Crocodile (*Crocodylus niloticus*) meal diets as a potential for replacement of fishmeal protein in commercial production of Mozambique tilapia (*Oreochromis mossambicus*)

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Abstract

Fishmeal production is mainly sourced from the forage fish species. Fish caught for fishmeal production potentially represents a loss in producing higher trophic level species in the ecosystem. Low stock abundance reduces ecosystem services such as food provisioning to other elements of the ecosystem. Increasing demand, unstable supply, and the high price of the fishmeal with the expansion of aquaculture made it necessary to search for alternative protein sources. Crocodiles are farmed mainly for producing skins used in the production of high-quality fashion accessories. However, the demand for crocodile meat, especially in South Africa, is very low and strict regulations are imposed onto the industry about the use and disposal of crocodile carcasses. This study was conducted to assess the nutritional value of crocodile meals and their suitability as a fishmeal replacement in animal feeds, especially fish. Systematic review and meta-analysis results showed the gap that some animal by-products, including crocodile meat, had not been assessed as protein sources in aquaculture or animal feeds. Different size groups of fish are not considered in studies when testing different alternatives for fishmeals. The nutritional values of *Crocodylus niloticus* derived meal obtained in the current study is of comparable quality for use in aquaculture feeds, compared to by-products meal quality reported for meal derived from bovine bones and meat, feathers, blood and other poultry by-products. There were similarities in the gross feed conversion ratio for fry and the specific growth rate for fingerlings of *Oreochromis mossambicus* among all the experimental diets fed. That means the *Crocodylus niloticus* meal is a suitable animal protein source for replacing fishmeal in *Oreochromis mossambicus* diets. Some haematological parameters such as red blood cells count, and haemoglobin concentrations were significantly different among *Oreochromis mossambicus* fed crocodile-based and commercial diets. However, platelets count, haematocrit value, mean cell volume, mean cell haemoglobin, and mean cell haemoglobin concentrations were not significantly different among all diets fed. More future studies are recommended for different levels of *Crocodylus niloticus* meal in other fish species, different size groups, and haematological parameters. This study provides new information to the aquaculture industry regarding reducing supply constraints imposed by high cost and competitive uses for fishmeal and waste management on crocodile farms.

Preface

The research contained in this dissertation was completed by the candidate while based in the Discipline of Ecological Science, School of Life Sciences of the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, Pietermaritzburg campus, South Africa between 2017 and 2021, under the supervision of Professor Samson Mukaratirwa and Dr Gordon O'Brien. The research was financially supported by Agribusiness Development Agency and the College of Agriculture, Engineering and Science, University of KwaZulu-Natal.

The contents of this work have not been submitted in any form to another university and, except where the work of others is acknowledged in the text, the results reported are due to investigations by the candidate.

Candidate signature: [redacted] Date: February 2022

I certify that the above statement is correct.

Supervisor: Professor Samson Mukaratirwa

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As the candidate's supervisor I have approved this thesis for submission.

Co-supervisor: Dr Gordon O'Brien

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As the candidate's Co-supervisor I have approved this thesis for submission.

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Declaration 2-Publications

Details of contribution to publications that form part and/or include research presented in this thesis.

Authors contributions for publications 1-4: R.L.R.; S.M., and G.O.B, conceived the papers. R.L.-R. collected data, analysed data, and wrote the papers. S.M. and G.O.B, reviewed the manuscripts and provide valuable comments.

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Publication 3 (Chapter 4)

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Conference Presentation

Luthada-Raswiswi R.W*, Mukaratirwa S and, O'Brien G. Can crocodile meal be used in poverty alleviation? ECOHEALTH INTERNATIONAL: Towards the establishment of an African chapter. Durban ICC, 13-14 November 2018. (**Poster**).

Signature: 

Signed: Rendani Winnie Luthada-Raswiswi

February 2022

DEDICATION:

This thesis is dedicated to Ndisalelani Raswiswi and Pfarelo Raswiswi for their endless love and patience throughout my studies. Mrs. Eunice Luthada and Mr. Mackson Luthada, for all the sacrifices they made for me and my siblings. Mr. Thomas Luthada, for always being interested in my schoolwork. I know you will read from the cover to the last page.

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LIST OF ABBREVIATIONS

ADA	Agribusiness Development Agency
ANOVA	Analysis of Variance
AREC	Animal Research Ethics Committee
AOAC	Association of official Analytical Chemists
CI	Confidence interval
CM	Crocodile meal
V	Constant
Df	Degrees of freedom
℃	Degree Celsius
EC	Eastern Central
esV	Effect summary
EDTA	Ethylenediaminetetraacetic acid
FCR	Feed conversion ratio
Fl	Femtolitres
FM	Fishmeal
FW	Final weight
g	Grams
g/Dl	Grams per deciliter
GFCR	Gross feed conversion ratio
Ht	Haematocrit
Hb	Haemoglobin
I^2	Heterogeneity index
Q	Heterogeneity test
h	Hour

IW	Initial weight
IFFO	International Fishmeal and Fish Oil Organization
L	Liters
LT	Low temperature
MCHC	Mean cell haemoglobin concentration
MCV	Mean cell volume
MCH	Mean corpuscular haemoglobin
Mt	Metric tonnes
ml	Milliliters
N	Nitrogen
NSM	Norsea mink
NE	Northeast
NW	Northwest
NR	Not recorded
H_0	Null hypothesis
n	Number
Es	Outcome
%	Percent
PLT	Platelets
P	Probability
PCD	Purchased commercial diets
PUFA	Polyunsaturated fatty acids
R	Rand
RBC	Red blood cells

NaoH	Sodium hydroxide
SA	South Africa
SE	Southeast
SGR	Specific growth rate
ST	Standard
SD	Standard deviation
SE	Standard error
F	Statistic
W	Study weight
H_2SO_4	Sulfuric acid
SR	Survival rate
FAO	The Food and Agriculture Organization of the United Nations
U.S. A	United State of America
Var	Variance
G	Weight gain
W*es	Weighted effect size
W	Western
WC	Western Central
WBC	White blood cell

CHAPTER ONE

Thesis introduction

1.1. Introduction

Fishmeal is a dry powdered material produced from species of pelagic fish that are captured primarily for producing fishmeal and fish oil (Hardy and Tacon 2002). According to Miles and Champman (2006) and Khan et al. (2012), fishmeal is a common word for a feed ingredient that is rich in nutrients and used primarily in diets for domestic animals, sometimes used as a high-quality organic.

High-quality protein content, well-balanced essential amino acids profile, high essential fatty acids content, minerals, vitamins, good digestibility, and high palatability are characteristics that make fishmeal an excellent protein source in animal feeds (Kritsanapuntu and Chaitanawisuti, 2015). Furthermore, fishmeal offers major benefits to animal health, including improved immunity against diseases, higher survival rates, growth, and reduced incidences of deformities (FAO, 1986). As a result, aquaculture has been utilizing most of the fishmeal produced globally (IFFO, 2011; Banchis, 2018).

There are three significant fishmeal sources: i., fish stocks explicitly harvested for fishmeal production purposes, ii. By-catches from other fisheries. iii. trimmings and offal leftover from fish processed for human consumption (unpalatable or fast spoiling) (Miles and Chapman, 2006). However, fish stocks species differs among countries (Table 1.1), and although they are destined for fishmeal and fish oil production, most of them were harvested to an optimal yield level, with no room for further expansion in 2002 (Table 1.2).

According to Rahman et al. (2016), raw materials of fishmeal are processed by heating, pressing, separation, evaporation and drying. Heating condenses the protein, breaks fat deposits, and releases oil and water. Pressing improves the meal quality and decreases the moisture content of the press cake as much as possible. The drying process removes sufficient water from the wet and unstable mixture of press cake to form stable fishmeal.

The nutritional value of fishmeal varies, mainly depending on the species of fish or sources of input, place of harvest and the addition of salt for preservation (Khan et al., 2012). According to Sheng et al. (2017), the variations are mainly embodied in the sensory indexes

such as colour, smell, and nutritional indices such as crude protein, crude fat, crude ash, calcium, phosphorus and acid values, volatile base nitrogen, lysine, and methionine, which have an impact on fishmeal quality. Tankikitti et al. (2016) reported that the quality of fishmeal depends mainly on the quality of raw materials and the processing method used in production. Fishmeal is divided into grades (Table 1.3) depending on the quality defined by the following criteria: percentage of protein, odour, Total Volatile Basic Nitrogen, and percentage of humidity (Achavanuntakul et al., 2014).

Fishmeal production is mainly sourced from the forage fish species (Alder et al., 2008). Forage fish are threatened and stressed by many factors, including climate change, ocean acidification, habitat loss, fishing pressure, pollution, and increased demand for forage fish-based feed for aquaculture and agriculture (Enticknap et al., 2011). Survival, growth, reproduction, and distribution of forage fish directly affect fluctuating fishmeal production (Figure 1.1).

Pig and poultry production has traditionally used fishmeal since 1960. The growth of the aquaculture industry has increased fishmeal demand, and since 1980, aquaculture has been consuming more fishmeal than other industries (Figure 1.2).

Table 1.1. Fish species used in production of fishmeal in different countries (Miles and Champman, 2006; Cashion et al. 2017; Zhao et al. 2021).

Country of production	**Main fish species used in fishmeal production**	**Scientific names**
Chile	Pacific sardine	*Sardinops sagax*
	Anchovy	*Engraulidae*
China	Anchovy	*Engraulidae*
	Sardine	*Sardinella aurita*
	Herring	*Tenualosa toli*
Denmark	Pout	*Trisopterus esmarkii*
	Sandeel	*Ammodytes*
	Sprat	*Clupea sprattus*
European union	Blue whiting	*Micromesistius poutassou*
	Herring	*Clupea harengus*
	Sandeel	*Ammodytes*
	Sprat	*Sprattus sprattus*
Iceland	Capelin	*Mallotus villosus*
	Herrings	*Clupea harengus*
	Blue whiting	*Micromesistius poutassou*
Japan	Sardine/Pilchard	*Sardina pilchardus*
Norway	Capelin	*Mallotus villosus*
	Herrings	*Clupea harengus*
	Blue whiting	*Micromesistius poutassou*
Peru	Peruvian Anchoveta	*Engraulis ringens*
South Africa	Pilchard	*Sardina pilchardus*
Thailand	Atlantic menhaden	*Brevoortia tyrannus*
U.S.A.	Menhaden	*Brevoortia tyrannus*
	Pollack	*Gadus chalcogrammus*

Table 1.2: Stock status for fish destined for fishmeal and fish oil production in 2002 (Alder et al., 2008).

Target stock	**Scientific names**	**Food and Agriculture Organization area**	**State of exploitation in 2002**
Atlantic menhaden	*Brevoortia tyrannus*	NW Atlantic FAO21	Fully exploited
		WC Atlantic FAO31	Fully exploited
Gulf menhaden	*Brevoortia patronus*	WC Atlantic FAO31	Fully exploited
Atlantic mackerel	*Scomber scombrus*	NE Atlantic FAO27	Fully exploited
Blue whiting	*Micromesistius poutassou*	NE Atlantic FAO27	Overexploited
Norway pout	*Trisopterus esmarkii*	NE Atlantic FAO27	Fully exploited
Sand eels/sand lances	*Ammodytidae*	NE Atlantic FAO27	Fully exploited
Atlantic herring	*Clupea harengus*	W Atlantic FAO21	Underexploited, fully exploited, recovering
		NE Atlantic FAO27	Fully exploited
European sprat	*Sprattus sprattus*	NE Atlantic FAO27	Fully exploited
		Mediterranean and Black Sea FAO37	Depleted
Capelin	*Mallotus villosus*	NE Atlantic FAO27	Fully exploited
Chub mackerel	*Scomber japonicus*	EC Atlantic FAO34	Fully exploited
South African anchovy	*Engraulis capensis*	SE Atlantic FAO47	Fully exploited
Horse mackerel	*Trachurus trachurus*	SE Atlantic FAO47	Moderately exploited, fully exploited
Pilchard	*Sardina pilchardus*	SE Atlantic FAO47	Fully exploited
Pacific herring	*Clupea pallasii*	NW Pacific FAO61	Unknown
Pacific saury	*Cololabis saira*	NW Pacific FAO61	Fully exploited
Japanese sardine (anchovy)	*Engaulis japonicus*	NW Pacific FAO61	Fully exploited
Peruvian anchoveta	*Engraulis ringens*	SE Pacific FAO87	Recovering, overexploited
South American pilchard	*Sardinops sagax*	SE Pacific FAO87	Fully exploited, overexploited
Chilean jack mackerel	*Trachus murphyi*	SE Pacific FAO87	Fully exploited, overexploited
Hake	*Merluccius capensis*	SE Pacific FAO87	Fully exploited, overexploited, depleted

NW-Northeast; WE-Western Central; NE-Northeast; W-Western; EC-Eastern Central; SE-Southeast

Table 1.3: Fishmeal grades according to quality defined by Achavanuntakul et al. (2014). 1st grade = Low Temperature (LT), 2nd grade = Norsea Mink (NSM), and 3rd grade = Standard (ST).

Graded	**1st grade**	**2nd grade**	**3rd grade**
Protein (Not more than)	60 percent (%)	55%	50%
Ash (Not more than)	26%	28%	30%
Salt (Not more than)	3%	3%	3%
Humidity (Not more than)	10%	10%	10%
Remaining (Not less than)	2%	2%	2%

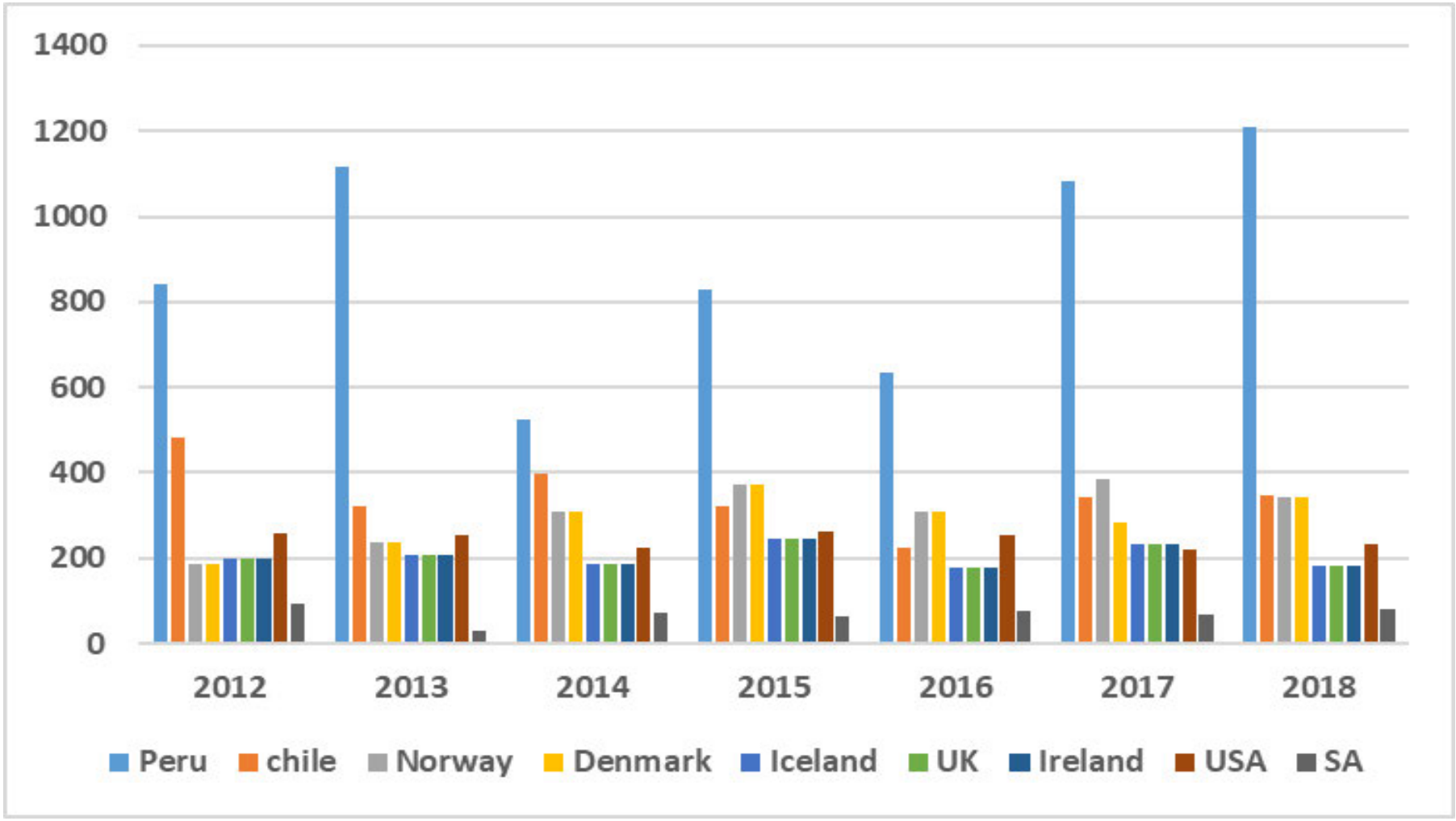

Figure 1.1: Production of fishmeal (000 Metric tonnes) for selected countries between 2012 and 2018. Data obtained from Banchis, (2018).

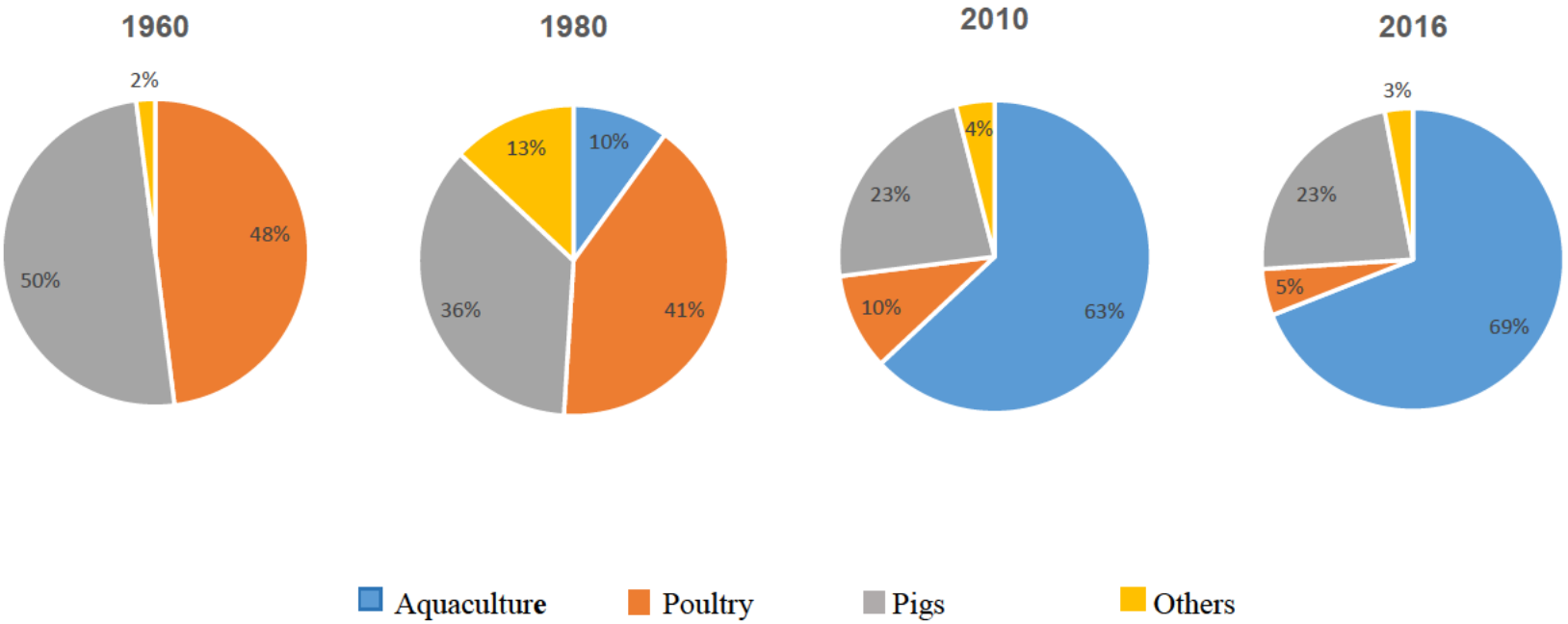

Figure 1.2: World fishmeal consumption by sector between 1960 to 2016. Data obtained from International Fishmeal and Fish Oil Organization (IFFO) (2011) and Banchis (2018).

1.2. Problem statement

Fishmeal production is mainly sourced from the forage fish species (Alder et al., 2008). Forage fish are described as the prey for other animals to eat (Alder et al., 2008). Forage fish species play an essential role in marine ecosystems because they transfer energy from primary producers (e.g., plankton) to higher trophic-level species, including large fish, marine mammals, and sea birds. According to Pikitch et al. (2014), fish caught for fishmeal production potentially represent a loss in production of higher trophic level species in the ecosystem. Low stock abundance reduces ecosystem services such as food provisioning to other elements of the ecosystem.

Fishmeal is the most expensive component of aquaculture feeds because of its competing use as a feed ingredient for other livestock species (Rana et al., 2009). Seventy-five percent of the world fish stocks used for fish meal production are currently considered fully exploited or overexploited, including much small pelagic fish (Tacon and Metian, 2008). Increasing demand, unstable supply, and the high price of the fishmeal with an expansion of aquaculture developed quench to search for alternative protein sources. According to Kritsanapuntu and, Chaitanawisuti (2015), numerous studies have shown that animal by-product meals arising from the processing of slaughtered farm livestock offer great potential for use as dietary fishmeal replacements within aquaculture feed.

1.3. Justification of research

Crocodiles are farmed mainly for producing skins used to produce high-quality fashion accessories (Ashton, 2010). Like in fish farming, the increase in production costs in this industry forced the farmers to look at alternative means of increasing profitability (Hoffman et al., 2000). Meat and tourism are becoming more important as income sources from crocodile farming for skins. However, the demand for crocodile meat, especially in South Africa, is very low and strict regulations are imposed onto the industry about the use and disposal of crocodile carcasses. According to Hoffman et al. (2000), crocodile meat is used as unprocessed for crocodiles in farms because the processing of crocodilian meat for human consumption involves public health regulations which include the design, construction, operation of abattoirs, food safety standards, and procedures explicitly established for the processing of crocodilians (Monalis and Webb, 2016). Abattoir facilities are costly to build, maintain and operate. Furthermore, managing abattoirs come with additional responsibilities related to packaging, labelling, shipping, and record-keeping (Luxmoore, 1992). Considering the grades

of fishmeal reported in Table 1.3 and the composition of the Nile crocodile (*Crocodlus niloticus*) carcass and meat characteristics reported by Hoffman et al., 2000, we think crocodile meat meal could be suitable alternative for fishmeal. However, the nutritional value to be determined in the third chapter of this study will report whether crocodile meal will be a suitable source to replace fishmeal or not.

Food Agricultural Organization, (FAO) (2013) reported that the world's demand for proteins of animal origin is expected to double by 2050. New initiatives are required to produce the necessary quantities of high-quality protein (Boland et al., 2013). There are no published studies on crocodile meat as a fishmeal replacement in fish or animal feeds. The study aims to assess the nutritional value of crocodile meat and its suitability as a fishmeal replacement in animal feeds, especially fish. If suitable, using crocodile meal in aquaculture will benefit the aquaculture industry by reducing supply constraints imposed by high cost and competitive uses for fishmeal. That could also translate into less dependence on marine-derived protein sources that are currently being over-exploited. The study's findings will be used in the production of more fish as a source of protein to affordable to resource-poor communities and contribute to poverty alleviation and food security. Furthermore, the findings and recommendations on the use of crocodile meat will be more beneficial to crocodile farmers who are finding it costly to dispose of crocodile meat as a by-product on South African commercial crocodile farms.

1.4.The objectives and null hypotheses of the study were to:

1. Conduct a systematic review and meta-analysis on animal protein sources used to substitute for fishmeal in aquaculture diets. (Null hypothesis (H_0); there is no difference in animal protein sources used to substitute fishmeal in aquaculture diets).
2. Determine nutritional values/profile of the meal derived from different parts of crocodile carcasses and compare results with other animal by-products meals used in aquaculture. (Null hypothesis (H_0), Nutritional values/profile of crocodile meal is not different from levels recommended for fishmeal and other animals by-products meals used in aquaculture).
3. Formulate crocodile meal-based diets as a fishmeal replacement for Mozambique tilapia (*Oreochromis mossambicus*) and evaluate the effects of crocodile-based diets compared to commercial diets on growth performance, feed utilization of and body composition. (Null hypotheses (H_0), i). There is no difference in growth performance, feed utilizations, and survival rates of *Oreochromis mossambicus* fed diets with

Crocodylus niloticus meat meal replacing fishmeal of different size groups. ii) There is no difference in feed costs among diets with fishmeal and those with crocodile meat meal.

4. Evaluate the effect of crocodile meal-based diets as fishmeal replacement on haematological parameters of *O. mossambicus*. (Null hypothesis (H_0), There is no difference in haematological parameters of *O. mossambicus* fish fed diets with crocodile meal).

1.5. **References**

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CHAPTER TWO

Animal Protein Sources as a Substitute for Fishmeal in Aquaculture Diets: A Systematic Review and Meta-Analysis

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2.1. Abstract

Fishmeal is the main source of dietary protein for most commercially farmed fish species. However, fishmeal prices have been raised even further because of competition with domestic animals, shortage in world fishmeal supply, and increased demand. Increased fishmeal prices have contributed to the quest for alternatives necessary to replace fishmeal as a global research priority. A literature search was conducted using these terms on Google Scholar and EBSCOhost, fishmeal replacement in fish feeds, fishmeal alternatives in fish feeds, animal protein sources in aquaculture, insects in fish feeds, terrestrial by-products, and fishery by-products. To calculate the variation between experiments, a random effect model was used. Results indicated that different fish species, sizes, and inclusion levels were used in the various studies and showed that the use of insects, terrestrial by-products, and fishery by-products has some limitations. Despite these drawbacks, the use of animal protein sources as a replacement for fishmeal in fish diets has had a positive impact on the feed conversion ratio, variable growth rate, final weight, and survival rate of different types of fish species of different size groups. Findings also showed that some animal by-products had not been assessed as a protein source in aquaculture or animal feeds, and future studies are recommended.

Keywords: Aquaculture**;** animal protein sources; fish; fishmeal; feeds

2.2. Introduction

In terms of species cultured and production systems used, aquaculture is a diverse industry [1]. According to [2], by producing fish with minimal environmental impact and maximum benefit for society, aquaculture is predicted to contribute more effectively to economic development, international food safety, nutritional well-being, and poverty reduction. Regardless of the cultivated systems within which fish are grown and species involved, fish production, growth, and health depend totally on a supply of adequate nutrients both in quantity and quality [3]. The quality of the protein ingredient used in feed formulation is generally known to affect the nutritional value of fish diets produced [4]. According to [5], aquaculture production (66 million tons) exceeded global beef production (63 million tons) for the first time in 2012. Increased aquaculture production means that aquaculture is produced more than half of the fish consumed by humans worldwide [6]. The demand for feed resources, particularly for prime quality protein fishmeal, has increased because of the global supply of fish as aquaculture production increases [3].

For both carnivorous and omnivorous species used in aquaculture, fishmeal has been used as an essential protein source, and many aquaculture formulations/feeds have a higher percentage of fishmeal than feeds of other animal species [7]. Fluctuations in supply, price, and quality of fishmeal present considerable risks because fishmeal is dependable solely on people's ingredients. Therefore, the identification, development, and utilization of fishmeal alternatives in aquaculture diets remain a high priority as a risk reduction strategy [8]. Competitive price, full availability, ease of handling, shipping, storage, and use in feed production are features that a candidate ingredient must possess to be a viable alternative feedstuff to fishmeal in aquaculture feeds [9]. Additionally, it should have high protein content, favorable amino acid profile, high nutrient digestibility, low fiber levels, starch, non-soluble carbohydrates, which are nutritional characteristics [9].

The more expensive fishmeal has been replaced by several sources of plant protein, single-cell protein, and animal protein in part or in full [10]. Due to higher protein and lipid content, superior essential amino acids, and excellent palatability, animal protein sources have commonly been considered ideal substitute protein sources to replace fishmeal in formulating fish diets [11, 12]. According to [13], animal-derived protein demand is expected to double by 2050 globally. Furthermore, future needs for both food and feed are expected to grow by 70%. According to [14], to provide the mandatory quantities of high-quality protein to fulfill the

increasing demand, new initiatives are needed. Several animal protein sources from insects, land by-products and fisheries by-products have been evaluated as possible feed ingredients in fish production [15, 16, 17, 18, 19]. However, no documented studies comparing animal protein sources in diet and control diet. The study was aimed to conduct a systematic review and meta-analysis of published articles on animal protein sources used in aquaculture and assess the results of recommended diets against the control diet.

2.3. Materials and Methods

A systematic search of published literature on Google Scholar and EBSCOhost from 1999 to 2019 was carried out using the following terms or phrases: fishmeal replacements in fish feeds, fishmeal alternatives in fish diets, animal protein sources in aquaculture, insects in fish feeds, terrestrial by-product, and fishery by-products. By reading through the titles and abstracts, the papers were found and screened. In addition, of the selected articles, the reference and bibliographic lists were screened as potential leads to additional relevant studies for inclusion. In Endnote reference manager version x7.7.1 (Clarivate Analytics, Philadelphia, PA, USA), full-text articles for studies including animal protein sources, were retrieved and managed. An article was included in the review if published between 1999 and 2019 and reported on 3 or all 4 of the following on experimental animals: Specific growth rate, final weight, feed conversion ratio, and survival rate. Studies with less than 4 protein levels tested, and those with no standard error on results were excluded. Furthermore, editorial material, book chapters, and conference papers were excluded. Meta-analysis was conducted for final weight, specific growth rate, feed conversion ratio, and survival rate, separately in a Microsoft Excel Spreadsheet using formulas and procedure described by [20] as follows after entering study Authors and year, events, and sample size for each study included:

1. Calculated the outcome (es) = number of events/the sample size
2. Calculated Standard Error (SE) = Square root of the outcomes/sample size
3. Variance (Var) =SE^2
4. Computed the individual study weights (W) = $1/SE^2$
5. Computed each weighted effect size (W*es) =each effect size multiplied by study weight
6. W*es^2 and W^2 were calculated.

All values of each variable were added to have the sum.

7. Calculated Q = $\sum(W*ES^2)-[\sum(W*ES)]^2/\sum W$, Q test to measure heterogeneity among studies. I^2 index = (Q-degree of freedom (df)/Q*100, was calculated to quantify heterogeneity, Degree of freedom (df) was calculated as the total number of studies minus 1. If values of I^2 index were 0%, ≤25%, 50%, or 75%, the I^2 index was interpreted as no, low, moderate, or high heterogeneity, respectively.
8. Decided on the effect summary model. Random Effect Model was used because we assumed that the variability in studies was not due to sampling errors only but also in the population of effects. Furthermore, the Random Effect Model was used to measure the variability between studies, considering that other studies, which were not included in the meta-analysis at hand, could be unpublished, ignored in the systematic literature quest, or to be conducted in the future [21]. The weight of each study was adjusted with a constant (V) = Q-df)/$\sum W-(\sum W^2/\sum W)$. However, we computed w^2 first and then the sum of $w^{2,}$ ($\sum W^2$), which was not computed yet.
9. New weight for each study was calculated using $W_v = 1/ (SE^2 + V)$.
10. Weighted effect size (W*es), $W*es^2$, Wv^2, Q_v, and I^2_v were computed using the new weight (W_v) as in steps 5–8.
11. Calculated the effect summary as $es_v = \sum(Wv*es)/\sum W_v$ and standard error as $SEes_v = \sqrt{1/\sum W_v}$
12. The lower and upper confidence intervals were calculated as $es_v - (1.96*SEes_v)$ and $es_v + (1.96*SEes_v)$, respectively.
13. Figures in results (excluding Figure 2.1) were drawn using the weights, prevalence, and confidence intervals calculated above.

2.4. Results

One-thousand-and-thirty-articles were obtained from search engines and additional records identified through other sources. Thirty articles were removed as duplicates after initial screening. Based on their names and abstracts, seven-hundred-and-eighty- three publications were omitted because they did not follow the requirements of reporting on three or all four of the following on experimental animals: specific growth rate, final weight, feed conversion ratio, and survival rate, have four or more protein levels tested, and others have no standard error on results. Eligibility was evaluated for two-hundred-and-seventeen articles, and eighteen articles were included in the systematic review and meta-analysis (Figure 2.1).

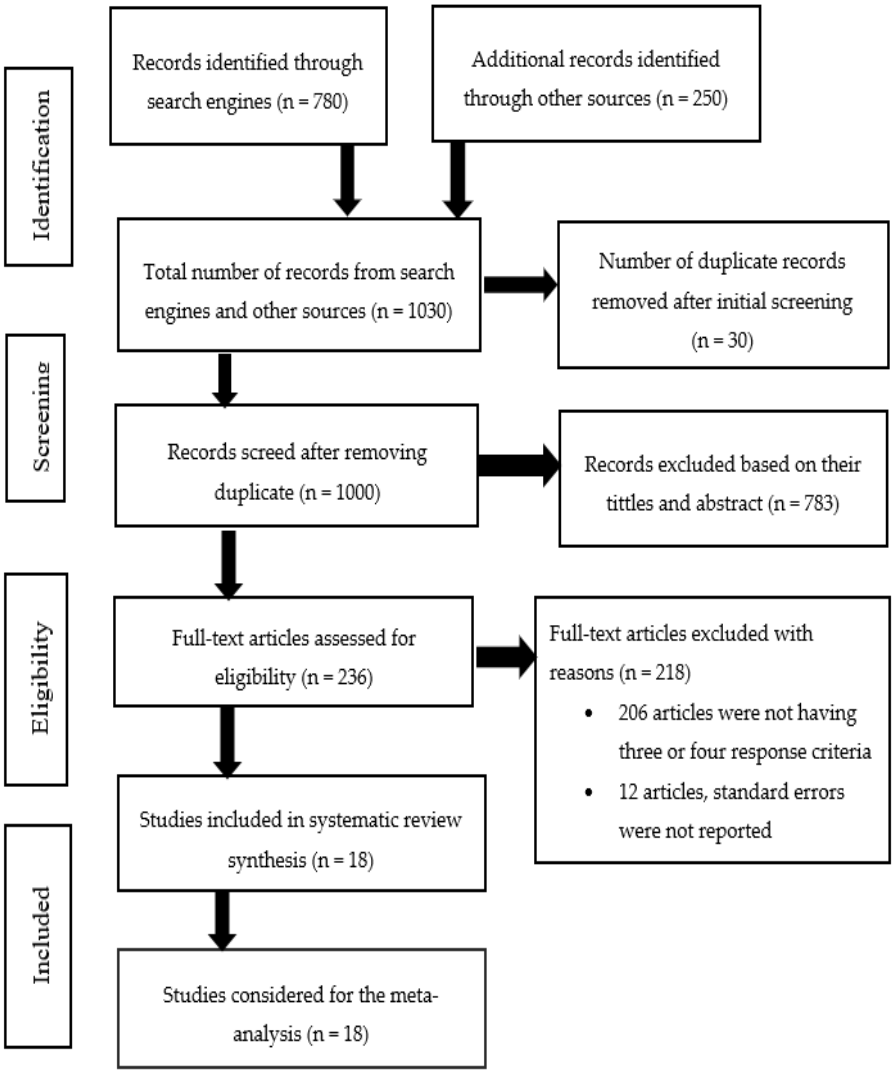

Figure 2.1. Flow chart of the study selection process for systematic review and meta-analysis of animal protein sources as a fishmeal replacement in aquaculture diets.

2.4.1. Fish Species Used and Recommended Levels of Animal Protein Sources

Results from the review articles showed that animal protein sources replacing fishmeal ranged from insects (Mopane worms (*Imbrasia belina*), grasshoppers (*Zonocerus variegatus*), field crickets (*Gryllus bimaculatus*), blowfly maggot (*Chrysomya megacephala*), black soldier fly (*Hermetia illucens*) and superworm (*Zophobas morio*), terrestrial animal by-products (fermented feather meal, feather meal, poultry by-products, meat and bone meal, and blood meal), and fishery by-products (fish silage, shrimp head meal and krill meal) (Table 2.1). Furthermore, a variety of fish species such as *Oreochromis mossambicus*, *Clarias gariepinus*, *Oreochromis niloticus*, *Sparus aurata*, *Dicentrarchus labrax*, *Scophthamus maeotinus*, *Lutjanus guttatus*, *Ophiocephalus argus*, Red tilapia (*O. mossambicus* × *O. niloticus* × *Oreochromis aureus*), and *Acipenser glueldenstaedtii* (which were not selected but reported because it is important to know when reporting for protein sources used) have been used. Animal protein sources inclusion levels in the diets ranged from 0%, 5%, 10%, 20%, 25%, 30%, 40%, 50%, 60%, 75%, to 100%. Recommended levels of animal protein sources in feeds were 20% for feather and shrimp head meal for *C. gariepinus*, 20% of meat and bone meal for *Op. argus*, 25% of superworm, poultry by-product and grasshopper meal for *L. guttatus* and *C. gariepinus* respectively, 30% of krill meal for *A. glueldenstaedtii*, 20–50% of fermented feather meal for *O. niloticus*, 50% of poultry by-products and fish silage for *O. niloticus* and Red tilapia (*O. mossambicus* × *O. niloticus* × *O. aureus*), respectively, 60% of mopane worm meal for *O. mossambicus* and 100% of field cricket meal for *C. gariepinus*.

Table 2.1. Summary of studies that assessed animal protein sources as a fishmeal replacement in fish diets in aquaculture. Final weight (FW in grams), specific growth rate (SGR in percentage (%)), feed conversion ratio (FCR), and survival rate (SR in %) were used as the assessment parameters to measure response.

Protein Sources Replacing Fish Meal	Fish Species	Recommended Levels of Feed (%)	Duration of Experiment (Days)	Feeding Frequency (Times/Day	Initial Weight IW (g)	Outcomes for Recommended Levels				References
						FW (g)	SGR (%)	FCR	SR (%)	
Insects										
Mopane worm (*Imbrasia belina*)	*Oreochromis mossambicus*	60	51	2	242.40	1221.10	3.16	1.25	100	[22]
Grasshopper (*Zonocerus variegatus*)	*Clarias gariepinus*	25	56	2	1.32	5.75	2.64	1.51	100	[23]
Field Cricket (*Gryllus bimaculatus*)	*Clarias gariepinus*	100	56	2	4.82	19.50	2.32	2.20	93.30	[24]
Blowfly Maggot *(Chrysomya megacephala)*	*Oreochromis sp.*	100	60	2	3.0	10.63	2.02	1.34	80.0	[25]
Black soldier fly *(Hermetia illucens)*	*Salmo salar*	66	112	2	1386	3721	0.9	1.1	NR	[26]
Superworm *(Zophobas morio)*	*Oreochromis niloticus*	25	56	2	5.57	10.11	1.02	1.25	100	[27]
Terrestrial animal by-products										
Fermented feather meal	*Oreochromis. niloticus*	25–50	84	2	122.81	222.35	NR	1.73	100	[15]
Feather meal	*Clarias gariepinus*	20	28	2	2.85	NR	7.89	1.34	88.89	[18]
Poultry by-products	*Lutjanus guttatus*	25	84	3	11.0	36.17	1.43	1.20	100	[28]
Poultry by-products	*Oreochromis niloticus*	50	84	NR	0.88	10.19	2.70	1.40	100	[17]
Poultry by-product	*Dicentrarchus labrax*	60	70	3	0.73	8.28	3.52	2.24	94	[29]
Poultry by-product	*Scophthalmus maeoticus*	25	60	2	18	29.38	0.18	0.91	100	[30]
Poultry by-product	*Oreochromis niloticus*	100	120	2	1.5	54.3	2.99	1.34	NR	[31]
Blood meal	*Clarias gariepinus*	50	86	2	10.32	66.50	1.03	0.86	100	[32]
Meat and bone meal	*Ophiocephalus argus*	20	70	3	12.11	138.67	3.48	1.24	94.2	[33]
Fishery by-products										

Fish silage	Red tilapia (*Oreochromis mossambicus* × *Oreochromis niloticus* × *Oreochromis aureus*)	50	84	NR	2.18	28.05	3.04	1.35	NR	[34]
Shrimp head meal	*Clarias gariepinus*	20	84	NR	12.1	32.8	1.19	2.50	NR	[35]
Krill meal	*Acipenser glueldenstaedtii*	30	200	NR	483	NR	0.56	1.10	83	[36]

NR = Not recorded.

2.4.2. Values for Final Weight, Specific Growth Rate, Feed Conversion Ratio, and Survival Rate

Values for final weight, specific growth rate, feed conversion ratio, and survival ratio for recommended levels of animal protein sources in feeds for different fish species are shown in Table 2.1. Assessment of the initial and final weights for all recommended levels of animal protein sources fed showed weight gain for all fish species involved in the experiments (Table 2.1). The specific growth rate ranged from 0.56% to 7.89%. Feed conversion ratios of 1.25, 1.51, and 2.20 were reported for *O. mossambicus*, *C. gariepinus*, and *C. gariepinus*, which were fed insect meal (*I. belina*, *Z. variegatus*, and *G. bimaculatus*), respectively. For terrestrial by-products (fermented feather meal, feather meal, poultry by-products, poultry by-products, and meat and bone meal), feed conversion ratios of 1.73, 1.34, 1.20, 140, and 1.24 were obtained for *O. niloticus*, *C. gariepinus*, *L. guttatus*, *O. niloticus*, and *Op. arg*us, respectively. Feed conversion ratios of 1.35, 2.50, and 1.10 were obtained in Red tilapia (*O. mossambicus* × *O. niloticus* × *O. aureus*), *C. gariepinus*, and *A. glueldenstaedtii* fed fishery-by products (fish silage, shrimp head meal, and krill meal), respectively. Survival rate ranged from 83% to 100%, except for Red tilapia (*O. mossambicus* × *O. niloticus* × *O. aureus*) and *C. gariepinus*, which were fed fish silage and shrimp head meal, respectively, where the survival rate was not reported.

2.4.*3. Meta-Analysis*

For meta-analysis, data from studies analyzed were grouped into final weight, specific growth rate, feed conversion ratio, and survival rate (Table 2.2, which summarizes results shown in Figures 2.2, Figure 2.3, Figure 2.4, and Figure 2.5). Samples analyzed were 1335, 1430, 1450, and 1307 for final weight, specific growth rate, feed conversion ratio, and survival rate, respectively. Results showed the overall effect size of 9015 (95% confidence interval (CI) 6110058.3 to 6110177.58), 10 (95% CI 32 to 21), 10 (95% CI 24 to 13), and 546 (95% CI 350 to 572) for final weight, specific growth rate, feed conversion ratio, and survival rate, respectively (Figures 2.2, Figure 2.3, Figure 2.4, and Figure 2.5). Effect summary for all Figures 2.2, Figure 2.3, Figure 2.4, and Figure 2.5 do not touch or cross the center line, meaning that meta-analysis results indicate a statistically significant difference. The level of heterogeneity observed were $I^2 = 99.70\%$, $I^2 = -17.73\%$, $I^2 = -25.79\%$, and $I^2 = 101.08\%$ for final weight, specific growth rate, feed conversion ratio, and survival rate, respectively (Table 2.2).

Table 2.2. Weights, prevalence (95 % CI), effect summary, I^2 index, and degree of freedom for final weight, specific growth rate, feed conversion rate, and survival rate for different studies included in the meta-analysis.

	Final Weight		Specific Growth Rate		Feed Conversion Ratio		Survival Rate	
Reference	**Weight**	**Prevalence (95% CI)**	**Weight**	**Prevalence (95% CI)**	**Weight**	**Prevalence (95% CI)**	**Weight**	**Prevalence (95% CI)**
[23]	21	47 (31–168)	50	23 (32– 19)	60	17 (26–9)	1	927 (637–839)
[15]	2	1041 (779–978)	-	-	174	12 (21–14)	4	500 (301–503)
[17]	50	39 (28–170)	170	12 (29–23)	225	9 (22–14)	4	500 (301–503)
[18]	-	-	56	27 (26–25)	101	15 (18–18)	3	435 (228–430)
[36]	709	141 (61–138)	1,851,851.8	0.054 (26–26)	1,021,450.5	0.1 (24–11)	11869.4	8 (94–108)
[28]	7	228 (2–197)	166	9 (32–20)	180	8 (22–13)	2	663 (433–635)
[34]	13	149 (70–129)	128	16 (27–24)	327	6 (20–15)	-	-
[35]	13	157 (65–129)	354	6 (30–22)	153	13 (19–17)	-	-
[22]	-	-	3236	3 (26–25)	7299	1 (20–15)	100	100 (21–181)
[24]	17	88 (70–134)	138	11 (31–20)	73	21 (27–8)	3	604 (380–1085)
[33]	12	334 (112–311)	466	9 (26–25)	1214	3 (20–15)	17	231 (83–286)
[25]	9.09	106 (76–122)	49.59	20 (33–18)	74.63	13 (27–8)	1.25	800 (524–726)
[26]	0.002	124,030 (85,539,850–85,540,050)	10.01	30(58–6)	8.14	37 (50–15)	-	-
[27]	0.01	101 (76–122)	98.03	10(35–16)	78.13	13 (27–8)	1	1000 (702–905)
[29]	76.92	33 (24–174)	178.57	14 ((26–25)	277.77	9 (20–14)	6.65	376 (199–401)
[30]	7.72	196 (31–168)	1275.51	1 (30–22)	243.9	6 (24–11)	2.27	667 (435–637)
[31]	181.82	54(49–150)	3448.28	3 (26–25)	7692.3	1 (19–17)	-	-
[32]	2.17	554 (269–495)	140.85	9(34–18)	169.49	7 (26–10)	1.44	833 (569–771)
Effect summary	9015 (6,110,058.3–6,110,177.58)		9.9 (24–13)		10 (32–21)		546 (350–572)	
Random effect model (I^2)	99.40		−7.73		−27.791		101.08	
Degree of freedom (df)	15		16		17		13	

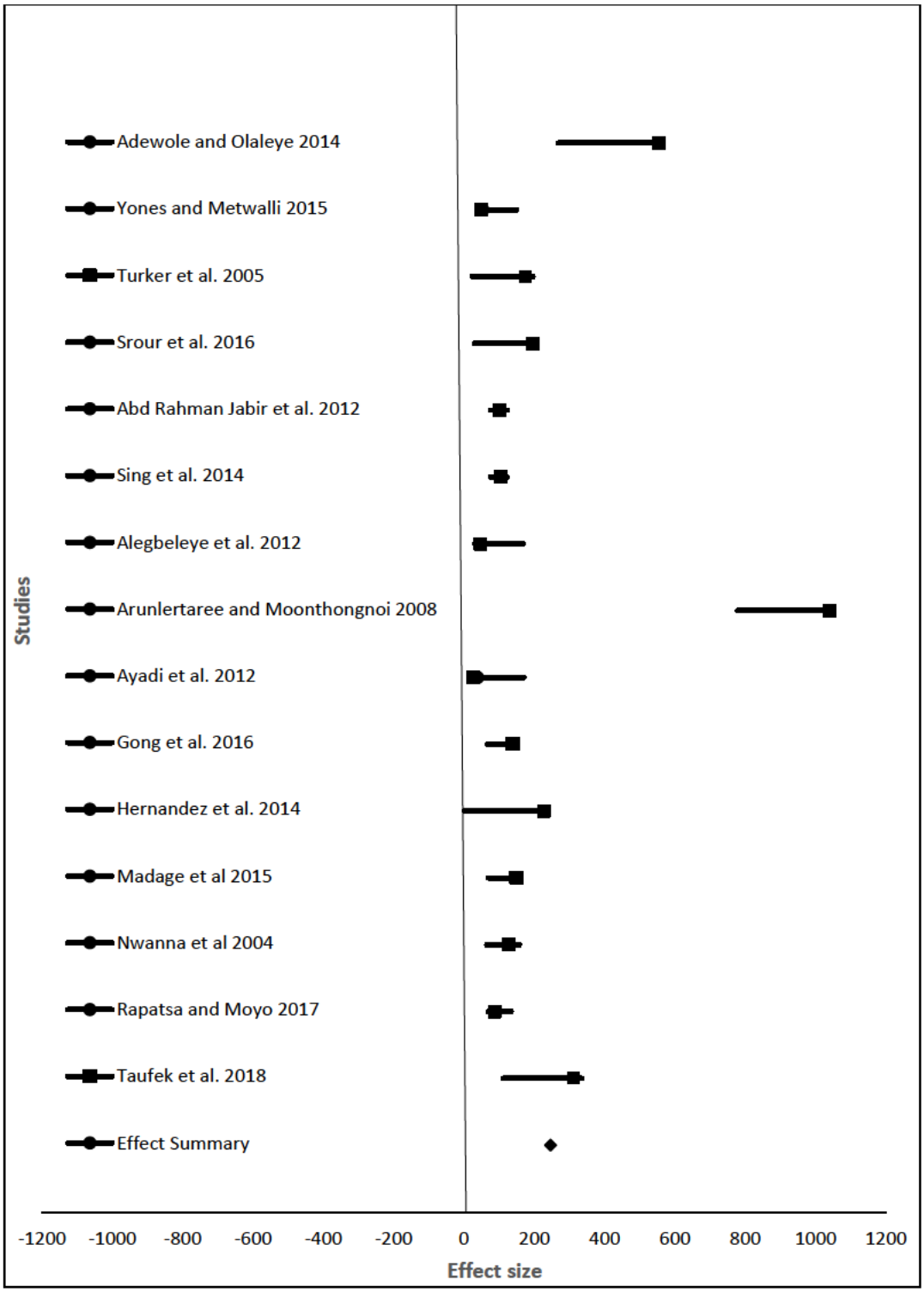

Figure 2.2. The effect size of final weight (%) of fish from different studies fed different animal protein sources compared to fishmeal as a protein source.

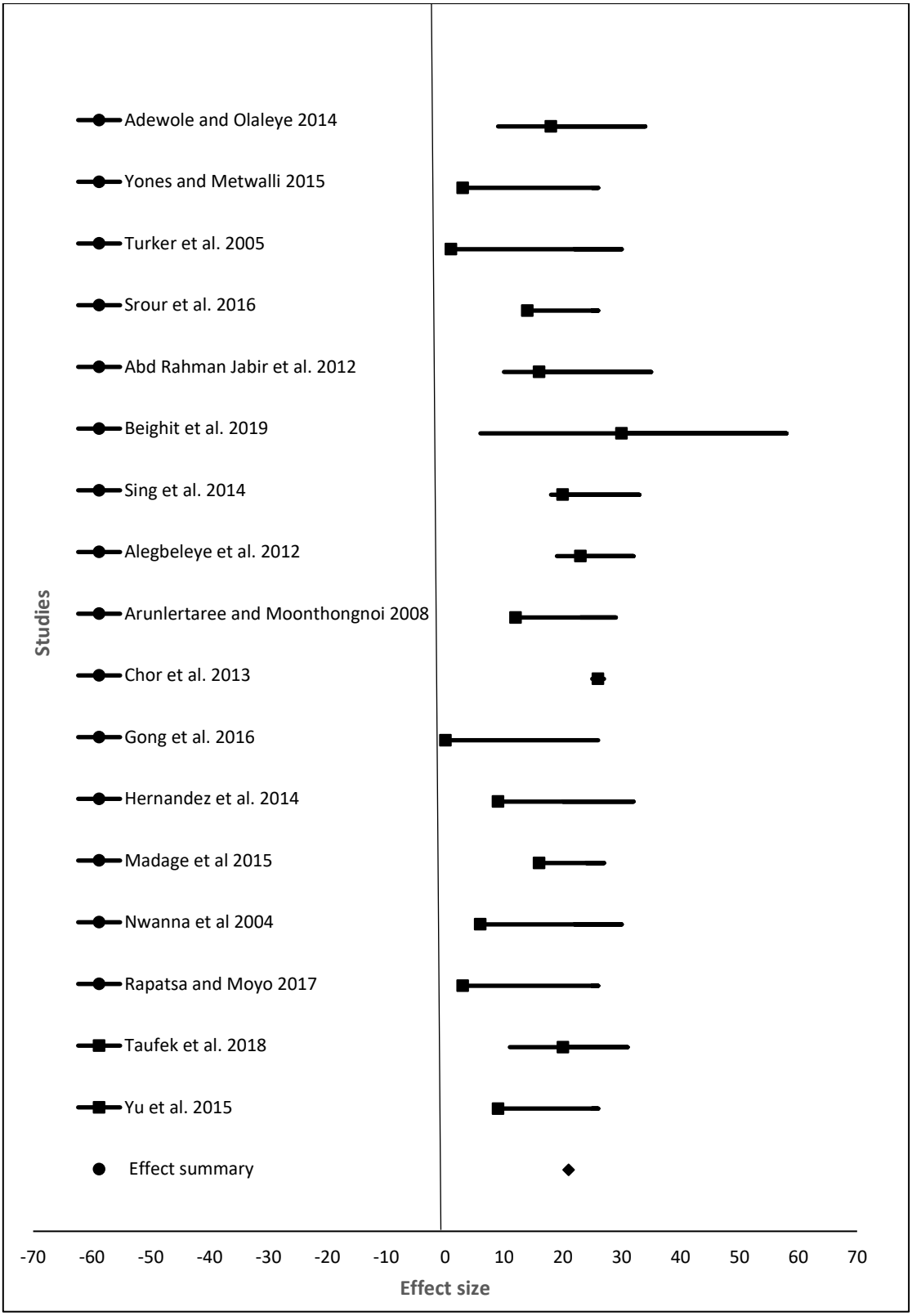

Figure 2.3. The effect size of specific growth rate (%) of fish from different studies fed different animal protein sources compared to fishmeal as a protein source.

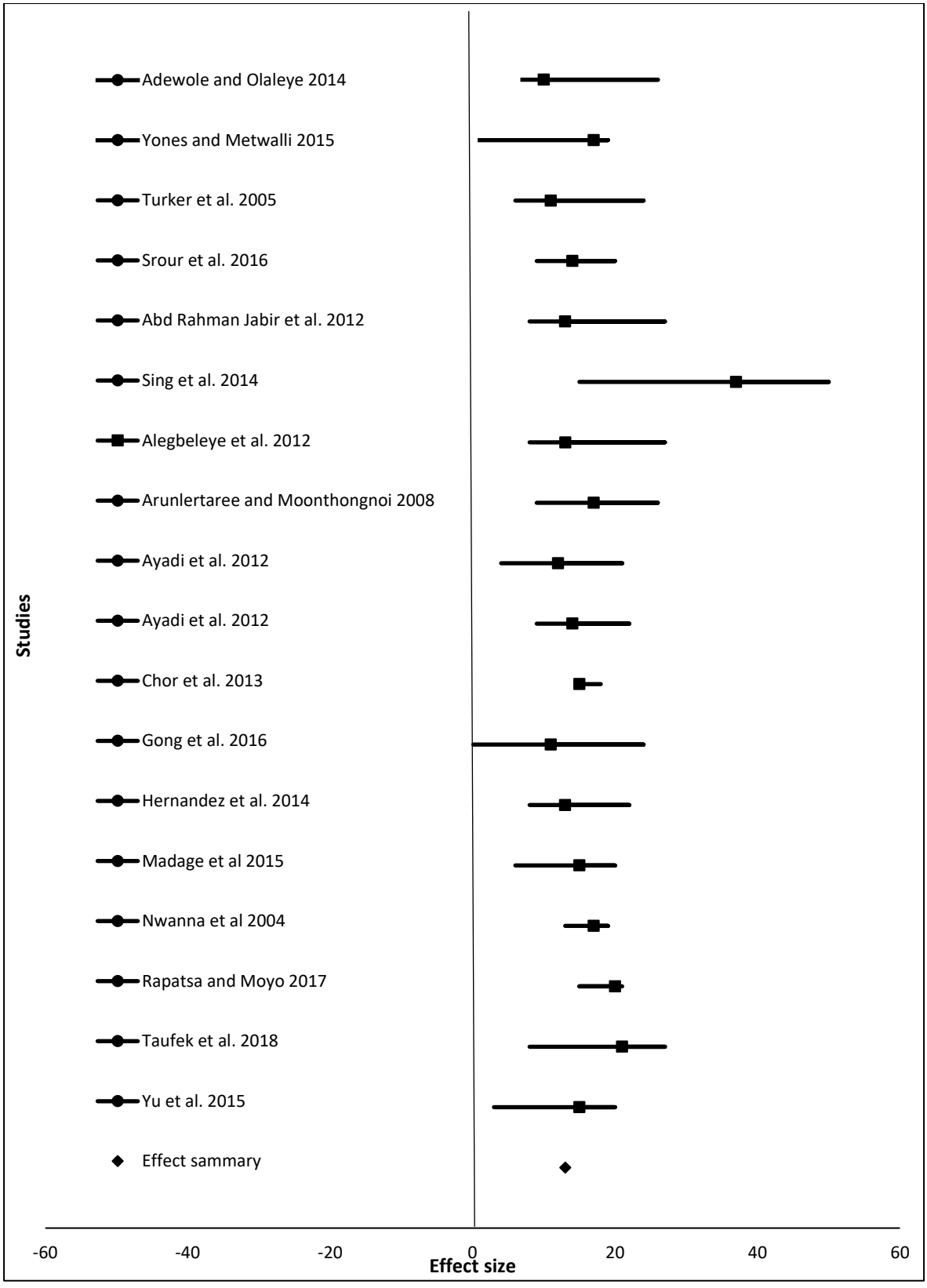

Figure 2.4. The effect size of feed conversion ratio (%) of fish from different studies fed different animal protein sources compared to fishmeal as a protein source.

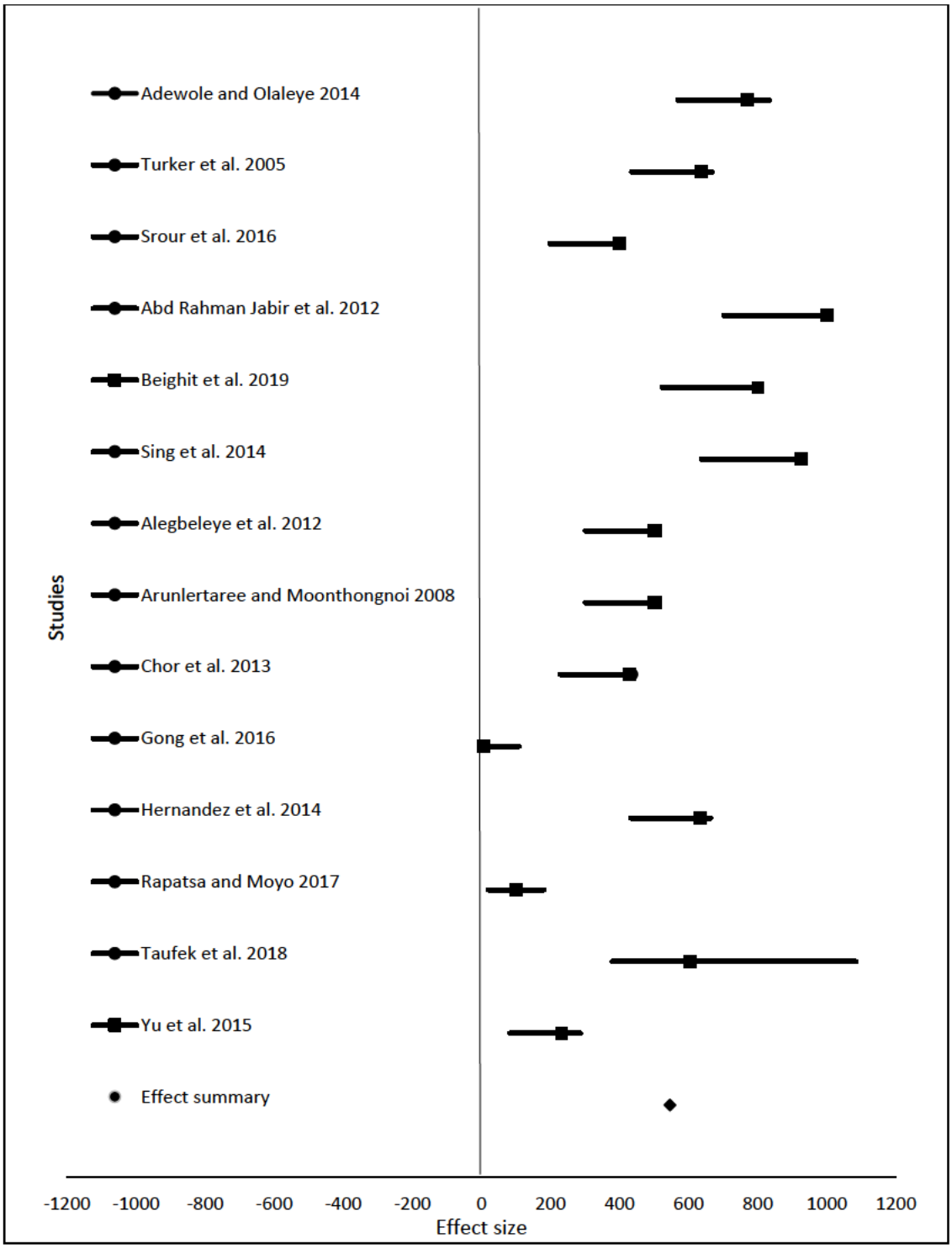

Figure 2.5. The effect size of survival rate (%) of fish from different studies fed d different animal protein sources compared to fishmeal as a protein source.

2.5. Discussion

From the results of this review, a variety of fish species, sizes, and inclusion levels have been used in aquaculture (Table 2.1). A variety of fish species, sizes, and inclusion levels may be because aquaculture is an incredibly diverse industry in terms of species cultured and production systems used [1]. Different fish species have different nutrients requirements [37], which affect the level of protein source inclusion in tested diets. According to [38], human health benefits, competitive price, fish safety, efficiency, customer acceptance, minimal contamination, and ecosystem stress are factors in selecting feeds.

Growth performance measured by final weight and specific growth rate showed that excess protein could not be used efficiently for growth because of growth energy used for the deamination and excretion of absorbed excess amino acids. After all, each fish species had a specific protein limit [39]. According to [40, 41, 42, 43], when dietary protein levels increase, the feed conversion ratio decreases. Results from this review indicated that *O. mossambicus* and *C. gariepinus* fed insect meal (*I. belina*, *Z. variegatus* and *G. bimaculatus*), respectively, converted their feeds efficiently. Both freshwater and marine fish species utilize insects as part of their natural diet [44]. Insects are rich in amino acids, lipids, vitamins, and minerals [45], and they do not require arable land, water, or energy to reproduce [46]. Besides, insects are more natural to replicate, have a higher growth rate, and very effectively transform low-grade or organic matter into high-value protein quite efficiently [44, 47]

Recommended levels reported for insect meal in this review shows that a total fishmeal replacement has not been successful. Results support findings reported by [44], who suggested dietary unbalance or deficiencies as the main reason. According to [48], limitations of using insects include their (i) varying nutritional value, which is dependent on the species, stage of development, and the substrate used to feed the insect, (ii) low concentration of sulfur-containing amino acids, and (iii) absence of eicosapentaenoic and docosahexaenoic.

Oreochromis niloticus, *C. gariepinus*, *L. guttatus*, *O. niloticus*, and *Op. argus* also efficiently converted terrestrial by-products (fermented feather meal, feather meal, poultry by-products, poultry by-products, and meat and bone meal). Like other animal protein sources, fishery by-products (fish silage, shrimp head meal, and krill meal), fed to Red tilapia (*O. mossambicus* × *O. niloticus* × *O. aureus*), *C. gariepinus* and *A. glueldenstaedtii*, respectively resulted in acceptable feed conversion ratios of 1.35, 2.50, and 1.10, respectively. Survival rates ranged from 83% to 100%, except for Red tilapia (*O. mossambicus* × *O. niloticus* × *O. aureus*) and *C. gariepinus*, which was not reported.

Fermented feather meal, blood meal, poultry by-products, feather meal, meat and bone meal are some of the terrestrial animal by-products used in aquaculture diets [15, 16, 17, 18, 19]. Terrestrial by-products have been reported to have great potential as fishmeal replacement because they are readily available, economical sources of protein and have more complete amino acid profiles than vegetable proteins [23]. The use of feather meal in aquaculture feeds is limited by the fact that fish are unable to digest it. Lysine, methionine, and isoleucine have been reported as limiting essential amino acids in poultry by-products, meat and bone meal, and blood meal, respectively [22]. Consumer acceptance is the primary constraint on the use of rendered animal products [23].

Fishery by-products are products generated from fishery industries [41]. Skin and fins, scales, heads and bones, viscera, and muscle trimmings are the main by-products produced in fishery industries with (1–3%), (5%), (9–15%), (12–18%), and (15–20%), respectively [41].

Scanty information is available for these by-products as a fishmeal replacement in fish feeds as they are considered waste [7]. Limiting factors of using fishery by-products include the cost of the collection of fish waste, timely processing, and quality control [49]. Furthermore, fish waste varies highly in its physical nature and proximate composition; and some fish waste such as from seafood is only available during the fishing season [17].

One of the advantages of meta-analysis is to increase the sample size. Samples analyzed in this study were 1335, 1430, 1450, and 1307 for final weight, specific growth rate, feed conversion ratio, and survival rate, respectively. Sample size differs due to the number of studies (16, 17, 18, and 14 for final weight, specific growth rate, feed conversion ratio, and survival rate, respectively) included in the meta-analysis. Results for final weight, specific growth rate, feed conversion ratio, and survival rate (Figures 2.2, Figure 2.3, Figure 2.4., and Figure 2.5), shows that there is a statistically significant difference among studies (the overall effect size of the overall effect size of 9015 (95% confidence interval (CI) 6110058.3 to 6110177.58), 10 (95% CI 32 to 21), 10 (95% CI 24 to 13), and 546 (95% CI 350 to 572) for final weight, specific growth rate, feed conversion ratio, and survival rate, respectively. The level of heterogeneity (I^2 index) was very high for both the final weight and survival rate with values 99.98 and 101.08, respectively. There was no heterogeneity for both specific growth rate and feed conversion ratio, as their values for I^2 index were I^2 = -25.79% and I^2 = -17.73%, respectively. Final weight, specific growth rate, feed conversion ratio and survival rate of fish in experiment or in farming in general are affected by many factors such as age of fish, fish species, stocking density, feeding level and frequency, protein source, and water quality parameters such as water temperature, dissolved oxygen, and pH as shown in Table 2.1, variety

of fish species, size, inclusion levels, recommended levels of protein found were reported, and these are the reasons our meta-analysis indicated heterogeneity in studies. Despite all the heterogeneity observed, these animal protein sources have shown positive effects on feed conversion ratio, specific growth rate, final weight, and survival of different fish species of different size groups.

2.6. Conclusion

Despite the limitations in the use of insects, terrestrial by-products, and fishery by-products as replacement of fishmeal, these animal protein sources have shown positive effects on feed conversion ratio, specific growth rate, final weight, and survival of different fish species of different size groups. However, future studies have recommended to (i) identify a fishmeal replacement that has no limitations, (ii) assessing the suitability of readily available animal meat or by-products as fishmeal replacement.

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2.8. References

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CHAPTER THREE

Nutritional value of the Nile crocodile (*Crocodylus niloticus*) meal for aquaculture feeds in South Africa.

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3.1. Abstract

The Nile crocodile (*Crocodylus niloticus*) aquaculture industry, primarily for the production of skins, is amongst the largest aquaculture industry in sub-Saharan Africa and produces a range of meat waste products. The aim of this study was to evaluate the nutritional value of raw and cooked meal derived from different parts of *Crocodylus niloticus* carcasses as a potential source of protein in animal feed production, especially fish. Proximate composition of major nutrients such as moisture, crude protein, crude fat, crude fibre, ash, and selected minerals were analysed in October-November 2018 for comparison with other meal sources. Results indicated that *Crocodylus niloticus* derived meal is of a comparable quality for use in aquaculture feeds, compared to by-product meal quality reported for meal derived from bovine bones and meat, feathers, blood and other poultry by-products. Crocodile meal is hypothesised to be a suitable fishmeal replacement in the production of aquaculture feeds.

Keywords: aquaculture, fish nutrition, fish production, proximate composition

3.2. Introduction

The Nile crocodile (*Crocodylus niloticus*) of the family Crocodylidae is a widely distributed carnivorous reptile occurring throughout sub-Saharan Africa (Fergusson 2010). It is the largest and most widely farmed species in Africa and is the only crocodilian species found in South Africa (Botha 2005). The reptile uses a wide array of freshwater habitat types, including rivers, lakes, swamps, estuaries and others such as wetlands (Leslie and Spotila 2001). Populations in South Africa are threatened by disturbance to wildlife stressors associated with cattle and human activity near nesting areas (Combrink et al. 2016), alien plants (primarily *Chromolaena odorata*) (Leslie and Spotila 2001) and water pollution resulting in disease (Ashton 2010; Ferreira and Pienaar 2011; Woodborne et al. 2012). Other threats include habitat loss, indirect anthropogenic effects including water resource development, prey reduction and hunting for the artisanal trade in leather goods (Fergusson 2010).

The aquaculture of *C. niloticus* has been established more than 25 years ago in southern Africa (Tosun 2013). Commercial production of *C. niloticus* in the region is of noticeable economic and ecological importance. According to Flint et al. (2000); Nogueira and Nogueira-Filho (2011), culturing of crocodiles can be used for enhancement of wild populations in selected areas, creation of jobs, environmental education programmes and collection of biological data on captive species and tourists attraction.

The *C. niloticus* production industry traditionally focuses on producing skins used in the production of high-quality fashion accessories (Ashton 2010). The increase in production costs in this industry has forced the farmers to look at alternative means of increasing profitability in this industry (Hoffman et al. 2000). Tourism and meat production were identified as the major components of skin production. However, the demand for crocodile meat, especially in South Africa is very low and strict regulations are imposed onto the industry pertaining to the use and disposal of crocodile carcasses. Although (Hoffman et al. 2000), reported that crocodile meat produced can either exported or sold to the restaurant trade or used as unprocessed crocodile feed for other crocodiles on the farm, processing of crocodilian meat for human consumption always involves the farmer in strictly regulated abattoir management and additional responsibilities relating to packaging, labelling shipping and record keeping (Luxmoore 1992). Furthermore, abattoirs facilities are costly to build, maintain and operate. The difficulty and expense involved in meeting the requirements of hygienic meat production has prompted farmers to dispose tons of whole crocodile carcasses.

According to FAO et al. (2013), the world demand for proteins of animal origin is expected to double by 2050. New initiatives are required to produce the necessary quantities of high quality protein (Boland et al. 2013). There is a lack of published information on chemical composition of and associated nutritional value of crocodile carcass derived meal for aquaculture feeds. The aim of this study was to evaluate the chemical composition of and associated nutritional value of meal derived from different parts of *C. niloticus* carcass and compare with other meal used in aquaculture feeds. This research is part of an effort to diversify the use of crocodile meat by enhancing the knowledge of the chemical composition of *C. niloticus* meal and using meat as processed product while closing the gap in proteins of animal origin.

3.3. Material and methods

3.3.1. Sample preparation and analysis

Ten carcasses from 4 years old crocodiles were collected from an abattoir at Albert falls Crocodile farm in Pietermaritzburg, KwaZulu-Natal province, South Africa. Each carcass was divided into legs, torsos, and necks and then meat, fat and bones separated. Meat was then transported to University of KwaZulu-Natal, Pietermaritzburg campus for analysis. Meat samples taken from the legs, neck, and torso were placed in polyethylene bags, vacuum sealed and placed in a water bath at 75ºC for 50 minutes to have a cooked sample for all parts. Thereafter the samples, still in bags were cooled under running water at 25ºC for 40 minutes (Hoffman et al. 2000). Then sample of raw lean meat and cooked meat were taken and dried in the oven at 100 ºC for three hours and then grinded using a coffee blender. Sieved through a 1.0 mesh micron sieve. Samples of raw meal and cooked meal of legs, torsos, necks, raw mixture, and cooked mixture were analysed in October- November 2018 at Soil Science and Animal Science departments at University of KwaZulu-Natal, Pietermaritzburg Campus for proximate analysis. All samples were replicated four times. Nitrogen (N) content was determined on a Leco TruMac^{R} Carbon Nitrogen Sulfur elemental analyser using Dumas's combustion. Crude protein was calculated as N x 6.25. Crude fat content was determined using Soxhlet method as described in AOAC Official method 920.39 (Horwitz 1975). Crude fibre was determined as loss of ignition of dried lipid-free residues with 1.25% H_2SO_4 and 1.25% NAOH solutions using the filter bag technique with ANKOM Fibre analyser 200. Moisture content was determined using an air-circulated oven at 95ºC for 72 hours. Ash content was

determined by burning pre-weighed samples in muffle furnace at 550ºC overnight as described in AOAC Official method 942.05. Minerals were determined using the Fast Sequential Atomic Absorption Spectrometer (AA280FS)(Paul et al. 2014), after ashing samples.

3.3.2. Statistical analysis

Statistical analyses were performed using SPSS software Version 25. The Shapiro –Wilk test was used to check if the data was normally distributed. One-way analysis of variance (ANOVA) was used to test for significant differences at a significant level of α=0.05 between the means of the treatment. The results were considered significantly different at a probability of (p) <0.05. Where there was a significant difference in means, Tukey's multiple comparison test was used to compare the variance among the means.

3.4. Results

There were significant differences ($P < 0.05$, ANOVA) in crude protein contents of the meals from different parts of *C. niloticus* (Table 3.1). The protein contents of meals from raw leg and cooked leg were significantly higher ($P < 0.05$, Tukey test) than meals from raw neck, raw torso, cooked neck, cooked torso, and cooked mixture. The raw mixture protein content was similar to the cooked leg and other parts but significantly lower than the raw leg (Table 3.2). High protein content that ranged from 81 to 85% for raw meal and 78 to 84.5% for cooked meal were obtained (Table 3.2).

There was a significant difference ($P < 0.05$, ANOVA) in crude fat, crude fibre, and ash content of meal from different parts of *C. niloticus* (Table 3.1). The cooked mixture meal had significantly higher ($P < 0.05$, Tukey test) fat content than the meal from raw leg, raw neck, raw torso, raw mixture, cooked leg, and cooked neck. The fat content for cooked torso was similar to cooked mixture Crude fat contents ranged from 3.63 to 8.46% for raw meal and 6.22 to 8.75% for cooked meal (Table 3.2). Crude fibre contents of the meal from cooked neck were significantly higher ($P < 0.05$, Tukey test) than meal from the raw leg, raw neck, raw torso, raw mixture, cooked leg, cooked torso, and cooked mixture. Crude fibre values ranged between -0.03 to 0.04% for raw meal and -0.02 to 0.26% for cooked meal (Table 3.2). Ash content ranged from 2.41 to 3.2 for raw meal and 2.66 to 3.83% for cooked meal (Table 3.2).

There was no significant difference in moisture content of the meal from all different parts of *C. niloticus* (Table 3.1). Mean values ranged from 8.73 to 12.40% for raw meal and 11. 0450 to 17.74% for cooked meal (Table 3.2).

There was no significant difference in Iron and Copper content of meal among different parts of *C. niloticus* (Table 3.3). Mean values ranged from 0.12 to 0.22% for raw meal and 0.16 to 0.24% cooked meal for Iron (Table 3.4). Mean values for Copper ranged from 0.04 to 0.09% for raw meal, and 0.01 to 0.27% for cooked meal (Table 3.4).

There was a significant difference in Potassium, Sodium, Calcium, Magnesium, Zinc, and Aluminium contents of *C. niloticus* meal from different parts (Table 3.3). Highest potassium value was observed in the meal from the raw leg. Sodium in the meal from cooked neck, was significantly higher ($P < 0.05$) than raw leg, raw neck, and raw mixture but similar to the meal from the raw torso, and cooked mixture. Magnesium in all raw meals were similar but significantly higher ($P < 0.05$, Tukey test) than cooked leg and cooked neck meal. Zinc was significantly higher ($P < 0.05$, Tukey test) in meal from the raw leg than all other parts. Cooked leg meal had significantly lower Aluminium than all other parts.

Table 3.1: Proximate analysis of major nutrients of the Nile crocodile *(Crocodylus niloticus*) meal from different parts. DF=Degree of freedom between groups= 7, within= 24, F= F Statistic, and P= probability

Major nutrients	F	P
Crude protein	33.620	<0.001
Crude fat	17.068	<0.001
Crude fibre	12.218	<0.001
Ash	2.830	0.027
Moisture	1.857	0.122

Table 3.2: Mean crude protein, moisture, crude fat, crude fibre, ash, and overall average for raw and cooked meal from leg, neck, torso, and mixture of three parts (leg, neck, and torso) of the Nile crocodile, *(Crocodylus niloticus)*. Values are means (± Standard Deviation) of four replicates for each part

Components[1]	Raw Leg	Raw Neck	Raw Torso	Raw Mixture	Cooked Leg	Cooked Neck	Cooked Torso	Cooked Mixture
Crude protein	85.06 ± 0.25^{a}	82.11 ± 0.17^{b}	81.05 ± 1.30^{bc}	83.04 ± 0.14^{bd}	84.55 ± 1.69^{ad}	80.02 ± 0.39^{c}	82.03 ± 0.23^{bce}	78.16 ± 0.30^{e}
Moisture	12.40 ± 0.88	9.75 ± 1.18	8.73 ± 2.72	9.78 ± 0.35	17.74 ± 10.81	12.03 ± 2.18	11.04 ± 1.17	12.19 ± 0.47
Crude fat	3.63 ± 0.26^{a}	8.45 ± 0.35^{b}	4.12 ± 0.07^{ac}	4.48 ± 2.86^{ad}	6.22 ± 0.12^{bcd}	8.22 ± 0.10^{bc}	8.13 ± 0.42^{bcde}	8.75 ± 0.34^{e}
Ash	3.24 ± 0.41^{a}	3.32 ± 0.46^{ab}	2.74 ± 0.41^{ab}	2.41 ± 0.42^{b}	3.23 ± 0.16^{ab}	3.08 ± 0.32^{ab}	2.83 ± 0.58^{ab}	2.66 ± 0.01^{ab}
Crude fibre	0.01 ± 0.07^{a}	0.04 ± 0.06^{ac}	-0.03 ± 0.06^{ab}	0.04 ± 0.04^{ab}	-0.02 ± 0.07^{ab}	0.26 ± 0.02^{b}	0.11 ± 0.02^{ac}	0.04 ± 0.04^{a}

[1]Mean values ± standard deviation in the same row with the same superscripts are not significantly different ($p > 0.05$)

Table 3.3: ANOVA results for selected minerals composition of the Nile crocodile, *(Crocodylus niloticus*) meal from different parts. DF=Degree of freedom between groups= 7, within= 24, F= F statistic, and P= probability

Minerals	F	P
Calcium	25.813	<0.001
Sodium	7.313	<0.001
Zinc	6.849	< 0.001
Potassium	5.377	0.001
Magnesium	4.043	0.005
Aluminium	2.473	0.046
Iron	2.172	0.074
Copper	0. 915	0.512

Table 3.4: Mean (±Standard Deviation) of selected minerals for raw and cooked meal from leg, neck torso and mixture of three parts (leg, neck, and torso) of the Nile crocodile, *(Crocodylus niloticus*). Values are means (± SD) of four replicates for each part

Minerals[1]	Raw Leg	Raw Neck	Raw Torso	Raw Mixture	Cooked Leg	Cooked Neck	Cooked Torso	Cooked Mixture
Potassium	39.67 ± 0.30^{a}	38.20 ± 1.77^{a}	37.23 ± 1.69^{a}	32.90 ± 6.15^{ab}	32.11 ± 5.72^{ab}	34.60 ± 2.60^{ab}	34.71 ± 1.67^{ab}	27.66 ± 1.07^{b}
Sodium	11.24 ± 0.16^{a}	8.72 ± 0.61^{b}	10.98 ± 0.42^{a}	11.17 ± 1.28^{ac}	9.27 ± 1.34^{ab}	11.44 ± 0.18^{ac}	9.67 ± 0.69^{ab}	10.66 ± 0.32^{ac}
Calcium	1.42 ± 0.06^{a}	2.30 ± 0.02^{b}	2.11 ± 0.03^{b}	1.93 ± 0.07^{b}	1.86 ± 0.33^{abc}	1.40 ± 0.05^{ad}	1.73 ± 0.33^{abc}	2.80 ± 0.18^{bc}
Magnesium	1.54 ± 0.08^{a}	1.69 ± 0.12^{a}	1.62 ± 0.08^{ab}	1.49 ± 0.08^{a}	1.41 ± 0.20^{ab}	1.50 ± 0.05^{abc}	1.78 ± 0.14^{abc}	1.59 ± 0.08^{abc}
Zinc	0.35 ± 0.05^{a}	0.15 ± 0.09^{b}	0.20 ± 0.02^{bc}	0.22 ± 0.01^{bc}	0.21 ± 0.02^{bc}	0.27 ± 0.02^{c}	0.20 ± 0.03^{bc}	0.23 ± 0.02^{bc}
Iron	0.22 ± 0.02	0.12 ± 0.02	0.21 ± 0.05	0.21 ± 0.02	0.16 ± 0.01	0.21 ± 0.01	0.24 ± 0.11	0.18 ± 0.05
Aluminium	0.13 ± 0.03^{ab}	0.10 ± 0.02^{ab}	0.15 ± 0.02^{ab}	0.16 ± 0.01^{ab}	0.16 ± 0.02^{ab}	0.17 ± 0.03^{a}	0.15 ± 0.02^{ab}	0.16 ± 0.03^{ab}
Copper	0.09 ± 0.03	0.07 ± 0.02	0.06 ± 0.04	0.04 ± 0.01	0.01 ± 0.03	0.01 ± 0.02	0.01 ± 0.03	0.27 ± 0.05

[1]Mean values ± standard deviation in the same row with different superscripts are significantly different ($p<0.05$)

Table 3.5: Comparison of proximate composition (%) of different nutrients from raw and cooked *Crocodylus niloticus* meal, fishmeal to be used in fish feeds preparation (tested by SGS South Africa (Pty) Ltd and produced by Pioneer Fishing), and the recommended values for fishmeal of different grades adapted from Tacon, Metian et al. (2009)

Nutrients	**Tacon, Matien et al. (2009) values**			**Values from this study**		
	Grade 1	**Grade 2**	**Grade 3**	**Raw**	**Cooked**	**Fishmeal**
Moisture (%) maximum	10	10	10	10	13	9
Crude protein (%) minimum	60	50	40	83	78	69
Crude lipid (%) maximum	8	10	11	5	8	10
Ash (%) maximum	2	3	4	3	3	13
Hard and sharp solid materials	Not permitted					

Table 3.6: Quality specification for purchasing by-products meal from selected by products as recommended by Davis, (2015) and that of crocodile (*Crocodylus niloticus*) meal from the study

Parameters	% Values as recommended by Davies, (2005)					Crocodile meal from this study	
	Meat bone meal	Meat meal	Poultry by-product meal	Feather meal	Blood meal	Raw	Cooked
Moisture (%) maximum	10	10	10	10	10	10	13
Protein (%) Minimum	50 or as specified	55 or as specified	58	80	85	83	78
Fat (%)	10	10	11	5	0.5-2.0	5	8
Crude fibre (%) Maximum	3	3	3	4	2	0.02	0.09
Ash (%) Maximum	-	-	18	4	5	3	3

3.5. Discussion

The chemical composition of meal derived from *C. niloticus* carcasses including nutritional value were evaluated for consideration to use as fishmeal replacement in aquaculture feeds. According to Gatlin et al. (2007), the candidate ingredient to be considered as suitable to replace fish meal must be a widely available, have a competitive price, be ease to produce, handle, ship and store for use in feed production. Furthermore, it must possess certain critical nutritional characteristics, such as low levels of fibre, starch, especially non-soluble carbohydrates and anti-nutrients, and have a relatively high protein content, favourable amino acid profile, high nutrient digestibility and reasonable palatability (Gatlin et al. 2007).

According to (Ahn 2014), moisture content in feedstuff is an important factor for sale, purchase, transportation, and storage. Furthermore, high moisture content can result in moulding and shorten the shelf life of the meal. Recommended maximum moisture content for different grades of fishmeal (Tacon et al. 2009) and for quality specification for purchasing by-products meal such as meat bone meal, meat meal, feather meal, blood meal and poultry by-product meal is 10% (Davis 2015). Furthermore, fishmeal to be used in fish feeds preparation for Chapter 4 had 9% moisture content. *Crocodylus niloticus* derived meal tested in this study has averages of 10% for raw and 13% cooked moisture content which is within maximum recommended range reported by Tacon et al. (2009) and Davis (2015), as there were no significant differences in raw and cooked meal from different parts of *C. niloticus* carcasses.

Results from the present study include significant differences in crude protein from *C. niloticus* meal derived from different parts. The content was higher than 60% in all parts (which is the highest minimum recommended level for grade 1 fishmeal reported by Tacon et al. 2009) and 69% for fishmeal to be used in fish feeds preparation for growth experiment, Chapter 4. Proteins are regarded as the major growth-promoting factor in feed, excess protein not utilized efficiently for growth are used for deamination and excretion of excess amino acids absorbed (Jauncey 1982). Animal proteins source are considered good-quality proteins since they contain a good balance of essential amino acids.

According to Craig and Helfrich (2009), fats are high-energy nutrients that can be utilized to partially spare protein in aquaculture feeds. Furthermore, fats supply about twice the energy as proteins and carbohydrates (Craig and Helfrich 2009). A recent trend in fish feeds is to use higher

levels of lipids/fats in the diet. Although increasing dietary lipids may help reduce the high costs of diets by partially sparing protein in the feed, problems such as excessive fat deposition in the liver can decrease the health and market quality of fish (Craig and Helfrich 2009). Crocodile meal analysed in this study had less than 10% crude fat, which is within maximum recommended for different grades of fishmeal (Tacon et al. 2009) and 10% reported by SGS South Africa (Pty) Ltd for fishmeal to be used in fish feeds preparation for growth experiment in Chapter 4.

Fibre is known to provide physical bulk to the feed (De Silva and Anderson 1994). Furthermore, a certain amount of fibre in feed permits better binding and moderates the passage of feed through the alimentary canal. According to De Silva and Anderson (1994), it is not desirable to have a fibre content above 8-12% range in diets for fish because excessive fibre content results in lower digestibility of nutrients. The analysed crude fibre content of meal from different parts of crocodile under study were within the safe dietary limit for fish.

Minerals are inorganic elements necessary in the diet for normal body functions (Craig and Helfrich 2009). According to Watanabe et al. (1997), fish may derive these minerals from the diet and also from ambient water. Even though they are required in small quantities, minerals are important for skeletal formation, maintenance of colloidal system, regulation of acid-base equilibrium and biologically important compounds such as hormones and enzymes (Watanabe et al. 1997). If excess amounts of the elements are ingested or assimilated, toxicity may develop and resulting in crocodile meal being unsuitable as animal feeds.

Even though there were no significant differences in moisture and ash contents of raw mixture meal and cooked mixture meal, cooking had significant effect on the crude protein level and crude fat content. This may be due to heating, that causes the protein to denature and loses its nutritional value. Considering recommended proximate composition of fish meal of different grades (Tacon et al. 2009), fishmeal composition, and quality specification for purchasing by-product meal such as meat bone meal, meat meal, feather meal, blood meal and poultry by-product meal (Davis 2015), the results of currents study indicate that *C. niloticus* meal meet quality specifications and that means *C. niloticus* meal can be used as fishmeal replacer in aquaculture feeds. Raw mixture meal should be prioritized as it has more protein level.

3.6. Conclusion

Considering quality specification of by-product meal such as meat bone meal, meat meal, feather meal, blood meal and poultry by-product meal, our study showed that *C. niloticus* meal meet quality specifications for aquaculture feeds. Future studies should be aimed at determining the quality of crocodile meal in controlled animal feeding, by measuring growth performance, feed conversion ratio, health, physiology, and digestibility of feeds containing crocodile meal.

3.7. Acknowledgements

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CHAPTER FOUR

Fishmeal replacement with animal protein source (*Crocodylus niloticus* meat meal) in diets of Mozambique tilapia (*Oreochromis mossambicus*) of different size groups

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4.1. Abstract

Fish are generally known to change their nutritional requirements depending on their life stage and formulating feeds for different size groups to meet their dietary needs is essential. This study aimed to assess the potential of *Crocodylus niloticus* meat meal as an animal protein source replacing fishmeal in *Oreochromis mossambicus* diets. Ten fry (0.07 g $fish^{-1}$) were randomly assigned to three diets (0% (D1), 50% (D2), and 100% (D3) formulated, and each diet had three replicates. The fry were fed 10% body weight per day (BWd^{-1}) for 30 days. New diets (0% (D4), 50% (D5), and 100% (D6) were introduced, and the feeding rate was reduced to 5% BWd^{-1} for 48 days. After that, fish were fed 2% BWd^{-1} for 78 days the same diets used for fingerlings. All size groups were fed two portions of their daily ration at 10:30h and 15:30h. Our results point to the suggestion that *Crocodylus niloticus* meat meal may replace fishmeal for *Oreochromis mossambicus* as there were no significant differences in weight gains (G), specific growth rates (SGR), gross feed conversion ratios (GFCR), and protein efficiency ratios (PER) for fry fed different diets. Furthermore, there were similarities in Gs, SGRs, GFCRs, and PER in fingerlings and sub adult to adult fish fed D4 and D5. The cost analysis of ingredients used in diets with 50% and 100% *Crocodylus niloticus* meat meal indicated that, it was profitable to use this meat meal in diets of *O. mossambicus* of all groups. The profit index of 0.3 for fry, 0.8 for fingerlings, and 1.9 for subadult to adult for 100% fishmeal diets were lower than 0.4 and 0.5 for fry, 0.9 and 1.1 for fingerlings, and 2.3 and 2.9 for sub adult to adult fish fed diets with 50% and 100% crocodile meat meal, respectively.

Keywords: animal protein source, *Crocodylus niloticus* meal, fishmeal replacement, *Oreochromis mossambicus*, fish size

4.2. Introduction

Oreochromis mossambicus is a freshwater species that belongs to the family Cichlidae [1]. This species is currently vulnerable in Southern Africa [1, 2]. The vulnerability is due to the introduction of invasive species, such as *Oreochromis niloticus*, hybridization, habitat competition, and diseases as the main reasons for decreasing *O. mossambicus* in their natural habitat [3–9]. Therefore, it is essential to conserve this species through aquaculture. *Oreochromis mossambicus* is regarded as a good candidate for aquaculture, because of high fecundity, its ability to utilize both plant and animal nutrients for growth efficiently, increased meat quality and good consumer acceptance, potential to develop value-added fish products, and resistance to diseases [10–12]. However, the success of any aquaculture species depends on the supply of adequate nutrients, both in quantity and quality [13]. Due to the rapid growth of aquaculture, some resources, such as fishmeal, are limited.

Fishmeal production is mainly sourced from the forage fish species [14]. Forage fish are described as the prey for other animals to eat [14]. They play an essential role in marine ecosystems because they transfer energy from primary producers (e.g., plankton) to higher trophic-level species, including large fish, marine mammals, and sea birds [14]. Fish caught for fishmeal production potentially represent a loss in production of higher trophic level species in the ecosystem because low stock abundance reduces ecosystem services, such as food provisioning, to other elements of the ecosystem [15]. According to [16], numerous studies have shown that animal by-product meals arising from the processing of slaughtered farm livestock offer great potential for use as dietary fishmeal replacements within aquaculture feed. A review of animal protein sources used in aquaculture diets showed that some by-products, such as crocodile meat, have not been assessed as a fishmeal replacement [13], except for the recent study on juvenile dusky kob (*Argyrosomus japonicus*) by [17]. Although fish are known to change their nutritional requirement depending on their life stage [18], different size groups are not considered when these animal protein sources are evaluated in aquaculture. Furthermore, the nutritional values of the crocodile meat meal are comparable to other by-products, such as meat bone meal, meat meal, feather meal, blood meal, and poultry by-products meal used in aquaculture [19,20], and different grades of fishmeal [21]. The world's demand for proteins of animal origin is expected to double by 2050 [22].

Furthermore, fishmeal is the most expensive component of aquaculture feeds because of its competing use as a feed ingredient for other livestock species [23]. Seventy-five percent of the world's fish stocks used for fish meal production are currently considered fully exploited or overexploited, including many small pelagic fish [24]. Increasing demand, unstable supply, and the high price of the fishmeal with an expansion of aquaculture have resulted in the need to search for alternative protein sources.

Crocodiles are cultured mainly for producing skins used to create high-quality fashion accessories [25]. As in fish farming, the increase in production costs in this industry forced the farmers to look at alternative means of increasing profitability [26]. The major components of source of profitability are skin production and meat production and all these are related to tourism. However, the demand for crocodile meat, especially in South Africa, is low and strict regulations are imposed on the industry regarding the use and disposal of crocodile carcasses. According to [26], crocodile meat is re-used on farms as unprocessed feed for other crocodiles and public health regulations are enforced in processing crocodilian meat for human consumption. This involves the design, construction, operation of abattoirs, food safety standards, and procedures explicitly established for the processing of crocodilians, making the whole process less cost effective [27]. Furthermore, managing of the abattoirs come with additional responsibilities related to packaging, labelling, shipping, and record-keeping [28]. Hence, this study aimed to assess the potential of *Crocodylus niloticus* meat meal as an animal protein source, replacing fishmeal in the diet of *Oreochromis mossambicus*. We hypothesized that, i). there is no difference in growth performance, feed utilizations, and survival rates of *Oreochromis mossambicus* fed diets with *Crocodylus niloticus* meat meal replacing fishmeal of different size groups. ii) there is no difference in feed costs among diets with fishmeal and those with crocodile meat meal. To our knowledge, to date there are no studies that have been conducted targeting at replacing fishmeal with *Crocodylus niloticus* meat meal in diets of *Oreochromis mossambicus*. If using *Crocodylus niloticus* meat meal becomes suitable, the aquaculture industry will benefit by reducing supply constraints imposed by high cost and competitive uses for fishmeal. This could also translate into less dependence on marine-derived protein sources currently being over-exploited. The study's findings could be used to produce more fish as a source of protein for poor communities and contribute to poverty alleviation and food security. Furthermore, the findings and

recommendations on crocodile meat meal could be beneficial to crocodile farmers who are finding it costly to dispose of crocodile meat as a by-product for the skin in South Africa.

4.3. Materials and Methods

4.3.1. Processing crocodile meat into meal

Crocodile meat was purchased from Shallow Drift Crocodile Abattoir, South Africa. The meat was cut into small thin pieces, dried in an oven for three hours, ground using the laboratory blender (Model HQBTWTS3, Waring Commercial, Torrington Connecticut, United State of America), and then sieved through a 1.0-micron mesh. The resulting crocodile meal samples were taken to the Animal Science Department, University of KwaZulu Natal for proximate analysis, while some samples were sent to Stellenbosch University for amino acids analysis and the rest were stored in a heavy-duty plastic bag at room temperature until used for fish feeds preparation. Nitrogen (N) content was determined on a Leco TruMac^{R} Carbon Nitrogen Sulfur elemental analyzer using Dumas's combustion. Crude protein was calculated as N × 6.25. Crude fat content was determined using the Soxhlet method, as described in the AOAC Official Method 920.39 [29]. Crude fiber was determined as the loss of ignition of dried lipid-free residues with 1.25% H_2SO_4 and 1.25% NAOH solutions, using the filter bag technique with the ANKOM Fibre Analyzer 200. Moisture content was determined using an air-circulated oven at 95 °C for 72 h. Ash content was determined by burning pre-weighed samples in a muffle furnace at 550 °C overnight, as described in the AOAC Official Method 942.05. Minerals were determined using the fast sequential atomic absorption spectrometer (AA280FS) [30], after ashing the samples. The results are shown in Table 4.1, which also include fishmeal composition.

Table 4.1. Composition of raw mixture crocodile meal and fishmeal used in the formulations of *Oreochromis mossambicus* diets.

Nutrient (%)	**Raw mixture Crocodile meal**	**Fishmeal**
Crude protein	83.04	69
Moisture	9.78	9
Crude fat	4.48	10
Ash	2.41	13
Crude fiber	0.04	-
Selected Minerals (%)		
Potassium	32.90	-
Sodium	11.17	-
Calcium	1.93	-
Magnesium	1.49	-
Zinc	0.22	-
Iron	0.21	-
Aluminium	0.16	-
Copper	0.04	-
Essential Amino Acids (g/100g dry matter)		
Argenine	7.55	-
Histidine	4.88	-
Isoleucine		
Leucine	8.06	-
Lysine	7.27	6.32
Methionine	4.53	2.25
Phenylalanine	8.37	-
Threonine	8.37	-
Valine	4.32	-
Non-Essential Amino Acids (g/100g dry matter)		
Alanine	5.88	-
Asparagine	8.61	-

Glutamic acid	14.34	-
Glycine	5.82	-
Proline	3.06	-
Serine	4.27	-
Tyrosine	6.28	-

4.3.2. Diets

Six experimental diets (Table 4.2) were formulated for different size groups of O. mossambicus. D1 and D4 are diets with 0% of crocodile meat meal, D2 and D5 are diets with 50% fishmeal and 50% crocodile meat meal, D3 and D6 are diets with 100% crocodile meat meal. All the diets are for fry and fingerlings, respectively. D1, D2, and D3 for fry had 38% crude protein, D4, D5, and D6 for fingerlings had 32% crude protein. All experimental diets were prepared by pre-weighing all dry ingredients separately. The combined ingredients in a bowl, were mixed for fifteen minutes. The raw mixture meal was chosen over the cooked mixture meal because raw mixture meal had significantly higher protein level than cooked mixture meal. Furthermore, more time was required to produce cooked mixture meal. Eight ml of water was added per kg of dry ingredients to make a dough. The dough was pelleted using a hand-operated meat mincer, and the pellets were dried in the sun [31]. All diets were used as treatments. Samples of all diets were analysed for moisture, crude fat, and crude ash at the Animal Science Department at the University of Kwa-Zulu Natal using the procedures explained for crocodile meat meal, while some samples were sent to Stellenbosch University for amino acids analysis. The results of proximate analysis for experimental diets are presented in Table 4.2.

Table 4.2. Main ingrediets and proximate composition of the experimental diets

	Fry diets (D1-D3)			Fingerlings, sub-adult, and adult fish (D4-D6)		
Ingredients (g/kg)	**D1 (0% CM)**	**D2 (50%/50% FM/CM)**	**D3 (100% CM)**	**D4 (0% CM)**	**D5 (50%/50%FM/ CM)**	**D6 (100% CM)**
Fishmeal[1]	25.000	12.500	-	15.000	7.500	-
Maize[2]	20.000	20.139	20.279	30.000	30.000	30.000
Crocodile meal[3]	-	9.032	18.063	-	8.406	16.812
Soybean meal 46[4]	15.000	15.000	15.000	15.000	15.000	15.000
Canola seed meal[5]	15.000	15.000	15.000	15.000	15.000	15.000
Maize gluten 60[6]	10.000	10.000	10.000	8.324	6.436	4.548
Wheat bran[7]	8.601	9.174	9.746	4.936	4.264	3.592
Canola oil[8]	3.531	4.309	5.087	4.356	4.774	5.191
Monocalcium phosphate[9]	1.719	2.940	4.161	1.031	4.565	8.099
Vitamin premix[10]	0.800	0.800	0.800	0.800	0.800	0.800
Limestone[11]	-	-	-	3.528	2.244	0.959
L-lysine HCL[12]	0.349	1.107	1.864	2.024	1.012	
Total	100	100	100	100	100	100
Proximate composition (%)						
Moisture**	8.995	8.895	8.994	8.627	8.517	8.746
Crude protein*	38	38	38	32	32	32
Crude fat**	8.842	8.090	6.408	8.344	8.935	5.446
Ash**	7.536	7.632	7.413	9.185	9.674	10.949
DE (MJ/kg) *	13.426	11.707	9.988	12.840	11.320	9.800

Essential Amino acids (g/100g dry matter)						
Arginine***	2.82	2.87	3.05	2.36	3.31	2.83
Histidine***	2.03	1.94	1.90	1.63	2.27	1.61
Isoleucine***	1.53	1.56	1.63	1.30	1.45	1.45
Leucine***	3.36	3.54	3.79	3.00	3.13	2.99
Lysine***	2.04	3.24	4.13	3.02	1.52	2.11
Methionine***	1.26	1.22	1.32	0.97	1.29	1.12
Phenylalanine***	3.82	3.87	4.04	3.11	3.88	2.89
Threonine***	2.35	2.39	2.50	2.00	2.64	2.24
Valine***	1.93	1.93	1.94	1.67	1.82	1.73
Non-essential amino acids (g/100g dry matter)						
Alanine***	2.33	2.54	2.65	2.12	2.02	2.05
Asparagine***	3.24	3.85	4.14	3.08	3.34	3.21
Glutamic acid***	6.34	7.62	8.45	6.23	6.86	6.32
Glycine***	2.65	2.59	2.62	2.04	2.66	2.43
Proline***	2.16	2.28	2.47	1.95	1.99	1.90
Serine***	2.12	2.16	2.24	1.88	2.39	1.95
Tyrosine***	2.85	2.92	3.27	2.32	3.15	2.33

FM-Fishmeal, CM- Crocodile meat meal, [1]Pioneer Fishing, [2 + 8]Spar, [3] Shallow Drift Abattoir, [4] Irwing Soya,[5] Southern Oil (Pty)ltd, [6] Tongaat-Hulett, [7]Milmac, [9]Bragan Chemicals, [10] SA Premix, [11]Idwala Industrial Holdings (Pty) ltd, [12] Protea Chemicals, all from South Africa. *Taken from feed formulations. **Analysed at Animal Science department, University of KwaZulu Natal, South Africa. ***Analysed at Stellenbosch University, South Africa

4.3.3. Experimental fish

The newly hatched fry were purchased from the University of Zululand, Department of Zoology, South Africa and transported to the University of KwaZulu Natal. They were allowed to acclimatize in the experimental tanks for 14 days before the feeding trial. The fry were fed a commercial diet purchased at the local supplier Avi-Products (Pty)Ltd., Pietermaritzburg, South Africa, during the acclimation period. It was not possible to separate sexes into males or females to avoid fish breeding, a small -size animals were used. Furthermore, the formulated diets tested are to be used in commercial farming and using both sexes is essential.

4.3.4. Feeding experiment

Three parts study was conducted at Animal House, University of KwaZulu-Natal, Pietermaritzburg campus, South Africa. Ninety fry with an initial weight of 0.07 ±1 g $fish^{-1}$ were randomly assigned to three treatments as follows: D1(0% crocodile meat meal with 100% fish meal, D2 (50% crocodile meal and 50% fishmeal, and D3 (100% crocodile meat meal and 0% fishmeal, in nine glass tanks with a volume of 40 liters each. The study was conducted in triplicates, and aeration was supplied to fish tanks by air stones connected to air pumps to maintain the oxygen supply to the fish, and water was circulated using 400 L/h submersible water pumps that were in containers with gravel and sand as filters. Ten individuals were used in each of the three replicates totalling 30 *O. mossambicus* for each treatment. The feeding trial conditions were maintained at a temperature of 28± 2℃, and the light was set at 12h dawn and 12h dark cycle.

In Part 1, the fry were fed two portions of their daily feed ration (10%) [32], two times a day (at 10:30h and 15:30h) for 30 days. The planned feeding frequency for fry was four times a day as recommended by [33] but changed to 2 times per day due to COVID-19 restrictions.

After 30 days of feeding, in part 2, all fish were fed new three diets, which are D4, D5, D6 diets with the same crocodile meal percentage as D1, D2, and D3, respectively. The feeding rate was reduced to 5% of their body weight per day in each replicate and fed two times per day (at 10:30h and 15:30h) for 48 days.

After 48 days, in part 3, all groups were considered as sub-adults to adults, and the same fingerling diets (D4, D5, and D6) were fed at a feeding rate of 2% body weight per day, was fed to fish twice a day (at 10:30h and 15:30h) [34] for 84 days. Water quality parameters and

mortality were monitored throughout the experimental period and were within tilapia tolerable ranges.

4.3.5. Growth and feed utilization measurements and calculations

For all three parts, the fish were weighed individually on a weekly interval from each tank, and the mean wet weight for fish per tank was calculated. Fish weight measurements were carried out to determine the correct amount of feed fed to experimental tanks per week as fish grow. The weight gain (G), specific growth rate (SGR), growth feed conversion ratio (GFCR), protein efficiency ratio (PER), and survival rate (SR) were calculated using the following formulae as reported by [33-35].

A. Weight gain (G) (g) = Final Weight (FW) - Initial Weight (IW)
B. Specific growth rate (SGR) (%/day) = Ln (Final Weight) - Ln (Initial Weight)/experimental duration (days) x 100
C. Gross feed conversion ratio (GFCR) = Weight of food fed/ Weight gain
D. Protein efficiency ratio (PER) = Weight gain/protein fed
E. Survival rate (SR) (%) = Number of surviving fish/ number of fish stocked x 100

It was not possible to measure the total quantity of food not consumed, therefore, the gross feed conversion ratio (GFCR) was calculated instead of the feed conversion ratio (FCR), which is the ratio between feed intake and weight gain measured during the experimental period [36]. The results were reported according to different size groups as per feeding rate adjustment.

4.3.6. Feed cost analysis

Incidence cost and profit index were calculated as an economic indicator using the following formulae as described by [37], assuming that only ingredients costs are the only variable costs and operating costs are constant.

F. Incidence cost = $\frac{\text{Cost of feed}}{\text{Quantity of fish produced (kg)}}$

G. Profit index = $\frac{\text{local market value of fish}}{\text{Cost of feed}}$

The estimated market prices were ZAR3.00 for fry, ZAR6.00 fingerlings and ZAR15.00 for sub adult to adult fish per fish, based on the University of Zululand sale prices.

4.3.7. Statistical analysis

The SPSS software Version 27 [38] to analyze the data. We checked data for normality using The Shapiro-Wilk test. The means of the treatments were tested for significant differences using One-way analysis of variance (ANOVA) at a significance level of $\alpha=0.05$. The results were considered significantly different at a probability of $p<0.05$. Tukey's multiple comparison test was used to compare the variance among the means differentiation.

4.4. Results

4.4.1. Weight gain

The weight gains of *O. mossambicus* fry were not significantly different among all diets fed (Table 4.3). The weight gains for fingerlings, and sub-adult to adult fish were significantly different ($p< 0.05$, ANOVA) among the experimental diets fed (Table 4.3). The fingerlings fed D4 and D5 had significantly higher ($P < 0.05$, Tukey test) weight gain than those fed D6 diet. For the sub-adult to adult fish, the weight gain of those fed D4 was significantly higher than those fed D6, but similar to those fed D5. There were no significant differences ($P < 0.05$, Tukey test) in weight gains of sub adult to adult fish fed D5 and D6 (Table 4.3).

4.4.2. Specific growth rate

There were no significant differences in the specific growth rate of the fry fed different diets. There were significant differences ($P < 0.05$, ANOVA) in SGR of *O. mossambicus* fingerlings and sub-adult to adult fish. Significant differences ($P < 0.05$, Tukey test) were observed in fingerling fed D4 and D6. Those fed D5 had specific growth rate similar to fish fed both D4 and D6 diets. For the sub-adult to adult size group, the differences were among O. *mossambicus* fed D6, which was significantly lower than those fed D4 and D5 ($P < .05$, Tukey test).

4.4.3 Gross feed conversion ratio

There were no significant differences ($P > 0.05$; ANOVA) in GFCRs of *O. mossambicus* fry among all diets fed (Table 4.3). Gross feed conversion ratios of 2.19 (D1), 2.28 (D2), and 2.17 (D3) were recorded. However, GFCRs for fingerlings and sub-adult to adult size groups were significantly different ($P < 0.05$, ANOVA) among diets fed. The GFCR of 2.0 (D4) was significantly better ($P < 0.05$, Tukey test) than 3.18 (D5) and 5.2 (D6) for fingerlings of *O. mossambicus.* The GFCR of *O. mossambicus* sub-adult to adult fish fed the D5 diet was similar to those fed the D6 diet but significantly different ($P < 0.05$, Tukey test) from those provided D4 diet.

4.4.4. Protein efficiency ratio

The protein efficiency ratios for *O. mossambicus* fry were not significantly different among all diets fed. There were significant differences ($P < 0.05$, ANOVA) in PER for fingerlings and subadult to adult fish size groups. The PER was significantly lower for fingerlings and sub-adult to adult fish ($P < 0.05$, Tukey test) fed D6 than those fed D4, and D5.

4.4.5. Survival rate

There were significant differences ($P < 0.05$, ANOVA) in the survival rate of all size groups fed different diets. The survival rate for *O. mossambicus* ranged between 90% to 96.7% for fry, 90% to 93.3% for fingerlings, 68.57 to 89.63% for sub-adult to adult fish were observed.

4.4.6. Weekly mean weights of experimental groups

The results showed that the weekly mean body weight of *O. mossambicus* fed with different diets were significantly different ($P < 0.05$; ANOVA) from the second week of feeding to week four. However, there were no significant differences in weight gain among all diets from five to week nine. Then from week ten, differences in weight gains among the diets continued until week twenty-five when the experiment was terminated (Table 4.4). Regardless of all the differences, all fish in all groups increased their body weight. Initial mean weights were similar in all diets. Egg production was not part of the parameters measured. However, eggs were observed during the weekly mean weight measurements. In fish fed D4 (at week 17 and week 20), D5 (at week 19), and there were no eggs nor fry observed in D6 during the experimental period.

4.4.7. *Economic analysis*

All size groups fed diets with 50% fishmeal and 50% crocodile meal had consumed more feed than other groups (Figure 4.1a-c). The costs for known ingredients show that using 100% fish meal (D1 and D4) was more costly than using 50% (D2 and D5) and 100% (D3 and D6) crocodile meal (Table 4.3). The profit index indicates that it is profitable to use 100% and 50% crocodile meal in diets of *O. mossambicus* of all size groups.

Table 4.3. Mean initial weight (IW), final weight (FW), weight gain (G), specific growth rate (SGR), gross food conversion ratio (GFCR), protein efficiency ratio (PER) and survival rate (SR) of *Oreochromis mossambicus* fry fed for 30 days, fingerlings fed for 48 days, and sub-adult to adult fed for 84 days different diets with crocodile meal as fishmeal replacement. Values are means (±SD) of three replicates for each treatment. The results were significantly different at $p < 0.05$. Degree of freedom between groups = 2, within groups = 8, F = F Statistic, and P = Probability.

Fry performance					
Variables	**D1**	**D2**	**D3**	**F**	**P**
IW (g)	0.0837±0.007[a]	0.0743±0.005[a]	0.0700±0.011[a]	2.078	0.220
FW (g)	0.8090±0.138[a]	0.8487±0.516[a]	0.6840±0.495[a]	1.850	0.250
G (g)	0.7253±0.144[a]	0.7743±0.052[a]	0.8140±0.061[a]	1.5513	0.299
SGR (%/day)	7.5400±0.838[a]	8.1187±0.302[a]	7.6155±0.783[a]	0.648	0.560
GFCR	2.1933±0.814[a]	2.2833±0.238[a]	2.1650±0.064[a]	0.035	0.960
PER	0.0191±0.0038[a]	0.0204±0.0013[a]	0.0162±0.0016[a]	1.878	0.246
SR (%)	90.00±<0.001[a]	96.70±5.773[b]	90.00±<0.001[a]	144.40	<0.001
Incidence cost	11.36	8.75	8.27	-	-
Profit index	0.3	0.4	0.5	-	-
Fingerlings	**D4**	**D5**	**D6**	**F**	**P**
IW (g)	0.8090±0.138[a]	0.8487±0.516[a]	0.6840±0.495[a]	1.850	0.250
FW (g)	3.9740±0.224[a]	3.4367±0.616[a]	2.0910±0.169[b]	12.220	0.011
G (g)	3.1650±0.100[a]	2.5880±0.577[a]	1.4075± 0.119[b]	13.336	0.009
SGR (%/day)	3.3340±0.243[a]	2.8947±0.281[b]	2.3275±0.0168[b]	11.008	0.015
GFCR	2.0467±0.085[a]	3.1867±0.293[b]	5.2450±0.431[c]	81.809	<0.001
PER	0.0989±0.0031[a]	0.0809±0.0180[a]	0.0439±0.0037[b]	13.334	0.009
SR (%)	90.00±<0.001[a]	93.33±5.774[b]	90.00±<0.001[a]	77.45	<0.001
Incidence cost	1.96	1.90	2.50	-	-
Profit index	0.8	0.9	1.1	-	-
Subadult to adult	**D4**	**D5**	**D6**	**F**	**P**
IW (g)	3.9740±0.224[a]	3.4367±0.616[a]	2.0915±0.169[b]	12.220	0.011
FW (g)	10.7100±0.629[a]	8.4400±2.268[ab]	4.6250±0.502[b]	9.809	0.018
G (g)	6.7360±0.53[a]	5.0033±1.757[ab]	2.5335±0.333[b]	7.724	0.029
SGR (%/day)	1.1767±0.061[a]	1.0467±0.198[a]	0.9450±0.035[a]	2.032	0.225

GFCR	2.0367±0.200[a]	3.2967±0.545[b]	3.640±0.665[b]	8.578	0.024
PER	0.2102±0.0167[a]	0.1564±0.05492[ab]	0.0797±0.0104[b]	7.849	0.028
SR (%)	77.33±2.0817[a]	89.63±4.7035[b]	70.85±4.0501[a]	19.084	0.0025
Incidence cost	0.73	0.77	1.13	-	-
Profit index	1.9	2.3	2.9	-	-

Mean values ± standard deviation within same row with different superscripts are significantly different ($p<0.05$)

Table 4.4. Weekly mean weights (±SD) for *Oreochromis mossambicus* fed different diets for 25 weeks. Degree of freedom between groups = 2, within groups = 8, F = F Statistic, and P = Probability.

Weeks	**D1**	**D2**	**D3**	**F**	**P**
Initial weight	0.0837±0.007	0.0743±0.005	0.0700±0.011	2.078	0.220
Week 1	0.1247±0.172	0.1167±0.016	0.0920±0.008	2.895	0.146
Week 2	0.2480±0.033^{a}	0.2113±0.041^{ab}	0.1355±0.002^{b}	6.849	0.037
Week 3	0.4817±0.021^{a}	0.2923±0.102^{b}	0.2370±0.014^{b}	9.867	0.018
Week 4	0.8487±0.155^{a}	0.4387±0.027^{b}	0.3450±0.027^{b}	13.928	0.009
Week 5	0.8090±0.137^{a}	0.8487±0.052^{a}	0.6840±0.049^{a}	1.850	0.250
Week 6	1.0063± 0.060^{a}	1.0307±0.200^{a}	0.8370±0.068^{a}	1.362	0.337
	D4	**D5**	**D6**		
Week 7	1.3160±0.042^{a}	1.2587±0.212^{a}	1.0670±0.048^{a}	2.029	0.226
Week 8	1.6290±0.092^{a}	1.5953±0.216^{a}	1.3505±0.445^{a}	2.324	0.193
Week 9	2.0443±0.096^{a}	1.9787±0.223^{a}	1.6190±0.072^{a}	5.623	0.053
Week 10	2.6067±0.139^{a}	2.3803±0.306^{ab}	1.8005±0.094^{b}	8.485	0.025
Week 11	3.2800±0.235^{a}	2.8560±0.441^{a}	1.8875±0.121^{b}	11.501	0.013
Week 12	3.9740±0.224^{a}	3.4367±0.616^{a}	2.0915±0.169^{b}	12.222	0.012
Week 13	4.8200±0.164^{a}	4.1400±0.466^{a}	2.3350±0.125^{b}	12.583	0.011
Week 14	5.5600±0.403^{a}	4.3967±0.819^{a}	2.5250±0.219^{b}	16.260	0.006
Week 15	6.3567±0.580^{a}	4.7067±0.929^{ab}	2.7500±0.240^{b}	16.054	0.007
Week 16	6.7033±0.280^{a}	5.0500±1.058^{ab}	3.0900±0.184^{b}	15.970	0.007
Week 17	7.4100±0.302^{a} eggs	5.5133±1.210^{ab}	3.0650±0.177^{b}	16.548	0.006
Week 18	7.7867±0.448^{a}	5.9000±1.272^{a}	3.3300±0.226^{b}	16.177	0.007
Week 19	8.7133±0.520^{a}	6.4867±1.677^{ab} eggs	3.2800±0.269^{b}	13.994	0.009
Week 20	9.1267±0.615^{a} eggs	6.7733±1.708^{ab}	3.7500±0.382^{b}	12.882	0.012
Week 21	9.5233± 0.527^{a}	7.4000± 2.053^{ab}	3.9300± 0.339^{b}	10.322	0.017
Week 22	9.5733±0.665^{a}	7.8067±2.128^{ab}	4.2750±0.559^{b}	8.727	0.023
Week 23	9.6800±1.773^{a}	8.0333±2.073^{ab}	4.4900±0.424^{b}	8.755	0.023
Week 24	10.3433±0.885^{a}	8.3133±2.234^{ab}	4.5750±0.459^{b}	6.959	0.036
Week 25	10.7100±0.629^{a}	8.4400±2.268^{ab}	4.6250±0.502^{b}	9.737	0.018

Mean values ± standard deviation within same row with different superscripts are significantly different.

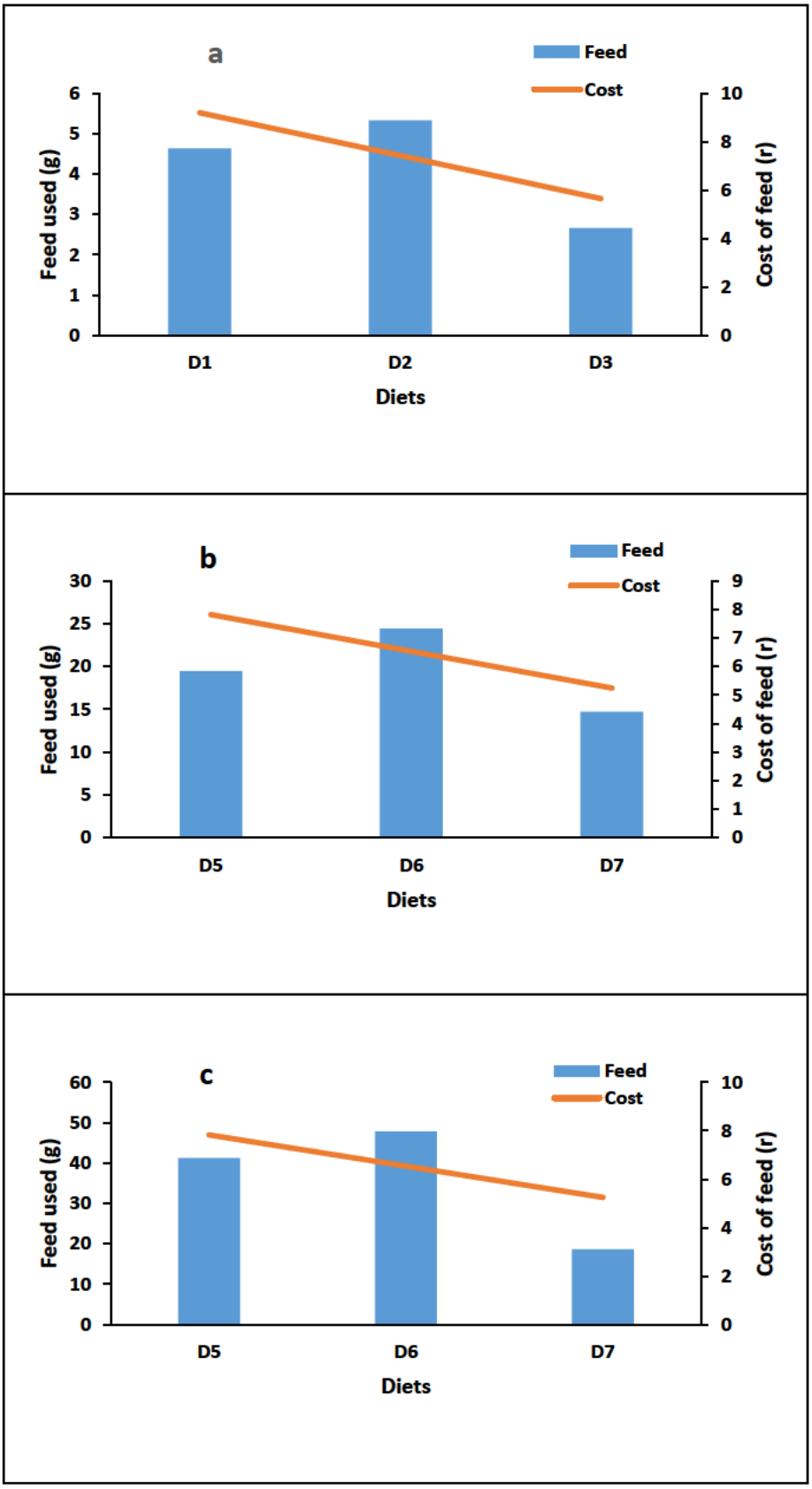

Figure 4.1: Feed used (grams) and cost of feed in South African rands (r) for (a) fry, (b) fingerlings, (c) sub adult to adult fish.

4.5. Discussion

Fish are generally known to change their nutritional requirements depending on their life stage [18] and formulating feeds for different size groups to meet their dietary needs is essential. Studies conducted on fishmeal replacement by animal protein sources, as reviewed by [13] did not consider different size groups of fish when testing various ingredients in the diets. Furthermore, the control diets used in the reviewed studies were not commercial feeds, and the diet formulations were used without the protein source tested [39-42].

Producing high quality fish, reducing the cost of feed, and minimizing the use of forage fish used in fishmeal production (pressure in the marine ecosystem) are the main reasons for the need to replace fishmeal which is the primary source used in animal feeds [43, 44]. To our knowledge, studies conducted on the evaluation of crocodile meals as a fishmeal replacement in aquaculture for different size groups of *O. mossambicus* diets are scanty. From our previous study on the nutritional value of the Nile crocodile meal, we recorded values comparable to other by-products from poultry such as feather meal, blood meal, and bone meal used in aquaculture diets [20].

The weight gain for fry was not significantly different among all diets. This may be because all the diets had similar crude protein level, moisture, and ash. However, fingerlings fed D6 had significantly lower weight gain than those fed D4 and D5. Sub adult to adult fish fed D4 had weight gain similar to those fed D5; the fish fed D6 had significantly lower weight gain than those fed D4 but similar to those fed D5. Diets with high-fat contents are known to have high concentrations of saturated fats and are characterized by high 18:2 n-6 polyunsaturated fatty acids (PUFA), which reduces the palatability of fish diets [17,45]. Our results are not in agreement with the findings of [46], who reported reduced growth performance, due to the high fat content in silver sea bream *Rhabdosargus sarba* fed a poultry by-product meal diet above the 25% substitution level. The D6 group had lower crude fat content and lower weight gain compared to those fed D4 and D5.

According to [47], fish growth rate decreases as they grow older. Our study results agree with [47]'s statement, as the SGRs of *O. mossambicus* fry ranged from 7.54% to 8.11%, and it was significantly higher than 2.89% to 3.33% for fingerlings and 0.94% to 1.17% for the sub-adults and adults. Similar results were reported by [48] for *O. niloticus* fry and young tilapia.

The feed conversion ratio determined by other authors is similar to GFCR in the current study, and it is defined as the amount of feed consumed to produce one unit of animal biomass gain [49]. Higher efficiency is indicated by lower GFCR values [50]. Gross feed conversion

ratios of 2.16 to 2.28 in fry, fingerlings, sub-adult, and adult were observed, except for D5 (3.18) and D6 (5.24) for fingerlings, and sub adult to adult fish. Regardless of fish species, initial weight, duration of the experiment, and feeding frequency used, our results are similar to those reported for the recommended levels of other animal protein sources, such as mopane worms (*Imbrasia belina*), grasshopper (*Zonocerus variegate*), field cricket (*Gryllus bimaculatus*), blowfly maggot (*Chrysomya megacephala*), super worm (*Zophobas morio*), fermented feather meal, feather meal, poultry by-products, fish silage, and shrimp head meal [40,51–56] used in aquaculture. Our GFCR values were also within the range of 1.0 to 2.4 reported for farmed fish species, such as Atlantic salmon (*Salmo salar*), Chanel catfish (*Ictalurus punctatus*), common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*), rainbow trout (*Oncorhynchus mykiss*), and shrimp species, such as giant tiger prawn (*Penaeus monodon*) and whiteleg shrimp (*Litopenaeus vannamei*) [49]. Lower efficiency was observed in higher GFCR values reported for D5 and D6 for fingerlings, sub-adults, and adults, possibly because the diets were changed from fry to fingerling diets, and the feeding rate was reduced from 10% to 5%, regardless of the size of the fish. Therefore, maybe 5% was just enough for fingerlings fed D5 and D6 diets to maintain their weight and not enough for them to grow.

The protein efficiency ratio measures how the protein source in a diet can provide the essential amino acids required by fish [37]. Regardless of their sizes, *Oreochromis mossambicus* requires 0.99 g/100g Methionine, 3.79 g/100g Lysine, 0.43 g/100g Tryptophan, 2.83 g/100g Arginine, 1.80 g/100g Tyrosine, 1.05 g/100g Histidine, 2.93 g/100g Threonine, 2.01 g/100g Isoleucine, 3.40 g/100g Leucine, and 2.50 g/100g Phenylalanine [37]. All our diets had less Methionine than required. This could be the main reason for the lower PER in D6, as it was lower in Methionine than those required by *O. mossambicus* which resulted in lower energy than the other diets.

The survival rates in all size groups from the study were higher than 50%. There were significant differences in all size groups. Fish fed D2 and D5, diets with 50% fishmeal and 50% crocodile meal had significantly higher survival rates than those fed D1, D3, D4, and D6. The mortality recorded was mainly due to fish jumping from tanks even though nets were used to cover the tanks. The lower survival rate in sub adult and adults may be because the fish stocked as fry were used throughout the experimental period, which was 25 weeks.

Even though the early sexual maturity of *O. mossambicus* is generally known [57], our study did not use monosex fish which are preferred in aquaculture projects [65], we used both males and females *O. mossambicus* because it was difficult to differentiate them as males or females at the start of the experiment as smaller fish were used. Furthermore, the tested diets

were formulated to be used in commercial production. Therefore, it is essential to use both sexes. Using both males and females in one tank resulted in fish breeding when they were still small. During the subadult and adult stage, eggs were first observed at the 100% fish meal without crocodile meal, and then at 50%/50% fishmeal and crocodile meal. There were no eggs nor fry observed at 100% crocodile meal-based diet. These results could mean that crocodile meal is a good source of protein that will delay sexual maturity in *O. mossambicus*. Tilapia females have a lower growth rate than males in general [59]. Even though it is a common feature of fish in a population to have variation in individual growth, in commercial fish culture, it is a drawback as size determines the price [60].

The costs for ingredients used in feed preparations were higher in diets with fishmeal than those with crocodile meal in all size groups in the current study.

4.6. Conclusion

There were no significant differences in weight gain, specific growth rates, gross feed conversion ratios or protein efficiency ratios of *O. mossambicus* fry fed different diets. Considering the similarities in Gs, SGRs, GFCRs, and PER in fingerlings and sub-adult to adult fish fed D4 and D5, the *Crocodylus niloticus* meat meal has the potential to substitute fishmeal for all size groups of *O. mossambicus*. The costs of ingredients used in the diets with 50% and 100% *Crocodylus niloticus* meat meal indicated that it was profitable to use this meal in diets of *O. mossambicus* of all size groups. There is a potential for obtaining crocodile meat for free, as it is considered waste to some crocodile farmers; therefore, it could be an economically viable alternative source of protein. Future studies are recommended in growth experiments considering monosex fish prioritized in aquaculture projects, testing other crocodile meal levels and fish health status by determining the hematological parameters.

4.7. Limitations

Using the same feeding frequency of two times per day for all size groups is regarded as a limiting factor for maximum growth, especially for fry and fingerlings, as they need to be fed more frequently.

Using the chemical composition of the fishmeal from supplier, as some essential, non-essential amino acids, and fatty acids were not included in their tests.

4.8. Recommendations

To minimize costs and maximize production efficiency, different size groups should be fed (feeding rates, feeding frequencies, and composition of diets) according to their sizes.

All essential, non-essential amino acids and fatty acids should be included in parameters to be analysed for animal protein source used.

Future studies should consider using commercial diets with same protein level as formulated diets.

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CHAPTER FIVE

Haematological parameters of Mozambique tilapia (*Oreochromis mossambicus)* fed diets with the Nile crocodile (*Crocodylus niloticus*) meal as a fishmeal replacement

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5.1. Abstract

Food shortage, increased production costs, investment loss, treatment costs, loss of employment opportunities, income, reduced consumer's confidence, and industry failures are socio-economic conditions that could affect farmers and communities due to fish diseases. Therefore, it is essential to monitor fish health. Haematological parameters are used to monitor fish health status. This study was aimed to determine the haematological parameters to assess the health status of Mozambique tilapia (*Oreochromis mossambicus)* fed crocodile meal-based diets compared with a commercial diet. Red blood cell (RBC) count, Haemoglobin (Hb) concentration, Haematocrit (Hct), Platelets (PLT) counts and white blood cell (WBCs) count, were analysed using the Coulter $A^{C\cdot}T$ 5diff Autoloader haematology analyzer, at the Physiology Laboratory, Westville Campus at the University of KwaZulu Natal. Mean Cell Haemoglobin Concentration (MCHC), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH) were calculated, and results were considered significantly different at $p<0.05$. Results showed that the following parameters (Hct, PLT, MCV, MCH, MCHC) evaluated in this study were not significantly different ($P>0.05$, ANOVA) among diets fed, whilst RBC and Hb were significantly different ($P<0.05$). Our results were within the ranges reported for other species such as *Oreochromis niloticus* and *Clarias gariepinus*. However, some mean values at different points were not within the range calculated in the current study. Therefore, more studies are recommended as this is the first study to report on haematological parameters of *Oreochromis mossambicus* fed crocodile-based diets compared to a commercial diet.

Keywords: Aquaculture, haematology, crocodile meal-based diets, *Oreochromis mossambicus*

5.2. Introduction

Aquaculture is the fastest-growing food-producing sector, Shefat 2018 and FAO 2020. Increased demands for fish as a source of protein, new technology advances, and awareness of the nutritional importance of fish are the main reasons for the industry's growth (Assefa and Abunna, 2018). The culture methods have shifted from extensive, semi-intensive to become more intensive for producing higher yields because of the expansion of the sector (Rico et al., 2012). Under intensive culture conditions, fish depend on feeds provided to attain their market sizes in a short period (Lall and Tibbertts, 2009). Feeds provided to fish contain the energy and essential nutrients (protein, lipids, carbohydrates, vitamins, and minerals) not for growth development only, but also for reproduction and health (Prabu et al., 2017; Adeosun et al. 2019). Excesses and deficiencies in nutrients can reduce growth rates or lead to nutritional diseases (Prabu et al., 2017). Some nutritional deficiency signs are shown in Table 5.1.

Nutritional and infectious diseases are considered a constraint in the development of the aquaculture industry, production, and expansion (Murray and Peeler, 2005, Fazio, 2019). Furthermore, fish diseases could result in social and economic impacts on human health, nutrition, and employment (Adam and Gunn, 2017). These could happen through (i) zoonotic pathogens transmitted from fish harvested in aquaculture to people via contact or as food, (ii) nutrition due to the negative impact on household food security because of losing food and income, and (iii) employment because of disease outbreaks resulting in excessive mortalities, increased production costs, treatment costs, and the poor market price of the fish that will force employers to retrench people (Adam and Gunn, 2017; Shefat and Karim, 2018; Tiamiyu et al. 2019; Alfred et al. 2020).

Waste products, toxins, gases, water, microorganisms, minerals, hormones, and nutrients are variety of constituents transported by blood in fish (Esmaeili, 2021). Therefore, I think replacing fishmeal with crocodile meat meal would affect blood parameters of *O. mossambicus*. Fish culturists or farmers may use an increase in mortality rate, abnormal swimming, reduction of appetite or lack of feeding, and changes in body colour abnormalities as an indication of presence of disease (Maita, 2007; Shefat and Karim, 2018). Immunological, morphological, and haematological examinations are used to evaluate fish health (Maita, 2007). Haematology or blood examination is a reliable way of monitoring the health condition of fish and provides reliable information on nutritional deficiencies, metabolic disorders, and chronic stress status before onset of clinical signs (Seibel et al. 2021).

Apart from nutritional diseases, environmental conditions, reproductive cycles, variations in fish activity, fish species, age, size, sex, feeding habits, feeding regime, stocking density, water quality, oxygen, water temperature, season, diet composition, laboratory techniques, anaesthesia, blood sampling, anticoagulants, blood storage, diluents and transportation were reported to have effects on haematological parameters of fish (Chaudhuri et al. 1986; Houston 1997; Luskova, 1998; Lim et al. 2000; Coz-Rakova et al. 2005; Osuique et al., 2005; Vazquez and Guerrero, 2007; Rafetnezhad et al., 2008; Adeyemo et al., 2009; Ferri et al., 2011; Witeska et al., 2015; Fazio et al., 2016; Sheik and Ahmed 2016, Ahmed et al., 2020).

Our previous study on crocodile meal nutritional values showed that the nutritional value of crocodile meal was comparable quality to other animal by-products and different fishmeal grades (Luthada-Raswiswi et al. 2019). Furthermore, crocodile meat meal in diets of *Oreochromis mossambicus* study (Luthada-Raswiswi et al. 2022) showed that crocodile meat meal used had the potential for being used as a protein source for *O. mossambicus* fry and fingerlings. However, no adverse health effects associated with the feeding of crocodile meal-based diets, have been reported for O. *mossambicus*. Therefore, the current study aimed to determine the haematological parameters of *O. mossambicus* fed crocodile meal-based diets as assessment of health status. We hypothesized that there is no difference in haematological parameters of *O. mossambicus* fed diets with crocodile meat meal.

Table 5.1: Dietary amino acids, vitamins, minerals and other deficiency signs and effects on haematological parameters for different fish species. (Tacon, 1992; Mohamed, 2001; Shefat and Karim, 2018)

Cause of disease	Species affected	Deficiency signs and symptoms	Haematological symptoms
Amino acids			
Lysine	*Oncorhynchus mykiss*	Dorsal /caudal fin erosion, increases mortality, and retarded growth.	NR
	Cryprinus carpio	Increase mortality.	NR
Methionine	*O. mykiss*	Cataract and retarded growth.	NR
	Salmor salar	Cataract	NR
Tryptophan	*O. mykiss*	Caudal fin erosion, lordosis, retarded growth, and scoliosis.	NR
	Oncorhynchus nerka	Cataract, scoliosis.	NR
Vitamins			
Vitamin A (Retinol)	*Oreochromis niloticus*	Abnormal swimming, blindness, eye, and fin haemorrhage, exophthalmia, high mortality, poor feed efficiency, poor growth, pot-belly syndrome, reduced mucus secretion, and restlessness	NR
Vitamin B_1	*O. niloticus*	Anorexia, increased serum pyruvate, light coloration, low haematocrit count, low red blood cell count, nervous disorder, poor growth, and poor feed efficiency.	NR
Vitamin B_3	Hybrid tilapia (*O. niloticus* x *O. aureus)*	Deformed snout, haemorrhage, gill oedema, fin, mouth, and skin lesions.	NR
Vitamin B_5 (Pantothenic acid)	*O. aureus*	Anaemia, fin erosion, haemorrhage, hyperplasia of epithelial cells of gill lamellae, poor growth, and sluggishness.	NR
Vitamin B_6 (Pyridoxine)	Hybrid (*O. niloticus* x *O. aureus)*	Abnormal neurological signs, anorexia, caudal fin erosion, convulsions, high mortality, mouth lesion, poor feed efficiency, and poor growth.	NR
Vitamin B6 (Pyridoxine)	*Heteropneustes fossilis*	Anaemia	Decreased haematocrit, decreased red blood cell, increases in MCH
Vitamin B_7	Hybrid tilapia (*O. niloticus* x *O. aureus)*	Poor growth	NR
Vitamin C (Ascorbic acid)	*O. niloticus*	Anaemia, exophthalmia deformity, fin erosion, gill deformity, haemorrhage, lordosis, operculum deformity, poor feed efficiency, poor growth, poor wood heeling, and scoliosis.	NR
Vitamin C (Ascorbic acid)	*Clarias gariepinus*	Biochemical dysfunctions, broken back syndrome, organ dysfunction, functional changes, morphological changes.	Decreases haemoglobin, decreases haematocrit, and decreases red blood cell count
Vitamin D (Cholecalciferol)	Hybrid tilapia (*O. niloticus* x *O. aureus)*	Low haemoglobin, poor feed efficiency, poor growth, reduced liver size	NR
Vitamin E (-tocopherol)	*O. niloticus*	Anorexia, depigmentation, fin haemorrhage, poor feed efficiency, poor growth, and muscle degeneration	NR

Folic acid	*O. niloticus*	Reduced feed intake and efficiency, and poor growth	NR
Folic acid	*Channa punctatus*	Anaemia	Decreases haemoglobin and increased MCV
Choline	Hybrid (*O. niloticus* x *O. aureus)*	Reduced blood tryglyceride, cholesterol, and phospholipid concentration and poor growth and survival	NR
Minerals			
Calcium		Increased mortality, slow growth rate, lordosis, scoliosis, skull deformities	NR
Copper	*C. carpio*	Cataract, reduced growth	NR
Iron	Ictulurus punctatus	hypochromic microcytic anaemia	Reduced Ht, reduced Hb, reduced MCV
Magnesium		Calcinosis, renal	NR
Manganese	*O. mossambicus*	Loss of equilibrium, mortality, and reduced growth and appetite	NR
Manganese	*O. mykiss*	Abnormal tail growth, cataract, reduced growth, and short body dwarfism	NR
Manganese	*C. carpio*	Reduced growth, and short body dwarfism	NR
Phosphorus	*C. carpio*	Abnormal calcification of ribs and soft rays of the pectoral fin, bone demineralization, cranial deformity, increased viscera fat,	NR
Other factors			
Rearing system	*O. mykiss*	NR	Higher red blood cells and higher haemoglobin
Transportation	*C. carpio*	NR	Increased red blood cells
Crowding stress	*Sparus curata*	NR	Increased red blood cells
Capture and handling	*Colossoma macropomum*	NR	Decreased red blood cells

NR-not reported

5.3. Materials and methods

5.3.1. Processing of crocodile meat into meal

Please refer to Chapter 4 (see 4.3.1).

5.3.2. Ingredients and experimental diets preparations

Please refer to Chapter 4 (see 4.3.2).

5.3.3. Experimental fish

Please refer to Chapter 4 (see 4.3.3).

5.3.4. Feeding experiment

Please refer to Chapter 4 (see 4.3.4). However, some fish had not yet reached 20 g which was regarded as the minimum size for blood collection in this study; we continued to feed them for two months after removing samples for body composition.

5.3.5. Sample collection

Fish were starved for twenty-four hours before sampling to prevent defecation during the procedure. Blood were collected from twelve live fish, and care was taken to avoid contamination of the sample with tissue fluids. Fish were sedated individually in a 5-l bucket containing 0.3 ml of Clove oil dissolved in 4ml ethanol. Fish were removed from the bucket once the righting reflex was lost and placed on a tray. Fish were held in dorsal recumbency with the left hand, and then the right hand was used to manipulate the heparinised syringe. Two ml of blood were collected with assistance from Principal Technician of Animal House, by piercing the vein located on the caudal peduncle using a U-100 insulin syringe and 0.33 (29G) x 12.7 mm needle. Each blood sample was placed separately in each sterile vacuum tube, containing 0.5 mg Ethylenediaminetetraacetic acid (EDTA) as an anticoagulant, and analysed for the following haematological parameters: RBCs counts ($x10^6/\mu L$), Hb concentrations (g/dL), Hct values, (%), Platelets counts ($10^3\mu L$) and WBCs counts ($x10^3\mu L$), were analysed using the Coulter $A^{C\cdot}T^{TM}$ 5diff Autoloader haematology analyzer, at Physiology Laboratory, Westville Campus at the University of KwaZulu Natal.

5.3.6. Laboratory tests.

Haemoglobin

The whole blood of 10 µL was diluted using 1700 µL of A^{C}T 5diff diluent to make a preliminary dilution ratio of 1:170. The volume of 42.5 µL was removed for making the RBC and Platelets dilutions. The 400 µL of A^{C}T 5diff Hgb Lyse and additional 400 µL A^{C}T 5diff diluent were used to make the final dilution of 1:250 that was used to determine the Hb. Haemoglobin concentrations were measured using the Spectrophotometric technique at a wavelength of 550nm.

Red blood cell counts (x10^6/µL) and platelets

The volume of primary dilution of 42.5 µL removed from haemoglobin determination was diluted further using 2500 A^{C}T 5diff diluent to make the secondary dilution ratio of 1:58.8. The final dilution for RBC and Plt results were calculated as 1:170x1:58.8 = 1:10,000. Then the final dilution was used to determine the RBC count and Plt count using the Coulter Principle method.

White blood cell counts

The whole blood of 10 µL was diluted using 2000 µL of A^{C}T 5diff WBC Lyse to make a dilution of 1:200. The final dilution of 1:200 was used to determine the WBC count using the Coulter Principle method.

Haematocrit

Hct values, (%) were determined by the sum of all the digitized pulses. The volume of the red blood cells analysed is directly proportional to the height of the pulse generated by the passage of a cell through the aperture.

5.3.7. Calculations

Mean Cell Haemoglobin Concentration (MCHC), MCV, MCH were calculated using the following formulae as described by Habib et al. (2012) and Naz et al. (2021).

MCHC (g/dL, grams per deciliter) = Hb%/Ht% x 100

MCV (fL, femtolitres) = Ht% /RBC million /mm^3 x 10

MCH (Pg, picograms) = Hb%/RBC million/mm^3 x 10.

5.3.8. Statistical analysis

Mean values of three replicates from each treatment for RBC, Hb, Hct, PLT, MCHC, MCV and MCH were subjected to one-way analysis of variance (ANOVA). Results were considered statistical significance at $p<0.05$. Where there were significant differences in means, Tukey multiple comparisons test was used to compare the variance among the means.

5.4. Results

Haematological parameters results of *O. mossambicus* fed crocodile meal-based diets are presented as mean values ± SD in Table 5.2. Haematological parameters reference ranges for fish species such as *Dormitator latifrons*, *D. labrax*, *Oncoryhynchus mykiss*, *Oreochromis niloticus*, *Clarias gariepinus*, *Cichlasoma dimerus*, *Morone hybrid*, *Oreochromis hybrid*, and *S. aurata* are shown in Table 5.3. Fish fed D7 had a 0.88 $x10^6$/µL mean value, which was significantly lower than 2.07$x10^6$/µL, and 2.12 $x10^6$/µL, RBC mean values for fish fed D5, and D6, respectively. Haemoglobin followed the pattern of RBC with a 6.67 g/dL mean value for fish fed D7 and was significantly lower than 10.77 g/dL (D5), 10.90 g/dL (D6), mean values.

No significant differences were observed in concentration of Ht, PLT, MCV, MCH, and MCHC in *O. moossambicus* fed crocodile meal-based diets and fishmeal diet. Haematocrit mean values ranged from 15.27% to 33.53%. All these mean values were within the 35%-55% ranges as there were no significant differences among the means. Platelets mean values range from 192.66 $x10^3$µL to 546.66 $x10^3$µL. MCV mean values ranged from 143.94 fL to 163.53 fL. All fish from all diets had higher MCV mean values than the range of 80 fL -100 fL reported for this species. MCH mean values ranged from 51.34 pg. to 136.92 pg. All MCH mean values were higher than the range of 26.0 pg to 34.0 pg reported in the current study. MCHC mean values ranged from 32.17 g/dL to 108.49 g/dL.

White blood cells were higher among all diets than the range of 4.0 $x10^3$µL - 6.20 $x10^3$µL calculated in current study.

Table 5.2: Mean values of haematological parameters of *Oreochromis mossambicus* fed diets with crocodile meat meal as fishmeal replacement. Values are means (±SD) of three replicates for each treatment. The results were significantly different at $p<0.05$. Degree of freedom between groups = 2, within groups = 8, F = F Statistic, and P = Probability.

Haematological parameters	**Units**	**Diet 5 (0% CM)**	**Diet 6 (50% FM/50% CM)**	**Diet 7 (100% CM)**	**Range**	**ANOVA**	
						F	**P**
Red blood cells (RBC)	$x10^6/\mu L$	2.07 ± 0.08^a	2.12 ± 0.02^a	0.88 ± 0.76^b	4.0-11.0	7.5077	0.0233
White blood cells (WBC)	$x10^3\mu L$	++++	++++	++++	4.00-6.20	-	-
Haemoglobin (Hb)	g/dL	10.77 ± 0.59^a	10.90 ± 0.17^a	6.97 ± 2.40^b	11.0-18.8	7.3037	0.0247
Haematocrit (Hct)	%	33.53±2.89	33.27±0.11	15.27±16.76	35.0-55.0	3.4115	0.1024
Platelets (PLT)	$x10^3/\mu L$	546.67± 334.86	192.66± 74.19	279.00 ±39.23	150-400	2.5728	0.1560
Mean Cell Volume (MCV)	fL	163.53±7.81	156.68±1.15	143.94±50.14	80-100	0.2884	0.7593
Mean Corpuscular Haemoglobin (MCH)	pg	51.90±0.81	51.34±1.37	136.92±111.51	26.0-34.0	1.7549	0.2511
Mean Cell Haemoglobin concentration (MCHC)	g/dL	32.17±1.12	32.76±0.63	108.49±103.48	31.0-35.0	1.6189	0.2740

++++ shows highest values that were not detected by the instrument

Table 5.3. Haematological parameters reference ranges for different fish species

Fish species	WBC	RBC	Hb	Hct	MCV	MCH	MCHC	References
Dormitator latifrons	21.011-49.059	1.177- 2.973	-	11.103-45.064	91.555-231.539	-	-	Ruiz-Gonzalez et al. 2020
D. labrax	30.90±8.35	3.51± 0.34	9.48±0.83	51.18±5.00	146.20±11.71	27.06±0.98	18.60±1.25	Fazio 2019
Oncorhynchus mykiss	20.10±0.94	1.53± 0.13	10.12±1.99	29.00±3.24	189.3±12.25	65.62±9.81	34.68±4.44	Fazio 2019
Oreochromis niloticus	829.33± 27.64	1.76±0.09	8.24±0.15	25.36±0.99	-	-	-	Fazio 2019
Oreochromis niloticus	-	0.7-28	6.58-15.98	15-45	12.36-528.57	5.07-120.86	19.84-87.73	Bittencourt et al. 2003
Clarias gariepinus	13.83-17.11	1.62-1.82	6.80-13.50	18.70-40.10	111.59-225.44	37.36-55.03	22.04-38.20	Adeyemo et al. 2012
Cichlasoma dimerus	6.64-18.59	1.68-4.27	5.23-8.33	-	70.14-198	14.51-40.59	17.45-30.31	Vazquez and Querrero 2007
Morone hybrid	12.1-13.1	3.15-4.22	7.3-9.4	29-36	78-102	19.25	22-27	Hrubec et al. 2001
Oreochromis hybrid	21.5-154.7	1.91-2.88	7.0-9.8	27-37	1.15 ±18.3	28.3-42.3	22-29	Hrubec et al. 2000
S. aurata	68.08±2.95	3.50±0.08	8.00±0.23	50.35±1.19	144.40±1.63	23.42±0.82	16.28±0.58	Fazio et al. 2019

5.5. Discussion

Fish for human consumption are produced from aquaculture (Assefa and Abunna, 2018), hence, public health safety is under critical threat due to nutritional diseases in fish (Shefat, 2018). According to Shefat (2018), it is difficult to detect clear signs early because nutritional deficiency signs are chronic in nature. Furthermore, haematological parameters depend on standard values and references for each species to be used as a biomarker (Parrino et al., 2018). This study is the first to report on haematological parameters of *O. mossambicus* which were fed crocodile meal-based and fishmeal diet.

The functions of RBCs are to transport oxygen to the tissues and returning carbon dioxide to the lungs in the body (Seibel et al., 2021). Other functions include gas exchange and homeostasis (Morera and MacKenzie, 2011; Shen et al., 2018; Olaniyi et al., 2020). Red blood cells are the first haematological parameter affected in a stressful situation (Olapade and Mariatu, 2015). Fish with low RBC numbers cannot transport a large amount of oxygen even though enough oxygen is available in the water and will experience a lack of oxygen (Yanuhar et al., 2021). Anaemia is also indicated by low RBC count (Tonya et al., 2008; Yanuhar et al., 2021). Above normal levels of RBCs are associated with stressed fish. The current study showed significant differences in RBC of *O. mossambicus* fed D7 and other diets. However, the RBC values observed in this study were within the reference ranges of $0.7x10^6$/µL to $27x10^6$/µL reported for the *Oreochromis* hybrid (Bittencourt et al. 2003), and 0.81 $x10^6$/µL to 3.73 $x10^6$/µL for *Cyprinus carpio* (Witeska et al. 2016). Meaning that replacing fishmeal with crocodile meal has no effect on the red blood cell counts of *O. mossambicus*.

Haemoglobin is the protein that transport iron-containing oxygen in the red blood cells of vertebrates except in fish of the family Channichthyldae, and it gives the blood cells its distinctive red colour (Etim et al., 2014). Transporting oxygen from the environment to cells, carbon dioxide and hydrogen ions produced metabolically in the opposite direction and increasing the carrying capacity of oxygen, carbon dioxide, and hydrogen ion are the functions of haemoglobin (Jensen et al., 1998). Higher Hb values indicate a more ability to resist disease infections as the blood has a higher oxygen-carrying capacity. In contrast, Hb deficiency decreases oxygen-carrying capacity, leading to anaemia (Akinrotimi et al., 2007). The current study results showed significant differences ($P < 0.05$) in Hb of *O. mossambicus* fed crocodile-based and fishmeal diet. Haemoglobin concertation was significantly lower in fish fed 100% crocodile meal-based diet (D7) than in other groups. However, all mean values obtained (6.97

-10.90 g/dL) were within reference intervals of (6.58-15.98 g/dL and 6.80-13.50 g/dL) reported for *O. niloticus* (Bittencourt et al. 2003) and *Clarias gariepinus* (Adeyemo et al. 2012), respectively.

Haematocrit measures the percentage of RBC in blood volume, and it is also called packed cell volume (PCV) (Witeska et al., 2022). Transporting nutrients and oxygen is the primary function of haematocrit. It depends on the size and the number of RBC (Witeska et al., 2022). High concentrations of haematocrit show that the transportation of nutrients and oxygen is better than in fish with a low haematocrit concentration. Haematocrit in fish ranges between 20% and 45% (Tonya et al. 2008). Fish with less than 20% of haematocrit are usually associated with anaemia. Whilst those with a haematocrit of 45% or greater are generally considered to have polycythemia (Tonya et al., 2008). The current study results were 33.53% (D5), 33.27(D6), and 15.27% (D7) and were not statistically significantly different among all diets fed. Even though D7 results were lower than the 20% lower limit (Tonya, et al, 2008), all other groups (D5 and D6) were within the ranges of 20% to 45% recommended by Tonya et al., (2008). Crocodile meat meal can replace fishmeal up to 50%.

Mean values obtained for platelets in the current study were 192.66 $x10^3$/µL to 546.67 $x10^3$/µL. Since there were no significant differences in mean values among the diets, all mean values were within the range of 150 $x10^3$/µL - 400 $x10^3$/µL calculated for this study. According to Witeska (2016), many studies conducted on fish do not perform platelets counts. Crocodile meal had no significant effect on platelets of *O. mossambicus*.

There were no statistically significant differences in MCV, MCH, and MCHC among *O. mossambicus* fed crocodile-based diets and those fed commercial diets. MCV is the average volume of RBCs (Oluwatobi and Solomon, 2017). High MCV values indicate macrocytic (large RBC size) anaemia, while low MCV indicate microcytic (small size RBC than the normal) anaemia (Oluwatobi and Solomon, 2017). Mean values of 143.94 fL to 163.53 fL obtained were higher than the range of 80fL-100 fL calculated in this study. However, all mean values were within ranges of 12.36 fL to 528.37 fL reported for *O. niloticus* (Bittencourt et al. 2003) and 111.59 fL to 225.44 fL for *Clarias gariepinus* (Adeyemo et al. 2012).

Mean corpuscular haemoglobin values fluctuate due to the lower concentration of haemoglobin in the RBC (Bittencourt et al., 2003). Mean values (50.64 pg to 127.92 pg) obtained were higher than the range calculated in this study (26.0 pg to 34.0 pg). However, except for D6, others were within the values of (5.07 pg -120.86 pg) reported for *O. niloticus*

(Bittencourt et al., 2003). Fish fed crocodile meal-based diets and those fed commercial diets had MCHC mean values ranging from 32.17 g/dL to 108.49 g/dL. These values were within the calculated range of 31.0 g/dL to 35.0 g/dL as there were not significantly different and range of 19.84 g/dL -87.73g/dL reported for *O. niloticus* (Bittencourt et al., 2003).

Fighting infections by producing or transporting and distributing antibodies in the immune response are the functions of white blood cells (Etim et al., 2014). Fish or animals with low WBC have a high risk of being exposed to disease infection. In contrast, those with high WBC counts can generate antibodies in the process of phagocytosis and is resistant to diseases and enhance adaptability to local environmental and prevalent disease conditions (Etim et al., 2014). The current study results showed higher WBC counts in all treatments than the ranges recommended. According to Michael et al. (2019), high WBC can result from increased production of WBCs in the lymphopoietic tissues and kidneys Furthermore, the WBCs might have been calculated together with thrombocytes because they have similar morphology to thrombocytes (Fazio et al., 2012).

5.6. Conclusion

Many factors are known to affect haematological parameters in fish. Most of the parameters (Hct, PLT, MCV, MCH, MCHC) evaluated in this study were not significantly different among diets fed, except the RBC and Hb for fish fed D7 that had significantly lower RBC and Hb than that fed D5, and D6. Our results were within the range reported for other species such as *O. niloticus* and *C. gariepinus* or fish in general. However, some mean values obtained were higher than the range calculated in the current study. Therefore, more studies are recommended as this is the first study to report on haematological parameters of *O. mossambicus* fed crocodile-based diets compared to a commercial diet.

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5.8. References

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CHAPTER SIX

General discussion

6.1. Introduction

Overfishing, which partly results from fish caught for fishmeal production, is a growing problem in the marine environment because the loss of fish in the ecosystem is linked to the increasing development of harmful algal bloom that reduces water quality, oxygen depletion, and pollution offshore (Barbier 2017). Alternative protein sources' search is necessary because of increasing demand, unstable supply, and the high price of the fishmeal with the expansion of aquaculture (Tacon and Metian, 2008; Boland et al., 2013; Hua et al., 2019; Luthada-Raswiswi et al., 2021). The Nile crocodiles (*Crocodylus niloticus*) are farmed mainly for producing skins used to produce high-quality fashion accessories in some countries in southern Africa, including South Africa (Ashton 2010). However, the demand for crocodile meat, especially in South Africa, is very low (Caldwell, 2021). The meat is considered waste, and strict regulations are forced on the industry to dispose of crocodile carcasses. Therefore, the study aimed to determine the nutritional value of the crocodile meal and its suitability as a fishmeal replacement in animal feeds, especially fish. The primary research questions were: what are the nutritional values of the crocodile meat meal? Are they comparable to other animal by-products meals used in aquaculture? Furthermore, can crocodile meal be used in animal feed/aquaculture as a substitute for fishmeal?

This study is divided into six chapters. Chapter One outlined the background to the research problem/s, specifying the research objectives and the significance of the research. Chapter Two presented a systematic review and meta-analysis of animal protein sources that substituted fishmeal in aquaculture diets. Chapter Three explained how the Nile crocodile (*Crocodylus niloticus*) meat was processed into meals. The nutritional values/profile of meals derived from different crocodile carcasses were determined, and results were compared with other animals' by-products meals and different fishmeal grades used in aquaculture. Chapter Four indicated formulated crocodile meal-based diets replacing fishmeal for Mozambique tilapia (*Oreochromis mossambicus*) of different size groups, and the effects of crocodile-based diets were investigated compared to commercial diets on growth performance, feed utilization, and body composition. Chapter five: haematological parameters of *O. mossambicus* fed crocodile-based diets, and commercial diets were determined to assess the health status of fish.

Chapter six: Summarized the overview of the study, significant research findings, concluding remarks and recommendations.

6.2. Research findings

Reviewed literature on various animal protein sources used in aquaculture diets and meta-analysis results showed that animal protein sources used in aquaculture range from insects, terrestrial by-products to fishery by-products. All these protein sources have limitations such as timely processing, quality control, seasonal availability, the cost of collecting fish waste; some fish cannot digest them. However, despite these drawbacks, animal protein sources used as fishmeal replacement positively impacted feed conversion ratio, specific growth rate, final weight, and survival rate of cultured fish. Furthermore, findings showed the gaps that some animal by-products, including crocodile meat, had not been assessed as a protein source in aquaculture or animal feeds. Although fish are known to change their nutritional requirement depending on their life stage (Wing-Keong and Romano, 2013), different size groups of fish are not considered when these animal protein sources are tested to be used in aquaculture.

This study presented the first report worldwide on the nutritional values of the crocodile meat meal. Results have revealed that nutrients such as moisture, crude protein, crude fat, crude fiber, ash, and selected minerals of *Crocodylus niloticus* derived meal had nutritional values of comparable quality for use in aquaculture feeds. Nutritional values obtained from this study were like those of other animal by-products such as meat bone meal, feather meal, blood meal, poultry by-products and different grades of fishmeal used in aquaculture. Practically, these results imply that crocodile meat meals can be used as a fishmeal substitute in aquaculture diets and reduce the pressure of depending on fishmeal only. Since some results are published (Luthada-Raswiswi et al., 2019; 2021) for the public, they also showed researchers (Abo-Taleb et al., 2021; Ang et al., 2021; Aya et al., 2021; Carvalho Pereira at al., 2021; Mdhluvu et al., 2021; Zhang et al., 2012; Joeng, 2022;) and crocodile farmers that they could process crocodile meat they store in their freezers as waste and use the meal as a fishmeal replacement in aquaculture or animal feeds.

The study reports no significant differences in weight gain, specific growth rates, gross feed conversion ratios or protein efficiency ratios of *O. mossambicus* fry fed different diets.

Considering the similarities in Gs, SGRs, GFCRs, and PER in fingerlings and sub-adult to adult fish fed D4 and D5, the *Crocodylus niloticus* meat meal has the potential to substitute fishmeal for all size groups of *O. mossambicus*. The costs of ingredients used in the diets with 50% and 100% *Crocodylus niloticus* meat meal indicated that it was profitable to use this meal in diets of *O. mossambicus* of all size groups.

Haematological parameters were determined to assess the health status of *Oreochromis mossambicus* fed crocodile meal-based diets compared with that fed a commercial diet. Results showed significant differences in red blood cells counts and haemoglobin concentrations. Fish fed a 100% crocodile-based diet had significantly lower red blood cell count and haemoglobin concentrations than those fed a commercial diet, and other crocodile meal-based diets. No significant differences were observed in haematocrit, platelets count, mean cell volume, mean corpuscular haemoglobin, and mean cell haemoglobin concentration. The mean values obtained for all parameters were within the ranges reported for *Oreochromis niloticus* reported by Bettencourt, (2003). Since some mean values obtained were higher than the ranges calculated in the current study, more studies are recommended as this is the first study to report on haematological parameters of *Oreochromis mossambicus* fed crocodile-based diets compared to a commercial diet.

6.3. Limitations

Using the same feeding frequency of two times per day for all size groups is regarded as a limiting factor for maximum growth, especially for fry and fingerlings, as they need to be fed more frequently.

Using the chemical composition of the fishmeal from supplier, as some essential, non-essential amino acids, and fatty acids were not included in the supplier's tests results.

6.4. Concluding remarks and recommendations

Producing high-quality fish, reducing the cost of feed, and minimizing the use of forage fish species in fishmeal production are the main reasons for replacing fishmeal in aquaculture. This study showed new information on how to process crocodile meat into the meal and that

the Nile crocodile (*Crocodylus niloticus*) derived meal had nutritional values of comparable quality compared to other animal by-products and different grades of fishmeal used in aquaculture. Furthermore, results showed that *Crocodylus niloticus* derived meal could be used as a fishmeal replacement for *Oreochromis mossambicus* fry and fingerlings. Future studies are recommended to use the feeding rates, feeding frequencies, and composition of diets according to fish sizes, to include all essential, non-essential amino acids and fatty acids as parameters to be analysed for animal protein source used, and to consider using commercial diets with same protein level as formulated diets.

6.5. References

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