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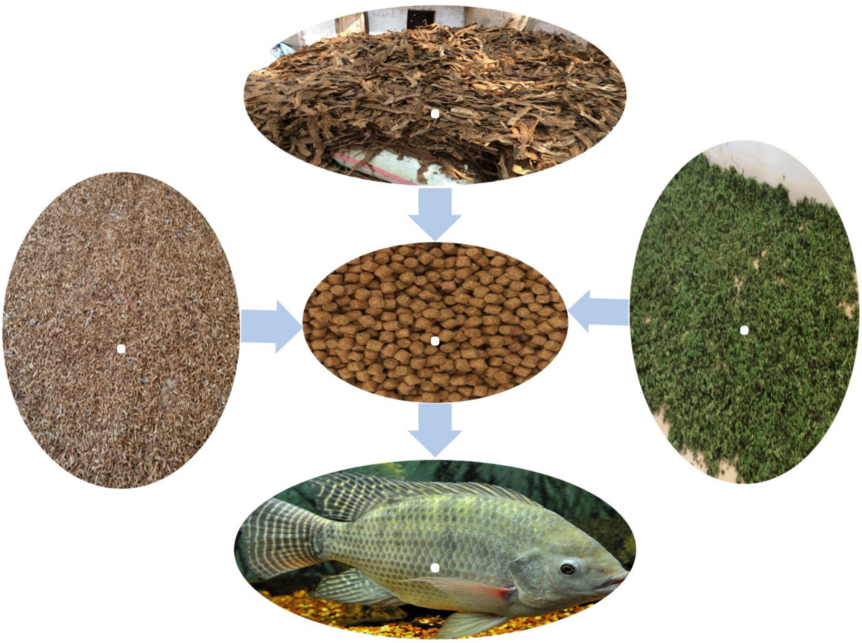
Francis Pius Mmanda



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Cover: Ingredients used as alternatives to fishmeal in feed for farmed Nile Tilapia *Oreochromis niloticus*
(Photo: Francis Pius Mmanda)

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Abstract

Fish farming has become popular in Tanzania in recent years, resulting in an increase in the number of fishponds. This has caused a corresponding demand for good-quality fish feeds at affordable prices, for sustainable aquaculture production and productivity. Fishmeal as a protein feed ingredient is expensive, so this thesis evaluated the nutritive value of locally available feed ingredients fed to tilapia, the most commonly farmed fish species in Tanzania. Commonly used local feed ingredients were identified through a field survey and their chemical composition was analysed. The digestibility of potential protein feed ingredients as an alternative to fishmeal was investigated in studies with Nile tilapia (*Oreochromis niloticus*), also studies on growth performance and carcass traits were studied in Nile tilapia fed with either fishmeal based diet or diets in which fishmeal protein was replaced with selected alternative feed ingredients. The feed costs of replacing fishmeal with alternative feed ingredients for tilapia were also determined.

Most tilapia fish farmers surveyed (n=202) relied on locally available ingredients to supplement tilapia diets. The farmers used either single ingredients or a combination of ingredients. Compositional analysis of these alternative feed ingredients showed that aquatic plants and agricultural by-products were good sources of trace minerals, particularly iron and iodine, while plant leaves were good sources of potassium. The protein digestibility of selected feed ingredients was found to be comparable to that of fishmeal. However, growth performance, feed intake and protein efficiency value of juvenile (male) Nile tilapia were higher with a fishmeal diet than with test diets where fishmeal protein was replaced (50% on dry matter (DM) basis) with cattle blood meal, fish frames meal, freshwater shrimp meal or brewery spent yeast meal. Apart from the cattle blood meal diet, there was no significant difference in feed conversion ratio between the fishmeal-based and test diets. Cost analyses for producing tilapia of standard Tanzanian market size (250 g) showed that 50% replacement of fishmeal (DM basis) with the selected test ingredients could reduce feed costs by 33%.

Keywords: Nile tilapia, feed ingredients, digestibility, chemical analysis, growth performance, feed costs

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Näringsvärde och användning av lokalt tillgängliga lågprisingredienser för tilapiaodling i Tanzania

Sammanfattning

Intresset för fiskodling har ökat i Tanzania under senare år och antalet fiskodlingar har blivit fler. Det har resulterat i en ökad efterfrågan på högkvalitativt fiskfoder till överkomligt pris, för att få en hållbar vattenbruksproduktion och produktivitet. Fiskmjöl som proteinfoderingrediens är dyrt, så denna avhandling har utvärderat näringsvärdet för lokalt tillgängliga foderingredienser som ges till tilapia, vilken är den vanligaste fiskodlingsarten i Tanzania. Genom en fältundersökning identifierades vanligt förekommande foderingredienser som används av fiskodlare i Tanzania och ingrediensernas kemiska sammansättning analyserades. Smältbarheten hos de foderingredienser med potential att ersätta fiskmjöl som proteinkälla i foder till fisk utvärderades i studier med Niltilapia (*Oreochromis niloticus*), även tillväxtprestanda och slaktkroppsegenskaper studerades hos Niltilapia som utfodrats med antingen fiskmjölsbaserat foder eller där fiskmjölet hade ersatts av de alternativa proteinfoderingredienserna. Kostnaden för foder innehållande fiskmjöl respektive alternativa ingredienser utvärderades också.

De flesta fiskodlare som intervjuades (n=202) matade den odlade fisken enbart med lokalt tillgängliga ingredienser. Odlarna använde antingen enskilda ingredienser eller en kombination av några få. Analys av näringsämnessammansättningen av dessa alternativa foderingredienser visade att vattenväxter och jordbruksbiprodukter var goda källor till spårmineraler, särskilt järn och jod, medan blad från växter var goda kaliumkällor. Proteinsmältbarheten för de utvalda ingredienserna var jämförbar med den för fiskmjöl. Tillväxt, foderintag och proteineffektivitetsvärde var dock högre hos juvenil Niltilapia (hanar) som fått fiskmjölsbaserat foder än då fiskmjölet var ersatt till 50 % med mjöl av nötkreatursblod, fiskrens, sötvattensräkor eller bryggerijäst. Förutom för foder baserat på nötkreatursblodmjöl var det ingen signifikant skillnad i foderomvandlingsförhållande mellan det fiskmjölsbaserade fodret och de testfoder som innehöll alternativa foderingredienser. Analyser av kostnaden för att producera Niltilapia i standardstorlek för den Tanzaniska marknaden (250 g) visade att genom att ersätta 50 % av fiskmjölet (torrviktsbasis) med de utvalda testingredienserna kunde foderkostnaden reduceras med 33 %.

Nyckelord: Niltilapia, foderingredienser, smältbarhet, kemisk analys, tillväxt, foderkostnad

Författarens adress: Francis Pius Mmanda, SLU, Institutionen för husdjurens utfodring och vård, Box 7024, 750 07 Uppsala, Sverige.

Dedication

To my wife *Mariana* and daughters *Maureen* and *Careen* for your encouragement, support and consolations

To my mother *Mariana,* nephews *Amadeus* and *Victoria* and sister *Angela* for your encouragement and consolations.

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Mmanda, F.P., Mulokozi, D.P., Lindberg, J.E., Norman Haldén, A., Mtolera, S.P.M., Kitula, R. and Lundh, T*. (2020). Fish farming in Tanzania: the availability and nutritive value of local feed ingredients. *Journal of Applied Aquaculture* 00(0), pp.1-20. DOI: 10.1080/10454438.2019.1708836

II. Mmanda, F.P., Lindberg, J.E., Haldén, A.N. and Lundh, T*. (2019). Mineral content in local feed ingredients used by fish farmers in four different regions of Tanzania. *Western Indian Ocean Journal of Marine Science 18*(2), pp. 1-9. https://www.ajol.info/index.php/wiojms/article/view/182758

III. Mmanda, F.P., Lindberg, J.E., Norman Haldén, A., Mtolera, S.P.M., Kitula, R. and Lundh, T (2020). Digestibility of local feed ingredients in tilapia (*Oreochromis niloticus*), determined on faeces collected by siphoning or stripping (*manuscript*).

IV. Mmanda, F.P., Lindberg, J.E., Norman Haldén, A., Mtolera, S.P. M., Kitula, R. and Lundh, T. (2020). Effect of diets formulated with locally available feedstuffs on growth performance and carcass traits in tilapia juvenile (*manuscript*).

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* Corresponding author.

The contribution of Francis Pius Mmanda to the papers included in this thesis was as follows:

I. Performed fieldwork with support from local government authorities (at the selected study sites) and co-authors. Performed chemical analysis with the support of laboratory technicians, and data analysis and interpretation with the support of the co-authors. Drafted manuscript with inputs from the co-authors and main supervisor, and corresponded with the journal.

II. Performed fieldwork, sample collection of local feed ingredients and laboratory mineral analysis with support from laboratory technicians and the co-authors. Performed data analysis and interpretation with support from the co-authors. Drafted the manuscript with inputs from the co-authors and main supervisor, and corresponded with the journal.

III. Performed the digestibility experiment and chemical analysis of feed ingredients, diets and faeces samples with support from local assistants, laboratory technicians and the co-authors. Performed data analysis and interpretation with support from the co-authors.

IV. Performed the growth performance experiment, chemical analysis of diets and whole-body fish, and fillet colour analysis with support from local assistants, laboratory technicians and the co-authors. Performed cost calculations of replacing fishmeal with test diets with the support of local assistants and the co-authors. Performed data analysis and interpretation with support from the co-authors. Drafted the manuscript with inputs from the co-authors.

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Abbreviations

A	Redness hue
AA	Amino acids
AD	Apparent digestibility
ADing	Apparent digestibility of the ingredients
ADWG	Average daily weight gain
AOAC	Association of Official Analytical Chemists
As	Arsenic
B	Yellowness hue
BM	Body mass
Br	Bromide
BSY	Brewery spent yeast
BSYD	Brewery spent yeast diet
BWT	Body weight mass
Ca	Calcium
CB	Cattle blood
CBD	Cattle blood diet
Cd	Cadmium
CFl	Cassava flour
CF	Crude fibre
Cl	Chlorine
Co	Cobalt
CP	Crude protein
Cr	Chromium
Cr_2O_3	Chromic or Chromium (III) oxide
CSC	Cotton seed cake
DIAAS	Digestible indispensable amino acid score
DM	Dry matter
DO	Dissolved oxygen
DW	Duckweed

DWD	Duckweed diet
EAA	Essential amino acids
ECI	Entire colour index
EE	Ether extract
FAO	Food and Agricultural Organization
FCR	Feed conversion ratio
Fe	Iron
FF	Fish frames (Nile perch skeletal remains)
FFD	Fish frames diet
FI	Feed intake
FM	Fishmeal
FMD	Fishmeal diet
FSHD	Freshwater shrimp diet
FW	Final weight
GE	Gross energy
Hg	Mercury
HIS	Hepatosomatic index
H_{mean}	Hue mean
H_t	Hue over time (t)
I	Iodine
IFFO	International Fishmeal and Fish Oil Organizations
IMS	Institute of Marine Sciences
IMS-MC	Institute of Marine Sciences, Mariculture Centre
IW	Initial weight
Kf	Condition factor
L	Lightness (of hue)
LM	Liver mass
M+C	Methionine + cysteine
MB	Maize bran
Mg	Magnesium
ML	Moringa leaf
MLD	Moringa leaf diet
MSH	Marine water shrimp
MSHD	Marine water shrimp diet
MT	Metric tonnes
N	Nitrogen
NEAA	Non-essential amino acids
N_f	Final fish number
NFE	Nitrogen-free extract
NH_3	Ammonia

N_i	Initial fish number
NO_2	Nitrite
NO_3	Nitrate
NRC	National Research Council
OM	Organic matter
P	Phosphorus
PER	Protein efficiency ratio
P_f	Final protein content
PG	Protein gain
P_i	Initial protein content
PI	Protein intake
PPV	Protein productive value
REFD	Reference diet
S/oil	Sunflower oil
SEM	Standard error of the mean
SFSC	Sunflower seed cake
SGR	Specific growth rate
SR	Survival rate
SUA	Sokoine University of Agriculture
TAFIRI	Tanzania Fisheries Research Institute
TAN	Total ammonium nitrogen
TBL	Tanzania Brewery Limited
TVLA	Tanzania Veterinary Laboratory Agency
URT	United Republic of Tanzania
USD	United States Dollars
Vit/Min	Vitamin and mineral premix
VSI	Viscerosomatic index
WG	Weight gain
WP	Wheat pollard

1 Introduction

Aquaculture is the world's fastest growing and most diverse food production sector, contributing around 17% of human total animal protein consumption globally in 2016 (FAO, 2018). Fish farming is continuously increasing worldwide to meet global market demand for fish and fishery products, driven by the increasing human population and over-exploitation of wild capture fisheries (FAO, 2014). In 2015, the contribution of fishery and aquaculture production comprised 50% of total animal protein for human consumption in countries such as Bangladesh, Cambodia, Sri Lanka, Indonesia, Sierra Leone, Ghana and some Small Island Developing States (SIDs), exceeding beef, pork, and poultry consumption (FAO, 2018). In the decade 2006-2016, average per capita fish consumption globally increased from 17.4 to 20.3 kg per year (FAO, 2018, 2012). The expansion of the industry is due to advances and innovations in modern technology and farming methods in terms of fingerling production, culture systems, culture methods and high-quality fish feed production (Tran *et al*., 2019). However, the majority of sub-Saharan countries (excluding Nigeria) continue to report low aquaculture production despite great potential for aquaculture development and past government efforts to assist fish farmers (Tran *et al.,* 2019). In 2016, the overall contribution of the sub-Saharan region to world aquaculture total production was only 0.75% (FAO, 2016). The slow growth of the aquaculture industry in sub-Saharan countries, including Tanzania, may be due to lack of skilled manpower, lack of quality feedstuffs and feed supply chains, use of outdated technology and methods, poor management and inadequate provision of advisory services for aquaculture (URT (United Republic of Tanzania), 2016). The most commonly cultured fish species in Tanzania is Nile tilapia (*Oreochromis niloticus*), followed by African catfish (*Clarius gariepinus*) (Chenyambuga *et al.,* 2014; Kaliba *et al.,* 2006). The suitability of Nile tilapia for fish farming is due to its fast growth rate, resistance to disease, ability to grow on a wide range of diets, high prolific rate, good carcass taste and ability to withstand a wide range of salinity levels (Gibtan *et al.*, 2008; Kapinga *et al.,* 2014).

In the past few years, there has been increasing interest in tilapia fish farming in Tanzania, with the number of earthen ponds increasing from 14,100 in 2016 (Kaliba *et al.,* 2006) to 26,445 in 2019 (URT, 2019). As a result, demand for good-quality fish diets has also increased. However, locally made and imported commercial feeds available in the country are expensive and not an option for smallholder fish farmers. Therefore, the majority of tilapia fish farmers opt to use locally available feed ingredients of both plant and animal origin as a nutritional supplement for their farmed fish (Chenyambuga *et al.,* 2014; Kaliba *et al.*, 2006). These ingredients include maize bran, kitchen leftovers, garden by-products, rice polish, banana leaves, sweet potato leaves, fishmeal and poultry by-products. The local ingredients used vary in nutritional value from one region to another, depending on climate conditions, geographical zone, soil type and seasonal production (NRC (National Research Council), 2011; Onyango *et al.,* 2019). However, information on the availability and potential nutritive value of local ingredients use in fish farming in Tanzania is limited. Therefore, there is an urgent for research on the availability and proximate composition of potential local ingredients that could be used as alternatives to fishmeal. In addition, investigations are needed on the effects on fish growth performance and production economics of using diets formulated with local feed ingredients, in order to support long-term development of sustainable fish production and productivity in Tanzania.

2 Background

2.1 Global aquaculture

In 2016, global fish production was 171 million metric tonnes (MT) (Figure 1), of which aquaculture production accounted for 46.8% (80 million MT), with an estimated value of USD 232 billion (FAO, 2018).

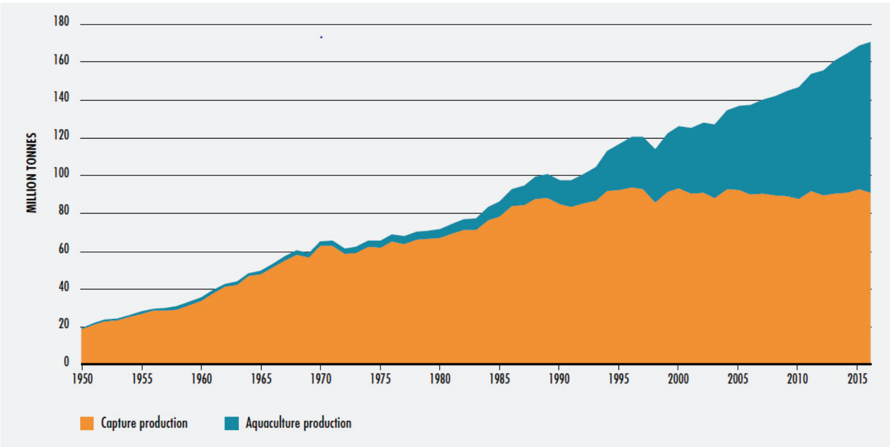

Figure 1: World capture fisheries and aquaculture production, 1950-2015. (Source: FAO, 2018)

The aquaculture industry is continuing to increase worldwide (FAO, 2018) with a world country average estimated/predicted growth rate of 3.72% in 2018, 4.1% in 2019, 4.50% in 2020, 4.83% in 2021 and 5.15% in 2022 (IFFO, 2018). Global food fish production in aquaculture is dominated by Asia, accounting for 89.4% of total production by volume in 2016, with China being the main producer (61.5%) followed by India (7.1%), Indonesia (6.2%), Vietnam (4.5%) and Bangladesh (2.8%) (FAO, 2018). Aquaculture production in Africa has also shown remarkably fast growth rate, to 2.5% of world aquaculture production in 2016 (FAO, 2018). The production is led by Egypt (1.7%), followed by Nigeria (0.4%). The contribution of all sub-Saharan countries excluding Nigeria accounted for 0.4% of total world aquaculture production in 2016 (FAO, 2018). Within sub-Saharan Africa, however, there was a seven-fold increase in aquaculture production over the decade 2004-2014, with an average growth rate of 21%. Most production was in inland aquaculture (98%), predominantly rearing indigenous tilapia and African catfish (FAO, 2016; Satia, 2017; Tran *et al.,* 2019). The main aquaculture producers in sub-Saharan Africa, accounting for 93% of total production in 2014, are Nigeria, Uganda, Ghana, Kenya, Zambia, Madagascar and South Africa (FAO, 2016; Satia, 2017) (Figure 2).

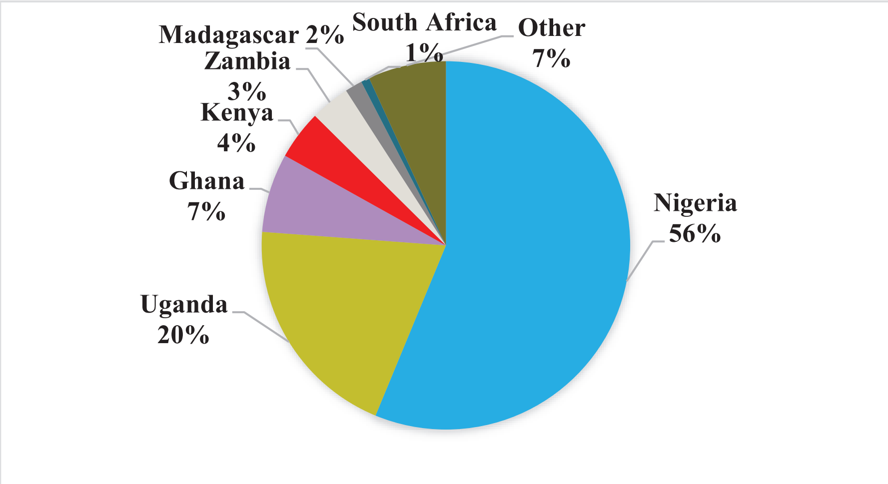

Figure 2: Top seven aquaculture producers in sub-Saharan Africa in 2014 by quantity (metric tonnes). (Source: FAO, 2016)

2.2 Aquaculture in Tanzania

Aquaculture has been practised in Tanzania since the 1950s (Mallya, 2007). However, the initial production stagnated due to poor farming methods and technologies, poor management and low availability of good-quality fish feeds (Mwanja & Nyandat, 2013; URT, 2016). Over several decades, farmed finfish and crustaceans were reared extensively or semi-intensively in Tanzania for subsistence, in ponds, tanks, hapas and cages, and were fed on locally available feed ingredients (Chenyambuga *et al.,* 2014; A.-F. M. El-Sayed, 2006; Kaliba *et al.,* 2006; Nguyen, 2008). However, aquaculture has gained in popularity in recent years, whereby fish farming has expanded from 19,860 earthen fishponds in 2012 to 26,445 in 2019, producing 3,364 MT of fish in 2012 and 18,019 MT in 2019 (URT, 2019) (Figure 3). According to Mulokozi *et al*. (2020), fish farming contributes on average 13% to household annual incomes.

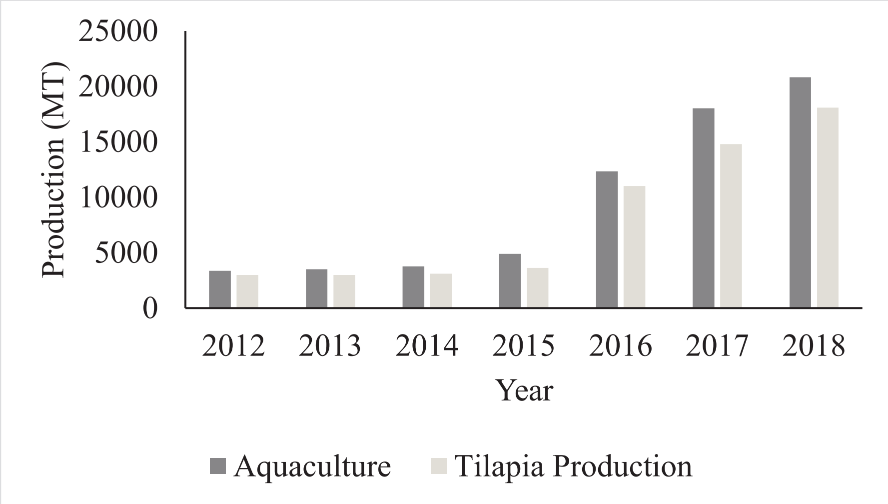

Figure 3: Trends in aquaculture and tilapia production in Tanzania, 2012-2018. (Source: URT, 2019)

The most common aquaculture operations in Tanzania are finfish, prawn, crab fattening and seaweed farming (URT, 2019). According to FAO (2018), Tanzania is among the top 10 producers of seaweed worldwide after Malaysia, producing about 119,000 MT of seaweed or 0.4% of total world aquatic plant production (FAO, 2018). The dominant finfish species farmed in the country are tilapia and catfish (URT, 2019), with Nile tilapia being the most cultured species, followed by African catfish (Chenyambuga *et al*., 2014; Kaliba *et al*., 2006; Mallya, 2007; URT, 2019). Tilapia fish farming has been steadily increasing and its contribution to aquaculture production has increased proportionally since 2012 (URT, 2019) (Figure 3). Semi-intensive mixed-sex monoculture of tilapia is the dominant fish farming practice (Kaliba *et al.,* 2006). However, the development of tilapia fish farming in Tanzania and across East Africa has been constrained for decades by lack of skilled manpower, poor-quality feeds and inadequate feed supply, poor management and lack of investment capital (Mwanja & Nyandat, 2013; URT, 2016).

2.3 Fish feed and production

The continuous expansion of tilapia fish farming operations in Tanzania has led to high demand for quality fish feeds. Although there are many local fish feed producers in the country (n=10), local fish feed production is still low compared with demand (Table 1). For example, locally-made fish feed production was 322.5 MT in 2019 and the combined production capacity of all local producers was 4635.7 MT (URT, 2019).

Table 1*: Production capacity and actual production of local fish feed producers in Tanzania in 2019*

S/N	Company/ Individual	Production capacity (MT yr^{-1})	Current production (MT, 2019)
1	Eden Agri Aqua Services	720	252.15
2	Igomelo Farm Ltd	0.25	-
3	Jans Aqua Centre	20	1.25
4	Tanfeed Ltd	168	21
5	Kise Farm	0.5	3.85
6	Kisima Farm	17	0.58
7	Salibaba Pellets Ltd	3600	8
8	Feed & Fingerlinks Co.Ltd	24	1.9
9	Aquasol Tz Ltd	80	33.77
10	Mother And Child Hope	6	-
	Total	*4635.75*	*322.5*

Source: URT (United Republic of Tanzania), 2019.

Therefore, the majority of large-scale fish farmers rely on imported fish feeds, which are of better quality than the locally produced feeds. A total of 520 MT of fish feed was imported to Tanzania from different countries worldwide in 2019, with the bulk supplied by Ruvu Farm Ltd in Zambia (Table 2).

The current market price for both local and imported fish feeds is USD 1.1-2.16 per kg, which is high and uneconomical for most Tanzanian fish farmers. It has been reported that feed costs account for over 50% of production costs in intensive and semi-intensive aquaculture (Kubiriza *et al.,* 2016; Madalla *et al.,* 2013; Watanabe, 2002). The high price of fish feeds is due to the use of expensive feed ingredients, such as fishmeal, which is an ideal protein source for most cultured fish species (El-Sayed, 2006; Kubiriza *et al*., 2016; NRC (National Research Council), 2011; Tacon & Metian, 2008). Due to the high production costs from using commercial fish feeds in Tanzania, most small-scale tilapia fish farmers rely on locally available feedstuffs for their cultured fish (Chenyambuga *et al.,* 2014; Kaliba *et al.,* 2006). The most commonly used local feed ingredient is maize bran, followed by Lake Victoria sardines, sunflower seed cake, rice bran and wheat pollard (Kaliba *et al.,* 2006). The ingredients are fed mixed or as single ingredients to the farmed fish. Moreover, due to the limited supply and high price of fishmeal, several alternative protein sources have been investigated worldwide (Kubiriza *et al.,* 2016; Madalla *et al.,* 2013; Mugo-Bundi *et al.,* 2015; Ozório *et al.,* 2012).

Table 2*: Imports and suppliers of fish feeds to Tanzania in 2019*

S/N	Import supplier company	Import supplier country	Amount (MT)
1	Ef Outdoor Ltd	Uganda	30
2	Konga Agribusiness Ltd	Mauritius	15.675
3	Petsville Ltd	Poland	0.398
4	Aquasol Tz Ltd	Zambia	8.725
5	Alphakrust Ltd	Malysia, India	47.118
6	Ruvu Fish Farm	Zambia, Thailand	110.1
7	Shazain Co.Ltd	Saudi Arabia	39.25
8	Leadway Co.Ltd	Zambia	0.56
9	Mpanju Fish Farm Ltd	Egypt	40
10	Puff & General Supply	Israel	25
11	Individual fish farmers	Uganda, Holland	180
12	Eden Agr.Aqua Ltd	Thailand	0.015
13	Sameki Ltd	Thailand	0.02
14	Zhongzhi Ltd	Vietnam	24
	Total		*520.861*

Source: URT, 2019.

2.4 Protein, carbohydrate and lipid sources

High-yielding, efficient aquaculture production requires high-quality feeds with a balanced protein content and ideal amino acid profile (A.-F. M. El-Sayed, 2006). Protein plays a vital role in growth, development and reproduction in fish (Cho & Kaushik, 1990; Gomes *et al*., 1995). In general, protein is the most expensive nutrient in fish feeds (Leal *et al.,* 2010). Fishmeal remains the major dietary protein source, comprising between 20 and 60 % of fish feed (De Silva & Anderson, 1995; Watanabe, 2002). Global fishmeal consumption in the aquaculture industry is high and has been increasing for several decades (A.-F. M. El-Sayed, 2006). The aquaculture sector is the main consumer of fishmeal and fish oil, accounting for about 70% of global consumption (Cashion *et al.,* 2017; FAO, 2014). In 2016, farmed tilapia accounted for about 17% of global fish consumption (FAO, 2018) (Figure 4). According to Malcorps *et al*. (2019), complete substitution of 20-30% of fishmeal in fish feeds could lead to increasing demand for freshwater (up to 63%), land (up to 81%), and phosphorus (up to 83%). Fishmeal has become an expensive feed ingredient all over the world, due to limited availability and competition between different animal production sectors (IFFO, 2017) (Figure 5).

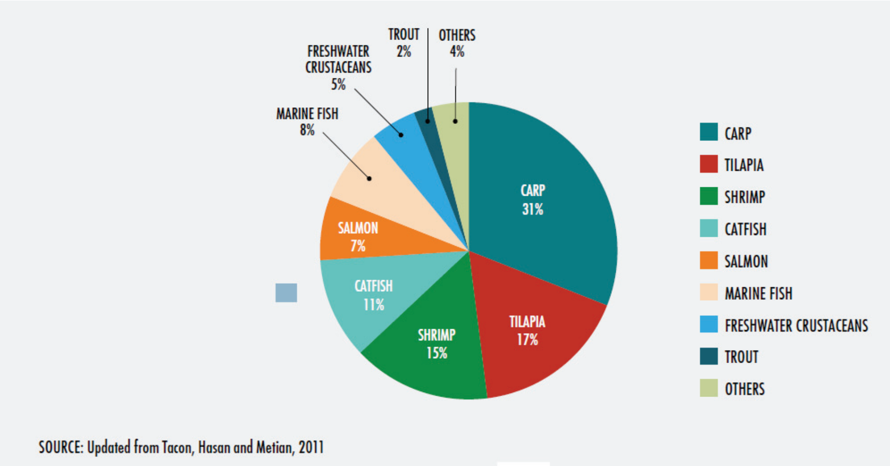

Figure 4: Global share of consumption of total aquaculture feed by species group (%). (Source: FAO, 2018)

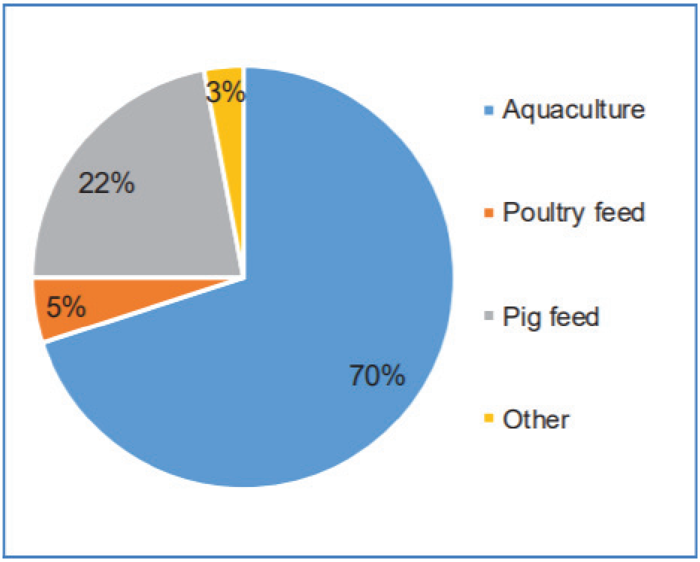

Figure 5: Global fishmeal usage (percentage by volume) by different production sectors in 2017. (Source: Marine Ingredients Organization; IFFO*)*

Decreases in availability and increases in the price of fishmeal and fish oil have prompted a search for sustainable protein ingredients as alternatives to fish-based products in aquaculture feeds (Garuso, 2015). The protein sources investigated can be categorised into animal by-products, agricultural by-products, plant leaves, aquatic plants and industrial by-products.

2.4.1 Animal by-products

Globally, the effects of several fishmeal alternatives of animal origin on the growth performance of cultured tilapia species have been studied over several decades. Mugo-Bundi *et al*. (2015) reported no negative impact on growth performance of tilapia when up to 100% of fishmeal in the diet was replaced with freshwater shrimp (*Caridina nilotica*). However, the protein and lipid content in the fish and the digestibility of the feed were found to be highest with a 20% replacement rate and decreased with increasing level of substitution of fishmeal with *C. nilotica* (Mugo-Bundi *et al*., 2015)). Other local ingredients investigated elsewhere include cattle blood (Bekibele *et al.,* 2013; Kirimi *et al.,* 2017), maggot fly (Devic *et al.,* 2013; Obeng *et al.*, 2015), poultry by-products (El-Sayed & Abdellah, 2012; Soltan, 2009; Yones & Metwalli, 2015), bone and meat meal (Mabroke *et al.,* 2013; Suloma *et al.,* 2013) and shrimp waste (Leal *et al.,* 2010). Animal by-products are high in crude protein and are therefore able to meet the protein requirements of tilapia for growth, reproduction and development (Abdel-Tawwab *et al.,* 2010). Moreover, ingredients of animal origin have an amino acid profile, particularly lysine, tryptophan, and methionine plus cysteine, which meets the essential amino acid requirements for fish growth and reproduction (NRC, 2011). However, few studies investigating the effects of ingredients of animal origin on growth performance in tilapia have been performed in Tanzania.

2.4.2 Agricultural by-products

Agricultural by-products are considered to be cheap protein sources to replace fishmeal in aqua-feed (Daniel, 2018). The agricultural by-products most commonly used as protein feed ingredients in fish feed are soybean meal, rapeseed meal, coconut seed cake and cotton seed cake (Cho & Slinger, 1979; El-Sayed, 1999; Storebakken *et al*., 1998). In addition, many agricultural by-products are used as energy feed ingredients in aqua-feeds, such as maize bran, rice polish, wheat pollard and rice bran (Kaliba *et al.,* 2006; Liti *et al.,* 2006). Sunflower oil and soybean oil have been used as the major sources of lipids in aqua-feeds (Azaza *et al.,* 2015; Bransden et al., 2003; Dalbir *et al.,* 2015; A. F.M. El-Sayed, 1998; Y. Li *et al.,* 2018). Other ingredients such as sunflower seed cake, rice polish, soybean full fat, cotton seed cake, maize bran and coconut seed cake also contribute to the lipid content in fish feeds (NRC, 2011; Ogello *et al.,* 2017). However, compared with ingredients of animal origin, most agricultural by-products contain high levels of indigestible organic matter in the form of insoluble plant fibres (Maina *et al.,* 2002; Naylor *et al.,* 2009). Moreover, they are often low in limiting amino acids, such as lysine, methionine and tryptophan (Gorissen e*t al.,* 2018). In addition, they may contain anti-nutritional factors that can affect the growth performance of fish (Gatlin *et al*., 2007; Maina *et al.,* 2002; Welker *et al.,* 2016). However, these anti-nutritional factors can be destroyed by heat processing and the limiting amino acids can be provided by supplementation with synthetic amino acids in formulated diets for Nile tilapia and catfish, without affecting their growth performance (Tacon & Metian, 2008).

2.4.3 Plant leaves

The main types of plant leaves commonly used as protein source alternatives to fishmeal in fish diets include leaves of moringa (*Moringa oleifera*) (Idowu *et al.,* 2017; Javid *et al.,* 2018; Madalla *et al.*, 2013; Puycha *et al.,* 2017), cassava (*Manihot esculenta*) (Chhay *et al.,* 2010; Madalla *et al.,* 2016; Nnaji *et al.,* 2010) and sweet potato (*Ipomoea batatas*) (Adewolu, 2008; Lochmann et al., 2013). The crude protein (CP) content of plant leaves found in Tanzania ranges from 311 to 349 g CP kg^{-1} dry matter (DM) in moringa leaves (Madalla et al., 2013) and 288 g CP kg^{-1} DM for unprocessed and 290 g CP kg^{-1} DM for processed cassava leaves (Madalla *et al.,* 2016). According to Lochmann *et al*. (2013), sweet potato leaves can be used as an energy source in catfish diets. However, plant leaves are high in fibre and other anti-nutritional factors that may hinder their potential for fish growth and may affect fish health (Francis *et al.,* 2001; Madalla *et al.,* 2016; Mbahinzireki *et al.,* 2001; Naylor *et al.,* 2009). There are many plant and weed species such as gallant soldier plant (*Galinsoga parviflora*), wandering jew (*Tradescantia zebrina*) and taro (*Colocasia esculenta*) which have leaves with good protein and mineral content, but their potential in fish diets has not yet been investigated (Munguti *et al.,* 2014).

2.4.4 Aquatic plants

The major aquatic plants that have been studied as replacements to fishmeal in fish diets include aquatic ferns (*Azolla* spp.) (Das *et al.,* 2018; Gangadhar *et al.,* 2015), duckweed (*Lemnoideae spp*.) (Noor *et al.,* 2000; Uddin *et al.,* 2007) and water lettuce (*Pistia stratiotes*) (Thy *et al.,* 2008). In general, these ingredients have a good nutrient profile with respect to protein, vitamins and minerals (Das *et al.,* 2018). However, due to seasonal availability, none of these ingredients is commonly used in commercial fish feed production. Moreover, available data indicate that high replacement of fishmeal with aquatic plants results in poor fish growth performance (Das *et al.,* 2018).

2.4.5 Industrial by-products

The major industrial by-products used for fish feed are brewery spent yeast and brewery spent grain. The crude protein content of brewery spent yeast available outside Tanzania ranges from 380 to 426 g CP kg^{-1} DM (Chollom *et al.,* 2017; Nhi, 2019), and that of brewery spent grain from 210 to 290 g CP kg^{-1} DM (Muthusamy, 2014). However, brewery spent grain is high in fibre (174 g kg^{-1}), which lowers its potential for use as a feed for cultured fish. No information is available on the nutritive value of brewery spent yeast produced in Tanzania, but brewery spent yeast generally has a low fibre content, with high digestibility compared with ingredients of plant origin, therefore offering potential for use in fish feed. However, a high level of fishmeal replacement by brewery spent yeast in fish diets can result in reduced growth performance in fish (Chollom et al., 2017). Therefore, at most 50% of fishmeal should be replaced by brewery spent yeast as a source of protein in fish diets (Chollom *et al.,* 2017; Ozório *et al.,* 2012; Zerai *et al.,* 2008).

2.5 Tilapia

Tilapia, a group of species in the family Cichlidae, order Perciformes, is one of the most common cultured fish species in the world, after carp (Cyprinidae family) (Watanabe, 2002). Nile tilapia, locally known in Tanzania as '*perege*' or '*sato*' is one of most widely cultured species of tilapia (A.-F. M. El-Sayed, 2006). It inhabits both freshwater and brackish water (Suresh & Lin, 1992), and can be easily identified by dark bands or stripes found on the body. The species is cultured all over Africa and is raised in tanks, concrete and earthen ponds, culture cages and canvas enclosures (Chenyambuga *et al.,* 2014; Kaliba *et al.,* 2006). More than 95% of fish farmers in Tanzania raise Nile tilapia (Kaliba *et al.,* 2006). The suitability of tilapia for fish farming relates to its biological, social and physiological factors (Gibtan *et al.,* 2008; Kapinga *et al.,* 2014; Madalla *et al.,* 2013). In Tanzania, tilapia has been found to have the best growth rate at salinities up to 15 ppt (Mapenzi & Mmochi, 2016). However, it has also been reported that water parameters, if not managed well, can have detrimental effects on the survival and growth performance of cultured tilapia species (Kapinga *et al.,* 2014).

2.6 Nutritional requirements of tilapia

Nutrients are dietary constituents considered physiologically important in body metabolism. They are essential for optimum growth, reproduction and health (Lall & Dumas, 2015). Tilapia, like other finfish and terrestrial animals, requires a balanced daily intake of protein, lipids, carbohydrates, minerals and vitamins to meet its nutrient and energy requirements. The quantitative nutrient and energy requirements of tilapia depend on age, size, physiological conditions and water temperature (Lovell, 1989; NRC, 2011). For example, juvenile Nile tilapia require a diet with high levels of protein, lipids, vitamins and minerals, but low in carbohydrates. Sub-adult and adult tilapia require a diet with high level of lipids and carbohydrates as their energy sources, and lower levels of protein for high growth rate (Lovell, 1989).

2.6.1 Protein and amino acid requirements

Proteins are complex biomolecules found in the cells and tissues of all animals and plants, and are indispensable for growth and maintenance of life (Lall & Dumas, 2015; Molina-Poveda, 2016). Although there are about 300 amino acids (AA) in proteins in natural sources, only 20 amino acids make up most proteins, each with different physical and chemical properties (Molina-Poveda, 2016). Of these 20 amino acids, 10 are defined as essential (EAA) and have to be provided by the diet as they cannot be synthesised by the animal. The other 10 amino acids are defined as non-essential amino acids (NEAA) as they can be synthesised by the animal. Amino acids are linked into chains by peptide bonds (Lall & Dumas, 2015) and cross-links between chains with sulfhydryl and hydrogen bonds (Molina-Poveda, 2016). Proteins are the most important component of the body of animals, representing 65-85% of the weight of fish and shrimp (Jauncey, 1982), and the protein quality is quantified by the digestible indispensable amino acid score (DIAAS) (Wolfe et al., 2016) or determined by the amino acid profile (Jauncey, 1982).

Fish require higher levels of protein for growth compared with terrestrial animals (Wilson, 1994) and protein is considered the most important constituent in fish diets (Ogunji & Wirth, 2002). The optimum level of crude protein in fish feed ranges from 25 to 50 (Lovell, 1989). However, the quantity of protein required varies considerably due to a number of factors, such as size and age of fish, water temperature, feed allowance, level of non-protein energy in the feed, quality of the protein, and feed availability in the culture environment (NRC, 2011; Santiago & Lovell, 1988).

The protein requirements of tilapia range from 40-45% CP for brood stock, 40-50% CP for fry/fingerlings and 28-32% CP for grow-out fish (Abdel-

Tawwab *et al.,* 2010; A.-F. M. El-Sayed, 2006; Stickney, 1997). Thus, the quantity of protein required for tilapia decreases as the fish grow to maturity. As an omnivorous fish species (NRC, 2011), tilapia can utilise both animal and plant materials for its nutrient supply. However, with respect to EAA requirements, feed ingredients of animal origin are more likely to meet the required amino acid profile (Madalla, 2008).

In order to support high fish performance and health, all EAA must be present in the diet in correct relative proportions for *in situ* protein synthesis to take place (Gatlin *et al.,* 2007). Estimated EAA requirements for tilapia have been reported by Santiago and Lovell (1988), Fagbenro (2000), Santiago (1985) and NRC (2011) (Table 3). Dietary protein deficiency in fish can result in severe growth retardation, depletion of body protein and amino acids, and low survival rate, and may be reflected in selected blood parameters (Ogunji & Wirth, 2002).

Table 3*: Amino acid requirements (%) of Nile tilapia (Oreochromis niloticus) reported in different sources*

Amino acid	Santiago & Lovell (1988)	Fagbenro (2000)	Santiago (1985)	NRC (2011)
Arginine	4.2	4.1	4.3	1.2
Histidine	1.7	1.5	1.5	1.0
Isoleucine	3.1	2.6	2.6	1.0
Leucine	3.4	4.3	3.5	1.9
Lysine	5.1	-	5.1	1.6
Methionine	2.7	1.3	2.2	0.7
Phenylalanine	3.8	3.2	5.0	1.1
Threonine	3.8	3.3	2.0	1.1
Tryptophan	1.0	0.6	0.5	0.3
Valine	5.1	3.0	3.0	1.5

2.6.2 Lipid requirements

Lipids, and their constituent fatty acids, are among the major organic components of fish, with carbohydrates being quantitatively much less important (Tocher, 2003). Fatty acids are essential sources of metabolic energy for growth, reproduction and movement in fish (Tocher, 2003). Moreover, fatty acids assist in absorption of fat-soluble vitamins, such as sterol and vitamins A, D, E and K (Lim *et al.,* 2011). These fat-soluble vitamins play integral roles in a multitude of physiological processes, such as vision, bone health, immune function and coagulation (Reddy & Jialal, 2018). Lipids, particularly phospholipids, are the major component of cellular structure and are responsible for maintenance of membrane flexibility and permeability (Lim *et al.,* 2011). The fatty acids are classified based on their chain length, degree of unsaturation (number of ethylenic or double bonds), and the position of their ethylenic bonds (Tocher, 2003) (Figure 6).

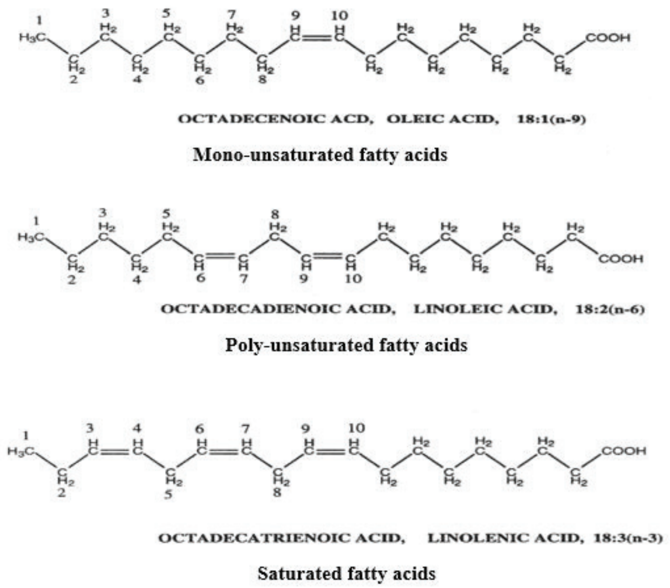

Figure 6: Chemical structure of 18-carbon saturated and monounsaturated fatty acids and of representative polyunsaturated fatty acids (PUFA) of the n-6 and n-3 series. (Source: Tocher, 2003)

Plant oils rich in C18:2 omega-6 fatty acids (*e.g*. soybean oil, maize oil, sunflower oil, rapeseed oil) have been reported to be good lipid sources for growth and reproductive performance of tilapia (Lim *et al*., 2011; Tocher, 2003). Other studies have reported dietary lipid sources of linoleic n-3 series fatty acids and omega-6 fatty acids to be essential for Nile tilapia, blue tilapia (*Oreochromis aureus)* and hybrid tilapia (*Oreochromis aureus* x *O. mossambicus*), as reflected in good growth and reproductive performance. However, growth response to omega-6 fatty acids is reported to be superior to the response to omega-3 series fatty acids in redbelly tilapia (*Tilapia zillii*) (Lim et al., 2011). Furthermore, better growth performance has been reported in tilapia when mixtures of plant and fish oil are used (El-Tawil et al., 2019).

The dietary lipid requirement for tilapia ranges from 10 to 15 % of DM, but the use of lipid levels above 15% may cause growth retardation and carcass lipid accumulation in juvenile hybrid tilapia (Chou & Shiau, 1996; He *et al*., 2015; Kefi *et al*., 2013). Although 4-5% dietary lipid in DM appears to be sufficient to meet the minimum lipid requirement of hybrid tilapia (Chou & Shiau, 1996; El-Marakby, 2006; He *et al*., 2015), a level of 14.9% of DM may be needed for maximum fish growth (Kefi *et al*., 2013). However, a dietary lipid content of less than 10% of DM is recommended for optimal growth in Nile tilapia (Chou & Shiau, 1996). Moreover, quantitative lipid requirements vary within fish species due to fish size. In juvenile blue tilapia, the dietary lipid requirement is estimated to be 7.5-10% of DM, compared with 5-6% of DM for grow-out blue tilapia (El-Marakby, 2006). In diets with a high protein content for fish larvae/fry, high levels of dietary lipids (10-12% of DM) are needed to maintain optimum protein: energy ratio and to optimise dietary protein utilisation (Lim *et al.,* 2011).

2.6.3 Carbohydrate requirements

Carbohydrates are organic substances that consist of carbon, hydrogen and oxygen, with the last two elements being present in the same ratio as in water (Blackstock, 1989). Carbohydrates are the main source of energy in the diet of many animals, including tilapia (Abro, 2014). They are categorised based on the constituent sugars, structure, composition, degree of polymerisation and glycosidic linkage to non-monomer carbohydrates (Englyst & Hudson, 1996). According to the degree of polymerisation (monomeric units), carbohydrates include sugars (1-2 units; glucose, sucrose, maltose, sorbitol and mannitol), oligosaccharides (3-9 units; dextrins, raffinose and starchylose) and polysaccharides (>9 units; amylose, cellulose, pectins and hydrocolloids). In addition, the chitin found in invertebrates (*e.g*. shrimps, krill) is a modified type of polysaccharide that contains nitrogen that is synthesised from units of N-acetyglycosamine (Lall & Dumas, 2015).

Carbohydrates are considered the least expensive form of dietary energy for fish and other animals. Nonetheless, the nutritional value of various forms of dietary carbohydrates and their utilisation vary among fish and between fish species and with carbohydrate source (Krogdahl *et al.,* 2005; Wilson, 1994). Fish are known to have a limited ability for digestion and metabolism of carbohydrates, and excessive intake of this nutrient may result in nutritional problems, such as reduced growth rate accompanied by poor feed utilisation, increased incidence of diseases and impaired anti-oxidation ability. (Hemre *et al.,* 2002). Fish are mainly reliant on protein and lipids as their major source of energy supply for the synthesis of other biologically important compounds (Lall & Dumas, 2015). Inclusion of dietary carbohydrates tends to have positive effects on growth (Watanabe, 2002), but if it is not provided appropriately it may have negative effects on nutrient utilisation, growth, metabolism and health

(Abro, 2014; X. Li *et al.,* 2012). In general, herbivorous fish utilise dietary carbohydrate more efficiently than carnivorous and omnivorous fish (Hemre *et al.,* 2002). On the other hand, warm-water omnivorous fish like tilapia use much higher levels of dietary carbohydrate than temperate-water carnivorous fish like salmonids (Wilson, 1994). The use of energy-yielding nutrients stimulates the secretion and action of certain hormones, particularly insulin and glucagon/glucagon-like peptides, in fish. However, growth hormones and insulin-like growth factor (IGF) are also influenced by environmental factors such as temperature and photoperiod that affect carbohydrate metabolism in fish (Lall & Dumas, 2015).

The optimal dietary carbohydrate requirements have not been demonstrated in fish (Lall & Dumas, 2015), but an optimum dietary carbohydrate supply is important for fish growth (Zhou *et al.,* 2013). According to Azaza *et al*. (2015), an increased dietary carbohydrate content improves metabolism and growth in tilapia. A dietary inclusion level of starch at 10-40% of DM is reported to support high growth rate in tilapia (Abro, 2014; Amirkolaie *et al.,* 2006).

2.6.4 Mineral requirements

All aquatic organisms require inorganic elements or minerals for their normal life processes (Santosh P. Lall, 2003). These life processes include formation of skeletal structure, maintenance of colloidal systems and regulation of acid-base equilibrium, and production of hormones, enzymes and enzyme activators (Chanda *et al.,* 2015). Calcium (Ca) and phosphorus (P) are essential for formation of the skeletal structures of the body and fish scales. Sodium (Na), potassium (K) and chlorine (Cl), along with phosphates and bicarbonate, are responsible for maintaining homeostasis and acid-base balance equilibrium. Iodine (I) is required for biosynthesis of thyroid hormones (Chanda *et al.,* 2015). Minerals are categorised as nutritionally essential major elements (*i.e*. Ca, K, magnesium (Mg), Na, and P), essential minor or trace elements (*e.g*. bromine (Br), iron (Fe) and iodine (I)), or toxic elements (*e.g*. mercury (Hg), cadmium (Cd), cobalt (Co) and chromium (Cr)) (Bhanderi *et al.,* 2016; Bhowmik *et al.,* 2012; Chiba, 2009; Das *et al.,* 2018; NRC, 2011).

Tilapia, like other finfish, obtain the mineral(s) they require from either the diet offered or from the surrounding water through the gills (Chanda *et al.,* 2015; Watanabe *et al.,* 1997). Dietary sources of essential major elements include ingredients of animal and terrestrial plant origin, *e.g.* animal by-products, plant leaves, and weeds contain potassium, shrimps contain sodium and prawns contain magnesium. The essential minor mineral elements are found in aquatic plants and agricultural by-products, *e.g*. soybean products contain iodine and mosquito fern and duckweed contain iron (Hertrampf & Piedad-Pascual, 2000). Major sources of calcium are limestone and seashells.

The mineral requirement of tilapia varies depending on stress exposure, age, physiological conditions, route of entry and form of mineral sources (Terech-Majewska, 2016). The phosphorus requirement range is reported to be 0.3-0.5, 2.1-7.1 and 7.6-7.9 g P kg^{-1} DM for blue tilapia, Nile tilapia and hybrid tilapia, respectively (Furuya *et al*., 2008; Phromkunthong & Udom, 2008; Robinson *et al*., 1987). The calcium requirement ranges from 1.7 to 10 g Ca kg^{-1} DM for blue tilapia and from 0.6 to 10.7 g kg^{-1} DM for hybrid tilapia (Robinson *et al*., 1987; Shiau & Tseng, 2007). The sodium requirement ranges from 1.5 to 1.6 g Na kg^{-1} DM for hybrid tilapia (Shiau & Lu, 2004). The magnesium requirement for Nile tilapia and its hybrids ranges from 0.03 to 3.2 g Mg kg^{-1} DM (Dabrowska *et al.,* 1989; Lin *et al.,* 2013). The requirement for iron ranges from 24.7 to 200 mg Fe kg^{-1} DM for Nile tilapia (Shiau & Hsieh, 2001) and from 85 to 160 mg Fe kg^{-1} for hybrid tilapia (Shiau & Su, 2003).

Iodine deficiency has been reported in soil in many places in Tanzania (Assey *et al.,* 2009). Therefore, the dietary content of iodine needs to be evaluated in order to meet the recommended minimum requirement of 2.8 mg I kg^{-1} DM for fish (Watanabe *et al.,* 1997). Limiting the supply of essential minerals in fish diets may lead to mineral deficiency, resulting in conditions such as anaemia, osteoporosis, stunted growth and genetic disorders (Bhandari & Banjara, 2015; Dato-Cajegas & Yakupitiyage, 1996). However, there is no information available about mineral concentrations in local feed ingredients used to supplement diets for fish and other animals in Tanzania, so evaluations of mineral concentrations in local feed ingredients are urgently needed.

2.7 Nutrient digestibility and growth performance in tilapia

2.7.1 Digestibility determination

Digestibility is a measure of the degree of absorption of nutrients in the digestive tract, whereby dietary carbohydrates, proteins and lipids are degraded into absorbable units in the form of monosaccharides, amino acids and fatty acids (Lall & Dumas, 2015). Determination of digestibility is one of the first steps in evaluating the nutritive value of a feed ingredient (Allan *et al.,* 2000). The nutritive value of a formulated feed depends on the digestibility of each ingredient and interactions among ingredients (Abro, 2014; Sørensen, 2012). However, the digestibility of a nutrient or energy is not a constant value like the chemical composition of the feed ingredient. Rather, the digestibility is influenced by several factors, including feeding and the individual fish (*e.g.* species, age, gut health, and physiological status) (Lall & Dumas, 2015; NRC, 2011).

The digestibility can be measured directly from the difference between intake and faecal output of a nutrient or energy source. However, quantitative total collection of faeces in water is not possible for fish, so an inert indicator like chromium or titanium (Ti) is added to the diet to allow estimation of the digestibility (Lall & Dumas, 2015). The digestibility of nutrients and energy in fish can also be evaluated by methods such as siphoning and stripping (Bureau *et al*., 1999; Cho & Slinger, 1979; El-Shafai *et al*., 2004; Montoya-Mejía *et al.*, 2017; NRC, 2011; Palupi *et al.*, 2020). The use of mechanical methods and siphoning for faeces collection have been associated with nutrient leaching, which can have implications for the digestibility estimates obtained (Storebakken *et al.,* 1998).

The apparent digestibility (AD) of nutrients and energy in feed samples can be determined according to Cho and Kaushik (1990) and that of ingredients can be computed according to Bureau *et al.* (1999) as follows:

i. Apparent digestibility of dry matter (AD_DM) (%) = 100 × [1- (% dietary chromic oxide / % faecal chromic oxide)]
ii. Apparent digestibility of nutrient or energy in diet (AD_Nutrient/energy) (%) = 100 × [1 – (% faecal nutrient / % dietary nutrient) × (% dietary chromic oxide / % faecal chromic oxide)]
iii. Apparent digestibility of a test ingredient ($AD_{test\ ingr.}$) (%) = 100* ($AD_{test\ diet}$ +[($AD_{test\ diet}$-$AD_{ref.\ diet}$) × (0.7×D ref / 0.3× $D_{test\ ingr.}$)]), where D_{ref} is percentage of nutrient in reference diet and $D_{test\ ingr.}$ is percentage of nutrient in test ingredient.

2.7.2 Dietary nutrient performance for tilapia fish growth

Quantitative direct measurement of feed intake in fish is difficult in aquaculture nutrition research (Glencross *et al.,* 2007). Instead, feed intake can be indirectly estimated from measurement of the growth performance, which reflects net nutrient deposition in the tissues of the fish body. In addition to feed intake, the growth response is influenced by several factors including life stages, fish size and species, physiological conditions, genotype and environmental factors (NRC, 2011).

The most commonly used growth performance indices are weight gain (WG), average daily weight gain (ADWG), specific growth rate (SGR), daily growth coefficient (DGC) and thermal growth coefficient (TGC) (Abdel-Warith *et al.,* 2019; Hassaan *et al.,* 2018; Ozório *et al.,* 2012; Vidakovic *et al.,* 2016). Good growth indicates efficient utilisation of the feed given. Therefore, efficient feed utilisation in cultured fish is another very important factor considered by the feed industry. According to Qi *et al*. (2012), the most commonly used feed utilisation indices for tilapia are feed conversion ratio (FCR), feed intake (FI), protein efficiency ratio (PEP) and protein productive value (PPV). According to de Verdal *et al*. (2018), the most widely used measure of fish production efficiency is FCR, which is calculated as the weight of feed administered over the lifetime of an animal divided by weight gain. Therefore, improving feed efficiency in fish is crucial at the economic, social and environmental level in order to develop more sustainable aquaculture production (de Verdal *et al.,* 2018). The FCR values reported for farmed tilapia range from 1.4 to 2.4 (Fry *et al.,* 2018). The efficient use of diets by farmed fish enhances fast fish growth rate and thus shortens the time until the fish attain the target market size, which lowers the operating costs and reduces environmental pollution due to lower waste output.

Globally, the effects of fishmeal alternatives on growth performance and feed utilisation in cultured tilapia species have been studied for many years (Al-Ghanim *et al*., 2017; Asma Ali Mohammed Abushweka, 2018; Aziza & El-Wahab, 2019; Kubiriza *et al*., 2016; Liti *et al*., 2006; Mugo-Bundi *et al.*, 2015; Soltan *et al*., 2008). However, information is limited on the impact of fishmeal alternatives on fish growth under Tanzanian conditions. The research performed to date mainly comprises studies performed on fly maggot meal (Hezron *et al*., 2019), cassava leaf meal (Madalla *et al.,* 2016) and moringa leaf meal (Madalla *et al.,* 2013). Therefore, further studies are needed on the nutritional value of locally available non-conventional feed ingredients that could potentially replace fishmeal in diets for tilapia. Greater knowledge of the nutritional properties of locally available non-conventional feed ingredients is important for the development of strategies to ensure long-term sustainable systems for tilapia production in Tanzania.

3 Aims of the thesis

The overall aim of this thesis was to evaluate the nutritive value of locally available feed ingredients fed to Nile tilapia (*Oreochromis niloticus*) for sustainable development of fish farming in Tanzania.
Specific objectives were to:

I. Assess the chemical composition of locally available feed ingredients used by tilapia fish farmers in various regions of Tanzania (Paper I)

II. Evaluate the mineral content of local feed ingredients used by fish farmers as a supplement to the diet of cultured fish in Tanzania (Paper II)

III. Investigate the potential nutritive value of selected feed ingredients, used by small-scale tilapia fish farmers, that are cheap and locally available in different regions of Tanzania (Paper III)

IV. Study the effect of diets formulated with locally available feed ingredients on growth performance and carcass traits in tilapia (Paper IV)

V. Perform a feed cost analysis of replacing fishmeal with locally available ingredients in feed for tilapia in Tanzania (Paper IV).

4 Materials and Methods

4.1 Brief outline of the studies performed

The work began with a field survey performed to evaluate the nutritive value of commonly used local feed ingredients and commercial fish feeds to supplement the diet of cultured tilapia. The most commonly used local feed ingredients and commercial feed samples were collected randomly from targeted fish farmers and/or animal feed centres located near fish farms. Before chemical analysis, the local feed ingredient samples were prepared according to the method described by Sindirações (2005) and Alimentaruis (2004). In brief, samples were sun-dried for 48 h, packaged and transported to the laboratory for proximate chemical analysis and amino acid analysis (Paper I) and for mineral analysis (Paper II). Two experiments were set up according to a complete random block design, with eight diets for a digestibility trial (Paper III) and five diets for a growth performance and carcass trait trial (Paper IV). These two experiments were conducted in plastic IBC tanks (each 1000 L) using brackish groundwater with salinity ranging from 2-5 ppt, with triplicate tanks of fish fed experimental diets for a period of 60 days in each experiment. The experimental fish (juvenile Nile tilapia) used for the digestibility assessment in Paper III were bought from local fish farmers located near the research facility (IMS-Mariculture Centre). For the growth trial (Paper IV), male Nile tilapia fry were bought in the coastal region (Ruvu Farm Ltd) and transported to the research centre in the Tanga region. In both experiments (Papers III & IV), the fish were acclimatised for two weeks prior to commencement of the experiment.

4.2 Field survey (Papers I & II)

The field survey was conducted in 24 districts within seven regions of Tanzania, on the Tanzanian mainland and on the island of Zanzibar, during the period January 2017-February 2018. The study sites were located between latitude 2°22'-11°20'S and longitude 32°50'-39°30'E, and were selected randomly based on existing geographical zones, high water resource potential, presence of large numbers of active tilapia fish farmers and availability of potential local feed ingredients (Figure 7). Data were collected using a structured questionnaire comprising questions concerning type of fish farm, farming system, farming methods and technology, local feed ingredients used, source of fish seeds, investment costs, stocking density, feeding practices, cost of feeds, type and sources of water used on the farm and other issues relating to aquaculture (Paper I). In addition, samples of a total of 30 local feed ingredients were collected during the field survey at four different geographical locations in Tanzania (Dar es Salaam, Morogoro, Mbeya and Mwanza regions) (Figure 7). These samples were analysed for proximate chemical composition (Paper I) and mineral content (Paper II).

4.3 Experimental facilities (Papers III & IV)

The experiments in Papers III and IV were carried out at the Institute of Marine Sciences Mariculture Centre (IMS-MC), located in Pangan, Tanga region, Tanzania (05°25´54.80"S; 38°57´28.87"E). Upon arrival, Nile tilapia juveniles in Paper III were acclimatised for two weeks in 15 plastic tanks (1000 L) while being fed a locally made diet composed of fishmeal as the primary protein source, followed by four days of starvation prior to beginning of the experimental trial. In paper IV, Nile tilapia fry were raised in quarantine tanks for two weeks, before being distributed randomly into seven tanks, each containing 900 L of water, where they were kept for one month and fed starter commercial feed (Aller parvo ex, 0-3 grade; 44% crude protein; Aller Aqua Group) four times a day. The fish were then acclimated for two weeks to a locally made reference diet (containing fishmeal), followed by four days of starvation prior to commencement of the experimental trial.

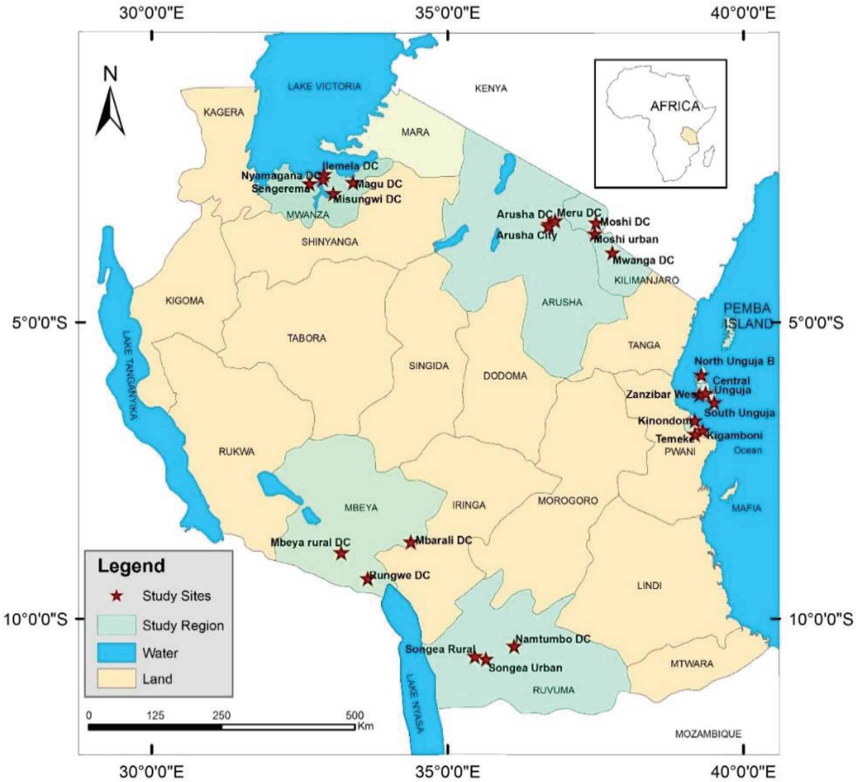

Figure 7: Map of Tanzania showing the location of the survey sites in the regions Dar es Salaam, Morogoro, Mbeya and Mwanza. (Source: modified from Google, IMS Database, 2016)

4.4 Test feed ingredients (Papers III & IV)

The reference diet contained fishmeal (*Rastrineobola argentea*) as the protein source in all cases. The local feed ingredients used in Papers III and IV were: blood from cattle (*Bos taurus*), freshwater shrimp (*Caridina nilotica*), marine shrimp (*Exhippolysmata oplophoroides*), fish frames (skeletal remains of Nile perch (*Lates niloticus*)), brewery spent yeast (*Saccharomyces cerevisiae*), moringa leaves (*Moringa oleifera*) and duckweed (*Lemna minor*). Fishmeal was supplied from Lake Victoria, while freshwater and marine shrimp were by-catches from Lake Victoria and Indian Ocean fisheries, respectively. Cattle blood was collected from cattle abattoirs, brewery spent yeast was obtained from a brewery (Tanzania Brewery Ltd, Dar es Salaam), and duckweed was collected from marsh areas and natural pools in Dar es Salaam. All the feed ingredients were sun-dried prior to feed formulation.

4.5 Experimental diets (Papers III & IV)

In Paper III, the diets (n=8) comprised one reference diet with fishmeal (FM) as the main protein source, and seven test diets containing 70% fishmeal and 30% test feed ingredient (*i.e.* 70:30 ratio) on a DM basis according to Cho and Slinger (1979). The seven test feed ingredients were: brewery spent yeast, moringa leaves, freshwater shrimp, marine shrimp, cattle blood, duckweed and fish frames. Other feed ingredients included to balance the nutrient content in the diets were maize bran, cotton seed cake, wheat pollard, sunflower seed cake, mineral and vitamin premix, cassava flour and sunflower seed oil. Approximately 0.5% (dry weight) of chromic oxide (Cr_2O_3) was included in the diets as an indigestible marker for assessment of digestibility.

In Paper IV, five experimental diets were used; one reference diet and four test diets. The reference diet contained fishmeal as the primary protein source, while the four test diets were formulated by substituting 50% of the fishmeal on a DM basis with brewery spent yeast, freshwater shrimp, cattle blood or fish frames (Table 4). The other inclusions were full fat soybean, sunflower seed cake, wheat pollard, maize bran, cassava flour and sunflower oil. The chemical composition of reference and test diets used in Papers III and IV is presented in Table 4. All the experimental diets used in both papers were produced by extrusion at Tanfeed International Ltd, Morogoro, Tanzania.

Table 4: *Composition (g kg^{-1}) of ingredients used in the reference diet (FMD) and in test diets containing brewery spent yeast (BSYD), moringa leaves (MLD), freshwater shrimp (FSHD), marine shrimp (MSHD), cattle blood (CBD), duckweed (DWD) and fish frames (FFD (Papers III & IV)*

	Paper III								Paper IV				
Ingredient[1]	[2]FMD	BSYD	MLD	FSHD	MSHD	CBD	DWD	FFD	FMD	BSYD	FSHD	CBD	FFD
FM	338	237	237	237	237	237	237	237	400	200	200	200	200
BSY	-	299	-	-	-	-	-	-	-	200	-	-	-
ML	-	-	299	-	-	-	-	-	-	-	-	-	-
FSH	-	-	-	299	-	-	-	-	-	-	200	-	-
MSH	-	-	-	-	299	-	-	-	-	-	-	-	-
CB	-	-	-	-	-	299	-	-	-	-	-	200	-
DW	-	-	-	-	-	-	299	-	-	-	-	-	-
FF	-	-	-	-	-	-	-	299	-	-	-	-	200
FFSB	-	-	-	-	-	-	-	-	190	290	380	40	300
CSC	159	111	111	111	111	111	111	111	-	-	-	-	-
SFSC	49.8	34.8	34.8	34.8	34.8	34.8	34.8	34.8	100	230	120	20	180
MB	199	139	139	139	139	139	139	139	90	20	40	270	40
WP	189	132	132	132	132	132	132	132	90	20	40	110	40
Cassava flour	29.9	20.9	20.9	20.9	20.9	20.9	20.9	20.9	80	20	20	90	40
Cr_2O_3	5	5	5	5	5	5	5	5	-	-	-	-	-
S/flower oil	19.8	13.9	13.9	13.9	13.9	13.9	13.9	13.9	20	20	0	50	0
Vit/Min	10	7	7	7	7	7	7	7	30	0[#]	0[#]	20	0[#]

[1]FM: fishmeal; BSY: brewery spent yeast; ML: moringa leaf; FSH: freshwater shrimp; MSH: marine shrimp; CB: cattle blood; DW: duckweed; FF: fish frames; FFSB: full fat soybean; CSC: cotton seed cake; SFSC: sunflower seed cake; MB: maize bran; WP: wheat pollard.

[2]FMD: fishmeal diet (reference); BSYD: brewery spent yeast diet; MLD: moringa leaf diet; FSHD: freshwater shrimp diet; MSHD: marine shrimp diet; CBD: cattle blood diet; DWD: duckweed diet; FFD: fish frames diet.

[#]The mineral content was covered by the ingredients used in the diet (Mmanda *et al.*, 2019).

4.6 Experimental conditions (Papers III & IV)

For the study in Paper III, 480 juvenile Nile tilapia fish with an average body weight (BW) of 23.6±0.3 g were purchased from a local tilapia fish farmer in Pangan. Prior to acclimatisation, the fish were evenly distributed into 48 plastic tanks (1000 L) with 10 fish per tank. The tanks were divided into eight groups, with six tanks per group. One group was assigned to the reference diet (control) and seven groups received test diets. In each group, three tanks were assigned for faeces collection using the siphoning method and three tanks for faeces collection using the stripping method. The trial was performed according to a change-over design with three tanks in each treatment group assigned for siphoning and three tanks assigned for stripping in the first experimental period, but swapped over to the other method in the second experimental period. The tanks were filled with 900 L of groundwater, with salinity ranging from 2 to 5 ppt. During the experiment, the tanks were cleaned weekly to improve visibility within the water column and discourage growth of algal bloom.

In Paper IV, 225 all male Nile tilapia juveniles (mean weight 1.47g) were evenly distributed into 15 tanks (each 1000 L) with 15 fish per tank. The tanks were divided into five groups, each with three tanks. The fish in each group were fed one of the five treatment diets in triplicate. The water source was groundwater obtained from a borehole. The water in the tanks was continuously aerated using air stones to ensure adequate oxygen supply to the experimental fish. Water quality parameters such as temperature, dissolved oxygen, pH and salinity were monitored on a daily basis, while ammonia, nitrite and nitrate concentrations were monitored twice per month.

4.7 Feeding and feed preparation (Papers III & IV)

In both Papers III and IV, most of the ingredients used, such as fishmeal, cattle blood, brewery spent yeast, wheat pollard, cotton seed cake, and maize bran, were obtained from a local feed ingredient supplier (Saimon's Animal Feeds Ltd, Dar es Salaam). Full-fat soy bean was obtained from Tanfeed International Ltd in Morogoro, freshwater shrimps from a local market in Morogoro, sunflower seed cake and sunflower seed oil from a local sunflower oil refinery in Morogoro, and mineral and vitamin premix from an animal feed shop in Morogoro. Chromium oxide was obtained from the Animal, Aquaculture and Range Sciences Laboratory, Sokoine University of Agricultural Sciences, Morogoro, and cassava flour (binder) was obtained from local food markets in Morogoro.

In Paper III, all ingredients used in diet formulation for digestibility were milled separately into powder form at a milling factory in Morogoro. The desired amount of ingredients was mixed thoroughly with the required amount of chromium oxide and cassava flour. Step-wise mixing was applied during diet preparation to ensure uniform distribution of chromium oxide into the formulated diet. The experimental diets were subjected to extrusion at a feed factory (Tanfeed International Ltd, Morogoro). Pelleted feed was produced using a pelleting extruder machine (DGP 60-HSHP1225, Zhengzhou, China) with a capacity of 100-150 kg h^{-1}, fixed with a 2 mm matrix at 120 -130 °C and high steam pressure of 350-450 kg L^{-1}.

In Paper IV, all ingredients were milled separately into powder form at milling factory in Morogoro. The appropriate amounts of ingredients were mixed according to formulated specifications for reference and test ingredients (Table 4), before being subjected to extrusion at a feed factory (Tanfeed International Ltd, Morogoro).

All feeds used in Papers III and IV were dried by spreading over a drying carpet in free air circulation for 48 h at the Tanfeed International Ltd warehouse, before being weighed, packed into sealed plastic containers and transported to research unit in Pangan, Tanga, for storage at 4 °C until further use.

The Nile tilapia used in Paper III (digestibility trial) were fed at 4% of BW, which corresponded to feeding to satiation once daily, in late morning (09:00 h) for fish groups assigned to the siphoning method, and twice per day, in late morning (09:00-10:00 h) and early afternoon (15:00-16:00 h) for fish groups assigned to the stripping method. In Paper IV (growth trial), the male Nile tilapia fingerlings were fed three times daily (at 08:00 h, 12:00 h and 15:00 h), with an average daily allowance of 10% of their BW for the first 30 days. They were then fed twice daily (08:00 h and 15:00 h) with an average daily allowance of 5% of their BW for the remaining 30 days of the experiment. In both experimental periods, the feed allowance corresponded to their apparent satiation. The feeding was closely monitored in each tank, and feed was adjusted every second week as fish grew, to ensure maximum growth. The amount of feed offered was measured and recorded for the entire experimental period.

4.8 Sample collection (Papers III & IV)

In fish assigned to the siphoning groups (Paper III), faecal matter samples were collected on a 100 µm nylon filter mesh 2 h prior to first feed waste material removal (14:00-17:00 h). The process was repeated daily throughout the experiment. For fish assigned to stripping groups (Paper III), faecal matter was collected (stripped) by pressing the belly of each experimental fish to cause faeces to be expelled from the gut (Nose, 1960). The experimental fish were stripped into separate sample collection containers twice a week. The procedure was repeated throughout the experimental period. All faeces samples collected from fish in each tank were pooled and kept frozen at -20 °C until further analysis.

In Paper IV, a total of five fish were randomly chosen from each experimental tank and their individual body weight (electronic balance) and total length (ruler) were recorded every two weeks throughout the experimental period. Total number and total weight of fish stocked in each tanks were also assessed every two weeks for the entire experimental period, for diet adjustment and survival rate evaluation. At the end of experimental period, three fish were randomly chosen from each tank for assessment of body weight, organ weight, hepatosomatic index (HSI) and viscerosomatic index (VSI). Before sampling, the fish were anaesthetised using clove oil (100 mg L^{-1}). For carcass quality determination, the body weight and length of the anaesthetised fish were recorded before filleting. The fish fillet samples were weighed and then stored in a freezer (-20 °C) before transport to Sokoine University Laboratory for fillet colour determination using a Konica Minolta Chroma meter (Minolta Chroma meter CR-400, Osaka, Japan).

4.9 Water quality monitoring (Papers III & IV)

In Paper III, water quality parameters were monitored weekly. Salinity was measured using a refractometer (RHS-10ATC, Shenzhen, China), while temperature and dissolved oxygen (DO) were measured using a DO meter (HI-8424N, 161 Kallang Way, Singapore).

In Paper IV, parameters such as pH, dissolved oxygen and temperature were measured on-site twice a day (09:00 h and 15:00 h) using a HQD portable meter (HQ40D & pH101, Loveland, Colorado, USA) and a hand-held refractometer (RHS-10 ATC, Shenzhen, China) for salinity. Ammonia, nitrate and nitrite concentrations in water samples from each culture tank were analysed twice per month using a photometer 7100 (Palintest 7100 photometer, Nottinghamshire, England). The reagents used during analysis were Palintest nitrite (Nitriphot No.1 & 2 tablets) for nitrite, Palintest ammonia (Ammonia No.1 & 2 tablets) for ammonia and Palintest nitrate (Nitratest powder, Nitricol tablets) for nitrate. Ammonia, nitrate and nitrite content of the water sample were determined by dilution techniques according to the manufacturer's instructions at 1:10; 1:20 and 1:10 ratio, with a calibration reading range of 0-1.0 mg L^{-1} N, 0-20 mg L^{-1} NO_3^- and 0-1500 mg L^{-1} NO_2^-, respectively. The concentration of unionised ammonia was calculated as a percentage of total ammonium nitrogen (TAN) according to Durborow *et al*. (1997).

4.10 Chemical analysis

In Papers I, III and IV, proximate analyses of ingredients, faecal matter samples, dietary treatments and whole-body fish samples were performed at Sokoine University, Morogoro, Tanzania, according to AOAC (1990). In brief, dry matter was determined by drying 2 g of sample (E 115, WTB binder 7200, Tuttlingen, Germany) at 105 °C overnight to constant weight. Crude protein content was analysed according to Kjeldahl (Pearson, 1999) using a 2200 Kjeltec auto distillation unit (Foss, Tecator, Sweden). Crude fat (ether extract, EE) content was determined with petroleum ether (ST 243 SoxtecTM, Hilleroed, Denmark). Crude fibre (CF) was determined using a fibre analyser (ANKOM 200 Fiber Analyzer, New York, USA). Ash content was determined as the residue remaining after incineration of 1 g of sample in a muffle furnace at 550 °C for 3 h (Nasim Al Mahmud *et al.,* 2012). Nitrogen-free extract (NFE) was calculated by subtracting the sum of moisture, CP, EE, CF and ash from 100 (Castell & Tiews, 1980).

In Paper II, mineral content was analysed at Sokoine University of Agriculture. For analysis of Ca, P, Mg, K, Na and Fe, 1.0 g of milled, homogenised sample was placed in a weighed porcelain crucible, which was then placed in an incinerator and ignited at 450 °C until white or grey ash was obtained (Jorhem, 2000). The ash was dissolved in 10 mL of 10% hydrochloric acid and the suspension was filtered (No. 1 Whatman ashless filter paper, GE Whatman Grade 40; 1440-090) before analysis. The filtrate was subjected to atomic absorption spectrophotometry (Atomic Absorption Spectrophotometer, UNICAM 199 AA Spectrometer, Cambridge CBI 2PX, England) for determination of Ca, Mg and Fe content with absorbance reading at 422.7 nm for Ca, 285.2 nm for Mg and 248.3 nm for Fe according to the manufacturer's instructions. Standard solutions for atomic absorption spectrophotometry of each mineral were prepared by serial dilution of an appropriate stock solution. The standard solutions for Ca ($CaCl_2$) contained 0, 5.0, 10.0, 15.0 and 20.0 mg Ca L^{-1}; the standard solutions for Mg ($MgCl_2.6H_2O$) contained 0, 0.5, 1.0, 1.5 and 2.0 mg Mg L^{-1}; and the standard solutions for Fe ($FeCl_3.6H_2O$) contained 0.0, 5.0, 10, 20, and 40 mg Fe L^{-1}.

For analysis of iodine content, 2.0 g of milled sample were placed in a 25 mL Erlenmeyer flask and 10 mL of deionised water were added. The mixture was shaken for 10 minutes using an orbital shaker (Multishaker, Baird & Tatlock, UK), diluted to 25 mL with deionised water, and filtered (No. 1 Whatman filter paper, GE Whatman Grade 40; 1440-090) before analysis. The content of K and Na in the filtrate was determined using a digital flame analyser (2655 Digital flame analyser, Chicago, USA) according to the manufacturer's instructions. Standard solutions for K and Na were prepared by serial dilution of an appropriate stock solution. The standard solutions for K contained 0.5, 2.5, 5.0 and 10.0 mg K L^{-1}, while the standard solution for Na contained 0.5, 2.5, 5.0 and 10.0 mg Na L^{-1}.

The concentrations of P and I were determined using an UV spectrophotometer (BIOMETE 6, WI53711, USA), with absorbance reading at 884.0 nm for P (AOAC, 1990) and at 665.6 nm for I (Narayana *et al*., 2006). The standard solutions for P (KH_2PO_4) contained 0, 0.1, 0.2, 0.4 and 0.8 ppm P, while the standard solutions for I (KIO_3) contained 0, 5.0, 10.0, 15.0, 25.0 and 30.0 mg I L^{-1}.

In Paper III, chromium oxide content in feed and faeces samples was determined at the Chemistry Laboratory, University of Dar es Salaam, by atomic absorption spectrometry (Varian AAS 240, Santa Clara CA 95051, USA) according to Hill *et al*. (1986). The samples were subjected to the wet acid digestion procedure according to Fenton and Fenton (1979). In brief, 250 mg of sample were weighed into a digestion tube (100 mL), followed by addition of 10 mL concentrated sulphuric acid (H_2SO_4) and 1 mL perchloric acid ($HClO_4$). The mixture was digested at 250 °C for 10-20 min until the contents turned yellow. Thereafter the sample was cooled and diluted to 50 mL with distilled water, followed by 20-fold dilution prior to chromium content determination. The dilute was analysed for Cr content by atomic absorption spectrometry (Varian AAS 240, Santa Clara CA 95051, USA) with absorbance reading at 357.9 nm. The standard stock solutions used were 2, 5 and 10 mg L^{-1} of chromium (III) oxide (Cr_2O_3).

4.11 Fillet colour determination (Paper IV)

In Paper IV, the muscle colour of Nile tilapia fillets was measured at Sokoine University Laboratory using a Konica Minolta Chroma meter (Minolta Chroma meter CR-400, Osaka, Japan) according to the manufacturer's instructions. The body weight and length of the anaesthetised fish were recorded before filleting. The fish fillet samples were weighed and then stored in a freezer (-20 °C) before transport to the laboratory for fillet determination. The muscle colour of tilapia was determined according to Lemmens (2014), where: L* describes lightless (L* = 0 is black and L* = 100 is white), a* is the intensity in red (a* >0) or the intensity in green (a* <0) and b* is the intensity in yellow (b* >0) or the intensity in blue (b* <0) (Figure 8).

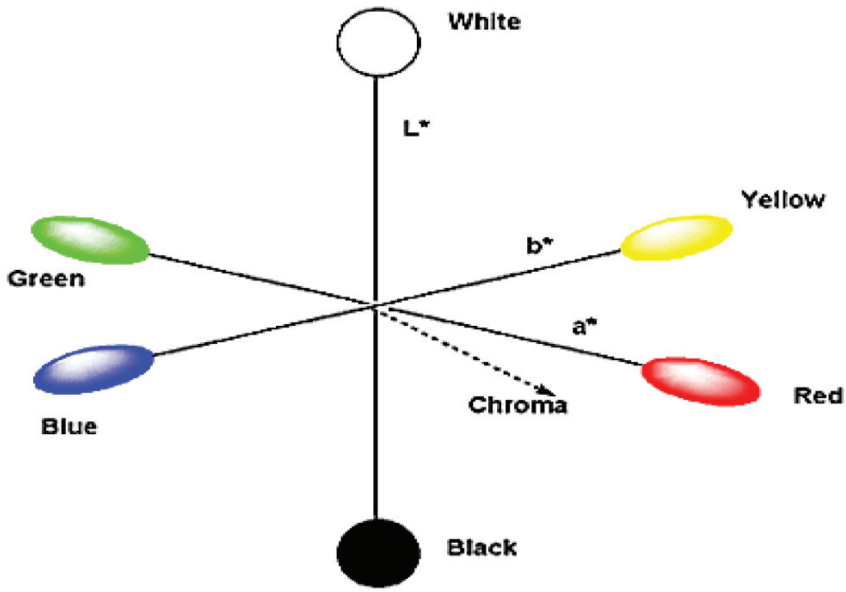

Figure 8: Tristimulus sphere indicating lightness (L*), colour intensities a* and b*. (Source: Lemmens, 2014)

4.12 Calculations (Papers III & IV)

Apparent digestibility (AD) of nutrients and gross energy (AD_GE) in test and reference diets in Paper III was determined according to Cho and Kaushik (1990) as follows:

i. AD_DM (%) = 100 × [1- (% Dietary chromic oxide/% Faecal chromic oxide)].

ii. AD_Nutrient (or Energy) (%) = 100 × [1 – (% Faecal nutrient/% Dietary nutrient) × (% Dietary chromic oxide/% Faecal chromic oxide)] (Cr_2O_3e values determined for faeces samples were normalised between sampling periods).

The AD of test ingredients ($AD_{test\ ingr.}$) was calculated using the equation proposed by Bureau *et al.* (1999):

$AD_{test\ ingr.}$ (%) = $AD_{test\ diet}$ + (($AD_{test\ diet}$-$AD_{ref.\ diet}$) × (0.7×D Ref/0.3× $D_{test\ ingr.}$))
where D_{ref} and $D_{test\ ingr.}$ are percentage of nutrient in reference (fishmeal) and in test ingredient, respectively.

In Paper IV, growth performance indices such as weight gain (WG), percentage weight gain (%WG), specific growth rate (SGR) and condition factor (Kf) were calculated according to Sveier *et al.* (2000) as follows:

WG = W_f-W_i; where W_f = final body weight and W_i = initial body weight

%WG = [(W_f-W_i)/Wf]*100

SGR = [(ln (W_f) - ln (W_i)/ T]* 100; where T= experimental period (days)

Kf = (TBW/TL^3)*100; where TBW = total body weight (g) and TL^3 = total length (cm)

Feed utilisation indices determined in Paper IV, *i.e.* feed conversion rate (FCR), protein efficiency ratio (PEP), survival rate (SR), protein productive value (PPV) and specific growth rate (SGR), were calculated according to Qi *et al.* (2012) as follows:

FCR = FI/WG; where FI = feed intake (g/day) and WG = weight gain (g/day)

PER = WG/PI; where WG = weight gain (g) and PI= protein intake (g)

SR = (N_f/N_i)*100; where N_f = final fish number and N_i = initial number of fish

PPV = PG/ PI; where PG = protein gain (g) and PI = protein intake (g)

SGR = 100 (ln P_f – ln P_i) /T; where P_i = initial protein content and P_f = final protein content.

In Paper IV, viscerosomatic index (VSI) and hepatosomatic index (HSI) were calculated according to Kubiriza *et al.* (2016) as follows:

VSI=100*(FVM/FBM); where FVM = fish visceral mass (g) and FBM = fish body mass (g)

HSI=100* (LM/BM); where LM = liver mass (g) and BM = body mass.

In Paper IV, fillet colour parameters lightness (L*), redness (A*), and yellowness (B*) were measured using a Konica Minolta Chroma meter (Minolta Chroma meter CR-400, Osaka, Japan) according to the manufacturer's instructions. Other parameters such as hue (colour tones), chroma (colour saturation), entire colour index (ECI) and hue angle were calculated according to Brown *et al*. (2016):

Hue = B*/A*; where B* = yellowness and A* = redness

Chroma = $\sqrt{(A^{*2}+B^{*2})}$, where B* = yellowness and A* = redness

ECI = C* Cos (H_t-H_{mean}), where C= chroma, H_t = hue over time (day) and H_{mean} is mean hue

Hue angle = Tan^{-1}(B*/A*), where B* = yellowness and A* = redness.

4.13 Statistical analysis

The observational data collected in Paper I were analysed using the SAS program (SAS (r) Proprietary Software 9.4 (TS1M3 DBCS3170) (Licensed to SLU - SAS KOMPLETT 64, Site 51302612). Cross-tabulation, determination of frequencies and chi-square tests were among the statistical analyses performed on the dataset.

In Paper III, the apparent digestibility values obtained for dry matter, organic matter (OM), crude protein and energy were subjected to one-way analysis of variance (ANOVA) within and between faeces collection methods, using SAS software (SAS version 9.4). Faeces collection method was considered a fixed effect in ANOVA, while diet was considered a random effect. Differences in AD values between diets were determined by multiple comparisons using the Tukey's test and were considered significant at $P<0.05$.

In Paper IV, the data collected during the growth performance trial were subjected to statistical analysis by one-way ANOVA using the SAS programme, version 9.4. Tank was considered a fixed effect, while diet was considered as a random effect. The values obtained in the trial (means and standard error) were compared by multiple comparison using the Tukey method and were considered significant at ($P<0.05$).

5 Main results

5.1 Field survey and chemical composition of local feed ingredients (Papers I & II)

5.1.1 Observational data (Paper I)

A total of 202 tilapia fish farmers, in seven regions of Tanzania, participated in the survey. The results showed that 81.5% of the respondents used local feed ingredients as a primary supplement diet for their cultured fish. The mostly commonly used ingredient was maize bran (28%), followed by Lake Victoria sardines (11%) and sunflower seed cake (11%) (Figure 9).

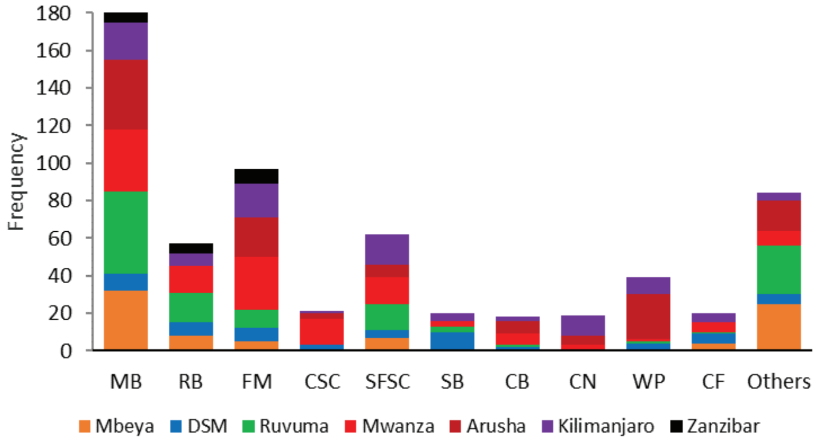

Figure 9: Frequency of use of different local feed ingredients by tilapia fish farmers (%, n=202) at seven study sites in Tanzania (DSM: Dar es Salaam). MB: maize bran; RB: rice bran; FM: fishmeal

(sardines; *Rastrineobola argentea*); CSC: cotton seed cake; SFSC: sunflower seed cake; SB: soybean; CB: cattle blood; CN: *Caridina nilotica* (freshwater shrimp); WP: wheat pollard; CFl: cassava flour.

5.1.2 Proximate composition analysis (Paper I)

The 30 selected local feed ingredients used by tilapia farmers that were analysed for proximate chemical composition were classified into the following five categories: Animal by-products; Agricultural by-products; Plant leaves and weeds; Aquatic plants; and Others (Table 5). In Paper I, the results indicated that the crude protein content in most local feed ingredients analysed was medium to high, while the crude fat (EE) content was high in some animal and agricultural by-products, but was medium to low in the other groups of ingredients. Moreover, agricultural by-products, aquatic plants and industrial by-products had a medium-high content of nitrogen-free extract (NFE), while the ash content varied between the feed ingredients analysed. The chemical composition of commercial fish feeds sold in Tanzania showed large variations in crude protein, crude fibre and crude fat (EE) content. The crude protein content ranged from 22.9 to 53.6%, but was mostly within the range 22.9-33.4% (Paper I).

5.1.3 Amino acids (Paper I)

Animal by-products, except for Nile perch fish frames, were high in lysine (3.6-6.4%), tryptophan (0.4-1.7%) and methionine + cysteine (1.4-2.6%) (Table 6). Agricultural by-products (*i.e.* soybean and full fat soybean), plant leaves and weeds (*i.e.* taro leaves, cassava leaves, sweet potato leaves and gallant soldier weed), aquatic plants (*i.e. Azolla* ferns and lettuce vegetable) and spent brewery yeast were intermediate in lysine (2.1-3.7%) and methionine + cysteine (0.4-1.9%), but high in tryptophan (0.2-1.5%).

Table 5: *Proximate composition of local feed ingredient categories (g kg^{-1} DM) (Paper I)*

Category	Ash	Crude protein	Crude fibre	Ether extract	NFE
Animal by-products	33-588	280-914	0-129	2-249	11-192
Agricultural by-products	35-216	93-437	47-244	49-175	92-684
Plant leaves and weeds	98-303	186-397	52-232	5-68	301-478
Aquatic plants	200-223	198-235	118-133	16-29	398-456
Industrial by-products	35-43	194-350	0-174	5-85	447-610

NFE = Nitrogen free extract

Table 6: *Amino acid composition of different feed ingredients (% of dry matter) (Paper I)*

Feed sample	Tryptophan	Lysine	Methionine + Cysteine
Earthworm	0.5	5.2	2.3
Housefly maggot	0.6	6.9	2.0
Cattle blood	1.8	5.3	2.7
Cock shrimp (marine)	0.7	6.7	2.1
Freshwater shrimp	0.6	6.3	2.0
Prawn head waste	0.5	5.6	1.5
Nile perch fish frames	0.0	0.1	1.3
Lake Victoria sardines	0.4	3.9	2.0
Soybean	0.4	3.4	2.0
Full fat soybean	0.4	2.8	1.7
Brewery spent yeast	0.5	3.8	1.4
Taro leaf	0.3	2.9	1.6
Cassava leaf	0.4	3.0	0.9
Gallant soldier plant	0.2	2.3	0.5
Lettuce vegetables	0.4	3.5	1.1
Sweet potato leaf	0.3	2.8	0.4
Azolla (aquatic fern)	1.6	2.6	1.3

5.1.4 Mineral content (Paper II)

Analysis of the mineral content in 26 local feed ingredient samples collected from fish farmers in different regions in Tanzania revealed a wide range of concentrations in different feed ingredients (Table 7). The highest level of P was found in fish frames (17.8 g kg^{-1}), the highest level of Ca in limestone (107.3 g kg^{-1}), the highest level of K in gallant soldier plant (51.0 g kg^{-1}), the highest level of Na in marine shrimp (11.7 g kg^{-1}), the highest level of Mg in prawn head waste (4.2 g kg^{-1}), the highest level of Fe in aquatic fern (*Azolla* spp.) (2355 mg kg^{-1}) and the highest level of iodine in full fat soybean (447 mg kg^{-1}).

Ingredients of animal origin, except for cattle blood, had a high content of P, Ca, K, Na, Mg, Fe and I (Table 7). Similarly, agricultural by-products contained high levels of P, K, Fe and I, but low levels of Ca, Na and Mg with the exception of wheat pollard, which contained high levels of Ca and Na (Table 7). With the exception of moringa leaves and lettuce vegetables, plant leaves and weeds showed a similar pattern to agricultural by-products, with high values for P, K, Fe and I, but low values for Na and Mg (Table 7). Aquatic plants were high in all minerals, except for Ca in aquatic fern and Mg in duckweed (Table 7). In general, the Ca content was higher in aquatic plants than in agricultural by-products. In the group 'Others', seashells and limestone showed a high content of Ca and Fe (Table 7).

Table 7: *Content (per kg DM) of different minerals in local feed ingredients used by tilapia fish farmers in Tanzania (Paper II)*

Sample	P (g)	Ca (g)	K (g)	Na (g)	Mg (g)	Fe (mg)	I (mg)
Animal by-products							
Cattle blood	0.9	<0.1	3.2	8.5	<0.1	202	3
Fly maggot	6.6	1.4	11.1	3.6	2.2	370	167
Sardines	10.9	7.6	10.8	2.4	1.3	142	118
Marine shrimp	8.3	5.4	12.4	11.7	3.2	97	58
Freshwater shrimp	9.9	16.3	11.3	4.7	1.4	328	294
Prawn head waste	12.3	26.6	5.9	5.5	4.2	223	63
Fish frames	17.8	18.5	3.9	4.7	1.6	94	14
Agricultural by-products							
Full fat soybean	4.5	0.6	16.0	<0.1	<0.1	401	447
Soybean	5.6	0.4	12.8	0.6	1.9	61	93
Sunflower seed cake	5.1	1.4	10.5	0.2	<0.1	146	66
Cotton seed cake	5.7	0.5	15.1	<0.1	<0.1	65	4
Maize bran	7.8	0.06	9.3	<0.1	<0.1	87	60
Rice polish	11.3	1.5	10.6	<0.1	<0.1	160	32
Wheat pollard	14.2	40.9	9.9	6.0	<0.1	78	41
Plant leaves and weeds							
Moringa leaf	3.2	8.4	14.0	0.8	3.6	95	84
Lettuce vegetables	3.4	10.6	51.5	6.9	3.8	838	137
Cassava leaf	4.7	1.6	51.1	<0.1	<0.1	245	165
Taro leaf	3.2	3.2	46.5	0.1	1.6	143	89
Gallant soldier plant	5.6	4.9	51.0	0.2	<0.1	217	13
Sweet potatoes	0.8	0.1	7.7	<0.1	<0.1	66	15
Aquatic plants							
Aquatic fern (Azolla)	5.8	1.5	31.5	5.6	1.9	2355	179
Water lettuce	4.8	13.6	33.2	4.3	0.9	229	77
Duckweed	14.3	44.8	9.3	6.7	<0.1	2265	60
Others							
Spent brewery yeast	3.3	1.2	3.7	0.2	0.3	52	157
Seashells	0.5	93.8	0.4	3.1	2.7	320	6
Limestone	0.2	107.3	0.5	0.5	1.1	316	8

5.2 Chemical composition of test diets (Papers III & IV)

The chemical composition of the test diets used in Papers III and IV is summarised in Table 8. In Paper III, the range of values was 96.9-222 g kg^{-1} DM for ash, 285-477 g kg^{-1} DM for crude protein, 47.8-82.9 g kg^{-1} DM for crude fibre, 76-113 g kg^{-1} DM for crude fat (EE) and 284-420 g kg^{-1} DM for nitrogen-free extract (NFE). In Paper IV, the reported proximate composition of the test diets was 137-170 g kg^{-1} DM for ash, 280-391 g kg^{-1} DM for crude protein, 70.5-108 g kg^{-1} DM for crude fibre, 88.2-128 g kg^{-1} DM for EE and 203-403 g kg^{-1} DM for NFE. The fixed proportion of test ingredient inclusion (30% of DM in Paper III and 50% of DM in Paper IV) in the experimental diets, in combination with the large variation in proximate chemical composition between test ingredients, resulted in a wide range of proximate chemical composition in the test diets (Table 8).

5.3 Feed digestibility in Nile tilapia (Paper III)

In Paper III, the apparent digestibility (AD, %) of DM, OM and CP was unaffected ($P>0.782$-0.901) by faeces collection method (*i.e.* siphoning or stripping) (Table 9). The correlation coefficient (r) between AD values for DM, OM and CP was 0.98, 0.99 and 0.93, respectively, following siphoning and stripping. The AD (%) of DM, OM, CP and gross energy (GE) in the test ingredients differed ($P<0.0001$). The AD (%) of DM and OM was lowest for diets containing brewery spent yeast (BSYD) and duckweed (DWD), followed in increasing order by diets containing moringa leaves (MLD), marine shrimp (MSHD), fish frames (FFD), freshwater shrimp (FSHD) and cattle blood (CBD). In general, the AD (%) of CP was high (>76%), but with a low value (72%) for the duckweed diet (DWD) (Table 9). The AD (%) of GE was closely correlated ($r=0.96$) with the AD of dietary organic matter.

Table 8: *Proximate chemical composition (g kg^{-1} DM) of test diets fed to Nile tilapia (Oreochromis niloticus) in Papers III and IV*

	Paper III					Paper IV				
Diet[1]	Ash	Crude protein	Crude fibre	Ether extract	Nitrogen free extract	Ash	Crude protein	Crude fibre	Ether extract	Nitrogen-free extract
FMD	121	322	68.2	102	387	145	308	70.5	107	370
BSYD	133	342	52.7	76	396	137	391	108	113	251
MLD	124	315	63.2	78.9	420	-	-	-	-	-
FSHD	219	366	51.4	80.2	284	170	367	94.1	110	259
MSHD	152	407	53.9	88.7	299	-	-	-	-	-
CBD	96.9	477	47.8	81.4	297	155	280	73.6	88.2	403
DWD	141	285	82.9	79.7	412	-	-	-	-	-
FFD	222	331	49.8	113	284	157	352	161	128	203

[1]FMD: Fishmeal diet (reference); BSYD: brewery spent yeast diet; MLD: moringa leaf diet; FSHD: freshwater shrimp diet; MSHD: marine shrimp diet; CBD: cattle blood diet; DWD: duckweed diet; FFD: fish frames diet.

Table 9: *Apparent digestibility (AD_, %) of dry matter (DM), organic matter (OM), crude protein (CP) and gross energy (GE) of the reference fishmeal diet (FMD) and test diets fed to Nile tilapia (Oreochromis niloticus) juveniles, determined on faeces collected by siphoning or stripping (Paper III)*

Diet	AD_DM	AD_OM	AD_CP	AD_GE
Siphoning				
FMD	25.5^{a*}	37.7^{a}	76.9^{a}	44.4^{a}
BSYD[1]	23.5^{a}	29.5^{b}	75.8^{a}	37.4^{b}
MLD	29.8^{ab}	38.4^{a}	78.4^{ab}	40.8^{ab}
FSHD	41.3^{c}	51.6^{c}	84.5^{b}	58.1^{c}
MSHD	33.3^{b}	41.3^{a}	79.5^{ab}	49.2^{d}
CBD	46.4^{d}	61.6^{d}	80.9^{bc}	78.0^{e}
DWD	21.6^{a}	27.9^{b}	73.1^{ad}	35.3^{b}
FFD	36.0^{b}	49.1^{c}	76.9^{a}	52.7^{d}
SEM[2]	1.07	1.09	0.69	0.93
P-value	<.0001	<.0001	<.0001	<.0001
Stripping				
FMD	27.6^{a}	39.6^{a}	75.5^{a}	-
BSYD	24.0^{b}	28.8^{b}	76.8^{a}	-
MLD	28.3^{a}	36.2^{ab}	78.5^{ab}	-
FSHD	40.3^{d}	51.3^{c}	83.6^{b}	-
MSHD	32.5^{c}	40.3^{a}	82.1^{b}	-
CBD	47.2^{e}	64.4^{d}	79.7^{ab}	-
DWD	23.6^{b}	30.1^{b}	72.4^{ac}	-
FFD	37.8^{d}	50.3^{c}	76.4^{ab}	-
SEM	0.81	0.87	0.76	
P-value	<.0001	<.0001	<.0001	
Comparison of faeces collection methods				
Siphoning	32.2	42.1	78.2	-
Stripping	32.6	42.6	78.1	-
SEM	1.72	2.31	0.77	-
P-value	0.782	0.828	0.901	-

[1]BSYD: brewery spent yeast diet; MLD: moringa leaf diet; FSHD: freshwater shrimp diet; MSHD: marine shrimp diet; CBD: cattle blood diet; DWD: duckweed diet; FFD: fish frames diet.

[2]SEM: standard error of the mean.

[*]Values within columns with different superscript letters are significantly different as determined by Tukey's test at P<0.05.

5.4 Performance in Nile tilapia (Paper IV)

The test diets used in Paper IV gave good growth rates (Figure 10). However, fish fed the reference fishmeal diet (FMD) showed the highest growth rate for the entire experimental period. This was confirmed by FMD giving the highest weight gain (WG), the highest average daily weight gain (ADWG) and the highest specific growth rate (SGR) in the entire trial (Figure 10, Table 10). The diets containing cattle blood (CBD), fish frames (FFD) and brewery spent yeast (BSYD) produced lower weight gain than the reference (fishmeal-based) diet and all test diets produced lower ADWG than the reference diet. The diets containing freshwater shrimp (FSHD) and brewery spent yeast (BSYD) produced higher SGR than the reference diet (Table 10). There were no differences in condition factor (Kf) or survival rate (SR) between treatments. The high growth rate observed in Paper IV presumably reflected the higher feed intake (FI) for the reference diet compared with other diets (Table 10). Nile tilapia also utilised the reference diet more efficiently than the other diets. However, there was no difference in protein productive value (PPV), viscerosomatic index (VSI) or hepatosomatic index (HSI) between treatments (Table 10).

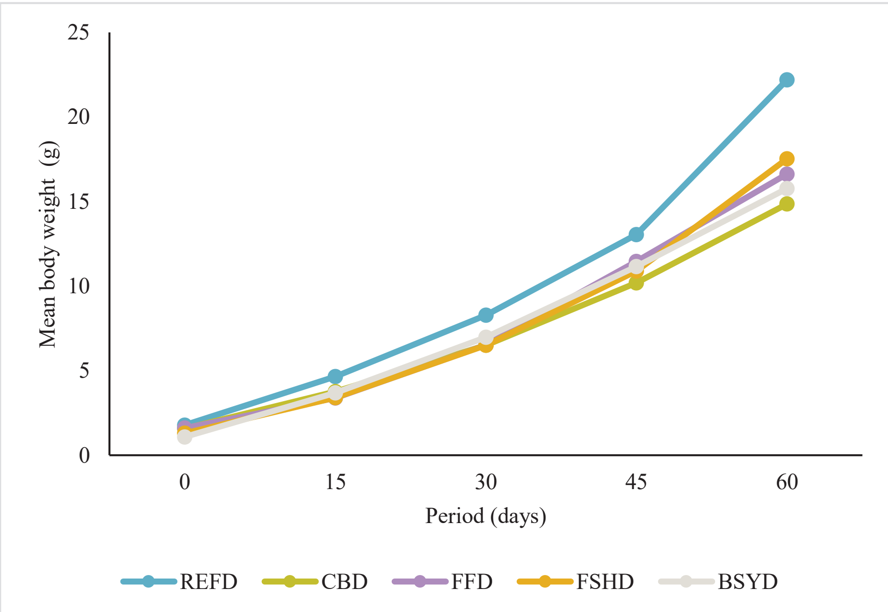

Figure 10: Growth performance over the 60-day feeding period of Nile tilapia (*Oreochromis niloticus*) fed the reference diet (REFD) and test diets containing cattle blood (CBD), fish frames (FFD), freshwater shrimp (FSHD) and brewery spent yeast (BSYD) (Paper IV).

5.5 Carcass quality and whole-body fish composition (Paper IV)

Colour analysis (Minolta Chroma meter, Osaka, Japan) of fish fillets showed no differences (*P*>0.05) between dietary treatments in lightness (L*; 39.7 ± 0.65), redness (A*; 1.96 ± 0.16), yellowness (B*; 5.24 ± 0.17), entire colour index (ECI; 2.91 ± 0.52), hue (3.11 ± 0.23), Chroma (5.65 ± 0.08) or hue angle (69.6 ± 1.49).

There were differences between the initial content of DM, CP, CF and EE and the final content of DM in whole-body fish for fish fed the reference diet and the diet containing freshwater shrimp (FSHD). Similar differences was observed in whole-body fish between initial CP content and the final content of CP on diets containing cattle blood (CBD), fish frames (FFD), freshwater shrimp (FSHD) and brewery spent yeast (BSYD), while initial CF differed from the final content of CF for all dietary treatments, and initial and final content of EE differed for diets FSHD and BSYD. There were no differences between the initial and final NFE value in whole-body fish for any of the dietary treatments.

5.6 Water quality (Paper IV)

Mean water temperature (°C) was higher (23.98 °C; P<0.002) for fish fed the reference diet (fishmeal-based) than for fish fed the test diets (23.91 °C). There were no differences (P>0.05) between treatments in other water quality parameters. The range of values obtained (mean ± SD) was 5.9-6.66.25 ± 0.58 mg L^{-1} for dissolved oxygen, 23.9-24.0 ± 0.04 °C for temperature, 8.7-8.9 ± 0.06 for pH, 3.7-3.8 ± 0.09 ppt for salinity, 1.1-1.9 ± 0.39 mg L^{-1} for ammonia, 3.7-5.8 ± 1.56 mg L^{-1} for nitrite and 55-60± 7.46 mg L^{-1} for nitrate.

5.7 Economic evaluation of replacing fishmeal with test feed ingredients (Paper IV)

The estimated costs of feed and of producing 1 kg weight gain in tilapia fed the reference and test diets in Paper IV are presented in Table 11. The greatest reduction in cost per kg feed was found for the diet with brewery spent yeast (BSYD, 32.9%), followed by the diets with cattle blood (CBD, 25.3%) and fish frames (FFD, 17.7%). Compared with the reference diet, the feed cost per kg fish weight gain was reduced by 26.2% for fish fed diet BSYD.

Table 10: *Growth indices, feed utilisation indices and somatic indices of Nile tilapia (Oreochromis niloticus) fed the reference diet (REFD) and test diets containing cattle blood (CBD), fish frames (FFD), freshwater shrimp (FSHD) and brewery spent yeast (BSYD) (Paper IV)*

Parameters[1]	REFD	CBD	FFD	FSHD	BSYD	SEM[2]	P-value
Growth indices							
IW (g fish^{-1})	1.78[ab*]	1.58[a]	1.61[a]	1.3[a]	1.08[ac]	0.11	0.014
FW (g fish^{-1})	22.2[a]	14.9[b]	16.6[b]	17.5[ab]	15.8[ab]	0.94	0.029
WG (g fish^{-1})	20.4[a]	13.3[b]	15.0[b]	16.2[ab]	14.7[b]	1.27	0.003
ADWG(g fish^{-1} day^{-1})	0.34[a]	0.22[b]	0.25[b]	0.27[b]	0.25[b]	0.02	0.004
SGR (%)	1.83[ab]	1.62[a]	1.69[a]	1.89[b]	1.94[b]	0.06	0.015
Kf (%)	1.80	1.74	1.69	1.80	1.68	0.05	0.337
SR (%)	100	100	100	100	100	0.00	1.000
Feed utilisation indices							
FI (g fish^{-1})	32.9[a]	26.9[b]	27.1[b]	27.2[b]	26.0[b]	0.99	0.005
FCR	1.61[a]	2.04[b]	1.8[ab]	1.7[ab]	1.77[ab]	0.1	0.072
PER	0.73 [a]	0.52 [b]	0.47 [b]	0.49 [b]	0.42 [b]	0.03	0.0004
PPV (%)	13.46	15.37	16.34	19.39	22.82	2.44	0.142
Somatic indices							
VSI (%)	8.36	11.5	9.94	9.98	9.6	0.75	0.145
HSI (%)	1.2	1.91	1.91	1.69	1.84	0.33	0.549

[1]IW: initial weight; FW: final weight; WG: weight gain; ADWG: average daily weight gain; SGR: specific growth rate; Kf: condition factor; SR: survival rate; FI: feed intake; FCR: feed conversion ratio; PER: protein efficiency ratio; PPV: protein productive value; VSI: viscerosomatic index; HSI: hepatosomatic index.
[2]SEM: Standard error of the mean.
*Values (means) within rows with different superscript letters are significantly different as determined by Tukey-Kramer at $P<0.05$.

Table 11: *Cost of feed[1] (USD kg^{-1}) and feed cost for producing one kg weight gain (WG) in Nile tilapia (Oreochromis niloticus) fed the reference diet (REFD) and test diets containing cattle blood (CBD), fish frames (FFD), freshwater shrimp (FSHD) and brewery spent yeast (BSYD) (Paper IV)*

Diet	Cost[2] (USD kg^{-1})	Relative to control (%)	Decrease in feed cost (%)	FCR	Feed cost (USD kg^{-1} WG)	Relative to control (%)	Decrease in feed cost kg^{-1} WG
REFD	0.80	100.00	0.00	1.61	1.27	100.00	0.00
CBD	0.59	74.68	25.32	2.04	1.20	94.63	5.37
FFD	0.65	82.28	17.72	1.80	1.17	91.99	8.01
FSHD	0.72	91.14	8.86	1.70	1.22	96.23	3.77
BSYD	0.53	67.09	32.91	1.77	0.94	73.76	26.24

[1]Feed costs per kg weight gain = FCR (kg feed/kg weight gain) ×costs of kg feed; exchange rate: 1 US dollar (USD) = 2292 Tanzanian shillings (TSHS).
[2]Local market price (TSHS/kg) for feed ingredients: fishmeal 3000, cattle blood 1000; fish frames 1500; fresh water shrimp 2000; brewery spent yeast 1000; full fat soybean 1500; sunflower seed cake 300; wheat pollard 500; maize bran 350; cassava flour 1200; sunflower seed oil 4000 and vit. & Min (mixture) 4000.

6 General Discussion

6.1 Chemical composition of local feed ingredients in Tanzania

More than 80% of 202 tilapia fish farmers surveyed reported that they use local feed ingredients as a primary dietary supplement for cultured tilapia (Paper I). However, the availability of these local feed ingredients varies from one region to another depending on production season, climate conditions, geographical zone and accessibility (Kaliba *et al*., 2006). Moreover, these ingredients were reported to be used as single or mixed ingredients to supplement the diet of cultured fish. The results were consistent with other findings for the region (Mwaijande & Lugendo, 2015). The most commonly used local feed ingredient in tilapia farming was reported to be maize bran, followed by Lake Victoria sardines, sunflower seed cake, rice bran and wheat pollard (Paper I). Similar observations have been made in previous studies in Tanzania and across the East African region (Chenyambuga *et al*., 2014; Munguti *et al*., 2012; Mwaijande & Prudence, 2015).

Furthermore, the chemical composition of the local feed ingredients analysed in this thesis (Paper I) was generally within the range reported in studies in other countries (Chiba, 2009; Mohanta *et al.,* 2016; Mugo-Bundi *et al.,* 2015; J. Munguti *et al.,* 2012; NRC, 2011). The content of crude protein was medium to high (300->500 g CP kg^{-1} DM) in animal by-products, most agricultural by-products, plant leaves and weeds, aquatic plants and industrial by-products. The crude fat (EE) content was high (>100 g kg^{-1} DM) in some animal by-products and some agricultural by-products. However, the content of EE in most other feed ingredients was medium to low (100 - <50 g kg^{-1} DM). The values obtained deviated from those reported previously for samples collected across East Africa, including Tanzania (Munguti *et al.,* 2012). The variation in chemical composition for similar feed ingredients between studies can be due to many factors, such as animal species, contamination, processing, handling, storage, climate conditions, production season, geographical zone, soil type and stage of maturity at harvest (NRC, 2011; Onyango *et al.,* 2019).

Moreover, most local animal by-product feed ingredients, except for Nile perch fish frames, contained high levels of lysine, tryptophan and methionine + cysteine (Paper I). On the other hand, agricultural by-products (*i.e.* soybean and full fat soybean), plant leaves and weeds (*i.e*. taro leaves, cassava leaves, sweet potato leaves and gallant soldier weed), aquatic plants (*i.e. Azolla* ferns and lettuce vegetables) and spent brewery yeast were intermediate in lysine and methionine + cysteine, but high in tryptophan (0.2-1.5%). This was consistent with findings reported in other studies performed in Spain (Barroso *et al*., 2014) and in Uganda (Kubiriza *et al*., 2016).

Analysis of the mineral composition of commonly used local feed ingredients (Paper II) showed high contents of phosphorus (P), calcium (Ca), potassium (K), sodium (Na), magnesium (Mg), iron (Fe) and iodine (I) in most animal by-products, which was in agreement with findings in previous studies worldwide (Chiba, 2009; Khan *et al.,* 2015; NRC, 2011). However, deviating results have also been reported for some minerals (Carter *et al.,* 2015; NRC, 2011; Odesanya *et al.,* 2011). Moreover, agricultural by-products and plant leaves and weeds showed high levels of K, but low contents of both Na and Mg, as found in previous studies done on pasture vegetation and supplement feedstuffs for domestic ruminants animal in both India and Kenya (Bhanderi *et al.,* 2016; Onyango *et al.,* 2019). However, deviating values have also been reported for commonly used plant leaves in fish farming and in the human diet (Awol, 2014; Dada & Oworu, 2010; Sun *et al*., 2014; Temesgen *et al*., 2016). A very high content of Fe (>2200 mg kg^{-1} DM) was found in aquatic ferns (*Azolla*) and duckweed. Moreover, water lettuce and duckweed showed a high content of Ca, confirming previous findings (Heaton, 2015; Titus & Pereira, 2006). Different growing conditions, genetic factors, geographical zone, differences in efficiency of mineral uptake and stage of maturity can explain differences between studies (Bhowmik *et al*., 2012; Mayer & Gorham, 1951; NRC, 2011; Onyango *et al*., 2019).

6.2 Experimental diets (Paper III & IV)

The fixed proportion of test ingredient (30% of DM) included in the experimental diets used in Paper III, in combination with the large variation in proximate chemical composition between these test ingredients, resulted in a wide range of proximate chemical composition in the test diets. The crude protein content of the dietary treatments tested ranged between 285 and 477 g CP kg^{-1} DM in Paper III and between 280 and 391 g CP kg^{-1} DM in Paper IV. In general, the content of crude protein was within the dietary CP requirement for tilapia reported in previous studies (El-Sayed & Teshima, 1992; Liti *et al*., 2006). Most of the commercial feed produced and supplied to tilapia fish farmers in Tanzania contains 238-334 g CP kg^{-1} DM (Paper I). However, higher dietary CP requirements for tilapia have been reported elsewhere (Abdel-tawwab, 2004; Abdel-Tawwab *et al.,* 2010; A.-F. M. El-Sayed, 2006). Moreover, conflicting results on the effect of dietary crude protein level on growth of Nile tilapia have been reported (Abdel-tawwab, 2004). The discrepancy in effects and in reported dietary crude protein requirements of growing tilapia could be due to culture facilities, fish size and age, quality of diet ingredients, environmental parameters and physiological status of the cultured fish.

6.3 Digestibility

It was found that fishmeal (reference), freshwater shrimp, marine shrimp, cattle blood, fish frames, brewery spent yeast, duckweed and moringa leaves, all collected in Tanzania, have acceptable protein digestibility for use in tilapia diet formulation (Paper III). However, the low energy digestibility observed for brewery spent yeast and moringa leaves may limit their use for young growing fish. Overall, there is great potential for using all seven feed ingredients tested to replace fishmeal in diets for tilapia in Tanzania. Variations in the quality and quantity of dietary nutrients may influence digestibility and growth performance in fish (Lundstedt *et al.,* 2004; Montoya-Mejía *et al.,* 2017). However, the digestibility of nutrients and energy differs from one fish species to another and even within fish species, depending on age, sex, species, water temperature and diet composition (NRC 2011).

6.4 Growth performance and feed utilisation

The results obtained in this thesis indicated that final body weight (FBW), total weight gain (WG) and average daily weight gain (ADWG) in tilapia juveniles were unaffected by the selected local feed ingredients used to replace fishmeal in the diet (Paper IV). Moreover, with the exception of poor feed conversion ratio (FCR) for the diet containing cattle blood, the FCR and protein efficiency ratio (PER) did not differ between the local feed ingredients tested in Paper IV. However, growth performance, FCR and PER were better with the fishmeal-based reference diet than with the diets where 50% of the fishmeal was replaced with local ingredients. This was related to the strong positive correlation coefficient (r=0.86) observed between feed intake and growth performance of the fingerlings in Paper IV. Moreover, there were no differences in survival rate and condition factor (Kf) between the dietary treatments in Paper IV. The similar Kf values indicated that all treatment diets had similar proportional impact on fish length in relation to weight. The high survival rate showed that the culture conditions during the study were good (Abdel-Tawwab *et al.,* 2010; Ahmad *et al.,* 2004; Ayisi *et al.,* 2017; Ighwela *et al*., 2011).

The values obtained for feed utilisation indices in Paper IV were within the range reported for Nile tilapia in previous studies (Abdel-Tawwab *et al*., 2010; Al-Souti *et al*., 2012; Fry *et al*., 2018; Soltan *et al*., 2017; Damusaru *et al*., 2019). The value of the somatic indices (VSI and HSI) obtained in Paper IV were also comparable with those in previous studies (Abdel-Tawwab *et al.,* 2010; Ayisi *et al.,* 2017; dos Santos *et al.,* 2019; Obirikorang *et al.,* 2016).

6.5 Proximate analysis of whole-body fish and carcass quality traits

Differences between initial and final whole-body chemical composition in tilapia were found in Paper IV, and have also been reported by others (Al-Souti *et al.,* 2012; A. F.M. El-Sayed, 1998; Goda *et al.,* 2007; Mugo-Bundi *et al.*, 2015). Variations in the whole-body chemical composition of fish can be explained by differences in deposition rate in muscle and/or different growth rates, different levels of nutrients in the diet and differences in the physiological ability of the cultured fish to convert diets into absorbable nutrients (Abdel-Tawwab *et al.,* 2006; Mugo-Bundi *et al.,* 2015). The initial whole-body crude protein levels were lower than those found at the end of the feeding trial (Paper IV), which was in agreement with Al-Souti *et al*. (2012). Moreover, the initial whole-body crude fat levels were higher than those found at the end of the trial (Paper IV). In contrast, initial and final whole-body ash and NFE content remained unchanged, which was in agreement with findings in other studies (Goda *et al.,* 2007; Mugo-Bundi *et al.,* 2015). The marked reduction in the whole-body content of crude fibre was a reflection of a decrease in the relative proportion of chitin and bones as the muscle mass increased with age. The differences in whole-body chemical composition were not reflected in any of the fillet colour parameters measured in Paper IV.

6.6 Water quality parameters

The water quality parameters recorded remained stable throughout the experimental period (60 days) and did not differ between treatments (Paper IV). The values were within the range reported in the literature for groundwater in Tanzania (Elisante & Muzuka, 2017; Makoba & Muzuka, 2019) and judged suitable for cultured tilapia (Balogun *et al.,* 2005; Makori *et al.,* 2017; Monsees *et al.,* 2017; Wang *et al.,* 2006). Water quality plays a significant role in the biology and physiology of fish (Cho & Kaushik, 1990). However, the quality of water varies from one region to another depending on the type and source of water used, farming system (tanks, cages or ponds), climate conditions, geology, geomorphology, land use and level of precipitation (Elisante & Muzuka, 2017; Makori *et al*., 2017; Nkotagu, 1996). Deviating water quality parameters in the rearing environment of cultured tilapia probably compromise fish health, result in retarded growth and cause mortality or harmfully physiological responses such as osmo-regulatory disturbances and kidney damage (Ruyet *et al.,* 1997; Wang *et al.,* 2006; Zeitoun *et al.,* 2016). However, the tolerance of cultured fish to water quality differences also varies, depending on fish health status, size and age of the fish, type of culture system used and climate conditions (Balogun *et al.,* 2005; Makori *et al.,* 2017; Wang *et al.,* 2006).

6.7 Economic evaluation

The feed cost analysis in Paper IV showed that using the test diets reduced the cost of fish feed by up to 33% relative to the reference diet, while the feed cost to produce 1 kg biomass of fish was reduced by up to 26%. Similar findings have been reported previously, *e.g*. in a study done in Egypt, Soltan *et al.* (2008) found that replacement of up to 45% fishmeal with a plant protein mixture in Nile tilapia diets reduced feed costs per kg diet by 11.4% and feed costs per kg weight gain by 6.7%. However, 100% replacement by the protein plant mixture was required to reduce the feed costs to 26% (Soltan *et al.,* 2008), whereas this level was achieved with 50% replacement of fishmeal with brewery spent yeast in this thesis. The results in this thesis indicate that replacing fishmeal in the diet of farmed tilapia can improve the production economics for small-scale fish farmers in Tanzania. In general, fish feed accounts for over 50% of the production costs in both semi-intensive and intensive aquaculture production system (A.-F. M. El-Sayed, 2004; Watanabe, 2002). In commercial fish production, fishmeal accounts for 20-60% of the feed cost (De Silva & Anderson, 1995). However, prior to this thesis work there was limited information on the economic value of fishmeal alternatives in feed for cultured tilapia (Ogello *et al.,* 2014).

7 General conclusions

- Novel information on the nutritive value of locally available feed ingredients from different regions of Tanzania is provided in this thesis (Paper I). This information will provide guidance on production of good quality feed at a low-cost. A balanced nutritional profile can be obtained by including more than one local feed ingredient in the diet of cultured tilapia.
- Wide ranges of mineral concentrations were detected in local feed ingredients used by tilapia fish farmers in Tanzania (Paper II). The data suggest that using two or more local ingredients in the diet may be sufficient to meet the mineral requirements of all cultured tilapia species and their hybrids, without the need for any mineral premix.
- The apparent digestibility (%) of dietary dry matter, organic matter and crude protein was unaffected by faeces collection method (*i.e.* siphoning or stripping) (Paper II).
- The dietary protein digestibility data obtained in this thesis suggested that all seven local feed ingredients tested (freshwater shrimp, marine shrimp, cattle blood, fish frames, brewery spent yeast, duckweed and moringa leaves) can be used to replace fishmeal without any reduction in protein value. However, the low energy digestibility of brewery spent yeast and moringa leaves may limit their use for young growing fish (Paper III).
- Diets fed to tilapia fingerlings where 50% of the fishmeal dry matter was replaced with alternative local feed ingredients (freshwater shrimp, cattle blood, fish frames, brewery spent yeast) resulted in efficient feed utilisation and proportional high growth rate (Paper IV).
- There were no differences in condition factor, survival rate and protein productive value between the reference and test diets. There were also no differences in hepatosomatic index and viscerosomatic index, or in

fillet quality traits in terms of chemical composition of whole-body fish and fillet colour evaluation, between the reference and test diets (Paper IV).

- No significant changes in water quality parameters were observed over a 60-days growth trial, and thus variations observed in fish growth performance and nutrient utilisation were not influenced by water quality parameters (Paper IV).
- Replacement of fishmeal in the diet reduced feed costs per kg feed and per kg body weight gain (Paper IV).
- The profitability of small-scale tilapia production in Tanzania can be improved by introduction of good quality, low-cost locally available feed ingredients as a replacement for fishmeal in feed.

8 Future perspectives

The number of fish farming operations in Tanzania is continuing to increase, which is leading to high demand for quality fish feed at affordable prices. This thesis investigated some cheap protein source ingredients locally available in Tanzania that had not been investigated previously. It was found that these feed ingredients could partially or completely replace fishmeal in tilapia diets to up to 50% of dry matter without affecting fish growth performance. In order to build upon the findings of this thesis, future studies should address the following issues relating to tilapia farming in Tanzania:

- The effects of diets containing local feed ingredients on optimal stocking density and feeding regime of tilapia. This information is needed to avoid undernourished cultured fish and environmental degradation linked to overfeeding.
- The impact of diets containing local feed ingredients on farms located in different geographical zones with different environmental conditions, particularly brackish water and fresh water. The culture environment can affect fish growth performance and feed utilisation
- The on-farm economic outcome of using diets containing local feed ingredients. An economic evaluation could help identify the pros and cons of using available non-conventional feed ingredients for long-term sustainable production and for economic profitability.

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Popular science summary

With an increasing global population, the demand for animal protein, including fish protein, is increasing. Wild catches of fish have reached an upper limit, so increases in the supply of fish protein will have to be achieved through fish farming. A major challenge to sustainable expansion of fish farming operations is limited access to sustainable high-quality feed at a reasonable cost. To date, the major feed ingredients in farmed fish feed have been fishmeal, fish oil and soybean meal. However, due to limited availability of and competition for these feed ingredients in the animal feed industry, the cost of fish feed is high.

Further development of the fish farming industry in Tanzania and in many other low-income countries will not be possible unless the availability of good-quality fish feeds at affordable prices can be increased. Use of expensive fishmeal and soybean meal as primary protein sources in farmed fish feed is not economically feasible for small-scale fish farmers. Therefore, the majority of fish farmers across the sub-Saharan region, including Tanzania, are reliant on locally available feed ingredients to feed their cultured fish.

Local feed ingredients, such as cattle blood from abattoirs, industrial by-products like brewery spent yeast and Nile perch skeletal remains (fish frames) are currently discarded and/or are available at a low cost in Tanzania. Agricultural by-products, such as maize bran, rice polish, wheat pollard and rice bran are also available at a low cost, especially during the crop production season. Aquatic plants like mosquito fern, water lettuce and duckweed can be found growing wild in marshes, pools and stagnant water during the rainy period, both in winter and spring. Freshwater and marine shrimps are available as by-catch from freshwater and marine sardine fishery, respectively. Before these cheap local ingredients can be used in fish feed in Tanzanian fish farming, the growth performance of fish fed diets containing these ingredients must be determined. The international literature indicates that freshwater shrimp (*Caridina nilotica*), cattle blood and brewery spent yeast can replace up to 50 %

of fishmeal in the diet of Nile tilapia (*Oreochromis niloticus*) without any adverse effect on growth and feed utilisation, whereas complete replacement (100 %) of fishmeal can reduce growth and impair feed utilisation. In general, however, very limited information is available on the potential nutritive value of non-conventional feed ingredients for fish farming. Hence, studies on the potential of locally available feed ingredients for use in diets for cultured fish, to replace expensive imported feed ingredients (*i.e.* fishmeal and soybean meal) are relevant. Some studies on the growth performance of Nile tilapia fed diets containing local feed ingredients such as moringa leaves have been performed in Tanzania. However, prior to this thesis, no information had been published on diets containing freshwater shrimp, cattle blood, brewery spent yeast or fish frames for small-scale tilapia production in Tanzania.

An initial field study showed that the majority (about 80 %) of over 200 small-scale fish farmers surveyed in different regions of Tanzania relied on locally available feed ingredients to feed their cultured tilapia. Analysis of the protein and amino acid content of these ingredients showed high to medium concentrations in animal and agricultural by-products. Analysis of the mineral content in selected local feed ingredients collected during the field survey revealed a wide range of values. The highest level of phosphorus was found in fish skeletons (fish frames), of calcium in limestone, of potassium in gallant soldier plant, of sodium in marine shrimp, of magnesium in prawn head waste, of iron in aquatic ferns, and of iodine in full fat soybean.

Growth experiments using tilapia were performed to investigate the nutritive value of the locally available feed ingredients used by fish farmers in Tanzania. Seven local feed ingredients with high protein and amino acid content were selected for the experiments and assessed for their potential to replace fishmeal in the diet of tilapia. These ingredients were: brewery spent yeast, freshwater shrimps, marine shrimps, cattle blood, moringa leaves, duckweed and fish frames. Their potential nutritive value was assessed in digestibility studies with Nile tilapia. The feed ingredients with the highest protein digestibility and with a suitable amino acid profile (cattle blood, freshwater shrimp, fish frames and brewery spent yeast) were tested in a growth performance study with Nile tilapia. In addition to growth performance, carcass traits were evaluated. Fishmeal was used in the reference diet, which was compared with test diets where 50 % of the fishmeal was replaced with the selected feed ingredients.

The results showed that good-quality tilapia feed can be produced by replacing up to 50 % of the fishmeal with non-conventional feed ingredients locally available in Tanzania (freshwater shrimp, cattle blood, fish frames and brewery spent yeast). The use of these alternative ingredients can also reduce the cost of feed by up to 33 % compared with a fishmeal-based diet, and can reduce the feed cost of producing one kg tilapia by up to 26 %. In addition, instead of feeding only one or two local ingredients to the fish, which is commonly practiced among small-scale fish farmers in Tanzania, the use of the complete balanced diets proposed in this thesis could result in that fish reach the desired market size faster. A shorter culture period leads to reduced amount feed used, which would balance the slightly higher cost of a complete fish feed compared to single ingredients with low protein content.

Thus, a change in feeding strategy for cultured tilapia in Tanzania would improve the profitability of small-scale production and also contribute to the long-term sustainability of production. However, large-scale on-farm studies are needed to identify critical and possible limiting factors related to feeding and management in culture systems based on feeding locally available feed ingredients.

Populärvetenskaplig sammanfattning

Med en ökande global befolkning ökar efterfrågan på animaliskt protein, inklusive fisk. Fångster från vildfisket har nått en övre gräns, vilket innebär att den ökande efterfrågan på fisk måste mötas genom ökad odling av fisk. En av de största utmaningarna för en hållbar expansion av fiskodlingen är den begränsade tillgången till miljövänligt och högkvalitativt foder till en rimlig kostnad. Hittills har de viktigaste ingredienserna i fiskfoder utgjorts av fiskmjöl, fiskolja och sojamjöl. På grund av begränsad tillgång till dessa foderingredienser, delvis beroende på konkurrens mellan olika djurslag inom djurfoderindustrin, blir kostnaden för fiskfoder hög.

Vidareutveckling av fiskodlingsindustrin i Tanzania och i många andra låginkomstländer kommer inte att vara möjlig om tillgången på högkvalitativt foder inte kan fås till överkomliga priser. Användning av fiskmjöl och sojamjöl som primära proteinråvaror i fiskfoder är inte ekonomiskt möjligt för småskaliga fiskodlare. Därför är majoriteten av fiskodlarna i regionen söder om Sahara, inklusive Tanzania, beroende av lokalt tillgängliga alternativa råvaror som kan ingå i foder till odlad fisk.

Lokala foderingredienser såsom nötkreatursblod från slakterier, fiskrens från nilabborre och industriella biprodukter i form av bryggerijäst kastas för närvarande och/eller är tillgängliga till en låg kostnad i Tanzania. Jordbruksbiprodukter som majskli, rispulver, vetekli och riskli finns också till en låg kostnad, särskilt under skördesäsongen. Vattenväxter som mossbräken, vattensallad och andmat kan växa vilt i våtmarker, fiskdammar och i stillastående vatten under regnperioden, både under vintern och på våren. Både söt- och saltvattensräkor finns tillgängliga som bifångster från fiske. Innan dessa billiga lokalt producerade ingredienser kan användas i foder till fisk i Tanzania måste tillväxtprestanda för fiskfoder som innehåller dessa ingredienser bestämmas. Den internationella litteraturen indikerar att sötvattensräka (*Caridina nilotica*), blod från nötkreatur och bryggerijäst kan ersätta upp till 50

% av fiskmjölet i foder till Niltilapia (*Oreochromis niloticus*) utan att negativt inverka på tillväxt eller foderutnyttjande, medan en 100-procentig ersättning av fiskmjöl med dessa alternativa ingredienser kan minska tillväxten och försämra foderutnyttjandet. Det finns mycket begränsad information om det potentiella näringsvärdet för icke-konventionella foderingredienser till odlad fisk. Följaktligen är det relevant att studera potentialen för lokalt tillgängliga foderingredienser, för användning i fiskfoder till odlad fisk, för att ersätta dyra importerade foderingredienser (dvs. fiskmjöl och sojamjöl). Endast ett begränsat antal tillväxtstudier på Niltilapia utfodrade med foder innehållande lokala foderingredienser (moringablad) har utförts i Tanzania. Före denna avhandling hade ingen information publicerats om fiskfoder för småskalig tilapiaproduktion i Tanzania som baseras på sötvattensräka, blodmjöl, bryggerijäst från nöt eller fiskrens.

En första fältstudie visade att majoriteten (cirka 80 %) av över 200 småskaliga fiskodlare som intervjuades i olika regioner i Tanzania förlitade sig på lokalt tillgängliga foderingredienser till odlad tilapia. Analys av protein- och aminosyrainnehållet i lokala foderingredienser visade på höga till medelhöga koncentrationer i animaliska produkter och i biprodukter från jordbruket. Analys av mineralinnehållet i utvalda lokala foderingredienser som samlats in under fältstudien avslöjade en stor spännvidd i mineralkoncentrationer. De högsta nivåerna för fosfor hittades i fiskrens, för kalcium i kalksten, för kalium i växten gängel, för natrium i saltvattensräkor, för magnesium i rens från räkor (räkhuvuden), för järn i vattenlevande ormbunksväxter och för jod i oprocessade sojabönor.

Tillväxtförsök med Niltilapia genomfördes för att undersöka näringsvärdet för de lokalt tillgängliga foderingredienserna som används som tillskottsfoder av fiskodlare i Tanzania. Sju lokala foderingredienser med högt protein- och aminosyrainnehåll valdes för experimenten och bedömdes med avseende på deras potential att ersätta fiskmjöl i foder till tilapia. Dessa ingredienser var: bryggerijäst, sötvattensräkor, saltvattensräkor, nötkreaturblod, moringablad, andmat och fiskrens. Deras potentiella näringsvärde utvärderades i matsmältningsstudier med Niltilapia. De foderingredienser som visade sig ha den högsta proteinsmältbarheten och lämplig aminosyraprofil (blodmjöl från nöt, sötvattensräka, fiskrens och bryggerijäst) testades sedan i en tillväxtstudie med Niltilapia. Förutom tillväxtprestanda utvärderades slaktegenskaperna. Fiskmjöl användes i en referensdiet som jämfördes med testdieter där 50 % fiskmjöl ersattes med de utvalda foderingredienserna.

Resultaten visade att fiskfoder av god kvalitet kan produceras genom att ersätta upp till 50 % av fiskmjölet med icke-konventionella foderingredienser som är lokalt tillgängliga i Tanzania (sötvattensräka, blodmjöl från nöt, fiskrens och bryggerijäst). Användning av dessa alternativa ingredienser kan också reducera foderkostnaderna med upp till 33 % jämfört med en fiskmjölbaserad diet och kan minska foderkostnaderna för att producera 1 kg tilapia med upp till 26 %. Resultaten indikerar också att, istället för att utfodra fisken med en eller två lokala foderingredienser som vanligtvis praktiseras bland småskaliga fiskodlare i Tanzania, kan användningen av de fullständigt balanserade dieterna som föreslås i denna avhandling leda till att fisken når den önskade marknadsstorleken snabbare. En kortare odlingsperiod leder till minskade mängder foder som används, vilket skulle balansera den något högre kostnaden för ett komplett fiskfoder jämfört med enstaka foderingredienser med lågt proteininnehåll.

En förändring i utfodringsstrategin för odlad tilapia i Tanzania skulle således förbättra lönsamheten i småskalig produktion och också bidra till en hållbar produktion på lång sikt. Emellertid krävs storskaliga studier på fiskodlingar för att identifiera kritiska och möjliga begränsande faktorer relaterade till utfodring och hantering i odlingssystem vid utfodring med lokalt tillgängliga foderingredienser.

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This thesis investigated good quality low-cost fish diets in which fishmeal was replaced by up to 50 % with potential local feed ingredients available in Tanzania. The fish diets were utilized efficiently without negative consequences for cultured Nile tilapia (*Oreochromis niloticus*), and reduced feed cost by up to 33 %. The results indicate that the economics of small-scale tilapia production in Tanzania could be improved by replacing fishmeal with locally available feed ingredients.

Francis Pius Mmanda received his postgraduate education at the Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences. He obtained his Master of Science degree in Aquaculture from Ningbo University in China.

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