EVALUATION OF *Cissus populnea* POWDERED STEM AS FEED BINDER FOR STABILITY, FLOATABILITY AND ITS INFLUENCE ON GROWTH, HAEMATOLOGY AND SURVIVAL RATE OF *Clarias gariepinus* JUVENILES

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NSU/MSC/AFM/0003/17/18

M.Sc AQUACULTURE AND FISHERIES MANAGEMENT

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, NASARAWA STATE UNIVERSITY, KEFFI IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE IN AQUACULTURE AND FISHERIES MANAGEMENT

DEPARTMENT OF AQUACULTURE AND FISHERIES MANAGEMENT

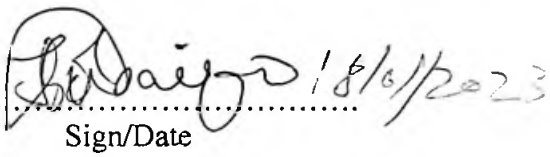
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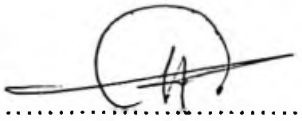
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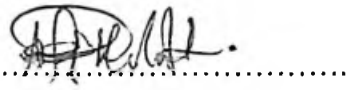
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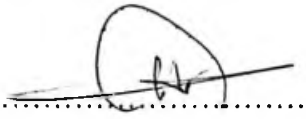
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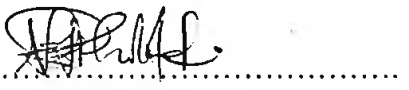
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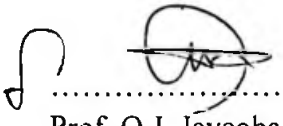
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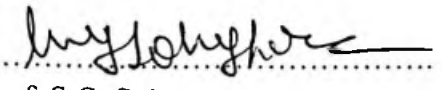
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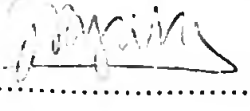
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DEDICATION

This dissertation is dedicated to the every member of my family and friends who have supported me through the course of embarking on this study.

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I also like to say thanks to late Barrister Timothy Onwude who supported me all the way until his unfortunate demise. May he continue to rest in perfect peace.

ABSTRACT

The use of Cissus populnea mucilage as a binder in the formulation of feed for Clarias gariepinus juveniles was investigated for water stability, floatability, growth response, haematology and survival rate at the demonstration fish farm of the Department of Aquaculture and Fisheries Management, Faculty of Agriculture, Nasarawa State University, Keffi. Five experimental feeds were formulated at different inclusion level of Cissus populnea (0g, 20g, 30g, 40g and 50g respectively /100kg) designated as DCP00, DCP20, DCP30, DCP40 and DCP50). Powdered Cissus populnea was bought from the market and used as binder in the formulation of the five experimental diets. The formulated experimental diets were pelleted in extruding machine and sun dried, while the water stability and floatability test were conducted for 50 and 60 minutes respectively. Three hundred Clarias gariepinus juveniles of the average weight sourced from a reputable fish farm in Lafia were acclimatized for seven days and later distributed into five treatments in three replicates. Water parameters of source of water used were also monitored. The fish were fed twice daily at 3% body weight per day for a period of 77days Percentage mean weight gain (of DCP40 and DCP50) had the highest values (2086.7±38.6and 2017±32.3 respectively) which were significantly different ($p<0.05$) from other treatments. Specific growth rate of DCP40 (4.00±0.02) and DCP50 (3.96±0.02) had the highest values significantly higher than other treatments. Feed conversion ratio had the lowest value in DCP40 (1.22±0.003) and DCP50 (1.22±0.006). Haematological results` shows white blood cells at DCP40 was not significantly different from the control while the Red blood cells at DCP40 and DCP50 was not significantly different from the control. The floatability shows that DCP50 92.963±0.642 is significantly different from other treatments. The feed water stability and floatability significantly increased with increase in inclusion of Cissus populnea ($p>0.05$). I highly recommend that Cissus populnea inclusion at 40-50g/100kg feed formulation is advisable for optimal growth and nutrient utilization.

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CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Aquaculture is today evolving into a viable and lucrative enterprise across the globe. This is not unconnected with advancements in science and technology which have promoted the push towards faster growth and higher yields of fish; particularly by way of enhancing and/or replacing natural food with prepared diets. Official report in recent years places Nigeria as Sub-Saharan Africa's largest producer of aquaculture finfish (FAO 2017).This steady rise in aquaculture production in the country has made it a subject of major concern for researchers. Today studies are gear towards determining alternative sources of nutrient for fish feed which will reduce competition with humans on the already established nutrient sources that humans heavily rely on for food (Adikwu 2003). Feed, as Lovell (1998) rightly observes, takes bulk of the cost of aquaculture. This makes it imperative for in-depth research into practical feeding and nutritional requirements of fish for successful fish farming.

Natural water bodies were traditional habitat of fish. Successful studies have revealed that they can as well be reared (Sanusi *et al.*, 2016). Fish farming simply means raising fish either for personal use, or for commercial purpose. The aim of fish farming as Sanusi *et al.*, (2016) explains, is to optimize the uses of land, water, source of food as well as generate income and employment for man. Adewuyi *et al.*, (2016) note that the increasing global population is leading to more need and clamor for livestock protein. In Nigeria for example, the average protein intake as Adewuyi *et al.*, (2016) unveils is low compare to FAO requirement of 65g/output/day. With this growing dependence on fish for animal protein provision, it has become very important to find better and efficient ways of feeding the fish to get optimal returns.

Fish according to Adikwu (2003) is rated as the major source animal protein. It contains very low cholesterol content because it is "white meat". The Low income earner regard it a relatively cheap substitute for beef and other livestock meat. Glaser (1999) observes that fish is fast becoming an essential dietary supplement for the low income earners who invariably rely on cheap food not minding the level of its nutrient. Carballo *et al.* (2008) note however that, there is a decrease in the growth of the world fish catches, over the last 20 years. Commenting on this decrease in world fish catches, Glaser (1999) avers that more than half of third world nations are not even able to meet the required animal protein from the consumption of fish. This decline in the world fish catches which invariably results from lack of improved technology has propelled the urgent need for increase fish production in Nigeria. In recent years, reports on fish farming unveil a steady rise in aquaculture production in Nigeria (Agboola, 2011).

With the steady rise in aquaculture, fish feed has to be made in a manner that they become so efficient that there would be minimal nutrient losses while optimal feed utilization is achieved. Steam-pelleted or extruded feed usually contain added binders (Lovell, 1998). These binders help to improve pellet floatability. Lovell (1998) adds that, there are varieties of binders used for this purpose. Among those are widely used are carboxylmethylcellulose, alginate and guar gum sodium/calcium bentonites, lignosulfonates and hemicellulose.

On essential quality parameter to be considered in the production of aquaculture diet is the pellet water stability (Sivagami and Ronald, 2015). High pellet water stability is achieved when fish feed are not easily lost in water as a result of early disintegration. Binders therefore help to ensure minimal disintegration and nutrient leaching while in the water until consumed by the fish (Sivagami and Ronald, 2015).

Nigeria is a nation naturally endowed with varieties of food crops. Among these are root tubers and cereal crops that are abundantly rich in natural starch. These locally grown food crops rich in natural starch can be harnessed for industrial agro-products for on-farm aquaculture feed production (Obaldo *et al.*, 2002). However these same root tubers and cereal crops are also heavily relied on for human food. It is on this note that this study beams its light on "*cissus populnea*"; a source of binder which is somewhat unconventional.

1.2 Statement of the Problem

Locally formulated fish feeds crumbles, sink easily and leaches the nutrients into water, thereby not conveying the balanced diet to the fish. The crumbling and leaching can also lead to water pollution and turbidity, making the aquatic environment not conducive for cultured fish. There is also great concern with fish feed stability and floatabilty when introduced in water as some due to their poor water stability disintegrates and waste also affecting the water quality.

This study will be focused on the evaluation of the efficacy of using *cissus populnea* as a binding agent to improve feed floatability, stability, digestibility, growth rate, survival rate and also its effect on experimenter fish hematology.

1.3 Objectives of the Research

The study's main objectives are to:

i. ascertain the floatability of fish feeds formulated using *Cissus populnea* as a binding agent;

ii. evaluate the feed stability in water using *Cissus populnea* as binding agent;

iii. investigate the effects the feed formulated using *cissus populnea* as organic binder on growth and growth pattern of *Clarias gariepinus*; and

iv. evaluate the haematology of *Clarias gariepinus* fed on feed formulated using *Cissus populnea* as organic binder.

1.4 Significance of the Study

The study will serve as a source of relevant information to individuals involved in fish feed formulation. It will also be of immense benefit to those who intend to venture into this field of enterprise. The result of this study will be of benefit to feed manufacturers and fish farmers; as it will provide an alternate source of increasing the stability and floatability of fish feed in water. A water stable pellet not only ensures wholesome delivery of nutrient to fish but also reduces the level of feed wastage. The immense benefit of such achievement to aquaculture operations in term of feed nutrient utilization cannot be over-emphasized. The economic benefit in any business venture is getting optimum returns on investments. In aquaculture this is achieved when every feed pellet counts and is converted into flesh by the fish.

1.5 Scope of the Study

This experimental feeding trial is expected to last a minimum of 12 weeks, and within the period of the experiment, evaluation will be made to access the efficacy and suitability of using *cissus populnea* as a feed binder in aquaculture diets.

CHAPTER TWO

LITERATURE REVIEW

2.1 Conceptual Frame Work

2.1.1 Fish Feed: A Conceptual Definition

Food is an essential requirement for all living organisms. For fish to survive and grow satisfactorily, they must be fed adequately in both quantity and quality. An aquaculture practitioner has the basic goal of getting the best utilization of feed fed to the fish. The feed has to be able to supply all the required nutrients to the fish for its healthy maintenance and ultimately ensure rapid growth of fish. In formulating fish feed, the benchmark requirement is to achieve a balanced diet containing in appropriate proportion; carbohydrates, protein, lipids, fibre, moisture, Ash (Steven *et al.*, 2019)

Nutrient availability is very essential for fish to perform optimally. Hence, a well compounded feed should have a very good binder to help achieve a very water stable pellet so as to erase the danger of nutrient leeching completely or to the barest minimum. An addition of vitamins and mineral premixes are also very essential in fish diets (Delbert, 2010).

2.1.2 Types of Fish Feed

Eriegha and Ekokotu (2017) note in their study that, a nutritionally balanced diet must be provided in order to grow a healthy stock of fish. This is why feed usually claims about 50% of the total production cost in aquaculture (Hasan, 2001). A wide variety of quality fish feed which are readily available in aquaculture has to be carefully considered. Different types of feed can be given to fish depending on their health advantage to the fish (Hartmut *et al.*, 2009)

2.1.3 Processed Feed

Processed feeds are those, manufactured basically to cater for the dietary need of fish. They can be dry feeds like: pellet, tablet, crumb and flake, form. Today, advancement in the field of aquaculture has made it possible to manufacture processed feed (Robb and Crampton, 2013).With the availability of processed feed, farmers and hobby fish-keepers now has different option available to make their choice from it terms of fish feed. Robb and Crampton (2013) points out that fish farmer use processed feed as staple food for their fish because they are rich in vitamins.

These scholars add that dry feeds that are processed vary in their sizes. This is mainly to make it useful to different types of fish. There are floating feed as well as sinking feed. Sinking feed is essentially to incorporate bottom feeders. Also commercially available are premium fish feeds as well as medicated fish feeds for feeding fish with specialized needs (Juli-Anne *et al.,* 2019)

2.1.4 Physical Properties of Processed Feed

There are several physical properties of processed feed and they are,

2.1.4.1 Pellet size

One of the physical properties of processed feed is the pellet size. Several research conducted reveal that feed should not be too large. According to Thorpe and Wankowski, (1979), the pellet size should be commensurate for easy consumption. Smith *et al.* (1995) in their study found that some fish like the Atlantic salmon can easily eat pellets that are big. This may not be so in the case of pellet that is small in size. There is a tendency for to reject such smaller pellets

2.1.4.2 Sinking speed

Also to be put into consideration is the speed at which pellet sinks. Scholars emphasize that the speed at which feed sink varies according to fish species (Menghe *et al.*, 2008). According to Menghe *et al.* (2008) floating pellets are good for catfish mainly to watch how they feed.

2.2 Concept of Fish Feed Binders

Binders, as defined by Sivagami and Ronald (2015) are products used to hold various feed component together not only to reduce disintegration and loss of nutrient but also to increase feed efficiency. These scholars add that binders perform three basic functions: it increases the hardness of feeds, aid the floatability of feed as well as increase water stability of pellets (Sivagami and Ronald 2015). The inclusion of a fish feed binder according to Sinha *et al.*, (2011) is necessary particularly to ensure water stable feed. There are varieties of substances with binding properties.

Reports from studies indicate that natural starch have binding properties which can be used to make solid pellets that can be stable in water as well as retains it nutritional values (Sinha *et al.*, 2011). Natural binders made from starch for example good source of nutrient for fish. There are as well immobile raw materials that have little dietary significance (Sinha *et al.*, 2011). Studies conducted on biopolymers reveal that pants and marine animals are main source of natural binders. Soy proteins, starch and cellulose etc., are among these biopolymers (Marina *et al.*, 2012).

According to Kalian and Morey, (2009) the feed industry has employed over 50 organic and inorganic binders. What the fish farmer choose as feed largely depends on how much money available. We need to bear in mind that bulk of the costs of operating a fish farm is consumed by feed. Feed's stability in water as well as its acceptability is among other factors influencing the formulation of aquatic feeds.

Binders are grouped into three categories namely:

1. Protein source binders
2. Carbohydrate source binders
3. Binders with no dietary benefit

On the basis of its nutritional values protein source binders should be more appropriate for a carnivorous species than carbohydrate. For herbivorous species, carbohydrate source binders would be more appropriate (De Silva and Anderson, 1995).

2.2.1 Carbohydrates

Polysaccharides as many scholars describe them are harmless and environment friendly biopolymers which are formulated by high molecular weight carbohydrates. Polysaccharides usually show peculiar physical-chemical properties by (Aspinall, 1982). They as well possess environmentally sustainable features.

2.2.2 Agar

This is found in red alga (seaweed) known as agarophyte (Usov 1998).It consists of agaropectin and Agarose. It contains high molecular weight of Agarose and low sulphate. In the case of Agaropectin, the sulphate level is high but low in molecular weight. Usov (1998) notes that Agarose produces gel in Agar.

2.2.3 Alginate

Aderibigbe and Buyana (2018) describes Alginate as a naturally occurring, anionic biopolymer, derived from brown sea weed.

2.2.4 Pectin

Pectin is a gelling agent; a thickener as well as a mouth-feel enhancer (Vanitha and Khan, 2019). It is a highly complex polysaccharide extracted from plant cell walls and functions as a form of structural cement for holding cell wall together. Fruits such as apples, oranges carrots etc. are rich in pectin (Stephen and Aspinall, 1983).

2.2.5 Chitosan

Chitosan is a cationic carbohydrate biopolymer extracted from chitin found in shell waste of crustaceans like crabs, shrimps and lobsters. Chitosan contains high level polysaccharide second only to cellulose (Johnson and Peniston, 1982).

2.2.6 Carrageenan

Carrageenan is natural thickening/gelling agent that comes from red seaweeds (Murano *et al.*, 1992).Carrageenan is usually extracted with water or aqueous alkali (Murano *et al.*,1992; Kalia, 2005).

2.2.7 Carboxymethylcellulose

Carboxymethylcellulose (CMC) is a water soluble and suspending agent derived from cellulose. According to the report of Codex Alimentarius Commission, (2016) Carboxymethylcellulose (CMC) is formed by its reaction with alkali and chloroacetic acid.

2.2.8 Guar gum *(Cyamopsis tetragonolobus)*

Cyamopsis tetragonolobus produces what in aquaculture is known as guar gum. *Cyamopsis tetragonolobus*is an India beans called guar which produces a gel-forming polysaccharide (guar gum) with thickening and stabilizing properties useful for food. Goldstein et al., (1973) notes guar gum is made up of mannose backbone with a galactose side chain which is

randomly placed on the mannose backbone with an average ratio of 1:2 galactose to mannose. The polymeric structure of guar gum containing numerous hydroxyl groups, have long since been exploited by industries. It has always been an important source of nutrition for humans as well as animals.

2.2.9 Proteins

The most widely used protein source feed binders are those gotten from wheat gluten and gelatin as well as urea formaldehyde. According to De Muylder *et al.,* (2008), binders of gelatin origin are substitute for gluten and urea formaldehyde. These researchers explain that these protein source binders are digestible. However, gluten's antigenicity could be a possible draw back to its usage (Shewry, 2009).

2.2.10 Gelatin

Gelatin is a mixture of water-soluble proteins, typically extracted through hydrolysis from animal hides, bone and tendon – collagen (Farris et al., 2009). The protein fraction of gelatin is almost entirely made up of amino acids joined by amide linkage to form a linear polymer. Gelatin is relatively cheap and biodegradable this makes it attractive for the formation of hydrogel. According to Farris et al. (2009), the structure of gelatin enables multiple combinations of molecular interactions.

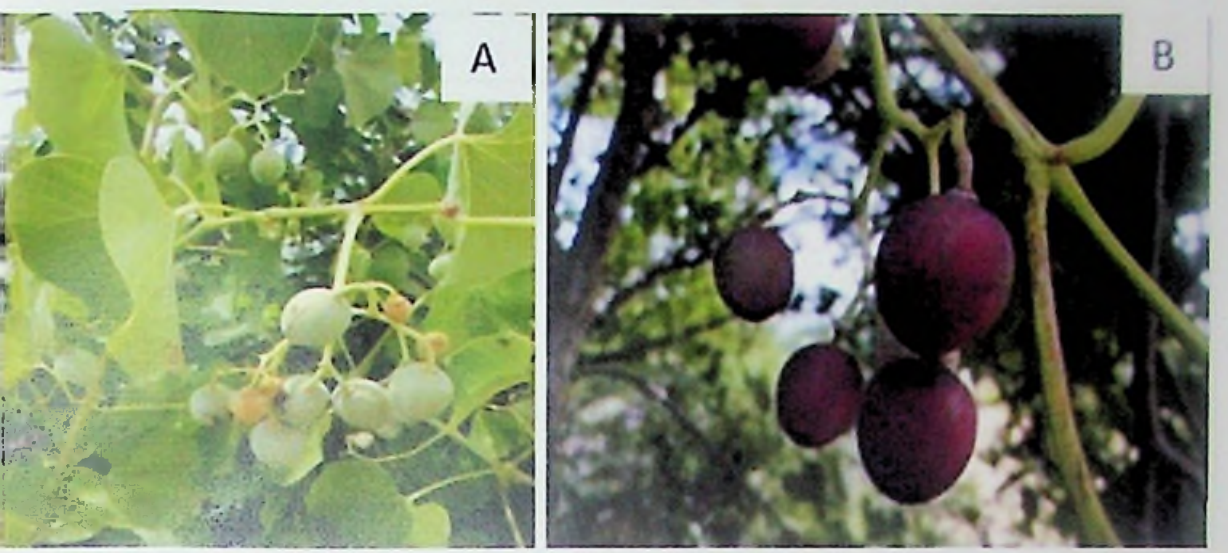
2.3 Theoretical Framework

2.3.1 *Cissus populnea*

From *Amplidaceae (Vitaceae)* family, *Cissus populnea*is a high semi-climbing West African shrub. It is widely found in countries like Senegal, Sudan, Uganda, Ethiopia and Nigeria (Hutchinson and Dalziel, 1958). The Igbo, Igala and Idoma tribes of Nigeria call it 'Okoho'. In Northern Nigeria, the Hausa of Kano and Zaria extraction refer to it as 'Dafaarua'; while it

is called 'latutuwa' among the Hausa in Katsina (Gbile, 1980). The Yoruba of Western Nigeria call it 'Ajara' or 'Orogbolo' (Olaolu Rotimi and Olaolu, 2018).

Agbo *et al.*, (2013) in their study present *Cissus populnea* as an edible wild plant rich in essential nutrients. The fruits as well as the stem bark can be used to prepare soup. It is an essential Ethno-medicinal herb (Olaolu Rotimi and Olaolu, 2018). Its inherent nutritional and phytochemical value has given *Cissus populnea* some extensive attention from the medical field. Iombor *et al.* (2019) however lamented that the plant is a bit under-utilized particularly in the agricultural and beverage fields.



Source: Ouoba (2018)

Plate 1: some section of the shoot system of *Cissus populnea* plant with immature fruits (A) and mature fruits (B)

Table 1: Proximate composition of *Cissus populnea powdered dry stem*

Nutrients	***Cissus populnea* stem (%)**
Moisture content	6.50
Ash content	5.37
Crude fibre	3.30
Crude protein	32.45
Crude fat	1.45
Carbohydrate	43.93

Source: (Adelakun *et al.*, 2012)

2.3.2 Taxonomy of *Clarias gariepinus*

Clarias gariepinus (African mud catfish)is of the clariidae family. It is characteristically aggressive with a high fecundity rate. *Clarias gariepinus* possesses a flexible phenotype with rapid growth, wide habitat preferences and is highly tolerance to extreme water conditions (Wing-Keong, 2019). Its ability to subsist on a wide variety of prey according to Wing-Keong (2019) can devastate indigenous fish and aquatic invertebrate populations.

C. gariepinus introduction into Asia has sparked off the rapid expansion of the hybrid catfish culture in the continent. The hybridized species is produced when the exotic male *C. gariepinus* is crossbred with local female *Clariid species*. Studies reveal that the resultant hybrids possess high growth rates, high resistance to disease (from paternal genes) as well as high flesh quality and taste (from maternal genes).These hybrids have become very popular with fish farmers and have almost completely replaced the native *Clariid* catfish aquaculture in Asia countries particularly Thailand (Poompuang and Na-Nakorn, 2004). This has not only given a great boost to the aquaculture of *Clariid* catfishes in many Asian countries but also positively impacted the livelihoods of many catfish farmers.

2.3.2.1 Taxonomic Tree

Domain: Eukaryota

Kingdom: Metazoa

Phylum: Chordata

Subphylum: Vertebrata

Class: Actinopterygii

Order: Siluriformes

Family: Clariidae

Genus: Clarias

Species: Gariepinus

Description of *Clarias gariepinus*

Clarias gariepinus have cylindrical body with scaleless skin. The head is bony and flattened with small eyes, elongated spineless dorsal fin and four pairs of barbels around a broad mouth (Van, 1995). The upper surface of the head of adult *C. gariepinus* is coarsely granulated while that of the young fish is smooth (Van, 1995). The anal, caudal and dorsal fins are not united. The male *C. gariepinus* has a distinct sexual papilla which is not present in the female. This sexual papilla is located immediately behind the anal opening of the male fish.

The body of *Cgariepinus* is greyish-black in colour while the underside of the head and body is creamy-white in colour (Van, 1995). There is a distinct black longitudinal band on each side of the ventral surface of the head of adult *C. gariepinus.* This is absent in young ones of less than 9 cm long. Larger *C. gariepinus* of more than 9 cm are mottled with an overall grey-khaki colour. Van, (1995) notes that, skin coloration of this species of fish is known to change slightly according to substrate and light intensity in culture systems.



Plate 1: *Clarias gariepinus*

2.3.3 Distribution of *Clarias gariepinus*

Clarias gariepinus is an African mud catfish found in much of the inland waters of Africa. They are today endemic in countries in Asia Minor, like Syria, Southern Turkey and Israel etc. (Van, 1995). Studies in recent years reveal that *C. gariepinus* can now be found in countries in Europe and much of South-East Asia and East Asia countries (Wing-Keong, 2019).

C. gariepinus as earlier noted, is highly tolerance to extreme water conditions. It can therefore be cultivated in areas with a tropical climate, areas with access to geothermal waters or with the use of heated recirculating water systems. It is a hardy fish that can be densely stocked in low oxygen waters. This makes it ideal for culture in areas with a limited water supply. *C. gariepinus* is regarded as the freshwater species with the widest latitudinal range in the world. This is not unconnected with its air-breathing ability, high fecundity, fast growth rate, resistance to disease and high feed conversion efficiency (Wing-Keong, 2019).

CHAPTER THREE

MATERIALS AND METHODS

3.1 The Study Area

This research was carried out at the Department of Aquaculture and Fisheries Management Experimental Farm, Nasarawa State University, Keffi, Shabu-Lafia Campus located within latitudes 8^0 35'N and longitudes 8^0 32' E, altitude 181.53m above sea level with a mean temperature of 34^0C, relative humidity of 40-86% and average daylight of 9-12hrs. (NIMET, 2019).

3.2 Source of Experimental Plants

Powdered *Cissus populnea* was bought from the market and used as binder in the formulation of experimental diets.

3.3 Collection and Acclimatization of Experimental fishes

Three hundred Juvenile *Clarias gariepinus* fish was purchased from a reputable farm in Lafia and was acclimatized for seven (7) days and in five (5) treatments with three (3) replicates for each treatment. On the seventh day of acclimatization the fish was starved for twenty-four (24) hours and the experimental diets were introduced on the eight day, the feed trial began on the eight day.

3.4 Feed Ingredients and Diets Preparation

The feed stuffs were obtained from markets and farms in Lafia and environs, the procured feed stuffs were pulverized into fine powder with the use of the hammer mill. The diet was formulated to provide 40% crude protein. Diets for each *Cissus populnea* treatment were designated as DCP0 – DCP5 (0g, 20g, 30g, 40g, 50g) depending on the level of inclusion of

Cissus populnea. (DCP= Diet *Cissus populnea*). Feed ingredients were weighed according to the percentage feed as fed in the formulation. The feed was prepared and pelleted using the extruding machine, the pellet size suitable for the *Clarias gariepinus* juvenile was achieved using the 2mm pellet die. The feed pellets were sun-dried immediately to constant moisture level required for storage.

Table 2: Composition of Experimental Diet used for the Study

Ingredients	DCP00	DCP20	DCP30	DCP40	DCP50
Fishmeal	12.37	12.37	12.37	12.37	12.37
Maize flour	9.69	9.69	9.69	9.69	9.69
Soya bean	34.27	34.27	34.27	34.27	34.27
Cassava flour	4.98	4.98	4.98	4.98	4.98
Wheat offal	4.98	4.98	4.98	4.98	4.98
Vitamin c	0.39	0.39	0.39	0.39	0.39
Methionine	0.49	0.49	0.49	0.49	0.49
Lysine	0.49	0.49	0.49	0.49	0.49
GNC	32.34	32.34	32.34	32.34	32.34
Total	**100**	**100**	**100**	**100**	**100**
C.	**0**	**20**	**30**	**40**	**50**
***populnea*(g)**					

3.5 Experimental Procedure and Design

The experimental fish were stocked at the rate of twenty juveniles per treatments and three replicates for each treatment. Five treatments were set up. Experimental fish were fed morning and evening (8am-4pm) with the formulated experimental diets for twelve weeks.

3.6 Evaluation of Floatability

The level of buoyancy for each test diets was determined by placing samples containing 10 pellets replicated 3 times in a plastic bowl containing five litres of water each for the period of, between 0-50 minutes and number of pellets afloat was recorded at every 10minutes interval. The mean number of floating pellets at the end was recorded. Timing was done using stop watch. The number of pellets afloat was recorded accordingly.

$$\% \text{ Feed afloat} = \frac{\text{Final number of pellets afloat}}{\text{Initial number of pellets dropped in water}} \text{ X } 100$$

(Misra *et al.*, 2002)

3.7 Evaluation of Water Stability

The water stability was evaluated by putting an exact number of pellets in a plastic containing water and care was taken so as not to alter the integrity of the pellet. Equal number of pellets from each treatment was put in water between an interval of 10 mins to 60 mins after which they were removed and dried to minimum constant weight. The weight was then taken and removed from the initial weight.

$$\%\text{water stability} = \frac{\text{Weight of retained whole pellets}}{\textit{initial total weight of pellets}} \text{ x } 100$$

3.8 Evaluation of Growth Parameters

Growth parameters was assessed in terms of body weight gain (WG), Percentage Weight Gain (PWG), Specific Growth Rate (SGR), Food Conversion Ratio (FCR).

- **Weight Gain (WG)**

$$WG = final\ weight - initial\ weight$$

- **Percentage Weight Gain**

$$PWG = \frac{final\ mean\ weight - initial\ mean\ weight}{initial\ mean\ weight} \times 100$$

- **Specific growth rate (SGR)**

$$SGR = \frac{Ln\ final\ weight - Ln\ initial\ weight}{T} \times 100$$

Ln =Natural log to base e

T= Period of experiment

- **Food conversion ratio (FCR)**

$$FCR = \frac{Total\ feed\ consumed\ (g)}{weight\ gain\ (g)}$$

3.9 Evaluation of Survival Rate

Evaluation of Survival will be done using the formula below;

- **Survival Rate (SR)**

$$SR = \frac{number of fish\ at\ the\ end\ of experiment}{number\ of\ fish\ at\ the\ onset\ of\ the\ experiment} \times 100$$

3.10 Evaluation of Experimental fish Haematology

At the end of the feeding trial, fish from all treatments were taken to lab for haematological analysis. The blood analysis was determined in compliance with the method described by Svobodova *et al.* (1991). They are;

Blood Analysis.

Blood Cell count

Haemoglobin estimation

Packed cell volume

Mean corpuscular volume (MCV)

Mean corpuscular haemoglobin (MCH)

3.11 Data Analysis

The experimental Data were subjected to one-way analysis of variance (ANOVA). Duncan multiple range test was employed in separating the means at ($p<0.05$).

CHAPTER FOUR

RESULTS

4.1 Effects of Processed *Cissus populnea* powdered dry stem on water stability of formulated fish feed

The result of the water stability of processed *Cissus populnea* stem of formulated fish feed is presented in Figure 1. The result shows that after 10 minutes, there was no significant difference ($p>0.05$) between treatments as they all maintained same high water stability. (DCP00 97.416±0.649) and (DCP40 98.531±0.631). At 20 minutes of exposure of the formulated feed to water, the results shows there was significant difference ($p<0.05$) between the treatments with the highest percentage stability recorded in DCP30 (98.160±1.681) and the lowest in DCP20 (95.588±0.028). After 30 minutes of exposing the feed to water there was also significant difference ($p<0.05$) among treatments as the stability of the feed from DCP30 (98.160±1.681) which had the highest score and DCP00 (95.580±1.882) which recorded the lowest score. At 40 minutes there was no significant difference ($p<0.05$) between treatments, as all treatments were statistically around the same percentage stability but DCP40: 94.489±1.075 recorded the highest percentage and DCP00: 92.621±1.689 recorded the lowest. At 50 minutes the statistical analysis showed the treatments had no significant difference ($p>0.05$), though DCP20 90.814±1.642 had the lowest score and DCP50 92.963±0.642 had the highest score.

The results generally show that stability significantly reduce with increase in the duration of time.

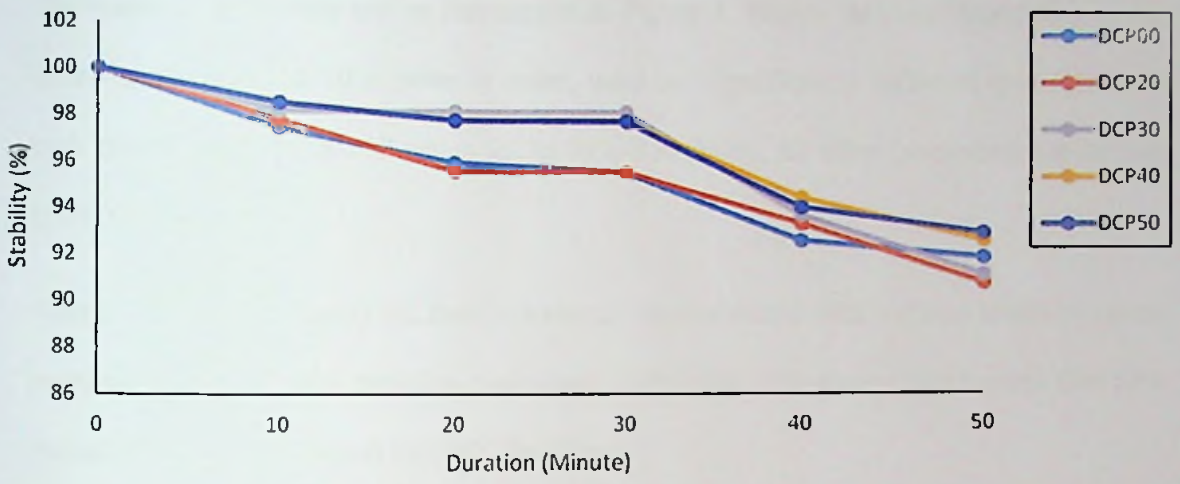


Figure 1: Stability of formulated fish feed with *Cissus populnea* powdered dry stem as binder measured against time.

4.2 Effect of processed *Cissus populnea* powdered stem on Floatability of Formulated Fish feed

The result of floatability test as represented in Figure 2. Shows that the floatability of the different treatments at 10 minutes in water, were not significantly different ($p>0.05$) from each other except for DCP00 which fell to 86% floatability, all other treatments maintained 100% floatability.

After 20 minutes of placing the feed in water all the treatments with various levels of *cissus populnea* powdered stem inclusion maintained 100% floatation except the control diet (0% inclusion of *cissus populnea*) had 66% floatation.

At 30 minutes the treatments showed no significant difference ($p>0.05$) between treatments,DCP30, DCP40 and DCP50 maintained 100% floatation while DCP00 was at 66% floatability and DCP20 dropped to 96.67% floatability.

After 40 minutes of the feed in water DCP00 dropped to 57% floatabiity and DCP20 maintained 96.67% floatability, showing a significant difference ($p<0.05$) from DCP30, DCP40 and DCP50 which maintained 100% floatability.

At 50 minutes in water the feed showed significant difference ($p<0.05$) amongst treatments with only DCP30 and DCP50 maintaining 100% floatability while DCP00 had dropped to 46% floatabilty, DCP20 and DCP40 were at 96.67% floatability.

At 60 minutes there was significant difference ($p<0.05$) DCP50 maintained 100% floatability, DCP40, DCP30 and DCP20 were at 96.67% floatability but DCP00 dropped to 42.33% floatability.

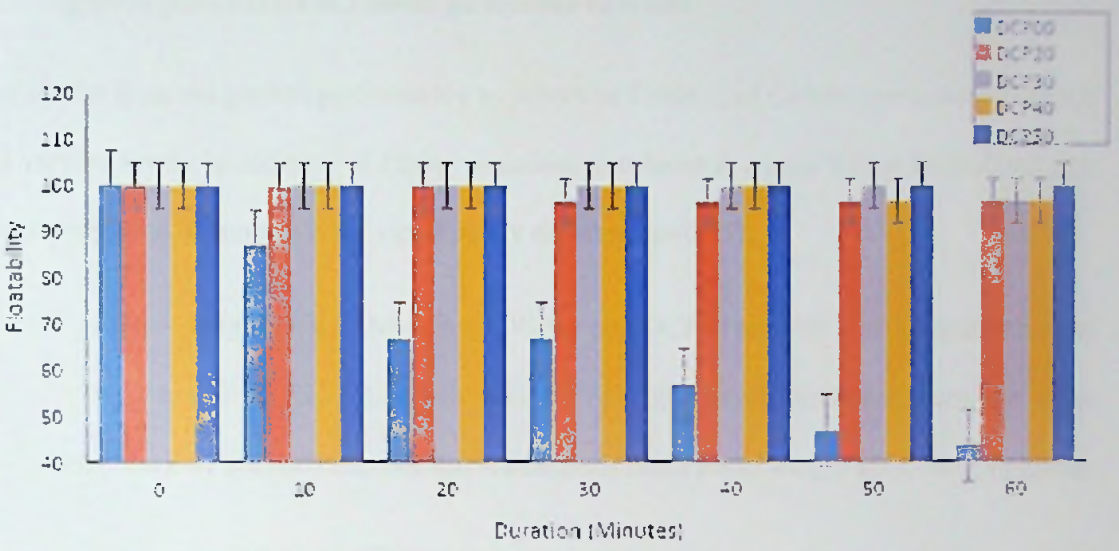


Figure 2: The effect of processed *Cissus populnea* on Floatability of Formulated Fish feed

4.3 Evaluation of *Cissus populnea* powdered dry stem as binder on survival and growth parameters in *Clarias gariepinus* Juvenile

The results from the growth performance as shown in Table 3; of *Clarias gariepinus* juvenile fed varying levels in addition of *Cissus populnea* powdered dry stem to the formulated fish diet showed that treatments were significantly different ($p<0.05$).

Percentage mean weight gain (%MWG) of DCP40 and DCP50 recorded the highest values of 2086.7±38.6g and 2017±32.3g, which was highly significantly different from the other treatments; however DCP00 recorded the lowest percentage mean weight gain 1809.3±29.7g.

Specific growth rate (SGR) also follow the same pattern as the percentage weight gain, with DCP40 and DCP50 having the highest specific growth rate of 4.00±0.02 and 3.96±0.02 respectively which was significantly higher than all other treatments. However DCP00 still had the lowest specific growth rate of 3.83±0.02.

Results show that the survival was significantly good for all the treatments, DCP00 96.67% survival rate which is the highest survival rate while DCP40 recorded the lowest survival rate with 88.33% survival.

Feed conversion ratio(FCR) results showed no significant difference ($p>0.05$) between DCP00, DCP20 and DCP30 which had higher value of feed conversion ratios, but was significantly different from DCP40 and DCP50 which had the lowest value of feed conversion

Feed efficiency ratio (FER) followed the same trend as FCR with DCP00, DCP20 and DCP30 significantly different ($p<0.05$) from DCP40 and DCP50. DCP50 recorded the highest value 82.31±0.38and DCP30 has the least value 79.43±0.79.

Protein efficiency ratio (PER) recorded the highest value for DCP50 which was not significantly different ($p>0.05$) from DCP40, however they were both significantly different from DCP00, DCP20 and DCP30. DCP30 recorded the lowest value 0.0198 ± 0.00.

4.4 Growth Pattern

The results for the growth pattern as represented in Figure 3. Shows *Clarias gariepinus* juveniles fed with formulated fish feed containing varying levels of *cissus populnea* powdered dry stem in the different treatments were significantly different ($p<0.05$).

Table 3: Evaluation of *Cissus populnea* mucilage binder on survival and growth parameters in *Clarias gariepinus* Juvenile.

Growth Parameters	DCP00	DCP20	DCP30	DCP40	DCP50
Final weight(g)	89.59 ± 1.52^{b}	89.83 ± 0.98^{b}	93.26 ± 0.93^{b}	103.83 ± 1.95^{a}	99.81 ± 1.55^{a}
Initial weight(g)	4.69 ± 0.15^{a}	4.59 ± 0.14^{a}	4.68 ± 0.07^{a}	4.74 ± 0.01^{a}	4.71 ± 0.03^{a}
Weight gain(g)	84.89 ± 1.51^{b}	85.24 ± 0.98^{b}	88.57 ± 0.93^{b}	99.08 ± 1.94^{a}	95.09 ± 1.55^{a}
Mean weight gain (g)	18.09 ± 0.30^{b}	18.57 ± 0.21^{b}	18.91 ± 0.19^{b}	20.87 ± 0.39^{a}	20.17 ± 0.32^{a}
(%) Mean weight gain	1809.3 ± 29.7^{b}	1857.4 ± 21.1^{b}	1891.5 ± 19.8^{b}	2086.7 ± 38.6^{a}	2017 ± 32.3^{a}
Specific growth rate	3.83 ± 0.02^{b}	3.86 ± 0.01^{b}	3.88 ± 0.01^{b}	4.00 ± 0.02^{a}	3.96 ± 0.02^{a}
Total feed intake (g)	106.19 ± 1.48^{c}	106.89 ± 0.87^{c}	111.51 ± 1.31^{bc}	120.88 ± 2.63^{a}	115.55 ± 2.54^{ab}
Feed conversion ratio	1.25 ± 0.006^{a}	1.25 ± 0.005^{a}	1.26 ± 0.013^{a}	1.22 ± 0.003^{b}	1.22 ± 0.006^{b}
Feed efficiency ratio	79.94 ± 0.39^{b}	79.74 ± 0.31^{b}	79.43 ± 0.79^{b}	81.97 ± 0.16^{a}	82.31 ± 0.38^{a}
Protein in feed	40.00 ± 0.00^{c}	40.10 ± 0.00^{d}	40.10 ± 0.00^{c}	40.10 ± 0.00^{b}	40.20 ± 0.00^{a}
Protein intake	4247.5 ± 59.2^{c}	4289.2 ± 34.9^{c}	4471.5 ± 52.5^{bc}	4847.5 ± 105^{a}	4645 ± 102^{ab}
(%) Survival rate	96.67 ± 1.67^{a}	95.00 ± 0.00^{a}	93.33 ± 1.67^{a}	88.33 ± 1.67^{b}	93.33 ± 1.67^{a}
Stocking density	*60*	*60*	*60*	*60*	*60*
Culture period(days)	*77*	*77*	*77*	*77*	*77*

Means that do not share the same letter are significantly different

DCP00 = Diet *Cissus populnea* at 0.00g inclusion, DCP20 = Diet *Cissus populnea* at 20g inclusion, DCP30 = Diet *Cissus populnea* at 30g inclusion, DCP40 = Diet *Cissus populnea* at 40g inclusion, DCP50 = Diet *Cissus populnea* at 50g inclusion

+

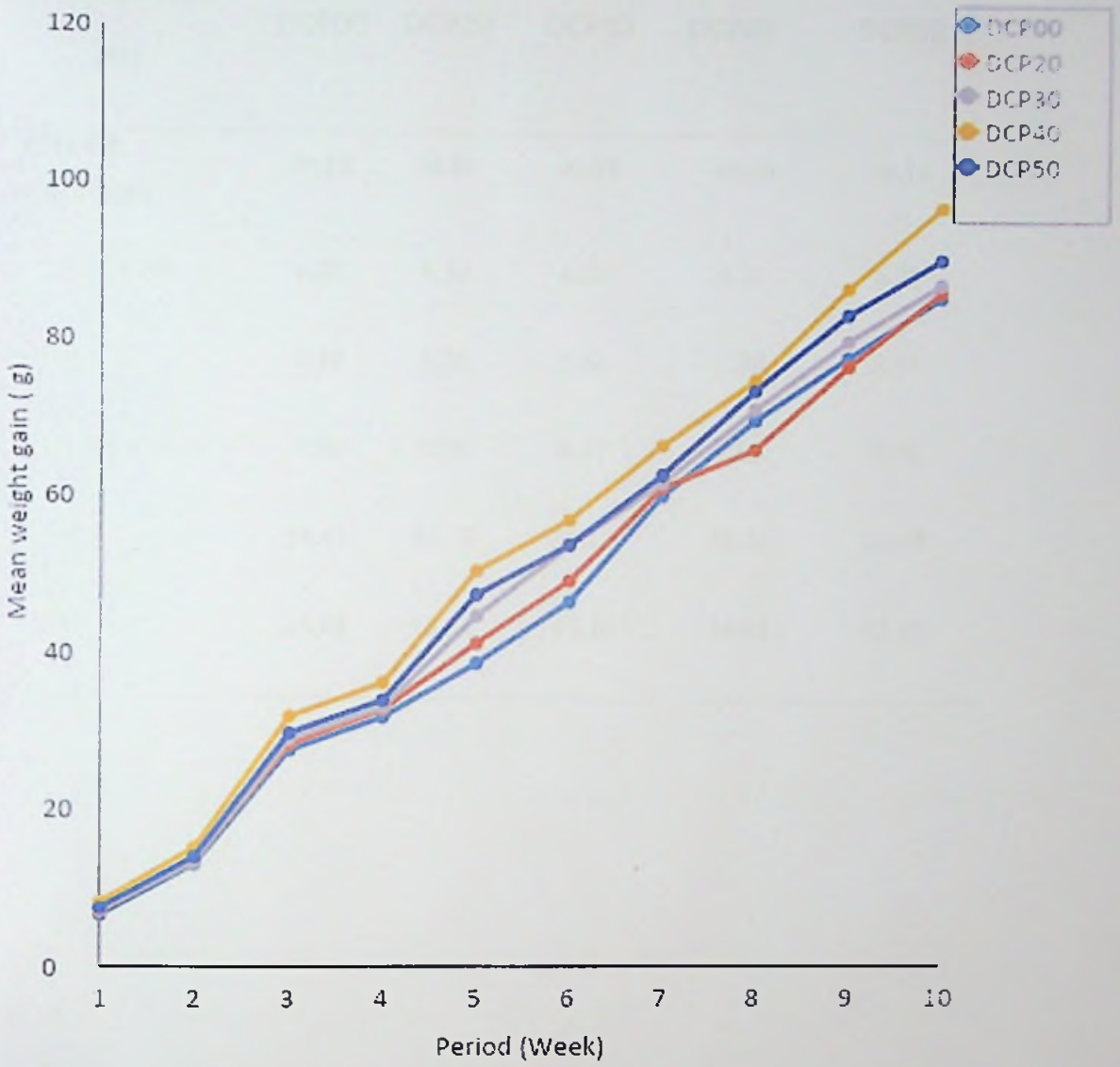


Figure 3: Growth Pattern of juvenile *Clarias gariepinus* fed feed with varying levels of *Cissus populnea* powdered dry stem in the formulated fish diet.

Table 4: Proximate Analysis of experimental diets used for the study.

PARAMETER (%)	DCP00	DCP20	DCP30	DCP40	DCP50
CRUDE PROTEIN	39.11	40.00	40.09	40.10	40.16
MOISTURE	6.25	6.30	6.28	6.21	6.29
ASH	3.59	3.25	3.46	3.30	3.55
CRUDE FIBER	0.96	0.98	0.97	1.05	0.96
LIPID	34.61	33.18	36.10	35.33	36.42
NFE	15.48	16.31	13.10	14.01	12.62

4.5 Evaluation of the effect of *Cissus populnea* on the haematology of experimental fish.

The result of the haematology is shown in Table 5: the Haemoglobin content (Hb) was significantly different ($p<0.05$) among treatments with DCP00 recording the lowest 5.53 ± 0.83 and DCP20 recording the highest value 8.93 ± 0.15. Packed cell volume (PCV) were not significantly different ($p>0.05$) from each other. White blood cells (WBC), the result showed that DCP50 recorded the highest value 95.13 ± 0.85 and this was significantly different from the result of all other treatments. All the other treatments showed no significant difference from each other. Red blood cells (RBC) results showed significant difference between treatments DCP20 had the highest number of red blood cells 2.90 ± 0.10 and DCP00 recorded the lowest number 2.00 ± 0.10. DCP30, DCP40 and DCP50 were significantly different from the other treatments.

Mean corpuscular haemoglobin concentration (MCHC) results showed significant difference among treatments with DCP00 recording the lowest value 27.33 ± 4.74 and DCP20 recorded the highest value 41.11 ± 7.60

Mean corpuscular volume (MCV) was significantly different from each other. DCP40 was highest with 112.74 ± 6.30 and DCP20 was lowest with 77.03 ± 16.92

Mean corpuscular haemoglobin (MCH) showed significant difference among the treatments DCP40 was highest with 35.47 ± 3.97 and DCP00 was lowest with 27.60 ± 3.05

Platelets show high significant difference among treatments DCP30 had the highest number 7.00 ± 0.20 and DCP20 was lowest with 5.00 ± 0.10

Table 5: Evaluation of the effect of *Cissus populnea* on the haematology of experimental fish.

PARAMETER	DCP00	DCP20	DCP30	DCP40	DCP50
Hb(g dL^{-1})	5.52±0.83^{c}	8.93±0.15^{a}	8.20±0.26^{ab}	7.53±0.45^{b}	7.37±0.51^{b}
PCV(%)	20.33±1.52^{a}	22.33±4.93^{a}	24.33±0.58^{a}	24.00±1.00^{a}	21.00±1.00^{a}
PLT(x$10^{9}L^{-1}$)	5.10±0.20^{c}	5.00±0.10^{c}	7.00±0.20^{a}	6.00±0.10^{b}	4.23±0.21^{d}
RBC(x10^{12} L^{-1})	2.00±0.10^{c}	2.90±0.10^{a}	2.60±0.20^{b}	2.13±0.15^{c}	2.10±0.20^{c}
WBC(x$10^{9}L^{-1}$)	85.87±1.76^{b}	87.27±0.75^{b}	86.50±0.79^{b}	86.33±0.83^{b}	95.13±0.85^{a}
MCV(f L)	102.01±11.93^{a}	77.03±16.9^{b}	93.96±7.39^{ab}	112.74±6.30^{a}	100.47±8.71^{a}
MCH(Pg)	27.60±3.05^{b}	30.83±1.3^{ab}	31.62±1.54^{ab}	35.47±3.97^{a}	35.26±3.73^{a}
MCHC(g dL^{-1})	27.33±4.74^{c}	41.11±7.60^{a}	33.72±1.54^{abc}	31.40±1.73^{bc}	35.06±0.92^{ab}

Means that do not share the same superscript are significantly different from each other.

Table 6: Experimental water source quality parameters

Water parameter	Treatments				
	DCP00	**DCP20**	**DCP30**	**DCP40**	**DCP50**
Temperature(^{0}C)	**27.43**±0.04	**27.43**±0.04	**27.43**±0.04	**27.43**±0.04	**27.43**±0.04
pH	**7.45**±0.02	**7.45**±0.02	**7.45**±0.02	**7.45**±0.02	**7.45**±0.02
Total alkalinity(mg-1)	**15.21**±0.03	**15.21**±0.03	**15.21**±0.03	**15.21**±0.03	**15.21**±0.03
Dissolved oxygen(mg-1)	**8.20**±0.03	**8.20**±0.03	**8.20**±0.03	**8.20**±0.03	**8.20**±0.03
Carbon dioxide(mg-1)	**4.20**±0.01	**4.20**±0.01	**4.20**±0.01	**4.20**±0.01	**4.20**±0.01

CHAPTER FIVE

DISCUSSION

5.1 Effect of *Cissus populnea* mucilage on growth parameters

The results of Percentage Mean Weight Gain (%MWG) from the anova on growth performance showed that DCP40 and DCP50 recording the highest values in their percentage mean weight gain (%MWG) agree with that reported by Idowu and Afolayan (2013) which stated that the percentage weight gain increases with increase in protein levels of the diets, this therefore means that when the binder is effective protein that gets to the fish increases its weight gain.

Specific growth rate (SGR) also follow the same pattern as the percentage weight gain, with DCP40 and DCP50 having the highest specific growth rate can also be attributed to higher protein inclusion in the diets. This result corroborates with the report of Olaniyi (2009) whose study reveals that the higher the SGR and the smaller the FCR values, the better the feed quality. Adikwu (2003) also reported that a lower FCR value implies efficient feed utilization by fish. DCP40 and DCP50 with the highest protein inclusion had the best conversion of feed

Survival rate (SR) of this studies results shows that the survival ranged between 96.67% and 88.33% survival, it shows an improvement to that which was reported by Idowu and Afolayan (2013) on supplementing of fish feed with maggot meals at varying levels.

Feed efficiency ratio (FER) was higher for the diets with higher inclusion of *Cissus populnea*. This will likely be as a result of the binding effect that enables the formulated nutrients get to the fish.

These results showed that the *Cissus populnea* mucilage binder in treatments enhances nutrient utilization which is reflected in improved weight gain, feed conversion ratio, protein efficiency ratio, feed conversion efficiency and specific growth rate. Growth increased as level of inclusion increased up to DCP40 and thereafter decreased, this is similar to results obtained for dietary inclusions of ethanolic extract of *G. kola* seed in *C. gariepinus* brood stocks by Dada and Ikuenerowo (2009) in which the authors found that the weight gain increased as amount of extract increased up to 1.0 g/kg and decreased thereafter.

5.2 Effect of *Cissus populnea* powdered dry stem on stability of feed in water

The stability of fish feed in water can be achieved by triggering the gelatinizing activity of starch especially those of plant origin. However, if stability in water is insufficient the inclusion of a binding agent during manufacturing is necessary to reduce dry matter loss not to exceed 10% after 60 mins of immersion as stated by Cuzon *et al.* (1994). All experimental diets followed the trend to be termed highly water stable as they all achieved more than 90% water stability.

5.3 Effect of *Cissus populnea* mucilage on water floatation of feed

The feed was observed to have maintained a floatation period of over 60 minutes in agreement with Falayi and Sadiku (2013) who reported a high floatation period of 75 minutes in feed formulated with yeast as floating agent.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study revealed that the floatability of fish feeds formulated using 50g/100kg *Cissus populnea* as binding agent was best among other treatments as it remained afloat for over 50 minutes.

The stability in water of fish feeds formulated using 50g/100kg *Cissus populnea* as binding agent presented the best result.

The feed treatment having 40g/100kg of *Cissus populnea* as organic binder was best among the treatments, as it performed best on growth and haematology of the *Clarias gariepinus* juveniles.

The survival rate of *Clarias gariepinus* fed the experimental diets were excellent as the feed showed no adverse effect on the fish.

6.2 Recommendation

Cissus populnea holds great potential for the aquaculture industry in feed preparation. The ingredient as a binding agent could be used efficiently to produce floating feeds without any adverse effect on the stability of the feed in water. The use of *Cissus populnea* is recommended for the aquaculture feed industry as shown by results from the study indicating high feed conversion ratio, as well as high fish survival rates.

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APPENDIX

EVALUATION OF *Cissus populnea* MUCILAGE BINDER ON SURVIVAL AND GROWTH PARAMETERS IN *Clarias gariepinus* JUVENILE

One-way ANOVA: inwg versus trt

Source	DF	SS	MS	F	P
trt	4	0.0424541	0.0106135	110.40	0.000
Error	10	0.0009613	0.0000961		
Total	14	0.0434154			

One-way ANOVA: Fwg versus trt

Source	DF	SS	MS	F	P
trt	4	479.77	119.94	18.05	0.000
Error	10	66.44	6.64		
Total	14	546.21			

One-way ANOVA: WG versus trt

Source	DF	SS	MS	F	P
trt	4	472.83	118.21	17.87	0.000
Error	10	66.16	6.62		
Total	14	538.99			

One-way ANOVA: MWG versus trt

Source	DF	SS	MS	F	P
trt	4	16.022	4.006	14.57	0.000
Error	10	2.750	0.275		
Total	14	18.772			

One-way ANOVA: PWG versus trt

Source	DF	SS	MS	F	P
trt	4	160224	40056	14.57	0.000
Error	10	27499	2750		
Total	14	187723			

One-way ANOVA: SGR versus trt

Source	DF	SS	MS	F	P
trt	4	0.06429	0.01607	14.94	0.000
Error	10	0.01076	0.00108		
Total	14	0.07505			

One-way ANOVA: TOTAL FEED versus trt

Source	DF	SS	MS	F	P
trt	4	454.4	113.6	10.22	0.001
Error	10	111.1	11.1		
Total	14	565.5			

One-way ANOVA: FCR versus trt

Source	DF	SS	MS	F	P
trt	4	0.0051308	0.0012827	26.36	0.000
Error	10	0.0004865	0.0000487		
Total	14	0.0056174			

One-way ANOVA: FER versus trt

Source	DF	SS	MS	F	P
trt	4	21.998	5.499	26.19	0.000
Error	10	2.100	0.210		
Total	14	24.098			

One-way ANOVA: SUR Rate versus trt

Source	DF	SS	MS	F	P
trt	4	116.67	29.17	4.38	0.027
Error	10	66.67	6.67		
Total	14	183.33			

One-way ANOVA: P IN FEED versus trt

Source	DF	SS	MS	F	P
trt	4	0.0600000	0.0150000	*	*
Error	10	0.0000000	0.0000000		
Total	14	0.0600000			

One-way ANOVA: P INTAKE versus trt

Source	DF	SS	MS	F	P
trt	4	756036	189009	10.57	0.001
Error	10	178871	17887		
Total	14	934907			

One-way ANOVA: PER versus trt

Source	DF	SS	MS	F	P
trt	4	0.0000012	0.0000003	23.03	0.000
Error	10	0.0000001	0.0000000		
Total	14	0.0000013			

Effect of processed *Cissus populnea* on floatability of formulated fish feed

One-way ANOVA: 0Minutes versus trt

Source	DF	SS	MS	F	P
trt	4	0.0000000	0.0000000	*	*
Error	10	0.0000000	0.0000000		
Total	14	0.0000000			

One-way ANOVA: 10Minutes versus trt

Source	DF	SS	MS	F	P
trt	4	0.0000000	0.0000000	*	*
Error	10	0.0000000	0.0000000		
Total	14	0.0000000			

One-way ANOVA: 20Minutes versus trt

Source	DF	SS	MS	F	P
trt	4	26.67	6.67	1.00	0.452
Error	10	66.67	6.67		
Total	14	93.33			

One-way ANOVA: 30Minutes versus trt

Source	DF	SS	MS	F	P
trt	4	40.0	10.0	0.75	0.580
Error	10	133.3	13.3		
Total	14	173.3			

One-way ANOVA: 40Minutes versus trt

Source	DF	SS	MS	F	P
trt	4	40.0	10.0	0.75	0.580
Error	10	133.3	13.3		
Total	14	173.3			

One-way ANOVA: 50Minutes versus trt

Source	DF	SS	MS	F	P
trt	4	40.0	10.0	0.50	0.737
Error	10	200.0	20.0		
Total	14	240.0			

One-way ANOVA: 60Minutes versus trt

Source	DF	SS	MS	F	P
trt	4	93.3	23.3	1.17	0.382
Error	10	200.0	20.0		
Total	14	293.3			

Effect of processed *Cissus populnea* on water stability of formulated fish feed

One-way ANOVA: g/0minutes versus trt

Source	DF	SS	MS	F	P
trt	4	0.00933	0.00233	0.88	0.512
Error	10	0.02667	0.00267		
Total	14	0.03600			

One-way ANOVA: g/10Minutes versus trt

Source	DF	SS	MS	F	P
trt	4	2.781	0.695	1.21	0.367
Error	10	5.763	0.576		
Total	14	8.543			

One-way ANOVA: g/20Minutes versus trt

Source	DF	SS	MS	F	P
trt	4	16.99	4.25	2.30	0.130
Error	10	18.45	1.84		
Total	14	35.44			

One-way ANOVA: g/30Minutes versus trt

Source	DF	SS	MS	F	P
trt	4	19.77	4.94	3.25	0.059
Error	10	15.21	1.52		
Total	14	34.98			

One-way ANOVA: g/40Minutes versus trt

Source	DF	SS	MS	F	P
trt	4	6.06	1.51	0.77	0.568
Error	10	19.63	1.96		
Total	14	25.69			

One-way ANOVA: g/50Minutes versus trt

Source	DF	SS	MS	F	P
trt	4	10.37	2.59	0.95	0.474
Error	10	27.21	2.72		
Total	14	37.58			