GREEN ALGAE (*Cladophora glomerata*) AS A COMPONENT AND AN ALTERNATIVE FISH FEED FOR NILE TILAPIA (*Oreochromis niloticus*)

A Research Study

Presented to the Faculty of the

Regional Science High School for Region I

Bangar, La Union in Partial fulfillment of

their Requirements in Research IV

By

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July 2023

INDORSEMENT

This research entitled, **GREEN ALGAE (Cladophora glomerata) AS A COMPONENT AND AN ALTERNATIVE FISH FEED FOR NILE TILAPIA (Oreochromis niloticus),** prepared and submitted by **Peter John N. Haboc and Azhton Maverick S. Sibayan,** in partial fulfillment of the requirements for the subject, **Research IV**, has been examined and is recommended for Oral Examination.

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This is to certify that the research entitled, **GREEN ALGAE (Cladophora glomerata) AS A COMPONENT AND AN ALTERNATIVE FISH FEED FOR NILE TILAPIA (Oreochromis niloticus),** prepared and submitted by **Peter John N. Haboc and Azhton Maverick S. Sibayan,** and in partial fulfillment of the requirements for the subject, **Research IV,** has been examined and is recommended for Oral Examination.

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ACKNOWLEDGEMENT

The researchers would like to thank those people who have become a big part of this study, the researchers would not make it without them.

First, to our **Almighty God**, who gave the researchers strength to finish this study and for never allowing the researchers to give up.

To their **Parents**, for their love, prayers, caring, unfailing support, and continuous encouragement throughout the study.

To their **principa**l, **Mrs. Nancy G. Hoggang**, for the pieces of advice and for encouraging the researchers to finish this study on time.

To the **Head of the Science Department**, **Mrs. Aurelia S. Garcia**, for her patience, dedication and encouragement to the researchers and research advisers.

To their **research adviser**, **Mr. Jake L. Mantilla** for giving the researchers the opportunity to conduct this study and for the continuous support for the study, for his patience, motivation, and knowledge. Also, for his double effort in checking the manuscripts and for making the study better.

To the **panel members** during the pre-oral defense, **Mrs. Anna T. Valdez**, **Mrs. Herma D. Acosta**, and **Mr. Leonard Levi L. Suguitan,** to the **panel members** during final -oral defense, **Mr. Genaro D. Omo, Mrs. Herma D. Acosta** and **Mr. Bernardo D. Mayrena Jr.**, for their suggestions during the defense and recommendations that helped to improve the study.

To their **class advisers**, **Mr. Bernardo Mayrena**, **Mrs. Ricel D. Mifa**, **Mrs. Imelda Canumay** and **Mr. Jake L. Mantilla,** for helping and supporting the researchers and motivating them to make their study better.

To the participants, for their time and effort, for their full cooperation, and for giving information that is helpful in finishing, this study.

To their friends and classmates, for contributing great ideas and advice during the conduct of the study.

And to all whom the researchers forgot to mention, thank you very much for your support, this study will not be a success without you.

-The Researchers

DEDICATION

The researchers would like to offer this humble research paper to the Almighty Father for His unending love as He became her source of strength all throughout this study. Lord God, she presents to you the fruit of her hard work to be blessed with your grace that it may become as one of her beautiful symbols of Your goodness.

Likewise, it is dedicated to her loving and supportive parents and friends who served as her inspiration and provider of endless piece of advice and never-ending words of encouragement.

Also, for the financial support, and sacrifices during the development of this work.

This piece of work is also dedicated to the beneficiaries, particularly to the fruit harvesters and sellers, as they've served as the motivation of the researcher to venture the study.

And lastly, to herself for having the determination in accomplishing this work, and for never getting tired of this journey.

- The Researchers

RESEARCH ABSTRACT

TITLE:	**GREEN ALGAE (Cladophora glomerata) AS A COMPONENT AND AN ALTERNATIVE FISH FEED FOR NILE TILAPIA (Oreochromis niloticus)**
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KEY WORDS: Green algae, Growth of Tilapia, fish feeds

Abstract: This study aimed to determine if Green algae (*Cladophora glomerata*) is efficient in increasing the length and weight of Nile Tilapia. The research was to see if Green algae (*Cladophora glomerata*) is acceptable as fish feed, so in doing this, the researchers had 2 treatments to test the Green Algae to Nile Tilapia and had 5 fishes per replicate with no trials. Moreover, the treatment 0 which is the commercialized organic fish feeds, yielded the best result with a mean result of 16.38cm in length and 78.13g in weight. Also, there is no significant difference between the commercialized feeds and the treatments in terms of their length due to the T-value of 0.23 being less than the T-critical of 2.78. In addition, there is also no significant differences between the treatments in terms of their weight as well because of the T-value of 0.12 being less than the T-critical of 2.78. Finally, it is therefore concluded that Green algae (*Cladophora glomerata*) is effective as an alternative fish feed since there is no significant difference between the two treatments. It can be investigated for its efficacy in the growth of the fishes.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER I

INTRODUCTION

Nowadays, a good amount of nutrition in animal production systems is significant for economical production of a healthy, high-quality product. Nutrition is a big asset in fish farming because 50 percent of the variable cost comes from fish feeds. For the past few years, fish nutrition has advanced remarkably creating a new, stable commercial diet that promote optimal fish growth and health (Craig, S., et al., 2017). However, the researchers used Green algae (*Cladophora glomerata*) as an alternative to fish feeds and fed it to Nile tilapia (*Oreochromis niloticus*).

Aquaculture is one of the most active livestock sectors, with Nile tilapia (Oreochromis niloticus) farming accounting for 8% of total aquaculture. However, the sustainability of the industry depends on developing cost-effective and environmentally friendly feeds. This study aimed to reduce the protein content of juvenile tilapia diets and minimize the environmental impact of the diet while maximizing biological efficiency. Growth trials were performed on five isoenergetic plant protein-based diets with reduced crude protein levels. (Teodósio, R., et al. 2019)

The development of more sustainable feed ingredients and food supply chains, and the sustainability of livestock ecosystems, require scientific justification for the integration of alternative protein ingredients into new or existing animal feeds.Specific Cladophora glomerata (C. glomerata) has a rich spectrum of bioactive components reflected in its chemical composition. Such biomass is therefore suitable for use as a feed additive in the prism of today's question. Filamentous green algae C. Glomelata thrives in nutrient-rich

waters, especially slow-moving rivers, and forms large communities. However, the flowering caused by C. glomerata is an assemblage mainly composed of only one type of green algae, thus reducing biodiversity, reducing the recreational value of water bodies, and increasing ecological and economic impact. cause adverse effects. By harvesting excess macroalgal biomass from water bodies and integrating it into feed production, the waste is adapted as a raw material, creating a sustainable production chain. (Nutautaitė, M., et al. 2021)

The study of Ansari, F.A., et al. (2021), the aquaculture industry is an efficient producer of edible protein and is growing faster than any other food sector. Fish feed has a direct impact on the quality, potential health benefits, and cost of the fish produced. Fishmeal (FM), Fis-Oil (FO), and herbal supplements, which are primarily used in fish feed, face challenges of low availability, low nutritional value, and high cost. The costs associated with aquaculture feed account for 40-75% of aquaculture production costs and are one of the major market drivers for the thriving aquaculture industry. Microalgae are major producers in the aquatic food chain. Microalgae are continuously expanding in renewable energy, pharmaceutical pigments, wastewater treatment, food and feed industries. The major components of microalgal biomass are proteins containing essential amino acids, lipids containing polyunsaturated fatty acids (PUFAs), carbohydrates, pigments, and other bioactive compounds. As such, microalgae can be used as an essential and viable alternative feed component in aquafeeds. Recently, live algal cultures, whole algae, and lipid-extracted algae (LEA) in fish diets were tested for growth, bioactivity, and nutritional value.

One of the major problems that fishers are facing that the experimenters' study can help with is the algal bloom. Harmful algal blooms (HABs), which contribute to both fisheries consequences and shellfish or fish poisoning owing to toxic algae, have long been documented in Asian coastal waters, particularly in East and Southeast Asia. HAB-related issues have long been a source of societal and economic concern. Despite ongoing efforts to research various elements of harmful algae, fresh instances of fish mass mortality due to previously unknown species at specific locales have continued to emerge over the last decade. These occurrences show that HAB species have recently expanded or been introduced into the Asian region. Progress in HAB research and contemporary challenges concerning hazardous algae are discussed in this chapter, along with descriptions of newly identified HAB species from Asia. (Furuya, K., et al., 2018).

The objective of this study was to determine if Green algae (*Cladophora glomerata*) are a good alternative for commercial fish feeds for Nile Tilapia. The fulfillment of study benefits the people like fish farmers and people who work at agricultural agencies. It is also beneficial to fish enthusiasts to know that there is a cheaper alternative to commercial feeds. In addition, it may also serve as a basis for future researchers to explore more in the discovery of algae fish feeds.

This study is being conducted at the researchers' homes from December 21, 2022 to January 21, 203. The study was delimited only to 2 treatments. Specifically, it is only focused on the use of Green algae.

This study aimed to determine if Green algae have the potential to be an alternative to commercial fish feeds.

Specifically, this study sought answers to the following questions:

1. What bioactive component is present in algae which makes it suitable to be used as an ingredient in making fish feed?
2. Which among the treatments yielded the best result?
    a.) *T0- Commercialize*
    b.) *T1- 100% of dried green algae*
3. Is there a significant difference among the treatments in terms of: weight: and length of the fish?

To better discuss the content of this research paper, the following terms are defined as how they are used in this study.

Green algae (*Cladophora glomerata*) – Cladophora glomerata is one of the most widely distributed and abundant macroalgae in freshwaters worldwide

Algal bloom – is a rapid increase or accumulation in the population of algae in freshwater or marine water systems. One of the problems faced by fishermen is when algae rapidly start to spread in the area.

Nile Tilapia (*Oreochromis niloticus*) – is a species of tilapia, a cichlid fish native to the northern half of Africa and the Levante area, including Israel, and Lebanon. It is the more common species of Tilapia that is found in our area.

Aquaculture – is the breeding, rearing, and harvesting of fish, shellfish, algae, and other organisms in all types of water environments. It is very common within the agricultural department in the government.

Aquafeed – generally known as fish feeds, is the food given to fish in fish farms to enhance the growth of the fish in terms of its weight, and length.

CHAPTER II

METHODOLOGY

This chapter revolved around the methods and processes that are used throughout the conduct of the study. Subsequently, it includes the research design, materials and equipment, data gathering process, data management, and ethical considerations.

Research Design

The study utilized the use of the post-test only experimental design specifically observation method to assess the effectiveness of the treatment in the growth of Nile Tilapia (Oreochromis niloticus), an experiment is very significant in isolating specific variables in order to determine whether they have some effect on a particular phenomenon. The experimental group fed by the treatment and both groups were measured afterwards. This type of experimental design is one of the more reliable research designs for investigating effect of the independent variable and it is convenient to use.

The research was to see if Green algae (*Cladophora glomerata*) is acceptable as fish feed, so in doing this, the researchers had 2 treatments to test the Green Algae to Nile Tilapia and had 5 fishes per replicate with no trials. This study was tested in a localized and controlled area where no outside variables can interfere with the experiment and for about a specific amount of time, the researchers measured the length and weight of the fishes in each replicate. All extraneous variables were controlled as always.

Materials and Equipment

Algae samples are used to conduct the experimentation process. The 10 kg of Green Algae was gathered from the ponds in Paratong, Sta. Lucia, Ilocos Sur. The sample was then dried and crushed thoroughly using a sledgehammer. 6 nets were used in the experimental set up. 10 kg of each treatment separated in 2 different containers. 30 Nile Tilapia fishes were used in the set-up with 5 fish per net. A meter stick was used to measure the parameters of the experimental set-up.

General Procedure

Gathering of Materials

Algae samples are used to conduct the experimentation process. The 10 kg of Green Algae was gathered from the ponds in Paratong, Sta. Lucia, Ilocos Sur. The sample was then dried and crushed thoroughly using a sledgehammer.

Preparation of Treatments

Table 1. Treatments

Treatments	Composition
Treatment 0	Commercialize
Treatment 1	100% Green algae

Fish were fed at least twice a day (at 9:00 a.m. and 3:00 p.m.) or three times a day (at 9:00 a.m., 12:00 p.m. and 4:00 pm). However, young fish or fry should be fed at least five times a day (at 8:00 am, 10:00 am, 12:00 pm, 2:00 pm and 4 p.m.) as their stomachs are still very small. Feeding rates decrease as fish grow. Fish can

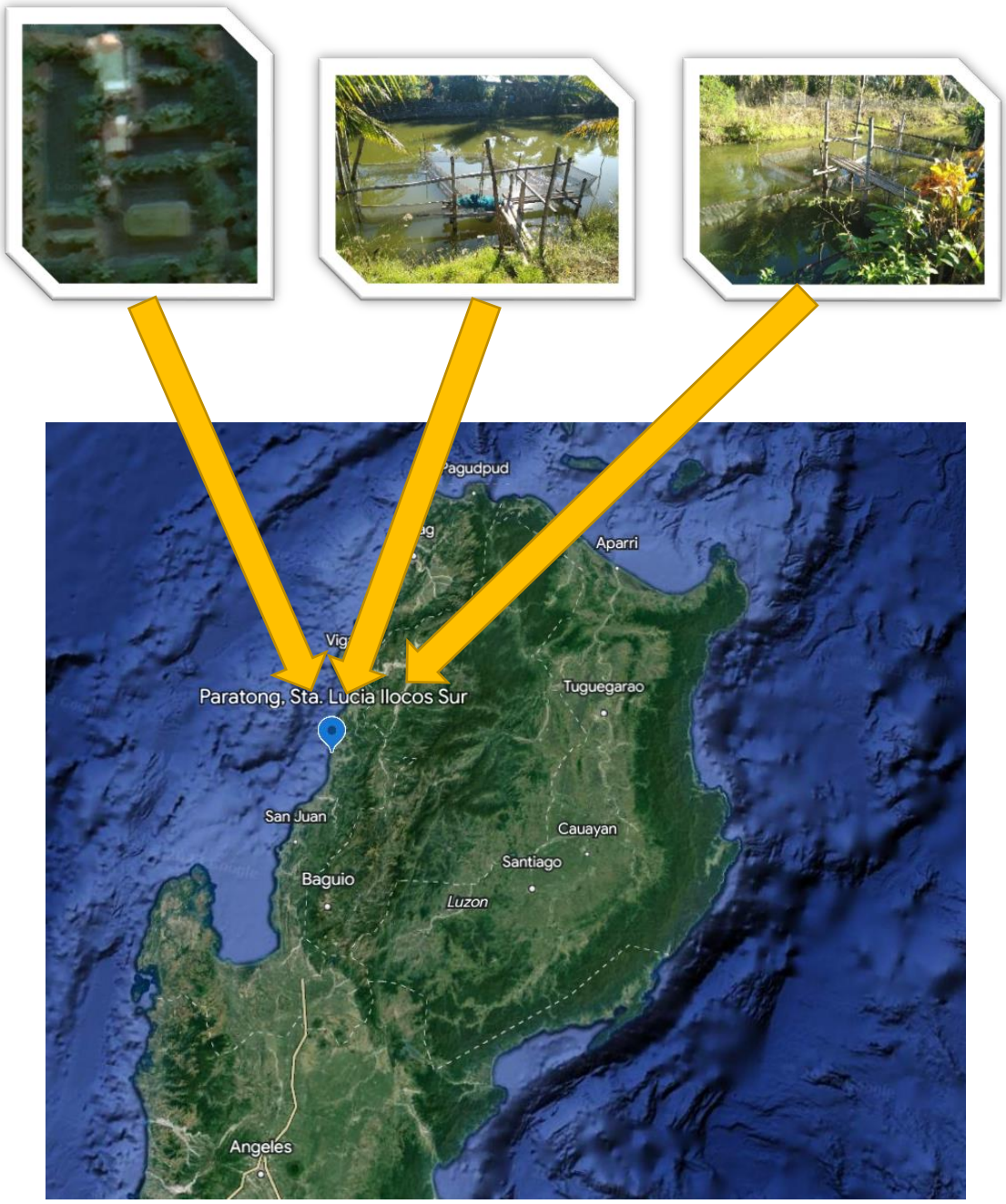
be fed 8% of their weight per day in the early stage, gradually decreasing to 3% in the growth stage and 2% in the final stage. However, the size of the granules increases as the fish grows. It is recommended to feed the fish when the weather is good and not when it is raining. During the feeding process, fish were checked visually to see if they are eating and are active. If they were not eating, stop feeding and check the water quality (Mushagalusa J., et al., 2021).

Acclimatization of Animal Samples

The researchers acclimatized the fishes for one day after it was bought for the fishes to adapt with the environment. This helped in feeding the fishes easily since the fishes were now comfortable with its' surroundings.

Preparation of Experimental Site

The researchers contained the fish samples in a 6m x 2m area of the fish pond which was done by measuring a certain area in which the nets are large enough to fit in and each treatment were separated from one another. Each treatment's environment is the same with a temperature around 28 degrees Celsius. Each treatment had 3 nets each representing the replicates. Treatment 0 fishes had an initial length of approximately 12.4cm, 13.42cm, and 16.12cm respectively while Treatment 1 had an initial length of approximately 12.16cm, 13.42cm, and 16 cm. In weight, Treatment 0 had an initial weight of 32g, 39.8g, and 63.6g respectively while Treatment 1 had an initial weight of 30.6g, 38.6g, and 62g respectively.



Geographical Location: Aerial View of Luzon showing Paratong, Sta. Lucia, Ilocos Sur, where the place of the experimentation is located (17°06’59”N 120°26’20”E).

Testing the Treatments

The researchers tested the treatments by feeding them about twice a day, a cup or treatment per feeding session of a day (1 cup of the treatments in the morning and another one in the afternoon) and we will measure the samples after a month.

Data Management

The first statement of the problem was responded through researching of the algae sample. The detection of components such as pure hydrated silica within a layer of organic, carbon-containing material was released. The second statement of the problem was answered by getting the length of fish in centimeters and the weight in grams. Likewise, the third statement of the problem was determined by comparing the length of fish among the treatments.

Ethical Considerations

Throughout the conduct of the study, there are some ethical concerns that should be observed by the researcher. First of all, paraphrasing the gotten article to avoid plagiarism and also considered in citing the authors properly in American Psychological Association (APA) style. Secondly is that the researchers made sure to prevent in manipulating or fabricating any data presented and in doing so made a reliable and unbiased study based in the experimentation.

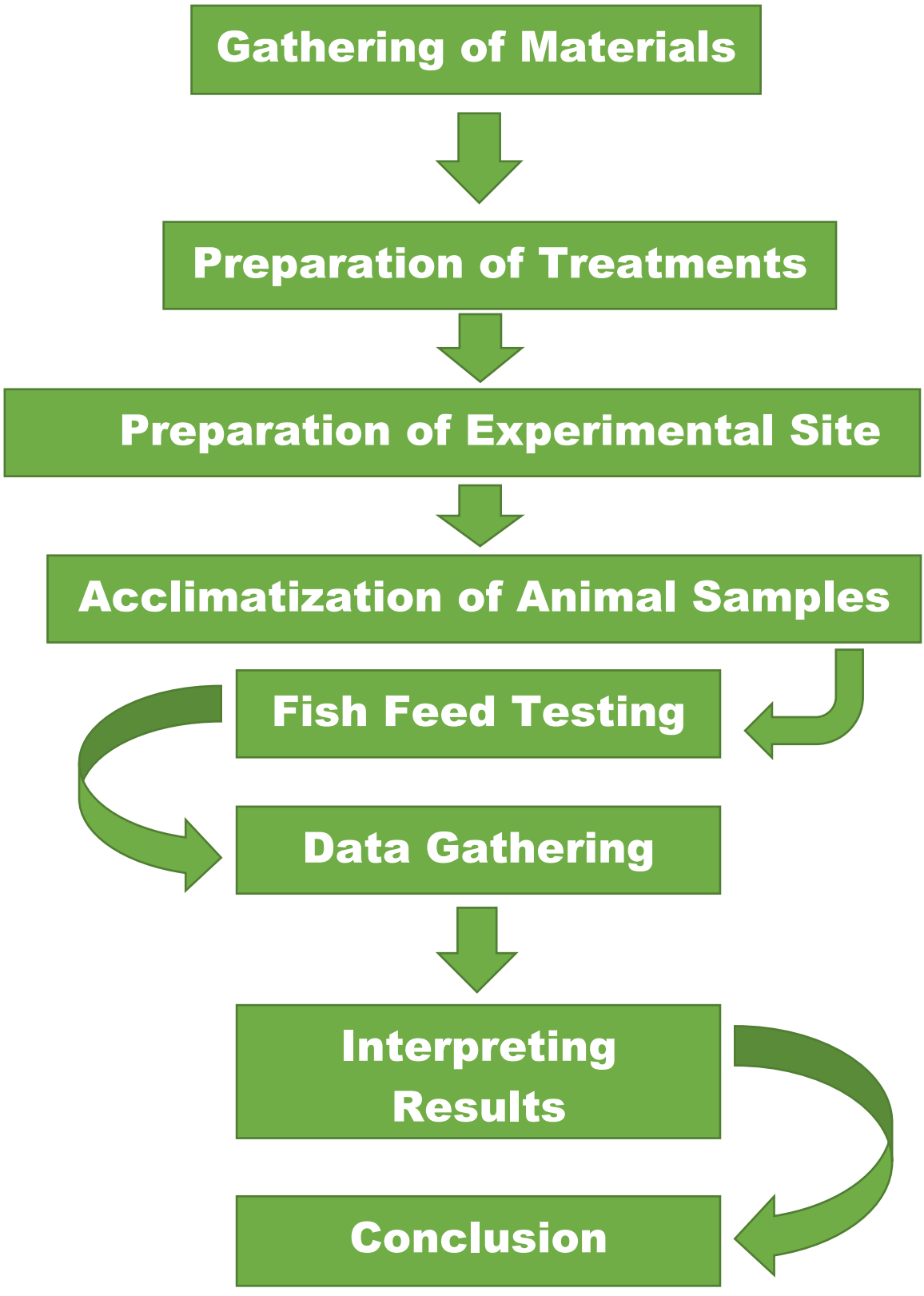


Figure 1. Flowchart of General Procedure

CHAPTER III

RESULTS AND DISCUSSION

This chapter shows the results on the analysis and interpretation on the presence of significant differences among the treatments based from the experiment.

Table 2. Phytochemical Analysis of Green Algae (*Cladophora glomerate*)

Phytochemicals		**Extracts**	
		Observations	**Results**
Alkaloids	**Wagner's test**	**Reddish brown solution precipitate**	**Present**
	Mayer's test	**No yellow coloured precipitate**	**Absent**
	Dragendroff's test	**Red coloured precipitate**	**Present**
	Hager's test	**Yellow coloured precipitate**	**Present**
Flavonoids	**Lead acetate test**	**Formation of yellow colour**	**Present**
	Alkaline reagent	**Formation yellow colour precipitate**	**Present**
Phytosterols	**Salkowski's test**	**No golden yellow colour precipitate**	**Absent**
	Libermam Burchard's test	**No brown ring**	
Diterpenes	**Copper acetate test**	**Emerald green colour**	**Present**
Carbohydrates	**Molisch's test**	**Violet ring at the junction**	**Present**
	Benedict's test	**Orange red precipitate**	**Present**

	Fehling's test	**Red coloured precipitate**	**Present**
Glycosides	**Modified Borntrager's test**	**No formation of rose-pink colour precipitate**	**Absent**
	Legal's test	**Pink to blood red colour precipitate**	**Present**
Saponins	**Froth test**	**Thin layer of foam**	**Present**
	Foam test	**Foam produced persists for 10 minutes**	**Present**
Phenols	**Ferric chloride test**	**No formation of bluish black colour precipitate**	**Absent**
Tannins	**Gelatin test**	**No formation of white colour precipitate**	**Absent**
Proteins and amino acids	**Xanthoproteic test**	**No formation yellow colour precipitate**	**Absent**
	Ninhydrin test	**No formation of blue colour precipitate**	**Absent**

Based on the results of the phytochemical analysis on Table 3, it shows that phytochemicals such as alkaloids, flavonoids, diterpenes, carbohydrates and saponins. Carbohydrates specifically is what contributed more in the growth of the Nile Tilapia since this component is one of the essential nutrients in their growth.

Table 3. Mean Result of the Experimentation on the Length of Fishes (cm) after one month

Treatments	Initial Mean or Average per Treatment	Final Mean or Average per Treatment
Treatment 0	**13.98**	**16.38**
Treatment 1	**13.78**	**16.11**

The table 4 shows experimentation of the different treatments in terms of its length. As shown from the table, treatment 0 which is the organic commercialized fish feeds and it yielded the best result in increasing the length of fishes having a mean length of 16.38cm while treatment 1 yielded a mean length of 16.11cm.

Table 4. Mean Result of the Experimentation on the Weight of Fishes (g) after one month

Treatments	Initial Mean or Average per Treatment	Final Mean or Average per Treatment
Treatment 0	**45.13**	**78.13**
Treatment 1	**43.73**	**76.07**

The table 5 shows experimentation of the different treatments in terms of its weight. As shown from the table, treatment 0 which is the organic commercialized fish feeds and it yielded the best result in increasing the weight of fishes having a mean weight of 78.13g while treatment 1 yielded a mean weight of 76.07g.

Table 5. Statistical Result on the Length of the Fishes

T-test
T-value 0.23 < Critical value 2.78

Based on the result of the statistical analysis conducted using T-test, it was gleaned that there is no significant difference between the commercialized feeds and the treatments. Since the results showed in the table that the T-value which is 0.23 is less than the Critical value which is 2.78.

Table 6. Statistical Result on the Weight of the Fishes

T-test
T-value 0.12 < Critical value 2.78

Based on the result of the statistical analysis conducted using T-test, it was gleaned that there is no significant difference between the commercialized feeds and the treatments. Since the results showed in the table that the T-value is 0.12 which is less than the Critical value which is 2.78.

CHAPTER IV

CONCLUSIONS AND RECOMMENDATIONS

This study aimed to determine if Green Algae (*Cladophora glomerata*) is efficient in increasing the length and weight of Nile Tilapia. Based on the results and findings gathered from the treatments with their respective $12m^2$ area, it was concluded that Green Algae (*Cladophora glomerata*) contain a significant amount of proteins and fiber for fish growth which shows that it is effective in fish growth. However, the organic commercialize fish feed had a slightly better result.

Moreover, the treatment 0 which is the organic commercialized fish feed yielded the best result with a mean result of 16.38cm in length and 78.13g in weight. Also, there is no significant difference between the commercialized feeds and the treatments in terms of their length and weight. Finally, it is therefore concluded that Green Algae (*Cladophora glomerata*) is a sufficient alternative for organic commercialized fish feeds.

Based on aforementioned conclusions, the researchers recommend the use of other species of algae as a substitute to Green Algae (*Cladophora glomerata*) to see the effectiveness of other species. The researchers would also want to recommend trying it on other species of fish if it is the main focus to see if it is effective to other kinds.

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APPENDICES

APENDICES

Appendix Table 1. Two-tailed T-test result on the Length of the Tilapia

	Treatment 0	*Treatment 1*
Mean	16.38	16.10666667
Variance	2.1852	1.930533333
Observations	3	3
Hypothesized Mean Difference	0	
Df	4	
t Stat	0.233362	
P(T<=t) one-tail	0.413468	
t Critical one-tail	2.131847	
P(T<=t) two-tail	0.826936	
t Critical two-tail	2.776445	

Appendix Table 2. Two-tailed T-test result on the Weight of the Tilapia

	Treatment 0	*Treatment 1*
Mean	78.13333333	76.06666667
Variance	371.2933333	484.2533333
Observations	3	3
Hypothesized Mean Difference	0	
Df	4	
t Stat	0.122379651	
P(T<=t) one-tail	0.454250262	
t Critical one-tail	2.131846786	
P(T<=t) two-tail	0.908500524	
t Critical two-tail	2.776445105	

Appendix Table 3. Raw Data of Length(cm) Gathered from both Treatments

Time of Measurement	Treatment 0			Treatment 1		
Before Experimentation	12	13.5	16	12.5	13	16
	12.6	13	16.3	12	13.3	16.2
	12.3	13.2	15.8	12.3	13.5	15
	13	13.4	16.5	11.8	13.8	16.1
	12.1	14	16	12.2	13.2	15.8
After Week 1	13	13.5	16.5	14	13	16
	13	13	17	14	14	17
	13	13.2	16	14.2	14	15.8
	14	13.5	17.2	12	14.5	16.5
	14	14.5	16.7	14	13.2	16
After Week 2	14	14.5	16.5	14	14	17
	14.5	14.2	17.3	14.1	14.3	18
	14.5	14.3	16.5	15.5	14.5	16
	14.8	14.5	17.5	13	15	17.2
	15	15	17	14.2	14.2	17
After Week 3	14.1	15.7	17	14.2	14.8	17
	14.6	15.3	18	14.5	15.3	19
	14.5	15.6	17.5	16	16.5	16.5
	15	16.2	18.5	13.5	16.5	18
	15.5	17	17.5	14.5	15.3	17
After Week 4	14.5	16	17.5	14.5	14.8	17
	14.8	15.5	18.2	14.5	15.5	19
	15	16	17.5	16.1	16.8	16.6
	15.2	16.5	19	14.5	17	18
	15.5	17	17.5	14.5	15.5	17.3

Appendix Table 4. Raw Data of Weight(g) Gathered from both Treatments

Time of Measurement	Treatment 0			Treatment 1		
Before Experimentation	30	39	63	32	37	63
	32	37	65	30	38	65
	31	38	60	31	39	58
	37	41	67	29	41	64
	30	44	63	31	38	60
After Week 1	45	47	82	46	40	63
	55	43	89	47	47	86
	48	44	69	54	49	58
	58	47	95	33	57	72
	45	62	84	46	44	60
After Week 2	48	59	84	48	44	79
	62	52	95	49	55	101
	51	54	90	57	60	60
	50	61	97	39	66	82
	66	64	90	48	51	66
After Week 3	50	62	94	51	53	84
	56	62	90	55	55	118
	54	67	90	59	89	79
	55	70	99	45	82	94
	65	83	84	55	55	83
After Week 4	54	68	99	56	67	91
	57	65	101	54	60	125
	63	66	95	60	84	90
	65	78	103	55	91	98
	68	92	98	55	61	94

PLATES

Plate 1. Treatment 0

Plate 2. Treatment 1

Plate 3. Measuring T0 & T1 fishes before Experimentation

Plate 4. Feeding T0 & T1 fishes in the 1st Week

Plate 5. Gathering of the fishes in T1 after the 1st Week

Plate 6. Gathering of the fishes in T0 after the 1st Week

Plate 7. Measuring T0 & T1 fishes after 1st Week

Plate 8. Feeding T0 & T1 fishes in the 2nd Week

Plate 9. Gathering of the fishes in T1 after the 2nd Week

Plate 10. Gathering of the fishes in T0 after the 2nd Week

Plate 11. Measuring T0 & T1 fishes after 2nd Week

Plate 12. Feeding T0 & T1 fishes in the 3rd Week

Plate 13. Gathering of the fishes in T1 after the 3rd Week

Plate 14. Gathering of the fishes in T0 after the 3rd Week

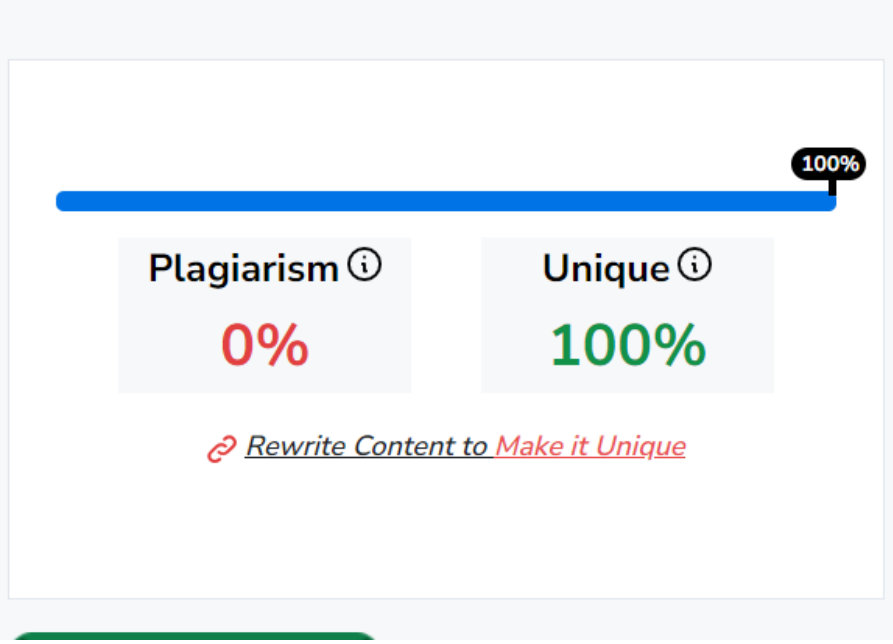
Plate 15. Measuring T0 & T1 after 3rd Week

Plate 16. Gathering of the fishes in T1 after the 4th Week

Plate 17. Gathering of the fishes in T0 after the 4th Week

Plate 18. Measuring T0 & T1 after 4th Week

Plagiarism Checker



Result

Extra Word Count Statistics

Syllables	1306	Average Sentence Length (word)	13.7
Sentences	48	Syllables Per Word(s)	2
Unique Word(s)	348 (53%)	Paragraph(s)	5
Average Word Length (characters)	5.7	Difficult Word(s)	328 (50%)
Reading Time	4 mins	Speak Time	6 mins

Daily Log

Activities	Date Started	Date Finished	Details
Research Proposal	September 13, 2022	September 13, 2022	A proposal of the research plan to find improvements on the study and to know whether the study should be continued or not.
Improving the Research Paper	November 20, 2022	April 19, 2023	The study was improved and now called as the: The Usage of Green Algae (Cladophora glomerata) as a Component and an Alternative Fish Feed for Nile Tilapia (Oreochromis niloticus)
Making Chapter I	November 20, 2022	November 20, 2022	The researchers worked on the Chapter I after gathering all the information
Making Chapter II	November 20, 2022	November 20, 2022	The researchers worked on the Chapter II after knowing all information
Gathering all the materials	December 18, 2022	December 19, 2022	Materials that are needed in the study were procured and gathered by the researcher.
Preparation of Treatments	December 18, 2022	December 18, 2022	Samples of algae were collected and dried.
Reconducting the Experiment	December 21, 2022	January 21, 2023	Reconducting the experiment for data to be more accurate and less bias.

Measuring of Nile Tilapia Before Experimentation	December 21, 2022	December 21, 2022	Measuring the initial length and weight of the Nile Tilapia per replicate.
Week 1 Experimentation	December 21, 2022	December 27, 2022	Start of feeding the Nile Tilapia
Measuring of Nile Tilapia After Week 1	December 28, 2022	December 28, 2022	Measuring the length and weight of Nile Tilapia after a week
Week 2 Experimentation	December 28, 2022	January 3, 2023	2nd week of feeding the Nile Tilapia
Measuring of Nile Tilapia After Week 2	January 4,2023	January 4, 2023	Measuring the length and weight of Nile Tilapia after 2 weeks
Week 3 Experimentation	January 4, 2023	January 10, 2023	3rd week of feeding the Nile Tilapia
Measuring of Nile Tilapia After Week 3	January 11, 2023	January 11, 2023	Measuring the length and weight of Nile Tilapia after 3 weeks
Week 4 Experimentation	January 11, 2023	January 17, 2023	4th week of feeding the Nile Tilapia
Measuring of Nile Tilapia After Week 4	January 18, 2023	January 18, 2023	Measuring the length and weight of Nile Tilapia after 4 weeks
Formulation of Chapter III and Chapter IV	February 12, 2023	February 12, 2023	With all the results and findings, Chapter III and IV were formulated.
Finalization of Manuscript	April 24, 2023	April 25, 2023	The manuscript was finalized by the researcher.
Pre-Oral Defense	April 27, 2023	April 28, 2023	Pre-Oral defense was conducted in order for the research adviser to assess the results of the study and to see improvements on the presentation.

CURRICULUM VITAE

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