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COVER LETTER

Dear **Editor-in-Chief**,

I hereby attach the original research article in the hope that it will be published in the journal *Advances in Animal and Veterinary Sciences (AAVS)*. The writing team says thank you

**Title:**

Effect of Guava Fruit Filtrate Supplementation during Brangus Bull Semen Chilled-storage on Improving Spermatozoa Quality

**Author(s) name:**

Sumadiasa<sup>1\*</sup>, Yuliany<sup>1</sup>, and Lukman<sup>1</sup>

**Afiliasi:**

<sup>1</sup> Faculty of Animal Science University of Mataram, Majapahit Street, number 62, Gomong, Selaparang, Mataram Lombok, 83125, Tel: (0370). 633007, West Nusa Tenggara, Indonesia  
\* Corresponding author : Faculty of Animal Science University of Mataram, Majapahit Street, number 62, Gomong, Selaparang, Mataram Lombok, 83125, Tel: (0370). 633007, West Nusa Tenggara, Indonesia. No. Hand Phone: +62 8175734057, Email : iwlanuss@unram.ac.id

**Statements:**

This manuscript has not been published and is not being considered for publication in any other journal or other type of publication (including web hosting) by either me or my co-authors, with the novelty of the research:

Utilization of guava fruit filtrate in semen extender to improving spermatozoa quality of the bulls after sex activity break for long time. It is found that addition of 10% guava fruit filtrate to Citrate with 10% egg yolk extender can maintain the quality of bulls spermatozoa until 7 days of storage and fit to applied for artificial insemination (AI)

Mataram Lombok Indonesia, 9<sup>th</sup> November, 2022


Sincerely yours

Sumadiasa

 Publications


 Galley Proof (0)

 Published (0)


Submissions Being Processed						
S no	Manuscript ID	Journal Name	Manuscript Title	Submitting Author	Date Submitted	Status
1	MH2021109081100	Advances in Animal and Veterinary Sciences	Effect of Guar Fruit Filtrate Supplementation during Brangus Bull Semen Chilled-storage on Improving Spermatozoa Quality	Dr I Wayan Sumadana	09 Nov 2022	With Editorial Office 

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M

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Sab, 12 Nov 00.28

Dear Dr I Wayan Sumadiasa,

Your Manuscript ID MH20221109081100 with title "Effect of Guava Fruit Filtrate Supplementation during Brangus Bull Semen Chilled-storage on Improving Spermatozoa Quality" has passed the initially quality checks and is now transferred to the Editor of the journal to be considered for review process.

This is a standard procedure for all the new and revised submissions. The editor will evaluate/assess your manuscript and either will proceed to a decision or send it for a review. This email is for your information only and there is nothing for you to do at this moment. We will keep you updated with further information.

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Your Manuscript in Advances in Animal and Veterinary Sciences is awaiting Editor's decision

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M

**Manuscript Handler**

Kam, 24 Nov 15.39 (10  
hari yang lalu)

Dear Dr I Wayan Sumadiasa,

We have received reviewer's reports or editor's assessments for your Manuscript ID MH20221109081100 with title Effect of Guava Fruit Filtrate Supplementation during Brangus Bull Semen Chilled-storage on Improving Spermatozoa Quality in Advances in Animal and Veterinary Sciences.

The manuscript is with Editor to make final decision.

This decision may take some time as it is being discussed with the Editorial Board members. Not hearing the decision on your manuscript indicates that the decision is not yet agreed. As soon as the decision is committed, you will be informed.

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## Researcherslinks: Decision on Manuscript ID MH20221109081100

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M

**Manuscript Handler**

24 Nov 2022 15.39 (10 hari  
yang lalu)

Thu, 24 Nov 2022, 07:39 AM

Dear Dr. I Wayan Lanus Sumadiasa,

We have received the reports from our reviewers on your manuscript, "Effect of Guava Fruit Filtrate Supplementation during Brangus Bull Semen Chilled-storage on Improving Spermatozoa Quality", which you submitted to *Advances in Animal and Veterinary Sciences* with MH20221109081100.

Based on the received comments, your manuscript could be reconsidered for publication, should you be prepared to incorporate Major Revisions.

The comments and requests of the Editor and the Peer Reviewers are included below. Please share this information with all coauthors of the manuscript.

Editor's Comments:

- Review the peer review comments and requests carefully, and edit the manuscript accordingly.
- Include a separate point-by-point response file addressing the reviewers comments along with an explanation of any request of the editor or the reviewers that you do not address in your revised manuscript. Your list of responses should be uploaded as a Cover Letter in addition to your revised manuscript.
- Please colour (e.g. red in contrast to black text) all changes in the revised manuscript, without such coloured changes the manuscript may be returned or rejected.
- Verify the placement and accuracy of each reference in your manuscript as well as the accuracy of all of the values in your tables and figures.
- Please ensure that all author's names and their affiliations are placed correctly.
- Make every effort to address the remaining concerns and to resubmit your manuscript. If you anticipate an additional delay, or if you do not wish to resubmit your manuscript, then please notify us as soon as possible.
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Reviewer(s) Comments to Author:

Reviewer: 1:

Comments to the Author

The title should be re-write based on the study objective.

Introduction has been written well, however it is still lack the information regarding the content of guava especially to expect in maintaining the quality of semen.

Methods also has been written well but some statements need reference (See comments) as well as

results and discussion.

[Download additional comments](#)

Reviewer: 2:

Comments to the Author

Title: it does not represent all aspects of the research. The research is dealing with the supplementation of Guava extract on the different extenders. Moreover, the experiment is done using bulls that have a sexual break for a long time

Introduction :

- a. The author does not provide the reason for selecting Guava extract as a source of Vit C as an antioxidant. The author also does not explain the possibility of the different effects of Guava extract being different among the different extenders (interaction).
- b. The review on the use of different sources of Vit C as an antioxidant is generally lacking
- c. In the text, it is mentioned that semen was collected from bulls that have had sexual breaks for a long time. Therefore, this must be stated in the introduction

Materials and Methods :

- a. The authors need to explain in detail the parameters measured, e.g. abnormality, motility, etc.
- b. Evaluation of the semen quality does not include day 0 (immediately after dilution)
- c. The statistical package nor the used model are not defined

Result and discussion

- a. From the evaluation of fresh semen it was found that sexual breaks for a long time did not affect semen quality. However, the author discussed the negative effect of prolonged sexual breaks. It should be otherwise.
- b. The author did not provide evidence(s) from other studies that sexual break affects semen volume and quality!
- c. The author did not discuss the ability of different extenders with or without GP in terms of how long the extenders can keep the quality of diluted semen.
- d. In most cases, the discussion and conclusion are drawn based on day 7, but not the other days.
- e. The result of the experiment actually showed that the addition of GF reduced sperm motility when GF was added to the extender (both TRIS and Citrate base) !! A significant positive effect was only obtained in T4 in which the proportion of egg yolk was reduced and partly replaced with GF. The author should discuss why this occurs and the mechanism behind this finding.

Conclusion :

Should be rewritten according to the findings

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## Response Letter

December 08, 2022

To  
Editor In Chief of Advances in Animal and Veterinary Sciences

Dear Editor in Chief of AAVS

**Subject: Submission of revised paper with title** “Effect of Guava Filtrate Supplementation in Tris and Citrate-Based Extenders on Spermatozoa Quality of Brangus Bull after Sex-rest” **with manuscript number** MH20221109081100

We have carefully reviewed the comments and have revised the manuscript accordingly. Our responses are given in a point-by-point manner below In the Table 1. esponse Letter

Table 1. Author Responses to the Reviewer Comments

Reviewer Comments	Author Response
<b>Reviewer: 1:</b>	
<b>The title</b> should be re-write based on the study objective.	According to the comments, the title have been changed as presented in line 3 to 4
<b>Introduction</b> has been written well, however it is still lack the information regarding the content of guava especially to expect in maintaining the quality of semen.	According to the comments, revisions have been made as presented in line 24 to 25, 52, 60 to 62
<b>Methods</b> also has been written well but some statements need reference (See comments) as well as results and discussion.	According to the comments, the statements have been added as shown in line 81 to 82, 94 to 95.
<b>Reviewer: 2:</b>	
<b>Title:</b> it does not represent all aspects of the research. The research is dealing with the supplementation of Guava extract on the different extenders. Moreover, the experiment is done using bulls that have a sexual break for a long time	According to the comments, the title have been changed as presented in line 3 to 4.
<b>Inttroduction :</b> a. The author does not provide the reason for selecting Guava extract as a source of Vit	According to the comments, revisions have been made as presented in line 64 to 75.

C as an antioxidant. The author also does not explain the possibility of the different effects of Guava extract being different among the different extenders (interaction).	
b. The review on the use of different sources of Vit C as an antioxidant is generally lacking	According to the comments, different sources of Vit C as an antioxidant have been added as presented in line 71 to 75.
c. In the text, it is mentioned that semen was collected from bulls that have had sexual breaks for a long time. Therefore, this must be stated in the introduction	According to the comments, different sources of vitamin C as an antioxidant have been added as presented in line 24 to 30.
<b>Materials and Methods :</b>	
a. The authors need to explain in detail the parameters measured, e.g. abnormality, motility, etc.	According to the comments, the explanation of parameters measured have been added as presented in line 140 to 143.
b. Evaluation of the semen quality does not include day 0 (immediately after dilution)	According to the comments, evaluation of the semen quality in day 0 have been added as presented in line 136 to 139.
c. The statistical package nor the use model are not defined	Correction according to the comments have been presented in line 145 to 147.
<b>Result and discussion :</b>	
a. From the evaluation of fresh semen it was found that sexual breaks for a long time did not affect semen quality. However, the author discussed the negative effect of prolonged sexual breaks. It should be otherwise.	Correction according to the comments have been presented in line 154.
b. The author did not provide evidence(s) from other studies that sexual break affects semen volume and quality!	Correction according to the comments have been presented in line 155 to 158, and 160 to 169.
c. The author did not discuss the ability of different extenders with or without GP in terms of how long the extenders can keep the quality of diluted semen.	According to the comments, evaluation of the semen quality in day 0 have been added as presented in line 136 to 139.
d. In most cases, the discussion and conclusion are drawn based on day 7, but not the other days.	According to the comments, evaluation of the semen quality in day 0 have been added as presented in line 206 to 208
e. The result of the experiment actually showed that the addition of GF reduced sperm motility when GF was added to the extender (both TRIS and Citrate base) !! A significant positive effect was only obtained in T4 in which the proportion of egg yolk was reduced and partly replaced with GF. The author should discuss why this occurs and the mechanism behind this finding	According to the comments, the explanation of positive effect of T4 extender have been presented in line 226 to 233.  <b>Others correction have been presented in some line of these responses</b>
<b>Conclusion :</b> Should be rewritten according to the findings	Correction according to the comments have been presented in line 325 to 329.

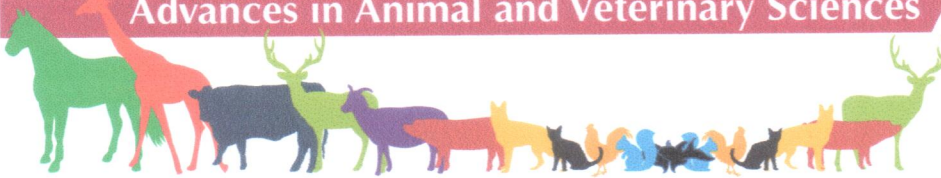
We hope the revised version is now suitable for publication

Sincerely yours

Corresponding author

I Wayan Lans Sumadiasa

Faculty of Animal Science University of Mataram, Majapahit Street, number 62,  
Gomong, Selaparang, Mataram Lombok, 83125, Tel: (0370). 633007, West Nusa  
Tenggara, Indonesia.

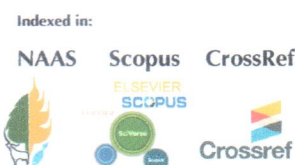
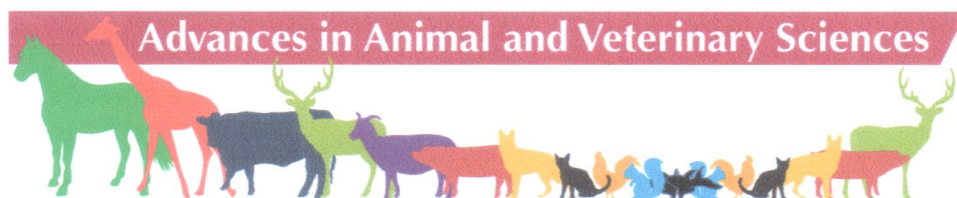


## Effect of Guava Filtrate Supplementation in Tris and Citrate-Based Extenders on Spermatozoa Quality of Brangus Bull after Sex-rest

<b>Journal</b>	Advances in Animal and Veterinary Sciences
<b>Manuscript ID</b>	MH20221109081100-R1
<b>Manuscript Type</b>	Research Article
<b>Area of Interest</b>	Husbandry
<b>Date Submitted by the Author</b>	Thu, 08 Dec 2022, 07:45 AM
<b>Complete List of Authors:</b>	<ul style="list-style-type: none"><li>■ Dr I Wayan Sumadiasa, University of Mataram, Indonesia</li><li>■ Prof Enny Yuliani, University of Mataram, Indonesia</li><li>■ Dr Lukman Lukman, University of Mataram, Indonesia</li></ul>



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## Response Letter

December 16, 2022

To  
Editor in Chief of Advances in Animal and Veterinary Sciences

Dear Editor in Chief of AAVS

**Subject: Submission of revised paper with tiitle** “Effect of Guava Filtrate Supplementation in Tris and Citrate-Based Extenders on Spermatozoa Quality of Brangus Bull after Sex-rest” **with manuscript number** [MH20221109081100](#)

We have carefully reviewed the comments and have revised the manuscript accordingly. Our responses are given in a point-by-point manner below in the Table 1.  
esponse Letter

**Table 1. Author Responses to the additional Comments**

Additional Comments	Author Response
<b>Comment in line 6:</b> <del>determines</del> should be written to <del>determine</del>	The word has been revised in accordance with the comment
<b>Comment in line 79:</b> <del>time</del> should be written to <del>times</del>	The word has been revised in accordance with the comment
<b>Comment in line 81:</b> Add “both” before macroscopically	The statement has been added in accordance with the comment

We hope the revised version is now suitable for publication

Sincerely yours

Corresponding author

I Wayan Lans Sumadisa

Faculty of Animal Science University of Mataram, Majapahit Street, Number 62, Gomong, Selaparang, Mataram Lombok, 83125, Tel: (0370). 633007, West Nusa Tenggara, Indonesia.

Eb

1 ORIGINAL ARTICLE,

2

3 Effect of Guava Filtrate Supplementation in Tris and Citrate-Based Extenders on  
4 Spermatozoa Quality of Brangus Bull after Sex-rest

5

6 **Abstract** | The objective of this study was to ~~determines~~ the effect of guava fruit filtrate (GF)  
7 supplementation in Tris and Citrate-based extenders on spermatozoa quality of Brangus bull  
8 after sex-break. Semen samples (twelve ejaculates) were diluted in Tris-egg yolk (TEY)  
9 without GF (control group, T1), TEY with 10% GF (T2), Citrate-egg yolk (CEY) with 10%  
10 GF (T3), Citrate with 10% egg yolk and 10% GF (T4), then chill-storage. The variables quality  
11 evaluated include spermatozoa progressive motility, visibility, and abnormality. Spermatozoa  
12 quality was observed every 24 hours until the spermatozoa motility reached at least 40%. The  
13 results showed that progressive motility and viability on the fourth day storage in extender  
14 Citrate-egg yolk (CEY) with 10% egg yolk and 10% GF were  $50.4 \pm 1.6\%$  and  $57.1 \pm 1.6\%$ ,  
15 significantly higher ( $P < 0.05$ ) than control, TEY with 10% GF and Citrate with 10% egg yolk.  
16 The spermatozoa abnormality was  $12.9 \pm 0.3\%$ , the lowest in extender Citrate with 10% egg  
17 yolk and 10% GF on the fourth-day storage. In conclusion, supplementation of GF to extender  
18 improves the spermatozoa quality of Brangus bull after sex activity break for a long time.  
19 Moreover, the best quality of spermatozoa is found in supplementing 10% GF to Citrate with  
20 10% egg yolk extender.

Commented [A1]: determine

21 **Keywords** | Brangus bull, chilled storage, extender, guava, semen

22

23 INTRODUCTION

24 Brangus has grown in several regions for transfer genetics superior to local cow through  
25 artificial insemination (AI), including in West Nusa Tenggara Province. However, in recent

## MATERIALS AND METHODS

The research material was the semen of three Brangus bulls aged 4.5 to 6.5 years belonging to the Artificial Insemination Center of West Nusa Tenggara Province at Banyuwilek. The semen was collected with an artificial vagina each six **time** per bull. The ejaculate obtained was taken to the Reproduction Laboratory, Faculty of Animal Husbandry, University of Mataram, for evaluation **macroscopically** and microscopically. **The distance of the laboratory from the AI center is about 7 km by freeway, which takes about 8 to 10 minutes.** Experimental animals in this study have received approval from the Animal Ethics Committee with the Register Number: 09/UN18.F2/EC/2021.

Commented [A2]: times

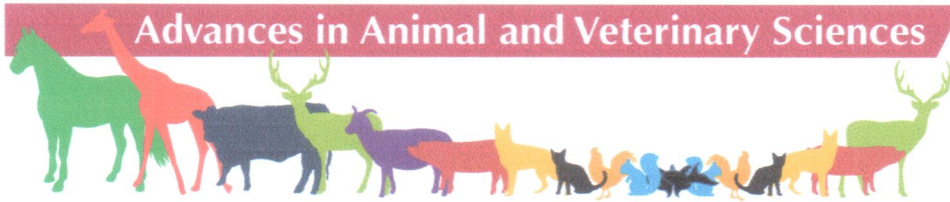
Commented [A3]: Add "both" before macroscopically

The first stage of the study was to assess the quality of fresh semen based on the results of the macroscopic and microscopic evaluation. The leading indicators used for the feasibility standard of fresh semen for processing in this study were semen volume ranging from 2 to 7.5 ml, progressive motility and viability of spermatozoa at least 70%, maximum abnormality 20% and minimum concentration 500 million cells per ml of semen, in accordance with Ghirardosi et al. (2018), Andrabi et al. (2008), Asadpour et al. (2011) and Suhardi et al. (2020). The second stage used an experimental method with a completely randomized design (CRD) consisting of four extender treatments for chilled storage. Treatment T1 consisted of tris-egg yolk (TEY) without GF as a control group, T2 was TEY with 10% GF, T3 was Citrate-egg yolk (CEY) with 10% GF, **and T4 was Citrate with 10% EY and 10% GF (a half of EY was substitute with GF).** Each group of semen samples was repeated six times.

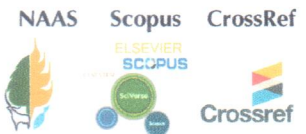
### PREPARATION OF THE STANDARD STOCK OF GF

Guava was cleaned with water at 37 °C dried with a tissue, juiced, and then dissolved with aquabidest with a ratio of 1: 2. The solution was centrifuged for 10 minutes at a speed of 3500 rpm. The supernatant resulting from centrifugation was filtered with a millipore membrane (Sartorius stedim Minisart®) at 0.42 µm and continued at 0.2 µm. The filtrate (filtration liquid)





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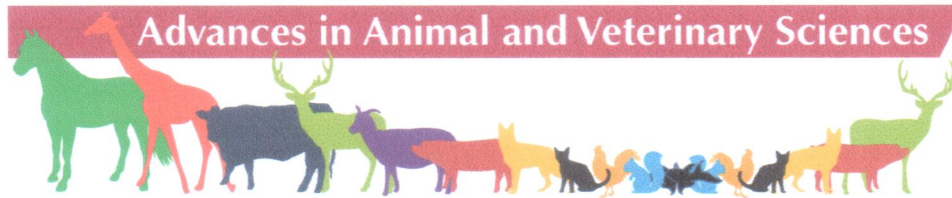


## Effect of Guava Filtrate Supplementation in Tris and Citrate-Based Extenders on Spermatozoa Quality of Brangus Bull after Sex-rest

<b>Journal</b>	Advances in Animal and Veterinary Sciences
<b>Manuscript ID</b>	MH20221109081100-R2
<b>Manuscript Type</b>	Research Article
<b>Area of Interest</b>	Husbandry
<b>Date Submitted by the Author</b>	Fri, 16 Dec 2022, 02:41 AM
<b>Complete List of Authors:</b>	<ul style="list-style-type: none"><li>■ Dr I Wayan Sumadiasa, University of Mataram, Indonesia</li><li>■ Prof Enny Yuliani, University of Mataram, Indonesia</li><li>■ Dr Lukman Lukman, University of Mataram, Indonesia</li></ul>



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Sab, 17 Des 20.28 (2 hari yang lalu)

Sat, 17 Dec 2022, 12:28 PM

Dear Dr. I Wayan Lanus Sumadiasa,

It is a pleasure to accept your manuscript entitled "Effect of Guava Filtrate Supplementation in Tris and Citrate-Based Extenders on Spermatozoa Quality of Brangus Bull after Sex-res" in its current form for publication in the Advances in Animal and Veterinary Sciences.

Your article is now being processed for formatting, copy editing and final publication. You will be informed for each step and we will contact you when we need any further information or material.

Thank you for your fine contribution. On behalf of the Editors of the Advances in Animal and Veterinary Sciences, we look forward to your continued contributions to the Journal.

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Reviewer(s) Comments to Author:

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*This document certifies that the manuscript listed below is accepted for publication:*

### Effect of Guava Filtrate Supplementation in Tris and Citrate-Based Extenders on Spermatozoa Quality of Brangus Bull after Sex-rest

Journal	Advances in Animal and Veterinary Sciences
Manuscript ID	MH20221109081100-R2
Manuscript Type	Research Article
Area of Interest	Husbandry

Complete List of Authors:	<ul style="list-style-type: none"><li>■ Dr I Wayan Sumadisa, University of Mataram, Indonesia</li><li>■ Prof Enny Yuliani, University of Mataram, Indonesia</li><li>■ Dr Lukman Lukman, University of Mataram, Indonesia</li></ul>
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