1, line 14-20)

5. First letter of keywords should be in Capital and design in an alphabet format.
   Authors response: We have mentioned detail keywords according to the suggestion and author guidance of Journal (page 1, line 21)

Introduction

1. The section has been written in good manner.
   Authors response: Thank you very much

2. The conclusion of the section need to be improvised.
   Authors response: We have revised and mentioned sentences in the end Introduction of manuscripts (page 3, line 71)

3. English and grammar are weak.
   Authors response: We have revised grammarly and English language of manuscript through proof read by expert proof reader, as we also have mentioned in the Acknowledgment (page 10, lines 279-280)

Materials and methods

1. The average weight of the experimental animals should be written as, 'weight + SE/SD'.
   Authors response: We have mentioned "±" in the Animal section of the Materials and Methods in manuscript (page 3, line 84).

2. Units (ml or mL should be followed informally throughout the manuscript)
   Authors response: We have mentioned consistent according to Author guidance of Journal (page 3, lines 90 and 93): "mL and L"

3. Feeding details are missed
   Authors response: We have mentioned the feeding detail in the Materials and Methods (page 4, line 99 and page 5, lines 129-132).

4. SPSS version used for the data analysis is very old, try at least SPSS Version 18 or above.
   Authors response: We have revised and used SPSS new version according to the suggestion in the Materials and Methods of manuscript (page 5, line 142)

5. The sentence restructuring is required in several places of MM section
   Authors response: We have revised the sentences in the Materials and Methods of manuscript (page 3, lines 76-81; lines 83-84; lines 89-93; page 4, lines 94-123; page 5, lines 140-142).

6. English needs to be cross-checked from an expert.
   Authors response: We have revised grammarly and English language of manuscript through proof read by expert proof reader, as we also have mentioned in the Acknowledgment (page 10, lines 279-280)

Results

1. Authors did not mention the significance symbol in few places.
   Authors response: We have mentioned some significant symbol in the Results (page 5, line 151 and page 6, lines 153-165): "($P<0.05$)"

2. English need to be improved
   Authors response: We have revised grammarly and English language of manuscript through proof read by expert proof reader, as we also have mentioned in the Acknowledgment (page 10, lines 279-280)

Discussion

1. I suggest to incorporate few more references in the First paragraph.
   Authors response: We have added and mentioned sentences and references in the Discussion of manuscript (page 6, lines 174-183 and page 7, lines 209-2010): "Probiotics have been used …" and "The use of the laser has also been widely applied chicken (Binawati, 2008) and sheep (Herdis, 2010)."

2. It may be more authentic if the discussion section will arrange in the sub-heading format'.
   Authors response: We have previously stated that in this study the main treatment was carried out on the fish broodstocks, including the treatment of probiotic-fermented feed and not directly on fish fry, so we found it difficult to separate the Discussion section into different sub-heading as shown in the results. Therefore, we did not make sub-heading for the Discussion section in manuscript.

Conclusion

1. The section need to be improvised
   Authors response: We have revised sentences in the Conclusion of manuscript (page 9, lines 269-271).

Reviewer 2

Title

1. From the results of your study, the title is more suitable to use the word "evaluation"
   Authors response: We have revised the Title with addition the word "Evaluation" (page 1, line 1): "Evaluation of …"

Abstract

1. Check grammarly.
   Authors response: We have revised grammarly and English language of manuscript through proof read by expert proof reader, as we also have mentioned in the Acknowledgment (page 10, lines 279-280).

Materials and Methods

1. Can you explain the status of "immature" in this article? Why done you just write down "level of gonad maturity I" is it possible to parent immature?
   Authors response: We have revised and mentioned sentences in the Materials and Methods of manuscript (page 3, line 83): "The I gonadal maturity stage male and female broodstocks …"

2. Is this really “induction”? Not “laser firing”? due to its role as a biostimulator or of endocrine glands

   Authors response: We have revised and mentioned word “firing” instead of the word “induction” such as Title etc. in the manuscript.

3. How much is the given dose of laser firing (joule)?.

   Authors response: We have mentioned dose of laser firing in the Materials and Methods of manuscript (page 4, line 103): “The laser was used a diode soft-laser of 15 mW with a wavelength of 532 nm equivalent with 1.125 Joule …”

Discussion

1. The discussion is futher enriched, can be included studies on land animals (e.g. ducks, mice, chickens, etc.)

   Authors response: We have added and mentioned sentences and references in the Discussion of manuscript (page 6, lines 174-183 and page 7, lines 209-2010): “Probiotics have been used …” and “The use of the laser has also been widely applied chicken (Binawati, 2008) and sheep (Herdis, 2010).”

Conclusion

1. It looks expensive (1 unit)? So it will be difficult to implement to farmer or breeder

   Authors response: According to our opinion, soft-laser equipment as an investment is not expensive. The price of one unit of Diode soft-laser (not He-Ne soft-laser) that we use is around USD 691. We recommend that this soft-laser equipment can be used the farmers (hatcher/breeder) group together and alternately. This equipment uses a charging system so that it is more efficient and effective for sustainable than the use of hormone induction that has been done so far.

2. Do not abbreviate: hatching rate, ….

   Authors response: We have revised sentences in the Conclusion of manuscripts (page 9, lines 271-272).

Reviewer 3

Abstract

1. And laser alone.

   Authors response: In this study, we did not perform treatment of a single laser firing. Study on the effect of laser firing alone have been carried out in previous studies, as references have also been mentioned in the Introduction of manuscript (page 3, lines 64-68).

2. This part need to rewrite and expansion.
   Authors response: We have revised methodology in the in the Abstract of manuscript (page 1, lines 9-14): "First study, …"

3. Please report in statistic form
   Authors response: We have revised and mentioned statistic form from the results in the Abstract of manuscript (page 1, lines 15-19): "(*P*<0.05)"

Introduction

1. Weak literature review.
   Authors response: We have added some study-related references in the Introduction of manuscript (page 2, lines 36-62): "Bromage (1995) stated ..."

2. The authors need to add references related probiotics and reproduction status not growth or immunity.
   Authors response: We have added references and have mentioned sentences in the Intorduction of manuscript (page 2, lines 50-59): "Another important role of probiotics ..."

3. Too long sentences and no reference??!!
   Authors response: We have revised and mentioned sentences and have added references in the Introduction of manuscript (page 3, line 64-68): "Laser firing for 15 seconds (dose of 1.125 Joule) at the reproductive point, precisely at 2/3 of the ventral body with the frequency of once in two weeks, both a single treatment and combination with different feed protein have optimal accelerated gonadal growth, development, and maturation time of catfish broodstocks (Hariani & Kusuma, 2019; Hariani et al., 2020)."

Materials and methods

1. Summarize the name of place of study.
   Authors response: We have revised and mentioned in the Materials and Methods of manuscript (page 3, line 76): "This study was conducted at the UPT PTPBP2KP Malang …".

2. When you are talking about probiotics always you should mention strain not species
   Authors response: In this study, we did not use homemade probiotics. However, we only use finished probiotics products (Probio-7) that are sold in the market with the existing composition label as we have mentioned in the Materials and Methods of manuscript (page 3, lines 89-93): "… using a commercial probiotic on the market (Probio-7) …".

3. The authors need to add laser as a single treatment while are not seen in the study.

Authors response: In this study, we did not perform treatment of a single laser firing. Study on the effect of laser firing alone have been carried out in previous studies, as references have also been mentioned in the Introduction of manuscript (page 3, lines 64-68): "Laser firing for 15 seconds …"

4. Suggest to the authors: You have two varliable 1- fish sex and 2- experimental treatments.
   Authors response: In this study no factorial treatment was carried out. However, we did a follow-up study. The main treatment was carried out in the First study, while in the Second study, we only used treated female fish. We have mentioned this in the Abstract of manuscript (page 1, lines 9-13): "First study, laser firing …" and in the Materials and Methods of manuscript (page 3, lines 77-80): "This study was carried out ..." and page 4, lines 94 and 111.

5. Why you did not use two-way ANOVA?s.
   Authors response: In this study, we used one-way ANOVA and not two-way ANOVA, because in this study no factorial treatment was carried out. However, we did a follow-up study. The main treatment was carried out in the First study, while in the Second study, we only used treated female fish. We have mentioned this in the Abstract of manuscript (page 1, lines 9-13): "First study, laser firing …" and in the Materials and Methods of manuscript (page 3, lines 77-80): "This study was carried out ..."

Thus authors responses on comments, corrections, and suggestions of reviewers, we expect the reviewers were pleased and understand it and we hope that this article will be corrected further. Thank you very much.

Best regards,

Akhmad Taufiq Mukti

akhmad taufiq mukti <akhmad-t-m@fpk.unair.ac.id>

Revision request for your manuscript

2 messages

Turkish Journal of Fisheries and Aquatic Sciences <info@trjfas.org> Mon, Sep 6, 2021 at 1:23 PM
To: akhmad-t-m@fpk.unair.ac.id

ISSN: 1303 - 2712
E-ISSN: 2149 - 181X

Turkish Journal of FISHERIES and AQUATIC SCIENCES

Dear Associated Prof.Akhmad Taufiq Mukti,

The Editors have now assessed the reviewer response and have concluded that, in its present form, the manuscript (20344) is not yet ready for publication in the journal. You will find the relevant reviewer comments and editorial notes on the online system. Acceptance of the paper is contingent upon effectively revising the work by taking these comments into serious consideration, and by responding or rebutting them in detail within the 30 days.

We ask you to submit your revision through the online system.

Please upload the file containing your revised manuscript. The rebuttal letter should be placed in “cover letter” section. Please note that you should submit your revised letter by clicking on “Submit Revision” link, not as a new manuscript.

If you have any problem please send an e-mail to info@trjfas.org

Sincerely,

Esen ALP ERBAY
Manager Editor
Turk. J. Fish & Aquat. Sci.
info@trjfas.org
+(90) 462 341 1053

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akhmad taufiq mukti <akhmad-t-m@fpk.unair.ac.id> Fri, Sep 10, 2021 at 9:18 PM
To: Turkish Journal of Fisheries and Aquatic Sciences <info@trjfas.org>

Dear
Editor in Chief

Excuse me. To date, we did not find any comments or suggestions in the system for revisions, so we do not understand what minor revisions are required.
Thank you.

Best regards,
[Quoted text hidden]
--
Dr. Akhmad Taufiq Mukti
Assoc. Prof. Genetics and Reproduction of Aquatic Organisms
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Kampus C Unair, Jl. Mulyorejo, Surabaya 60115
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akhmad taufiq mukti <akhmad-t-m@fpk.unair.ac.id>

Revision request for your manuscript

akhmad taufiq mukti <akhmad-t-m@fpk.unair.ac.id> Fri, Sep 10, 2021 at 9:18 PM
To: Turkish Journal of Fisheries and Aquatic Sciences <info@trjfas.org>

Dear
Editor in Chief

Excuse me. To date, we did not find any comments or suggestions in the system for revisions, so we do not understand what minor revisions are required.
Thank you.

Best regards,
[Quoted text hidden]
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Dr. Akhmad Taufiq Mukti
Assoc. Prof. Genetics and Reproduction of Aquatic Organisms
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akhmad taufiq mukti <akhmad-t-m@fpk.unair.ac.id>

(no subject)

akhmad taufiq mukti <akhmad-t-m@fpk.unair.ac.id> Thu, Sep 16, 2021 at 12:35 PM
To: Turkish Journal of Fisheries and Aquatic Sciences <info@trjfas.org>

Dear
Editor in Chief

Excuse me. To date, we did not find any comments or suggestions in the system for revisions, so we do not understand what minor revisions are required.
Thank you.

Best regards,

--
Dr. Akhmad Taufiq Mukti
Assoc. Prof. Genetics and Reproduction of Aquatic Organisms
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akhmad taufiq mukti <akhmad-t-m@fpk.unair.ac.id>

First Reminder for Revision

2 messages

Turkish Journal of Fisheries and Aquatic Sciences <info@trjfas.org> Mon, Sep 20, 2021 at 1:24 PM
To: akhmad-t-m@fpk.unair.ac.id

ISSN: 1303 - 2712
E-ISSN: 2149 - 181X

Turkish Journal of FISHERIES and AQUATIC SCIENCES

Dear Akhmad Taufiq Mukti,

This is an automatic system message. We would like to remind you that your revisions or rebutting on the manuscript entitled "Evaluation of probiotic-fermented feed addition and laser firing to accelerate mature broodstocks and seed productions of African catfish (Clarias gariepinus)" have not been received although it has been 14 days since the request was sent to you. Your manuscript will be rejected if we will not receive the revised manuscript within 16 days. You can submit your revision through the online system.

The site is located at http://www.trjfas.org/ Please upload the file containing your revised manuscript. The rebuttal letter should be placed in “cover letter” section. Please note that you should submit your revised letter by clicking on “revision requested” link, not as a new manuscript.
If you have any technical problem please send an e-mail to info@trjfas.org

Sincerely.

TrJFAS Editorial Office
info@trjfas.org

---

akhmad taufiq mukti <akhmad-t-m@fpk.unair.ac.id> Tue, Sep 21, 2021 at 1:18 PM
To: Turkish Journal of Fisheries and Aquatic Sciences <info@trjfas.org>

Dear
Editor in Chief
Turkish Journal of Fisheries and Aquatic Sciences

Until now, we are not aware of the minor revisions that we should revise in the manuscript because there are no clues regarding those minor revisions, including in the system.

However, we are enclosing herewith a revised manuscript entitled “Evaluation of probiotic-fermented feed addition and laser-firing to accelerate mature broodstocks and seed productions of African catfish (Clarias gariepinus)” submitted to the Turkish Journal of Fisheries and Aquatic Sciences for evaluation again. Thanks for the corrections and suggestions to our paper. We expect the editor to be pleased and understand it and we hope that this article will be corrected further.

Thank you very much.

Best regards
[Quoted text hidden]
--
Dr. Akhmad Taufiq Mukti
Assoc. Prof. Genetics and Reproduction of Aquatic Organisms
(Aquaculture Biotechnology)
Department of Aquaculture
Faculty of Fisheries and Marine

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Confirmation for revised manuscript

1 message

Turkish Journal of Fisheries and Aquatic Sciences <info@trjfas.org> Tue, Sep 21, 2021 at 1:08 PM
To: akhmad-t-m@fpk.unair.ac.id

ISSN: 1303 - 2712
E-ISSN: 2149 - 181X

Turkish Journal of
FISHERIES and
AQUATIC SCIENCES

Dear Associated Prof.Akhmad Taufiq Mukti,

This is to acknowledge receipt of your revised manuscript entitled Evaluation of probiotic-fermented feed addition and laser firing to accelerate mature broodstocks and seed productions of African catfish (Clarias gariepinus). You can learn the stage of your manuscript in the review process through the author center. Thank you.

Sincerely.

http://www.trjfas.org/

User ID: akhmad-t-m@fpk.unair.ac.id
Password: https://www.trjfas.org/submit/forgot.php

TrJFAS Editorial Office
info@trjfas.org

Info: This is an automatic system message.

akhmad taufiq mukti <akhmad-t-m@fpk.unair.ac.id>

First Reminder for Revision

akhmad taufiq mukti <akhmad-t-m@fpk.unair.ac.id> Tue, Sep 21, 2021 at 1:18 PM
To: Turkish Journal of Fisheries and Aquatic Sciences <info@trjfas.org>

Dear
Editor in Chief
Turkish Journal of Fisheries and Aquatic Sciences

Until now, we are not aware of the minor revisions that we should revise in the manuscript because there are no clues regarding those minor revisions, including in the system.

However, we are enclosing herewith a revised manuscript entitled “Evaluation of probiotic-fermented feed addition and laser-firing to accelerate mature broodstocks and seed productions of African catfish (Clarias gariepinus)” submitted to the Turkish Journal of Fisheries and Aquatic Sciences for evaluation again. Thanks for the corrections and suggestions to our paper. We expect the editor to be pleased and understand it and we hope that this article will be corrected further.

Thank you very much.

Best regards
[Quoted text hidden]
--
Dr. Akhmad Taufiq Mukti
Assoc. Prof. Genetics and Reproduction of Aquatic Organisms
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LETTER OF RESPONSE TO REVIEWERS

MANUSCRIPT TITLE: Evaluation of probiotic-fermented feed addition and laser-firing to accelerate mature broodstocks and seed productions of African catfish (*Clarias gariepinus*)

MANUSCRIPT ID : TRJFAS-19303

Until now, we are not aware of the minor revisions that we should revise in the manuscript because there are no clues regarding those minor revisions, including in the system.

Thus authors responses on comments, corrections, and suggestions of reviewers, we expect the reviewers were pleased and understand it and we hope that this article will be corrected further. Thank you very much.

Best regards,

Akhmad Taufiq Mukti

akhmad taufiq mukti <akhmad-t-m@fpk.unair.ac.id>

Manuscript Desicion for authors

1 message

Turkish Journal of Fisheries and Aquatic Sciences <info@trjfas.org> Wed, Sep 22, 2021 at 12:37 PM
To: akhmad-t-m@fpk.unair.ac.id

ISSN: 1303 - 2712
E-ISSN: 2149 - 181X

Turkish Journal of
FISHERIES and
AQUATIC SCIENCES

Dear Author,

The manuscript titled "Evaluation of probiotic-fermented feed addition and laser firing to accelerate mature broodstocks and seed productions of African catfish (Clarias gariepinus)", which has been submitted for evaluation by Associated Prof.Akhmad Taufiq Mukti to the TrJFAS and of which you are an author of has been reviewed by reviewers from relevant scientific fields. The outcome of this review is stated below for your attention.

The decision of the journal's editorial office: Accepted

We wish you success with your future studies and thank you for your interest in our journal.

Best regards.

Editorial Office
Turk. J. Fish. & Aquat. Sci.

akhmad taufiq mukti <akhmad-t-m@fpk.unair.ac.id>

Result of the Manuscript Evaluation

1 message

Turkish Journal of Fisheries and Aquatic Sciences <info@trjfas.org> Wed, Sep 22, 2021 at 12:37 PM
To: akhmad-t-m@fpk.unair.ac.id

ISSN: 1303 - 2712
E-ISSN: 2149 - 181X

Turkish Journal of
FISHERIES and
AQUATIC SCIENCES

Dear Associated Prof.Akhmad Taufiq Mukti,

I am pleased to inform you that your manuscript entitled "Evaluation of probiotic-fermented feed addition and laser firing to accelerate mature broodstocks and seed productions of African catfish (Clarias gariepinus)" has been accepted for Vol 22 (the year 2022). The issue number will be assigned later.

Your proof paper will be listed on "Early View" page on our website. You can track your manuscript via the link below:

http://trjfas.org/content.php?id=48

If there is any mistake in your manuscript, please send your corrections on pdf file by e-mail to editor@trjfas.org

Meanwhile, you're required to send us 3 photographs and a short video/videos about your study to share on our social media accounts. This is not optional, but mandatory. The preferable format for video clips is MP4, and for images either JPEG, GIF, or PNG (maximum file size per image is 5MB, and 3MB for animated gifs)." (Please if you have one, send us your twitter name with this email in order to mention you on our twitter page)

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*Please notice that Turkish Journal of Fisheries and Aquatic Sciences reserves the right to reject a paper if it becomes apparent that there are serious problems with its scientific content, or the publishing policies of the journal have been violated.

Best wishes

Esen ALP ERBAY
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+(90) 462 341 1053